In situ incubations highlight the environmental constraints on soil organic carbon decomposition

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Abstract
We use root exclusion plots, subsurface gas sampling and in situ diffusivity measurements to quantify in situ soil organic carbon (SOC) decomposition dynamics within separate depth-dependent soil pools (0 and 35 cm). We contrast these measurements with observations of temperature–decomposition potentials, generated from laboratory incubations of the same soils at optimal moisture levels and native temperatures. The decomposition–temperature response was similar at different depths in the field, but every gram of soil C at 35 cm was more than 100 times less active in decomposition than surface soil. These depth-related variations were not evident in decomposition potentials generated from aerobic laboratory incubations, highlighting the importance of environmental physical factors in constraining soil organic carbon decomposition. At depth, physical protection of SOC could match or even override the importance of quality and temperature in determining the future stability of deeper, recalcitrant pools.

Keywords: soil organic matter, soil carbon storage, SOC decomposition dynamics

1. Introduction
Evaluating soil organic matter (SOM) responses to perturbations in the physical and biological environment is critical to understanding how the terrestrial–atmospheric carbon balance may shift in the face of climate change. Increases in primary production are expected to increase SOM inputs; however, this will be offset by increases in the rates of SOM decomposition due to elevated soil temperatures [1–3]. Whether the balance will lie in a net gain or loss of soil carbon is a topic of current debate [3–6], especially the response of different soil pools to changes in soil temperature over the long term.

A variety of techniques are used to assess SOM decomposition as a function of temperature. Modeling studies have provided useful estimates of long-term SOM turnover processes [7] and contradictory results may be a result of assumptions about how different pools of SOM respond to temperature over time. Studies which assume a single soil carbon pool and uniform turnover rate [5] are unlikely to produce results consistent with those which assume multiple pools with different turnover times [4]. Incubation experiments have also provided critical evidence [8, 9], but are conducted under non-native conditions which may explain differences in predicted temperature response [10]. In addition, the perturbation of soils in such experiments may alter soil structural properties that might otherwise play an important role in the temperature response [11, 12]. Laboratory incubations, therefore, may provide optimal decomposition responses at a given temperature, rather than an accurate representation of true rates of field decomposition.

A recurrent theme in this debate is the accurate representation of natural processes and conditions, pointing to a need for further clarification of temperature–decomposition relationships in situ, where soil physical properties and environment can be preserved. In situ approaches are valuable, because in addition to SOM quality, there are a suite of...
environmental factors that contribute to the actual response of SOM to climate, vegetation and land use changes [1].

Soil profiles represent natural vertical gradients along which we might expect controlling environmental factors to impede potential rates of decomposition with increasing depth. It may be possible to quantify the role of environmental limitations on SOM decomposition along these gradients in situ by examining depth-specific decomposition rates [2] in soils where CO₂ is generated solely from microbial activity. Obtaining realistic estimates of subsurface microbial respiration rates through depth has, however, been hampered to date by issues surrounding the magnitude of gas diffusivity values used in calculation methods. The production of CO₂ at each depth is assumed to be the difference between the flux across soil layers. The flux (F) for each layer is determined from Fick’s law in one dimension:

\[ F = -D \frac{\partial C}{\partial z}, \]

where \( D \) is the diffusivity (m² s⁻¹), \( C \) is the CO₂ concentration (g m⁻³) and \( z \) is depth (m). In temperate soils where soil volumetric water content is subject to large annual fluctuations (50% to <10%), \( D \) can vary annually by 100 or 1000 times [13]. In contrast, the annual variability in CO₂ concentration may only be a factor of 3 [14]. As a result, tightly constrained diffusivity values must be used to calculate within-soil CO₂ fluxes and production.

Detailed examinations of vertical in situ decomposition processes therefore require novel approaches in root-free soils that combine accurate estimates of diffusivity with corresponding vertical SOM-C concentration measurements, CO₂ concentration and soil climatic instrumentation—all at a single location. Soil plots where gas data, diffusivity data and corresponding temperature and moisture data are being measured collectively provide opportunities to develop and test hypotheses about how environmental factors ultimately control SOM decomposition processes in discrete subsurface soil layers.

Here we examine the temperature response of vertically distinct SOM decomposition at sites where root exclusion is made possible by trenching or removal of vegetation. We examine decomposition–temperature relationships in shallow and deep soil zones using a newly developed in situ diffusivity measurement system and a subsurface sampling approach [15], and compare these relationships to those developed for soils incubated under laboratory conditions in the range of native temperatures. Laboratory incubations can be considered to represent the temperature–decomposition dynamics under ideal conditions, and hence a potential, without the environmental constraints of factors that might otherwise limit decomposition rates. We use differences between the laboratory incubations (potential) and in situ field measurements (actual) as an indication of the role environmental controls play in decomposition dynamics at experimental plots located within a temperate zone forested soil. To our knowledge, this is the first study to combine field and laboratory estimates of subsurface CO₂ production to evaluate the relative control of substrate quality and environmental constraints on decomposition.

| Site   | Depth (cm) | Soil C (%) | Soil C/N (g/g) | Mean moisture (cm³ cm⁻¹) |
|--------|------------|------------|----------------|-------------------------|
| TR     | 0          | 3.49       | 37(2)          | 0.273                   |
| TR     | 35         | 1.54       | 19(1)          | 0.273                   |
| CC     | 0          | 5.67       | 21(3)          | 0.342                   |
| CC     | 35         | 2.58       | 16(3)          | 0.342                   |

2. Methods

This study was conducted in sandy textured soils in a temperate mixed forest located in Lakevale, Nova Scotia, Canada 45°45'11"N, 61°57'21"W. In 2003, a 40 ha section of the forest was clearcut and in 2003 2 m² experimental plots were established in both the clearcut (CC) and mature intact forest (TR). Both sites are equipped with above- and below-ground instrumentation following Beltrami [16] to monitor temperature and moisture. The CC treatment was left free of slash, regrowth has been curtailed by regular weeding and we assume that fresh carbon inputs since harvesting are negligible. In the intact forest, we employed root exclusion techniques [17, 18] to mimic the absence of root activity of the CC soils. In 2003, a 50 cm deep trench was dug around the TR plot and lined with a vapor barrier to prevent root ingrowth and to minimize lateral CO₂ gradients. The plot was left for one year to allow for recovery from the respiratory burst initiated by the presence of freshly severed roots. In each plot, 50 cm long subsurface equilibration tubes [14] were installed horizontally in the mineral soil at 0, 2.5, 5, 10, 20, 35 and 50 cm from a temporary pit at the side of the plot. Soil samples were collected during installation of subsurface samplers for elemental analyses (C and N) using an elemental analyzer (Euro-vector). We assume that differences in soil profile C concentrations between sites (table 1) are the result of natural spatial variability.

In 2004, we sampled subsurface CO₂ concentrations weekly from May to August by drawing samples from the equilibration tubes for lab analysis using a LI-7000 gas analyzer, in continuous flow configuration. Carbon dioxide concentrations were used to construct CO₂ concentration–depth profiles to calculate heterotrophic subsurface CO₂ production at two depths (0 and 35 cm) using a simplistic multi-layered diffusion model based on Fick’s law ([2] and references therein). Soil gas diffusivity was parameterized using in situ measurements collected under a wide range of soil moisture contents with a membrane probe and automated continuous flow system [15]. The total error in CO₂ production estimates is roughly 2%, expressed as the RMSE of all associated uncertainties including analysis (1%), sampling, handling (3–4%), diffusivity measurement (1%, [15]) and soil moisture reflectometers used for diffusivity–moisture curves (Campbell CS616, 1.5%). While soil CO₂ concentrations follow typical vertical patterns in soil profiles amongst plots at these sites, they are also spatially variable [14], a reflection of variability in both gas transport rates and CO₂ production. Nevertheless, typical variations in subsurface
Figure 1. Relationships between soil CO₂ production (PCO₂) and soil temperature at surface (0 cm) and depth (35 cm) for the trenched plot at the mature intact forest (TR) and clearcut (CC) forest, with 95% confidence intervals indicated by the dotted lines.

3. Results and discussion

3.1. In situ temperature response of SOC pools

The temperature dependence of SOC decomposition for different depths (figure 1) indicated no apparent difference in the temperature response of surface pools at either site, with overlapping 95% confidence intervals on exponential regressions. At depth, we observed sluggish CO₂ production rates overall, and, at the CC site, a high temperature dependence. We expected that any observed depth differences would be linked to SOM quality or to in situ differences in microbial community dynamics, and subsequent sections address their possible underlying causes.

3.2. Field in situ rates of SOC decomposition

The decomposition rate per unit kg of soil C, observed and normalized for soil temperature (10 °C), allows comparison of the activity of each gram of soil C, in shallow and deep pools, and for plots where environmental factors, including soil temperature, may differ as a consequence of clearcutting (figure 2). Respiration of TR and CC deep layers are comparable, and every gram of soil C is >100 times less active in decomposition than at the surface, indicating high recalcitrance, and/or the importance of conditions that limit microbial activity (or access to substrate) at this depth. While within-plot variabilities in CO₂ concentrations were high, and prevent differentiation of observed temperature-dependent decomposition rates between sites at a given depth, these data highlight the depth-dependent differences in decomposition rates at both sites.

3.3. Potential decomposition versus observed field decomposition

Decomposition rates of surface soils are of similar magnitudes in the field and under laboratory conditions (figure 2). Most interestingly, though, while we observe decomposition rates in the deep soil profile that are many times (>100×) slower than at the surface, incubation of these soils yield contrasting results (figure 2), even after correcting for differences in carbon content. Incubation of these soils in the range of native temperatures and under optimal conditions of moisture suggest the soils have the potential to respire at much greater rates than...
observed in the field, and in fact at rates that are comparable on a per gram of carbon basis to the surface measurements. Rates of decomposition are in the reported range for surface (0–10 cm, 600–900 mg C–CO$_2$ g C$^{-1}$ d$^{-1}$) and subsurface (35–50 cm, 120–800 mg C–CO$_2$ g C$^{-1}$ d$^{-1}$) mineral soils incubated around 20°C [21–23] and our laboratory incubation results are consistent with incubation studies such as Fang et al [6] that have shown far smaller differences (∼2–4×) between surface and deep soil decomposition rates. Incubations did not show the dramatic differences in decomposition rates between depths, or the wide range of $Q_{10}$s that we observed in situ (figure 2), suggesting that SOM quality differences may, in fact, be negligible relative to differences imposed by environmental conditions in the field.

In situ, many physical mechanisms of SOM protection such as texture, aggregation/occlusion, soil moisture, oxygen availability and compaction [1] act to limit potential respiration rates observed in laboratory incubations. On an individual basis, it is not well understood how these factors might manifest themselves in the microbial community with respect to decomposition rates and the temperature sensitivity of decomposition. In addition, soil parameters may co-vary with temperature to obscure the pure temperature response. For example, at our sites temperature and moisture are strongly coupled and early season saturation strongly inhibits soil respiration, but when temperatures rise, soils dry, pores become aerobic and high soil gas diffusivities help replenish consumed oxygen. Together, these factors boost decomposition rates over short time and temperature intervals, as reflected in the field $Q_{10}$s (figure 2) and are unlikely to represent a pure temperature response. While the CC plot experienced higher temperatures (see figure 1), mean moisture values for the study period were also higher for this plot, due to reduced transpiration rates associated with harvesting of the vegetation.

Although in situ SOM decomposition studies must be approached and interpreted carefully, they should assume an important role in questions pertaining to future soil organic carbon stability, providing contrast and context to the decomposition potentials and climatic responses observed during laboratory incubation. These results suggest that in situ depth-dependent environmental factors may match or even override the importance of carbon quality in determining SOM stability, and that these effects could be disproportionately important for slower cycling pools which dominate at greater depths.

4. Conclusions

The goal of this study was to examine decomposition rates and temperature responses in vertically distinct, root-free soils both in situ and in the laboratory in order to quantitatively evaluate the role environmental limitations may play in SOM decomposition dynamics.

Deep soil layers (35 cm) at these sites produced >100 times less CO$_2$ per gram of organic C than surface (0 cm) layers, suggesting critical differences in controls on decomposition through depth. Contrasting results from laboratory incubations lead us to question the role of SOM quality as a major control on in situ decomposition rates, and confirm that real-world dynamics are complex and unique to the in situ environment. Of particular importance may be physical mechanisms of protection that limit microbial activity and/or substrate availability. We propose that these factors could match or even override the importance of SOM quality in determining the future stability of deep, recalcitrant carbon pools at these and other study sites. While past soil carbon research has been overwhelmingly focused on biochemical processes, generating realistic predictions of C sequestration or C loss will require that new research also targets soil physical factors as a critical determinant of decomposition rate.

Although covariant factors can make the interpretation of data more complicated, in situ studies provide the only hope of capturing true decomposition–climate responses. We recommend that in situ and laboratory research be conducted in tandem to (1) confirm that measured potential laboratory responses are in fact observed in the field and (2) determine the extent to which additional physical factors influence organic matter pool stability.

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