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Short Communication

Physical protection regulates microbial thermal responses to chronic soil warming

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ABSTRACT

Climate warming can affect the temperature sensitivity of microbial activity and growth efficiency, possibly explained by changes to microbially unavailable carbon (C) protected within in soil aggregates. We assessed physical protection by crushing macroaggregates (250–2000 μm) and microaggregates (<250 μm) isolated from mineral soils exposed to 27 years of experimental warming (+5 °C). We hypothesized that removal of physical protection would increase microbial activity and reduce C use efficiency (CUE). We found that crushing increased microbial respiration and biomass turnover rate, but did not affect CUE. We also hypothesized that long-term warming would reduce the effect of physical protection on microbial activity, and that physical protection would attenuate microbial temperature sensitivity in heated compared to control soils. We found that long-term warming was associated with a smaller effect of physical protection for microbial respiration but with a larger effect for biomass turnover rate in macroaggregates. Physical protection reduced the temperature sensitivity of respiration but enhanced the temperature sensitivity of microbial biomass turnover rate in heated compared to control soils. Our work shows that long-term warming has contrasting effects on how microbial respiration, biomass turnover rate, and their thermal responses are mediated by physical protection within soil aggregates.

Climate warming accelerates soil organic matter (SOM) decomposition through several mechanisms, including enhanced microbial activity, altered microbial physiology (e.g., growth efficiency and biomass turnover), and potentially, declines in the physical protection of SOM within soil aggregates (Bailey et al., 2013; Conant et al., 2011). Physical protection makes SOM inaccessible to microbes and suppresses SOM decomposition (Six et al., 2002; Waring et al., 2020) and thus has the potential to attenuate the temperature responses of microbial carbon (C) cycling processes (Gillabel et al., 2010; Plante et al., 2009). The quantity and quality of occluded SOM within aggregates may also affect temperature responses of SOM decomposition (Wankhede et al., 2020; Waring et al., 2020). Microbial C use efficiency (CUE) can also affect soil C loss, depending on SOM quality and accessibility to microbes (Frey et al., 2013). However, it remains unclear how physical protection of SOM affects microbial thermal responses to long-term warming, especially in different soil aggregate size fractions.

Long-term warming may reduce the protective capacity of aggregation and thus alter microbial thermal responses. SOM can be occluded within aggregates or bound to mineral surfaces (Bandyopadhyay, 2020; Liu et al., 2021; Tian et al., 2015) and this physical protection is generally weaker at lower SOM levels (Goebel et al., 2009). Long-term warming reduces SOM stocks (Frey et al., 2008; Melillo et al., 2017; Pold et al., 2017), with potential feedbacks to physical protection mechanisms. Removal of physical protection, however, may accelerate biomass turnover rate and reduce CUE due to overflow respiration caused by imbalance in stoichiometry (Manzoni et al., 2012) or by increased enzyme production to decompose complex substrates (Geyer et al., 2019). Soil organic matter in macroaggregates has less physical protection than microaggregates (Poeplau et al., 2017; Six et al., 2002), more large pores, and easier nutrient movement (Liu et al., 2021; Waring et al., 2020). Because prior field studies have observed of adaptation of temperature sensitivity (Bradford et al., 2008; Melillo

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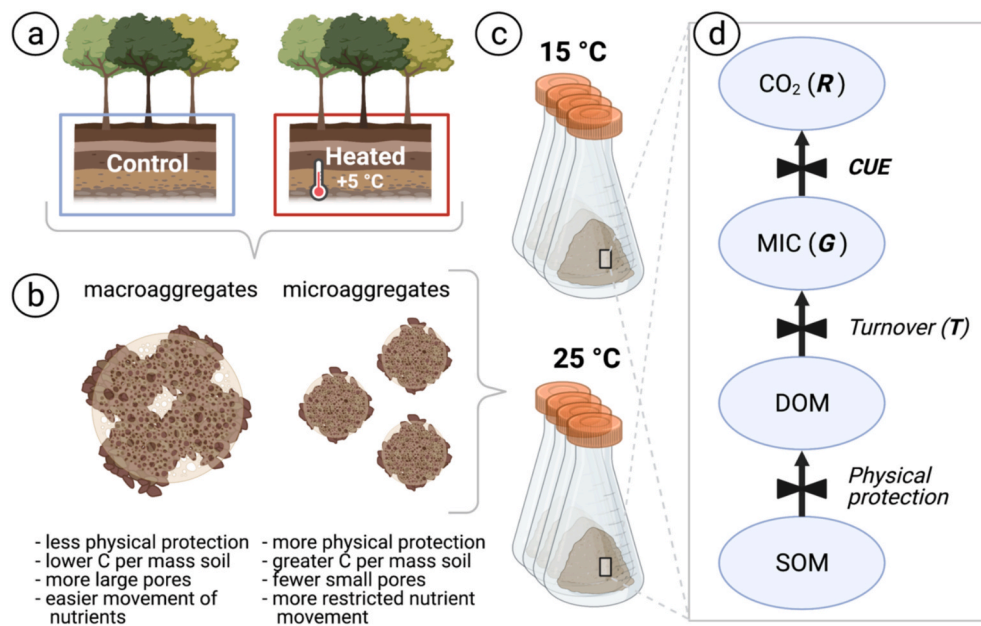


Fig. 1. Conceptual diagram to understand how chronic warming affects on microbial activity in soil compartments that vary in physical protection. (a) Mineral soils were collected from a long-term field warming experiment at Harvard Forest, where control soils were sampled alongside soils that have been heated 5 °C above ambient temperatures for 27 years. (b) Soils were optimal-moisture sieved to separate microaggregates (<250 μm) and macroaggregates (250–2000 μm). (c) Aggregates were incubated at two temperatures to understand temperature sensitivity of microbial activity and growth efficiency. (d) Microbial activities were measured to understand how soil organic matter (SOM) was made microbially available as dissolved organic matter (DOM), consumed as part of microbial biomass turnover rate (T) to increase growth (G), with microbial carbon use efficiency (CUE) modulating how much CO₂ was respired (R).

Table 1
Hypothesized and experimental results for the effect of physical protection on microbial activities in different soil aggregates after long-term warming.

Microbial response	Parameter	Hypothesized results				Experimental results			
		MA (0.25-2 mm)		MI (<0.25 mm)		MA (0.25-2 mm)		MI (<0.25 mm)	
		Control	Heated	Control	Heated	Control	Heated	Control	Heated
Activity	R	++	+	+++	++	++	+	++	+
	G	++	+	+++	++	0	0	0	0
	T	++	+	+++	++	+	++	+	++
	CUE	--	-	---	--	0	0	0	0
Q ₁₀	R	+	0	++	+	-	0	-	0
	G	+	0	++	+	0	0	0	0
	T	+	0	++	+	0	+	0	+
	CUE	+	0	++	+	+	0	0	0

Note: MA, macroaggregates; MI, microaggregates; R, respiration; G, growth rate; T, microbial biomass turnover rate; CUE, carbon use efficiency. +, -, and 0 indicate the positive, negative, or nil effect of crushing (vs intact; as an index of physical protection); blue and red cells indicated negative and positive effects of physical protection; more symbols (+ or -) and darker colors indicating stronger effects of physical protection.

et al., 2017), microbial activity in macroaggregates may be more responsive to long-term warming.

Here, we used aggregate crushing as an index of physically protected SOM (Bischoff et al., 2017) in an experiment to investigate how protection of SOM in different sizes of aggregates affects microbial responses to long-term warming (Fig. 1). Because physical protection tends to include more complex C compounds, we hypothesized that 1) removal of physical protection would increase microbial activities (respiration, growth, biomass turnover rate) but reduce CUE. Because chronically warmed soils tend to have lower microbial biomass and more complex SOM after long-term warming, we further hypothesized that 2) soils exposed to long-term warming have a smaller effect size of physical protection on microbial activities and CUE compared to control plots. Finally, due to declined substrate availability with warming (Table 1), we hypothesized that 3) the effect size of physical protection on the temperature sensitivity (Q₁₀) of microbial activities would be

smaller in the heated compared to control plots.

Mineral soil samples (0–10 cm; pH 4.7, sandy Typic Dystrudepts) were collected (steel core; 5.0 cm dia.) from 27-year heated (+5 °C) and ambient control plots (6 × 6 m; four replicates) at the Harvard Forest Long-Term Ecological Research (LTER) site (Petersham, MA, USA) (Melillo et al., 2017). The soil was sieved (<2 mm) and allowed to dry under controlled conditions (4 °C) to a constant moisture (~10%) to facilitate isolation of microaggregates (<250 μm) and macroaggregates (250–2000 μm) and to minimize disturbance effect on microbial communities (Bach and Hofmøckel, 2014; Liu et al., 2021; Tian et al., 2015). Half of the aggregates were crushed using a mortar and pestle, and the other half were kept intact. Aggregates were preincubated at 40% water holding capacity (WHC) at 15 or 25 °C for one week to account for the disturbance effect of crushing, so that effects of physical protection could be isolated (Liu et al., 2021). Crushed and intact aggregates were amended with H₂¹⁸O (97 atom%) or with natural abundance water to

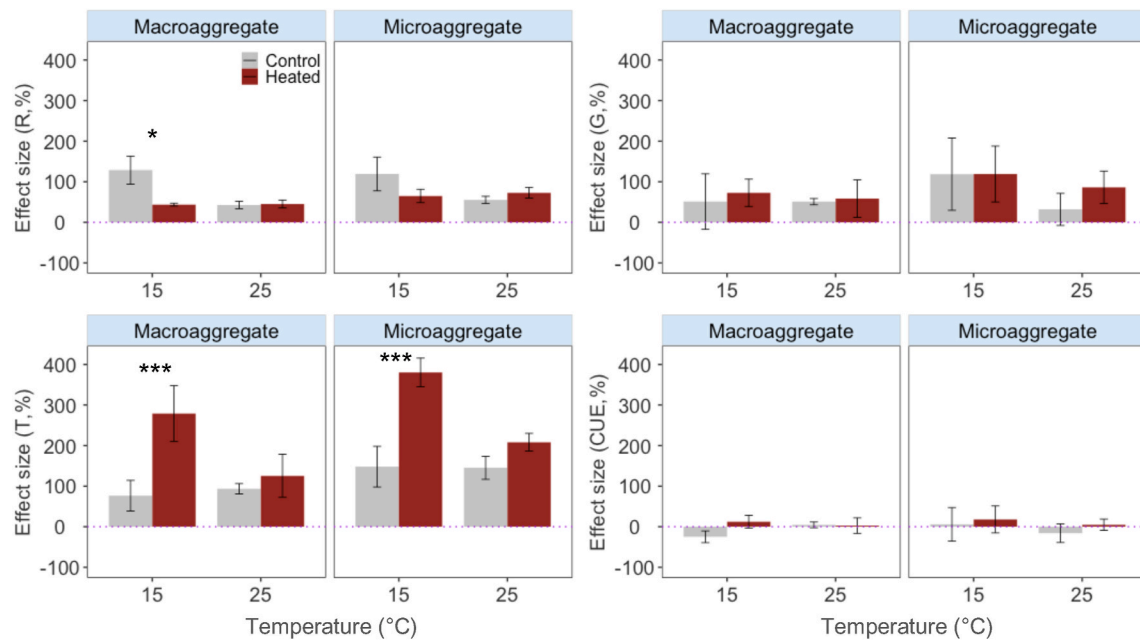


Fig. 2. Microbial responses to removal of physical protection as mediated by aggregate size and incubation temperature over long-term warming. R, respiration; G, growth; T, turnover rate; CUE, C use efficiency. Data are shown as effect size=(crushed – intact)/intact \times 100%. * and *** indicate significant warming effects ($P < 0.05$ and 0.001). Error bars show error of means ($n = 4$).

reach 60% WHC, and incubated 24 h at 15 or 25 °C. 30 mL of gas samples were taken from a 27 mL Hungate tube using a 60 mL syringe at the beginning and end of incubation, and measured by a CO₂ analyzer (Model 906, Quantek Instruments, Grafton, MA, USA). Respiration was calculated using a mass balance equation (gas volumes and concentrations) following the ideal gas law, with additional details being available in Liu et al. (2021). Microbial biomass C (MBC) was measured at the beginning of incubation, using the chloroform fumigation extraction method with modifications (Liu et al., 2020): 2.0 g soil was extracted with 10 ml K₂SO₄ (0.5 M) before and after chloroform fumigation (24 h), and dissolved organic C (DOC) was measured using a TOC-L analyzer, and MBC was the difference in DOC concentrations before and after chloroform fumigation using a factor of 0.45 (Liu et al., 2017). Soil DNA was extracted (Qiagen), and microbial growth rate was estimated using the MBC:DNA ratios from newly formed DNA, which was calculated from excess ¹⁸O in the labeled against non-labeled DNA samples using a conversion factor of 31.9% (Geyer et al., 2019; Liu et al., 2021; Papp et al., 2018). CUE was calculated as the ratio of growth to organic C uptake (respiration + growth); and biomass turnover rate was calculated by dividing growth (per day) by MBC (Domeignoz-Horta et al., 2020; Liu et al., 2021). Mass-specific respiration and mass-specific growth were calculated by dividing respiration and growth with MBC. Data were checked for normality and log-transformed as needed. Treatment effects were tested using linear mixed models (with experimental field plots as the random effect) and the post hoc test (*glht*) in R (version 4.0.0; R Core

Team, 2020). Variance partitioning was calculated from repeated ANOVA with plots as a random effect (Liu et al., 2020, 2021). The effect of physical protection (effect size) is defined as (crushed – intact)/intact \times 100%.

Our first hypothesis was that removal of physical protection by crushing would increase microbial activity but decrease growth efficiency. We found that crushing increased microbial respiration and biomass turnover rate, especially at 15 °C (Figs. 2 and S1). Crushing also increased mass-specific respiration and mass-specific growth (Figs. S2 and S3). However, crushing did not affect CUE. This suggests that the quality of substrates released by crushing is similar to the quality of those remained protected within intact aggregates, which has been previously observed (Guan et al., 2018). As expected, crushing increased DOC concentrations (Figs. S2 and S3), supporting our assumption that aggregates protect soil C (Navarro-García et al., 2012). Yet, crushing reduced microbial biomass, suggesting possible contributions of microbial necromass to elevated respiration (Balesdent et al., 2000; Gregorich et al., 1989). Microaggregates had greater DOC concentrations and slower microbial biomass turnover rates than macroaggregates in both intact and crushed soils (Figs. S1 and S3), consistent with previous observations that microaggregates SOM is less accessible to microbes than macroaggregates SOM (Bach et al., 2018; Bailey et al., 2013; Fox et al., 2018; Goebel et al., 2009; Liu et al., 2021).

Our second hypothesis was that soils exposed to long term warming have a smaller effect size of physical protection on microbial activities

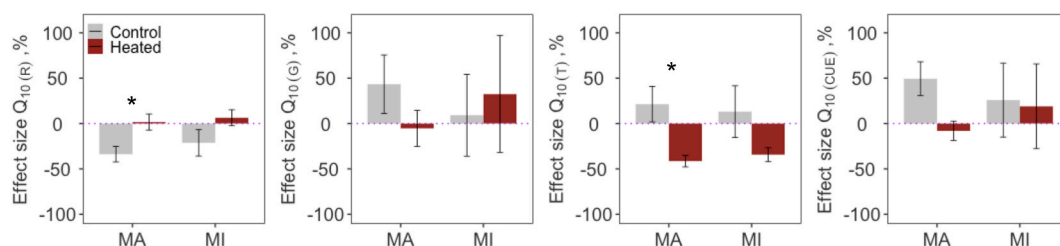


Fig. 3. Microbial thermal responses (temperature sensitivity, Q10) to removal of physical protection as mediated by aggregate size over long-term warming. R, respiration; G, growth; T, turnover rate; CUE, C use efficiency. MA = macroaggregates and MI = microaggregates. Data are shown as effect size=(Q10 of crushed – Q10 of intact)/Q10 of intact \times 100%. * indicates significant warming effects ($P < 0.05$). Error bars show error of means ($n = 4$).

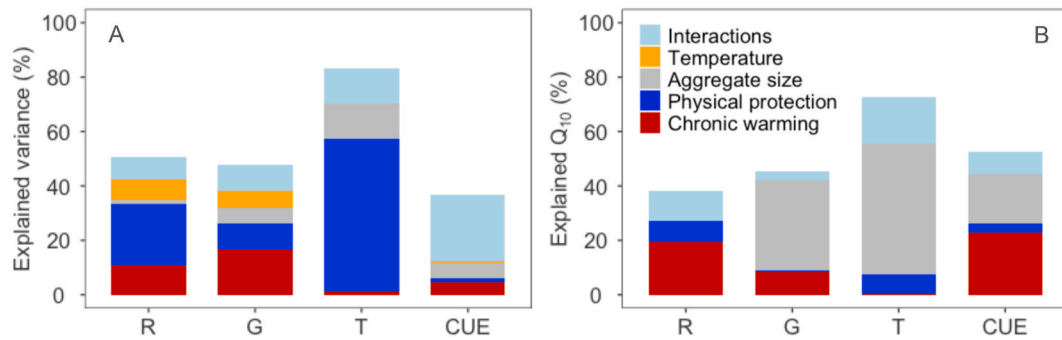


Fig. 4. Variance of microbial activities (A) and temperature sensitivities (B) explained by treatment factors (Chronic warming, control and heated; Aggregate size, 250–2000 and < 250 μm ; Physical protection, intact and crushed; Temperature, incubation temperature at 15 and 25 $^{\circ}\text{C}$). R, respiration; G, growth rate; T, turnover rate; CUE, C use efficiency.

and CUE compared to control plots. Long-term warming was associated with a smaller effect of physical protection on respiration, but with a larger effect on microbial biomass turnover rate, especially at 15 $^{\circ}\text{C}$ (Fig. 2 and Table 1). This is consistent with prior observations that chronic warming affected microbial thermal responses and elicited a decline in microbial biomass (Liu et al., 2021; Melillo et al., 2017; Pold et al., 2017). Long-term warming enhanced the positive crushing effect on mass-specific growth and enhanced the negative crushing effect on MBC (Fig. S2), but did not impact the crushing effect on CUE (Fig. 2), consistent with prior studies that the quality between aggregate-protected and unprotected substrates is similar in the heated and control soils (Guan et al., 2018; Waring et al., 2020).

Our third hypothesis was that the effect size of physical protection on temperature sensitivity (Q_{10}) of microbial activities would be smaller in the heated compared to control plots. The effect of physical protection on temperature sensitivity of respiration was smaller in the heated compared to control plots, while the effect on temperature sensitivity of microbial biomass turnover rate was larger (Fig. 3 and Table 1). Crushing reduced the temperature sensitivity of respiration in the control soils but showed little effect in the heated soils (Fig. S4). The effect of physical protection on temperature sensitivity of CUE was similar between heated and control soils, and the effect on temperature sensitivity of mass-specific growth was larger in the heated than control soils (Figs. 3 and S5). These variable effects of physical protection on microbial thermal responses might be associated with substrate limitation (Liu et al., 2021; Pold et al., 2017), microbial adaptation in utilizing complex substrates (DeAngelis et al., 2015; Pold et al., 2016), and changes in microbial substrate accessibility and community structure (Bach et al., 2018; Fox et al., 2018) after nearly three decades of soil warming.

Microbial activities and their temperature responses were driven by different environmental factors (Fig. 4). Respiration was driven mainly by physical protection and its temperature sensitivity was driven mostly by long-term warming. Microbial growth and biomass turnover rate were driven by long-term warming and physical protection, respectively (Fig. 4A), but their temperature sensitivities were both driven by aggregate size (Fig. 4B). Temperature sensitivity of CUE was driven by long-term warming and aggregate size. Physical protection also explained most of variance in mass-specific respiration and mass-specific growth (Fig. S6). These findings suggest that physical protection and aggregate size regulate microbial activities and their thermal responses to long-term warming (Liu et al., 2021; Poepplau et al., 2017).

After nearly three decades of warming, the effect of physical protection on temperature sensitivity of respiration was reduced but the effect on temperature sensitivity of biomass turnover rate was increased, especially in macroaggregates. This suggests that physical protection mediates microbial thermal responses in soil aggregates that have lower SOM and microbial biomass but greater substrate accessibility to microbes (Liu et al., 2021). Our findings suggest that physical protection

differentially regulate microbial activities and their thermal responses among soil compartments, with little effect on the efficiency with which microbes utilize SOM after long-term warming.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2021.108298>.

References

- Bach, E.M., Hofmockel, K.S., 2014. Soil aggregate isolation method affects measures of intra-aggregate extracellular enzyme activity. *Soil Biology and Biochemistry* 69, 54–62. <https://doi.org/10.1016/j.soilbio.2013.10.033>.
- Bach, E.M., Williams, R.J., Hargreaves, S.K., Yang, F., Hofmockel, K.S., 2018. Greatest soil microbial diversity found in micro-habitats. *Soil Biology and Biochemistry* 118, 217–226. <https://doi.org/10.1016/j.soilbio.2017.12.018>.
- Bailey, V.L., McCue, L.A., Fansler, S.J., Boyanov, M.I., DeCarlo, F., Kemner, K.M., Konopka, A., 2013. Micrometer-scale physical structure and microbial composition of soil macroaggregates. *Soil Biology and Biochemistry* 65, 60–68. <https://doi.org/10.1016/j.soilbio.2013.02.005>.
- Balesdent, J., Chenu, C., Balabane, M., 2000. Relationship of soil organic matter dynamics to physical protection and tillage. *Soil and Tillage Research* 53, 215–230. [https://doi.org/10.1016/S0167-1987\(99\)00107-5](https://doi.org/10.1016/S0167-1987(99)00107-5).
- Bandyopadhyay, P.K., 2020. Functional behaviour of soil physical parameters for regulating organic C pools. In: Ghosh, P.K., Mahanta, S.K., Mandal, D., Mandal, B., Ramakrishnan, S. (Eds.), *Carbon Management in Tropical and Sub-tropical Terrestrial Systems*. Springer, Singapore, pp. 233–247. https://doi.org/10.1007/978-981-13-9628-1_14.
- Bischoff, N., Mikutta, R., Shibistova, O., Puzanov, A., Silanteva, M., Grebennikova, A., Fuß, R., Guggenberger, G., 2017. Limited protection of macro-aggregate-occluded organic carbon in Siberian steppe soils. *Biogeosciences* 14, 2627–2640. <https://doi.org/10.5194/bg-14-2627-2017>.
- Bradford, M.A., Davies, C.A., Frey, S.D., Maddox, T.R., Melillo, J.M., Mohan, J.E., Reynolds, J.F., Treseder, K.K., Wallenstein, M.D., 2008. Thermal adaptation of soil microbial respiration to elevated temperature. *Ecology Letters* 11, 1316–1327. <https://doi.org/10.1111/j.1461-0248.2008.01251.x>.
- Conant, R.T., Ryan, M.G., Ågren, G.I., Birge, H.E., Davidson, E.A., Eliasson, P.E., Evans, S.E., Frey, S.D., Giardina, C.P., Hopkins, F.M., Hyvönen, R., Kirschbaum, M.U.F., Lavallee, J.M., Leifeld, J., Parton, W.J., Steinweg, J.M., Wallenstein, M.D., Wetterstedt, J.Å.M., Bradford, M.A., 2011. Temperature and soil organic matter

- decomposition rates – synthesis of current knowledge and a way forward. *Global Change Biology* 17, 3392–3404. <https://doi.org/10.1111/j.1365-2486.2011.02496.x>.
- DeAngelis, K.M., Pold, G., Topcuoglu, B.D., van Diepen, L.T.A., Varney, R.M., Blanchard, J.L., Melillo, J., Frey, S.D., 2015. Long-term forest soil warming alters microbial communities in temperate forest soils. *Frontiers in Microbiology* 6, 1–13. <https://doi.org/10.3389/fmicb.2015.00104>.
- Domeignoz-Horta, L.A., Pold, G., Liu, X.J.A., Frey, S.D., Melillo, J.M., DeAngelis, K.M., 2020. Microbial diversity drives carbon use efficiency in a model soil. *Nature Communications* 11, 3684. <https://doi.org/10.1038/s41467-020-17502-z>.
- Fox, A., Ikoyi, I., Torres-Sallan, G., Lanigan, G., Schmalenberger, A., Wakelin, S., Creamer, R., 2018. The influence of aggregate size fraction and horizon position on microbial community composition. *Applied Soil Ecology*. <https://doi.org/10.1016/j.apsoil.2018.02.023>.
- Frey, S.D., Drijber, R., Smith, H., Melillo, J., 2008. Microbial biomass, functional capacity, and community structure after 12 years of soil warming. *Soil Biology and Biochemistry* 40, 2904–2907. <https://doi.org/10.1016/j.soilbio.2008.07.020>.
- Frey, S.D., Lee, J., Melillo, J.M., Six, J., 2013. The temperature response of soil microbial efficiency and its feedback to climate. *Nature Climate Change* 3, 395–398. <https://doi.org/10.1038/nclimate1796>.
- Geyer, K.M., Dijkstra, P., Sinsabaugh, R., Frey, S.D., 2019. Clarifying the interpretation of carbon use efficiency in soil through methods comparison. *Soil Biology and Biochemistry* 128, 79–88. <https://doi.org/10.1016/j.soilbio.2018.09.036>.
- Gillabel, J., Cebrian-Lopez, B., Six, J., Merckx, R., 2010. Experimental evidence for the attenuating effect of SOM protection on temperature sensitivity of SOM decomposition. *Global Change Biology* 16, 2789–2798. <https://doi.org/10.1111/j.1365-2486.2009.02132.x>.
- Goebel, M.-O., Woche, S.K., Bachmann, J., 2009. Do soil aggregates really protect encapsulated organic matter against microbial decomposition? *Biologia* 64, 443–448. <https://doi.org/10.2478/s11756-009-0065-z>.
- Gregorich, E.G., Kachanoski, R.G., Voroney, R.P., 1989. Carbon mineralization in soil size fractions after various amounts of aggregate disruption. *Journal of Soil Science* 40, 649–659. <https://doi.org/10.1111/j.1365-2389.1989.tb01306.x>.
- Guan, S., An, N., Zong, N., He, Y., Shi, P., Zhang, J., He, N., 2018. Climate warming impacts on soil organic carbon fractions and aggregate stability in a Tibetan alpine meadow. *Soil Biology and Biochemistry* 116, 224–236. <https://doi.org/10.1016/j.soilbio.2017.10.011>.
- Liu, X.J.A., Finley, B.K., Mau, R.L., Schwartz, E., Dijkstra, P., Bowker, M.A., Hungate, B. A., 2020. The soil priming effect: consistent across ecosystems, elusive mechanisms. *Soil Biology and Biochemistry* 140, 107617. <https://doi.org/10.1016/j.soilbio.2019.107617>.
- Liu, X.J.A., Pold, G., Domeignoz-Horta, L.A., Geyer, K.M., Caris, H., Nicolson, H., Kemner, K.M., Frey, S.D., Melillo, J.M., DeAngelis, K.M., 2021. Soil aggregate-mediated microbial responses to long-term warming. *Soil Biology and Biochemistry* 152, 108055. <https://doi.org/10.1016/j.soilbio.2020.108055>.
- Liu, X.J.A., Sun, J., Mau, R.L., Finley, B.K., Compson, Z.G., van Gestel, N., Brown, J.R., Schwartz, E., Dijkstra, P., Hungate, B.A., 2017. Labile carbon input determines the direction and magnitude of the priming effect. *Applied Soil Ecology* 109, 7–13. <https://doi.org/10.1016/j.apsoil.2016.10.002>.
- Manzoni, S., Taylor, P., Richter, A., Porporato, A., Ågren, G.I., 2012. Environmental and stoichiometric controls on microbial carbon-use efficiency in soils. *New Phytologist* 79–91. <https://doi.org/10.1111/j.1469-8137.2012.04225.x>.
- Melillo, J.M., Frey, S.D., DeAngelis, K.M., Werner, W.J., Bernard, M.J., Bowles, F.P., Pold, G., Knorr, M.A., Grandy, A.S., 2017. Long-term pattern and magnitude of soil carbon feedback to the climate system in a warming world. *Science* 358, 101–105. <https://doi.org/10.1126/science.aan2874>.
- Navarro-García, F., Casermeiro, M.Á., Schimel, J.P., 2012. When structure means conservation: effect of aggregate structure in controlling microbial responses to rewetting events. *Soil Biology and Biochemistry* 44, 1–8. <https://doi.org/10.1016/j.soilbio.2011.09.019>.
- Papp, K., Mau, R.L., Hayer, M., Koch, B.J., Hungate, B.A., Schwartz, E., 2018. Quantitative stable isotope probing with $H_2^{18}O$ reveals that most bacterial taxa in soil synthesize new ribosomal RNA. *The ISME Journal* 1. <https://doi.org/10.1038/s41396-018-0233-7>.
- Plante, A.F., Six, J., Paul, E.A., Conant, R.T., 2009. Does physical protection of soil organic matter attenuate temperature sensitivity? *Soil Science Society of America Journal* 73, 1168–1172. <https://doi.org/10.2136/sssaj2008.0351>.
- Poeplau, C., Kätterer, T., Leblans, N.I.W., Sigurdsson, B.D., 2017. Sensitivity of soil carbon fractions and their specific stabilization mechanisms to extreme soil warming in a subarctic grassland. *Global Change Biology* 23, 1316–1327. <https://doi.org/10.1111/gcb.13491>.
- Pold, G., Billings, A.F., Blanchard, J.L., Burkhardt, D.B., Frey, S.D., Melillo, J.M., Schnabel, J., Diepen, L.T.A. van, DeAngelis, K.M., 2016. Long-term warming alters carbohydrate degradation potential in temperate forest soils. *Applied and Environmental Microbiology* 82, 6518–6530. <https://doi.org/10.1128/AEM.02012-16>.
- Pold, G., Grandy, A.S., Melillo, J.M., DeAngelis, K.M., 2017. Changes in substrate availability drive carbon cycle response to chronic warming. *Soil Biology and Biochemistry* 110, 68–78. <https://doi.org/10.1016/j.soilbio.2017.03.002>.
- R Core Team, 2020. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Six, J., Callewaert, P., Lenders, S., De Gryze, S., Morris, S.J., Gregorich, E.G., Paul, E.A., Paustian, K., 2002. Measuring and understanding carbon storage in afforested soils by physical fractionation. *Soil Science Society of America Journal* 66, 1981–1987. <https://doi.org/10.2136/sssaj2002.1981>.
- Tian, J., Pausch, J., Yu, G., Blagodatskaya, E., Gao, Y., Kuzyakov, Y., 2015. Aggregate size and their disruption affect ^{14}C -labeled glucose mineralization and priming effect. *Applied Soil Ecology* 90, 1–10. <https://doi.org/10.1016/j.apsoil.2015.01.014>.
- Wankhede, M., Ghosh, A., Manna, M.C., Misra, S., Sirothia, P., Rahman, M.M., Bhattacharyya, P., Singh, M., Bhattacharyya, R., Patra, A.K., 2020. Does soil organic carbon quality or quantity govern relative temperature sensitivity in soil aggregates? *Biogeochemistry* 148, 191–206. <https://doi.org/10.1007/s10533-020-00653-y>.
- Waring, B.G., Sulman, B.N., Reed, S., Smith, A.P., Averill, C., Creamer, C.A., Cusack, D.F., Hall, S.J., Jastrow, J.D., Jilling, A., Kemner, K.M., Kleber, M., Liu, X.J.A., Pett-Ridge, J., Schulz, M., 2020. From pools to flow: the PROMISE framework for new insights on soil carbon cycling in a changing world. *Global Change Biology* 1–13. <https://doi.org/10.1111/gcb.15365>.