Qualitative and quantitative response of soil organic carbon to 40 years of crop residue incorporation under contrasting nitrogen fertilisation regimes

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Abstract. Crop residue incorporation (RI) is recommended to increase soil organic carbon (SOC) stocks. However, the positive effect on SOC is often reported to be relatively low and alternative use of crop residues, e.g. as a bioenergy source, may be more climate smart. In this context, it is important to understand: (i) the response of SOC stocks to long-term crop residue incorporation; and (ii) the qualitative SOC change, in order to judge the sustainability of this measure. We investigated the effect of 40 years of RI combined with five different nitrogen (N) fertilisation levels on SOC stocks and five SOC fractions differing in turnover times on a clay loam soil in Padua, Italy. The average increase in SOC stock in the 0–30 cm soil layer was 3.1 Mg ha^{-1} or 6.8%, with no difference between N fertilisation rates. Retention coefficients of residues did not exceed 4% and decreased significantly with increasing N rate ($R^2 = 0.49$). The effect of RI was higher after 20 years (4.6 Mg ha^{-1}) than after 40 years, indicating that a new equilibrium has been reached and no further gains in SOC can be expected. Most (92%) of the total SOC was stored in the silt and clay fraction and 93% of the accumulated carbon was also found in this fraction, showing the importance of fine mineral particles for SOC storage, stabilisation and sequestration in arable soils. No change was detected in more labile fractions, indicating complete turnover of the annual residue-derived C in these fractions under a warm humid climate and in a highly base-saturated soil. The applied fractionation was thus useful to elucidate drivers and mechanisms of SOC formation and stabilisation. We conclude that residue incorporation is not a significant management practice affecting soil C storage in warm temperate climatic regions.

Additional keywords: carbon sequestration, soil fractionation, straw incorporation.

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Introduction

The baseline soil organic carbon (SOC) stocks in cultivated mineral soils are often low compared with those in soils under grassland or forest (Freibauer *et al.* 2004). Increasing the SOC level in cultivated soils is important for soil fertility and ecosystem resilience under a changing climate (Lorenz and Lal 2015). Moreover, the potential to sequester atmospheric carbon dioxide (CO_2) in those soils to mitigate climate change is high. Lal (2004) estimated that the carbon (C) sink capacity of agricultural and degraded soils at global scale accounts for 50–66% of historic C losses due to land use change, amounting to 42–78 Gt. The SOC stocks are determined by the balance between C inputs and outputs. The capacity of farming activities to decrease or decelerate the C loss from the soil, through decreased heterotrophic respiration, leaching and erosion, may be limited and reduced to the option of changing the tillage regime (Bernacchi *et al.* 2005). A more effective way to increase SOC stocks may be to maximise C inputs (Christopher and Lal 2007; Kätterer *et al.* 2012). Suggested

and widely applied input-related management practices that have shown to significantly affect SOC stocks include: addition of biosolids such as manure, compost and crop residues, cultivation of cover crops used as green manure, improved crop rotations with the inclusion of perennial and deep rooting crops, and optimising mineral fertilisation to obtain the highest possible net primary productivity (Lal 2004; Smith *et al.* 2005). Aboveground crop residues such as straw have frequently been found to contribute less than 10% to the SOC pool, although the variability among sites and studies is not sufficiently explained (Lehtinen *et al.* 2014). The relatively low C sequestration efficiency and the potential use of straw as a renewable energy source are generating debate about the most climate-smart and sustainable use of this resource (Poeplau *et al.* 2015*a*), of which an estimated four billion tonnes are produced annually (Chen *et al.* 2013). Despite the large number of studies worldwide examining the effect of straw incorporation or removal on soil C, little is known about: (i) the long-term (>20 years) effect of this management practice; and (ii) the

(ii) qualitative changes in SOC over time. To compare the sustainability of straw incorporation or removal, it is essential to understand the temporal dynamics of SOC accumulation. Well-documented, long-term agricultural experiments are needed to determine this, but are scarce or published only in poorly accessible national reports. In a global review on residue incorporation effects on SOC, only 33% of all experiments cited had a duration exceeding 20 years (Lehtinen *et al.* 2014).

Nitrogen (N) fertilisation typically increases net primary production (NPP) and thus the total amount of crop residues returned to the soil. Furthermore, Kirkby *et al.* (2013) showed that adding straw to the soil together with N leads to a greater increase in SOC compared with straw addition alone. This increase in the 'retention coefficient' of straw (Poeplau *et al.* 2015*a*) is explained by increased C use efficiency of microbes under balanced nutrition than under N deficiency, which can lead to N mining at the cost of labile C (Fontaine *et al.* 2004; Murphy *et al.* 2015). It is well established that microbes need nutrients for biosynthesis (Scheu 1993), so stoichiometric balance of $C:N:P:S$ is required to form soil organic matter (Kirkby *et al.* 2013). Therefore, N fertilisation can have dual positive effects on SOC.

Soil C fractionation is performed to isolate functional pools with distinct ecological properties, such as turnover time (von Lützow *et al.* 2007). Such characterisation of SOC composition is needed to initialise or validate C turnover models or to understand how changes in land use or management influence the pool distribution and therefore the stability of SOC stocks. For instance, Poeplau and Don (2013) investigated the effect of land use changes on SOC stocks and found similar absolute effects of cropland conversion to forest or grassland and no effect of grassland afforestation. However, they also found that aggregate formation was promoted in grassland soils, while soils under forest accumulated a major proportion of newly-formed SOC in the particulate organic matter (POM) fraction, which is acknowledged to be the most labile form. They concluded that grassland establishment might be a more sustainable option for SOC sequestration than afforestation. Fractionation of SOC might be helpful to predict the long-term effect of straw incorporation on SOC stocks, and to identify abundant stabilisation pathways.

In this study, we evaluated the effect of long-term (40 years) straw incorporation on SOC stocks and fractions under five different rates of N fertilisation, examining the following questions: (i) how much of the added straw was retained in the soil? (ii) how did N fertilisation influence retention of straw carbon in soil? and (iii) how did straw incorporation alter the composition of SOC (distribution in different fractions) and was this affected by N fertilisation?

Material and methods

Study site

The long-term field trial used in this study is located in north-east Italy, in the experimental farm of the University of Padua (Veneto Region, NE Italy 45°21′N, 11°58′E). The soil is classified as a Fluvi-calcaric Cambisol (FAO, http://www.fao.org/soils-portal/soil-survey/soil-classification/fao-legend/key-to-the-fao-soil-units/en/, accessed 16 August 2016), with 29% clay,

37% silt and 34% sand (clay loam). At the start of the experiment (1966), the carbonate content in the topsoil (0–30 cm) was 33%, soil pH was 7.8, bulk density was 1.44 g cm^{-3}, SOC content was 1.8% and $C:N$ ratio was 8.3. The climate is subhumid, with a mean annual precipitation of 850 mm, mean annual temperature of 12.8°C and the highest monthly average temperature in August (22.3°C) and the lowest in January (2.3°C). A more detailed description of the site and an earlier time series of SOC can be found in Lugato *et al.* (2006). The experiment was laid out in a randomised block design with four replicates. Each block had three residue treatments (RR: residues removed; RI: residues incorporated, RIC: residues incorporated and chicken manure added), which were factorially combined with five different N fertiliser rates (0, 60, 120, 180, 240 kg N ha^{-1} year^{-1}). In this paper, only RR and RI are compared. All treatments, including the 0N treatment, received 150 kg P$_2$O$_5$ and 150 kg K$_2$O each year. Between 1966 and 1983 continuous maize was grown, and from 1984 maize has been rotated with sugar beet and soybean and, more seldom, potato, tomato, sorghum and winter wheat (all grown between 1–4 times since 1984).

Soil sampling, fractionation and analysis

For this study, we used archived soils from the year 2006, 40 years after the start of the experiment, as well as measured values for 1966, 1982, 1986 and 1993 published by Lugato *et al.* (2006). Soil sampling in the experimental plots was performed after the growing season (September to October), but before ploughing. In each plot, five randomly located soil samples at some distance from the plot edges were taken with an auger of 5 cm diameter to a depth of 30 cm and mixed to one composite sample per plot. After sampling, the samples were air-dried and sieved to 2 mm and air-dried in a dark room. Crop yield and straw biomass were measured annually in each plot. A C concentration of 45% was assumed when calculating residue-derived C inputs. In the RR treatments, it was assumed that 10% of the residues were left as stubble.

SOC fractionation was performed as initially described by (Zimmermann *et al.* 2007) and refined by Poeplau *et al.* (2013). First, 30 g dry soil was mixed with 150 mL deionised water in a 250-mL beaker glass and treated with ultrasound using a total energy of 22 J mL^{-1} at an output power of 30 W. The resultant dispersion was wet-sieved over a 63-µm sieve to separate the fine silt and clay (SC) fraction from a coarse fraction (>63 µm). An aerosol pump spray was used to flush the sample with a fixed amount (2000 mL) of water. The suspension was centrifuged for 30 min at 1000*g*. An aliquot of decanted water (~200 mL) was filtered through a 0.45 µm membrane filter and analysed as one fraction for dissolved organic carbon (DOC) in a liquid analyser. The SC fraction and the coarse sieve residues were dried at 60°C and weighed. The coarse fraction (>63 µm) was further separated into a heavy sand and aggregates (SA) fraction and a light particulate organic matter (POM) fraction by density fractionation with sodium polytungstate (SPT) solution at a density of 2 g cm^{-3}. After adding SPT to the coarse soil, the sample was centrifuged for 15 min at 1000*g* and allowed to stand for several hours until the POM fraction was entirely floating on the SPT. The POM was then carefully transferred to sieve bags with a mesh size of 50 µm (Poeplau and Don 2013). The entire density fractionation step was repeated once to ensure complete

separation of the SA and POM fractions. Also, the SA fraction was transferred to sieve bags, which had the advantage that soil material could be easily washed under the deionised water tap to remove SPT and subsequently quickly dried and weighed without changing the vessel. Finally, a chemically resistant SOC (rSOC) fraction was isolated from the fine SC fraction by oxidation with 6% sodium hypochlorite (NaOCl). The NaOCl solution was first adjusted to pH 8 using hydrochloric acid (HCl) and then 50 mL of the solution was added to a 1 g subsample of the SC fraction. After 18 h of oxidation at room temperature, the sample was centrifuged (15 min, 1000g) and decanted. This procedure was repeated twice and after each oxidation and decanting step the sample was washed once with 50 mL deionised water. To prevent waterlogging in the fine-textured soil pellet, a vortex shaker was used every time liquid was added to the soil to thoroughly mix liquid and solid, ensuring complete oxidation and cleaning. The fractionation procedure thus yielded five fractions, two fast cycling (POM, DOC), one intermediate (SA), one slow cycling (SC-rSOC) and one passive (rSOC). All solid fractions and a bulk soil subsample were milled and successively analysed for total C and N by dry combustion in an elemental analyser (LECO CNS 2000, St. Joseph, Michigan, USA). Carbonate C was determined using the same instrument after pretreatment overnight at 550°C. Organic C was then determined as the difference between total C and carbonate C.

Statistics

Linear mixed effect models were used to investigate the difference between RI and RR (fixed effect) across the different N rates (random effect) (Pinheiro *et al.* 2009), as well as the difference between N rates (fixed effects) across the different residue treatments (random effects) at a significance level of $P < 0.05$. This analysis was conducted for the bulk SOC and SOC in all fractions, separately. As a post-hoc test, the general linear hypotheses multi-comparison of means function (*glht*) of the R package *multicomp* was used, with the specification 'Tukey'. To investigate the effects of RI and N fertilisation on total SOC quality, the difference in relative fraction distribution of RI and RR was tested by multivariate analysis of variance (MANOVA) with either residue treatment or N rate as the factor.

Results

Effect of residue incorporation and N fertilisation on total SOC stocks

The long-term incorporation of crop residues had a significant positive overall effect on total SOC ($P = 0.001$), while the difference was not significant for any individual N fertilisation level. After 40 years of treatment, the average SOC stock in RR was 45.7 Mg ha^{-1}, while the average SOC stock in the RI treatments was 48.8 Mg ha^{-1} (Table 1). Thus, residue incorporation increased the SOC stock by 3.1 Mg ha^{-1} or 6.8%. Average annual SOC sequestration was 78 kg ha^{-1} year^{-1}. The average difference in C input due to residue incorporation was 2.3 Mg ha^{-1} year^{-1}. Thus, on average, only 3.3% of the total residue-derived C had been stabilised in the soil.

Nitrogen fertilisation increased NPP (Fig. 1) by 27%, 43%, 48% and 51% for 60N, 120N, 180N and 240N, and the amount of residues increased accordingly. The average harvest index (HI) was 043 ± 0.02 with the unfertilised treatments (0N) having a slightly lower HI of 0.39. The difference in NPP between the unfertilised treatments and the high N rates increased only in the first decade and remained relatively constant thereafter. However, the SOC stocks in both the RR and RI treatments were not influenced by N level (Fig. 2), which did not interact with residue management. The difference in SOC stock between RI and RR at the highest N rate (240N) was 2.9 Mg ha^{-1}, while the difference at the lowest N level (0N) was 3.1 Mg ha^{-1}, despite the difference in annual C input between RI and RR for these two N extremes being 0.43 Mg ha^{-1} year^{-1}. Consequently, the retention coefficient for residue-derived C did not increase with N fertilisation, but was in fact highest (4%) in the 0N treatments (Fig. 3, $y = -0.0037x + 3.84$).

SOC sequestration due to residue incorporation lasted for 23 years (Fig. 4). The average SOC stock difference between RI and RR decreased from 4.6 Mg ha^{-1} year^{-1} in 1989 to 3.1 Mg ha^{-1} year^{-1} in 2006 (Fig. 4). However, this was not related to less residue input after the rotation change, as the average estimated difference in C input between RI and RR was 2.37 Mg ha^{-1} year^{-1} in the period 1966–85 and 2.34 Mg ha^{-1} year^{-1} in the period 1986–2006. However, the inter-annual variability in residue-derived C inputs, which is a function of NPP, increased considerably (Fig. 1). In the first

Table 1. Soil organic carbon (SOC) stocks in all SOC fractions and in bulk soil for treatments with residues removed (RR) and residues incorporated (RI), averaged over all N fertilisation rates tested ($n = 20$) with standard deviations (s.d.), the resulting absolute and relative change after residue incorporation and proportion of total SOC in each fraction for the two residue treatments in 2006

POM, particulate organic matter; DOC, dissolved organic carbon; SA, sand and stable aggregates; SC-rSOC, NaOCl-oxidisable silt and clay sized SOC; rSOC, resistant SOC; *, indicates significance at $P < 0.05$

Fraction	RR (Mg ha^{-1})	s.d.	RI (Mg ha^{-1})	s.d.	Absolute change (Mg ha^{-1})	Relative change (%)	Proportion of total SOC (%) RR	RI
Bulk	45.8	2.5	48.8	3.6	3.1	6.7*		
POM	1.1	1.1	1.1	1.3	0.0	–0.2	2.4	2.3
DOC	0.7	0.2	0.9	0.3	0.2	25.2*	1.6	1.9
SA	2.0	0.8	2.0	1.2	0.0	0.3	4.3	4.1
SC-rSOC	24.3	2.5	27.1	3.6	2.8	11.7*	53.1	55.6
rSOC	17.6	1.9	17.6	2.7	0.0	0.0	38.5	36.1
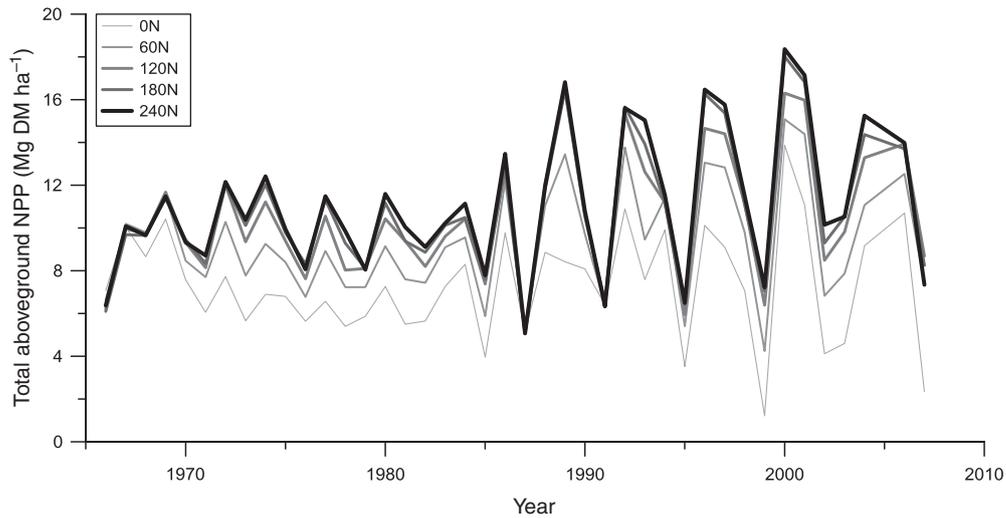
Fig. 1. Time series of total aboveground net primary production (NPP) at the five different nitrogen (N) fertilisation rates tested.
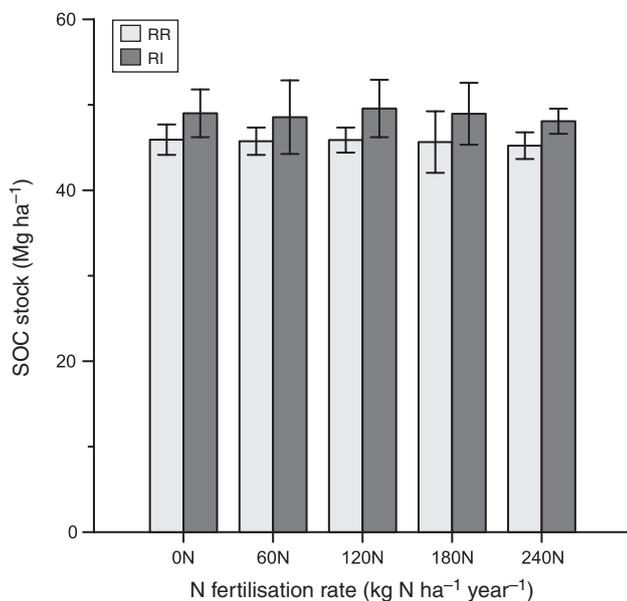
Fig. 2. Soil organic carbon (SOC) stocks in the residue removed (RR) and residue incorporated (RI) treatments under five different N fertiliser rates in 2006.

Fig. 3. Retention of residue-derived carbon (C) in the soil (%) at different nitrogen (N) fertilisation rates, with regression line and coefficient of variation (R^2).

period (1966–85) the coefficient of variance was 27%, while in the second period (1986–2006) it was 45%.

Effect of residue incorporation and N fertilisation on distribution of SOC in fractions

On average for all samples, 92% of the total SOC stock was found in the silt and clay (SC) fraction (sum of fractions SC-rSOC and rSOC) (Table 1, Fig. 5). The coarse fractions SA and POM and the liquid DOC fraction contributed only 4%, 2.3% and 1.7% respectively to the total SOC stock. Furthermore, 93% of the total surplus of SOC due to

40 years of residue incorporation was found in the SC fraction (Table 1). Within the SC fraction, the greatest change in SOC stock (significant at $P < 0.001$) occurred in the more active SC-rSOC fraction, while the more passive rSOC on average did not change at all (Table 1, Fig. 5). The only additional fraction that significantly increased due to residue incorporation was DOC (25%, $P = 0.009$). MANOVA revealed only a slightly significant change in SOC quality due to residue management ($P = 0.073$). Nitrogen fertilisation did not systematically influence the distribution of SOC in different
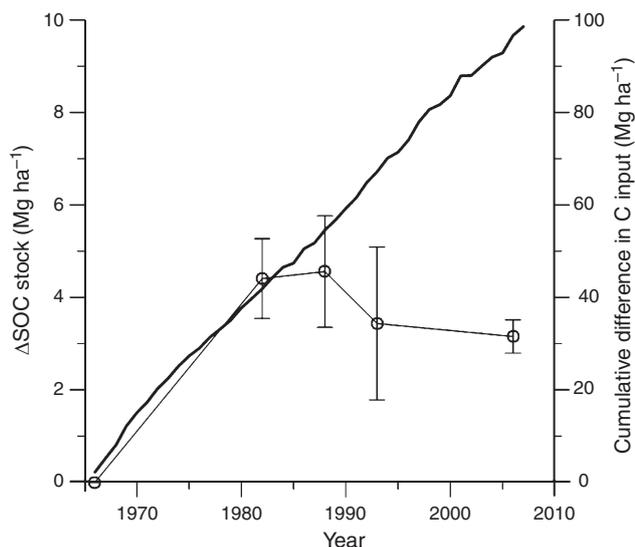
Fig. 4. Average difference in soil organic carbon stock (ΔSOC stock) over time (1966–2006) between treatments of residues incorporated (RI) and residues removed (RR) with standard deviations (y-axis) and cumulative difference in carbon input (z-axis).
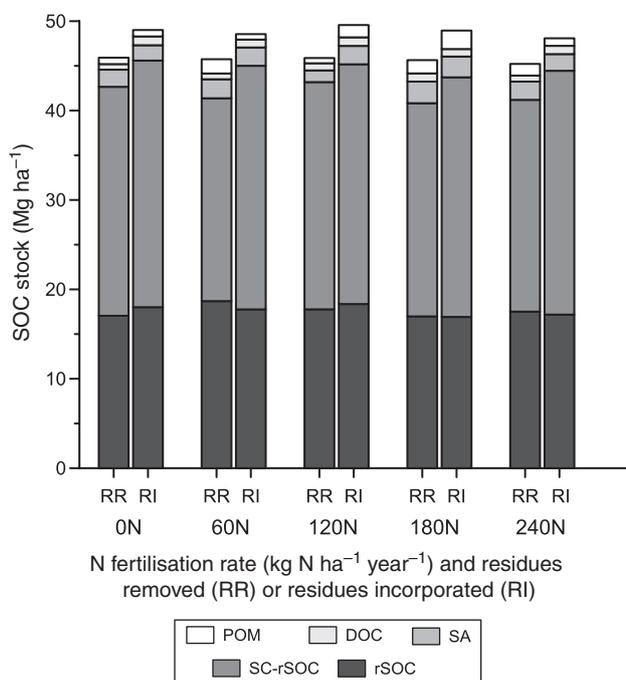
Fig. 5. Soil organic carbon (SOC) stock in different fractions for residues removed (RR) and residues incorporated (RI) treatments at different nitrogen (N) fertilisation rates after 40 years of residue incorporation (2006). For soil fraction abbreviations, see Table 1.

fractions or qualitatively influence the accumulation of residue-derived C (Fig. 5).

Discussion

The effect of 40 years of residue incorporation on SOC stocks was limited, with a total SOC stock change of 3.1 Mg ha^{-1} or

6.8%, which is within the range reported in other studies. For example, in a global meta-analysis, Lehtinen et al. (2014) found an SOC increase of 7%, averaged over 39 studies. Liu et al. (2014) found a slightly higher increase in SOC (12.8%) averaged over 176 studies. Overall, there were significant differences in the SOC stocks measured in the RR and RI treatments, but these were only apparent when the results were aggregated over all N levels. This indicates that the treatment effect was lower than the minimum detectable difference with four replicates (Knebl et al. 2015). The proportion of residue-derived C stabilised in the soil (retention coefficient) did not exceed 4%, which is within the range of reported retention coefficients. For example, Poeplau et al. (2015a) modelled SOC stock changes after residue incorporation in Swedish long-term experiments and found a retention coefficient of between 0 and 17%, depending on clay content. However, in 1986 and 1993, the difference in SOC stocks between RI and RR in the present study was higher (Fig. 3). For the same site, Lugato et al. (2006) calculated a mean annual sequestration rate of residue-derived SOC of 170 kg ha^{-1} year^{-1} for 1993, which is more than double the annual sequestration rate measured in this study for 2006 (78 kg ha^{-1} year^{-1}). Residue-derived SOC accumulation has not only stopped, but the absolute SOC stock difference between RI and RR has also decreased. Stagnation in SOC stock increase after a management change has often been reported to occur after 20–50 years (Franzluebbers and Arshad 1996; Lal 2004; Buysse et al. 2013; Poeplau et al. 2015a), which might be related to C saturation in some cases, or a delayed adjusted increase in output in other cases. In the present case, the levelling off cannot be explained by saturation, because SOC concentration only increased by 0.1%, to ~1.1% C, which is still low for a clay loam soil. Interestingly, the SOC stock difference between RI and RR started to decrease after the rotation was changed from maize monoculture to a diverse rotation of maize, sugar beet, soybean and occasionally potatoes, tomatoes, sorghum and winter wheat. This diversification caused high inter-annual variability in residue-derived C inputs, which had been fairly stable previously. However, it is not obvious how this increase in input variability might have directly influenced SOC dynamics, as on average the amount of residue-derived C inputs remained constant. It could be speculated that peak inputs entail a stronger priming effect than relatively constant inputs over time (Fontaine et al. 2004). Furthermore, a shift in input quality (e.g. lower C:N ratios of the incorporated residues) might have influenced its decomposition. Indirectly, the rotation change might have contributed to altering SOC dynamics in different ways, e.g. through. changes in the decomposition rate due to modifications to the micro-climate and soil moisture under the influence of vegetation cover, plant water use and other crop-specific factors (Fortin et al. 2011). However, a higher decomposition rate does not necessarily entail lower retention of the residue-derived C in the soil (Kirkby et al. 2013). After all, the decrease in ΔSOC from 1986 to 2006 was on average 1.5 Mg C ha^{-1}, which corresponds to a very low concentration change of 0.034% C. The observed difference in ΔSOC at the three sampling dates (1989, 1993 and 2006) might therefore be well within

the range of a possible measurement bias or random spatio-temporal *in situ* variation in SOC. A further increase in ΔSOC seems unlikely, however, even if the rotation changed back to maize monoculture, as the average annual input remained constant over time.

Surprisingly, no positive effect of N fertilisation on SOC stocks was evident, which is in contrast to numerous studies reporting positive responses of SOC stocks upon N addition, that are mainly explained by increased NPP (Alvarez 2005; Christopher and Lal 2007; Ramirez *et al.* 2012). The retention coefficient of residue-derived C slightly decreased with increasing N level, which is in contrast to the findings by Kirkby *et al.* (2014), who reported increasing SOC sequestration upon N addition. Numerous studies have shown that N in combination with labile C addition reduces the priming of older SOC (Craine *et al.* 2007; Griepentrog *et al.* 2014; Fisk *et al.* 2015). However the opposite, namely increased C mineralisation upon N addition, has also been observed (Reinertsen *et al.* 1984) and might be the most logical explanation for the results observed in the present study. Henriksen and Breland (1999) found clear catalysing effects of N addition on mineralisation of straw-derived C and attributed this to increased fungal growth and enzyme activity. The role of N and other major nutrients on SOC dynamics is still not entirely understood (Craine *et al.* 2007). In the present study, there were no indications of a positive effect of N availability on C sequestration. This might be related to the fact that the investigated site was rather N-rich with an initial average C : N ratio of 8.3 and an average C : N ratio of 10.7 in the unfertilised plots in 2006. Microbial metabolism was most likely not N-limited in any treatment.

On average, we found 92% of total SOC in the silt and clay (SC) fraction, which is a typical magnitude for agricultural soils. For example, Bol *et al.* (2009) found 67% and 23% of SOC stored in the clay and silt fraction, respectively, while Christensen (2001) reported that 50–75% and 20–40% SOC is usually attached to clay and silt particles, respectively, in temperate soils. Flessa *et al.* (2008) found 88% of SOC in the silt and clay fraction of two German arable soils and suggested that the main stabilisation mechanism is the formation of organo-mineral complexes. Compared with other land use types, such as grassland or forest, the proportion of SOC stored in this fraction is very high in arable soil (Steffens *et al.* 2011; Poeplau and Don 2013), while macro-aggregates do not play a significant role. Apart from the well-established negative effect of tillage on soil aggregation (Six *et al.* 1999), Wiesmeier *et al.* (2014) suggested that tillage might promote the formation of such organo-mineral complexes due to mixing of fresh organic material with unsaturated mineral surfaces. In an incubation experiment, Stemmer *et al.* (1999) showed that mixing residues and mineral soil resulted in higher SOC contents in the silt and clay fraction than applying residues on the surface of the mineral soil. In the present study, residue incorporation did not change the distribution of SOC in different fractions, as 93% of the additional SOC was found in the SC fraction. (Christensen 1987) reported similar results, but showed that more residue-derived C was stabilised in the silt than in the clay fraction, which could indicate the importance of silt-sized microaggregates. Similarly, Tiessen and Stewart (1983) found more rapid depletion of

SOC in the fine clay fraction (<0.2 µm) than in the coarse clay (0.2–2 µm) and fine silt (2–5 µm) fractions after a land use change from native prairie to arable land. In an *in situ* incubation experiment, Li *et al.* (2016) studied labelled residues mixed into mineral soil over two years and found that after 60 days, 61% of the applied residue C was left in the soil, with 75% of it still stored in particles >2000 µm and only 0.1% was stored in microaggregates <53 µm, which correspond to the SC fraction. After 780 days, 26% of the applied C was left in the soil, with only 39% of it stored in the >2000 µm particles and 2.4% stored in the SC fraction. This indicates that the transformation from undecomposed coarse particulate organic matter to stabilised, complexed C in the fine fraction occurs relatively rapidly. In the present study, the decomposition and transformation of residue C must have occurred faster, because the difference between RI and RR in the POM and SA fractions was marginal after approximately one year since the last residue incorporation.

Although the amount of residues >2000 µm was not determined in our study, we suggest that this might be related to: (i) the relatively warm and humid climate in Padua (ii) the annual soil disturbance by tillage; and (iii) the high soil pH of 7.8, all of which are known to stimulate microbial activity and C mineralisation. (Ordóñez-Fernández *et al.* 2007) found that 53% of wheat straw was left after 109 days at a site in Mediterranean climate conditions, which is similar to the amount of straw left (51%) after 180 days in the study by (Li *et al.* 2016). Thus, under such favourable conditions for C mineralisation, as found in the present study, decomposition and stabilisation of crop residue-derived C occurs very quickly. The higher DOC concentration in the RI (1.8% of the total SOC) as compared with the RR treatments (1.5%) identifies leaching as a potential output pathway of the amended residue-C during decomposition. The POM fraction or sand and aggregates >53 µm were therefore not meaningful for long-term C sequestration. Indeed, the ability to find management-induced differences in labile SOC fractions might be related to site-specific decomposition rates. If decomposition and transformation of SOC occurs faster than the input and/or sampling interval, no difference in fast-cycling SOC fractions will be determined. In a Southern German soil at a site with 6.5°C mean annual temperature and 800 mm precipitation, 60% of which falls in summer, Leifeld and Kögel-Knabner (2005) could not identify the POM fraction as an early indicator of land use change, while at a much dryer Chinese site (6.5°C and 450 mm precipitation) Xu *et al.* (2011) found significant increases in SOC in the POM fraction, but no significant differences in the bulk soil after two years of residue incorporation.

Within the SC fraction, all additional C was found in the NaOCl-oxidisable SC-rSOC fraction, while even after 40 years of residue incorporation, the non-oxidisable rSOC fraction did not change at all. This fits the fractionation method concept proposed by Zimmermann *et al.* (2007), who related the rSOC fraction to an inert SOC pool, which is assumed to be stable in this timeframe. The DOC fraction, which represents the water-extractable SOC during wet sieving, also increased significantly (by 25%), which exceeded the 6.9% increase in SOC in the bulk soil and indicates that SOC in the RI treatments was more

soluble. Thus, in total, the SOC stock became slightly more labile with long-term residue incorporation, which implies higher sensitivity to changes in management or environmental conditions.

In summary, the effect of long-term crop residue incorporation on SOC stocks in the soil studied here was relatively low, confirming reported effects in other long-term field experiments (Lemke *et al*. 2010; Poeplau *et al*. 2015a). A new equilibrium was reached after approximately two decades of residue incorporation (Fig. 5). The sequestered SOC might be sensitive to even minor management changes, as revealed by the decrease in ΔSOC after introducing a new crop rotation. Since most of the difference between RR and RI treatments was associated with the NaOCl oxidisable and DOC fractions, complete cessation of RI could lead to reasonably rapid loss of the accumulated SOC, but this hypothesis requires careful testing. The annual C sequestration rate of $78 \, kg \, ha^{-1} \, year^{-1}$ and findings in other studies suggest that the C sequestration potential of residue incorporation used in several continental projections ($0.7 \, Mg \, ha^{-1} \, year^{-1}$; Freibauer *et al*. 2004; Smith *et al*. 2005) represents a strong overestimation, at least for some soils, particularly when extrapolated to more than 20 years. Alternative uses of crop residues, such as bioenergy production, are currently being discussed. Considering the low effect of long-term residue incorporation on SOC stocks, removal of at least some residues might be the more climate-smart use of this resource (Lemke *et al*. 2010). The average amount of incorporated residues in this study was $5 \, Mg \, DM \, ha^{-1} \, year^{-1}$. The heating or fuel value of 1 Mg straw dry mass corresponds to 0.33 or 0.24 Mg diesel (Larson 1979; Lal 1997). When used for heating or as fuel, the crop residues from this study could annually replace 1.41 or 1.02 Mg C emitted from fossil sources. This is 18-fold and 13-fold more than would be compensated by SOC sequestration after incorporation. This is in agreement with calculations by Powlson *et al*. (2008), who predicted a 7-fold higher CO_2 saving potential when straw is used for electricity production. In a fertile, C-rich soil, Villamil *et al*. (2015) did not find consistent degradation of any soil property after seven years of residue removal and suggested that this practice does not cause any threat to the sustainability of the cropping system. However, this might only be true for fertile soils, as straw incorporation is acknowledged for its positive effects on numerous soil properties, such as soil structure, earthworm abundance, aggregate stability, and water-holding capacity (Karlen *et al*. 1994; Roldán *et al*. 2003). The relatively stable NPP in the unfertilised treatments over the study period in the experiment investigated here suggests high fertility. Therefore, the results of this study suggest that straw removal for bioenergy production would be more sustainable at sites with comparable characteristics. For SOC sequestration, other management practices would most likely be more effective. For example, Poeplau *et al*. 2015b found an average retention coefficient of 0.32 for cover crops, which is ~10-fold higher than found for crop residues in this study. Furthermore, a meta-analysis did not find any indication of saturation of SOC sequestration after 54 years of cover crop cultivation (Poeplau and Don 2015). Similarly, high coefficients have been found for farmyard manure and sewage sludge (Kätterer *et al*. 2011).

Conclusions

Forty years of crop residue incorporation significantly increased SOC stocks, but only during the first 20 years of residue incorporation. This indicates that a new equilibrium has been reached and no further SOC sequestration can be expected after about two decades under the prevailing conditions. The change in crop rotation, which occurred around the same time, might have influenced SOC dynamics to a certain extent, although the residue-derived C inputs did not decrease. Compared with other agricultural management practices, the long-term potential of crop residue incorporation to sequester SOC is rather limited in this particular clay loam soil. Complete decomposition of crop residues probably occurred within one year, as indicated by the equal size of the labile SOC pools in treatments with residues removed and residues incorporated. The silt and clay fraction was most important for SOC storage and sequestration, although SOC in this fraction might not be highly stable, as indicated by the response to a crop rotation change in 1984 and by complete NaOCl-oxidation of the accumulated SOC. Cessation of residue incorporation might therefore lead to rapid loss of much of the C sequestered by this management practice.

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