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Review

Carbon ($\delta^{13}C$) dynamics in agroecosystems under traditional and minimum tillage systems: a review

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Abstract. Following cultivation, substantial loss of soil organic matter occurs in surface soil layers. No-till is an agronomic practice to reverse or slow the loss of soil organic matter. We reviewed 95 research papers that used ^{13}C natural abundance of soils to quantify the impact of tillage on the C dynamics of cropping systems. New C (from current cropping systems) accumulated in the surface soil under no-till, whereas the most extreme cultivation (mouldboard ploughing) mixed new C throughout the soil. There was a decline in soil C with years of cultivation. Compared with land that had been tilled, no-till generally had little impact on the accumulation on soil organic C. Tillage and residue retention caused stratification in C stocks that depended on tillage depth, with the highest C concentrations and stocks found in the surface under no-till. Shifts in the $\delta^{13}C$ signature indicated significant exchange of 'new' C for the original (old) C. Tillage methods had no impact on the size and $\delta^{13}C$ signature of the microbial biomass pool. Change in $\delta^{13}C$ indicates that microbial biomass rapidly incorporates new carbon. The largest change in the $\delta^{13}C$ values ($\Delta^{13}C$) was observed in the coarse sand fraction, whereas the smallest change occurred in the clay fraction. Comparison of conventional vs no-till showed inconsistent results on the effect of tillage on C in the different particle size fractions. Natural ^{13}C abundance data show that no-till cropping systems do not result in increases in soil organic C in the top 0.30 m of soil.

Keywords: tillage, no-till, zero till, C_3, C_4, soil microbial biomass, $\delta^{13}C$, C sequestration.

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Introduction

The preferential fixation of ^{12}C over ^{13}C during photosynthesis results in the $\delta^{13}C$ signature of C_3 plants (Calvin cycle) being lower (more negative) than that of C_4 plants (Hatch-Slack pathway) (Smith and Epstein 1971; Farquhar *et al*. 1982, 1989; Lloyd and Farquhar 1994). In undisturbed systems, the soil mirrors the $\delta^{13}C$ signature of the dominant vegetation. When a C_3 forest is clear-felled for continuous cultivation of C_4 maize (*Zea mays*), for example, the soil $\delta^{13}C$ signature gradually changes towards that of the replacement C_4 vegetation.

The proportion of soil C derived from C inputs (crop or residue) can be calculated using an isotope end-member mixing model (Balesdent *et al*. 1987), with the assumptions of similar isotopic fractionation during humification of C_3 and C_4 plant residues (Dorodnikov *et al*. 2007), and a constant temporal ^{13}C abundance of the input C. Growing C_4 plants on a soil that has previously been under C_3 vegetation, or vice versa, can be considered as an *in-situ* labelling of the organic matter incorporated into the soil and is best used in the case of several successive cultivations (Balesdent *et al*. 1987). Based on measured changes in C stocks and $\delta^{13}C$ signatures, the turnover times of the original and replacement soil organic matter fractions can be estimated (Zach *et al*. 2006; Chalk *et al*. 2021).

Smith and Chalk (2020) reviewed the effect of tillage systems on nitrogen cycling by using ^{15}N tracing techniques and concluded that no-till had little impact on ^{15}N fertiliser recovery, or the accumulation of N released from the mineralisation of 'native' soil organic matter. However, the influence of tillage systems on the stability and turnover of soil organic matter in the soil profile was not considered. Additionally, Smith and Chalk (2020) found the effect of no-till was restricted to the surface soil layers (<0.05 m), but the ^{15}N studies could not confirm or refute the widely-held view that soil organic N increased under no-till, and by inference, soil organic C sequestration. New insights with respect to the influence of tillage on the stability and turnover of soil organic C may be obtained by measuring changes in the soil $\delta^{13}C$ signatures in systems that have undergone a C_3 to C_4 vegetation shift, where original vs replaced C pools can be identified *in situ*. We reviewed studies of the effects of tillage on shifts in ^{13}C natural abundance ($\delta^{13}C$) implemented for varying lengths of time following the clearing of native vegetation.

In several of the papers reviewed, C in a soil layer was reported as a concentration rather than an amount, and the key soil property, bulk density, was not reported. Consequently, it was not possible to convert C concentrations into C stocks in the various soil layers. Furthermore, nominal sampling depths vary among studies, because of choice, as well as disturbance caused by tillage, and comparisons of tillage effects using mass-based concentrations and a material coordinate (defined by integrating the bulk density profile down from the soil surface; Smiles 2009) cannot be made. That is, comparisons should be made on values adjusted to an equivalent depth based on the weight of soil being the same in the comparisons (Resh *et al*. 2002; Zach *et al*. 2006). The objective of the review is to examine C dynamics under traditional and minimal tillage systems using the *in situ* natural ^{13}C abundance technique, taking into account C stocks rather than C concentrations whenever possible. We do not review ^{13}C enrichment studies because pulse labelling of plants with $^{13}CO_2$ results in a non-uniform label (Sangster *et al*. 2010). Furthermore, such studies are short-term by nature and consequently do not provide information on long-term soil C dynamics.

$\delta^{13}C$ signatures of CO_2 and selected organic materials

Plants preferentially fix ^{12}C over ^{13}C and accordingly have a lower $\delta^{13}C$ value compared with atmospheric CO_2 (Farquhar *et al*. 1989; Lloyd and Farquhar 1994). The C_3 photosynthetic pathway (Calvin cycle) results in stored C depleted in $\delta^{13}C$ of ~18‰ relative to the atmosphere (Farquhar *et al*. 1982). Since 1975, $\delta^{13}C$ in the atmosphere is not constant and has steadily decreased (Francey *et al*. 1999; Keeling *et al*. 2017). Ice core records of atmospheric CO_2 show that it had a $\delta^{13}C$ value of approximately –6.5‰ (1387–1900 years; Francey *et al*. 1999), which has declined to around –8‰ (Keeling *et al*. 2005, 2017; Dean *et al*. 2014). The decline in $\delta^{13}CO_2$ is linked to the burning of fossil fuels derived from the organic remains of organisms (mainly plants) that lived millions of years ago. The $\delta^{13}C$ of oil or coal is like modern C_3 plants with values ranging from −32 to −21‰ for oil and −28 to −23‰ for coal relative to the Vienna Pee Dee Belemnite (VPDB) standard (Given 1984; Warwick and Ruppert 2016; Xu *et al*. 2017; Winiger *et al*. 2019). The annual trend in the decrease in atmospheric $\delta^{13}C$ is explained by the addition of CO_2 to the atmosphere that must come from the terrestrial biosphere and/or fossil fuels. As a result of the change in the atmospheric $\delta^{13}C$, carbon (C) inputs from plants are likely to be more depleted than in the past, thus increasing the uncertainty in subsequent C cycling calculations.

In general, the average $\delta^{13}C$ abundance for a range of organic materials of C_3 origin under a range of climates was –28.0 ± 1.9‰ (Balesdent *et al*. 1987, 1988, 1990, 1993; Natelhoffer and Fry 1988; Vitorello *et al*. 1989; Wanniarachchi *et al*. 1999; Desjardins *et al*. 1994; Cadisch *et al*. 1996; Andriulo *et al*. 1999; Gerzabek *et al*. 2001; Kaler *at al*. 2018), whereas the average value from C_4-derived organic sources was –12.5 ± 1.5‰ (Balesdent *et al*. 1987, 1990; Desjardins *et al*. 1994; Puget *et al*. 1995; Cadisch *et al*. 1996; Andriulo *et al*. 1999; Wanniarachchi *et al*. 1999; Hansen *et al*. 2004; Kristiansen *et al*. 2005; Fuentes *et al*. 2010). There are small differences in the isotopic composition of plants in different land use systems. For example, forest, –29.0 ± 1.8‰ (Natelhoffer and Fry 1988; Desjardins *et al*. 1994; Cadisch *et al*. 1996); grass and cereal, –28.4 ± 2.3‰ (Balesdent *et al*. 1990; Andriulo *et al*. 1999; Fuentes *et al*. 2010); organic wastes from animal housing such as manure, straw and slurries, –27.6 ± 1.5 (Gerzabek *et al*. 2001); and peat, –25.6‰ (Gerzabek *et al*. 2001).

Soil C stocks under tillage systems

Definitions of tillage systems are somewhat variable and were described by Smith and Chalk (2020). The systems and the degree of soil disturbance and mixing needs to be understood in order to make valid comparisons between different experiments. This is not the case in much of the literature. There is a need to use standard terminology in tillage experiments to quantify the impact of the tillage on incorporation of residues, tillage depth and soil bulk density. This will enable C and isotope stocks to be correctly determined on an area basis. Furthermore, soil C and ^{13}C stocks of different treatments should be compared on an equivalent mass of soil, not on a fixed depth basis (Smiles 2009; Lam *et al*. 2013).

The influence of mouldboard ploughing (MP), chisel ploughing (CP), conventional tillage (CT) and no-tillage (NT), except for soil disturbance during the seeding operation are in Fig. 1. Where possible, C stocks in the surface 0.3 m are presented because the cultivation methods disturbed the soil to different depths. For example, mouldboard ploughing inverts the soil to a depth of 0.2 m and moves organic material from the surface to depth.

There was a general decline in soil C concentrations and stocks with years of cultivation of virgin land (Lefroy *et al*. 1993; Jolivet *et al*. 1997; Andriulo *et al*. 1999), which can either be described by a single or double exponential decay model. Of the 10 studies that report C stocks (Vitorello *et al*. 1989; Desjardins *et al*. 1994; Balesdent *et al*. 1998; Andriulo *et al*. 1999; Collins *et al*. 1999; Clapp *et al*. 2000; Dolan *et al*. 2006; Jantalia *et al*. 2007; Fuentes *et al*. 2010; de Sant-Anna *et al*. 2017), time series data are lacking, especially for long-term cultivation studies. Carbon stocks showed a reduction in C when comparisons were made on the same mass of soil equal to that under 0.3 m of native vegetation (Jantalia *et al*. 2007). Whilst no-till systems reduced the C loss compared to conventional tillage, the largest losses occurred with mouldboard ploughing and/or twice-yearly tillage (Jantalia *et al*. 2007). Differences in the C stocks because of land-use change and no-tillage were predominately restricted to the surface soil, whereas mouldboard ploughing resulted in decreases in the C stock in the surface 0.25 m (Clapp *et al*. 2000; Layese *et al*. 2002; Huggins *et al*. 2007; Loss *et al*. 2012).

Ploughing and harrowing in annual conventional tillage systems promoted greater loss of soil C compared to no-till (Loss *et al*. 2016). In a 13-year continuous maize study, soil C stocks were found to be sensitive to tillage and harvest of the aboveground plant material (Clapp *et al*. 2000). Continuous maize grown after a low input mixture of lucerne (*Medicago sativa*) showed a small decrease in soil C in the annually tilled (MP, CP) treatments. In the no-till treatment, C stocks in the surface plough layer (0.15 m) remained unchanged when

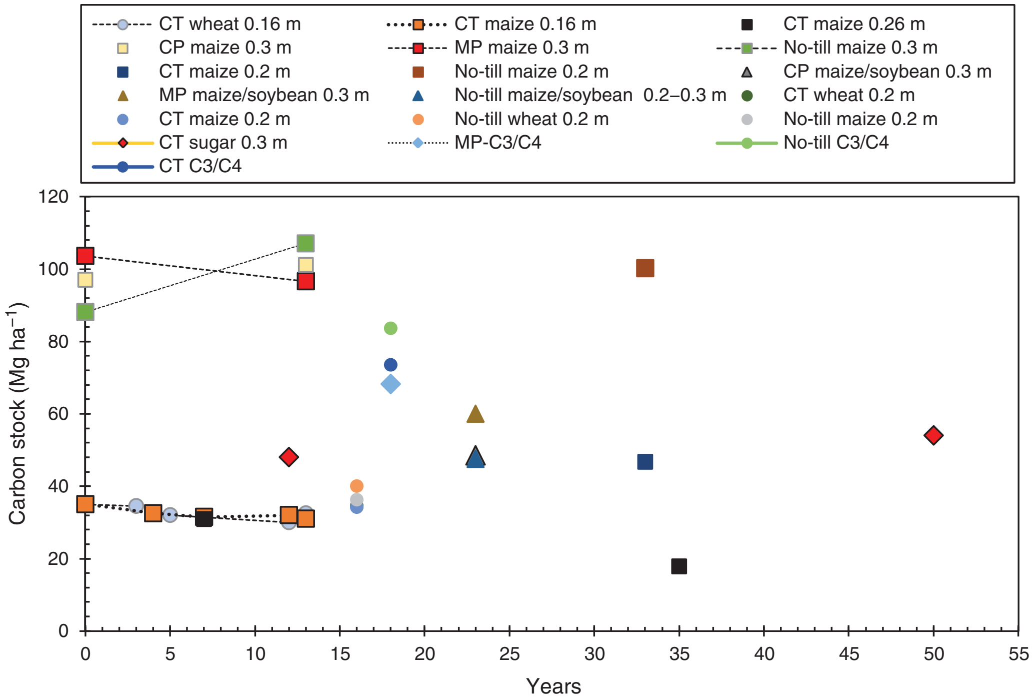

Fig. 1. C stocks in the surface 0.3 m under various tillage systems and crop rotations. Data are from Vitorello *et al*. (1989), Desjardins *et al*. (1994), Balesdent *et al*. (1998), Andriulo *et al*. (1999), Collins *et al*. (1999), Clapp *et al*. (2000), Dolan *et al*. (2006), Jantalia *et al*. (2007), de Sant-Anna *et al*. (2017) and Fuentes *et al*. (2010).

the stover was harvested but increased when the stover was returned (Clapp *et al*. 2000). In contrast, C stocks were unaffected by tillage (non-till compared to MP and CP) when summed to 0.5 m (Dolan *et al*. 2006), nor did tillage system affect the mineralisation of soil organic C (Liu *et al*. 2015).

Tillage and residue retention cause stratification in C stocks that are dependent on tillage type (depth) with the highest C concentrations and stocks found in the surface (<0.05 m) under NT (Balesdent *et al*. 1990; Andriulo *et al*. 1999; Machado *et al*. 2003; Allmaras *et al*. 2004; Dolan *et al*. 2006; Huggins *et al*. 2007). For comparison of the effect of tillage on soil C stocks, comparisons need to be made by sampling the tilled layer and ensuring that the cumulative mass of soil per unit area of cross-section is the same between the different treatments. Furthermore, soil C is responsive to the quantity of organic C applied, including the recycling of animal manures (Gerzabek *et al*. 2001), which in turn is influenced by climate, crop selection, agronomic management, fertilisation and residue management.

Soil δ^{13}C signatures under tillage systems

The δ^{13}C signatures of soil organic C under C_3, C_4 and mixed plant systems show end members ranging from –28‰ under C_3 vegetation to –15‰ from C_4 vegetation (Fig. 2). Mixed plant communities have δ^{13}C values that lie between these two end member signatures. In general, the δ^{13}C composition of the soil C tended to decrease with soil depth suggesting that the soil C at depth was predominately derived from C_3 plant material. The δ^{13}C values were lower than –12‰ in land use systems that supported C_4 plants, which is the value one would anticipate if the soil C was derived solely from C_4 plants. It should be noted that in all the papers reviewed, the C was determined on soil samples that had carbonates (CO_3) removed, thereby ensuring the δ^{13}C signature is that of organic C. Systems with a mixture of C_3 and C_4 plants have intermediate δ^{13}C values ranging from –17‰ to –22‰. The δ^{13}C values decreased for higher proportions of C_4 crops in the rotation, and for the length of time the C_4 crops were grown.

Although not shown in Fig. 2, the soil organic C declined from the surface to 1.0 m depth, while the turnover period of soil C below the cultivation depth increased with tillage. The δ^{13}C signature integrated for the profile reflects more closely the current vegetation under CT compared to NT (Machado *et al*. 2003), with significantly ‘newer’ C under CT compared to NT, which in CT extended below the cultivation depth. The difference between the two tillage systems reflects the retention of the fresh C in the surface layer (0.05 m), which declines rapidly with depth under NT. Under CT, the fresh C is distributed through the plough layer and declined below the plough layer. Tillage had no significant effect on the total amount of C present to 0.4m, but the effect was significant only when the surface soil (defined as 0.2 m) was considered (Machado *et al*. 2003). Furthermore, the result remained to be corrected after adjusting for different bulk densities and using the same mass of soil.

Stratification in soil C under the different tillage regimes is highlighted by δ^{13}C values, with the least negative values in the very surface soil. δ^{13}C values decreased under CP or MP,

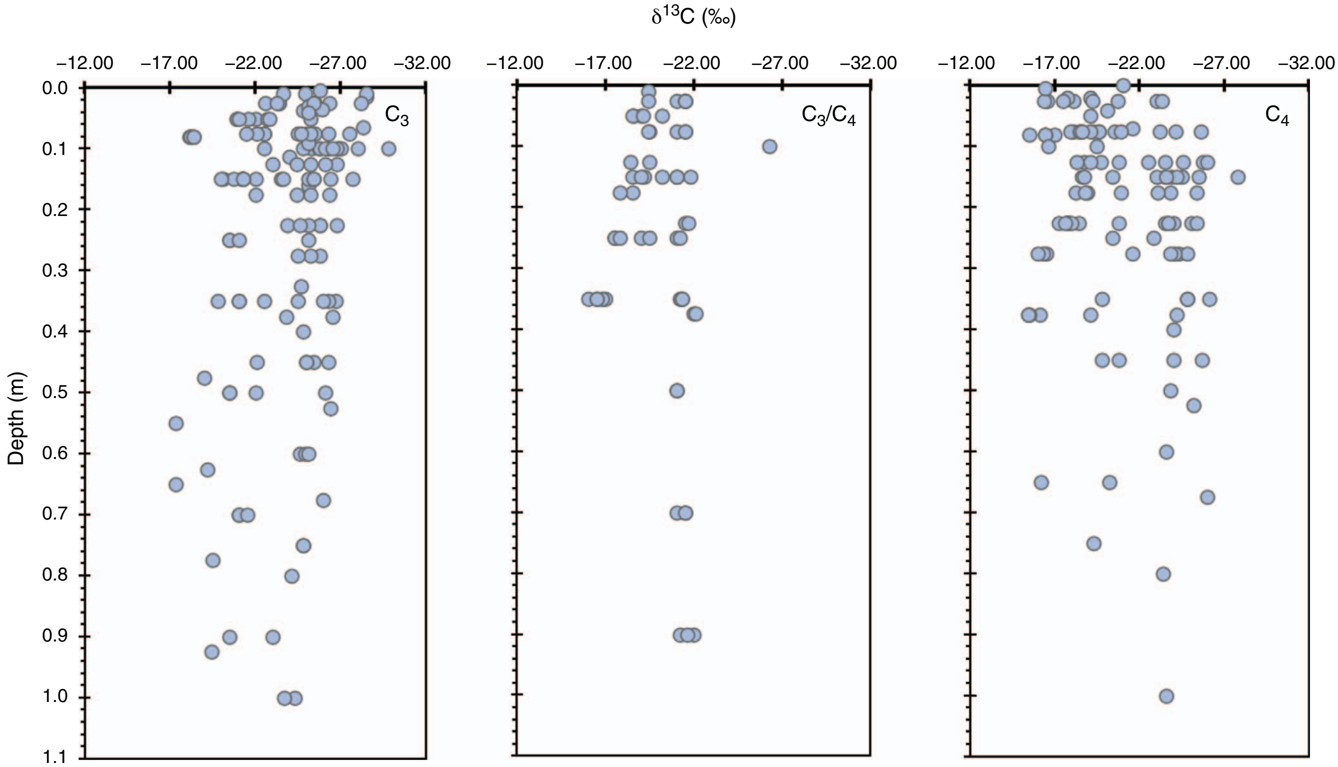

Fig. 2. Relative isotopic composition ($\delta^{13}C$) of the soil C under C_3, C_3/C_4 and C_4 plant systems. Data are from Balesdent *et al*. (1987, 1988, 1990, 1993), Natelhoffer and Fry (1988), Vitorello *et al*. (1989), Bonde *et al*. (1992), Desjardins *et al*. (1994), Gregorich *et al*. (1995), Cadisch *et al*. (1996), Andriulo *et al*. (1999), Wanniarachchi *et al*. (1999), Shang and Tiessen (2000), Gerzabek *et al*. (2001), Roscoe and Burrman (2003), Sisti *et al*. (2004), Antil *et al*. (2005), Jantalia *et al*. (2007), Fuentes *et al*. (2010), de Sant-Anna *et al*. (2017) and Muñoz-Romero *et al*. (2017).

which mixes plant residues throughout the depth of cultivation (Table 1). The stratification was most pronounced in the studies of Balesdent *et al*. (1990) and Layese *et al*. (2002) and to a lesser extent in Wanniarachchi *et al*. (1999) as shown in Fig. 3. The highest $\delta^{13}C$ values were reported by Balesdent *et al*. (1990) and the value (–16.4‰) was closer to that of the maize residue compared to the values under CT or superficial tillage. These results emphasise the need to sample to depth and make comparisons on adjusted soil C values based on the same mass of soil, if the effect of tillage practices on soil C stocks is to be accurately assessed, as discussed by Jantalia *et al*. (2007).

Whilst there was no large change in the C stocks when summed to a constant mass of soil (Balesdent *et al*. 1990; Andriulo *et al*. 1999; Clapp *et al*. 2000; Allmaras *et al*. 2004; Dolan *et al*. 2006), the shifts in the $\delta^{13}C$ signature indicated significant exchange of 'new' C for the original (old) C (Fig. 3; Zang *et al*. 2018). The data highlight the dynamic nature of the soil C and enable the contribution of the recently derived C to the total C stock in a soil layer and profile to be estimated. Furthermore, assuming soil organic C decomposition follows first order kinetics, the mean residence time of the soil C can be estimated from the mass balance and isotopic composition data (Balesdent and Mariotti 1996; Zang *et al*. 2018; Chalk *et al*. 2021).

Mean residence times under tillage systems

Mean residence time (MRT) is the average length of time that an element such as C spends in a given pool that is not uniform in composition. Each component or fraction of the pool changes at a different rate. The turnover of C is calculated using either a first order decay model (Six *et al*. 1998; Six and Jastrow 2002) or a double exponential decay model representing a rapid and then a slow change in the $\delta^{13}C$ signature of soil organic matter pools (Arrouays *et al*. 1995; Jolivet *et al*. 1997). The MRT is calculated as the reciprocal of the decomposition rate (Six and Jastrow 2002).

The effect of tillage on the MRT of C at soil depth intervals is summarised in Table 2. In general, MRTs increase with depth. In the surface soil, MRT values are quite variable, ranging from <10 to 100 years under cultivation systems. Conventional tillage and MP tended to have shorter MRT values compared to CP. NT systems had a similar spread in MRT values with the average value of 53 years in the surface 0.3 m of soil. The MRTs under NT were larger than those under MP and CT. Apparent difference in MRT among tillage systems integrate several variables, including intrinsic soil properties (e.g. pH and texture), the amounts of residues produced and incorporated into the soil and the method of calculation (Table 2).

Table 1. Effect of years of tillage and depth under maize rotation on $\delta^{13}C$ signatures of the soil organic C

Reference	Cultivation (years)	Tillage	Depth interval (cm) a	b	$\delta^{13}C$ (‰)
Balesdent *et al*. 1990	17	No-till	0	1	–16.4
			1	3	–17.7
			3	5	–20.1
			5	10	–22.6
			10	15	–23.5
			15	20	–23.8
			20	25	–23.7
			25	30	–24.1
		Conventional	0	5	–20.7
			5	10	–20.9
			10	15	–20.8
			15	20	–20.9
			20	25	–20.8
			25	30	–21.6
		Superficial	0	5	–19.2
			5	10	–19.1
			10	15	–19.7
			15	20	–23.1
			20	25	–23.7
			25	30	–23.8
Clapp *et al*. 2000	13	No-till	0	15	–17.9
			15	30	–17.2
		Chisel	0	15	–18.1
			15	30	–17.7
		Mouldboard	0	15	–18.6
			15	30	–17.8
Collins *et al*. 1999	33	Conventional	0	20	–16.6
		No-till	0	20	–19.5
Fuentes *et al*. 2010	16	No-till	0	5	–16.6
			5	10	–18.4
			10	20	–18.7
		Conventional	0	5	–18.1
			5	10	–18.0
			10	20	–18.6
Layese *et al*. 2002	13	No-till	0	5	–16.2
			5	10	–18.6
			10	15	–19.1
			15	20	–18.8
			20	25	–17.6
			25	30	–16.0
Layese *et al*. 2002	13	Chisel	0	5	–17.5
			5	10	–18.5
			10	15	–18.7
			15	20	–18.9
			20	25	–17.8
			25	30	–16.5
		Mouldboard	0	5	–18.1
			5	10	–18.5
			10	15	–18.3
			15	20	–18.2
			20	25	–18.0
			25	30	–16.3
Puget *et al*. 1995	23	No-till	2	3	–16.4
		Conventional	0	30	–20.5
		Superficial	0	10	–19.1

(*continued next page*)

Table 1. (*continued*)

Reference	Cultivation (years)	Tillage	Depth interval (cm) a	Depth interval (cm) b	$\delta^{13}C$ (‰)
Wanniarachchi *et al.* 1999	61	No-till	0	5	–23.3
			5	10	–25.7
			10	15	–25.8
			15	20	–25.4
			20	25	–25.4
			25	30	–24.8
		Conventional	0	5	–24.2
			5	10	–24.2
			10	15	–24.6
			15	20	–25.4
			20	25	–25.1
			25	30	–24.4

Table 2. Effect of tillage method on mean residence times of C in soil depth intervals (blank cells denote no data)
MP, mouldboard ploughing; CT, conventional tillage; CP, Chisel ploughing; NT, no-tillage; MRT, mean residence time; 1st, first order exponential decay; GCT, gross C turnover , which is soil organic C stock expressed as equivalent soil mass (g m^{-2})/annual C input (g m^{-2})

Reference	Tillage	Crop	Time (years)	Soil texture	Soil pH	Depth interval (m)	Method	MRT (years)
Collins *et al.* 1999	CT	Maize (*Zea mays*)	33	Loam		0.00–0.20	1st	96
						0.25–0.50	1st	125
						0.50–1.00	1st	243
Clapp *et al.* 2000	MP	Maize	13	Silt-loam	6.4	0.00–0.30	1st	64
	CP						1st	106
	NT						1st	70
Six *et al.* 2002	CT	Wheat (*Triticum aestivum*)-fallow; maize	5–26			0.00–0.20	1st	52
	NT						1st	80
Huggins *et al.* 2007	MP	Maize; soybean (*Glycine max*); Maize-soybean	14	Clay-loam		0.00–0.45	1st	32
	CP						1st	50
	NT						1st	44
Fuentes *et al.* 2010	CT	Wheat or maize	16	Clay-loam	6.5	0.00–0.20	GCT	7
	NT						GCT	9

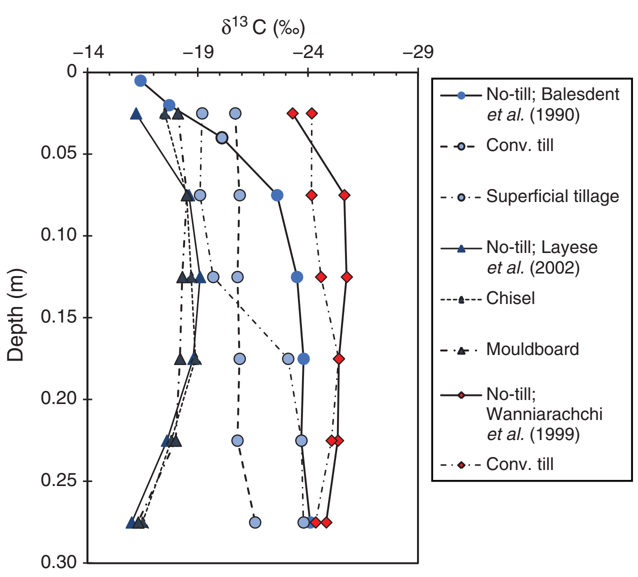

Fig. 3. The isotopic stratification of $\delta^{13}C$ (‰) in the surface 0.3 m of soil under no-till and different cultivation methods.

Distribution of ^{13}C among soil constituents

Microbial biomass

Change in microbial biomass is regarded as an early indicator of tillage and crop rotation effects on soil organic matter compared with total organic C or N measurements and is a potential index of C sequestration (Powlson and Jenkinson 1981; Carter 1986; Powlson *et al.* 1987; Balota *et al.* 1998; Sá *et al.* 2001; Potthoff *et al.* 2003). Furthermore, microbial biomass can be a valuable tool for understanding changes in soil carbon, nutrient availability, aggregate stability, and quality (Smith and Paul 1990; Doran and Parkin 1994; Brookes 1995; Sparling 1997; Dalal 1998).

It might be anticipated that tillage treatments such as MP or CT that incorporate residues may have a greater effect on MB than NT, where residues are largely left on the soil surface. However, the limited data available allow few definitive comparisons (Table 3). Generally, there were only small differences in MB-C under maize rotation between NT and MP treatments (Rochette *et al.* 1999), between NT and CT (Ryan and Aravena 1994, Ryan *et al.* 1995) and between ST, RT and MP (Angers *et al.* 1995).

Table 3. Effect of tillage regime on soil and microbial biomass (MB) C concentrations and their δ^{13}C signatures (blank cells denote no data)
ST, surface tillage; RT, ridge tillage; MP, mouldboard ploughing; NT, no-tillage; CT, conventional tillage; control, repacked soil cylinders without maize, or with maize after 35 or 70 days

Reference	Rotation	Time (years)	Tillage	C origin	Depth (cm)	Organic C (g m^{-2})	MB-C (g m^{-2})	δ^{13}C (‰) Soil	MB	C_4-MB (%)	Δ^{13}C (‰)
John *et al*. 2003	Maize (*Zea mays*)	42		Total	0–25	3654	21.6				
				C_3		3308	13.4				
				C_4		346	8.2				
				Total	25–50	4711	16.6				
				C_3		4046	11.2				
				C_4		666	5.4				
	Rye (*Secale cereale*)	116		Total	0–25	3830	21.9				
				Total	25–50	2561	12.5				
Qian and Doran 1996	Maize	25	Control			6025	86	–22.9	–22.9		
			35 days			6024	127	–22.8	–22.8		
			70 days			6121	139	–22.7	–22.6		
Rochette *et al*. 1999	Maize	2	MP		0–15		35		–22.9	8.1	
			NT				41		–22.9	10.2	
			Fallow				23		–24.7	0	
Ryan and Aravena 1994	Maize	6	NT		0–20		44[A]		–20.6	30	
			CT				65[A]		–16.9	55	
Ryan *et al*. 1995	Maize	3.3	NT		0–20						
		4					57[A]		–21.7	16	
		4.5					61[A]		–17.5	40	
		3.3	CT		0–20						
		4					62[A]		–20.2	27	
		4.5					74[A]		–17.2	47	
Angers *et al*. 1995	Maize (silage)	11	ST		0–8		50			16	
			RT				30			14	
			MP				30			19	
	Meadow		ST				100				
			RT				110				
			MP				120				
			Meadow				250				
Dijkstra *et al*. 2006	C_3 soil				0–10			–21.0	–19.4		1.6[C]
	C_4 soil							–17.0			1.5[C]
Muñoz-Romero *et al*. 2017	C_3 crops	27	NT		0–15			–25.1	–22.7[B]		
			CT					–24.9	–22.0[B]		

[A]Concentration (mg C kg^{-1} soil).
[B]Labile C not BM carbon.
[C]Δ^{13}C (‰) of microbial biomass calculated relative to total C for the soil.

However, after 4 to 6 years of continuous maize, a larger percentage of the total microbial biomass was derived from C_4-C under CT compared to NT management (Ryan and Aravena 1994; Ryan *et al*. 1995) (Table 3), which may reflect the degree of mixing of residues and soil under different tillage regimes. Microbial biomass varies seasonally according to crop growth, with an annual minimum before planting and a maximum during the growing season (Ryan *et al*. 1995). We therefore conclude that MB-C *per se* is a poor indicator of long-term changes in soil C under different tillage regimes, representing only 1–2% of soil organic C.

Soil organic C fractions

Tillage intensity generally increase the turnover rate of soil organic matter and decreases soil aggregate stability. The reduced aggregate stability and increased soil organic C decomposition is a function of the physical disturbance (Six *et al*. 1998). The turnover rate of the soil organic matter in the different particle size fractions has the potential to provide a basis for the partitioning the larger soil organic C pool into smaller functional pools. The changes in the soil δ^{13}C signature before and after cultivation of either C_4 or C_3 plants (Δ^{13}C) for different particle size fractions are in Fig. 4, where plant sequences of C_4 to C_3 or C_3 to C_4 are shown in the upper and lower panels, respectively. Changing from C_3 to C_4 vegetation cover, in general resulted in a decrease δ^{13}C values (positive shift in Δ^{13}C) and vice versa for a C_4 to C_3 vegetation change. In most studies, the largest change in the Δ^{13}C values was observed in the coarse sand fraction (200–2000 µm), whereas the smallest change occurred in the clay fraction. Collectively, these data confirm the slower turnover rate of soil organic C in clay (as evident by the lower

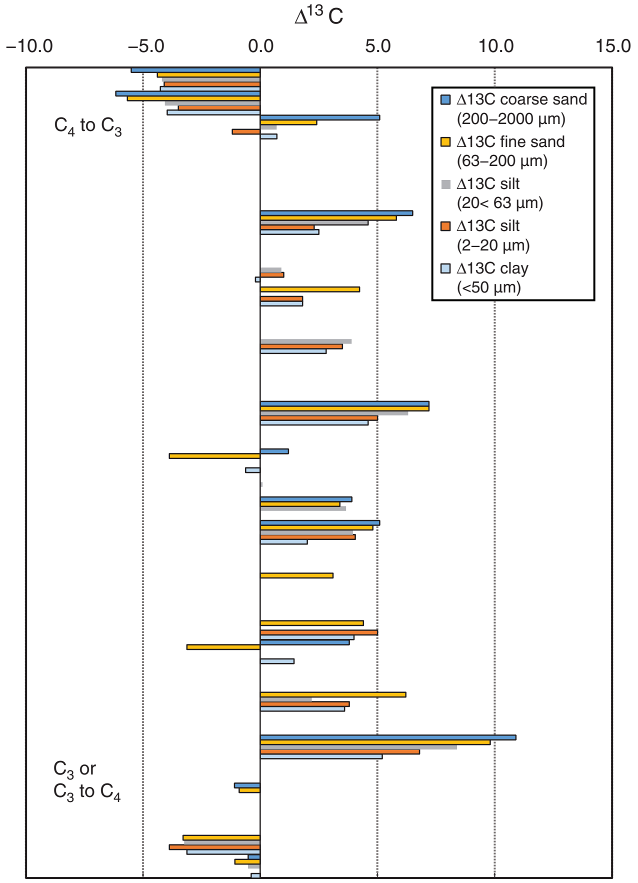

Fig. 4. Effect of particle size fractions on the change in the δ^{13}C signatures of organic C for different soil depths. Data are from Balesdent *et al.* (1988), Bonde *et al.* (1992), Desjardins *et al.* (1994), Gregorich *et al.* (1995), Puget *et al.* (1995), Jastrow *et al.* (1996), Six *et al.* (1998, 1999, 2002), Shang and Tiessen (2000), Gerzabek *et al.* (2001), Roscoe *et al.* (2001), Sá *et al.* (2001), Six and Jastrow (2002), Antil *et al.* (2005), Zach *et al.* (2006), Murage *et al.* (2007), Liang *et al.* (2014) and de Sant-Anna *et al.* (2017).

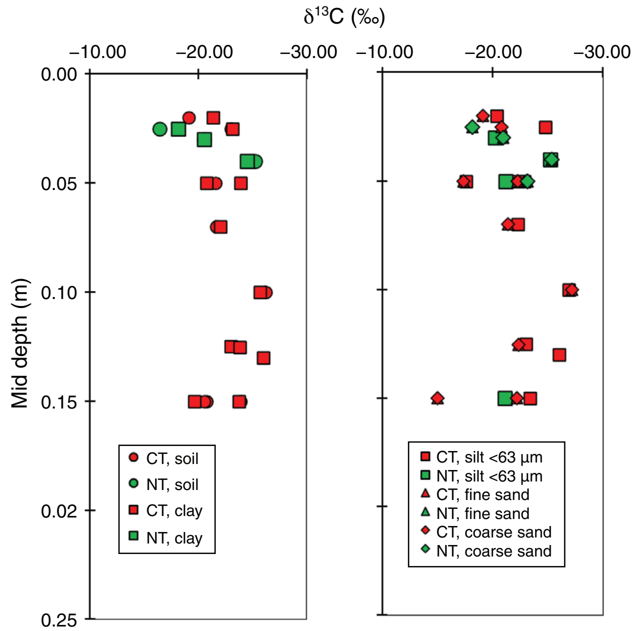

Fig. 5. δ^{13}C (‰) signatures of particle size fractions with soil depth for conventional (CT) and no-till (NT) systems (derived from a subset of data shown in Fig. 4).

replacement of the original C), the fastest turnover associated with coarse and fine sand, and intermediate values for silt fractions. Carbon in macroaggregates in general had a mean residence time 42 ± 18 years, whereas microaggregate-associated C has a mean residence time of 206 ± 95 years (Six and Jastrow 2002).

Comparison of CT (including mouldboard ploughing) to NT showed inconsistent results of the effect of tillage on C in the different particle size fractions (Fig. 5). In contrast, Six *et al.* (1999) reported greater C accumulation in NT compared to CT because of the slower turnover of C in macroaggregates under NT. According to Denef *et al.* (2007), the formation of macroaggregates is important to the long-term C stabilisation in NT systems. Formation of macroaggregates serves as an early indicator for potential total SOC sequestration caused by the greater stability and slowed turnover of C in macroaggregates. More frequent tillage may lead to the formation of small aggregates rich in labile C (Urbanek *et al.* 2011). The δ^{13}C signature in the surface soil (<0.01 m) suggests that C_4-C derived from maize is the dominant fraction of C in agroecosystems, accounting for up to 80% of the total soil organic C concentration (Jha *et al.* 2017).

The light fraction of soil organic C is dynamic and responds to short-term shifts in organic C storage. This pool has been shown to contain charcoal or a char component formed by the incomplete burning of organic residues (Skjemstad *et al.* 1994; Murage *et al.* 2007). Jantalia *et al.* (2007) estimated that 40% of soil C was charcoal in a soybean (*Glycine max*) rotation on an Oxisol in the Cerrado region of Brazil. The char or charcoal is generally composed of C from the original vegetation, and consequently may lead to misinterpretation of C turnover. For example, Murage *et al.* (2007) showed that the turnover of the light fraction of organic matter appears to be 2.5 times slower compared to the same pool without charcoal. Given that most of the cropping systems involve burning of residues, the char component should be removed from the light fraction when determining the changes to this pool. In a comparison of a soil under forest and under maize cultivation, the light fraction of soil organic matter had a shift in the δ^{13}C value (Δ^{13}C) of +10‰, indicating rapid mineralisation of C in the light fraction compared to the other particle size fractions (Gregorich *et al.* 1995). However, sample char or charcoal was not determined, and thus the turnover of the light fraction may have been overestimated.

The soil particulate organic matter fraction (POM) has been reported to be affected by cultivation of a forest soil (Balesdent *et al.* 1998), with the change being reached within the first few years after forest clearing. Six *et al.* (1999) suggest that free POM is mostly affected by plant C input rates. Generally, coarse and fine POM increased under NT, with C derived from C_3 plants being mainly associated with the finer compared to the coarse particles (Liang *et al*. 2014). The turnover time of POM increased with NT compared to CT, especially in the finer POM (53–250 μm) fraction, which largely controls aggregate stability (Six *et al*. 1998, 2002).

Role of carbon sequestration in climate change mitigation

The potential of C sequestration in soil to off-set the anthropogenic increase in atmospheric CO_2 has been discussed by several authors (e.g. Luo *et al*. 2011; Lam *et al*. 2013; Amundson and Biardeau 2018; Sun *et al*. 2020). The studies reported the impact of different tillage practices and rotations on changes in soil C stocks in different soil layers. Generally, the accumulation of C is the largest in the surface layer and declines exponentially with depth. Whilst the studies all use total C stocks, where possible, there is an opportunity to use knowledge of changes in the $\delta^{13}C$ isotopic signatures of soil C to confirm estimates of net and gross C changes that are likely to occur over longer times scales. The shifts in $\delta^{13}C$ signatures provide valuable information on the residence times of C in soil and assist in confirming that predictions of tillage and rotations on C storage are reasonable. Acton *et al*. (2013) reported a framework for soil C and isotopic mass balance modelling for forests that includes multiple C pools, including the vertical distribution and turnover of soil C. There is a need to include similar routines in agricultural system models used to evaluate the long-term impact of reduced tillage on soil C sequestration.

Conclusions

Generally, the literature shows that tillage has little impact on the accumulation on soil organic C when the comparisons are made on the same volume of soil that the different tillage systems influence. Ideally the comparisons should be made using mass of soil as the *x* coordinate to allow for differences in bulk density. Tillage, however, does cause stratification in soil C with the accumulation of the largest amounts of C in the surface layers under no-till systems. In contrast, the use of mouldboard ploughing that inverts the soil to depths of 20–30 cm distributes the new C throughout this layer. The relative ^{13}C signature of the carbon is an ideal natural *in situ* labelling technique that confirms the long-term fate of the C when it is incorporated into the soil. The technique is limited to systems where there is a change in the $\delta^{13}C$ signature of the newly applied land use system from that developed under the previous system. The shift in the $\delta\ ^{13}C$ signature is a powerful tool for quantifying the fate and turnover times of the different sources of C in the soil. These findings are consistent with the previous review by Smith and Chalk (2020) that showed tillage having little effect on soil N dynamics.

Soil C stocks are the balance between new C inputs in the form of crop residues and root biomass and the decomposition of the new and older soil C. Net primary productivity of the plant system regulates potential C input which in turn is constrained by climate, nutrients and agronomic management.

Conflicts of interest

The authors declare no conflict of interest.

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