1 **Nonnegligible role of warming-induced soil drying in regulating warming effect** 2 **on soil respiration**

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10 **Abstract**

11 Based on results of a 26-year soil warming experiment (soil temperature being
12 elevated by 5 °C) in a Harvard hardwood forest, Melillo et al. demonstrated a
13 four-phase pattern of long-term warming effect on soil respiration, while the
14 mechanisms were not fully elucidated because they neglected the indirect effect due
15 to warming-induced soil drying. By showing a significant correlation between
16 precipitation anomaly and inter-annual variation of warming effect on soil respiration,
17 we suggest a nonnegligible role of warming-induced soil drying in regulating the
18 long-term warming effect on soil respiration. Our analysis recommends further efforts
19 to consider both the direct and indirect (i.e., warming-induced soil drying) warming
20 effects to gain more in-depth understanding of the long-term soil C dynamics.

Understanding the long-term effect of climate warming on soil respiration is a prerequisite for the projection of future change in global soil carbon (C) pools and their consequent feedbacks to climate change. Based on results of a 26-year soil warming experiment (soil temperature being elevated by 5 °C) in a Harvard hardwood forest, Melillo et al. (1) proposed a hypothesis of four-phase pattern of soil warming effect on soil respiration ($\Delta R_s = R_{s\text{Heated}} - R_{s\text{Control}}$) and attributed it to soil substrate and microbial changes. However, the mechanisms were not fully elucidated because the authors improperly neglected the indirect effect due to warming-induced decline in soil moisture (Fig. 1).

Figure 1. A conceptual framework showing the direct and indirect warming effects on soil respiration. Precipitation buffers warming-induced soil drying and thus has an positive effect on inter-annual variation of ΔR_s . Negative feedbacks exist between C substrate availability and respirational C loss. Current experiment failed to track the direct and indirect warming effects on forest primary productivity (NPP) and consequent fresh soil C inputs due to a small plot size (6 x 6 m²) (as indicated by dashed lines). The symbols “+” in red and “-” in blue indicate positive and negative effects, respectively.

Experimental results have evidenced an essential role of soil moisture in regulating the warming effect on soil respiration (2-5). Lower soil moisture can

reduce ΔR_s , and even leads to negative ΔR_s , when soil moisture becomes extremely limiting to plant roots and soil microbial organisms (Fig. 1; 3-6). In addition to the direct warming effect (1), warming-induced soil drying (2-6) may indirectly reduce ΔR_s , especially in drier years (Fig. 1; 2-5). Moreover, soil moisture stress has been evidenced to reduce temperature sensitivity of soil respiration (7, 8). Warming-induced soil drying may have also contributed to a decrease in temperature sensitivity of soil respiration in heated plots, being observed to occur consistently across all four phases during the long-term soil warming experiment in Harvard forest [see Fig. 3 in Melillo et al. (1)]. Unfortunately, we cannot re-analyze the role of warming-induced soil drying due to unavailable soil moisture data (1).

As a primary source of soil moisture, precipitation is expected to buffer the soil moisture stress and result in a positive effect on inter-annual variation of ΔR_s (Fig. 1). By retrieving data on total annual precipitation (TAP) anomaly (showing no significant temporal trend, $p=0.94$) and ΔR_s from Melillo et al. (1), we tested the effect of TAP anomaly on inter-annual variation of ΔR_s . As ΔR_s showed a significant decrease over time ($\Delta R_s = -6.1547 \cdot \text{Year} + 12402$, adjusted $R^2 = 0.29$, $p=0.005$) due to long-term microbial C substrate depletion (Fig.1; 1, 9, 10), we thus used detrended (i.e., residual) ΔR_s [being calculated as, measured $\Delta R_{s_i} - (-6.1547 \cdot \text{Year}_i + 12402)$, where i indicates a specific year] to indicate the inter-annual variation of ΔR_s . As expected, we found a significantly positive effect of precipitation anomaly on the inter-annual variation of ΔR_s (slope = $0.15 \text{ g C m}^{-2} \text{ year}^{-1}$ per mm; adjusted $R^2=0.17$, $p=0.03$; Fig. 2). Our result suggests a significant role of soil moisture in regulating the warming effect on soil respiration. It further implies that warming-induced soil drying stress has very likely exerted a negative effect on the response of soil respiration to long-term warming treatment in Harvard forest.

Figure 2. Significant effect of total annual precipitation (TAP) anomaly (mm) on the inter-annual variation of warming-induced ΔR_s ($\text{g C m}^{-2} \text{ year}^{-1}$). Because ΔR_s showed a significant decrease over time ($\Delta R_s = -6.1547 \cdot \text{Year} + 12402$, adjusted $R^2 = 0.29$, $p=0.005$) due to soil C substrate depletion, we used detrended residual [being calculated as, measured $\Delta R_{s_i} - (-6.1547 \cdot \text{Year}_i + 12402)$, where i indicates a specific year] to indicate the inter-annual variation of ΔR_s . Note that our analysis excluded data for the years 1995, 2005, 2010 due to warming system failure.

The long-term experiment, with each plot covering a small area of $6 \times 6 \text{ m}^2$ (1), generally represented a scenario of soil C depletion under soil warming, while it failed to demonstrate the story in a whole C cycle perspective. The small plot size was not able to track the direct and indirect (i.e., warming-induced soil drying) warming effects on forest primary productivity (Fig. 1), which could consequently provide fresh C substrates for soil respiration (11). In that case, negative feedbacks between C substrate limitation and respirational C loss likely dominate a long-term reduction of ΔR_s (9, 10). By inducing a reduction of soil moisture (2-6, 12, 13), soil warming also indirectly regulates ΔR_s via inducing changes in the composition and activity of

microbial community (5, 14). For instance, warming-induced soil moisture stress may have contributed substantially to the declining trends of ΔR_s during the relatively drier periods 1997-2002 and 2015-2016 [see Fig. S2 in Melillo et al. (1)].

As the indirect effect due to warming-induced soil drying stress has not been addressed properly, the mechanisms of the four-stage pattern of long-term warming effect on soil respiration are likely not fully elucidated (1). Moreover, Melillo et al. (1) assumed that microbial respiration accounted for a constant proportion (approximately 2/3) of soil respiration, while they ignored temporal changes in the microbial proportion over the four-phase pattern of soil respiration. Therefore, in-depth understanding of the long-term warming effect on soil C dynamics calls for further efforts to consider both the direct and indirect (i.e., warming-induced soil drying) effects in a whole C cycle perspective at an ecosystem scale.

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