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Warming accelerates decomposition of decades-old carbon in forest soils

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Global climate carbon-cycle models predict acceleration of soil organic carbon losses to the atmosphere with warming, but the size of this feedback is poorly known. The temperature sensitivity of soil carbon decomposition is commonly determined by measuring changes in the rate of carbon dioxide (CO₂) production under controlled laboratory conditions. We added measurements of carbon isotopes in respired CO₂ to constrain the age of carbon substrates contributing to the temperature response of decomposition for surface soils from two temperate forest sites with very different overall rates of carbon cycling. Roughly one-third of the carbon respired at any temperature was fixed from the atmosphere more than 10 y ago, and the mean age of respired carbon reflected a mixture of substrates of varying ages. Consistent with global ecosystem model predictions, the temperature sensitivity of the carbon fixed more than a decade ago was the same as the temperature sensitivity for carbon fixed less than 10 y ago. However, we also observed an overall increase in the mean age of carbon respired at higher temperatures, even correcting for potential substrate limitation effects. The combination of several age constraints from carbon isotopes showed that warming had a similar effect on respiration of decades-old and younger (<10 y) carbon but a greater effect on decomposition of substrates of intermediate (between 7 and 13 y) age. Our results highlight the vulnerability of soil carbon to warming that is years-to-decades old, which makes up a large fraction of total soil carbon in forest soils globally.

climate feedback | soil organic matter | soil respiration | radiocarbon | soil incubation

The potential for carbon stored on land to become a source of carbon dioxide (CO₂) to the atmosphere in the 21st century is a key uncertainty in predictions of future climate. Global warming increases the rate of decomposition of soil organic carbon (C), a major loss pathway of C from the land surface to the atmosphere, thus contributing to the increase in atmospheric CO₂ and hence, global temperatures. However, how much of the estimated 3,000 Pg C (1) stored in soils globally is vulnerable to enhanced decomposition with warming is highly uncertain and difficult to assess (2). In particular, the temperature sensitivity of C cycling on decadal timescales is a key uncertainty controlling the size of potential soil C responses to warming (3). Although there are no global estimates of decadal-aged C, it makes up the majority of C in mineral soils in temperate forests (4). We took advantage of a decade-long, whole-ecosystem C-isotope label to isolate the effect of warming on decomposition of decades-old C in a laboratory incubation experiment.

The temperature sensitivity of decades-old C is difficult to observe using traditional approaches, such as response of CO₂ flux to experimental warming, because respiration is dominated by soil C cycling on fast timescales of 1 y or less. Previous studies using C isotopes to identify older C and assess its temperature sensitivity do not provide consistent results (recently reviewed in refs. 5 and 6). Most of these studies used a change in vegetation type (e.g., from C₃ to C₄ photosynthetic vegetation) as a means

to distinguish old and young C. However, such vegetation shifts also change the amount and quality of C inputs to soil, affecting the decomposition process and potentially confounding measurements of temperature sensitivity. In addition, most studies took place in agricultural soils and may not be representative of less managed systems. Other methods to determine the temperature sensitivity of slower-cycling C also have significant drawbacks. Extended incubation periods to deplete the soil of fast-cycling C pools can change the decomposition process through substrate limitation (7). The response of slow-cycling soil C to warming is difficult to detect on the timescales of manipulative experiments, and it may be affected by covarying factors along natural temperature gradients (6). Model-derived predictions of temperature sensitivity of C pools cycling on different timescales are highly sensitive to assumptions in underlying model structures, such as which parameters are temperature-sensitive (8). Moreover, any inference of temperature sensitivity from bulk CO₂ fluxes alone is difficult to relate to soil C destabilization processes, because respiration integrates across C pools stabilized by multiple interacting controls. Specifically, the mean residence time of different soil C pools is affected by both biology and physicochemical conditions, which are both likely to be temperature-sensitive (9). As a result, the effect of warming on the stability of soil C stocks is a topic of intense debate.

We investigated the temperature sensitivity of decades-old C by taking advantage of a whole-ecosystem C-isotope label in two temperate forest sites. Both sites had free air CO₂ enrichment (FACE) experiments, where atmospheric CO₂ concentrations in treatment plots are raised by fumigating with fossil-derived CO₂ that has a distinct C-isotope signature in ¹⁴C and ¹³C compared with background air [fumigation gas $\Delta^{14}\text{C}$ value of $-1,000\text{‰}$ compared with $50\text{--}100\text{‰}$ for background air (10) and $\delta^{13}\text{C}$ value of -43‰ compared with about -8‰ in background air (11, 12)]. Thus, C fixed by photosynthesis and incorporated into plant material and soil C under elevated CO₂ is isotopically distinguishable from previously existing soil C (FACE label). CO₂ enrichment began at both sites more than a decade before we sampled soils (Table 1), and therefore, the C-isotope label allows us to distinguish the contribution of decades-old C (pre-FACE C > 10 y) from the contribution of more recent C (FACE C < 10 y) to heterotrophic respiration during incubation using standard isotopic mixing models (13).

Author contributions: F.M.H., M.S.T., and S.E.T. designed research; F.M.H. performed research; F.M.H. and S.E.T. analyzed data; and F.M.H., M.S.T., and S.E.T. wrote the paper.

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Table 1. Site information

Site	Location	MAT (°C)	Mean annual precipitation (mm)	Planted (y)	+CO ₂ (y)	Vegetation	Soil type	Soil texture
Aspen	45°N 89°W	4.9	810	1997	1998–2009	<i>Populus tremuloides</i>	Ultic Hapludalf	Sandy loam
Duke	35°N 79°W	15.5	1,140	1983	1996–2010	<i>Pinus taeda</i>	Alfic Haplorthod	Clay loam

Although elevated CO₂ soils provide us with a unique opportunity to constrain how much decades-old C contributes to respiration by using the large difference in ¹⁴C and ¹³C between C fixed before and after FACE (Fig. 1), measurements of background levels of ¹⁴C in the nonenriched ambient CO₂ plots provide an additional age constraint. In ambient CO₂ plots, the ¹⁴C content of soil-respired CO₂ reflects the relative contribution of ¹⁴C fixed by photosynthesis into ecosystem C pools since aboveground nuclear weapons testing in the 1960s (Fig. 1). The atmospheric ¹⁴CO₂ signature has been declining by ~5‰/y in recent years (14), and therefore, the mean age of respired C—the mean time elapsed since respired C was fixed from the atmosphere—can be determined using a time-dependent, steady-state model and the atmospheric history of ¹⁴C (15). With the bomb-derived ¹⁴C label, we can detect differences between C fixed from the atmosphere from 1 y to several decades before the date of sampling.

We sampled soils from both ambient and elevated CO₂ treatment plots at two FACE sites (Aspen FACE, Rhinelander, WI; Duke FACE, Durham, NC) after they had been exposed to elevated CO₂ for 11 y. Both sites are temperate forest plantations on old agricultural soils, but they differ with respect to species, lifeform, and stand age (Table 1). At Aspen FACE, deciduous aspen clones were planted in monoculture in 1997, and CO₂ enrichment was initiated the next growing season. At Duke FACE, evergreen loblolly pines were planted in 1983, and CO₂ enrichment began when the trees were already 13 y old. We incubated surface mineral soils (0- to 15-cm depth) at their site mean annual temperatures (MATs; 5 °C and 15 °C, respectively) and under two warming treatments (+10 °C and +20 °C). Respired CO₂ was collected for determination of flux rates and ¹⁴C and ¹³C content of respiration.

The two isotope constraints allowed us to distinguish the contribution of soil C cycling on three different timescales—

years, decades, and intermediate between the two time periods—to CO₂ fluxes across incubation temperatures. Specifically, we tested a common assumption of global ecosystem models that all ages of soil C have similar temperature sensitivity. We can expect one of four possible outcomes.

- If the temperature sensitivity of C up to several decades old is greater than the temperature sensitivity of C of younger age, we would expect more enriched C-isotope values of respiration under higher temperature in both FACE and ambient CO₂ treatments, with (i) a gradual increase in $\Delta^{14}\text{C}$ of respiration from ambient CO₂ soils, reflecting greater decomposition of C fixed since 1960, and (ii) a rapid increase in $\Delta^{14}\text{C}$ of respiration from elevated CO₂ soils, reflecting increased contribution of isotopically distinct, decades-old C fixed before CO₂ enrichment.
- If the temperature sensitivity of C around a decade old is greater than the temperature sensitivity of the younger and older age classes, we would expect an increase in $\Delta^{14}\text{C}$ of respiration with warming from both FACE and ambient CO₂ treatments at a similar rate, reflecting relatively faster decomposition of 10-y-old C fixed during the CO₂ enrichment period, with slightly higher ¹⁴C content than the youngest C because of the gradual decline in atmospheric ¹⁴C in both CO₂ treatments over this time period.
- If the temperature sensitivity of the youngest C is greater than the temperature sensitivity of the two older age classes, we would expect a decrease in $\Delta^{14}\text{C}$ of respiration with warming from elevated CO₂ soils and ambient CO₂ soils.
- If the temperature sensitivity of all ages of C is similar, we would expect $\Delta^{14}\text{C}$ of respiration from elevated CO₂ and ambient CO₂ soils to remain constant across temperature treatments.

Results

Respiration Sensitivity to Warming. Warming consistently increased respiration rates from incubated surface soils for both CO₂ levels at the two sites ($P < 0.0001$ for temperature effect). Although respiration rates dropped with time (Fig. S1) in the Duke soils, the effect of temperature on respiration rate was consistent over the many months (up to 12 mo for Duke soils) of the experiment. The increase in respiration rates corresponded to a Q_{10} of 1.5–1.9 for Duke and 2.9–3.1 for Aspen. The elevated atmospheric CO₂ treatment also significantly increased fluxes ($P = 0.006$) and interacted with the temperature effect ($P = 0.044$) at the Aspen site, but it had no statistically significant effect on fluxes at Duke.

Temperature Dependence of Decades-Old C (Pre-FACE C). Isotopic signatures of the CO₂ respired in the incubations reflect the large influence of the isotopically depleted C fixed in the FACE treatments (Fig. 2). We used an isotopic mixing model to partition fluxes from the elevated CO₂ treatment into FACE-derived (<10 y) and pre-FACE (>10 y) pools using the FACE ¹⁴C label (SI Methods). In the FACE soils, roughly one-third of the C respired was fixed before the FACE experiment, regardless of temperature (Table 2). Warming increased the rate of losses from both pools, showing that decades-old C (>10 y) is vulnerable to immediate, enhanced losses on warming and has similar temperature sensitivity as younger FACE-derived (<10 y) C

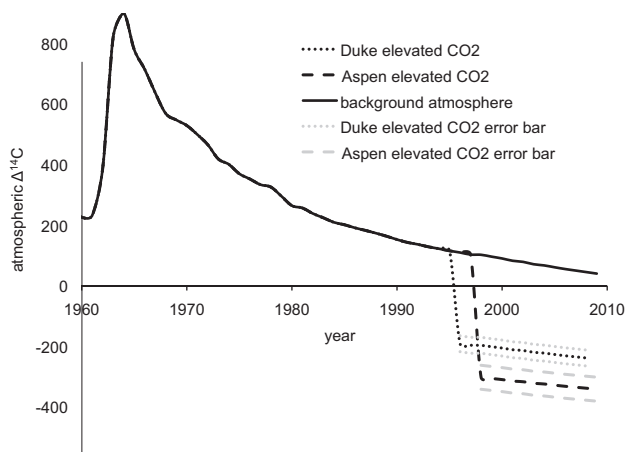


Fig. 1. Atmospheric ¹⁴C content by year. The solid line represents background atmosphere $\Delta^{14}\text{C}$ value since 1960, and the dashed and dotted lines represent atmosphere with FACE in elevated CO₂ plots at Aspen and Duke, respectively. Light gray lines represent the potential variability in ¹⁴C signature of the elevated CO₂ treatments based on the SD of CO₂ concentrations measured in elevated CO₂ plots (SI Methods).

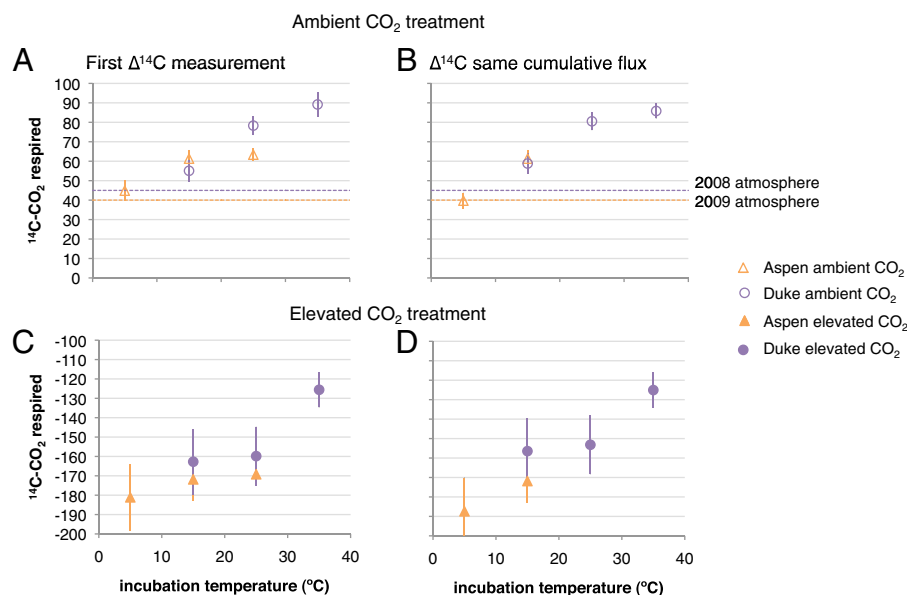


Fig. 2. Average $\Delta^{14}\text{C}$ values of CO_2 respired by incubated soils from Aspen ($n = 3$) and Duke ($n = 4$) FACE sites by incubation temperature (error bars are SEM). (A and B) Ambient CO_2 . (C and D) Elevated CO_2 . (A and C) $\Delta^{14}\text{C}\text{-CO}_2$ respired at the first flux measurement period. (B and D) Cumulative $\Delta^{14}\text{C}\text{-CO}_2$ respired to a given loss of initial soil carbon (0.11% for Aspen and 2.68% for Duke). Dashed lines indicate atmospheric $\Delta^{14}\text{C}$ values in the years of sampling at Aspen (2009) and Duke (2008) sites.

(Fig. 3). Isotopic partitioning using the $\delta^{13}\text{C}$ label also supports the conclusion that both FACE and pre-FACE C are equally sensitive (Table 2).

We quantified the temperature effect on partitioned fluxes with an exponential model, where the temperature sensitivity coefficient b defines the increase in flux per change in temperature and A is a constant that represents the basal reaction rate (8). Within each site, we observed no significant differences in b for C pools of different ages (Fig. 3 *Inset*). The flux of pre-FACE (>10 y) C was slightly more temperature-sensitive (but not statistically different) than the flux of FACE (<10 y) C. Values of A were always higher for FACE C than pre-FACE C, confirming that the model separated pools with different overall cycling rates.

Increase in Substrate Availability with Temperature. Along with increased fluxes, we observed an immediate shift in the $\Delta^{14}\text{C}$ sig-

nature of respired CO_2 from warmed soils relative to the site MAT control soils (Fig. 2 A and C). Warming increased the mean age of respired C, which was shown by the significant increase in $\Delta^{14}\text{C}$ of respiration with incubation temperature from the ambient CO_2 treatment at both Aspen ($P = 0.0454$) and Duke ($P = 0.0058$). For the elevated CO_2 soils, $\Delta^{14}\text{C}$ of respiration also tended to increase with warming, although this difference was not statistically significant because of greater variability in $\Delta^{14}\text{C}$ of respiration among replicates (Table 3).

To test whether this pattern was caused by rapid depletion of fast-cycling C substrates, we normalized the isotopes of CO_2 flux data by amount of C lost (rather than by time). This normalization allows us to compare the sources of the equivalent amount of C respired across temperatures (Fig. S2) (16). If the same substrates were used at all temperatures but more rapidly depleted under warming, we would expect the same C-isotope content for the equivalent amount of initial soil C

Table 2. Fraction of soil C stock (0- to 15-cm mineral soil) and respiration flux (first sampling) coming from pre-FACE (>10 y) C (\pm SEM) identified with ^{14}C and ^{13}C mixing models

	Stock ($f_{>10\text{ y}}$ by ^{13}C)	Temperature ($^{\circ}\text{C}$)	Respiration	
			$f_{>10\text{ y}}$ by ^{14}C	$f_{>10\text{ y}}$ by ^{13}C
Duke				
CO_2 ambient		15	0.33 (0.10)	
CO_2 ambient		25	0.31 (0.15)	
CO_2 ambient		35	0.27 (0.12)	
CO_2 elevated	0.62 (0.03)	15	0.29 (0.16)	0.38 (0.15)
CO_2 elevated		25	0.24 (0.16)	0.28 (0.07)
CO_2 elevated		35	0.28 (0.22)	0.42 (0.06)
Aspen				
CO_2 ambient		5	0.40 (0.31)	
CO_2 ambient		15	0.34 (0.13)	
CO_2 ambient		25	0.35 (0.12)	
CO_2 elevated	0.68 (0.07)	5	0.28 (0.12)	0.43 (0.21)
CO_2 elevated		15	0.27 (0.08)	0.44 (0.09)
CO_2 elevated		25	0.28 (0.10)	0.37 (0.11)

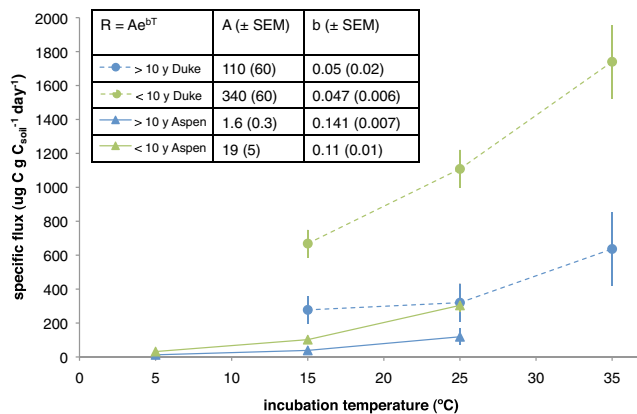


Fig. 3. Respired CO₂ (micrograms C grams C_{soil}⁻¹ day⁻¹) partitioned into >10 y C (pre-FACE) and <10 y C (FACE) using the ¹⁴C mixing model for Aspen (*n* = 3) and Duke (*n* = 4) elevated CO₂ soils. Average fluxes from each pool are shown, and error bars are SEMs calculated from propagated error in the model. *Inset* shows parameters (±SEM) from regression of respiration flux on temperature by $R = Ae^{bT}$.

respired. Instead, we observed a shift to higher $\Delta^{14}\text{C}$ values under warming (Fig. 2 *B* and *D*), which was similar in magnitude to the shift observed by comparing isotopes of flux at the same time in the incubation (Fig. 2 *A* and *C*). With this adjustment, $\Delta^{14}\text{C}$ respired under warming from ambient CO₂ soils was still significantly higher than from the MAT treatments, indicating that an isotopically distinct soil C source was used at higher temperatures.

The hypothesis of new substrate becoming available under warming is also supported by the observed decrease in flux rates over the incubation period. We modeled the change in CO₂ fluxes over time of incubation with a two-pool exponential equation to resolve an active pool (C_a), a slow pool (C_s), and their respective turnover rates (17). If substrate depletion was occurring in warmed soils, we would expect a more rapid initial decline of flux rates relative to the MAT treatment represented by an increase in the decay constant of the active pool (k_a). However, we found that increased respiration under warming in the early part of incubation is best modeled with an increase in the size of the active pool (C_a) with warming, whereas the decay constant (k_a) for that pool stays relatively constant (Table S1) in the Duke soils (flux data from Aspen soils did not fit the model). This pattern has been found in many studies, and it has resulted in an ongoing debate about whether temperature dependence can be in both the pool size terms and the rate constant (18–20).

These results suggest that increased substrate availability may be the key to the initial stages of the warming response.

Age of Respired C Substrates. From the ambient CO₂ treatment soils incubated at the site MAT, bomb ¹⁴C modeling estimates of the age of C respired were 2 y for Aspen (<1–5 y, 95% confidence interval) and 3 y for Duke (<1–6 y). Warming increased the mean age of C respired by 3–5 y at both sites relative to the MAT treatment (MAT + 10°C: +3 y at Aspen, +4 y at Duke; MAT + 20°C: +3.5 y at Aspen, +5 y at Duke). The young age of respired C agrees with the expectation that C with the fastest turnover time is metabolized early in incubation and that the youngest C dominates the heterotrophic respiration signal.

To confirm that additional substrate made available by warming was less than a decade old, we modified our original mixing model to include a warming-induced pool defined by the change in flux and $\Delta^{14}\text{C}$ -CO₂ respired from warmed soils over the MAT control soils (Fig. 4). Using data for the same cumulative C loss across temperatures, we assumed an equal contribution of <1-y C to fluxes in ambient CO₂ soils and the same $\Delta^{14}\text{C}$ end members at all temperatures, which enabled us to solve for the $\Delta^{14}\text{C}$ value of the warming-induced substrates. We estimate this pool to have a mean age of 7–13 y in Aspen and 9–12 y in Duke.

Discussion

Vulnerability of Decades-Old Soil C to Warming. In these two temperate forest soils, warming increased respiration of soil C more than a decade old fixed before the FACE treatment. Such decades-old C is a major component of organic matter in these soils and temperate forests more broadly (4), implying that a large portion of soil organic C is vulnerable to increased decomposition with global warming. C more than a decade old made up 70% of mineral soil C in the 0- to 15-cm depth that we incubated, but ~30% of the CO₂ respired from it. The difference implies that there is some component of the soil C stock, often referred to as passive or inert C, that is not contributing detectably to respiration (21, 22). We estimate that this passive pool makes up 6–47% of the C in the Aspen and <17% of the C at Duke (*SI Methods*). Hence, at least 53–94% at Aspen and >83% at Duke of the top 15 cm mineral soil C is vulnerable to increased decomposition with warming or 1,850–4,700 and 1,750–2,160 g C m⁻², respectively (*SI Methods*).

Temperature Sensitivity of Decades-Old Soil C Decomposition Is Robust Across Sites. Warming increased decomposition rates of decades-old C at both sites, despite large differences in overall soil cycling rates (Fig. 3 and Table 3). Aspen had much slower

Table 3. Mean CO₂ flux and isotope values (±SEM) from first sampling

	Temperature (°C)	flux _{μgC_{resp}gC_{soil}⁻¹d⁻¹}	$\Delta^{14}\text{C}$ -CO ₂	$\delta^{13}\text{C}$ -CO ₂
Duke				
Ambient CO ₂	15	775 (31)	55.1 (5.6)	-26.56 (0.2)
Ambient CO ₂	25	1,399 (76)	78.3 (4.8)	-26.68 (0.4)
Ambient CO ₂	35	2,682 (63)	89.2 (6.3)	-26.80 (0.2)
Elevated CO ₂	15	946 (89)	-162.7 (17.8)	-34.30 (0.9)
Elevated CO ₂	25	1,429 (72)	-159.8 (16.1)	-35.60 (0.4)
Elevated CO ₂	35	2,377 (188)	-125.6 (8.8)	-33.92 (0.3)
Aspen				
Ambient CO ₂	5	38 (7)	44.9 (5.2)	-26.29 (0.7)
Ambient CO ₂	15	116 (6)	61.3 (4.4)	-27.47 (0.3)
Ambient CO ₂	25	338 (16)	63.4 (3.2)	-27.45 (0.3)
Elevated CO ₂	5	45 (3)	-181.1 (17.0)	-34.08 (1.7)
Elevated CO ₂	15	141 (8)	-171.8 (11.0)	-34.50 (0.6)
Elevated CO ₂	25	423 (28)	-169.2 (16.3)	-35.33 (0.7)

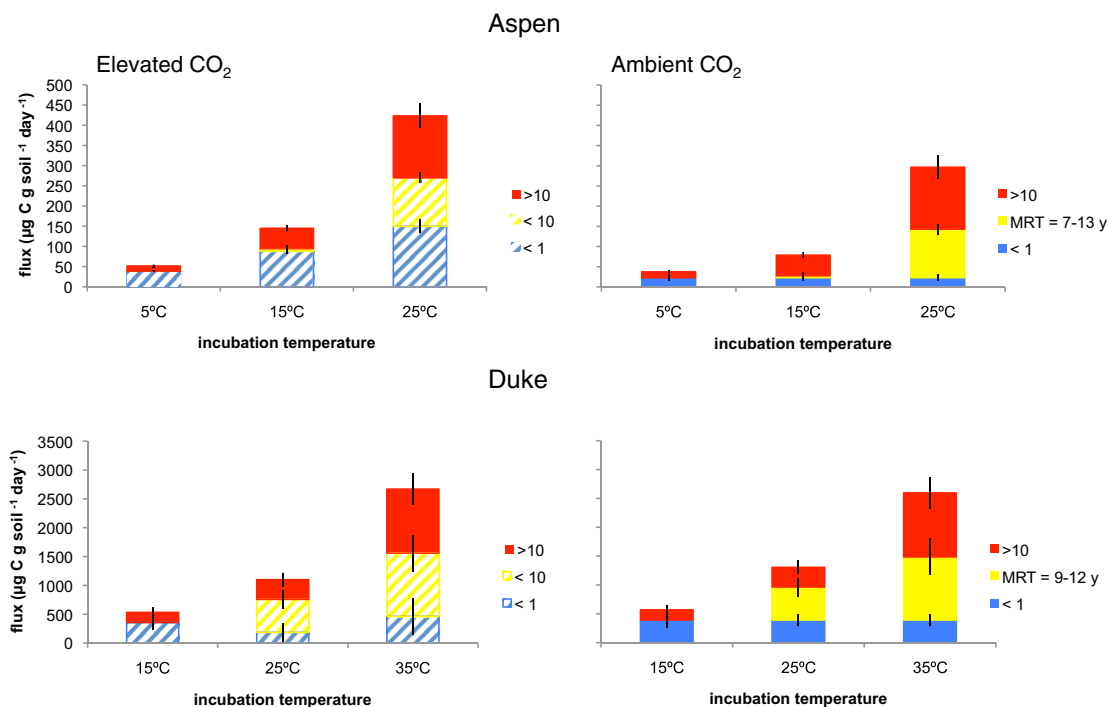


Fig. 4. Cumulative respired CO_2 (micrograms C grams soil $^{-1}$ day $^{-1}$) partitioned by modified isotopic mixing model. Solid bars represent soil CO_2 fluxes with the ^{14}C signature of the atmosphere, and hatched bars represent soil CO_2 fluxes with the ^{14}C signature of the FACE label. Colors represent the age of C: red, >10 y; yellow, intermediate-aged and warming-induced C; blue, <10 y for elevated CO_2 soils and <1 y for ambient CO_2 soils. Error bars represent SEMs propagated through the mixing model.

respiration rates than Duke (Table 3) (23) and less contribution of decades-old C to the soil C stock, which was indicated by bomb-derived ^{14}C in bulk ambient CO_2 soils ($\Delta^{14}\text{C}$ values of 51‰ at Aspen and 73‰ at Duke). The total amount of soil C and its distribution among physical fractions also differed greatly between the sites (Table S2) (24, 25). Specifically, there was a greater proportion of mineral stabilized C at Aspen FACE relative to Duke FACE. Along with the $\Delta^{14}\text{C}$ of bulk soils, this finding suggests a larger proportion of prebomb C and a larger overall pool of passive C not contributing to soil respiration at Aspen FACE than Duke FACE.

Nevertheless, the proportion of pre-FACE C in respiration and its response to warming was similar at both sites. For elevated CO_2 soils from Aspen and Duke, decades-old C comprised a surprisingly large 30% of respired CO_2 across all incubation temperatures. Based on the $\Delta^{14}\text{C}$ of heterotrophically respired CO_2 from the ambient CO_2 soils, the estimated mean age of respired C was 2–3 y; however, additional information from the FACE-labeled soils indicates that this finding is averaging of very young C with almost one-third that is more than a decade old. Other studies with in situ isotope labels from agroecosystems show that up to 66% of respiration derived from decades-old C [>45 y old (26); other studies: 0–21%>40 y (27); 52%>14 y old (28); 45%>26 y old (26)].

The age distribution of respired C at both sites for the control temperature and warming treatments was consistent for both types of C-isotope tracers. Specifically, the offset between $\Delta^{14}\text{C}$ – CO_2 respired in incubation and the $\Delta^{14}\text{C}$ value of the atmosphere in the year of sampling was nearly the same at both sites, indicating similar residence time of respired C. Thus, respirable C was more uniform between soils than overall C stocks. Importantly, the similar response to warming—measured by the effect of warming on both proportional contribution of decade-old C to respiration fluxes and the increase in $\Delta^{14}\text{C}$ respired—suggests that the sites have similar age distributions of C sources

contributing to respiration and perhaps, similar mechanisms of temperature response.

What Is Decadal-Aged C at These Sites? We used the C-isotope signatures of soil C components (e.g., roots, microbes, and physical soil fractions) to identify sources of respired CO_2 and particularly, decades-old CO_2 (Fig. 5). C that was recently de-

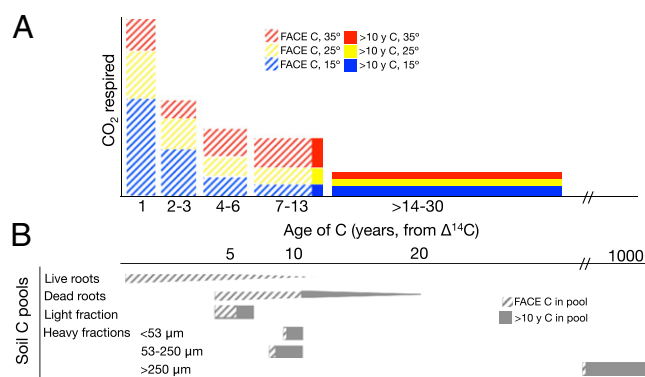


Fig. 5. Age distribution of C in Duke FACE respiration (A) and soil C pools (B). A gives our best estimate of the contributions of different age classes to respiration at the three incubation temperatures (i.e., blue bar shows respiration at 15 °C, yellow bar shows additional respiration at 25 °C above the 15 °C respiration rate, and red bar + yellow bar shows additional respiration at 35 °C). Hatched areas represent FACE-derived C, and solid bars represent C pre-dating the FACE experiment (>10 y C). Temperature sensitivity is similar between FACE and >10 y C, but it is higher for C aged 7–13 y, which is indicated by the relatively larger red bar, increasing the average age of respiration in warmer soils. B shows the ^{14}C -derived mean age of soil C pools. The hatched gray area represents FACE-derived C, and solid gray is C pre-dating the FACE experiment in each physically separated soil C fractions.

posited by roots, either as exudate or litter, is probably the source of most C respired from these soils, although visible roots were removed before incubation. Roots are increasingly recognized as the primary source of C to microbes in A horizon soils (29). The age of roots coincides with $\Delta^{14}\text{C}$ values of respired C at these sites; at Duke FACE, the mean age of roots was 4–6 y, with some roots >18 y old (30), and at Aspen FACE, the mean age of roots was 1–3 y old for <2-mm roots and 3–5 y old for >2-mm roots. Given these values, the time spent by C in structural root tissue was sufficient to give the respired CO_2 age without significant additional time in soil C; the inferred age of respired C may be a function of time spent in structural root tissue rather than soil C pools.

Although decay of root tissues may be a major component of respired CO_2 , it is not the primary source of decades-old C to respiration. Although root tissue containing pre-FACE C may have been present at Duke FACE, roots at Aspen FACE are composed only of FACE-derived C, because those trees experienced an enriched CO_2 atmosphere for their whole lives. The source of decades-old C to respiration is more likely to be C that is associated with minerals <250 μm in size, which were ~20% and ~30% of soil C at Aspen and Duke, respectively (24, 25). Temperature sensitivity of this fraction is consistent with the increase in respiration of both FACE-derived and pre-FACE C, because this fraction contains significant portions of both age classes of C and is the only soil C pool with enough bomb-derived ^{14}C to have caused the increase in $\Delta^{14}\text{C}$ of respiration with warming. The larger-size fraction (>250 μm) of mineral-associated C at Duke FACE has very low ^{14}C values in both elevated and ambient CO_2 treatments, suggesting very slow turnover and negligible contribution to respiration. Although we do not have $\Delta^{14}\text{C}$ measurements for physical soil fractions at Aspen FACE, incorporation of ^{13}C -depleted C from the FACE label in mineral-associated size fractions suggests similar C turnover patterns as observed in the Duke FACE soils (24).

Microbial biomass has a similar C isotopic signature to its C sources and respiration (29), but it is too small of a C pool in itself to be solely responsible for the observed respiration flux. Up to 9% of total soil C at Duke was respired as active-pool C in the warmest soils compared with living microbial biomass that is, at most, 4–5% of total C in these soils (31).

Another explanation for the large release of decades-old C is a disturbance effect; however, this reason is unlikely to be a full explanation in our experiment. A major criticism of the incubation method is that preincubation sample preparation may change soil C decomposition rates. Particularly if soils are sieved, previously protected, decades-old soil C may be exposed to microbial attack and vulnerable to degradation. However, potential disturbance effects are unlikely to yield similar results for both sites, because Aspen soils were sieved, whereas Duke soils were not. In addition, soil aggregation is probably not the mechanism of soil C protection in Duke FACE soils (13).

Increased Vulnerability of Intermediate-Aged (7–13 y) C with Warming. The central question of our experiment was whether decades-old C had different temperature sensitivity than faster-cycling C, because the feedback between soil respiration and climate warming in earth system models is particularly sensitive to this premise (32, 33). The FACE C-isotope label (both ^{13}C and ^{14}C) allowed us to unequivocally determine that decades-old C fixed before FACE had similar temperature sensitivity to C fixed during the last 10 y. However, integrating the bomb-derived ^{14}C label into the analysis, we identified a subtle difference in the age of C respired with increasing temperature. Specifically, we observed a parallel increase in $\Delta^{14}\text{C}$ of CO_2 respired with warming in both ambient and elevated CO_2 soils that was not caused by exhaustion of fast-cycling C. The similar increase in $\Delta^{14}\text{C}$ in both CO_2 treatments suggests that warming increased

the contribution of C fixed earlier in the decade since the FACE treatment began, reflecting the ~60‰ decline in atmospheric $\Delta^{14}\text{C}$ over the period of the FACE experiment in ambient CO_2 plots and an ~40‰ decline under enriched CO_2 , where the decline in background atmosphere $\Delta^{14}\text{C}$ was diluted by addition of FACE label C during CO_2 enrichment.

Our study is not unique in the finding that the ^{14}C content of respiration increased with warming. Two other incubations of forest soils with background levels of ^{14}C inferred higher temperature sensitivity of soil C with a similar age as the age in our study. In boreal forest soils, increased ^{14}C of respiration with warming corresponded to higher temperature sensitivity of decadal cycling C compared with annually and centennially cycling C pools (34). In a temperate forest soil, 25 °C of warming increased the ^{14}C -derived mean residence time of respiration by up to 4 y from 7.9 to 11.9 y (35). These findings show that warming increases the respiration of C up to several decades old (fixed during the postbomb period; i.e., post-1960).

With the additional time constraint of the FACE label, we can eliminate the possibility that C much older than 10 y was more temperature-sensitive than other ages of C. If this finding were the case, we would expect to observe a much more rapid increase in ^{14}C respired by elevated CO_2 soils than ambient CO_2 soils. From the similar rate of change in both types of isotope labels, we conclude that some portion of soil organic C aged 7–13 y responded disproportionately to warming. Different temperature sensitivity of this age of C does not contradict our finding of similar temperature sensitivity of decades-old and younger C; in fact, C of this age would be partitioned into both the FACE and pre-FACE C pools in the mixing model.

Higher temperature sensitivity of intermediate-aged C provides a potential explanation for inconsistencies between conclusions of previous studies that used C-isotope labels in soil to infer temperature sensitivities of different ages of C. Isotope label studies differ in the length of the labeling period before sampling, resulting in varying definitions of older C. If C with a similarly disproportionate temperature sensitivity and age is present in the soils of these studies, then inconsistent conclusions for temperature sensitivity of older C may depend on whether this C was included as part of the older or younger C pool. In shorter experiments, temperature sensitivity of this intermediate C is likely to be categorized as older C, and therefore, its disproportionate response to warming gives the appearance that older C is more temperature-sensitive [e.g., soils sampled after 5 y of label (36) or 14 y of label (29)]. In contrast, studies with a longer labeling period [e.g., labels of 26 and 45 y (26) or 33 y (16)] find equal temperature sensitivity between age classes. This finding suggests that, when this intermediate-aged C with higher temperature sensitivity is categorized as younger C, its response cannot be resolved from the temperature response of the majority of respiratory C substrate, resulting in equal apparent temperature sensitivity of the two age classes. The finding of equal sensitivity with longer label times suggests that the contribution of disproportionately temperature-sensitive C to the total flux is relatively minor.

The combination of the FACE isotope label and the bomb ^{14}C signal allowed us to identify the effects of warming on three different timescales of C cycling and avoid some confounding factors present in previous studies. The age constraint of the FACE label improved age estimates over those estimates from bomb-derived ^{14}C alone. Also, measurement of ^{14}C has advantages over the ^{13}C label used in most studies. Although $\Delta^{14}\text{C}$ data reported here are corrected for mass-dependent fractionation, the ^{13}C isotope may be affected by temperature-dependent kinetic fractionation by microbial respiration (37) or preferential use of ^{13}C -depleted substrate (38). Other confounding factors include differential substrate depletion between temperature treatments (39), differences in substrate conditions because of

seasonal effects (40), and differences in C cycling under C₃ and C₄ vegetation. In FACE experiments, manipulation of CO₂ concentrations may have altered decomposition rates, resulting in differences between CO₂ treatments at these sites (41, 42); however, this manipulation is unlikely to affect our results. Although the $\Delta^{14}\text{C}$ mixing model assumes similar decomposition rates of pre-FACE C between CO₂ treatments, model results are not sensitive to this term. In addition, the $\delta^{13}\text{C}$ mixing model gave similar estimates of the fraction of pre-FACE C and does not require the assumption that C cycling rates are similar between the two treatments.

What Potential Mechanisms Underlie the Observed Temperature Response? The similarity in temperature sensitivity of the two broad age classes suggests a common suite of mechanisms of soil C response to warming. This finding is consistent with the conceptual framework emerging from recent synthesis efforts (6, 9), which emphasizes different temperature controls over microbial respiration and supply of soil C to microbial respiration. In the short term, the temperature sensitivity of microbial respiration is the primary control of the temperature dependence of soil respiration. The constant proportion of decades-old and younger C respired across temperatures is likely determined by their fractional contributions to microbially assimilable C. Hence, the warming response could simply reflect faster respiration of assimilable C by microbes. Alternatively, it could mean that the availability of younger and older C sources was controlled by the same process or that their respective controls were similarly temperature-sensitive.

Although the temperature sensitivity of microbial respiration has been well-established, much less is known about temperature dependence of substrate supply to microbial respiration, which controls C availability in the long term. Multiple lines of evidence suggest that warming increased the supply of C of both age classes to microbes, including consistently higher flux rates over the whole incubation period, larger pools of actively cycling C, and increase in the mean age of respiration substrate at higher temperature. Previous incubation and litter decomposition studies also suggest that warming increases the fraction of soil C that is assimilable by microbes (43, 19, respectively).

Various potentially temperature-sensitive processes could influence substrate supply or cause an apparent change in supply in an incubation, such as shift in microbial community composition (44), change in microbial efficiency (45), increased turnover of microbial biomass (46), change in biochemical composition of soil C substrates respired (47), increased desorption of mineral-adsorbed organic C (6), and increased diffusion. In our study, increased substrate availability coincided with an increase in respiration of soil organic C with a mean age of 7–13 y, suggesting that a greater proportion of C of this age became available with warming. Some of these processes can be ruled out, because they would increase assimilation of substrates without a change in substrate age, such as increased diffusion, change in microbial efficiency, or increased turnover of microbial biomass.

Other mechanisms may be consistent with a change in the age of respired CO₂. A warming-induced shift in microbial community or enzyme production could change the use C of different ages (48); however, it is unlikely that such a shift would happen within the relatively short time period over which we collected CO₂ from these soils (7). Chemical kinetic theory, also known as the carbon quality temperature hypothesis (8, 49), provides a potential explanation for an increased contribution of slower turnover compounds because of higher temperature sensitivity of compounds with greater total bond strength, which is often associated with compounds that are more structurally complex (i.e., more chemical bonds) (47). If substrates with greater complexity are also retained in soils longer (i.e., become older) and warming disproportionately

promotes their decomposition, we would expect to see an increase in the mean age of respired CO₂ with increased temperature. However, the radiocarbon age of soil C is not necessarily indicative of biochemical stability (48), and we do not know the extent to which biochemical stability or activation energy of compounds per se controls C decomposability in mineral soils (50).

It is difficult to tease apart mechanisms in incubations such as this incubation or field respiration studies; heterotrophic respiration integrates over multiple C sources and reflects overlapping mechanisms of soil C stabilization. In addition, extended incubation periods have been criticized for their departure from *in situ* conditions (51, 52). Incubation isolates soils from sources of C input and results in rapid onset of substrate limitation to decomposers, which can modify the apparent response of respiration to warming (53). Substrate depletion was eventually observed in the incubation of Duke FACE soils at all temperatures, suggesting that the increase in amount of assimilable C under warming may not be sustained over time. It remains an open question whether increased substrate availability observed with warming in incubations would be sustained in a field setting or is the product of a finite, exhaustible pool as some studies suggest (54).

Modeling the Temperature Response of Soil C Decomposition. In many soil C models, temperature sensitivity is expressed exclusively in the rate constants of linear, donor-controlled soil C pools (55). When we modeled our data with this model structure, increased respiration was best simulated with an increase in the size of the active pool rather than a change in the rate constants. Indeed, including the effect of warming on substrate availability in current model structures would require a highly temperature-sensitive pool to rapidly transfer previously slow-cycling C to the fast pool. If this new warming-induced supply is rapidly depleted, then the flux from this highly temperature-sensitive pool may be transitory—a case we cannot determine with an incubation, because substrate limitation is observed at all temperatures. In that case, however, inferring the changes in respiration rates using only a temperature-sensitive rate constant may overstate the warming effect on the soil C stock.

If chemical bond strength (activation energy) were the fundamental limit to decomposition rates, then the Arrhenius equation of chemical kinetic theory (8) can be used to quantify increases in respiration substrate availability with warming. However, recent attempts to model this effect either explicitly (16) or implicitly (49) assumed that respiratory substrate stays constant under warming by parameterizing temperature sensitivity in the rate constants that directly control respiration rates. This approach predicts a more rapid loss of active pool C in warmed soils than soils at the MAT control temperature, which is counter to our findings. In contrast, our data suggest that the change in respiration rate with warming is more strongly controlled by substrate availability than temperature. As a result, caution must be taken in deriving parameter estimates in models from measurements of warming on respiration in incubations or field studies.

Earth system models are designed to predict future climate, but they still lack a predictive understanding of how much soil C is vulnerable on timescales of the next century. In this timeframe, the most important C response will come from C cycling on decadal timescales. Older pools (centuries to millennia) are also an important component of global soil C stocks (15), but their very long turnover times (and correspondingly slow decomposition rates) indicate that they will not have much effect on feedbacks in the 21st century and cannot be measured in incubation experiments in any case (56).

Our results indicate that large amounts of C (1,750–4,700 kg m⁻² in the top 15 cm of mineral soils at these two temperate

forest sites) were vulnerable to increased decomposition losses with warming. The fact that we saw similar results at the two sites, despite differences in soil C stabilization therein, suggests that the pattern we observed may apply more broadly. The importance of decadal-aged C to the large amount of C in forest soils globally suggests that soil C could become a source of atmospheric CO₂ under global warming.

A continuing challenge for models is to understand the unresolved mechanisms where C of different ages and stability can have the same temperature sensitivity. Although more research is needed to better incorporate soil C decomposition processes into models, our results suggest that we need models and experiments that explicitly separate the temperature sensitivity of microbial metabolism and the temperature sensitivity of substrate supply rather than parameterizing the temperature sensitivity of any particular compound or fraction.

Methods

We sampled the top 0–15 cm mineral soil from the Duke and Aspen FACE sites, which have been documented extensively elsewhere (57). These FACE experiments have a similar design, consisting of replicate 30-m diameter forested plots, one-half of which receive CO₂ fumigation (elevated CO₂ plots; +200 ppm above ambient) and one-half of which served as CO₂ fumigation control (ambient CO₂ plots). The evergreen plantation at Duke already had a closed canopy when FACE CO₂ enrichment began, whereas deciduous aspens were planted just as CO₂ enrichment began at Aspen FACE (Table 1).

We treated each plot as the level of replication for our laboratory incubation experiment (Duke $n = 4$, Aspen $n = 3$). Three soil cores per plot were sampled from Duke FACE in July of 2008, with each core assigned one of three temperature treatments (15 °C, 25 °C, or 35 °C) and incubated separately. Five cores per plot were sampled from Aspen FACE in July of 2009 and subsequently composited in the laboratory, with a subsample (~140 g) from each plot assigned to each of three temperatures (5 °C, 15 °C, and 25 °C). Before incubation, visible roots and rocks were removed (both sites) and sieved to 4 mm (Aspen only).

Field-moist samples were placed in glass jars with airtight lids fitted with a sampling port. The jar headspace was purged with CO₂ free air and then incubated continuously at one of three temperatures (site MAT, +10 °C, or +20 °C). Rates of CO₂ increase were measured on 2-mL aliquots headspace air by a LiCor 6252 infrared gas analyzer. Fluxes reported here are calculated as the total amount of CO₂ evolved by the soil on the time of isotope sampling. $\delta^{13}\text{C}-\text{CO}_2$ was measured by isotope ratio MS (Thermo Finnigan Gas Bench coupled to continuous flow Delta Plus) on a subsample of headspace air injected into He-filled vials to a target concentration of 3,500 ppm CO₂. When CO₂ concentrations were high enough for a ≥ 0.5 mg C subsample (>3,000 ppm), we collected headspace air by attaching 0.5-L stainless steel evacuated canisters to the lid sampling port. CO₂ was extracted from the canisters on a vacuum line, graphitized for ¹⁴C, and measured at University of California at Irvine's W. M. Keck Carbon Cycle Accelerator Mass Spectrometer (58).

To compare the same amount of respired C between temperature treatments, we chose a target amount of C equal to the total C respired at 35 °C for Duke (2.68% of initial C respired) and at 15 °C for Aspen (0.11% of initial C respired) at the first sampling period. We summed fluxes from the lower temperatures until reaching the target C loss (Fig. S2), solving for the amount of time to respire the same amount of C at each temperature (16).

C-isotope values for equivalent amounts of C were computed by linearly interpolating between isotopes measurements over this time period.

We estimated the age of respired C for ambient CO₂ soils by using a time-dependent, steady-state model of soil C that assumes that inputs to the soil have the same ¹⁴C signature as the atmosphere that year (15). The mean age of respired C is equivalent to the average time spent in the soil plus the length of time that C spent in plant tissue before it was deposited to the soil. In 2008 and 2009 (the years of sampling), the difference between pools with turnover times of 1 and 3 y is detectable—we would expect a 10‰ difference between respiration from these pools, which is greater than the combined errors from ¹⁴C analysis and spatial variability as determined from replicate samples of incubated ambient CO₂ soils (Table 3). To partition the contribution of FACE C vs. pre-FACE C to respired CO₂ flux, we used an isotopic mixing model independently at each temperature. We divided fluxes into pre-FACE (>10 y) flux and FACE-derived (<10 y) flux by assuming that flux of pre-FACE C and its ¹⁴C content was the same for both CO₂ treatments and applying isotopic end members, $\Delta^{14}\text{C}$ of the FACE atmosphere for FACE-derived C in elevated CO₂ soils, and $\Delta^{14}\text{C}$ of background atmosphere for recent C in ambient CO₂ soils. We applied a similar mixing model to ¹³C of CO₂ fluxes from elevated CO₂ soils alone to confirm these results. More details and equations can be found in *SI Methods*.

The temperature sensitivities of these two pools were quantified by assuming an exponential relationship between flux rates (R) and temperature (T) of the form $R = Ae^{bT}$, where A and b are parameters found by fitting the model to partitioned fluxes by incubation temperature for each site. The temperature dependence of R can be written $\frac{dR}{dT} = b \times R$, where b is the temperature sensitivity coefficient. Similarly, the temperature sensitivity parameter Q_{10} is the factor by which respiration rate increases with 10 °C of warming (e.g., $R = Ae^{10b}$).

Another way to define C pools with different turnover times and determine their temperature sensitivity is by modeling the change in flux rate over time at different temperatures (27). We fit a two-pool, first-order decay model of the form $R_t = k_a C_a e^{-k_a t} + k_s (1 - C_a) e^{-k_s t}$ to flux rates over time (17) for the Duke soils (in Aspen soils, there was no statistically detectable change in fluxes). We found the best-fit parameters k_a (decay rate of active pool), k_s (decay rate of slow pool), and C_a (proportional size of active pool) separately for each temperature treatment (59).

We report error as the SEM of experimental replicates or by propagating the error in isotope calculations (60). Reported P values are from comparisons of treatment means in ANOVA done using PROC GLM (unless t test was indicated), and exponential fits to data are done by PROC NLIN in SAS 9.2.

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