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PREDICTING SOIL ORGANIC CARBON IN AGROECOSYSTEMS UNDER CLIMATE CHANGE

Dynamic, Intermediate Soil Carbon Pools May Drive Future Responsiveness to Environmental Change

Susan E. Crow* and Carlos A. Sierra

Abstract

Accurately capturing dynamic soil response to disturbance effects in agroecosystem models remains elusive, thereby limiting projections of climate change mitigation potential. Perennial grasses cultivated in zero-tillage management systems hold promise as sustainable agroecosystems. High-yielding tropical C_4 grasses often have extensive rooting systems, and the belowground processes of root turnover, aggregate formation, and mineral stabilization drove rapid C accumulation after cultivation in a recent study. We sought (i) to understand and constrain the size and responsiveness of dynamic, intermediate-cycling C pools contributing to the observed C accrual rates, and (ii) to simulate C stocks over time under the disturbance of elevated temperature using soil incubation at multiple temperatures and physical fractionation via density and sonication. Three-pool transfer modeling of soil incubations revealed small pools of readily available (i.e., days to months) microbial substrate that were responsive to temperature, time since cultivation, and inputs. Larger, kinetically slow-cycling pools were more indicative of long-term (i.e., years to decades) changes in C stock and strongly connected to measured changes in physical fractions. Combining the sensitivity of readily available microbial substrate with three-pool transfer modeling of the physical fractions over time since cultivation revealed that dynamic transfers of inputs occurred between the free organic and aggregate-protected fractions, and from these fractions to the mineral-associated dense fraction. Under 5°C temperature elevation, increased transfer rates outweighed elevated decomposition losses to sustain soil C accrual into the future. To effectively plan managed landscapes and monitor sustainable agroecosystems for climate change mitigation, tools must incorporate the complexity of soil response to change.

Core Ideas

- Dynamic aspects of soil C are inadequately represented in most models.
- Intermediate-cycling pools are more responsive to disturbances than thought.
- Root inputs are rapidly moved from particulate debris to mineral-bound stable pools.
- Temperature increased the transfer rate to stable pools and sustained C accumulation
- Climate change mitigation projections must capture dynamic responses in agroecosystems.

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*Corresponding author (crows@hawaii.edu).

Projecting interactions of land use change and temperature on soil C balance during cultivation of perennial grasses, which are among the greatest contenders as feedstocks for sustainable renewable biofuels (DeLucia, 2016), is an increasingly important component of planning and monitoring renewable energy systems (Agostini et al., 2015). Net soil C balance is fundamentally a function of inputs and outputs (Olson, 1963); however, the underlying soil C pools and fluxes are dynamic and dependent on soil biological, chemical, and physical properties (Schmidt et al., 2011; Tiemann and Grandy, 2015). Commonly used tools for soil C simulation such as the Century and RothC models are effective in predicting long-term dynamics in total C stocks (Jenkinson et al., 1991; Parton et al., 1998) but falter in systems undergoing rapid change because of uncertainty in their representation of soil C pools and their dynamics. For instance, much attention has been given to the effects of temperature and moisture in controlling decomposition rates (Davidson and Janssens, 2006; Martinez-Moyano and Richardson, 2013), but less to environmental controls on transfers and transformations of C that result in stabilization or destabilization of soil organic matter. Further, it is well known in agricultural systems that soil C fractions are highly sensitive to management (Cambardella and Elliott, 1992, 1993), but most agronomic models do not allow for plasticity in transfer rates among pools. Better numerical models are needed that integrate measurable soil components with current conceptual understanding of the primary mechanisms controlling soil C stabilization and destabilization (Schmidt et al., 2011; Cotrufo et al., 2015) and to improve our projections of soil change in a rapidly changing environment (Lehmann and Kleber, 2015; Luo et al., 2016).

There is a global push for sustainable intensification of agricultural systems in response to increasing population and decreasing arable land availability and soil health (Godfray et al., 2010; Garnett et al., 2013; Campbell et al., 2014). The application of ecological principles to the management of agricultural ecosystem, or agroecology, is a fundamental component of developing sustainable food and fuel production systems (DeLonge et al., 2016). In Hawaii, about half of the prime agricultural land (~60,700 ha) is currently abandoned after the complete collapse of plantation agriculture, but programs are in place to increase the number of farmers, access to land, and area in cultivation for food, fiber, and fuel (see http://www.aiphawaii.com/index.html). This movement is consistent with a growing awareness of

S.E. Crow, Natural Resources and Environmental Management Dep., Univ. of Hawaii Manoa, 1910 East West Rd., Honolulu, HI 96822; C.A. Sierra, Max Planck Institute for Biogeochemistry, Hans-Knoll-Straße 10, 07745 Jena, Germany. Assigned to Associate Editor Hero Gollany.

the need to increase agricultural activity for food, fiber, and fuel within a robust framework of economic and environmental sustainability (Werling et al., 2014).

Previous work in Hawaii showed that soil C accumulation after cultivation of a grassy field for C_4 perennial grasses under ratoon (i.e., zero-tillage management) was driven primarily by rapid decomposition of root tissue with low lignin concentration (Sumiyoshi et al., 2016). After ratoon harvest, where aboveground biomass is removed ~10 cm from the soil surface and the grass is allowed to regenerate vegetatively, a subset of roots die and subsequently decompose. In the study by Sumiyoshi et al. (2016), most of the residue accumulated as soil C, as opposed to being lost from the system as CO_2. There is increasing evidence that active root-associated C cycling provides a mechanistic explanation for observed net soil C sequestration in temperate perennial grass systems (Anderson-Teixeira et al., 2013; Lange et al., 2015). In addition, interactions among root productivity, soil type, aggregation processes, and microbial communities explained cross-site variability in soil C accrual in temperate US perennial grass systems (Tiemann and Grandy, 2015), suggesting that more detailed understanding of the dynamics underlying the changes in total C stock is needed.

A follow-up study in perennial grasslands in Hawaii showed that, where no additional plant inputs were present, the aggregate-protected soil C pool declined over time, whereas in planted soils, the aggregate-protected pool accumulated C, specifically, root residues from the recently cultivated grass inputs (Crow et al., 2018). These data suggest the presence of a dynamic component to the aggregate-protected C pool that is sensitive on an annual timescale to changes in the soil environment and may affect the soil C response to future change. However, it remained unclear from the empirical data alone whether the short-term changes detected in the aggregate-protected pool would become stabilized there, be processed by microorganisms and transferred to another pool, or be lost from the soil.

Understanding dynamic components of what typically are considered intermediate-cycling C pools and constraining the factors that control the size and responsiveness of this dynamic component are critical to our ability to project changes in soil C pools under various land use, management, and climate change scenarios. Here, we investigated whether further evidence for the dynamic component of the aggregate-protected C pool could be derived from a comparison of multiple pool C models. We sought to constrain pool size and dynamics (including transfers and decomposition rates) with multiple approaches to modeling the soil C system and validating responsiveness to changes in temperature, inputs, and time. Soil incubation and physical fractionation data were used to generate multiple models simulating the soil C dynamics for the system undergoing change after cultivation of a grassland for the production of tropical perennial grasses.

A series of questions guided our investigation of dynamic components of intermediate-cycling pools:

- How responsive are C pool sizes and decomposition rates to temperature, inputs, and time after cultivation?
- How do the C pools derived from microbial metabolism during incubation relate to operationally defined physical fractions, and what is the fate of the fresh C inputs derived from perennial grass root and rhizosphere inputs as it enters dynamic C pools?
- What are potential changes in pool sizes and total C stocks projected by the interaction of land use and temperature change?

We hypothesized that a dynamic, intermediate-cycling C pool within the aggregate-protected fraction was responsive to change in plant inputs, providing substrate for microbial metabolism, and that this pool can effectively be represented within C pool models that combine information from various derivations of C pool information. This hypothesis was tested by measuring the C properties of a soil recently converted from grassland to cultivated land use and then comparing the initial properties to those of soils undergoing changes associated with plant inputs, time, and temperature. Controlled soil incubations, physical isolation of multiple pools, and model comparisons were used to develop different versions of a three-pool model that effectively simulates changes in C stock over time in response to change in inputs (no plant inputs vs. perennial grass root inputs).

Materials and Methods

Field Experiment

The field study was conducted on the island of Oahu at the Waimanalo Experiment Station, a research station run by the College of Tropical Agriculture and Human Resources at the University of Hawaii Manoa (21°20′11″ N, 157°42′46″ W). Mean annual temperature at the station is 24.6°C and mean annual precipitation is 938 mm (Giambelluca et al., 2013). The soil is classified as a Mollisol of the Waialua series (very-fine, mixed, superactive, isohyperthermic Pachic Haplustoll). Mollisols are high-fertility soils distributed globally in areas known for high productivity, are rich in organic matter, and critical for maintaining soil health to meet production needs and sustainability goals. Soil from the field site is high in organic matter, base saturation, and CEC, consistent with high natural fertility common to Mollisols. The mineralogy is mixed, with the presence of smectite and montmorillinite giving minor shrink–swell properties to the soil. The fine texture further contributes to a sticky, heavy clay soil characteristic when wet. Less than 8% of the land in Hawaii is classified as Mollisol, but it is an important agricultural soil across the islands (Deenik and McClellan, 2007). The land was grassland maintained regularly by the research station crew with mechanical trimming for at least 20 yr prior to the establishment of the study plots. A persistent, compacted plow layer suggests prior history under intensive cultivation. Cultivation of the field for the study began with preparation tillage using a disk tiller in November 2009.

As part of a larger experiment conducted for a productivity comparison trial for 25 cultivar of two species, *Megathyrsus maximus* Jacq. (Guinea grass), *Pennisetum purpureum* Schumach. (napiergrass), and *Pennisetum purpureum* × *Pennisetum glaucum* cross (pearl millet × dwarf napiergrass sterile hybrid, or napier hybrid), three cultivars of napiergrass and the napier hybrid were selected for this study. These four cultivars, Bana, Purple, Merkeron, and napier hybrid, were selected to potentially represent a range of productivity according to preliminary data for aboveground yield available at the time. Details of the field management are available in Sumiyoshi et al. (2016). Briefly, the grasses were grown from stem cuttings in four rows within a 2-m

× 3-m plot with a nonplanted buffer of 0.6 m between each plot. Plants were irrigated 3 d wk^{-1} until February 2012, when irrigation lines were cut. Although irrigation ceased, the plants continued to grow as a rainfed system during a typical rainy season. Fertilizer (granular 16–16–16) was added at a rate of 53 kg N ha^{-1}, 23 kg P ha^{-1}, and 44 kg K ha^{-1} when the grasses were first planted and a year later after a harvest. The grasses were ratoon harvested (i.e., cut ~10 cm above the soil surface, leaving the belowground root system intact) every 7 to 8 mo. As such, this constitutes a zero-tillage management system for the cultivation of tropical perennial grasses for forage or feedstock production for lignicellulosic biofuel or anaerobic digestion.

Each cultivar was grown in four replicate plots. Previous detailed work on ecosystem C components found few significant differences, even among the cultivars selected previously to represent a range of productivity (Sumiyoshi et al., 2016); therefore, we treated all grasses the same regardless of cultivar for a total sample size of 16 (see also Crow et al., 2018). Four 2-m × 3-m bare fallow plots were also established from the onset of the field trial and maintained throughout the experiment with no inputs. Periodic weeding occurred to maintain the plots in a state to receive no further plant inputs. Soil samples were collected from each plot in April 2010, August 2011, and July 2012 to determine changes to soil C pools over time since cultivation of the grassy field. Each time, four soil cores were collected with a 5-cm-diam. core sampler to a depth of 15 cm and composited for every plot. The samples were brought to the laboratory and air dried for 1 wk, then sieved to <2 mm and stored at room temperature. Root biomass was quantified during this sieving stage, with identifiable root fragments also picked out from the <2 mm soil if necessary. Therefore, soil samples were homogenized and root free.

Carbon concentration for each soil sample was determined after oven drying at 75°C and further homogenization with a ball mill (Retsch MM200 mixer mill) to pass through a 250-μm sieve by oxidative combustion on an elemental analyzer (Costech ECS 4010 CHNSO Analyzer, Costech Analytical Technologies). Total soil C pool size was determined by the equivalent soil mass method (Sumiyoshi et al., 2016) as the C stock within 150 Mg ha^{-1} of soil, which was roughly equivalent to the top 15 cm for this soil.

Laboratory Incubations

Carbon dioxide efflux was determined during a 191-d incubation of soil from each plot in a controlled environment set at 21 and 26°C. The lower temperature was the mean annual temperature for multiple field sites under study at the same time, and the higher temperature is ~2°C higher than the mean annual temperature of the site and corresponds with the projected global temperature increase range scenarios for Hawaii (Giambelluca et al., 2013; IPCC, 2014). The details of the incubation may be found in Crow et al. (2018), which reported the raw cumulative CO_2 efflux curves as a proxy for the amount of active C, readily accessible to microbes. Briefly, 10 g of soil (the dry weight equivalent) was brought to field capacity and placed in an incubation jar fitted with a septa and equilibrated for 48 h at the designated temperature within two C-controlled environment chambers (Model 6021-1, Caron Products & Services). The incubation jars were closed, and an initial sample of the headspace was taken and analyzed for CO_2 concentration (Clarus 580 Gas Chromatograph, PerkinElmer). The jars then remained closed to allow CO_2 to accumulate in the headspace until the next sampling day, when another sample of the headspace was taken and analyzed. Sampling occurred on Days 1, 2, 3, 6, 9, 14, 19, 24, 29, 34, 39, 49, 63, 79, 94, 108, 122, 136, 150, and 191. The initial concentration was subtracted from the final concentration for total CO_2 efflux, and cumulative values were calculated for each time point. Mean ± 1 SE is reported for four field-replicated samples.

Physical Fractionations: In Situ Carbon Pool Measurements

Physical C pools with different rates of turnover were isolated following the method of Golchin et al. (1994) using a combination of density and application of ultrasonic energy to separate soil fractions. By this method, a floating free light fraction was isolated, consisting of organic debris outside of soil aggregates and not mineral associated. Ultrasonic energy was used to further isolate an occluded light fraction consisting of organic debris and decomposed organic matter that was held within soil aggregates. Finally, mineral-associated C was isolated within the dense fraction. The details may be found in Crow et al. (2018), which reported the C pool size of density fractions as representations of unprotected organic debris (free light fraction), aggregate-protected organic matter (occluded light fraction), and mineral-associated organic C (dense fraction). Briefly, 20 g of soil (dry weight equivalent) was fractionated in a 1.8-g mL^{-1} solution of sodium polytungstate, and the free light fraction was isolated by gently shaking and removing the floating material by suction. Then, 150 J mL^{-1} of energy was input (calibrated 505 Sonic Dismembrator, Fisher Scientific, FB-505-110) to disrupt aggregates, and the subsequently floating material was recovered as the occluded light fraction. The dense fraction was recovered as the material that sank in the density solution. Each fraction was rinsed repeatedly to remove the sodium polytungstate, dried at 75°C, weighed, and analyzed for C concentration as described previously. Mean mass recovery was 99.2%, and C recovery was 93.2%.

Mathematical Models

We developed a model to conceptually combine and integrate various sources of information and represent the dynamic nature of various C pools. In each case, the soil is composed of three pools with different turnover (Fig. 1): fast (C_1), intermediate (C_2), and slow (C_3). In this conceptualization, C cycles are at different rates among the pools but can also be transferred among them simultaneously. This permutation represents hypothesized stabilization and destabilization processes (Sierra et al., 2012). A system of differential equations expressed the model generically:

$$\begin{pmatrix} dC_1/dt \\ dC_2/dt \\ dC_3/dt \end{pmatrix} = \begin{pmatrix} I \\ 0 \\ 0 \end{pmatrix} + \begin{pmatrix} -k_1 & \alpha_{1,2}k_2 & \alpha_{1,3}k_3 \\ \alpha_{2,1}k_1 & -k_2 & \alpha_{2,3}k_3 \\ \alpha_{3,1}k_1 & \alpha_{3,2}k_2 & -k_3 \end{pmatrix} \begin{pmatrix} C_1 \\ C_2 \\ C_3 \end{pmatrix} \quad [1]$$

with initial conditions

$$\begin{pmatrix} C_{01} \\ C_{02} \\ C_{03} \end{pmatrix} = C_0 \begin{pmatrix} \gamma_1 \\ \gamma_2 \\ \gamma_3 \end{pmatrix} \quad [2]$$

where k_i represents the cycling rate for each pool i, coefficient $\alpha_{i,j}$ represents the proportion of decomposed C from pool j transferred

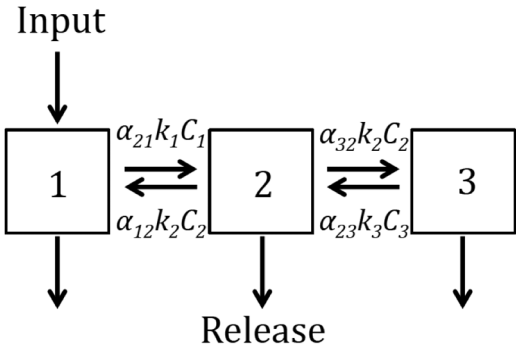

Fig. 1. Conceptual diagram depicting the structure of the model expressed in Eq. [1]. Each C pool (C_i) has its own decomposition rate (k_i), and transfers among pools are represented by the coefficients $\alpha_{i,j}$, from pool *j* transferred to pool *i*. Connections between Pools 1 and 3 are not shown for simplicity.

to pool *i*, and coefficient γ_i represents the proportion at which the total initial amount of C is split between the three pools. Total inputs of C to the soil are represented by *I*. This conceptual model was developed and tested previously for the in situ C measurements and reported on in Crow et al. (2018) to confirm the rapid, dynamic transfer of root-derived organic matter through multiple particulate C pools and into the mineral-bound pool at this site.

Here, we used the data from both the incubation experiments and from the in situ C measurements to fit the model of Eq. [1] and [2] using a two-step parameter optimization. First, to find the parameter set that minimizes the difference between model predictions and observations, the Nelder–Mead (Nelder and Mead, 1965) or the pseudorandom search optimization algorithms (Soetaert and Petzoldt, 2010) were used. Second, a Bayesian optimization using Markov chain Monte Carlo (Soetaert and Petzoldt, 2010) was run using the results from the initial optimization. We report the best parameter set obtained by the initial optimization and the uncertainty in parameter values and predictions obtained by the Bayesian procedure.

Simulations were run forward to 2016 to predict potential changes in C stocks and pools over time as an effect of land use and temperature change, and to assess whether the system would gain or lose C during this timeframe. The result of the baseline simulation for the in situ C measurements is reported in Crow et al. (2018). Here, we used the relative change in cycling rates from the incubation experiment to modify the cycling rates obtained in the optimization of the in situ data of density fractions (Table 1). More specifically, the ratio of decay rates at 26 to 21°C of the fast, intermediate, and slow pools observed in the incubation experiments was applied to the decomposition rates of the free light, occluded light, and dense fractions, respectively. In this manner we combined knowledge on the C storage in different density fractions, with their potential reactivity derived from incubations.

Results

Response of Carbon Pool Sizes and Decomposition Rates to Temperature, Time, and Inputs after Cultivation in Incubated Soils

To assess the temperature, time, and input response of kinetic C pools derived from the incubation experiments, we set the transfer values of $\alpha_{i,j} = 0$ in the optimization procedure, thereby simulating values only for the decomposition rates k_i and the partitioning coefficients γ_i (Table 2, see supplemental material for all data). Initially, a set of optimizations including all transfer rates for the incubation experiments was run. However, we obtained low values in all cases ($\alpha_{i,j} < 0.0001$), which indicated that these transfer rates were very low. So, to reduce the number of parameters to optimize and obtain a more parsimonious model, we proceeded to run the optimization without these transfer rates. These results are also consistent with the idea that, for relatively short-term incubation experiments, the dynamics of C transfers cannot be appropriately captured within the timeframe of these incubations.

At 21 and 26°C, the optimized model provided good fit to the observed data, with higher uncertainty in the predictions for the incubation at the lower temperature resulting from greater variability in respiration during incubation (Fig. 2). Independent of temperature, >98% of C was within a slow pool (that decomposed at decadal to millennial timescales), and <1% each was within an intermediate pool (that decomposed in 1–2 mo) and a fast pool (that decomposed in a few days) (Table 2, Fig. 3a–3d). In the initial soils (i.e., the control, bare treatment in 2010), a difference in incubation temperature of 5°C resulted in increases in decomposition rates for the fast and slow pools, and a decrease in the decomposition rate of the intermediate-cycling pool. The ratio between decay rates at 26 and 21°C [$k_i(T = 26)/k_i(T = 21)$], which demonstrated the relative change with temperature, was 1.87, 0.80, and 2.12 for the fast, intermediate and slow pools, respectively.

Each kinetic C pool responded differently to the elevated temperature treatment over the course of the incubation. In the initial soils, release of C from the fast pool in the warming treatment occurred as a result of the elevated decay rate, particularly at the beginning of the incubation (Fig. 3a). For the intermediate pool, the difference in CO_2 release between the two temperature treatments decreased during the first 100 d of incubation, and then both curves converged by the end of the incubation. For the slow pool, C release was always higher in the warming treatment (Fig. 3c). The total amount of release is therefore dominated mostly by contributions from the fast and intermediate pools at the beginning of the incubation and by contributions from the slow pool at later stages of the incubation (Fig. 3d).

Table 1. Three pool model parameter estimates for the best-fit starting model of the in situ C measurements (from Crow et al., 2018).

Sample	Parameter†						
	k_1	k_2	k_3	$\alpha_{2,1}$	$\alpha_{1,2}$	$\alpha_{3,2}$	$\alpha_{2,3}$ or $\alpha_{3,1}$
	yr^{-1}						
Bare	0.299	0.306	0.058	0.005	0.008	0.501	0.036
Planted	0.248	0.089	1.24×10^{-4}	0.485	0.601	0.890	0.985
Relative change (planted/bare)	0.829	0.291	0.002	97.000	75.125	1.776	27.361

† *k*, decomposition rate; $\alpha_{i,j}$, transfer rate from pool *j* to pool *i*. For the bare treatment, the best fit model includes a transfer $\alpha_{2,3}$; for the planted treatment, the best fit model includes a transfer $\alpha_{3,1}$.

Table 2. Incubation-based three-pool model parameter estimates and summary statistics for the best value and standard deviation (SD).

Sample	Incubation temperature	Parameter	k_1†	k_2	k_3	γ_1‡	γ_2	γ_3
	°C				d^{-1}			
Bare 2010	21	Best fit	0.335	0.038	1.70×10^{-5}	0.001	0.008	0.991
		SD	0.208	0.011	9.20×10^{-6}	5×10^{-4}	0.001	0.008
Bare 2010	26	Best fit	0.626	0.030	3.60×10^{-5}	0.001	0.011	0.988
		SD	0.216	0.006	5.20×10^{-6}	4×10^{-4}	0.001	0.011
Bare 2011	21	Best fit	0.389	0.018	1.35×10^{-5}	0.001	0.007	0.992
		SD	0.129	0.008	8.70×10^{-6}	2×10^{-4}	0.001	0.007
Bare 2012	21	Best fit	0.176	1×10^{-5}	3.94×10^{-5}	0.001	0.011	0.987
		SD	0.035	1×10^{-5}	4.60×10^{-6}	2×10^{-4}	0.009	0.011
Planted 2012	21	Best fit	0.395	0.024	1.97×10^{-5}	0.001	0.010	0.989
		SD	0.130	0.005	1.01×10^{-5}	2×10^{-4}	0.002	0.010

† k, decomposition rate.

‡ γ, pool size (proportion of total, between 0 and 1).

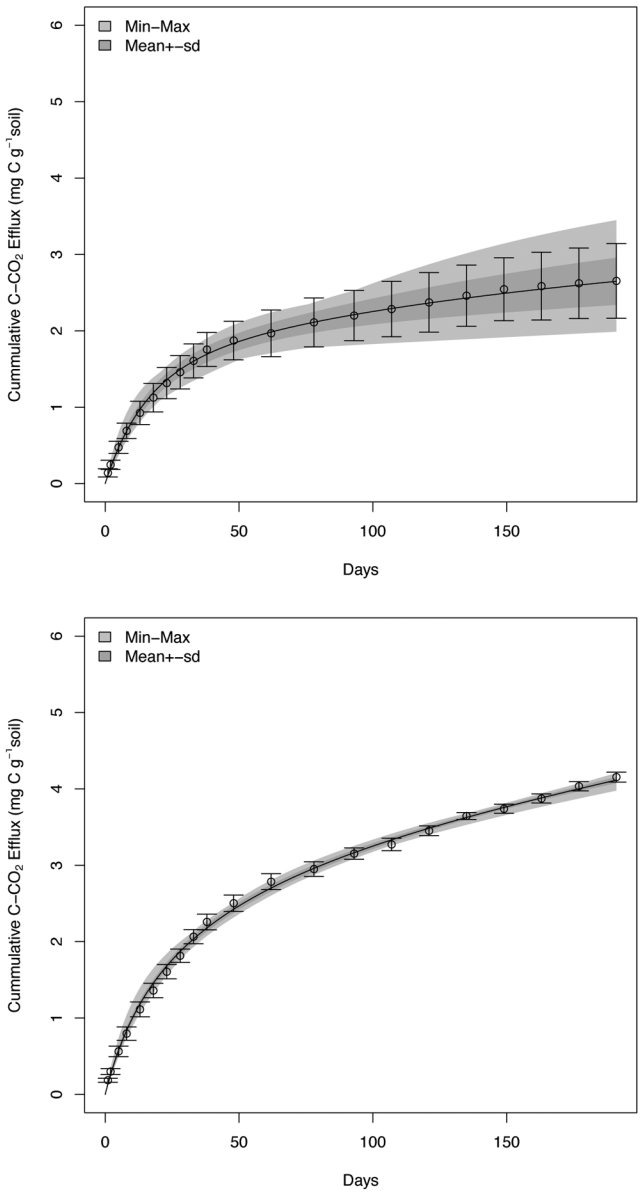

Fig. 2. Cumulative CO_2 efflux during 191-d incubation of the initial soil collected after preparation tillage and preplanting at 21 (top) and 26°C (bottom). Data points and error bars are mean ± 1 SE for the incubation of replicated field plots. Curve is the best fit for the three-pool model, gray areas are the SD, and 95% confidence interval of the mean model fit. Min., minimum; max., maximum.

Over time, the effect of no belowground plant inputs was evident in the 21°C incubation results. Two years without plant inputs to the soil resulted in a decrease of the contribution from the fast (Fig. 4a), intermediate (Fig. 4b), and slow (Fig. 4c) pools to the total release of C from the incubations (Fig. 4a). These results suggest that readily available C was lost from multiple soil pools in the field under the absence of C inputs, and during the incubation experiments, microbes relied mostly on C that is more difficult to access and therefore cycles at slower rates. Overall, the rates and amount of C release are largely decreased by the absence of C inputs over time (Fig. 4d).

In contrast with the absence of inputs, the addition of C through planting resulted in higher amounts of C released and increased decomposition rates (Table 2, Fig. 5a–5d). The fast (Fig. 5a) and intermediate pools (Fig. 5b) provide most of the contribution to the total amount of release (Fig. 5d), which most likely increases by the addition of fresh plant inputs and an active rhizosphere.

Kinetic Pools versus Physical Fractions

The C stock within the free and occluded light fractions (Fig. 6a–6d) was an order of magnitude greater than was found in the active and intermediate kinetic pools during incubation (Fig. 6e–6h). The vast majority of C was stored in the slow kinetic fraction in every case (Table 2), and the slow pool stock declined over time in the bare soil with no inputs (Fig. 6). Changes in the slow kinetic pool during incubation tracked most closely with changes in the free and occluded light fractions observed in the field in the absence of plant inputs. As reported in Crow et al. (2018), with no plant inputs, total C stock significantly declined over time as the free and occluded light fractions were depleted. After 2 yr, the effect of perennial plant root and rhizosphere inputs was evident in the maintenance of free light fraction and accumulation within the dense fraction (Fig. 6a vs. Fig. 6d).

Despite no change in relative size, decomposition rates increased in the active and intermediate kinetic pools after cultivation of perennial grasses (Table 2). The ratio between decay rates of planted and unplanted soils in 2012 [k_i(planted)/k_i(unplanted)] was 2.24 and 2.40 for the fast and intermediate pools, respectively, which demonstrates important dynamic responses in the decomposition and transfer of C through the intermediate kinetic pool in particular. In contrast, this ratio

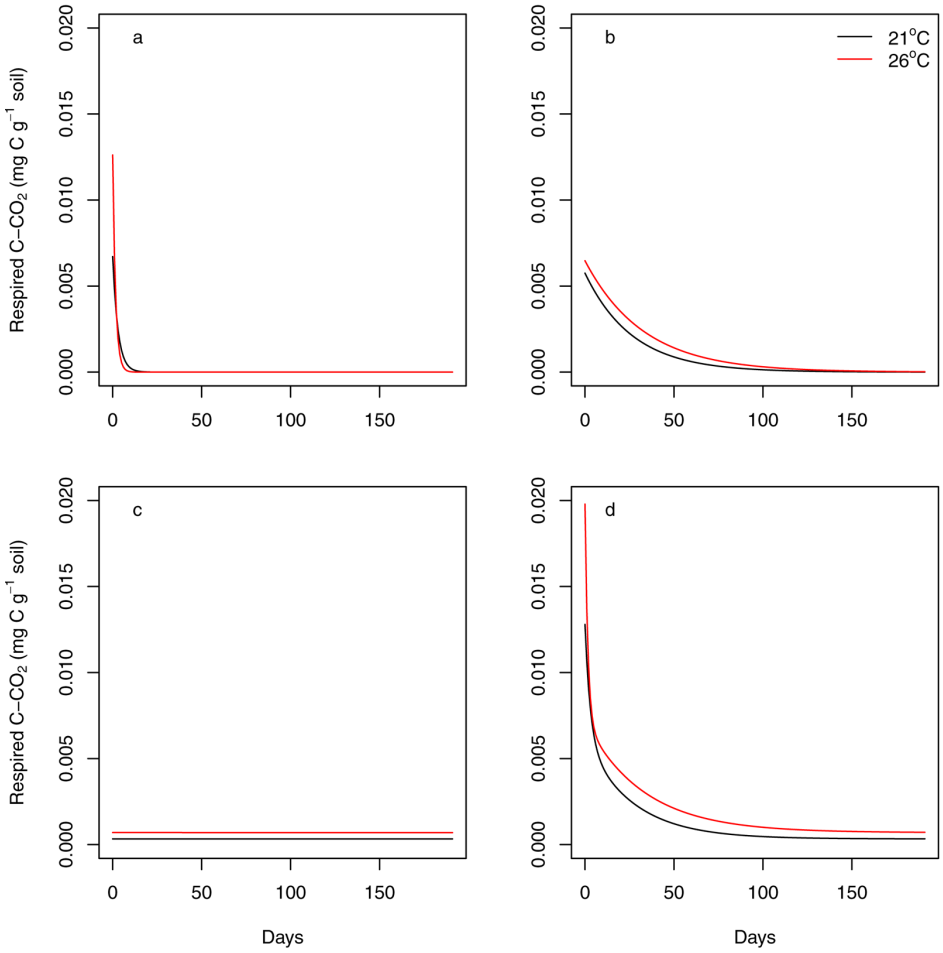

Fig. 3. Carbon released over time (days) for each of the pools calculated from the model using the incubation data of the initial soil collected after preparation tillage and preplanting. (a) Fast, (b) intermediate, (c) slow, and (d) total C release curves are shown for the 21 and 26°C incubations.

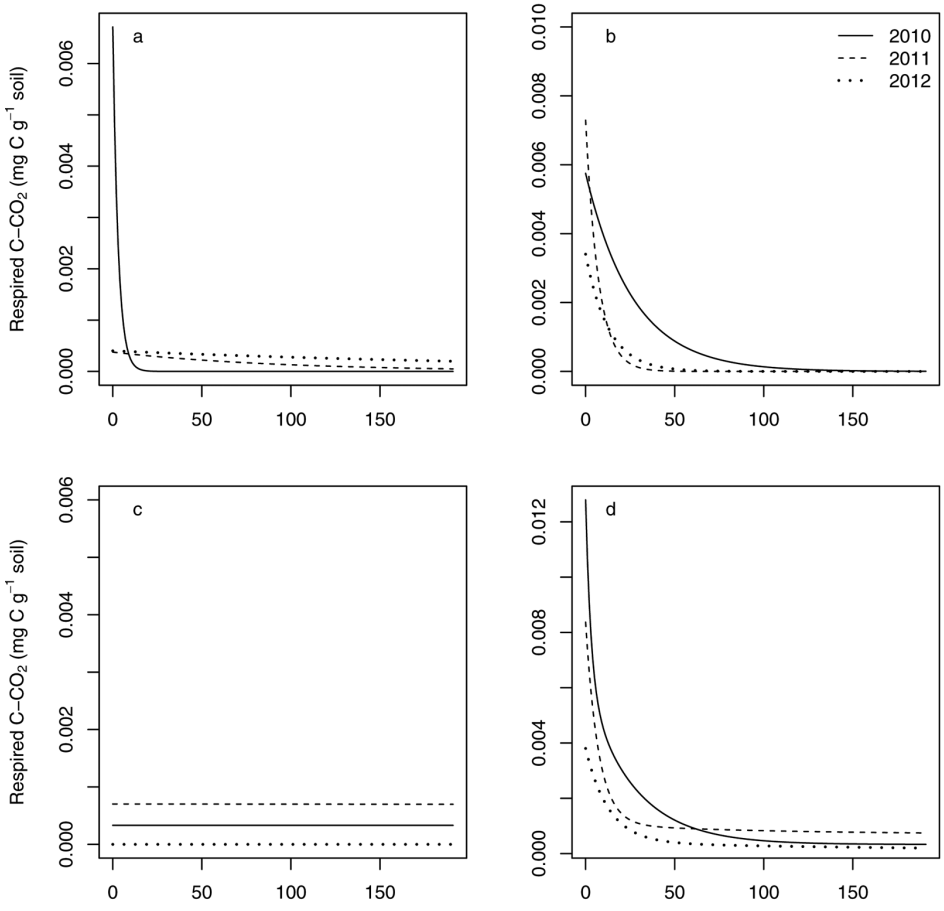

Fig. 4. Carbon release curves over time (days) derived from the incubation of initial soil and 2 yr with no further plant or rhizosphere inputs. (a) Active, (b) intermediate, (c) slow, and (d) total C release curves are shown for the baseline soil (2010) and subsequent collections of bare soil in 2011 and 2012.

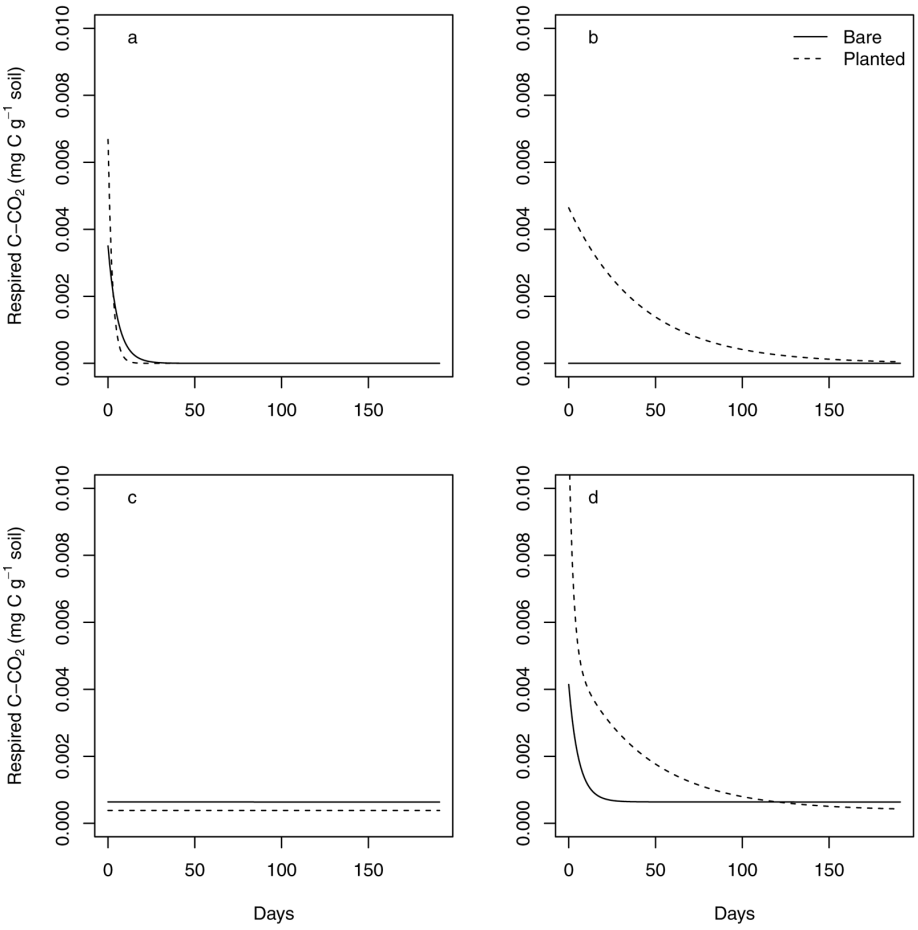

Fig. 5. Carbon release curves over time derived from the incubation of unplanted and planted soils. (a) Active, (b) intermediate, (c) slow, and (d) total C release curves are shown for the soils collected in 2012.

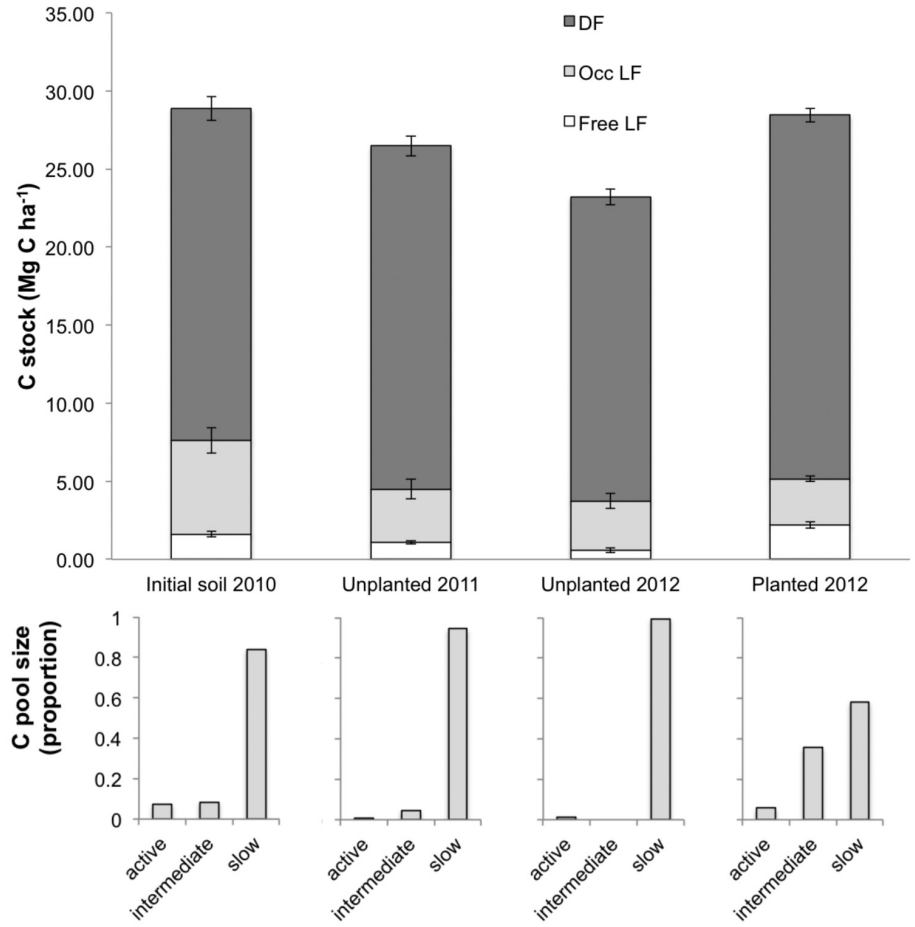

Fig. 6. Hierarchical comparison of C stocks within multiple pools derived from the density fractionation (physical fractions, top) and the incubation-based three-pool model (kinetic pools, bottom) for the initial soil in 2010, unplanted soil in 2011, unplanted soil in 2012, and planted soil in 2012. Values are means ± 1 SE of measurement for the physical fractions and the best fit value for the incubation-based modeled pools. DF, dense fraction; Occ, occluded; LF, light fraction.

was 0.5 for the slow pool, which is indicative of a net decrease in losses and gain of soil C as a result of planting (Fig. 6h).

Land Use and Temperature Effects Derived from Changes in Physical Fractions

The interaction between land use change and temperature leads to higher C stock losses in the bare treatment (Fig. 7a) and lower gains in the planted treatment (Fig. 7b). Increases in temperature by 5°C caused net increases in decomposition and therefore a higher amount of released C in both land uses. In the bare soil, losses were dominated by the dense fraction (Fig. 7c). In the planted treatment, losses were from a combination of the dense and free light fraction, although the free light fraction was exhausted rapidly with warming and did not contribute to C release in the projections (Fig. 7d). Because of gains in the occluded light fraction with temperature, the net reduction in C stock was reduced in the planted compared with the bare soils (Fig. 7d).

Discussion

Results from a set of incubation experiments with warming, as well as soil physical fraction data from a field experiment with plant input treatments, were used to parameterize different instances of a three-pool model, observe changes in parameter values due to treatments, and project soil C pools and fluxes with land use change and temperature. Changes in parameter values are indicative of changes in the primary mechanisms responsible for C storage in these soils affected by external perturbations. Although we did not observe these mechanistic changes directly, model results help us to understand the factors potentially driving future C stocks under environmental change.

Response of Carbon Pool Sizes and Decomposition Rates to Temperature, Time, and Inputs after Cultivation in Incubated Soils

Evidence for dynamic C pools and decomposition rates that were modified by warming and plant-derived C inputs emerged from this study. Results from the incubation experiment suggest a bigger role in long-term C balance for the slow-cycling pool than for the fast-cycling pool. The fast pool contributed in similar magnitudes to total respiration in the warming treatment and was rapidly exhausted after 2 yr with no C inputs. Additional respiration in the warming treatments was thus contributed mostly by the intermediate- and slow-cycling pools. These results suggest that the additional available energy under the warming treatment is used by microbes to access C that requires higher energy to mineralize under lower temperatures (Bosatta and Ågren, 1999; Craine et al., 2010a). Lower variability in respiration among samples and the resultant low uncertainty in the model at 26 versus 21°C suggest metabolic homogeneity in the microbial community members that adapted to higher incubation temperatures.

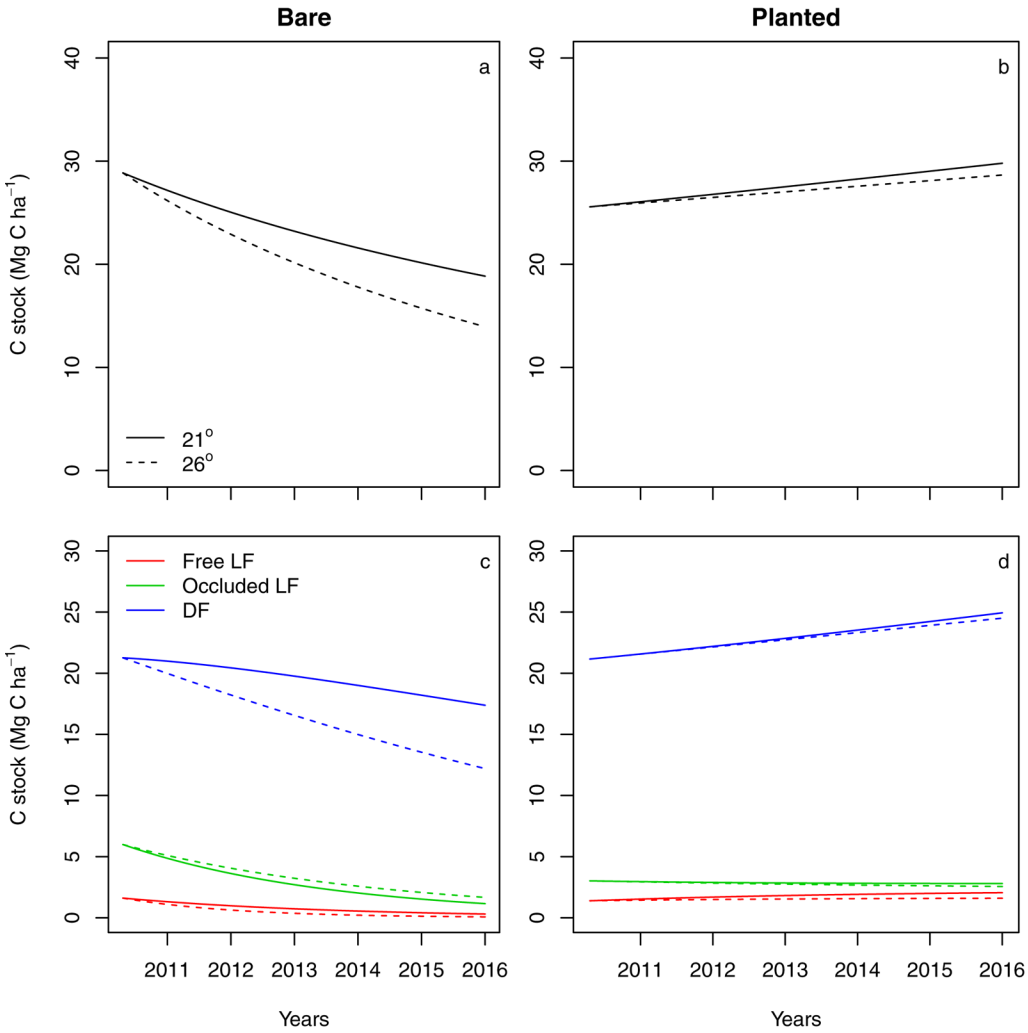

Fig. 7. Simulated changes in C stocks and C fractions over time as an effect of land use and temperature change with respect to initial state in 2010. LF, light fraction; DF, dense fraction.

Our results are consistent with other studies; for instance, in a previously uncultivated field in the upper Midwest of the United States, soil declined in the 2- to 8-mm aggregate class by >40% and CO_2 emissions doubled (Grandy and Robertson, 2006b). Subsequently, the authors warned that if C accumulates primarily in intraaggregate pools and these aggregates are broken down after tillage, then C will remain sequestered only if management changes such as zero-tillage are permanent (Grandy and Robertson, 2006a). In the tropics, this change can occur rapidly, and measureable declines in soil C concentration can be detected within months (Houghton et al., 1985). However, if soil processes promote the transfer of accrued organic matter from aggregates to more stable mineral pools, then losses will be less drastic and ecosystems may begin to aggrade C again after tillage (Sumiyoshi et al., 2016; Crow et al., 2018).

Kinetic versus Physical Fractions

Incubation-based kinetic fractions are highly sensitive indicators of readily available microbial substrate (from days to months) (Hamdi et al., 2013; Schädel et al., 2013), whereas physical fractions are an operational division of soil C into pools of varying turnover (from years to millennia) that encompass the integrative aspects of biological, chemical, and physical properties in soil (Crow et al., 2007). Elevated rates of decomposition in the small kinetic active and intermediate pools with cultivation suggested a supply of readily available substrate from the rhizosphere that drives transfers of C from one pool to another. Concurrent losses from the kinetic slow pool and the free and occluded light fractions over time with no inputs suggested a division between actively cycled and remnant, stable components of both light fractions. Just as losses occurred with no plant inputs, cultivated soils gained C overall, particularly within the free light fraction and the slow kinetic pool (which grew in size and the decay rate was cut in half), further reinforcing the connection between slow kinetic pools and the light fraction.

Within the most passive pool of both methods, clearly some component is, in fact, dynamic and responsive to change over a short (i.e., 3 yr in this study) timeframe, regardless of the fact that C in these fractions cycles at timescales of both decades and millennia. Transfers of C between pools facilitated by microbial activity, such as was captured within the incubations, appear to be a critical component to better understanding the rapid response of C in soil undergoing change.

Land Use and Temperature Effects Derived from Changes in Physical Fractions

Gains in the dense, mineral-bound fraction occurred as a result of the model-predicted transfer of C from the free organic debris and aggregate protected organic matter resulting in soil C accumulation with cultivation in this system. Although the kinetic active and intermediate pools were rapid responders to temperature and inputs, the kinetic slow pool is a greater integrator of the dynamic components of in situ C fractions that respond on the years-to-decades scale to change in land use and management. Dynamic transfers among these in situ pools drive soil C accumulation (or loss) after preparation tillage and cultivation in zero-tillage perennial grasses. Rapid response by the microbial community to change drives the transfers among pools that push the system to aggrade or degrade C.

Dynamic transfer of fresh belowground inputs through the light fractions to the dense fraction drove gains in the C balance on a years-to-decades timeframe; in planted systems, these transfers result in accumulation that is greater than losses expected from warming. Projected gains in the occluded light fraction with temperature mitigated losses from other pools. In particular, changes in transfer rates are a critical component of the belowground C cycle that has been little explored. Recently, there has been an increased interest in exploring changes in C use efficiency (Manzoni et al., 2012; Frey et al., 2013; Hagerty et al., 2014) with soil warming. Changes in microbial C use efficiency lead to changes in the proportion of processed C that is not respired and therefore is transferred among other pools. These changes are captured by the parameters α_{ij} in our model, and as we observed, these parameters can also change with changes in C inputs. Shifts in microbial community composition or abundance, C use efficiency, and the response to differences in inputs may explain the greater uncertainty derived from large variation among the soils incubated at the lower temperature compared with the higher. It is likely that different types of environmental change modify these transfer rates (Lange et al., 2015), and more research is needed to understand physiological mechanisms that lead to these changes.

The overall response of soil C stocks to disturbance events was the combination of changes in (i) decomposition rates, (ii) size of the pools that are being cycled at different rates, and (iii) transfers of microbially processed (i.e., decomposed) C among different pools. Many previous studies focused mostly on changes in decomposition rates, implicitly assuming that fraction sizes and transfer rates remain unaffected (Craine et al., 2010b; Hamdi et al., 2013). On the contrary, our results suggest that changes in decomposition rates are likely accompanied with changes in pool sizes and transfer rates. Changes in pool sizes can be explained by different mechanisms such as transformation of C quality by microorganisms or changes in activation energy with changes in temperature. Future research must acknowledge that environmental change will lead to other responses in belowground C cycling than just changes in decomposition rate. Holistic approaches are needed in this regard, and although modeling studies such as this help to explore some of these mechanisms, more field and laboratory experiments are needed to better disentangle the role of pool sizes and transfer rates in the context of global change.

Supplemental Material

Available online is supplemental material detailing the main results from the optimization of a three-pool model using two different sources of data: time series of CO_2 fluxes from incubations, and density fraction measured after land use conversion.

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