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Long-term zero-tillage enhances the protection of soil carbon in tropical agriculture

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Abstract

Contrasting tillage strategies not only affect the stability and formation of soil aggregates but also modify the concentration and thermostability of soil organic matter associated with soil aggregates. Understanding the thermostability and carbon retention ability of aggregates under different tillage systems is essential to ascertain potential terrestrial carbon storage. We characterized the concentration and thermostability of soil organic carbon (SOC) within various aggregate size classes under both zero and conventional tillage using novel Rock-Eval pyrolysis. The nature of the pore systems was visualized and quantified by X-ray computed tomography to link soil structure to organic carbon preservation and thermostability. Soil samples were collected from experimental fields in Botucatu, Brazil, which had been under zero-tillage for 2, 15 and 31 years, and from adjacent fields under conventional tillage. Soils under zero-tillage significantly increased pore connectivity whilst simultaneously decreasing interaggregate porosity, providing a potential physical mechanism for protection of SOC in the 0–20-cm soil layer. Changes in the soil physical characteristics associated with the adoption of zero-tillage resulted in improved aggregate formation compared to conventionally tilled soils, especially when implemented for at least 15 years. In addition, we identified a chemical change in composition of organic carbon to a more recalcitrant fraction following conversion to zero-tillage, suggesting aggregates were accumulating rather than mineralizing SOC. These data reveal profound effects of different tillage systems upon soil structural modification, with important implications for the potential of zero-tillage to increase carbon sequestration compared to conventional tillage.

Highlights

- Different tillage systems may affect SOC thermostability and C retention potentials of soil aggregates.
- SOC thermostability was characterised by Rock-Eval pyrolysis and pore systems were quantified by X-ray CT within aggregate size classes.
- Profound effects of zero versus conventional tillage upon soil structural modification were observed
- Important implications for zero-tillage to increase C sequestration versus conventional tillage.

KEYWORDS

no-till, Rock-Eval pyrolysis, soil carbon, soil structure, tillage, X-ray computed tomography

1 | INTRODUCTION

The quantity of CO_2 released to the atmosphere from agricultural soils is mainly dependent on the rate of soil organic carbon (SOC) formation versus decomposition (Trumbore, 1997). Conventional agricultural practices, such as ploughing/conventional tillage accelerate the loss of SOC by increasing the oxygen concentration in the soil profile, destroying soil aggregates and exposing organic carbon for mineralization (Liu, Herbert, Hashemi, Zhang, & Ding, 2006). Zero-tillage, an agricultural practice that minimizes soil disturbance, can increase soil aggregation and may preserve and/or accumulate SOC (Liu et al., 2006; Luo, Wang, & Sun, 2010a; West & Post, 2002), which is critical to meet global targets for soil carbon sequestration (Minasny et al., 2017). However, results from studies on the impact of zero-tillage on soil carbon storage to date have been inconsistent, reporting both significant increases and decreases compared with conventional tillage following adoption, depending on the methodology used (Luo, Wang, & Sun, 2010b). For example, studies that consider the top 20 cm, show an increase in carbon storage in zero-tillage compared to conventional tillage. However, in those studies taking into account soil layers deeper than 30 cm, there has typically been no overall significant difference in SOC between zero-tilled and conventionally tilled soils (Baker et al., 2007).

Few studies have characterized the chemical composition and thermostability of organic carbon in long-term zero-tilled soils; thus, there remains a large gap in our understanding of the mechanisms that control carbon storage and determine future susceptibility to mineralization or accumulation (Bongiorno et al., 2019; Sainepo, Gachene, & Karuma, 2018). The dynamic processes involved in the transformation of organic matter are highly sensitive to environmental conditions. In wet subtropical regions, organic matter decomposes at an annual rate of approximately 3.2%, over three times faster than in temperate zones (1.0%) (Nogueirol, Cerri, da Silva, & Alleoni, 2014). Changing from conventional tillage to zero-tillage alters the soil physical structure and influences the arrangement of solid particles (mineral and organic matter) and pores in which microbial decomposers, gases and soluble compounds are located (Mangalassery et al., 2014). The interactions between the organic matter and decomposing microorganisms, or their enzymes, drive organic matter mineralization at the micrometre scale. X-ray computed tomography (CT) offers a way to measure non-destructively the size and shape of the pores and their degree of connectivity, enabling assessment of the susceptibility of organic matter to mineralization (Galdos et al., 2019).

Soil carbon sequestration potential depends on the level of aggregation. It has been hypothesized that zero-tillage increases not only the proportion of macroaggregates but also the quantity of microaggregates formed within macroaggregates (Six, Elliott, & Paustian, 1999). Previous studies have shown that microaggregate formation within macroaggregates is crucial for long-term carbon sequestration, as microaggregates have a greater capacity to protect carbon from decomposition compared with macroaggregates (Coleman, Crossley, & Hendrix, 2007; Kumar, Rawat, Jitendra, Ashutosh, & Ashish, 2013). It is important not only to assess the quantity of organic carbon but also the lability within different aggregates. Turnover of labile organic matter occurs over intervals ranging from hours to years and is highly influenced by soil management practices, whereas stable organic matter turnover occurs on timescales ranging from decades to centuries (Feng, Plante, Aufdenkampe, & Six, 2014). Both fractions can be found in aggregates of all sizes and contribute to the regulation of organic carbon storage duration (Bongiorno et al., 2019). Advances in analytical techniques now permit the detailed description of the structure of organic carbon. Rock-Eval pyrolysis has previously been used to characterize organic matter thermostability in forest soils (Soucémarianadin et al., 2018), mountainous soils (Saenger, Cécillon, Sebag, & Brun, 2013) and tropical peatlands (Cooper et al., 2019; Girkin et al., 2019) but to date is rarely used in cropping-system soils under contrasting managements to understand carbon dynamics (Cécillon et al., 2018).

The aim of this study was to understand the relationship between soil physical protection of carbon in different aggregate size classes and identify the potential for further accumulation or release by assessing carbon thermostability under two contrasting agricultural systems. We hypothesize that (a) the minimized mechanical disturbance associated with zero-tillage will allow regeneration of the soil porous architecture over time and increase soil porosity through the development of continuous pores, (b) this will increase stable macroaggregates under long-term zero-tilled soils, (c) there will be a greater fraction of microaggregates formed within macroaggregates as the rate of macroaggregate formation and degradation is reduced under zero-tillage, and (d) zero-tilled aggregates will contain more organic carbon than conventionally tilled aggregates, with an increase in the "labile" proportion, as characterized by its thermostability, due to a reduction in aggregate porosity therefore limiting microbial access.

2 | MATERIALS AND METHODS

2.1 | Sites and soil sample collection

Soil samples were collected in November 2017 from the experimental site of the São Paulo State University (UNESP) at Botucatu, São Paulo, Brazil (22°46′S, 48°25′W). The climate is classified as mesothermal with dry winters, and the dry season is well defined from May to September, with yearly average rainfall of 1,450 mm, distributed typically between October and April. Soil samples were taken from six fields, of which three had been zero-tilled for different lengths of time and the other three were paired conventionally tilled fields (details are outlined in Table 1 and Figure 1). Zero-tilled soils had been managed this way for 2 (field 6), 15 (field 2) and 31 (field 4) years, whereas the conventionally tilled soils were subjected to annual mechanical turnover to a depth of 15–20 cm in fields 1 and 3, and to a depth of 10–15 cm in field 5. Below is a description of the management for the experimental fields.

Field 1: Tillage operations were carried out with a disc plough plus disc harrow at the 15–20-cm-deep layer. Maize was grown in the spring/summer (November through to March/April) and triticale in the autumn/winter (April through to August).

Field 2: Before 1997 the field was cultivated in a conventional tillage system for soybean and maize production. In 1997, zero-tillage was established and the crop rotation soybean/black oats/maize/triticale was used until 2002. From 2003 to 2018, soybean was grown in the spring/summer (November through to March/April), followed by triticale in the autumn/winter (April through to August).

Fields 3 and 4: The experimental field is part of a long-term study of conventional tillage and zero-tillage systems that begun in 1985. In the conventionally tilled field, the tillage was undertaken every year with a disc plough plus disc harrow at the 15–20-cm-deep layer. For both fields (3 and 4) the cropping history was the same and included wheat, black oats, yellow oats, maize, pearl millet, dry beans, brachiaria grass and safflower in the autumn/winter growing season, followed by maize and soybean in the spring/summer growing season.

Fields 5 and 6: These fields were part of an experiment where maize is grown intercropped with palisade grass. The area had been fallowed for many years before the experiment with a stand of mixed grasses. Maize was fertilized annually with 180 hg ha^{-1} of nitrogen (N), 53 kg ha^{-1} of phosphorus (P) and 100 kg ha^{-1} of potassium (K). From each field (Figure 1), employing simple randomized sampling, six intact soil cores (5 cm diameter × 30 cm depth) were collected with a manual core sampler that used

TABLE 1 Selected soil and management characteristics of experimental fields

Field	Management	Ploughing depth (cm)	Years in zero-tillage	Soil texture (% clay)	Crop rotation (2017/2018)	Grain yield (Mg ha^{-1})
1	CT	15–20		65	Triticale/maize	2.5/10.2
2	ZT		15	65	Triticale/soybean	2.0/1.8
3	CT	15–20		62	Maize/soybean	8.5/2.7
4	ZT		31	62	Maize/soybean	8.5/2.9
5	CT	10–15		62	Grasses/maize	6.0
6	ZT		2	62	Grasses/maize	6.0

Note: ZT and CT refer to zero-tillage and conventional tillage, respectively.

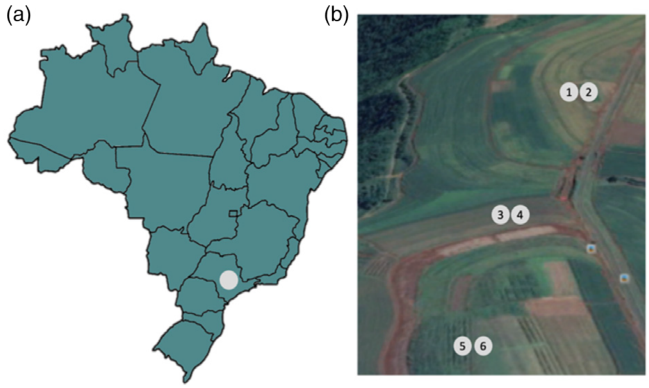

FIGURE 1 (a) Location of state São Paulo University, Botucatu campus and (b) the location of selected field sites

transparent sample liner tubes (Van Walt Ltd, Haslemere, UK) suitable for X-ray computed tomography (CT) imaging. The soil cores were first sealed with paraffin wax at the top and bottom to prevent movement during transit and then shipped to The University of Nottingham where all samples were stored at 4°C until analysis (within 2 weeks). To account for changes in bulk density between managements, soil samples were collected to a depth of 50 cm at 4-cm increments using a stainless-steel cutting cylinder (7 cm diameter × 4 cm height). Two cores were collected (0–4 cm and 1–5 cm) and a mean calculated. Simultaneously, subsamples of bulk soil samples were collected at each increment for measurements of soil organic carbon, aggregate size distribution and soil moisture content.

2.2 | X-ray computed tomography

Following sealing, samples were transported to the Hounsfield Facility at the University of Nottingham for X-ray CT scanning. Prior to this, a pilot study had been conducted, which involved preserving the samples as described here and then transporting them for X-ray CT scanning initially in Brazil and then again at the Hounsfield Facility in the UK. This confirmed that the addition of paraffin wax was an excellent approach for preventing sample disturbance during transit. The method was subsequently utilized successfully in Galdos et al. (2019) prior to this study.

Soil cores were scanned using a Phoneix v|tome|x M scanner (GE Measurement and Control Solution, Wunstorf, Germany), which allowed visualization and quantification of the soil porous architecture. Voxel resolution was set to 50 μm, with a potential energy of 160 kV and a current of 180 μA. Soil cores were scanned in three sections (i.e., depths), with a total scan time of 30 min per soil core. A total of 2,998 image projections were captured for each core. After scanning, each soil core was dismantled into two depths, 0–20 cm and >20 cm, and the soil passed through a series of sieves of 2, 0.25 and 0.053-mm aperture while subjected to horizontal shaking for 4 min at 250 rotations min^{-1}. Three randomly selected aggregates, retained between the 2 and 0.25-mm sieve per core at each depth, were scanned using a Phoneix Nanotom (GE Measurement and Control Solution), where voxel resolution was 1.51 μm, with a potential energy set at 90 kV and a current of 65 μA. A total of 1,440 projection images were collected with a total scan time per sample of 69 min. A longer scan time was required to achieve enhanced image quality at the higher resolution.

Scanned images were reconstructed at 32-bit using Phoneix Datos x 2 reconstruction software. Due to the irregularity of the soil surface between cores, the first 50 image slices from the top of each soil core were excluded from analysis. Scanned images were optimized to correct for any movement of the sample during the scan and noise was reduced using the beam hardening algorithm in Datos × 2, set at level 8. As a multi-scan routine was performed for the soil cores to enable a higher spatial resolution than scanning the whole column in a single scan with a larger field of view, VG StudioMax 2.2.5 was used to merge the three scans to obtain a single 3D volume for the complete core.

2.3 | Image analysis

Initial image analysis of the soil pore morphology was performed using *Image J* (Schneider, Rasband, & Eliceiri, 2012). A uniform region of interest (ROI) was defined for each soil core and aggregate; 50 × 50 × 50 mm and 0.500 × 0.650 × 0.480 mm, respectively, after scanning. Soil core ROIs were centrally positioned to limit inclusion of stones and any cracks that may have been introduced during the sampling procedure. Cubic ROIs for the aggregates were not possible because of their inconstant geometry, so the same ROI, the largest possible in all aggregates, was chosen and the coordinates of these regions were adapted for each image volume/sequence. Once ROIs had been determined, several stages of image processing occurred: (a) cropping image volume to the ROI, (b) enhancing the contrast/brightness by 0.35%, (c) application of a 2-pixel median filter, (d) converting the image volume to 8-bit format, and (e) saving the new image volume. Pore morphology within the soil cores and individual aggregates was analysed using the bin bi-threshold approach by Vogel and Kretzschmar (1996), using the open source software Quantlm (http://www.

quantim/ufz.de/). Measurements were taken for detectable porosity (referred to as total porosity), pore size distribution and pore connectivity. For a more detailed methodology please refer to Bacq-Labreuil et al. (2018).

2.4 | Dry aggregate size distribution

Subsamples of 100 g of field moist soils were sieved with an 8-mm sieve, air dried and stored at room temperature. The samples were placed on top of a nest of sieves, comprising 4, 2, 0.250 and 0.053 mm. Aggregates were slowly separated by horizontal oscillations (150 rounds per min) for 30 s. The aggregates remaining on each sieve were weighed and the dry mean weight diameter (dMWD) calculated:

$$dMWD = \sum_{i=l}^{n} xi\, wi,$$

where *wi* is the weight percentage of each aggregate size class with respect to the total sample and *xi* is the mean diameter of each aggregate size class (van Bavel, 1950).

The proportion of microaggregate (0.053–0.250 mm) weight within macroaggregates (0.250–2 mm) was calculated following Six, Elliott, and Paustian (2000). In brief, macroaggregates (10 g) were immersed in deionised water on top of a 0.25-mm screen and gently shaken with 50 glass beads (dia. = 3 mm). Continuous and steady water flow through the mesh ensured that microaggregates were immediately flushed onto a 0.053-mm sieve. Subsamples of the sieved aggregates and the microaggregates within macroaggregates (50 mg) were used for Rock-Eval pyrolysis.

2.5 | Rock-Eval 6 pyrolysis

Rock-Eval pyrolysis is a technique used to trace changes in bulk organic matter composition and degree of decomposition (Disnar, Guillet, Keravis, Di-Giovanni, & Sebag, 2003; Newell, Vane, Sorensen, Moss-Hayes, & Gooddy, 2016). It predicts soil carbon contents reliably and is an appropriate tool for assessing the vulnerability of SOC stocks to microbial degradation (Saenger et al., 2013; Soucémarianadin et al., 2018; Upton, Vane, Girkin, Turner, & Sjögersten, 2018). Surface (0–20 cm) and subsurface (20–50 cm) soil samples were analysed using a Rock-Eval 6 analyser. Freeze-dried powdered soil samples (60 mg) were heated at 300°C for 3 min before an increase in temperature to 650°C at a rate of 25°C min^{-1} in an inert N_2 atmosphere. Residual carbon was subsequently oxidized from 300 to 850°C at a rate of 20°C min^{-1}. The release of hydrocarbons during the two-stage pyrolysis process was detected by a flame ionisation detector, with an infrared cell detecting the release of CO and CO_2 during the thermal cracking of the organic matter. Rock-Eval analysis generated a range of standard parameters, including:

- Total organic carbon (TOC_{RE}), calculated from the sum of the carbon moieties (HC, CO and CO_2).
- The hydrogen index (HI mg HC g^{-1} TOC), a measure of hydrocarbons released relative to TOC, was calculated from S2 × 100/TOC_{RE}.
- The oxygen index (OI mg O_2 g^{-1} TOC), corresponding to the amount of oxygen released as CO and CO_2 relative to $TOC_{RE,}$ was calculated from S3 × 100/TOC_{RE}.
- S2 (mg g^{-1}), free hydrocarbons released on the thermal cracking of organic matter for temperatures up to 850°C.
- S3′ (mg g^{-1}), CO and CO_2 derived from oxygen-containing moieties, generally dominated by carbohydrates and lignins.

2.6 | Estimating carbon stocks

Total carbon of the bulk soil was determined from 20 mg of oven-dried, ball-milled soil combusted using a total element analyser (Flash EA 1112, CE Instruments, Wigan, UK). Soil carbon stocks were estimated by an equivalent soil mass (ESM) procedure using a cubic spline function to calculate stocks in multiple soil layers (Mg C ha^{-1}) within a defined area using several calculations, which can be shown in Wendt and Hauser (2013). This method quantifies and corrects for the fixed depth error associated with calculating carbon stocks as the product of soil bulk density, depth and concentration.

2.7 | Statistical analysis

All statistical analysis was performed using the R software (R version 3.4.1). If the residuals from fitted models did not appear consistent with a normal distribution, and showed any evidence of heteroscedasticity (with their variance increasing with the fitted value) then a Box-Cox transformation was applied, using the BOXCOX procedure in the MASS package for the R platform (Venables & Ripley, 2002). A linear mixed model was fitted on all primary variables, with management as a fixed effect. Depth was also considered as a fixed effect for those variables where properties were measured for different depth increments within sample cores. Field and pair were random effects in all models. For those variables where measurements were made on different depth increments within a core, then core was also a random effect. The random effects were nested: (a) between-core (within field), which groups together observations within the same core, (b) between-field within pair, which groups together observations in the same field (and so acknowledges that these are not independent randomizations of the treatments, and (c) between-pairs, which reflects the paired fields in each zone. Linear mixed models were fitted using the nlme package for the R platform (Pinheiro et al., 2014). The table in Appendix S1 shows the outline analysis of variance tables for the different variables with the partition of degrees of freedom.

This study examines soil properties in paired fields from long-established trials. A limitation to this is the length of time for which the zero-tilled paired field has been managed, which differs markedly between the pairs and is not a replicated factor. The observations are therefore pseudo-replicated with respect to time, and effects of time contribute to the between-pair random effect. We therefore cannot make inferences about effect of length of time under zero tillage because this is confounded with other factors that may differ between the pairs. As time might have substantial effects, which might interact with the management and depth differences, this could result in heteroscedasticity. As noted above, evidence for heteroscedasticity was examined in the residuals of the fitted model, and data transformed as necessary.

3 | RESULTS

3.1 | Effect of management on soil pore structure

3.1.1 | 3D image assessment at core scale

There were several differences in soil pore characteristics between zero-tilled and conventionally tilled fields (Figure 2). At the pore size class 0.19 mm there was a significant difference in pore size distribution between zero-tilled and conventionally tilled soils, whereby 20% of the total porosity in conventionally tilled soils was accounted for by pore size 0.19 mm compared to 14% of total porosity in zero-tilled soils ($F_{1,2} = 25.0$, $p = 0.038$, Figure 2a).

There was a significant difference in pore connectivity between zero-tilled and conventionally tilled managements at 0.06 and 0.11 mm diameter ($F_{1,2} = 113.7$, $p = 0.009$ and $F_{1,2} = 121.5$, $p = 0.008$, respectively; Figure 2b). Soils that had been in zero-tillage for 15 and 31 years had a higher degree of connected pores compared with soils in the paired conventionally tilled fields. There was no significant difference in total porosity between zero-tilled and conventionally-tilled fields ($F_{1,2} = 0.41$, $p = 0.587$). However, it is notable in Figure 2c that the mean porosity in the conventionally tilled field is markedly larger than that in the zero-tilled field for the pair with zero-tillage for 2 years. There is little difference between the treatments with respect to porosity for the pair with 15 years under zero-till, and porosity is notably larger in the field under zero-tillage for 31 years than in its pair under conventional tillage. This is suggestive of an interaction between management and time in the effect on soil porosity, which we are unable to examine formally here because of the lack of true replication of treatments composed of a factorial combination of these two factors.

3.1.2 | Soil aggregate properties

There was no significant difference between the managements with respect to mean weight diameter (MWD) ($F_{1,2} = 9.52$, $p = 0.091$). However, there is evidence for a difference in MWD between the depth increments ($F_{4,136} = 19.71$, $p < 0.0001$). Furthermore, there was significant change in MWD with depth differences between the managements ($F_{4,136} = 76.18$, $p < 0.0001$). Figure 3a shows that under zero-tillage MWD is generally larger near the surface than at depth, whereas the converse is true under conventional tillage.

There was a significant difference in mM (the proportion of microaggregates within macroaggregates) between zero and conventionally tilled managements ($F_{1,2} = 28.0$, $p = 0.034$; Figure 3b). The proportion of mM was largest at the sites that had been under zero-tillage for 15 and 31 years compared with their adjacent conventionally tilled soils.

3.1.3 | 3D image assessment at aggregate scale

Porosity in macroaggregates significantly differed between the two managements ($F_{1,2} = 20.8$, $p = 0.045$; Figure 4a,b), with conventionally tilled macroaggregates having an average total porosity of 10.1% compared to 4.0% in zero-tilled macroaggregates. The porosity in macroaggregates decreased from 6 to 4 to 2% in macroaggregates that had been in zero-tillage for 2, 15 and 31 years, respectively.

These was no evidence for a difference in pore size distribution and cumulative pore size distribution between zero-tilled and conventionally tilled fields. Further, it is notable in Figure 4c that soil aggregates under zero-tillage for 15 and 31 years of treatment had a larger

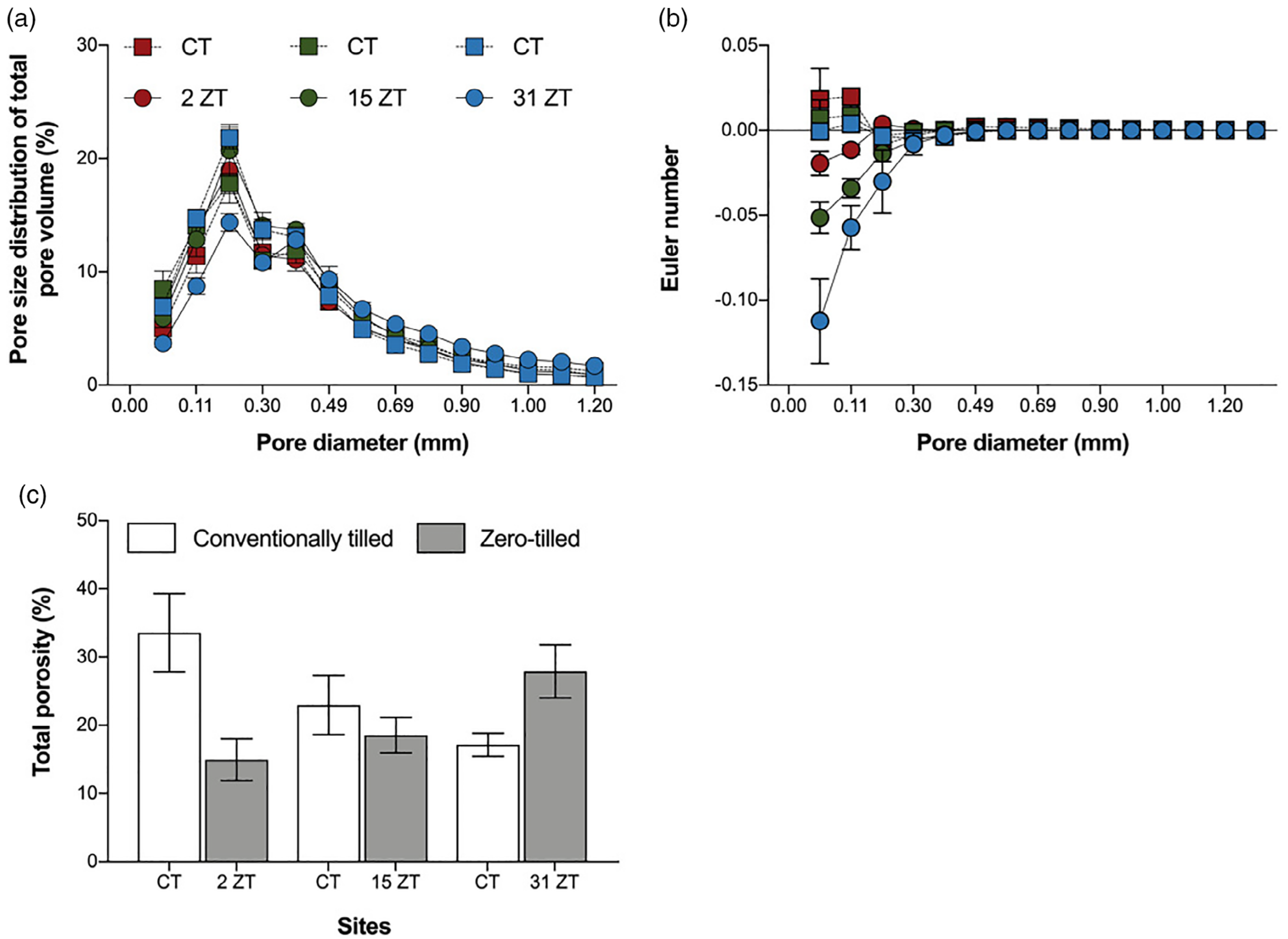

FIGURE 2 Soil pore characteristics of soils under different tillage systems at the core scale (50 μm resolution). (a) Pore size distribution of total pore volume, (b) the Euler number and (c) total porosity of each management. Means ± 1 standard error of the mean (*SEM*) ($n = 6$). CT is conventional tillage and ZT is zero-tillage. The red data points refer to fields 5 and 6, the green correspond to fields 1 and 2 and the blue data points refer to fields 3 and 4 as outlined in Table 1

percentage of the total porosity accounted for by smaller pores (<5.97 μm) compared with conventionally tilled soils, where a larger percentage of total porosity was accounted for by larger pores (>11.83 μm).

3.2 | Effect of management on soil carbon storage

3.2.1 | Land-use effects on aggregate-associated carbon

When we considered the cumulative soil carbon stock over all three depth increments, there was a significant difference between the treatments ($F_{1,2} = 20.67$, $p = 0.045$). Analysis of the components of the stock by depth increment showed a significant effect of depth ($F_{2,68} = 254.00$, $p < 0.0001$) and a significant interaction of treatment with depth, with the distribution of the soil carbon stock within the profile differing between the treatments ($F_{2,68} = 4.66$, $p = 0.013$).

Between the three aggregate size classes, there was no significant difference in TOC %. However, it is notable in Figure 5a, that analysis of the components of TOC % by depth showed a significant effect of depth ($F_{1,16} = 121.10$, $p < 0.0001$) and a significant interaction of treatment with depth; the concentration of TOC % within the macroaggregates differs between the treatments ($F_{1,16} = 36.07$, $p = <0.0001$). In addition, in both microaggregates and microaggregates within macroaggregates there was a significant effect of depth ($F_{1,16} = 139.79$, $p = <0.0001$ and $F_{1,16} = 64.28$, $p = <0.0001$).

In surface aggregates (0–10 cm), the largest increase in TOC % was in 31-year zero-tilled soils, where TOC increased from 1.4 to 2.2% in the macroaggregates (Figure 5a), from 1.4 to 2.1% in the microaggregates (Figure 5b)

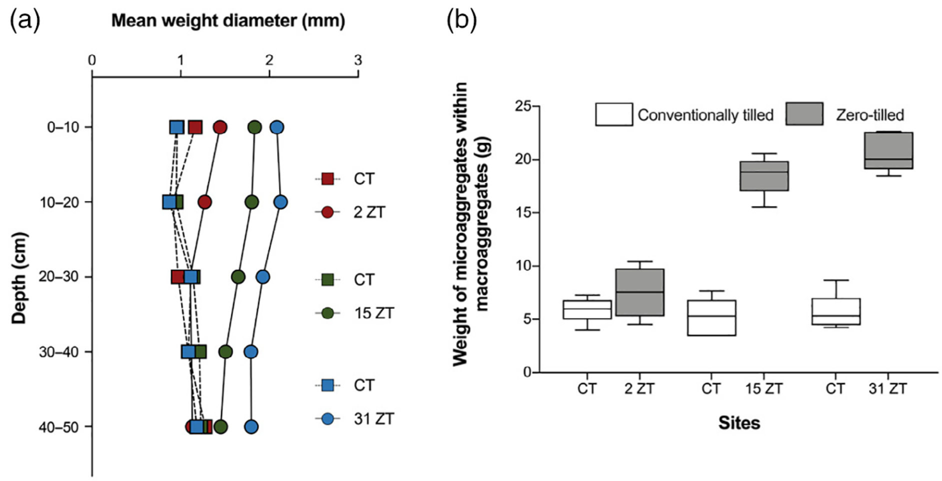

FIGURE 3 Soil aggregate characteristics under conventional tillage and zero-tillage. (a) Mean weight diameter throughout the soil profile to a depth of 50 cm (standard errors of the mean (*SEM*s) ranged from 0.007 to 0.05 mm) and (b) the weight of microaggregates (53–250 μm) within macroaggregates (250–2,000 μm) to a depth of 50 cm. Means ± 1 *SEM* ($n = 6$). CT is conventional tillage and ZT is zero-tillage. The red data points refer to fields 5 and 6, the green correspond to fields 1 and 2 and the blue data points refer to fields 3 and 4 as outlined in Table 1

and from 1.5 to 2.0% in the microaggregates formed within macroaggregates (Figure 5c).

4 | INFLUENCE OF TILLAGE METHODS ON THERMOSTABILITY OF SOIL ORGANIC CARBON

When we considered the S2 values within the macroaggregates across tillage treatments, there was a significant difference ($F_{1,2} = 19.35$, $p = 0.0480$). Similarly, the S3 values significantly differed between tillage practices in microaggregates ($F_{1,2} = 299.00$, $p = 0.0033$).

5 | DISCUSSION

5.1 | Influence of tillage management on soil structural properties

The absence of mechanical disturbance to invert topsoil increased soil surface bulk density and reduced soil porosity (Figure 2c) when soils had been under zero-tillage for 2 years. Matula (2003) and Romaneckas, Romaneckiene, Šarauskis, Pilipavicius, and Sakalauskas (2009) also observed that a reduced soil porosity decreased water infiltration rates and noted a significantly lower root mass measured compared with conventionally tilled soils. The surface soil condition under zero-tillage can impede root development and the growth of the main root axes (Martínez, Fuentes, Silva, Valle, & Acevedo, 2008).

However, in line with our first hypothesis, that "minimized mechanical disturbance associated with zero-tillage will regenerate the soil pore architecture through the development of continuous pores", we show soil structure under long-term zero-tillage (15 and 31 years) improved considerably, with a significant increase in soil porosity and pore connectivity (Figure 2b,c). Galdos et al. (2019) also found that soil under zero-tillage for 30 years had larger connected pores than neighbouring conventionally tilled soils in Brazil. The absence of soil inversion and disturbance in zero-tilled systems increases bioturbation, in which soil organisms, including microbes, rooting plants and burrowing animals, alter the soil structure and contribute to the development of a system of continuous pores (Dignac et al., 2017; Piron et al., 2017).

A soil's porosity, which influences the water-holding capacity, gas exchange and microbial activity, is usually greater under conventional tillage compared to soil recently converted to zero-tillage (Skaalsveen, Ingram, & Clarke, 2019). However, most similar studies generally consider only the topsoil, and do not include measurements below the plough layer (c. 25–30 cm). Our study considers the differences in soil structural properties down to 50 cm. Indeed, in recently converted zero-tilled soils (2 years), the total porosity decreased by 7% in the top 50 cm, but after 31 years the total porosity was 13% greater than in the paired conventionally tilled soils (Figure 2c). It has been shown (Hangen, Buczko, Bens, Brunotte, & Hüttl, 2002; He et al., 2009) that long-term zero-tilled soils can have much deeper percolation, due to more favourable conditions for burrowing soil

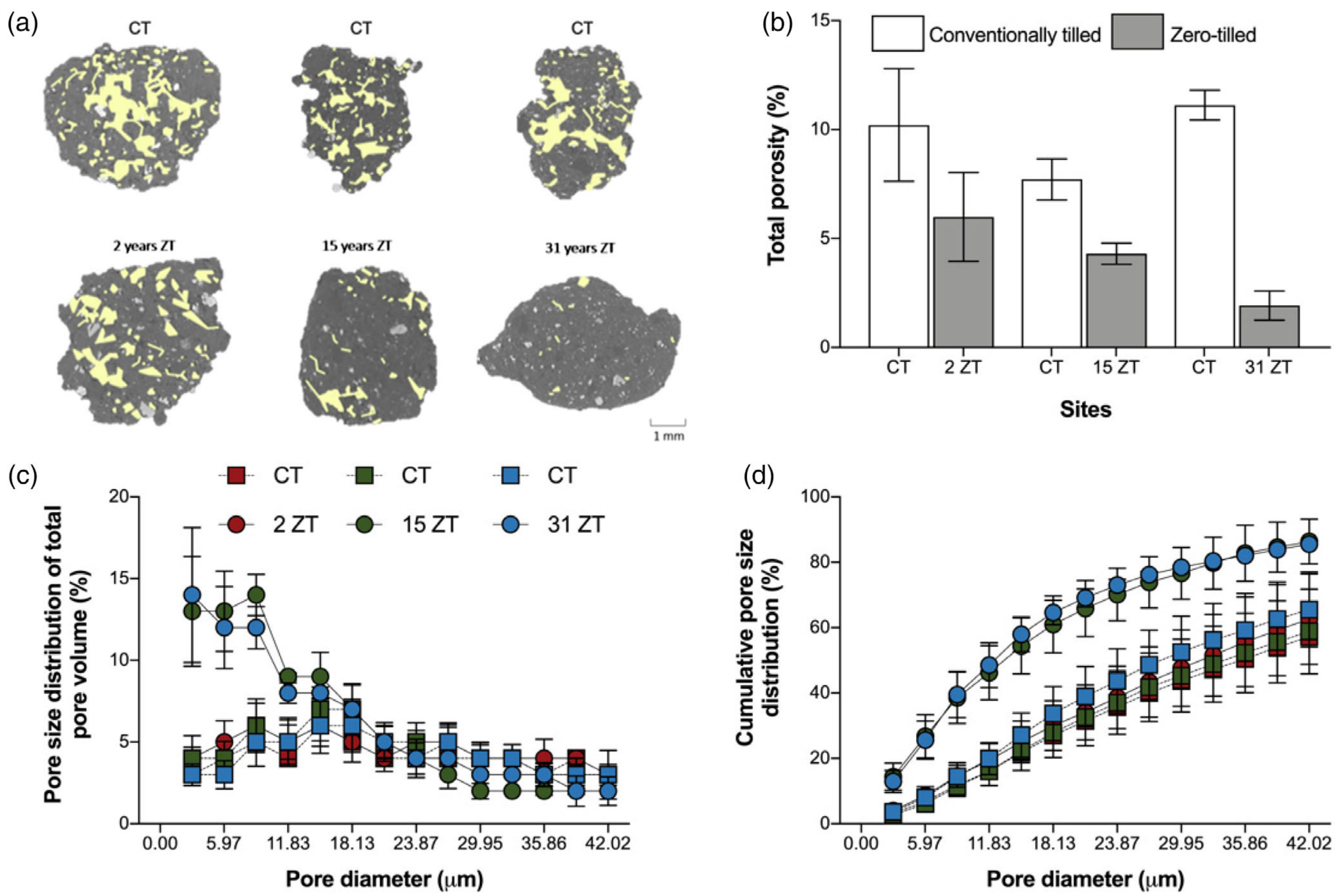

FIGURE 4 Soil pore characteristics of soils under different tillage systems in macroaggregates at 1.5-μm resolution in the top 20 cm. (a) Representative microtomographic images for soil macroaggregates under different tillage systems, where yellow is identified as the pore space, (b) total porosity, (c) pore size distribution of total pore volume and (d) cumulative pore size distribution. Means ± 1 standard error of the mean (*SEM*) (***n*** = 3). CT is conventional tillage and ZT is zero-tillage. The red data points refer to fields 5 and 6, the green correspond to fields 1 and 2 and the blue data points refer to fields 3 and 4 as outlined in Table 1

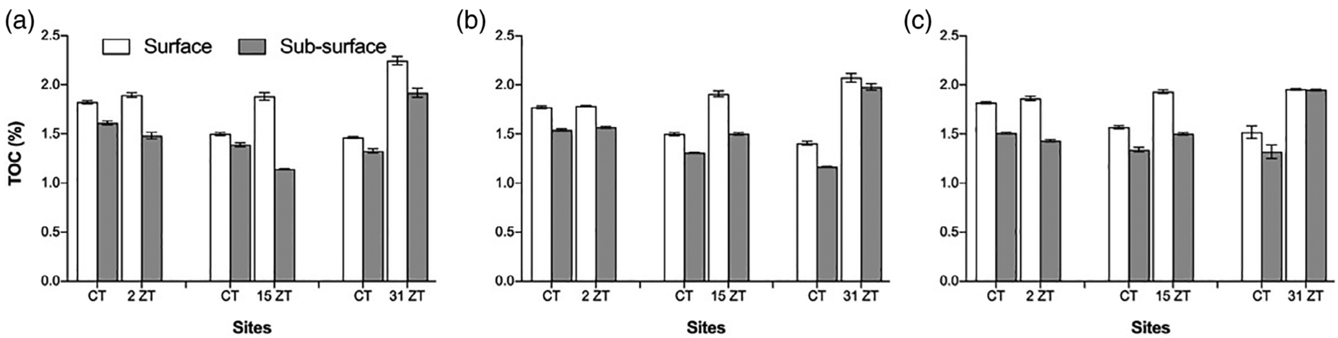

FIGURE 5 Aggregate-associated organic carbon under different tillage systems in (a) macroaggregates, (b) microaggregates and (c) microaggregates within macroaggregates. Results are shown for the soil surface (0–10 cm) and sub-surface (40–50 cm). Means ± 1 standard error of the mean (*SEM*) (*n* = 3). CT is conventional tillage (fields 5, 1 and 3) and 2 ZT, 15 ZT and 31 ZT represent 2, 15 and 31 years in zero-tillage, respectively

animals, providing deep vertical macropores and increasing the soil porosity. Our results demonstrate that the soil structure regenerates under long-term zero-tillage and increases the connectivity of pores, which will impact root growth, water dynamics and soil gas fluxes.

5.1.1 | Effect of tillage management on soil aggregate formation and structure

Changes in the soil physical characteristics associated with the adoption of zero-tillage generally resulted in improved aggregate formation, especially when implemented for at least 15 years across the soils we examined. Figure 3a shows the effect of different tillage systems on aggregate stability down to 50 cm and supports our second hypothesis, that "zero-tillage will result in a greater number of stable macroaggregates, which will increase in long-term zero-tilled soils". In general, aggregates are considered "stable" if the mean weight diameter is >1.3 mm and stable aggregates play a central role in protecting pools of carbon and nitrogen, providing microhabitats for microorganisms, and are important in ensuring the soil is resilient against wind and water erosion (Blaud, Menon, van der Zaan, Lair, & Banwart, 2017). Our results indicate that long-term zero-tillage improved aggregate stability in the top 50 cm compared with conventionally tilled soils (Figure 3). It is likely that this is through a reduction in soil disturbance as a result of discontinuing mechanical inversion and an increase in surface crop residue cover enhances the organic binding agents, preventing soil erosion. In this study, it was observed in the conventionally tilled soils that the aggregates were considered either "unstable" or "median/medium" stability. The exposure of the soil surface in conventionally tilled soils results in aggregates breaking apart due to continuous drying and wetting cycles (Devine, Markewitz, Hendrix, & Coleman, 2014; Kou et al., 2012). However, the incorporation of crop residue into conventionally tilled soils also promotes aggregate stability (Zhang et al., 2012).

In contrast to results from the core scale, whereby porosity increased with time under zero-tillage (Figure 2c), porosity decreased at the macroaggregate scale with time under zero-tillage (Figure 4a,b). Very few studies have assessed the impact of zero-tillage at the aggregate scale, focusing primarily on the larger soil "core" scale. A significant reduction in aggregate porosity would provide a crucial mechanism for soil macroaggregates to provide physical protection of organic matter by minimizing microorganism mineralization. On the other hand, the encapsulated organic carbon within these aggregates may slow any additional decomposition, primarily due to lack of microbial penetration into aggregates (Lehmann & Kleber, 2015). Fungi occur more abundantly in association with macroaggregates and differences in the microbial community compositions have not only been reported for different aggregate size fractions, but also for the aggregate interior versus exterior. This demonstrates that different-size aggregates and their opposing associated pore structures, nutrient availabilities and chemical conditions can favour some microbial groups over others (Kihara et al., 2012). In addition, zero-tillage is coupled with a reduction in macroaggregate turnover, thereby increasing the microbial abundance in large macroaggregates and the associated organic carbon (Six et al., 2000). Furthermore, zero-tillage can improve microbial growth by stabilizing soil temperature and moisture, thereby supplying environmental conditions that promote both the abundance and diversity of fungi and bacteria and a favourable fungal:bacterial biomass ratio (Wagg et al., 2018).

5.1.2 | Effect of tillage management on aggregate-associated soil organic carbon

Aggregates from zero-tilled soils had a larger TOC content compared with conventionally tilled aggregates (Figure 5), supporting our fourth hypothesis, that "zero-tilled aggregates contain more organic carbon than conventionally tilled aggregates". During conventional tillage, the disruptive shearing breaks down soil aggregates (of any size class), and the organic material that was held within them is mineralized by microbial organisms, eventually decreasing the organic carbon content of the soil. In addition, the smaller carbon content in conventionally tilled soil can be partially explained by the reduced probability that the carbon of plant residues will transform into organic matter. In these systems, the carbon balance (input vs. output) does not always support carbon sequestration (Haddaway et al., 2017).

We observed that the larger carbon content within zero-tilled aggregates translated into a greater total carbon content throughout the soil profile (0–50 cm) (Figure 5a). Beare, Hendrix, Cabrera, and Coleman (2010) and Six, Conant, Paul, and Paustian (2002) also found higher carbon concentrations in surface samples from zero-tilled soils than from conventionally tilled soils. However, they found no significant differences in carbon content between zero and conventional tillage in deeper soil layers (>20 cm). The large increase in carbon stocks in superficial layers in the zero-tilled soils can be explained by minimal soil disturbance, which allows greater protection of carbon from microbial attack in the aggregates, reducing the organic matter mineralization rate (Franzluebbers, 2010; Lal, 2015; Ruis & Blanco-Canqui, 2017). Previous research has proposed an even distribution of carbon throughout the soil profile in conventionally tilled soils, compared to the larger carbon concentration found only in surface soils in zero-tillage due to deposition of crop residues, with carbon content decreasing with depth (Powlson et al., 2014). In agreement with our results (Figure 6a–c), Sisti et al. (2004) measured greater carbon content in zero-tilled soils that had been under this management for the greatest length of time, showing long-term zero-tillage can significantly increase the organic carbon content of soil, an effect that was significantly greater in zero-tilled than in conventionally tilled soils across 0–100-cm depths. An increase in organic carbon throughout the soil profile in zero-tilled soils has previously been attributed to the increase in SOC in stable macroaggregates (Song et al., 2016), and is partly due to bioturbation, specifically via the surface deposition of soil by anecic earthworms, which are known to move material to lower soil horizons over time (Blouin et al., 2013). In addition, with incorporation of soil tillage and plant residues, the decomposition of fresh organic material is favoured by soil aeration, which increases the oxidation of labile organic matter (Reicosky, 1995). Differences in carbon stocks at greater depths can be explained by better root growth conditions under zero-tillage (Galdos et al., 2019). Sisti et al. (2004) showed that increased carbon accumulation in zero-tillage soil below 30 cm depth could be explained by greater root density when compared with conventional tillage. Bodey et al. (2010) showed a strong correlation between carbon accumulation at 0–30 cm and 0–100 cm in ferralsols. Sá et al. (2014) compared the carbon stocks in the soil profile in long-term tillage systems in a Brazilian Oxisol and observed that in the 20–40-cm layer the carbon stocks in zero-tillage were 15 Mg ha^{-1} higher than those in conventional tillage.

It has been proposed that rates of soil carbon sequestration reduce as the soil carbon stock approaches a new steady state (i.e., when soil carbon inputs approximate soil carbon outputs) and the soil carbon sink is saturated (Paustian, Collins, & Paul, 1997; West & Six, 2007). The proposed time period necessary for SOC to attain a steady state varies between studies, ranging from 10 years (Qin, Dunn, Kwon, Mueller, & Wander, 2016) to 100 years (Sauerbeck, 2001), depending on climate and soil type. In our study, the data suggest that a steady state was reached after 15 years under zero-tillage (Figure 6).

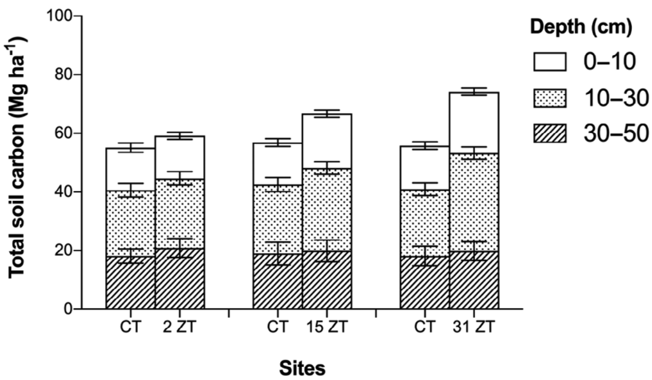

FIGURE 6 Cumulative carbon stocks and carbon content with depth in conventional and zero-tilled soils to a depth of 50 cm, calculated on an equivalent soil mass basis. CT and 2 ZT are fields 5 and 6, CT and 15 ZT are fields 1 and 2, and CT and 31 ZT correspond to fields 3 and 4. CT is conventional tillage and ZT is zero-tillage; TOC, total organic carbon

Highlighting the importance of long-term management strategies, Six and Paustian (2014) postulated that the proportion of microaggregates formed within macroaggregates (mM) can serve as a robust indicator for changes in SOC under different management practices over a decadal time scale. Significantly, greater SOC in the mM fraction of zero-tilled soils has been identified in numerous experiments across varying environmental contexts, signifying that a considerable proportion of the difference between zero and conventional tillage could be driven by carbon associated with the mM fraction (Arshad et al., 1990). Our results confirm our third hypothesis, that "there will be a greater fraction of microaggregates formed within macroaggregates in zero-tilled soils, as the rate of macroaggregate formation and degradation is reduced under zero-tillage". The majority of macroaggregates in conventionally tilled soils are destroyed upon mechanical disturbance; therefore, any formation of microaggregates within macroaggregates is also destroyed. The increased amount of soil organic carbon in the mM fraction in long-term zero-tilled soils, in addition to the increase in mean weight diameter, indicates stronger potential for soil aggregation and accumulation of SOC under zero-tillage compared with conventional tillage.

5.1.3 | Impact of tillage methods on thermal stability of soil organic carbon

It has been speculated that the additional carbon found in zero-tilled soils is predominantly in labile forms (plant residues and particulate organic carbon) that could be decomposed if zero-tillage practices ceased and the soil reverted back to conventional tillage (Powlson et al., 2014). Our results confirm this is the case for the soil sampled from the surface after zero-tillage for 2 years. Soils under zero-tillage are generally cooler and wetter compared to nearby conventionally tilled soils and receive less mechanical disturbance, which leads to slower macroaggregate turnover rates and more labile soil organic matter in macroaggregates (Salem et al., 2015). On the other hand, it may also be expected that the retention of crop residues provides more favourable soil moisture conditions for SOC decomposition to occur (Corbeels et al., 2016). Broadly in line with our fourth hypothesis, "zero-tilled aggregates will contain more organic carbon than conventionally tilled aggregates, with an increase in the 'labile' proportion, as characterized by its thermostability, due to a reduction in aggregate porosity therefore limiting

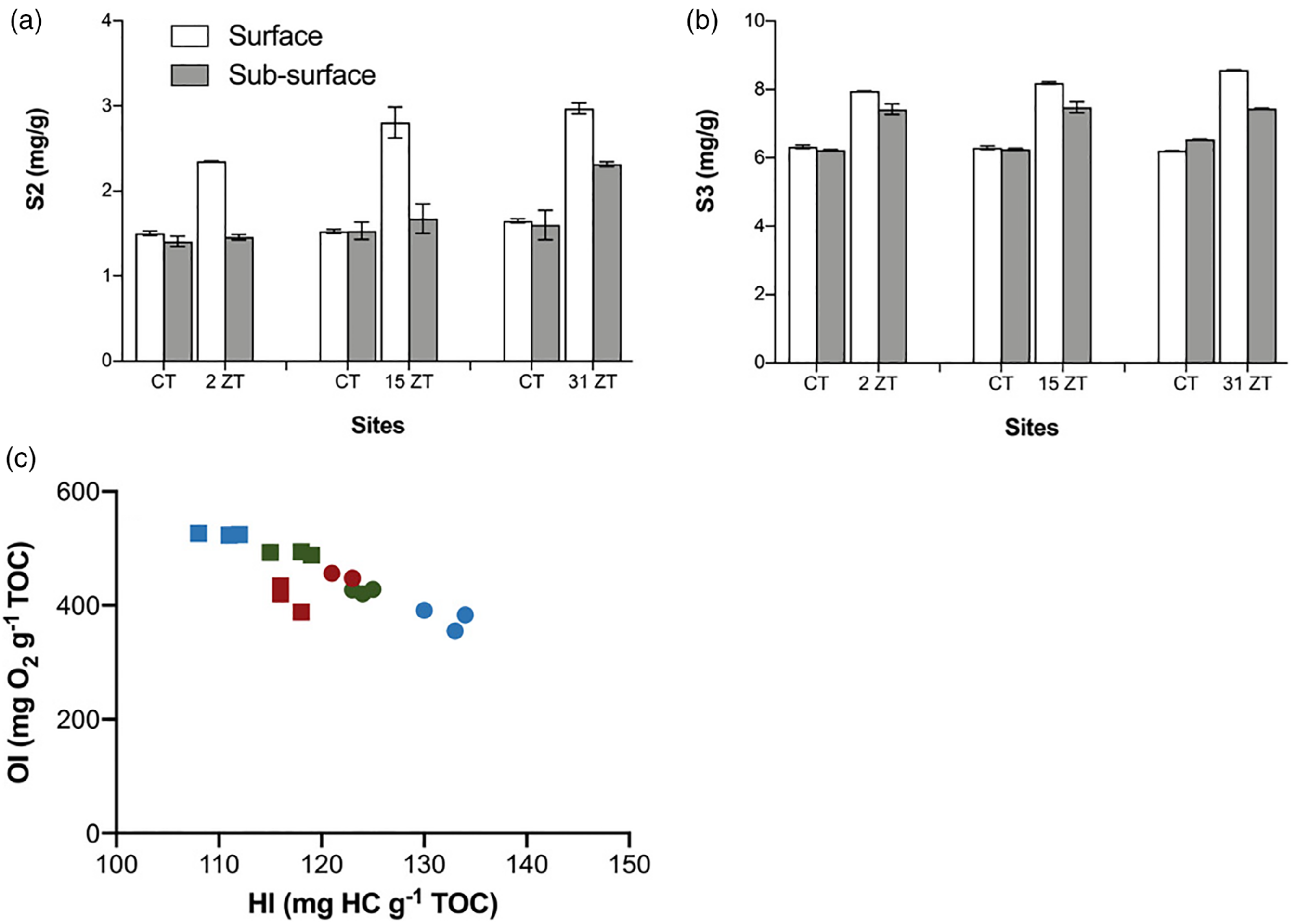

FIGURE 7 Selected Rock-Eval 6 parameters across different tillage practices in surface and sub-surface soils. Where (a) shows the S2 values (free hydrocarbons released on the thermal cracking of organic matter for temperature up to 850°C) and (b) shows the S3 values (CO and CO_2 derived from oxygen-containing moieties, generally dominated by carbohydrates and lignins). (c) HI versus OI plot, which indicates the level of humification within the macroaggregates across the tillage practices. CT and 2 ZT are fields 5 and 6, CT and 15 ZT are fields 1 and 2 and, CT and 31 ZT correspond to fields 3 and 4. CT is conventional tillage and ZT is zero-tillage

microbial access," soils that had been managed under zero-tillage for 15 and 31 years were characterized by a progressive increase in S2 in surface and subsurface soil macroaggregates (Fig. 7a). Increasing time under zero-tillage was associated with higher HI and lower OI, suggesting that surface soils were characterized by a progressive increase in the proportion of labile carbon (Fig. 7c).

Recently added organic matter is generally incorporated into macroaggregates, with subsequent slow movement of carbon into macroaggregates at 40–50 cm, driven by a combination of root dynamics and earthworm activity (Fonte et al., 2007). Macroaggregates are more susceptible to disruption by cultivation and environmental perturbations (e.g., wet–dry cycles) than are microaggregates (Tisdall & Oades, 1982). For example, organic matter lost from cultivated grasslands is largely from macroaggregates (Elliott, 1986). S3 also progressively increased with time under zero-tillage in microaggregates (Fig. 7b). This material generally has a lower C:N ratio and is rich in humic materials and compounds resistant to microbial degradation (Tisdall & Oades, 1982; Turchenek & Oades, 1979; Oades, 1988). The chemical composition of this type of carbon is more resistant to decomposition by microorganisms, and tends to turn over more slowly, from decades to centuries. In addition, the lower proportion of labile carbon in conventionally tilled systems is likely to occur due to the reduced deposition of crop residues, the greater microbial accessibility and the aeration of the soil. In zero-tilled soils, as the residues are left on the soil surface they are less susceptible to microbial attack (Wang et al., 2020).

Overall, these findings demonstrate both a progressive increase in total carbon under zero-tillage, including increases in labile carbon, and the gradual incorporation of more recalcitrant carbon in microaggregates.

6 | CONCLUSIONS

After a period of 31 years, our measurements suggest zero-tilled management improved the soil structure through an increase in porosity and pore connectivity (as revealed by X-ray CT imagery) and increased the stability of soil aggregates. In addition, zero-tillage increased the soil organic carbon content in all aggregate size classes in comparison to conventionally tilled soils. The increased soil disturbance by conventional tillage accelerated the soil organic carbon decomposition by altering physical protection and/or mineralizing the labile forms of carbon in soil aggregates. Furthermore, long-term zero-tillage (15 and 31 years) further increased carbon stocks and altered the chemical composition of the organic carbon stored, which reduced the susceptibility of mineralization to any disturbance. Our results demonstrate the potential of zero-tillage to increase carbon sequestration when practised in the longer term compared to paired conventionally tilled soils. However, additional studies, taking into account soils that have been in zero-tillage for at least 10 years and with an increased number of spatial observations, are urgently required for the potential of zero-tillage to underpin any future policy recommendations.

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AUTHOR CONTRIBUTIONS

Hannah Cooper: Conceptualization; data curation; formal analysis; investigation; methodology; writing-original draft; writing-review & editing. **Sofie Sjogersten:** Conceptualization; investigation; methodology; supervision; writing-review & editing. **Richard Lark:** Formal analysis; methodology; writing-review & editing. **Nicholas Girkin:** Formal analysis; investigation; methodology; writing-original draft; writing-review & editing. **Christopher Vane:** Formal analysis; writing-review & editing. **Juliano Calonego:** Data curation; writing-review & editing. **Ciro Rosolem:** Data curation; writing-review & editing. **Sacha Mooney:** Formal analysis; funding acquisition; methodology; supervision; writing-review & editing.

CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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