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Nagy, R Chelsea
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Brando, Paulo
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RESEARCH ARTICLE

Soil Carbon Dynamics in Soybean Cropland and Forests in Mato Grosso, Brazil

R. Chelsea Nagy[1,2,3] [ID], Stephen Porder[1] [ID], Paulo Brando[4,5] [ID], Eric A. Davidson[5,6] [ID], Adelaine Michela e Silva Figueira[7] [ID], Christopher Neill[1,2,5] [ID], Shelby Riskin[1,2] [ID], and Susan Trumbore[8,9] [ID]

[1]Ecology and Evolutionary Biology, Brown University, Providence, RI, USA, [2]Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA, USA, [3]Earth Lab, University of Colorado Boulder, Boulder, CO, USA, [4]Instituto de Pesquisa Ambiental da Amazônia, Brasília, Brazil, [5]Woods Hole Research Center, Falmouth, MA, USA, [6]Appalachian Laboratory, University of Maryland Center for Environmental Science, Frostburg, MD, USA, [7]Natural Science Program, Federal University of Western Pará, Santarém, Brazil, [8]Max Planck Institute for Biogeochemistry, Jena, Germany, [9]Earth System Science, University of California, Irvine, CA, USA

Abstract Climate and land use models predict that tropical deforestation and conversion to cropland will produce a large flux of soil carbon (C) to the atmosphere from accelerated decomposition of soil organic matter (SOM). However, the C flux from the deep tropical soils on which most intensive crop agriculture is now expanding remains poorly constrained. To quantify the effect of intensive agriculture on tropical soil C, we compared C stocks, radiocarbon, and stable C isotopes to 2 m depth from forests and soybean cropland created from former pasture in Mato Grosso, Brazil. We hypothesized that soil disturbance, higher soil temperatures (+2°C), and lower OM inputs from soybeans would increase soil C turnover and deplete C stocks relative to nearby forest soils. However, we found reduced C concentrations and stocks only in surface soils (0–10 cm) of soybean cropland compared with forests, and these differences could be explained by soil mixing during plowing. The amount and $\Delta^{14}C$ of respired CO_2 to 50 cm depth were significantly lower from soybean soils, yet CO_2 production at 2 m deep was low in both forest and soybean soils. Mean surface soil $\delta^{13}C$ decreased by 0.5‰ between 2009 and 2013 in soybean cropland, suggesting low OM inputs from soybeans. Together these findings suggest the following: (1) soil C is relatively resistant to changes in land use and (2) conversion to cropland caused a small, measurable reduction in the fast-cycling C pool through reduced OM inputs, mobilization of older C from soil mixing, and/or destabilization of SOM in surface soils.

1. Introduction

The conversion of tropical forest to pasture and cropland has fundamentally altered the global C cycle (Baccini et al., 2012; Houghton, 2005; Houghton et al., 2012). Driven by an increasing global demand for beef and grain from a growing human population (Boucher et al., 2012; Brown, 2009), tropical deforestation to date has largely been for conversion to pasture and the C consequences of such conversions have been extensively documented (De Camargo et al., 1999; Fujisaki et al., 2015; Neill et al., 1997; Powers et al., 2011). Although pasture remains a widespread use of tropical land, in the past two decades land has been increasingly converted to highly mechanized agriculture of commodity crops like soybeans (Galford et al., 2011; Laurance et al., 2014; Nepstad et al., 2006; Morton et al., 2016) and little is known about the transition to industrial soy production, which now covers large swaths of the southern Amazon and Cerrado. This transition, which is playing out on deep, highly weathered soils, may be a substantial C source to the atmosphere, but little work has attempted to quantify soil C changes, particularly the changes that occur deeper in the soil profile.

Conversion of tropical forests to agriculture can influence soil C storage and soil organic matter (SOM) dynamics in several ways. First, the amount, depth distribution, and quality of litter inputs to soil C can change as annual crops or pasture grasses replace trees (Davidson et al., 1995; Fisher et al., 1994; Harrison et al., 1993). Second, decomposition rates may increase because of higher temperatures in agricultural soils and physical soil disturbance from management practices such as tillage, which mixes soils and breaks up soil aggregates (Harrison et al., 1993; Matson et al., 1997; Murty et al., 2002; Six et al., 1999). The decomposition of fast-cycling young C often increases with conversion to agriculture, and old forest-derived C is often destabilized after cultivation (Dieckow et al., 2009; Don et al., 2011; Houghton, 2003; Powers et al., 2011; Trumbore, 1997). Third, there is less water transpired from agricultural compared with forest soils (Bosch &

Hewlett, 1982; Coe et al., 2011; Hayhoe et al., 2011; Hibbert, 1967; Neill et al., 2013), which could reduce water limitation of decomposition. Finally, physical removal of SOM by increased erosion can remove C and nutrients, particularly in tilled soils (Harden et al., 1999; Lal et al., 2004; Quinton et al., 2010; Smith et al., 2016), though the fate of this C downstream of farm fields is not well understood (Huth et al., 2012).

Previous work in the Amazon has generally found that the conversion of forest to pasture leads to soil C gains because of high inputs from pasture grasses (Fujisaki et al., 2015; Neill et al., 1997; Powers et al., 2011; Trumbore, 2000). Soil texture controls C storage capacity (Desjardins et al., 2004; Feller & Beare, 1997; Parton et al., 1987; Six et al., 2002), with fine-textured soils having greater C capacity (Angers et al., 2011), but coarse-textured soils had slightly greater C increases with pasture use in the Amazon (Desjardins et al., 2004). Crop-based agricultural use in the Amazon has in some cases led to C losses, particularly under frequent tilling which can enhance decomposition by breaking up aggregates and mixing surface soil C into deeper soil layers (Carvalho et al., 2010; Durigan et al., 2017; Maia et al., 2010; Rittl et al., 2017). Less is known about changes in deep soil C after conversion to intensive agriculture at the scale of that in the southern Amazon, and deep soils are a focus of the work described here.

Changes in land use and C storage are also influenced by climate, and combining projections of changes in climate and land use increases complexity and uncertainty in predictions of future soil C stocks (Ciais et al., 2013; Friedlingstein et al., 2013). Warmer temperatures are expected to increase SOM decomposition rates (Davidson & Janssens, 2006; Eglin et al., 2010; Trumbore, 1997; Trumbore et al., 1996). However, decomposition is also limited by soil moisture and substrate availability (Davidson & Janssens, 2006; Giardina & Ryan, 2000; Hursh et al., 2016); thus, future changes in soil C remain poorly constrained. Despite these uncertainties, climate models suggest that tropical soils will store less C in a warmer climate (Intergovernmental Panel on Climate Change, 2014). Coupled projections of land use and climate change suggest a cumulative loss of soil C in tropical Latin America and Africa of 2.75 Pg C M km^{-2} between 2000 and 2100 or 27.5 Tg C M km^{-2} yr^{-1} during this 100 year period (Eglin et al., 2010).

Turnover of SOM can be estimated from respiration rates and $\Delta^{14}CO_2$ from long-term laboratory incubations, modeling of soil C pools using soil C stocks and $\Delta^{14}C$, and changes to SOM $\delta^{13}C$ (Ehleringer et al., 2000; Torn et al., 2009; Townsend et al., 1995; Trumbore, 2009). In laboratory incubations, the rate of CO_2 production provides an estimate of the amount of fast-cycling C and the mean C residence time in soils (Torn et al., 2009). Respiration of C in the early stages of incubation is dominated by an active pool and rates decline over time as the active pool diminishes and the respired C comes increasingly from the slower cycling pool (Figure S1 in the supporting information Townsend et al., 1995). Because soils are an open pool that exchanges C with the atmosphere through continual organic inputs from plants and removal through decomposition, $\Delta^{14}C$ data of bulk soil and respired CO_2 can be used with models to estimate the mean turnover time and transit time of soil C (Figure S1) (Torn et al., 2009; Trumbore et al., 2016). Models that best fit the data include at least an active (or fast) pool that cycles on an annual to decadal timeframe and a passive (or slow) pool that turns over in centuries or millennia (Baisden et al., 2013). Stable isotope analysis of C ($\delta^{13}C$) can also be used to assess turnover of SOM following a change from C_3 to C_4 vegetation or vice versa (Ehleringer et al., 2000; Navarrete et al., 2016; Neill et al., 1997; Torn et al., 2009). Because C_3 plants have lower $\delta^{13}C$ values than C_4 plants because of greater fractionation during photosynthesis, replacement of C_3 trees with C_4 pasture grasses increases soil $\delta^{13}C$ and allows for calculation of turnover times (Ehleringer et al., 2000; Neill et al., 1997; Pendall et al., 2010; Torn et al., 2005).

We used a combination of these approaches to constrain C cycling and determine if a decade of intensive soybean cultivation following two decades of pasture use in Mato Grosso, Brazil, altered soil C storage and SOM turnover. We focused on the changes that occurred in croplands created from former pasture because this represented the majority of current Mato Grosso soybean cropland (Macedo et al., 2012) and because the changes of soil C with pasture use in the Amazon have received considerable attention. We tested three hypotheses about the relationship between this region's land use history and the fate of soil C in this landscape: (1) that total soil C stocks would be lower and soil respiration rates would be higher in soils from soybean cropland compared with native forest, (2) that faster C turnover and lower C inputs would deplete the pool of fast-cycling C in soybean cropland soils, and (3) that the most depleted C stocks and the smallest pools of fast-cycling C would occur in fields that were planted with soybeans for the longest time. We also expected soil texture to be important for C storage and changes in C storage with pasture and agricultural use.
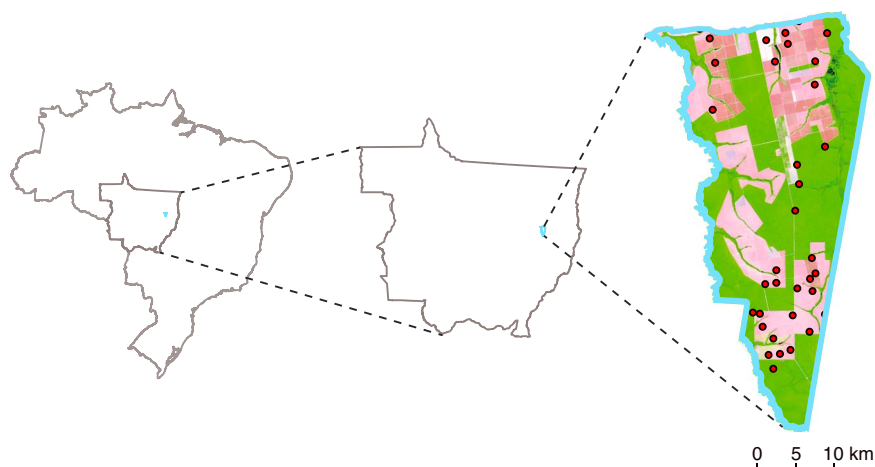
Figure 1. Location of forest and soybean soil samples collected from Tanguro Ranch (blue outline) in the state of Mato Grosso, Brazil. Soil sample locations (red dots) shown on top of a Landsat image (bands 5, 4, and 3) from June 2011 where forest appears green and agriculture fields appear pink to white.

2. Materials and Methods

2.1. Site Description

We worked on Tanguro Ranch, an 800 km^2 soybean farm in the Brazilian state of Mato Grosso (13°04'35.39"S, 52°23'08.85"W). Approximately 400 km^2 of Tanguro Ranch was cleared for pasture between 1976 and 1992 (Figure S2a), planted with C$_4$ *Brachiaria brizantha* (a widely-planted pasture grass in Amazônia) and subsequently converted to soybean production between 2003 and 2010 (Figure S2b) (Riskin et al., 2013). To initiate soybean cultivation, vegetation in the pastures was burned and soil was tilled to 30–40 cm for the first 2–3 years of cultivation, after which tilling ceased (Riskin et al., 2013). Soybean fields were fertilized annually with phosphorus (~50 kg ha^{-1} yr^{-1}) and limed (~1,500 kg ha^{-1} yr^{-1}) every 2–3 years (Riskin et al., 2013). Soy was planted in October or November and harvested in March or April. Crop residues were not removed or otherwise altered in soybean fields, with the exception of the years in which the fields were limed, then the crop residues were disked into the soil. Fields were bare or had a sparse cover crop of millet during the non-cropping season. The remaining forests on Tanguro Ranch are closed-canopy evergreen forests that are intermediate in stature between more humid rain forests to the north and Cerrado, or savanna, vegetation to the south (Ivanauskas et al., 2004).

Tanguro Ranch is at 320–390 m above sea level on the Brazilian Shield on Precambrian gneisses of the Xingu Complex (Figueira et al., 2016). The dominant soils of this region are classified as Latossolos-vermelho-amarealo-distrófico in the Brazilian system and Oxisols (Haplustox) in the U.S. Department of Agriculture system (Figueira et al., 2016; Neill et al., 2013). Soils are greater than 10 m deep, acidic (pH of native forest soil is ~3.9), and have a sandy clay texture (mean 43% clay) (Neill et al., 2013). The region has a mean annual temperature of 25°C and mean annual precipitation of about 1,770 mm yr^{-1} (Rocha et al., 2014). Rainfall is highly seasonal: from May to August, rainfall is <10 mm month^{-1} (Rocha et al., 2014). Surface soils in soybean cropland are ~2°C hotter and 5% drier than in forests in both wet and dry seasons (O'Connell, 2015; Silvério et al., 2015).

2.2. Sample Collection

In July 2013, we collected soil samples with an auger from five depths (0–10, 10–20, 40–50, 90–100, and 190–200 cm) at 35 sites: 7 forest sites and 28 locations in soybean cropland (Figure 1). Of the 28 soybean sites, 7 were converted to soybean cultivation in each of the years 2003, 2004, 2007, and 2008. These same 35 sites had been sampled in 2009 using the same methodology. A full description of the site selection procedure is outlined in Appendix S1. Soils were air dried for a minimum of 48 h and sieved (<2 mm) then stored in a cool, dry location until analysis. Gravel (>2 mm) is rare in these soils. We collected one sample to measure soil bulk density (BD) from each site (at 0–10, 10–20, 40–50, and 90–100 cm) and one deeper sample (at 190–200 cm) from three of the seven sites in each land cover and age category by hammering a steel cylinder

(diameter = 4.7 cm, length = 5.0 cm) horizontally into the side of the soil pit at each depth. We analyzed all surface soils (0–10 cm) (forest $n = 7$, soybean cropland $n = 28$) and four complete profiles (to 2 m depth) in forest and soybean cropland for $\Delta^{14}C$, and all samples at all depths ($n = 35$ sites × 5 depths) for % C and $\delta^{13}C$.

To examine the longer-term effects of agricultural use and to compare the evolution of radiocarbon as well as C stocks over time (Baisden et al., 2013; Schrumpf & Kaiser, 2015; Torn et al., 2009; Trumbore, 1997), we also used a subset (forest = 4 sites at 5 depths, soybean cropland = 2 sites at 5 depths) of archived samples collected in January–February 2009 from the same 35 sites. We analyzed this subset of samples for bulk soil % C, $\delta^{13}C$, and $\Delta^{14}C$.

2.3. Soil Incubations

We selected a subset of soils collected in 2013 for incubation (forest $n = 15$, soybean cropland $n = 21$; Appendix S1). For each of these samples, we incubated ~100 g of soil in 1 L mason jars with modified lids fitted with Suba-Seal silicone septa. We wet each sample to approximate field moisture conditions (10% surface soil volumetric moisture) and incubated them at 26°C. Field moisture conditions varied from 7% to 17% throughout the year in forest surface soils at the field site (Rocha et al., 2014). We sampled the headspace in the jars at increasing time intervals between sampling (days after start of incubation = 1, 3, 5, 7, 11, 18, 28, 40, 61, and 89) to determine rates of CO_2 evolution (μg C g soil^{-1} d^{-1}). We used a syringe to extract 10 mL of gas from the headspace volume and injected it into a Shimadzu GC14A gas chromatograph to determine the CO_2 concentration. When concentrations of CO_2 in the headspace air reached ~20,000 ppm (2%), we collected a sample by attaching and opening an evacuated 100 mL serum bottle to the incubation jar. After sample collection, we vented the jars to release the accumulated CO_2, then resealed the jars. If the sample had not reached 20,000 ppm after ~3 months, but had a concentration above the minimum sample size of 2,000 ppm (the detection limit for accelerator mass spectrometry (AMS) at the University of California, Irvine), we collected the sample in evacuated 100 mL serum jars.

2.4. Sample Analysis

We analyzed CO_2 from soil incubations and a subset of bulk soil samples (all surface soils and eight complete profiles) for ^{14}C and ^{13}C at the Keck Carbon Cycle AMS facility at the University of California, Irvine. Before analysis, pH of homogenized soil was tested to check for the presence of soil carbonates from liming of agricultural soils. The pH of all soil samples ranged from 4.3 to 6.9. Soil $\Delta^{14}C$ was not significantly related to soil pH ($p = 0.48$). As an additional test for the potential effect of added lime on soil C, we also acidified several samples and compared the radiocarbon signature with and without carbonates. We found no difference in soil $\Delta^{14}C$ between acidified and nonacidified replicates (data not shown).

To analyze for $\Delta^{14}C$, soil samples were sealed in evacuated quartz tubes with CuO and Ag and combusted at 950°C for 2 h. The CO_2 from this combustion was purified and reduced to graphite and measured by AMS (Southon et al., 2004; Xu et al., 2007). Reporting of ^{14}C data as $\Delta^{14}C$ corrects for mass-dependent fractionation using $\delta^{13}C$ values measured by the AMS (not reported here; Stuiver & Polach, 1977). For more precise measurement of $\delta^{13}C$, we used a small aliquot of the CO_2 evolved during combustion for the ^{13}C measurement by continuous flow isotope ratio mass spectrometry (IRMS) using a GasBench II ConFlo Interface to a Thermo Delta Plus isotope ratio mass spectrometer (Xu et al., 2007).

The remainder of the 2013 bulk soil samples (27 sites × 4 depths) was analyzed for ^{13}C and % C at the Marine Biological Laboratory Stable Isotope Laboratory in Woods Hole, Massachusetts, using a Europa 20-20 continuous-flow isotope ratio mass spectrometer interfaced with a Europa ANCA-SL elemental analyzer.

2.5. Calculations and Modeling

Carbon stocks were calculated from C concentrations as

$$C_{stock} = C_{conce} \times BD \times d \times 10,000 \qquad (1)$$

where C_{stock} is the C content (g C m^{-2}) within a sampled depth interval, C_{conce} is the C concentration (g C g^{-1}), BD is the bulk density (g cm^{-3}), and d is the depth increment of the sample (cm). To calculate the cumulative C storage of the soil profile to 2 m depth, the mean depth-weighted C storage in between sampled depths (i.e., 20–40, 50–90, and 100–190 cm) was calculated and added to the C storage of the sampled depths (0–10, 10–20, 40–50, 90–100, and 190–200 cm).

To account for changes in bulk density that resulted from land use and management practices between soybean cropland and forest soils, we also calculated the C stocks based on equivalent mass as opposed to equivalent volume. To do this, we applied a correction to the surface soil (0–10 cm) C content from soybean cropland as follows:

$$C_{stockadj} = C_{stock} \times \frac{BD_{forest}}{BD_{soy}} \tag{2}$$

where $C_{stockadj}$ is the adjusted C stock based on equivalent mass, C_{stock} is the C stock calculated from equation (1), BD_{forest} is the mean bulk density of forest soils from 0 to 10 cm, and BD_{soy} is the mean bulk density of soybean cropland soils from 0 to 10 cm (Davidson & Ackerman, 1993). This correction assumes that compaction only occurs in the surface soils (Davidson & Ackerman, 1993). We also included an adjusted cumulative C storage to 50 cm depth (to approximate the depth of soil mixing during tillage) that includes the C stock based on equivalent mass for 0–10 cm from equation (2).

We also calculated the cumulative C-mass weighted $\Delta^{14}C$ and $\delta^{13}C$ to 50 cm depth to account for mixing in the soil profile as

$$\Delta^{14}C_{mw} = \frac{\sum_{i=0}^{i=50} Cmass_i \times \Delta^{14}C_i}{\sum_{i=0}^{i=50} Cmass_i} \tag{3}$$

where $\Delta^{14}C_{mw}$ was the cumulative C-mass weighted $\Delta^{14}C$, i represented each sampled depth interval, $\Delta^{14}C_i$ was the sample measurement by AMS for each depth interval, and C_{mass} was the depth interval * bulk density *C stock as calculated by equation (1). Analogously,

$$\delta^{13}C_{mw} = \frac{\sum_{i=0}^{i=50} Cmass_i \times^{13}C_i}{\sum_{i=0}^{i=50} Cmass_i} \tag{4}$$

where $\delta^{13}C_{mw}$ was the cumulative C-mass weighted $\delta^{13}C$, i represented each sampled depth interval, $\delta^{13}C_i$ was the sample measurement by IRMS for each depth interval, and C_{mass} was the depth interval * bulk density *C stock as calculated by equation (1).

To estimate the inputs of C_4 pasture grasses versus C_3 trees or soybeans vegetation into soils, we used a simple mixing model to calculate the percent C_4 or percent C_3-C in the 2009 and 2013 bulk soil samples:

$$C_{stock} \times \delta^{13}C_{tot} = (C_f \times \delta^{13}C_f) + (C_p \times \delta^{13}C_p) \tag{5}$$

where C_{stock} was the C stock in the depth interval as calculated by equation (1), C_f and C_p were the relative proportions of forest- and pasture-derived C, and $\delta^{13}C_{tot}$, $\delta^{13}C_f$, and $\delta^{13}C_p$ were the $\delta^{13}C$ values of total soil C, forest-derived C, and pasture-derived C, respectively. In surface soils (0–10 cm), we used a C_3 end-member of $\delta^{13}C = -28‰$ based on measurements in forest soils and a C_4 end-member of $\delta^{13}C = -12‰$ based on published $\delta^{13}C$ values of *Brachiaria* spp. litter (-11.4‰ to $-13.3‰$ Mosquera et al., 2012; Schweizer et al., 1999). To account for $\delta^{13}C$ enrichment with increasing depth in the soil profile, the mean measured enrichment between each depth interval from the forest sites was applied to the end-members used at each depth for all sites. This resulted in C_3 end-members that ranged from $-28‰$ at the surface to $-23.2‰$ at 2 m and C_4 end-members that ranged from $-12‰$ at the surface to $-7.2‰$ at 2 m depth. This simple model assumed a pure C_4-$\delta^{13}C$ signal in pasture grasses rather than a mixture of C_4 and C_3 inputs. We also did not include in this model any effect of past C_4 vegetation such as Cerrado encroachment during historical climatic conditions (Sanaiotti et al., 2002; Victoria et al., 1995).

We used a two pool model, with fast and slow pools, to estimate turnover times and pool sizes in forest soils (Trumbore et al., 1996). The inputs to each pool were calculated as

$$I_i = \frac{C_{tot} \times f_i}{tt_i} \tag{6}$$

where I_i represented the inputs to pool i (i = fast or slow), C_{tot} was the mean soil C content of that depth interval in forest, f_i was the pool size as a fraction of C_{tot}, and tt_i was the turnover time of pool i. The amount of C in each pool was calculated as

$$C_i = C_{i,t-1} \times \left(1 - \left(\frac{1}{tt_i} \times \Delta t\right)\right) + I_i \times \Delta t \tag{7}$$

Table 1

Mean ± Standard Error C Concentration (%), Bulk Density (g cm^{-3}), and C Storage (g C m^{-2}) in Forest and Soybean Cropland Bulk Soil in Samples Collected in 2013

Depth (cm)	Concentration (%)		Bulk density (g cm^{-3})		C storage (g C m^{-2})	
	Forest	Soybean cropland	Forest	Soybean cropland	Forest	Soybean cropland
0–10	2.4 (a) ± 0.4	1.2 (b) ± 0.4	1.2 (b) ± 0.1	1.5 (a) ± 0.0	2830 (a) ± 170	1740 (b) ± 190
10–20	1.0 (a) ± 0.1	0.9 (a) ± 0.1	1.4 (b) ± 0.1	1.6 (a) ± 0.0	1380 (a) ± 190	1560 (a) ± 110
40–50	0.7 (a) ± 0.1	0.6 (a) ± 0.0	1.5 (a) ± 0.1	1.6 (a) ± 0.0	1180 (a) ± 300	1090 (a) ± 110
90–100	0.6 (a) ± 0.2	0.4 (a) ± 0.0	1.4 (a) ± 0.1	1.4 (a) ± 0.0	1120 (a) ± 480	770 (a) ± 130
190–200	0.5 (a) ± 0.2	0.4 (a) ± 0.0	1.2 (a) ± 0.1	1.4 (a) ± 0.0	730 (a) ± 290	840 (a) ± 200
Total to 200 cm	1.0 (a) ± 0.1	0.7 (b) ± 0.0	1.3 (b) ± 0.0	1.5 (a) ± 0.0	22,800 (a) ± 6200	19,400 (a) ± 2450

Note. Total profile to 200 cm includes a depth-weighted estimate of the C storage between the sampled intervals. Significant differences ($p < 0.05$) between forest and soybean soils are indicated by different letters.

where C_i was the C content in pool i, $C_{i,t-1}$ was the C content in pool i at the previous time step, and Δt was the time elapsed in the previous time step. Built into the model is a function that simulates the lag time spent by a C atom in living vegetation (the time between C fixation and litter deposition to the soil) so that the Δ^{14}C in the litter is equal to the value of the current year minus the lag (number of years). For tropical forests this lag time is about 7–8 years (Trumbore, 2006), so we set the lag time to 7 years. The model uses observed values of C content and Δ^{14}C of respired CO_2 and bulk soil as inputs. Undoubtedly, bulk soil and CO_2 each contain portions of fast and slow cycling C, but bulk soil is dominated by slowly cycling C whereas respired CO_2 comes primarily from faster cycling C (Figure S1) (Torn et al., 2009; Trumbore et al., 2016). We adjusted pool sizes and turnover times of the two model pools to match bulk soil and CO_2 data from samples collected in 2013. The model assumes that C_{tot} is at steady state in forests. Based on previous studies (Baisden et al., 2013; Trumbore et al., 1996), we tested a range of potential pool sizes for the fast pool (as a proportion of the total C at that depth interval) as follows: for surface soils: 0.1 to 0.6, for 40–50 cm: 0.01 to 0.1, and at 190–200 cm: 0.001 to 0.015. Correspondingly, we tested a range of slow pool values from 0.4 to 0.9 of total C in surface soils, 0.9 to 0.99 of total C at 40–50 cm, and 0.985 to 0.999 of total C at 190–200 cm. For each of these pool size combinations, we used the "solver" function in Excel to calculate the best fit of the turnover times of the two pools while minimizing the sum of squared deviation between the model and observed values of CO_2 and bulk soil Δ^{14}C.

2.6. Statistical Analyses

All statistical analyses were done in R version 3.2.2 (R Core Team, 2015). Because we expected the length of soybean cultivation and percent clay to be important for soil C, we ran simple linear and multiple regression with Δ^{14}C, δ^{13}C, or the C stock as the response variable and years of soybean cultivation and percent clay as predictor variables. If a significant relationship was found with any of the response variables and percent clay, we then ran analyses of covariance (ANCOVAs) to control for differences in clay content while looking for differences between treatments (soybean cropland versus forest). We also tested for differences in C stocks, δ^{13}C, and Δ^{14}C using analyses of variance (ANOVAs) among treatments of different number of years in soybean cultivation and in all soybean cropland versus all forest sites. We ran these ANOVAs at individual depths, for the total soil profile (to 2 m), and for the cumulative C-mass weighted value to 50 cm depth to account for soil mixing that occurred during tillage. We tested the assumptions of linear regression, ANOVA, and ANCOVA including normally distributed residuals. When the data were nonnormally distributed, transformations were attempted and if this did not improve normality, equivalent nonparametric statistical tests were used (e.g., quantile regression: "quantreg" R package, Kruskal-Wallis: base R, nonparametric ANCOVA: "sm" R package).

3. Results

3.1. Bulk Soil C Stocks and Respired CO_2

Soil C concentration was significantly higher in forest than soybean cropland soils at 0–10 cm but not at other depths (Table 1). The higher C concentrations at 0–10 cm led to higher mean C concentration across the 2 m profile (Table 1). Soil C stocks were also significantly higher in forest than soybean cropland at 0–10 cm. Calculations of C stocks at 0–10 cm based on equivalent mass rather than volume corroborated a

Table 2

Mean ± Standard Error C Evolved as CO_2 (μg C g $soil^{-1}$ d^{-1}) Over 89 Days From Soil Incubations by Land Use and Depth (cm)

	CO_2 evolved (μg C g $soil^{-1}$ d^{-1})	
Depth (cm)	Forest	Soybean cropland
0–10	35 (a) ± 4	12 (b) ± 1
40–50	1.4 (a) ± 0.3	1.6 (a) ± 0.2
90–100	0.44 (a) ± 0.09	0.55 (a) ± 0.11
190–200	0.52 (a) ± 0.10	0.65 (a) ± 0.11

Note. Significant differences between forest and soybean cropland soils are indicated by different letters.

significant reduction in C storage in soybean cropland compared with forest soils (Table S1). However, no significant difference ($p = 0.38$) was found between C stocks in forest and soybean cropland soils to 50 cm depth as calculated based on equivalent mass (Table S1). Similarly, total C stocks to 2 m did not significantly differ between forest and soybean cropland soils: mean C storage in forest soils to 2 m was 22,800 g m^{-2} compared with 19,400 g m^{-2} in soybean cropland soils (Table 1).

We found a significant negative relationship of C concentrations ($p = 0.002$) and C storage ($p = 0.04$) with the number of years in soybean cultivation. Mean C stocks at 0–10 cm were lowest in sites converted to soybean cropland in 2007 and highest in forest. Mean C stocks in forests were 2,830 ± 170 g C m^{-2}, and C stocks for soybean cropland were 1,650 ± 490 g C m^{-2} (for soybean cropland since 2003), 1,580 ± 190 g C m^{-2} (for soybean cropland since 2004), 1,160 ± 380 g C m^{-2} (for soybean cropland since 2007), and 2,570 ± 230 g C m^{-2} (for soybean cropland since 2008). Across all depths, linear regression indicated no significant relationship between C concentration and percent clay ($p = 0.10$) and between C stock and percent clay ($p = 0.06$).

In surface soils (0–10 cm), CO_2 evolution rates were significantly higher ($p < 0.0001$) in forest compared with soybean cropland soils (Table 2). Even after accounting for mixing of soil profiles to 50 cm, soybean cropland soils had significantly lower rates of CO_2 evolution ($p < 0.0001$) following up to a decade of cultivation. Rates of CO_2 evolution were very low in deep soils (190–200 cm) in both forest and soybean cropland (Table 2).

3.2. Soil $\Delta^{14}C$

Bulk soil $\Delta^{14}C$ was significantly lower ($p = 0.0003$) in soybean cropland than forest soils at 0–10 cm (forest mean = 107.8 ± 4.8‰, soybean cropland mean = 77.4 ± 10.8‰), even after accounting for differences in percent clay ($p = 0.04$). Multiple regression indicated that $\Delta^{14}C$ at 0–10 cm was not related to years in soybean cultivation ($p = 0.14$) or to percent clay ($p = 0.66$). Calculations of mixing in the top 50 cm indicated no significant difference in the cumulative C-mass weighted $\Delta^{14}C$ in forest soils compared with soybean cropland soils ($p = 0.26$; Table S2). In deeper soils (>50 cm), $\Delta^{14}C$ did not differ between forest and soybean cropland soils (Figure 2a) and was not related to the length of time in soybean cultivation across all depths ($p = 0.95$). At most sites, the change in bulk soil $\Delta^{14}C$ from 2009 to 2013 was negative (lower $\Delta^{14}C$ in 2013 than 2009), but positive changes occurred in both forest and soybean cropland and the variation in the difference among sites increased with depth (Figures 2b and S3). Across all depths, bulk soil $\Delta^{14}C$ was not significantly related to percent clay in 2009 or 2013. There was more variation in bulk soil $\Delta^{14}C$ in low clay sites (18–30% clay, $n = 4$) than in high clay sites (43–55% clay, $n = 4$) (Figure 3).
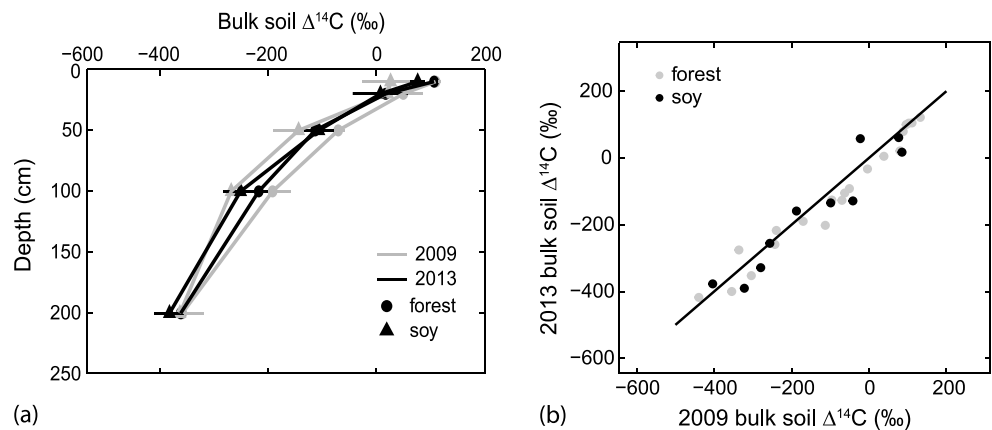

Figure 2. (a) Mean ± standard error bulk soil $\Delta^{14}C$ measured in soils collected at the same forest and soy sites in 2009 and 2013 to 2 m depth. At 0–10 cm depth, mean forest $\Delta^{14}C$ was significantly higher than mean soybean cropland $\Delta^{14}C$ ($p < 0.0001$). (b) Comparison of bulk soil $\Delta^{14}C$ (‰) from samples collected at the same sites in 2009 and 2013 across all depths. The solid black line is the 1:1 line.
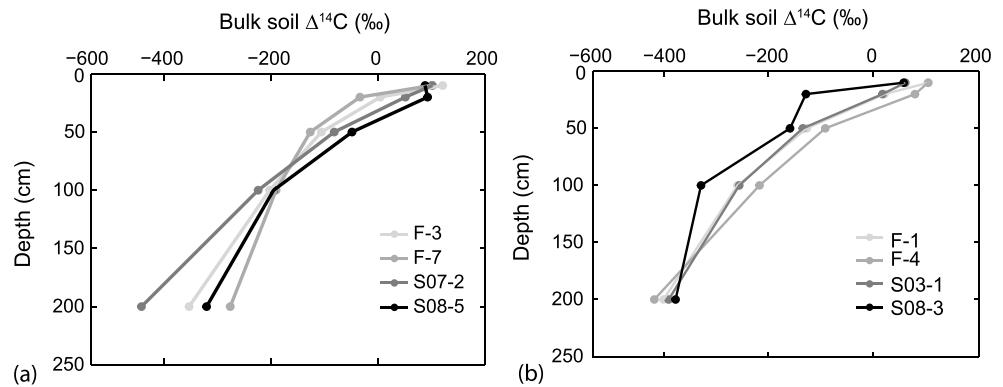
Figure 3. Bulk soil $\Delta^{14}C$ of soils collected in 2013 to 2 m depth in (a) low and (b) high clay sites. Each profile represents one sampling location. Locations starting with "F" are forested; locations starting with "S" are agricultural and the number after "S" is the two-digit year that it was converted to soybean cropland (e.g., S07-5 is a soybean cropland location that was converted in 2007); the number after "-" was randomly assigned to each point (1–7) for each treatment. Clay content ranged from 18 to 30% in low clay sites and 43 to 55% in high clay sites.

The CO_2 released from incubated soybean cropland soils from 0 to 10 cm had significantly lower ($p = 0.002$) $\Delta^{14}C$ than CO_2 released from forest soils (Table 3). Additionally, the $\Delta^{14}C$ of CO_2 that accounted for mixing in the top 50 cm of soil was significantly lower in soybean cropland soils than forest soils ($p = 0.01$). The $\Delta^{14}C$ of respired CO_2 was not significantly related ($p = 0.10$) to the length of time in soybean cultivation. The $\Delta^{14}C$ of CO_2 in both soybean cropland and forest soils was higher than bulk soil $\Delta^{14}C$ at 40–50 and 90–100 cm, while the opposite was true at 0–10 cm (Figure 4).

Results from the two-pool model indicated that in forest soils at 0–10 cm, the turnover time of the faster pool was approximately 1.0 to 1.6 years while the turnover time of the slower pool ranged from 29 to 75 years (Table 4 and Figure S4). At deeper depths, where the proportion of slower cycling C was greater than in surface soils, both pools turned over on timescales of centuries or millennia (Table 4). Turnover of forest soil C at 190–200 cm ranged from 4,952 to 5,770 years for the slower pool (Table 4).

3.3. Soil $\delta^{13}C$

Bulk soil $\delta^{13}C$ was significantly higher in soybean cropland compared with forest soils from the surface to 20 cm (soils collected in 2009) or 50 cm depth (soils collected in 2013), and indicated a lasting influence of pasture grasses (Figure 5a). Calculations of mixing in the top 50 cm indicated a significant difference in the cumulative C-mass weighted $\delta^{13}C$ in forest soils compared with soybean cropland soils ($p = 0.02$; Table S2). Bulk soil $\delta^{13}C$ was significantly ($p < 0.0001$) related to the number of years in soybean cultivation. Sites that had been in soybean cropland for fewer years tended to have higher $\delta^{13}C$ at 0–10 cm (mean values of $-28.5 \pm 0.2‰$ in forest and $-23.3 \pm 0.3‰$, $-24.2 \pm 0.4‰$, $-23.7 \pm 0.4‰$, and $-23.0 \pm 0.4‰$ in areas converted to soybean cultivation in 2003, 2004, 2007, and 2008), but this relationship was not linear. The change in bulk soil $\delta^{13}C$ from 2009 to 2013 tended to be negative near the soil surface but varied in magnitude and direction in both forest and soybean cropland sites and indicated high heterogeneity among samples collected from within a few meters of the same site in 2009 and 2013 (Figures 5b and S5). Mean bulk soil $\delta^{13}C$ of all soybean cropland soils at 0–10 cm in 2013 was $-23.6‰$ compared to $-23.1‰$ in 2009.

The $\delta^{13}C$ of CO_2 from 0 to 10 cm was significantly higher ($p < 0.0001$) in soybean cropland soils ($-18.8 \pm 0.2‰$) compared with forest soils ($-27.3 \pm 0.7‰$). The $\delta^{13}C$ of CO_2 was not significantly ($p = 0.48$) related to the number of years in soybean cultivation.

The mean % C_4-C in the soybean cropland sites (all former pastures) across all depths was $12 \pm 4\%$ in 2009 and $14 \pm 2\%$ in 2013. We did not detect a decrease in the percent of C_4-C in soybean cropland soils over time as expected and the standard error was larger than the

Table 3

Mean ± Standard Error $\Delta^{14}C$ (‰) of C Evolved as CO_2 in Soil Incubations by Depth (cm)

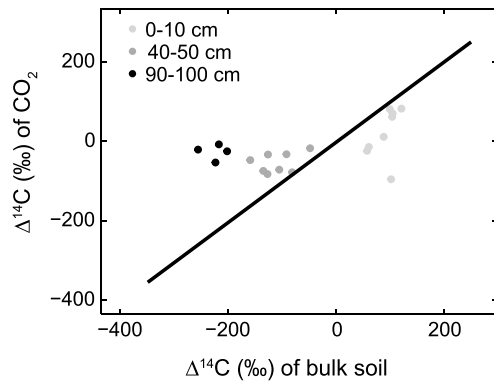| Depth (cm)	$\Delta^{14}C$ of CO_2 evolved (‰)	
	Forest	Soybeans
0–10	73.8 (a) ± 4.9	−28.5 (b) ± 17.9
40–50	−55.0 (a) ± 12.9	−54.3 (a) ± 14.1
90–100	−16.4 (a) ± 8.7	−37.1 (a) ± 16.3

Figure 4. Bulk soil $\Delta^{14}C$ versus respired CO_2 $\Delta^{14}C$ by depth of soils collected in 2013 at forest and soybean cropland sites. The solid black line is the 1:1 line.

change (increase) in the mean. The mean percent C_4-C in the forest sites across all depths was $0.0 \pm 1.1\%$ in 2009 and $0.3 \pm 0.9\%$ in 2013.

4. Discussion

4.1. Resilience of C Stocks to Large Changes in Land Use and Microclimate

Contrary to our first hypothesis, after nearly three decades of pasture followed by a decade of intensive soybean cultivation, we found evidence of differences in soil C stocks between forest and soybean cropland in surface soils only. These differences can be partially explained by tillage and soil mixing at the time of conversion to soybean cropland. Previous studies have found that tropical forest conversion to pasture can increase, decrease, or have no effect on soil C stocks, while use as cropland often leads to losses of soil C (Don et al., 2011; Fujisaki et al., 2015; McGrath et al., 2001; Powers et al., 2011). Using the predicted loss of 0.00275 Tg C km^{-2} from soils in Latin America from 2000 to 2100 (Eglin et al., 2010) as a rough estimate (while acknowledging the caveats that rates of C emissions and land use change could be nonlinear or variable over the next 100 years), we expected stocks to be on the order of 275 g C m^{-2} less in soybean cropland soils after a decade of cultivation. This was not detectable here, even after accounting for differences in bulk density between forest and soybean cropland soils.

These results suggest a resistance of these soils to extensive changes in land use and associated changes in soil microclimate under current minimum tillage management. The increase in the rate of decomposition of SOM associated with a 10°C increase in soil temperature (Q_{10}) is commonly assumed to be around a factor of 2 (Davidson & Janssens, 2006). Thus, we would expect that the 2°C soil temperature increase observed in surface soils at Tanguro Ranch (O'Connell, 2015) and in the Xingu Basin (Silvério et al., 2015) would be accompanied by least a 15% increase in the rate of decomposition of OM. We did not observe this across the total soil C stocks. It is possible that the decomposition rate only increases in the smaller fraction of more labile C but leaves the majority of more recalcitrant C unchanged on the timescale of two decades (Davidson & Janssens, 2006; Liski et al., 1999; Melillo et al., 2002) and that this reduces the magnitude of change to overall soil C stocks, but this hypothesis remains unresolved (Fang et al., 2005; Xu et al., 2014).

Several of our results indicate that C inputs from soybean plants were low. First, there was at least a several per mil difference between forest and soybean cropland soils in the $\delta^{13}C$ signature in bulk soil to 50 cm depth (Figures 5a, 5b, and S5) and in the CO_2 respired from surface soils. If soybean inputs were substantial, the C_4 signature of the pasture grasses on $\delta^{13}C$ would have declined between 2009 and 2013 after replacement of pasture with soybean cropland, but the C_4-C in soils from soybean cropland sites did not change over time (Figures 5a, 5b, S5). Thus, even though a fairly large fraction of the C in the 0–10 or 0–20 cm depths had a turnover time fast enough to acquire the $\delta^{13}C$ signature of C_4 pasture grasses, the turnover time was still slow enough, or the new inputs from soybean plants were small enough, that the signature remained measurable 5–10 years after the return of C_3 vegetation in cropland. These findings were supported by field observations of a small litter pool in soybean fields following harvest and low inputs of soybean litter at Tanguro Ranch estimated at 65 g C m^{-2} yr^{-1} (Figueira et al., 2016). With a larger sample size in 0–10 cm than we used, Figueira et al. (2016) found a small but significant decrease in $\delta^{13}C$ from 2009 to 2013 of soil under soybean cropland (mean values of −23.5 in 2009 and −24.5 in 2013).

Table 4
Modeled Pool Sizes (Fraction of Total C) and Turnover Times (Years) From a Two Pool Model of Forest Soils Based on $\Delta^{14}C$ of Evolved CO_2 and Bulk Soil and C Stocks in That Depth Interval

Depth interval (cm)	Pool 1 C/ Total C	Pool 2 C/ Total C	Turnover time (years): pool 1	Turnover time (years): pool 2
0–10	0.1	0.9	1.0	74.9
0–10	0.2	0.8	1.4	69.6
0–10	0.3	0.7	1.5	63.4
0–10	0.4	0.6	1.6	55.8
0–10	0.5	0.5	1.6	45.9
0–10	0.6	0.4	1.5	28.8
40–50	0.01	0.99	46.1	1377
40–50	0.04	0.96	121.9	1432
40–50	0.08	0.92	190.7	1496
40–50	0.1	0.9	219.2	1527
40–50	0.15	0.85	280.3	1606
40–50	0.2	0.8	331.8	1688
190–200	0.001	0.999	<1	4952
190–200	0.003	0.997	<1	4966
190–200	0.008	0.992	20.9	5031
190–200	0.01	0.99	26.4	5053
190–200	0.015	0.985	37.7	5105
190–200	0.1	0.9	<1	5770

Note. Pool sizes and turnover times were adjusted for the two pools at 0–10 cm, 40–50 cm, and 190–200 cm.

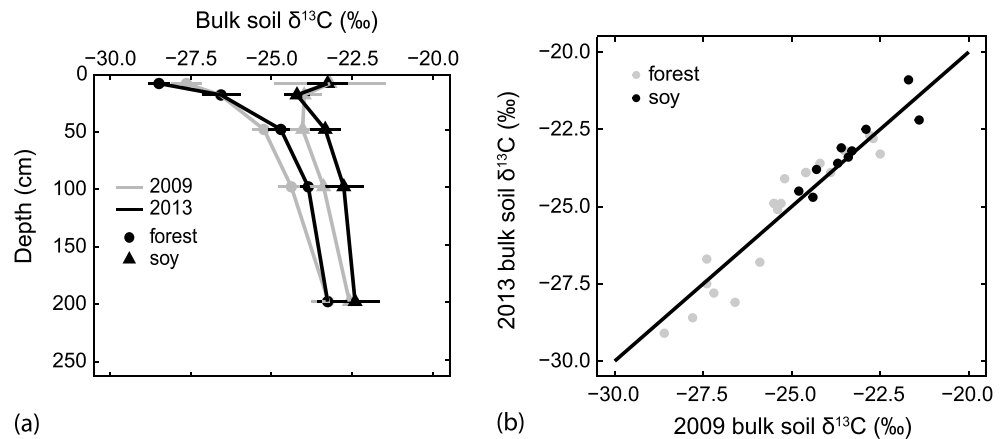**Figure 5.** (a) Mean ± standard error bulk soil $\delta^{13}C$ measured in soils collected at the same forest and soybean cropland sites in 2009 and 2013 to 2 m depth. (b) Comparison of bulk soil $\delta^{13}C$ (‰) from samples collected at the same sites in 2009 and 2013 across all depths. The solid black line is the 1:1 line.

4.2. Effects of Mixing and Soil Compaction

In addition to the potential mechanism of C loss in surface soils from reduced soybean OM inputs, the changes in surface soil C could also be caused by mixing with deeper soil depths. Consistent with our second hypothesis, the $\Delta^{14}C$ data indicated that young C was respired and/or mixed deeper in the soil profile, leading to an older average age of bulk soil C under soybean cropland (Figure 2a) and lower rates of CO_2 evolution in incubations of soybean cropland surface soils (Table 2). The $\delta^{13}C$ indicated the presence of residual C_4-derived C as deep as 20 or 50 cm (Figure 5a), which could indicate the influence of either downward mixing or the residual effect from past inputs from deep-rooted pasture grasses. Tilling in these soils occurred to 40 cm depth so the potential for mixing of old and young soil C was high in the upper soils following tillage. However, old (pre-bomb) C was respired in CO_2 in soils at 0–10 cm and 0–50 cm depth in excess of what could be attributed to mixing alone (Table 3). Management practices associated with cultivation, specifically tillage, can break up soil aggregates and expose OM to microbes and oxidation (Six et al., 1999; Torn et al., 2009), which could change the apparent age of both bulk soil C and respired CO_2.

The errors associated with C stock calculations in croplands can be large because soil bulk density is difficult to measure accurately (Holmes et al., 2012; Taalab et al., 2013; Veldkamp, 1994), particularly in the upper soil horizons in seasonally dry soils, or in soils that are tilled and impacted by machinery. To observe changes in C stocks following land conversion, the changes must be large enough to detect (De Camargo et al., 1999; Figueira et al., 2016) despite errors associated with bulk density that can be up to 5% of the uncertainty in C stocks (Holmes et al., 2012). According to the projected losses of 27.5 g C m^{-2} yr^{-1} through the year 2100 (Eglin et al., 2010), we were looking for a small change in a large pool that may be below our detection limit. It is possible that error associated with measurement of bulk density masked some of the difference in C stocks by introducing additional variability or that the influence of land conversion on C stocks has been small. Our small errors in bulk density, together with significant differences in C concentrations and stocks found only in surface soils and not at deeper soil depths, even after accounting for soil compaction (Table S1), points toward the conclusion of low rates of change in soil C stocks.

4.3. The Effect of the Length of Cultivation

The duration of soybean cultivation did not have a large effect on soil C, contrary to our third hypothesis. The lowest C storage was in areas converted to soybean cropland in 2007, rather than 2003 or 2004, and did not fit with the expected pattern that longer time in soybean cropland would cause greater C reductions. This suggested that reduction in C stocks of surface soils was not a linear process or that initial conditions varied across sites and was related in some way to pre-existing soil properties or past management. It would require very large changes in soil C to detect the effect of this chronosequence of years in soybean cultivation within the soybean cropland, and the chronosequence approach assumes that all other factors are equal among

sites. We know that some of the sites converted to soybean cropland in 2007 had lower clay content (Riskin et al., 2013) and thus lower C storage in surface soils (Figueira et al., 2016). However, even with clay content as a covariate, the effect of years in soybean cultivation was not statistically significant in surface soils (Figueira et al., 2016). Significant negative relationships of C content and $\delta^{13}C$ with the years in soybean cultivation were observed, but the greatest differences were between forest and soybean cropland soils.

4.4. Multiple Controls on Tropical Soil C

We observed heterogeneity in C dynamics within forest soils (Figure 2a). Inherent soil properties, such as clay content, are important controls on C dynamics (Powers et al., 2011; Torn et al., 2009). However, the influence of percent clay on C stocks, C concentrations, $\delta^{13}C$, and $\Delta^{14}C$ across soil depths was inconsistent. The strong vertical structure down the profile in $\Delta^{14}C$ but not in percent clay indicated that a combination of percent clay and depth controlled $\Delta^{14}C$ changes. Climate can also be an important control of C processes, but these sites all had a similar regional climate (although differences in microclimate between soybean cropland and forest were pronounced).

4.5. Stability of Deep Soil C

Soils of the Brazilian Shield are typically highly weathered and very deep (Neill et al., 2013; Nepstad et al., 1994; Trumbore, 2000) and the small stocks of relatively older C at 2 m depth, compared with surface soils, produced low levels of CO_2 from respiration (Table 2). Simple calculations of the time to respire all of the C in deep soils indicated a turnover time of decades, yet the ^{14}C age of the C respired was on average older, suggesting that incubation conditions enhanced destabilization or that rates would slow over time as the more available C was respired. We measured declining rates of CO_2 evolution during the 3 month incubation period (data not shown), consistent with previous studies (Follett et al., 2007; Swanston et al., 2002).

Older C in deeper soils at 1–2 m did not exhibit differences in C stocks, CO_2 production, or C_4 vegetation signature, despite the change in land use over three decades. The lack of change may be related to the relative stability of Fe- and Al-stabilized SOM at deeper depths. Associations of organic C and primary and secondary minerals, such as Fe and Al oxides and hydroxides, are important for soil aggregation and SOM stabilization (Sollins et al., 1996; Torn et al., 2009). The respiration of C that was younger than the bulk soil C in deeper soils (Figure 4) was consistent with previous studies (Phillips et al., 2013) and indicated that there was some older substrate that was more protected or less available. Because both inputs and respiration were low in deep soils, mean turnover times were very slow.

Changes in regional climate caused by land use and global climate forcings, including increasing atmospheric CO_2 concentrations, warmer temperatures, and increasing periods of drought, have the potential to alter C cycling in the Amazon if large C pools are responsive to climatic changes (Oliveira et al., 2013). The 2°C soil warming that has resulted from land use change here (O'Connell, 2015; Silvério et al., 2015) may be larger than the effects caused by climate change directly, and reductions in transpiration in the agricultural soils mean that they remain wetter at depth than forest soils (Neill et al., 2013). We found that large amounts of C in deeper soils remained unaffected despite dramatic changes in land use over three decades. Thus, more work is needed to refine long-term projections of the effects of climate and land use change on soil C dynamics in the Amazon.

5. Conclusions

In summary, conversion of seasonally dry tropical forests to pasture and cropland in the southern Amazon region of cropland expansion led to only small changes in soil C dynamics. While organic inputs from African pasture grasses such as *Brachiaria* spp. can meet or exceed those from forest trees, our data suggested that inputs from soybeans in the last decade must have been low relative to those from trees or pasture grasses under forest or pasture. Differences in C storage below the top 10 cm were not detected and mixing to 50 cm depth could account for some differences in both C storage and bulk soil $\Delta^{14}C$ between forest and soybean cropland soils. However, lower rates of CO_2 production and lower $\Delta^{14}C$ of CO_2 indicated that there was a loss of young C or destabilization of old C in the top 50 cm. With continued low OM inputs from soybeans and hotter local and regional temperatures, further reductions in C stocks would be possible if these large C pools were responsive. However, we found that C stocks in these soils were mostly resistant to these drivers and that C stock changes in the coming decades are likely to be modest if similar crop

management practices are used. Programs like Brazil's Low-Carbon Agriculture Plan that provides lines of credit for following best agricultural management practices can help reduce C losses from expanding tropical cropland in the Amazon (Angelo, 2012; de Moraes Sá et al., 2017).

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