



# Impact of agricultural management on soil aggregates and associated organic carbon fractions: analysis of long-term experiments in Europe

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**Abstract.** Inversion tillage is a commonly applied soil cultivation practice in Europe, which often has been blamed for deteriorating topsoil stability and organic carbon (OC) content. In this study, the potential to reverse these negative effects in the topsoil by alternative agricultural management practices are evaluated in seven long-term experiments (running from 8 to 54 years the moment of sampling) in five European countries (Belgium, Czech Republic, Hungary, Italy and UK). Topsoil samples (0–15 cm) were collected and analysed to evaluate the effects of conservation tillage (reduced and no tillage) and increased organic inputs of different origin (farmyard manure, compost, crop residues) combined with inversion tillage on topsoil stability, soil aggregates and, within these, OC distribution using wet sieving after slaking. Effects from the treatments on the two main components of organic matter, i.e. particulate (POM) and mineral associated (MAOM), were also evaluated using dispersion and size fractionation. Reduced and no-tillage practices, as well as the additions of manure or compost, increased the aggregates mean weight diameter (MWD) (up to 49 % at the Belgian study site) and topsoil OC (up to 51 % at the Belgian study site), as well as the OC corresponding to the different aggregate size fractions. The incorporation of crop residues had a positive impact on the MWD but a less profound effect both on total OC and on OC associated with the different aggregates. A negative relationship between the mass and the OC content of the microaggregates (53–250 µm) was identified in all experiments. There was no effect on the mass of the macroaggregates and the occluded microaggregates (mM) within these macroaggregates, while the corresponding OC contents increased with less tillage and more organic inputs. Inversion tillage led to less POM within the mM, whereas the different organic inputs did not affect it. In all experiments where the total POM increased, the total soil organic carbon (SOC) was also affected positively. We concluded that the negative effects of inversion tillage on topsoil can be mitigated by reducing the tillage intensity or adding organic materials, optimally combined with non-inversion tillage methods.

## 1 Introduction

Soil tillage by mouldboard ploughing, which inverts and mixes the soil, is a classic conventional agricultural practice in temperate agricultural systems, including most of Europe. This soil cultivation technique is used to prepare the soil for seeding, to reduce soil compaction, to control weeds and crop residues and to improve water and nutrient availability for plants (Cannell, 1985; Peigné et al., 2018; Schneider et al., 2017; Hobson et al., 2022). However, inverting the topsoil, especially by mouldboard ploughing, is blamed for deteriorating the stability and arrangement of the soil aggregates (soil structure) with subsequent negative effects on soil organic matter (SOM) preservation and sequestration potential (Six et al., 2000a; Six and Paustian, 2014). For this reason, introducing agricultural practices that counter the adverse effects of mouldboard tillage on soil structure and SOM will contribute to sustainable agricultural management and climate change mitigation (Cooper et al., 2021).

The protection of SOM in the soil aggregates has been proposed as an important stabilization mechanism. According to Oades (1984), macroaggregates are initially formed around fresh plant material (i.e. particulate organic matter (POM)) entering the soil through physical, chemical and biological processes. As the organic matter within macroaggregates ( $> 250 \mu\text{m}$ ) decomposes, an internal structure develops consisting of microaggregates ( $53\text{--}250 \mu\text{m}$ ) within which the SOM is better protected (i.e. occluded SOM (mM-C)) as compared with non-occluded SOM (Ananyeva et al., 2013; De Clercq et al., 2015; Denef et al., 2001; King et al., 2019). When macroaggregates break down, for instance due to wetting–drying cycles, the microaggregates are released (Six et al., 2000a; Denef et al., 2001). However, agricultural practices such as inversion tillage may break down soil macroaggregates prematurely, which results in the release of unstable microaggregates and leads to increased SOM accessibility for decomposition (Kotronakis et al., 2017; Six et al., 2000a). For this reason, the fraction of microaggregates held within the macroaggregates (mM) has been proposed as an indicator to monitor present and potential soil structural and SOM changes caused by management practices (Denef et al., 2004, 2007; Kong et al., 2005; Six et al., 2000a; Six and Paustian, 2014).

Tillage effects on soil structure and especially topsoil SOM are a point of controversy in the literature. Several studies have observed an increase in macroaggregates under non-inversion and/or zero till as compared with conventional inversion tillage agriculture (Jat et al., 2019; Paustian et al., 1997; Six et al., 2000b). Reduced soil disturbance is often accompanied by an increase in SOC in the topsoil (Apostolakis et al., 2017; Fuentes et al., 2012; Giannakis et al., 2014; Karlen et al., 2013; Virto et al., 2012) or even the whole soil profile (Cooper et al., 2021; Varvel and Wilhelm, 2011) espe-

cially when it is combined with increased inputs from cover crops or intercrops (Boddey et al., 2010; Fuentes et al., 2009; Minasny et al., 2017). This increase is mainly explained by differences among the practices in terms of organic input accumulation in the topsoil (Andruschkewitsch et al., 2014; Virto et al., 2012) and reduced mineralization rates. Other studies have reported limited or no effects of reduced or no tillage on SOM or even negative effects in some cases, especially when the entire soil profile was considered (Blanco-Canqui and Lal, 2008; Camarotto et al., 2020; Du et al., 2017; Haddaway et al., 2017; Piccoli et al., 2016; Meurer et al., 2018). This was explained mainly by the concentration of organic materials in the top and/or surface layer due to no redistribution through inversion tillage in the deeper soil layers. Reduced and zero tillage have also been claimed to cause other agricultural risks like increased soil compaction, impaired root development, increased weed and disease pressure leading to more pesticide use and, possibly, lower crop yields and poor crop establishment (Berner et al., 2008; Ogle et al., 2012; Peigné et al., 2018; Piccoli et al., 2021; Van den Putte et al., 2010). Overall, while tillage reduction or cessation seems to alleviate the negative effects on soil structure and topsoil SOM, it also removes some of its beneficial effects especially in the short term. Kay and Vandenberg (2002) and Sartori et al. (2022) speculated that in the short term, soil compaction would be expected as a result of tillage absence and traffic load, while crop yield and soil structure stabilization are expected in the mid and long term ( $> 15$  years) (Rusinamhodzi et al., 2011) as a result of greater biological activity and SOM redistribution.

While soil tillage increases the turnover rate of OM, a supply of organic materials should compensate for the losses. Under conventional agriculture, the addition of exogenous organic materials, like compost or manure, could offset the negative effects of inversion tillage on soil structure (Williams et al., 2017) while maintaining the beneficial ones. Gross and Glaser (2021) concluded that regardless of tillage intensity, manure application can increase the SOC stocks and that animal manure led to a greater SOC increase compared with green manure or plant-derived organic amendments (e.g. straw). While compost and animal manure additions are found to improve soil aggregation and increase both the total and the aggregate associated OC in croplands receiving conventional tillage, the inorganic fertilization did not result in such improved soil fertility (De Clercq et al., 2016; Kotronakis et al., 2017; Lin et al., 2019; Yin et al., 2016). Minasny et al. (2017) reviewed several studies and found that organic amendments (such as compost or manure) lead to a SOC accumulation of on average  $0.5 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  while residue incorporation leads to accumulation of on average  $0.35 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$ . Incorporation of crop residues increased total SOC from 2.7 % up to 18.2 % (Bolinder et al., 2020) when compared with residue

removal as well as increased the SOC within the different aggregate fractions (Zhao et al., 2018) and improved aggregation (Zhang et al., 2014). The effects of different residue management practices are highly connected with the tillage methods that are combined with them, thus leading to controversial findings when the latter is overlooked. Paul et al. (2013) concluded that tillage or residue management alone does not affect SOC, but when the residues are incorporated into the soil in the 15–30 cm layer, the SOC content increases whereas the aggregate stability is not improved. On the other hand, Li et al. (2020) identified non-inversion tillage (breaking the upper soil layers without any mixing or inverting) coupled with residue retention (when compared with conventional inversion or zero tillage with or without residue retention) as the optimum system to increase SOC stocks in the 0–30 cm soil layer.

Management effects on SOC and soil aggregation vary in the literature possibly due to differences in the climatic conditions and/or soil properties between studied regions (Pan et al., 2021), as well as variations in the application and incorporation rates of the organic materials. Methodological differences between studies during sampling or analysis processes (Poeplau et al., 2018) may also be responsible for the controversial responses reported. For this reason, studies that investigate the impact of multiple and combined agricultural practices on several regions under a common methodological framework are needed. Such studies are in general scarce due to the logistical constraints associated with field experiments, and available studies in different countries are difficult to compare due to differences in objectives and analysis methods of heterogeneous field experiments. Meurer et al. (2018) identified the lack of documented required data for meta-analysis of such studies as another limiting factor.

SOM is a complex mixture that can be separated into multiple pools with distinct functional properties (Schrumpf et al., 2013; Trumbore, 2009). The separation of SOM into particulate organic matter (POM) that consists of plant-derived, relatively not decomposed light fragments, and mineral-associated organic matter (MAOM) that consists of mostly simple molecules, has been proposed for many decades (Cambardella and Elliott, 1992; Poeplau et al., 2018). Lavalley et al. (2020) suggested the applicability of SOM separation into POM and MAOM for the investigation of management effects on carbon cycling. Coupling the separation of SOM in OM occluded in different aggregation fractions and in POM and MAOM within each aggregation fraction can help in elucidating the effects of tillage intensity and soil amendments on SOM stability.

In this study, we investigated the effects of three agricultural practice categories on soil structure and SOC distribution in four aggregate size fractions and one subfraction (i.e. occluded microaggregates (mM)). The same field and laboratory protocols were used for the analysis of seven long-term experiments in five European countries. The agricultural practice categories were (i) soil cultivation including

zero, minimum and conventional tillage, (ii) crop residue incorporation or removal and (iii) addition of exogenous organic or inorganic material including manure, compost and NPK fertilizers.

Our hypotheses were that in the topsoil:

- Inversion tillage reduces the mass of water-stable (large) macroaggregates and of SOC stabilized in macroaggregates and microaggregates, and this effect increases with increasing tillage intensity (from zero to conventional mouldboard ploughing).
- Matured exogenous organic fertilization (i.e. manure and compost application), increases the mass of water-stable macroaggregates and increases SOC in all aggregate fractions, even under inversion tillage.
- Incorporation of the previous crop residues increases the mass of water-stable macroaggregates and occluded POM due to higher additions of fresh plant-derived organic matter, and thus leads to higher SOC stabilized in both occluded and free microaggregate, compared with the practice of crop residue removal.
- Increased organic inputs and lower soil disturbances lead to more water-stable mM and corresponding SOC (mM-C) (i.e. the mass of mM and occluded OC exhibit a linear relationship).

Our objectives were therefore: (i) to evaluate the effects of the different treatments/practices on the topsoil structural stability and SOC, (ii) to determine the soil mass and OC distribution in four water-stable aggregate size fractions (i.e. large macroaggregates:  $LM > 2000 \mu\text{m}$ ; macroaggregates:  $2000 < M < 250 \mu\text{m}$ ; microaggregates:  $250 < \text{micro} < 53 \mu\text{m}$ ; clay and silt domains:  $s\&c < 53 \mu\text{m}$ ), (iii) to determine the effects of the practices on the occluded microaggregates (mM) and the corresponding OC (mM-C) and (iv) to determine the OC in POM and MAOM in the bulk soil and the aggregation fractions.

## 2 Methodology

### 2.1 Study site description

For our objectives, we took the option to combine several long-term experiments and to have a wide range of soil management practices and combinations rather than just concentrating on only one practice. Logistically, to handle seven experiments from five countries by the same methods, sampling under identical conditions and laboratory analysis in the same laboratory, we had to concentrate on the 0–15 cm topsoil. Thus, topsoil samples were collected from different long-term agricultural experiments (8–54 years) with different treatments. (The towns, countries, coordinates, main soil type and climate of the sites are given in Table A1 taken from Panagea et al., 2021.) The long-term experiments were set up

independently from one another with different objectives and under different environmental conditions. Nevertheless, they offer the possibility to explore a wide range of representative management practices and pedo-climatological conditions across Europe as they cover a wide gradient both west–east and north–south (Fig. 1). For each country, the field experiment included management practices (and intensities) that were adapted to the local conditions and commonly applied by local farmers, but also intensities that were considered extreme, to evaluate the maximum effects (e.g. 45 Mg ha<sup>-1</sup> compost annually in BE).

For this research, a subset of the experimental treatments was selected to include various treatment categories to test our hypotheses (Table 1). This resulted in 79 experimental plots under 26 treatments. The first category includes primarily treatments with different soil cultivation intensities (CZ, HU\_2, UK), the second category focuses on the addition of different types of exogenous organic materials (BE, IT\_1c, IT\_1p, HU\_1), in cropping systems that are conventionally tilled, and the third category deals with the incorporation of crop residues (HU\_1, IT2\_c, IT\_2l), also in systems under conventional inversion tillage. The experiments in Italy are conducted on two different soil types each and, in this study, were analysed as separate experiments: a clay and an initially peaty soil for IT\_1 (i.e. IT\_1c and IT\_1p) and a clay and a loamy soil for experiment IT\_2 (i.e. IT\_2c and IT\_2l). The selected treatments per experiment are presented in Table 1. At the five study sites, an identical sampling and analysis procedure was performed for determining the soil aggregates size fraction distribution, the OC content in each size fraction and the separation of SOM into POM and MAOM (Fig. 2). For consistency reasons, all laboratory analyses were conducted in the same laboratory with the same equipment, by the same person. This is particularly important for the analysis of the aggregates.

## 2.2 Aggregate separation

Field-moist topsoil samples were taken with a sharp shovel in a Z-shape sampling design within each experimental plot from the upper 15 cm of soil, mixed and directly broken to pass a < 8 mm sieve. Soil samples were stored in plastic containers to avoid compaction during transport and then stored in the refrigerator until air drying. All samples were air dried and stored in a dark and dry place at room temperature.

Aggregate separation was done by wet sieving, as prescribed by Elliott (1986) and described in Six et al. (2000b). A 100 g subsample of 8 mm-sieved and air-dried soil was submerged in demineralized water for 5 min on a 2000 µm sieve. Aggregates were separated by moving the sieve up and down mechanically 50 times in 2 min. The > 2000 µm fraction was backwashed in a glass jar and the < 2000 µm fraction was transferred into the next sieve (250 µm) and sieved with the same methodology. This procedure was repeated with a 53 µm sieve. All aggregate fractions were oven

dried (50 °C), until dried, weighted and stored for further fractionation and analysis. The aggregates were differentiated into large macroaggregates (> 2 mm) (LM), macroaggregates (250 µm<sup>-2</sup> mm) (M), microaggregates (53–250 µm) (micro) and silt- and clay-size particles (< 53 µm) (s&c). The procedure was replicated three times for each sample from each experimental plot and the averaged values were used for the following analysis.

## 2.3 Determination of occluded microaggregates

The isolation of the microaggregates held within the macroaggregates was done by a microaggregate isolator using the method proposed by Six et al. (2000a). A 10 g subsample of macroaggregates was placed in the microaggregate isolator on top of a 250 µm mesh sieve, immersed in demineralized water and shaken with 50 glass beads (4 mm diameter). Steady and continuous water flow helped the microaggregates to flush on a 53 µm sieve. When only water was flushed in the sieve, the material on the 53 µm sieve was sieved to ensure that the isolated microaggregates were water stable, backwashed in a glass jar, oven dried (50 °C), weighed and stored. The material on the 250 µm sieve was also backwashed in a glass jar, oven dried (50 °C), weighed and stored for further analysis.

## 2.4 Size fractionation of SOM to MAOM and POM and sand correction

A size fractionation approach was used to differentiate SOM from its components, i.e. MAOM and POM as described in Cambardella and Elliott (1992), and to determine the sand content of all aggregate fractions > 53 µm using the method of Elliott et al. (1991). Specifically, the average upper size limit specification from MAOM at 53 µm was used which is also considered as the lower limit for the sand fraction. An aliquot of 5 g from each aggregation fraction was dispersed in 20 mL sodium hexa-metaphosphate (5 g L<sup>-1</sup>) (0.5 %), for 18 h at 180 rpm, and sieved through a 2000 µm sieve to remove possible small stones and a 53 µm sieve to determine sand content. The fraction remaining in the latter sieve was backwashed in a glass jar, oven dried (105 °C) until constant mass and weighed. The procedure was replicated twice for each sample and the average value was used for the following analysis. By dispersing and sieving the fractions through the 53 µm sieve, the fraction remaining in the sieve was considered sand and the POM component of the SOM and the fraction that passed through the sieve was taken as the MAOM component and s&c fraction.

The relevant aggregate weight percentages were corrected for their sand content to allow comparisons between soils with different sand contents. The sand-corrected fraction proportions were calculated as proposed in Six et al. (2002).

The soil stability was assessed using the mean weight diameter (MWD) (Eq. 1) which is the most widely used index,

Table 1. Details of the treatments in the various experiments.

Code	Treatments	Tillage System	Sampling (month, year)	Years applied	Repl. (#), design	Main crop type
CZ	Conventional: conventional ploughing (turning of stubble-furrow opener at 10 cm, mouldboard plough at 22 cm) (control) Minimum: minimum tillage (turning of stubble-furrow opener at 10 cm, 30 % of crop residues remain on the soil surface) Zero: no tillage (all residues remain on the soil surface)	Depending on the experimental treatment	Nov 2018	23	4 <sup>b</sup>	Oil rapeseed, winter wheat, peas
HU_2	Conventional: deep winter ploughing (27–28 cm) + secondary tillage (control) Minimum: disking just before drilling (< 15 cm) Shallow: shallow winter disking (< 15 cm) + secondary tillage	Depending on the experimental treatment	Nov 2018	46	4 <sup>b</sup>	Winter wheat, maize
UK	Conventional: ploughing at 25 cm (control) Direct drilling: direct drilling of the seeds into previous crop residues	Depending on the experimental treatment	Apr 2019	8	3 <sup>a</sup>	Winter wheat, wheat, oat
BE	No organic: no organic fertilization (control) 45m3-yearly: 45 Mg ha <sup>-1</sup> compost* applied every 3 years 15m-yearly: 15 Mg ha <sup>-1</sup> compost* applied yearly 45m-yearly: 45 Mg ha <sup>-1</sup> compost* applied yearly	Conventional tillage up to 23 cm and bed preparation according to the crop type	Oct 2019	22	4 <sup>a</sup>	Winter wheat, carrots, sugar beet, potatoes
HU_1	NPK: Only mineral fertilization and removal of straw (control) NPK + FYM: 35 Mg ha <sup>-1</sup> 0.5 % N, farmyard manure application every 3 years and removal of straw NPK + STR: Straw and stalk incorporation completed with 10 kg mineral N** for each Mg straw per ha	Conventional tillage: shallow stubble tillage, ploughing (27 cm) in autumn, secondary tillage and seedbed preparation	Nov 2018	35	3 <sup>b</sup>	Maize, winter wheat, winter barley
IT_1c	Unfertilized: no organic or mineral fertilization (control) Manure L1: 20 Mg ha <sup>-1</sup> manure applied annually** Manure L2: 40 Mg ha <sup>-1</sup> manure applied annually**	Shovelling–inversion tillage (0–20 cm) each autumn after the removal of crop residues.	Nov 2018	54	2 <sup>a</sup>	Maize, winter wheat, potato, tillage radish (winter cover crop), ryegrass, silage maize
IT_1p	Unfertilized: no organic or mineral fertilization (control) Manure L1: 20 Mg ha <sup>-1</sup> manure applied annually** Manure L2: 40 Mg ha <sup>-1</sup> manure applied annually**	Shovelling–inversion tillage (0–20 cm) each autumn after the removal of crop residues.	Nov 2018	54	2 <sup>a</sup>	Maize, winter wheat, potato, tillage radish (winter cover crop), ryegrass, silage maize
IT_2c	Residue remov.: removal of the previous crop residues (control) Residue incorp.: burial of the previous crop residues	Shovelling–inversion tillage (0–20 cm) each autumn	Nov 2018	48	3 <sup>a</sup>	Maize, winter wheat, potato, ryegrass, silage maize, tillage radish
IT_2l	Residue remov.: removal of the previous crop residues (control) Residue incorp.: burial of the previous crop residues	Shovelling–inversion tillage (0–20 cm) each autumn	Nov 2018	48	3 <sup>a</sup>	Maize, winter wheat, potato, ryegrass, silage maize, tillage radish

<sup>a</sup> Randomized complete block design. <sup>b</sup> Split plot randomized complete block design. \* VFG Compost C/N ≈ 12 \*\* Farmyard manure from dairy cows (20 % dry matter, 0.5 % N, 0.25 % P<sub>2</sub>O<sub>5</sub>, 0.7 % K<sub>2</sub>O)

defined as the sum of the weighted mean diameter of the aggregate size fractions where the weighting factor of each fraction is the proportion of the total sample weight for each fraction (Nimmo, 2013):

$$\begin{aligned} \text{MWD}(\mu\text{m}) = & \frac{8000\mu\text{m} + 2000\mu\text{m}}{2} \text{LM} \% \\ & + \frac{2000\mu\text{m} + 250\mu\text{m}}{2} \text{M} \% \\ & + \frac{250\mu\text{m} + 53\mu\text{m}}{2} \text{micro} \% \\ & + \frac{53\mu\text{m}}{2} \text{s\&c} \% \end{aligned} \quad (1)$$

## 2.5 Soil organic carbon determination

Soil organic carbon content (SOC), which is the largest and easiest component of SOM to quantify, was used in this study. The SOC content was determined by dry combustion and mass spectrometry elemental analysis (Carlo-Erba EA 1110, Thermo Scientific). A representative subsample of each fraction was taken with a soil sample splitter, ground and weighed into an Ag capsule. To determine only the carbon present in organic form, carbonates were removed with the addition of HCl (35%). After drying at 40 °C for 24 h, the soil samples were loaded into the autosampler for combustion with oxygen with the presence of chromium trioxide (catalyst). The mass percentage was determined after quantification by infrared absorption spectroscopy of the organic carbon (OC) that reacted to carbon dioxide (CO<sub>2</sub>).

## 2.6 Statistical analysis

One-way analysis of variance (ANOVA) (Webster, 2007) was carried out using R-Studio with R version 3.6.1 (R Core Team, 2019; RStudio Team, 2016) to test for differences between treatments. Estimated marginal means by factors were computed by the least squares method using the package “emmeans” (Lenth, 2020). All graphs were produced with the package “ggplot2” (Wickham, 2016). The statistical significance was checked at  $p < 0.05$ . The assumptions of normality and homoscedasticity of the residuals were assessed by visual inspection of the  $Q-Q$  plots and plots of the normalized residuals against the fitted values.

# 3 Results

## 3.1 Soil stability

The soil structure stability of the sand-corrected aggregates represented by the MWD (Fig. 3) was a sensitive index reflecting management changes. In CZ, the zero-tillage treatment had almost double MWD in comparison with the minimum or conventional tillage; the same applied to the HU\_2 experiment between the shallow and conventional tillage. The UK experiment presented the highest MWD values of

all the experiments monitored up to 3000 μm for the direct drilling (zero tillage) treatment, which was significantly higher than the conventional tillage.

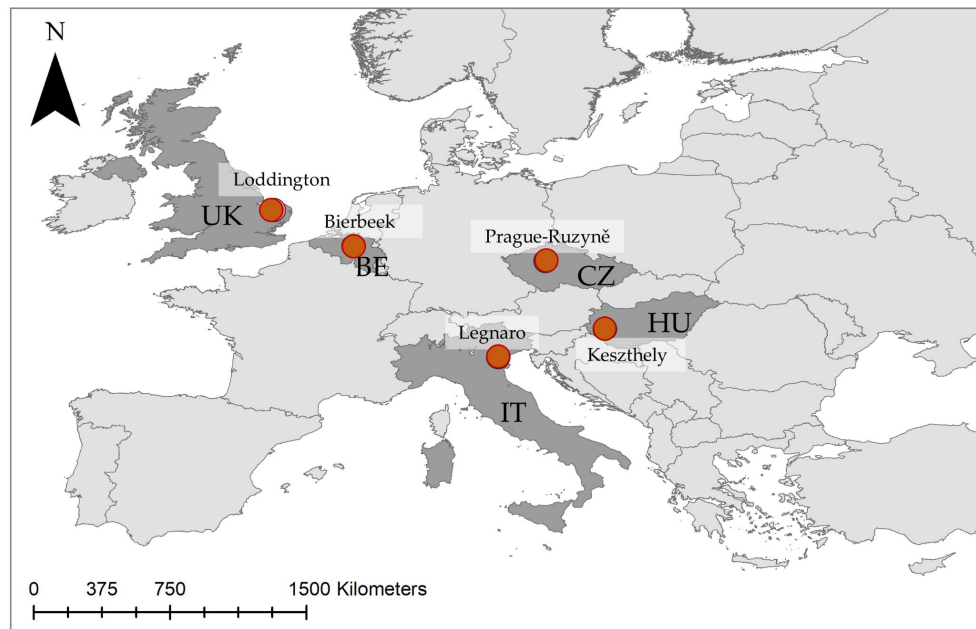
The addition of animal manure or compost led to higher MWD, but differences among the treatments were only statistically significant in the BE experiment. Adding 45 Mg ha<sup>-1</sup> of VFG compost annually for 22 years led to an MWD of about 1200 μm, which was significantly higher compared with the addition of 45 Mg ha<sup>-1</sup> triannually (one-third of the previous treatment) and from no addition of compost. In the IT\_1c, IT\_1p and HU\_1 experiments, even after 54 and 35 years respectively, there were no significant differences among the treatments, but the visual trends were consistent, i.e. adding manure increased the MWD. In the HU\_1 experiment the incorporation of the crop residues led to significantly higher MWD compared with the NPK-only treatment. Similarly, in the IT\_2c and IT\_2l experiments, the incorporation of residues resulted in higher values of MWD, but the differences were statistically significant only in the IT\_2l.

## 3.2 Soil organic carbon

The total SOC (Fig. A1) expressed in its two main fractions (i.e. POM and MAOM) (Fig. 4) ranged between 10 g C kg<sup>-1</sup> soil and 70 g C kg<sup>-1</sup> soil (1%–7%) among all experiments. In all cases when significant increases in the total POM were identified as a result of management, the total SOC was also increased. The treatments with the minimum soil disturbance in CZ and HU\_2, and the treatments where manure or compost were applied in BE, IT\_1c, presented significantly higher values of total POM and MAOM in the topsoil than the treatments where conventional inversion tillage took place or when they were unamended. The retention of the crop residues did not lead to an increase in the topsoil MAOM when compared with their removal, while the incorporation of straw significantly increased the POM in HU\_1.

## 3.3 Aggregate weight and organic carbon distribution

Reduced tillage intensities caused statistically significant differences in the mass distribution of water-stable large macroaggregates and microaggregates, with those causing less soil disturbance resulting in more water-stable large macroaggregates but fewer microaggregates (Fig. 5). The macroaggregates between 250 and 2000 μm did not present significant differences in the CZ and HU\_2 experiments, but they differed significantly in the UK experiment in which the conventional tillage resulted in more water-stable macroaggregates than the direct drilling treatment. Different rates of manure or compost applications caused significant differences (in the case of BE and IT\_1p) or an increasing trend when the organic input level was higher in the large macroaggregates. In the BE experiment, an abundance of water-stable microaggregates was also observed in the treatment without



**Figure 1.** Map with the location of the long-term experiments.

addition of compost compared with those with addition. In the HU\_1 experiment, the application of FYM resulted in more macroaggregates and fewer microaggregates than when only mineral fertilizer was applied. In addition, when the straw was incorporated in the soil, we found significantly more water-stable macroaggregates and less microaggregates than when FYM and mineral fertilizer, or only mineral fertilizer, was applied. The residue retention in IT\_2c and IT\_2l did not result in significant differences (except the microaggregates fraction in IT\_2l) in the aggregate size fractions.

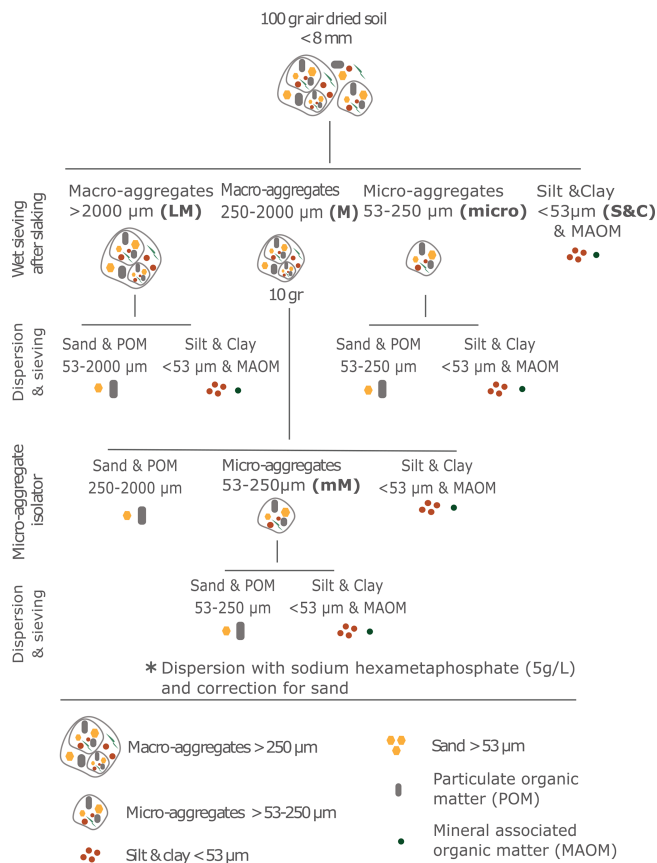
The SOC content in each aggregate fraction after the sand correction is illustrated in Fig. 6. In the CZ experiment, zero tillage resulted in higher OC contents than conventional tillage in every aggregate fraction except the large macroaggregates, although this fraction exhibited the highest OC content up to  $35 \text{ g C kg}^{-1}$  soil. Interestingly, the minimum tillage resulted in the same OC content with the zero tillage in the macroaggregates fraction. In the microaggregates fraction, minimum tillage presented significantly lower values than the zero tillage and significantly higher OC content than the conventional tillage. In general, the HU\_2 experiment followed the same trend as the CZ. Shallow disking resulted in statistically higher OC than conventional tillage in all fractions apart from the large macroaggregates. In the UK experiment, significant differences between the two treatments were only present in the microaggregates and s&c fractions, with the direct drilling presenting higher OC content (more than  $40 \text{ g C kg}^{-1}$  soil in the microaggregates fraction).

In the compost or manure experiments, i.e. BE, IT\_1c and IT\_1p, the OC content in the aggregates fractions (Fig. 6) responded as the total OC (Fig. 4). The higher application

rates led to higher OC contents in the different fractions. In the BE experiment, the annual application of  $45 \text{ Mg ha}^{-1}$  compost led to significantly higher OC in the macroaggregates and microaggregates fractions compared with every other treatment. In the IT\_1c experiment, addition of manure led to significantly higher OC among all the treatments in the macroaggregates fraction. In the microaggregates and large macroaggregates fractions, there was large variability between the experimental plots and no significant differences were found. Similarly, in the IT\_1p experiment, where even though the OC content within the different fractions went up to about  $80 \text{ g C kg}^{-1}$  soil, the in-between experimental plots' variability did not allow for safe comparisons among the treatments. In the experiments where the treatments dealt with the residues management, i.e. HU\_1, IT\_2c and IT\_2l, we did not observe significant differences among the treatments even after 48 years of application.

### 3.4 Mass and OC dynamics in the occluded microaggregates

Based on our observations, we found that tillage intensity and OM additions (i) influenced the quality of macroaggregates in terms of OC content, but not their mass, and (ii) led to a negative relationship between OC content and aggregate mass in the microaggregates fraction. We therefore isolated the microaggregates within macroaggregates (mM) to understand better the underlying processes. The analysis did not show significant differences among the treatments in all categories on the amount of mM (data shown in Fig. A2), but it did show significant differences in their OC content (mM-C)



**Figure 2.** Aggregate and SOM separation scheme used in this study. For each fraction the different soil particles and relevant organic matter components as considered in this research are presented. This separation scheme was designed following a combination of methods and concepts described in Cambardella and Elliott (1992), Elliott et al. (1991) and Six et al. (2000a).

(Fig. 7). Over all study sites, their OC content ranged from 10 to 47 g C kg<sup>-1</sup> mM. In the CZ experiment significantly more mM-C was observed in the zero tillage in comparison with the conventional, while in the HU\_1 there was significantly more occluded OC in the shallow disking plus secondary tillage compared with the other two treatments (i.e. shallow disking alone and conventional deep ploughing). In the UK experiment, OC content in the mM fraction did not differ among the treatments. In the BE experiment, only the annual addition of 45 Mg ha<sup>-1</sup> of compost led to increased OC content in the mM fraction compared with the other application rates. Both in the IT\_1c and IT\_1p experiments, the occluded OC was higher when manure was added than when it was not, but the difference was statistically significant only for IT\_1c. The residue retention led to more mM-C only in the HU\_1 experiment.

When comparing the differences in each OC component among the treatments (Fig. 8), the same trends with the total OC were followed and observed. In the BE experiment when

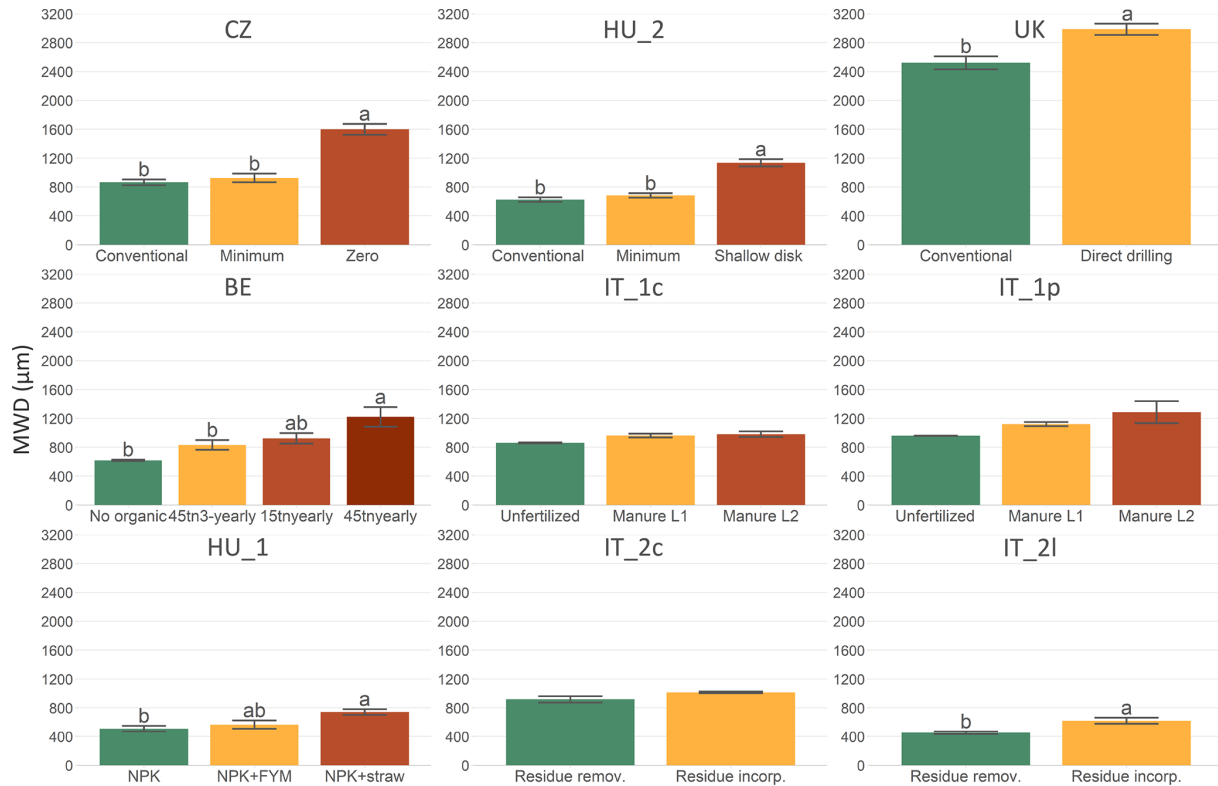
45 Mg ha<sup>-1</sup> of compost were added, the MAOM was double compared with the treatments with no addition of compost and statistically significantly higher than the other two treatments with lower amounts of compost addition. In the IT\_1c experiment when animal manure was added (both levels), the MAOM was significantly higher than in the unfertilized treatment. The same results were observed in the HU\_1 experiment. Comparing the treatment that included only application of mineral fertilizers with those that got either manure or residues, we observed that the MAOM was significantly higher in the case of farmyard manure addition and retention of the residues.

#### 4 Discussion

Improving the topsoil structure and increasing the SOM through improved soil management, while ensuring agricultural production and farmers' income, constitutes an important challenge in the agricultural sector (Dignac et al., 2017). Restoring degraded agricultural soils via natural attenuation (i.e. set aside) demands long periods of fallow. Especially in regions with low primary production (Apostolakis et al., 2017), this is not compatible with ensuring farmers' income or societal objectives for food production. This is especially important for regions with stagnating yields or with yields that are considerably lower than their potential (Wiesmeier et al., 2015; Schils et al., 2018) due to land mismanagement and poor soil quality, and for regions with low primary production. For this reason, additional measures are needed.

In this study, we explored the potential to reverse the negative effects of inversion tillage in the topsoil structure and SOM by applying different management practices that have the potential to improve the soil. To test our hypotheses, we exploited a network of ongoing long-term experiments running from 8 to 54 years at the moment of sampling. We used common sampling and sample-analyses protocols, as these factors can contribute to the strong controversies in the effects of practices demonstrated in the literature (Blanco-Canqui and Lal, 2008; Haddaway et al., 2017; Karlen et al., 2013; Sheehy et al., 2015; Meurer et al., 2018). The practices evaluated included the addition of soil amendments, such as compost or manure, and the management of crop residues combined with inversion tillage, and the reduction of tillage intensities (from minimal to zero). These practices control both the spatial and temporal distribution of OM inputs into the soil and regulate the sensitivity of OM to mineralization, affecting the OM stocks (Jastrow et al., 2007). Such practices can (i) reduce OM mineralization by minimizing disturbances, (ii) promote aggregation and (iii) increase the SOC content and aggregate stability. On the other hand, they might also enhance the decomposition of old OC due to the addition of fresh OM inputs (e.g. residues), i.e. the priming effect (Fontaine et al., 2007; Liu et al., 2020).





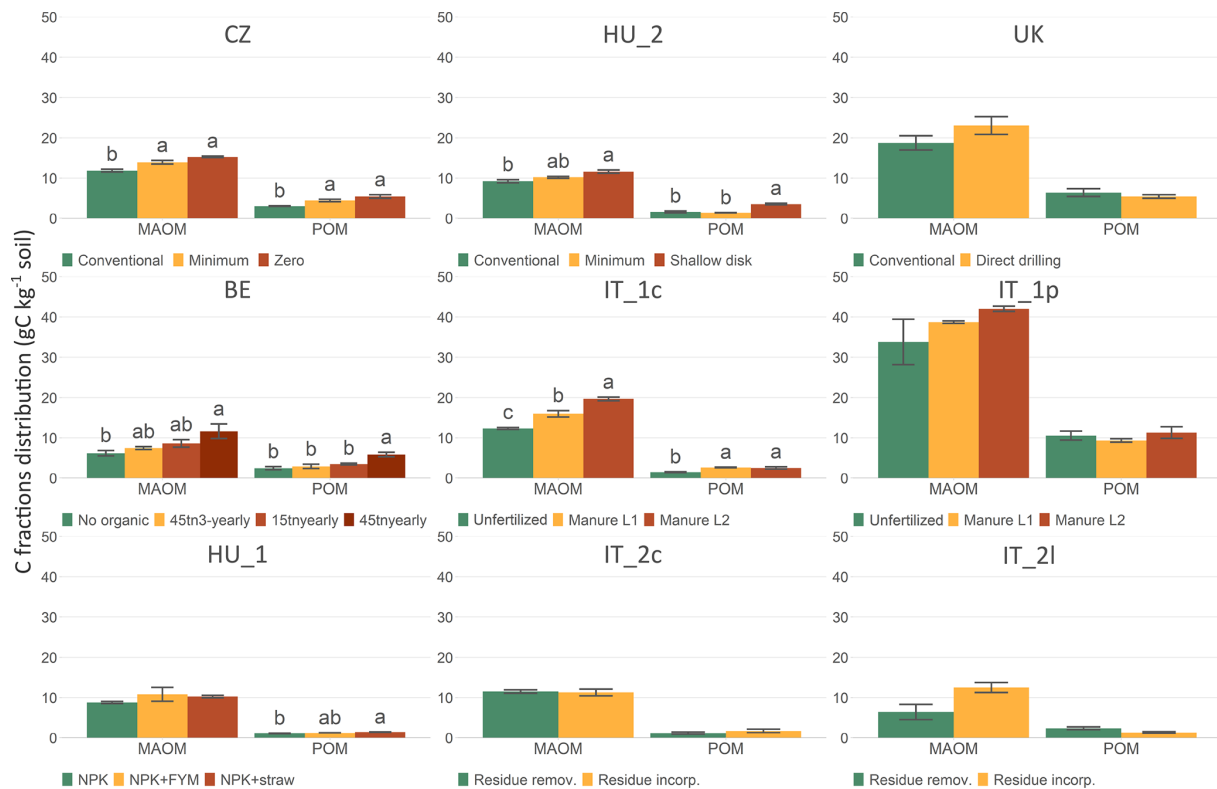
**Figure 3.** Mean weight diameter (MWD) of sand free aggregates in the topsoil (0–15 cm) for each study site. (See also Table 1 for the codes and a description of the treatments.) The error bars represent the standard error. Within an experiment, bars with a different letter differ significantly according to Tukey's test ( $p < 0.05$ ).

Generally, it was observed that inversion tillage in CZ, HU\_2, and UK and less organic inputs in BE, IT\_1, HU\_1 and IT\_2, deteriorated the topsoil aggregate stability and decreased the topsoil SOC. Moreover, inversion tillage and less organic inputs led to fewer large macroaggregates with no profound differences in the OC content within them, and mainly no differences in the macroaggregates quantity but a significantly lower OC content in these macroaggregates. Finally, these practices resulted in more microaggregates, but carbon depleted, and a small increase in the s&c fraction weight but with reduced OC content.

The results obtained after soil separation into aggregates and the determination of their OC content confirm our first hypothesis that inversion tillage reduces the mass of water-stable large macroaggregates (Fig. 5) and the SOC stabilized in macroaggregates and microaggregates (Fig. 6) including the mM (Fig. 7). However, as also observed in some other studies (Modak et al., 2020; Piazza et al., 2020), we did not observe a decrease in the mass of macroaggregates under inversion tillage as initially hypothesised. The tillage systems that reduced soil disturbances to a minimum or zero level and avoided excessive soil inversion (e.g. CZ, UK and HU\_2) improved the topsoil stability, increased the mass of large macroaggregates, did not affect the mass of the macroaggregates and reduced the mass of all other fractions. They

increased the OC in all fractions in the 0–15 cm soil layer. The mass decrease in the large macroaggregates and the reduction in the SOC in the aggregates fractions in conventionally tilled soils has been presented in several studies (Devine et al., 2014; Jat et al., 2019; Mikha and Rice, 2004; Sheehy et al., 2015), but most of them also found a reduction in the macroaggregates mass (Jat et al., 2019; Mikha and Rice, 2004; Mondal and Chakraborty, 2022; Plaza-Bonilla et al., 2010; Song et al., 2019; Zheng et al., 2018). This can be linked to the seasonal soil aggregate stability variation depending on the soil management, climatic conditions, root stage development, microbial activity and freezing–thawing processes (Dimoyiannis, 2009; Yang and Wander, 1998; Batista et al., 2022; Jirků et al., 2010), which favoured the formation and preservation of large macroaggregates at the expense of macroaggregates.

Our second hypothesis dealt with the addition of exogenous material which already underwent decomposition before the application on the soil, in fields in BE, IT\_1c, IT\_1p and HU\_1 where conventional inversion tillage was the main soil cultivation practice. The initial hypothesis was that matured exogenous organic material increases the mass of water-stable macroaggregates and SOC in all aggregates fractions even if combined with inversion tillage. Indeed, under most conditions, adding matured exogenous organic ma-



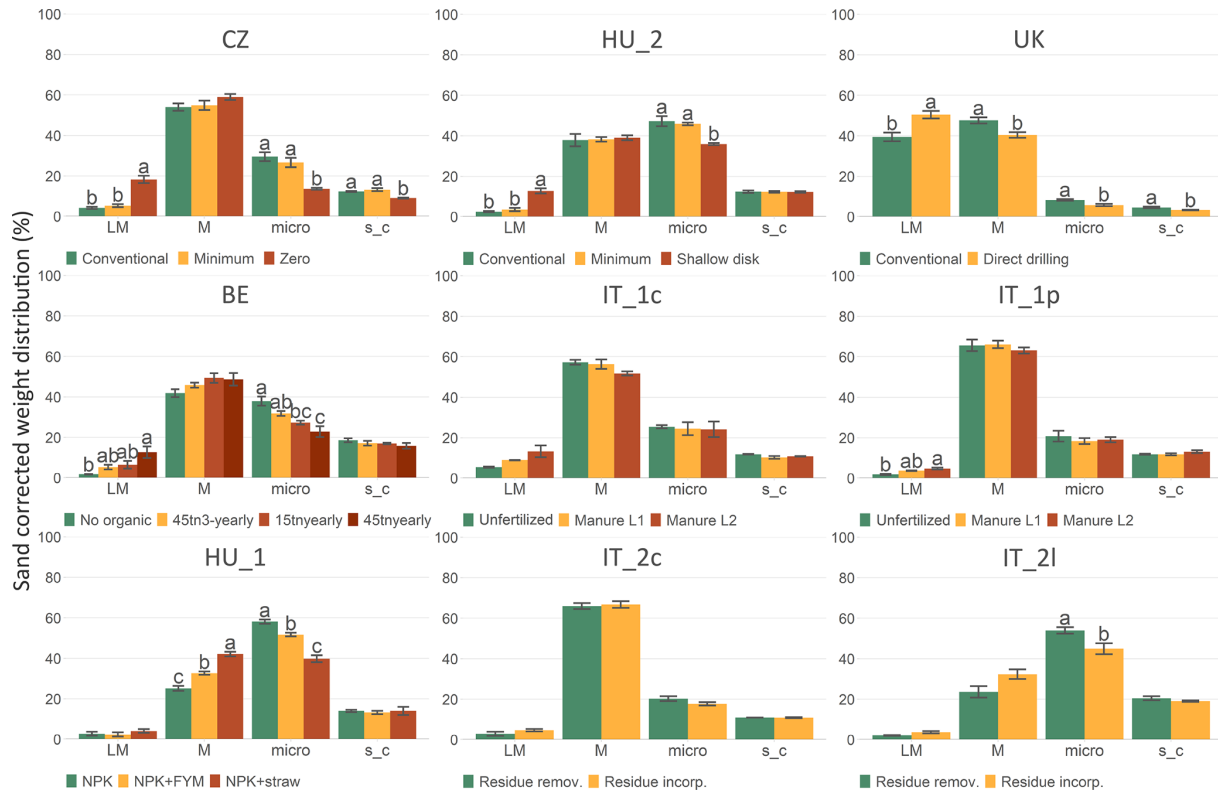
**Figure 4.** Total topsoil (0–15 cm) SOC separated in its components for each study site (see Table 1 for the codes and a description of the treatments). MAOM stands for mineral associated organic carbon and POM for particulate organic carbon. The error bars represent the standard error. Within an experiment and fraction, bars with a different letter differ significantly according to Tukey's test ( $p < 0.05$ ).

terial, such as compost or manure, increased substantially the OC content within all aggregate fractions as well as the total OC in the topsoil, even if combined with inversion tillage (De Clercq et al., 2016; Lin et al., 2019; Mikha and Rice, 2004; Zhang et al., 2021). Interestingly, we did not observe an increased mass of water-stable macroaggregates as initially hypothesized and demonstrated in previous studies (Mikha and Rice, 2004; Wen et al., 2021; Wortmann and Shapiro, 2008). Results similar to ours were obtained by Lin et al. (2019) and Zhang et al. (2021) who even observed a reduction in the macroaggregates and an increase in the large macroaggregates after manure fertilization. Nevertheless, addition of exogenous organic material increased the mass of the large macroaggregates as also observed by Mikha and Rice (2004) which, despite being a small fraction, is important for maintaining a good soil structure. The presence of macroaggregates controls the distribution of macro-pores that influence the water flow particularly near the soil surface. It is also associated with better pore connectivity, movement and storage of gases, water, heat and nutrients, enhanced biological activity and root penetration.

The addition of exogenous organic sources had a pronounced impact on soil structure and SOM regardless of the tillage intensity as also observed by Gross and Glaser (2021). On the other hand, the effects of crop residue left on the soil

or incorporated into the soil varied among the different study sites. Based on our findings in all experiments sampled in our study that dealt with residue management, our third hypothesis that incorporation of the crop residues will lead to an increase in the mass of macroaggregates and the occluded POM cannot be supported. Even if the stability of the soil improved, with several experiments to present statistically significant increase in the MWD as also observed by Modak et al. (2020) and mass of macroaggregates, the corresponding SOC contents and the total SOC were not affected positively by the incorporation of fresh organic materials as had been reported by Powlson et al. (2011). Similar results were obtained by Lin et al. (2019) while other studies demonstrated a parallel increase in aggregates mass (large macroaggregates, macroaggregates and microaggregates) and respective OC contents (Fuentes et al., 2012; Karlen et al., 1994; Zhao et al., 2018). This may be because the exogenous organic materials (compost in BE or manure in IT\_1) were at least partially decomposed and therefore in a more stable form (more resistant soil organic matter) (Berti et al., 2016) and of a higher quality (low C/N ratio) (Castellano et al., 2015). In contrast, the crop residues were not previously decomposed, and they decomposed fast when applied to the soil (Struijk et al., 2020).

In addition, there are concerns and evidence (Dignac et al., 2017; Fontaine et al., 2007; Wang et al., 2015) that the addi-



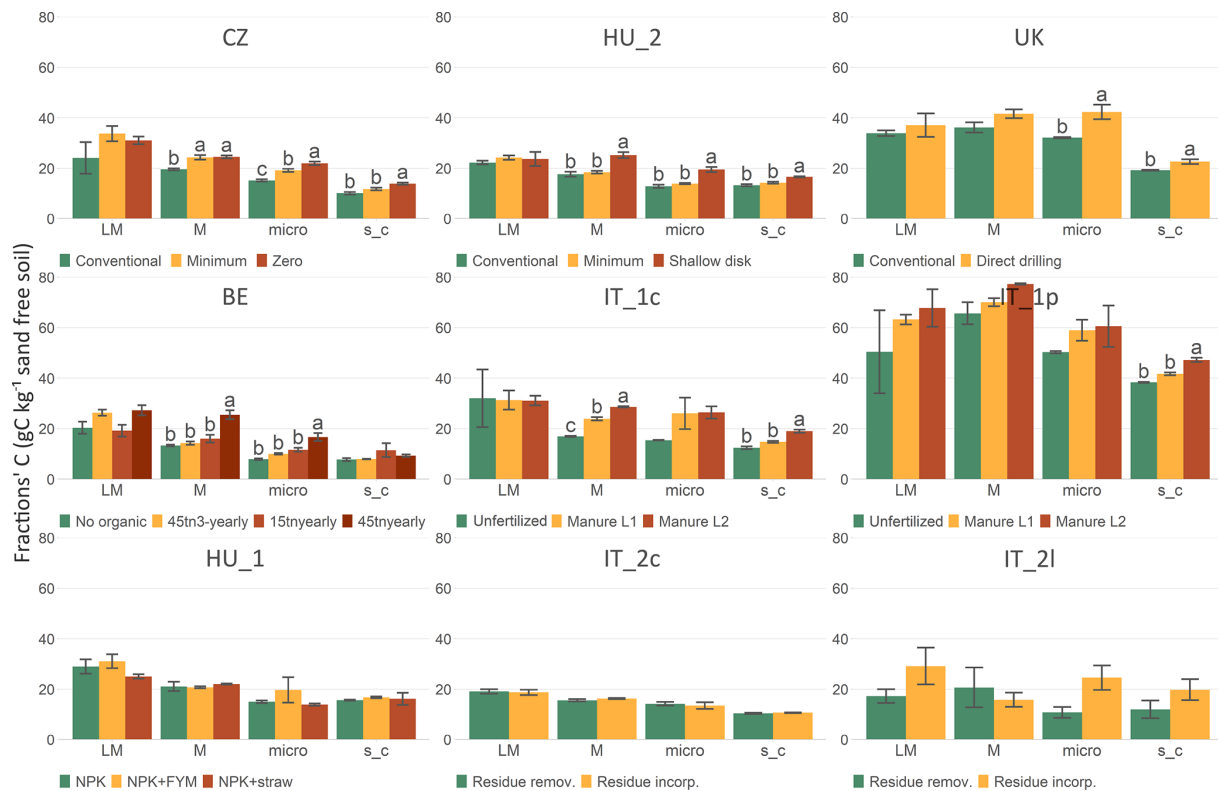
**Figure 5.** Weight distribution of sand free aggregate fractions for each study site. (Codes and description of the practices are presented in Table 1.) The error bars represent the standard error. Within an experiment and fraction, bars with a different letter differ significantly according to Tukey's test ( $p < 0.05$ ).

tion and incorporation of fresh organic material combined with conventional deep ploughing may trigger the microbial demand for carbon. This may lead to increased mineralization and consumption of the old existing OC in the soil (i.e. priming effect). Nevertheless, the priming effect, input quality and decomposition rate seem to play a role only in the short term (days to year), as in the long term (decades) there is evidence from Cardinael et al. (2015) and Thomsen et al. (2013) that does not show differences in the SOC retention, regardless of the initial organic material quality. The combination of residue retention with zero (Pu et al., 2019), minimum tillage, or shallow ploughing could be more effective for SOC storage increase even in the short term ( $< 6$  years) (Li et al., 2020) compared with conventional tillage practices (Dal Ferro et al., 2020; Fuentes et al., 2012; Luo et al., 2010; Xu et al., 2019).

Reduced or no tillage keeps fresh organic material concentrated on the soil surface or in the topsoil (Andruschkewitsch et al., 2014; Virto et al., 2012) and keeps the roots intact increasing the POM concentration. Commonly, the roots contribute more to SOC storage than the above-ground inputs, due to an approximately double efficiency in conversion into stable SOC compared with above-ground biomass (e.g. crop residue) (Berti et al., 2016; Kätterer et al., 2011; Rumpel and

Kögel-Knabner, 2011). Reduced topsoil disturbances promote aggregation and subsequently provide adequate time for the added fresh POM to become protected within large aggregates and further to become stable in the occluded microaggregates (Six et al., 2000a). These processes lead to reduced mineralization not only in the zero tillage systems but also under reduced tillage in several cases (Chen et al., 2019; Ghimire et al., 2017).

An important aspect is to unravel the soil processes responsible for long-term OC storage and, more importantly in the agricultural sector, the SOC components that are sensitive to management changes. This allows identification of the changes of management effects, which could be either positive or negative towards a desired soil composition and structure. The physically protected OC within the microaggregates fraction has been identified as the second most protected after the OC chemically bonded to the soil mineral particles. A linear negative relationship between the microaggregates fraction weight percentage and their carbon content was detected in this study ( $F$ -stat: 15.61;  $p$  value: 0.000171), as also found in agricultural or native soils (Six et al., 2000b) under different levels of disturbances and organic material addition. This indicates that the increasing cultivation intensity and the lack of organic materials lead to a loss of carbon-



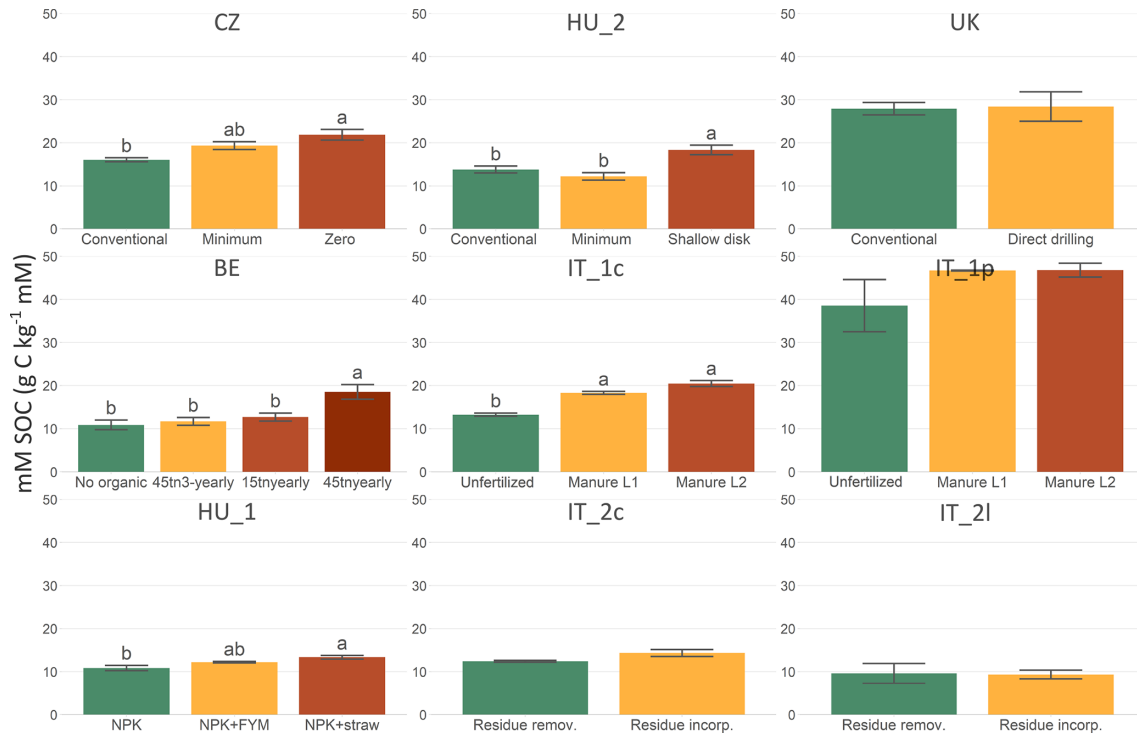
**Figure 6.** The SOC content in each aggregate fraction expressed in a sand free soil for each study. (Codes and description of the practices are presented in Table 1.) The error bars represent the standard error. Within an experiment and fraction, bars with a different letter differ significantly according to Tukey's test ( $p < 0.05$ ).

rich macroaggregates and to an increase in microaggregates which are, however, C-depleted.

In most of the investigated experiments, the mass of both the macroaggregates (except for UK and HU\_1) and occluded microaggregates were not affected by the different management practices or their intensities. On the other hand, the total SOC ( $\text{g C kg}^{-1}$  soil), macroaggregate C and mM-C ( $\text{g C kg}^{-1}$  mM) were in most cases positively affected when the tillage was reduced, or when more organic materials were added. Therefore, we can partially reject our fourth hypothesis stating that increased organic inputs and reduced soil disturbances will lead to more mM and corresponding SOC (mM-C). Increased SOC inputs and lower soil disturbances indeed led to more mM-C but not necessarily to more water-stable mM. The parallel change of the occluded microaggregates with the corresponding OC content (i.e. more mM-C when the mM are increasing) has been proposed by Six et al. (2000a) and supported by several other studies (King et al., 2019; Modak et al., 2020; Piazza et al., 2020; Zhao et al., 2018); however, our findings do not support this hypothesis. Similarly, Andruschkewitsch et al. (2014) and Denef et al. (2007) also concluded that there is no concomitant change in specific cases. Thus, the relationship between mass of occluded microaggregates and corresponding OC, as well as

the applicability of this concept to different soils and under various management practices, should be further evaluated.

In the experiments with significant differences in the mM-C ( $\text{g C kg}^{-1}$  mM) (Fig. 7), the differences were reflected by the different occluded carbon components, i.e. mM-MAOM ( $\text{g MAOC kg}^{-1}$  mM) and mM-POM ( $\text{g POC kg}^{-1}$  mM) (Fig. 8), as well as the total SOC content ( $\text{g C kg}^{-1}$  soil) (Fig. 4). In the treatments in which the soil was less disturbed compared with those that were under conventional tillage, both the MAOM and POM were higher, as also found by Modak et al. (2020). Taking into account that the quality of the macroaggregates and the occluded microaggregates depends on the POM (Six et al., 1998), which is trapped initially within the macroaggregates, together with the above-mentioned results on their mass we suggest that the total soil OC content not dependent on the macroaggregates quantity but rather on their quality in terms of POM which is trapped within them. In the experiments which included addition of organic materials like manure or compost, an increase was found only in the MAOM component. In contrast, in the experiments that incorporated the fresh residues in the soil, no changes in the MAOM nor in the POM were identified. POM is mainly plant derived and has been proven in other studies to increase when the residues are retained or



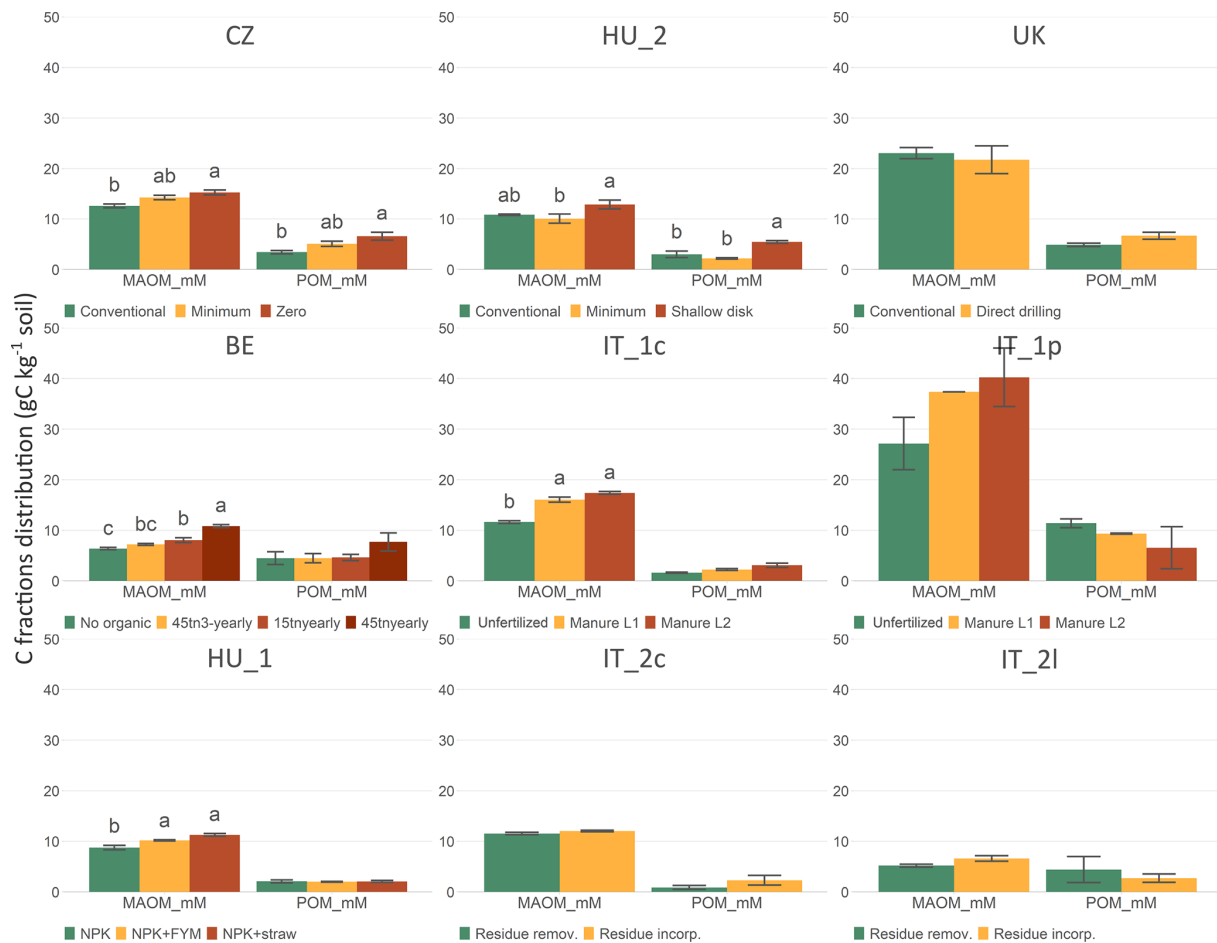
**Figure 7.** The SOC content in the microaggregates held within the macroaggregates fraction (mM-C). (Codes and description of the practices are presented in Table 1.) The OC content is expressed in g C per kg of occluded microaggregates. The error bars represent the standard error. Within an experiment, bars with a different letter differ significantly according to Tukey's test ( $p < 0.05$ ).

incorporated in the soil (Modak et al., 2020) or if manure is applied (Wen et al., 2021). In our research these practices were combined with inversion conventional tillage, and thus we suggest that the time that the macroaggregates remain intact is crucial for the labile POM to stabilize within the occluded microaggregates.

An important difference found between exogenous (matured) material and fresh crop residue in our results is that the first can possibly counteract the inversion tillage effects on topsoil OC while the latter cannot. In the case of matured exogenous organic material, as in BE there is an increasing trend of SOC with increasing compost doses under conventional tillage, which is visible throughout all treatments but is only statistically significant at the highest dose. This does not mean that there is no effect at lower doses. However, because of the great field variability, statistical differences are often difficult to demonstrate. That is the reason why the extremely high dose for Belgium of  $45 \text{ Mg ha}^{-1}$  compost to the plots every year has been included, even if from a practical point of view it is not feasible to be implemented in large-scale agricultural fields. Apart from the impracticability at field scale, though, the application of high doses of organic fertilizers is not always preferred. It may lead to several other negative effects, such as leaching or accumulation of nutrients with subsequent negative effects on plants, soil, water, microorganisms and climate change mitigation (Song et al.,

2017; Lazcano et al., 2021). In the case of IT\_1 both levels of manure application in the clay soil increased the total soil OC when compared with the unfertilized field (no nitrogen of any nature). Again, the option of not fertilizing the fields is neither preferred nor common in the current agricultural systems. It should be stressed that, even if fields under mineral fertilization (NPK fertilization but no manure) had been compared with fields that received manure, differences in the OC content probably will not be noticeable, as in Italy the mineral fertilization alone seems not able to maintain the SOC levels (Lugato et al., 2010).

Our results coming from the analysis of diverse cropping systems across five European countries give strong indications about the effects of the common factor, i.e. the tillage method in the topsoil structure and its dynamics. Nevertheless, our analysis focused only on the top 15 cm soil layer, and this is a limitation that should not be overlooked in the interpretation of the results. The concentration of the residues in the topsoil and the lack of mixing of these residues because of reduced or no tillage throughout the deeper soil horizons have been linked to constraints regarding the maintenance of OC level in the deeper soil horizons. Some authors claim that the SOC balance over the whole soil profile remains unchanged (Blanco-Canqui and Lal, 2008; Chen et al., 2019; Haddaway et al., 2017) or even decreases (Meurer et al., 2018). Nevertheless, there is recent evidence that the adop-



**Figure 8.** The OC content in each SOM component in the mM size fraction in each study site. (Codes and description of the practices are presented in Table 1.) The OC content is expressed in g C per kg of occluded microaggregates. The error bars represent the standard error. Within an experiment and fraction, bars with a different letter differ significantly according to Tukey's test ( $p < 0.05$ ).

tion of zero or reduced tillage systems also maintains better the existing SOC levels in the deeper horizons (Cooper et al., 2021; Varvel and Wilhelm, 2011), so increasing the levels of SOC in the topsoil and keeping the subsoil SOC content unchanged leads to a net SOC increase in the system (Cooper et al., 2021).

Based on the above-mentioned results, we agree partly with the suggestion of Chenu et al. (2019) that the best option to increase SOC is to increase the inputs as opposed to decrease the outputs through mineralization. However, we need to stress that a combination of increased organic inputs coupled with less intensive tillage methods that will stop or reduce the further release of SOC seems more optimal and realistic. Adding the parameter of reduced tillage intensity, the time required for the microaggregates to be formed properly within the macroaggregates and the OC to get stabilized within them is considered. In addition, most probably by reducing the depth and intensity of tillage, the priming effect will be weakened in systems with the addition of fresh OM, leading to higher SOC stocks. This proposal should be val-

idated with further studies including factorial treatments of several tillage intensities and different organic materials, ideally over broad environmental conditions.

## 5 Conclusions

We analysed seven long-term experiments in five European countries to evaluate soil management practices that could inverse the negative effects of inversion conventional tillage on topsoil structural stability and consequently the SOC content. Following testing several hypotheses and considering the limitation of the sampling depth we conclude that:

- Inversion tillage reduces the mass of topsoil water-stable large macroaggregates, and the OC stabilized in the macroaggregates and microaggregates, and this effect increases with increasing tillage intensity, but it does not affect the mass of macroaggregates.
- Addition of matured exogenous organic materials increases in most cases the topsoil soil OC content in all

aggregate fractions even under inversion tillage, but it does not increase the mass of water-stable macroaggregates.

- Incorporation of the previous crops residues into the soil does not increase the mass of the topsoil water-stable macroaggregate nor the occluded POM and does not influence in a consistent way the OC content in the occluded nor in free microaggregates.
- Increased organic material inputs and reduced soil disturbances do not lead to more water-stable occluded microaggregates, but they do lead to increased OC within them (mM-C).

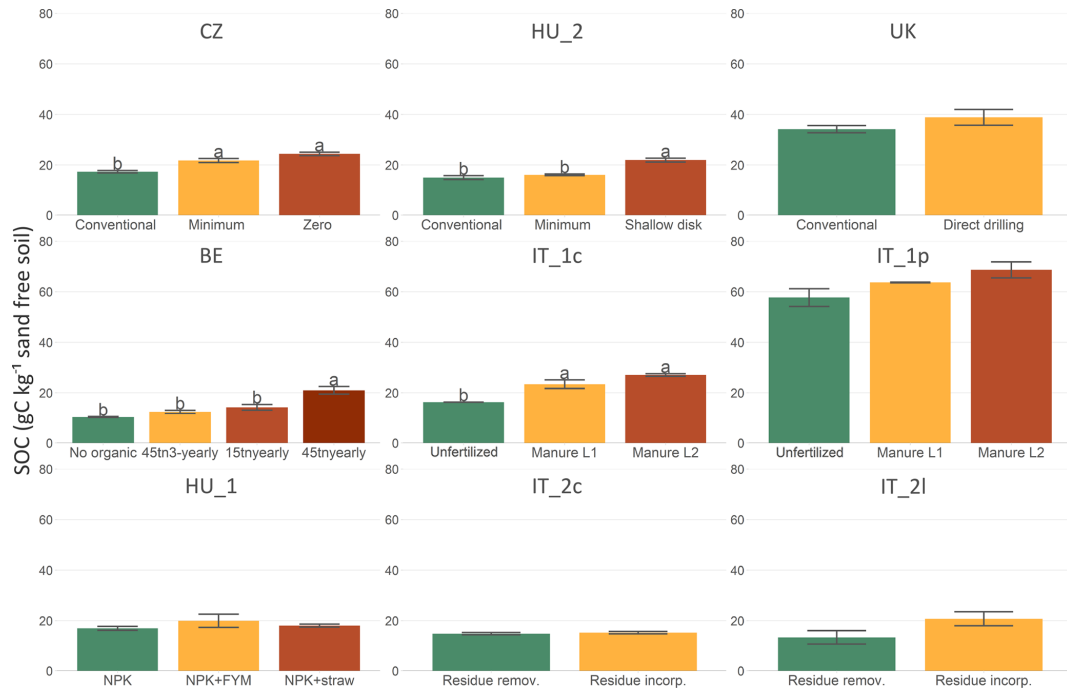
We propose that conventional inversion soil tillage affects topsoil structure in a two-fold way: (i) mainly by disrupting the aggregation cycle, thus limiting the time for the development of a stable structure and, subsequently, the time for SOM stabilization within the occluded microaggregates, and (ii) by increasing the decomposition of OM, thus limiting the OM that is available to promote aggregation. Therefore, we suggest that there are two ways to protect and/or mitigate soil degradation caused by conventional inversion tillage in the topsoil: (i) to reduce the tillage intensity and allow for the soil structure to build and to stabilize SOM, and (ii) to increase the OM inputs, combined with non-inversion tillage practices, in order to increase the SOC stocks and diminish the decomposition of old OC. By these ways, the topsoil structural stability, as well as the quality of the topsoil in terms of OC content, improves with a subsequent positive effect on the topsoil functions.

#### **Appendix A: Study sites details and supporting data**

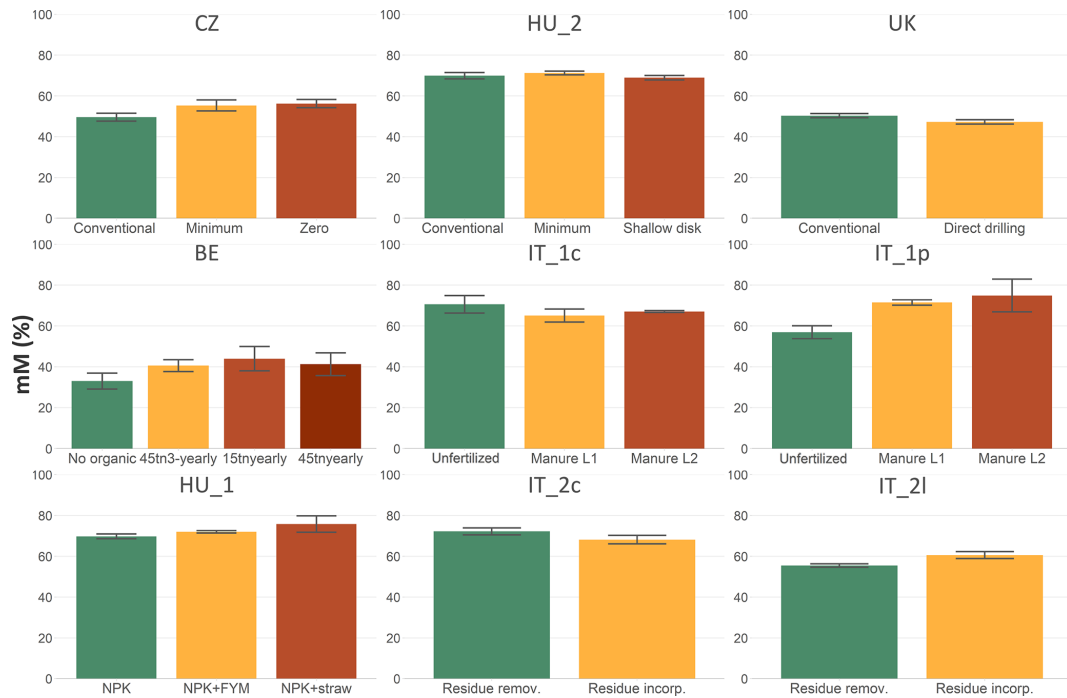
**Table A1.** Description of the study sites and experiments. Table taken from Panagea et al. (2021).

Code	Town, country	Coordinates (decimal degrees)	Agro-climate zone (Ceglár et al., 2019)	Start of experiment	Soil type	Name of experiment	Reference
CZ	Prague-Ruzyně, CZ	50.0880 14.2980	Continental	1995	Silt loam	Tillage trial	Mühlbachová et al. (2015)
HU_2	Keszthely, HU	46.7346 17.2302	Pannonian	1972	Silt loam	Soil tillage systems in wheat and maize bi culture	Hoffmann and Kismányoky (2001)
UK	Loddington, UK	52.6089 0.83257	Maritime north	2011	Clay loam	Soil biology and Soil health	–
BE	Bierbeek, BE	50.8244 4.79605	Maritime north	1997	Silt loam	VFG compost trial	Tijs et al. (2014)
HU_1	Keszthely, HU	46.7332 17.2295	Pannonian	1983	Silt loam	Organic and inorganic fertilization trial (IOSDV)	Kismányoky and Tóth (2013)
IT_1c	Legnaro, IT	45.3506 11.9497	Maritime south	1964	Silty clay loam	Organic and mineral fertilization trial	Giardini (2004)
IT_1p	Legnaro, IT	45.3506 11.9497	Maritime south	1964	Peat 18% OC initially	Organic and mineral fertilization trial	Giardini (2004)
IT_2c	Legnaro, IT	45.3507 11.9498	Maritime south	1970	Silty clay loam	Nitrogen fertilization and crop residue trial	Giardini (2004)
IT_2l	Legnaro, IT	45.3507 11.9498	Maritime south	1970	Silt loam	Nitrogen fertilization and crop residue trial	Giardini (2004)





**Figure A1.** Topsoil (0–15 cm) organic carbon expressed in sand free soil for each study site. The OC content is expressed in g C per kg of sand free soil. The error bars represent the standard error. Within an experiment, bars with a different letter differ significantly according to Tukey’s test ( $p < 0.05$ ).

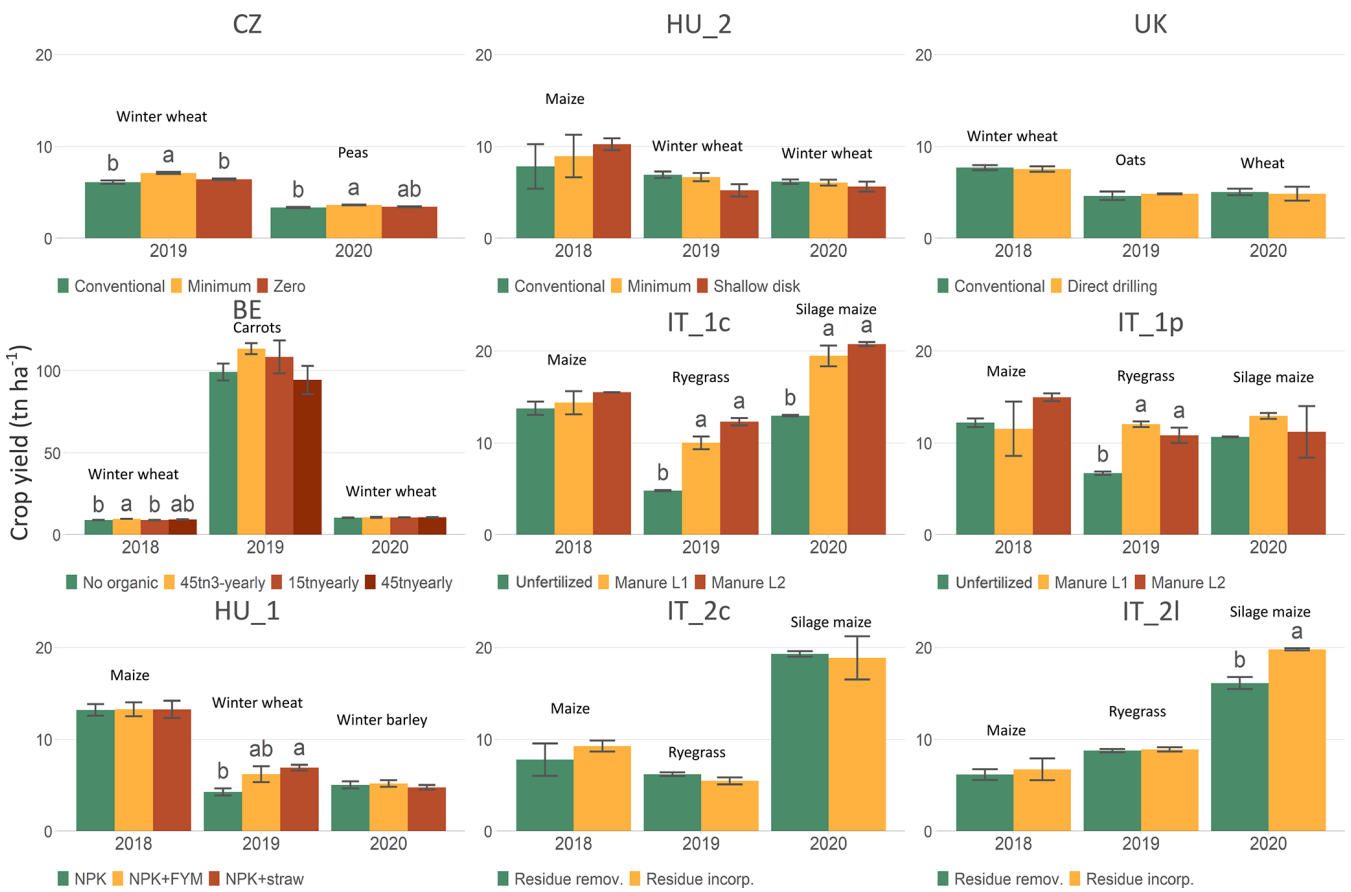


**Figure A2.** Mass percentage of the occluded microaggregates within the macroaggregates (mM). The mM mass is expressed as percentage of macroaggregates. The error bars represent the standard error. Within an experiment, bars with a different letter differ significantly according to Tukey’s test ( $p < 0.05$ ).

**Appendix B: Crop yield results**

The different treatment categories did not present specific patterns or consistent differences when it came to crop yield (Fig. B1). In the tillage experiments, only the treatments in the CZ experiment caused statistically significant differences. Specifically, both in 2019 and 2020 the minimum tillage yielded more than the conventionally tilled field. Zero tillage did not cause a yield loss when compared with conventional tillage. Addition of manure caused a statistically significant increase in the IT\_1c and the IT\_1p (only in 2019) when compared with the unfertilized plots. Addition of compost in BE did not cause significant differences in yields. Finally, the incorporation of residues created only a small increase in individual cases such as during 2019 in HU\_1 and 2020 in the IT\_2l experiments.

None of the practices evaluated in this research caused a yield reduction opposing the results of Song et al. (2019), and this offers opportunities for a possible adoption from farmers, but our results also showed that there is little potential for adopting practices that improve the topsoil SOC and stability, to increase the crop yields. Individual similar field experiments have shown a positive relationship between SOC and crop yield (D’Hose et al., 2014); however, similar to our results Vonk et al. (2020) found a poor association between yield and SOC for common crops in Europe and highlighted the need for a diversified strategy when it comes to motivating farmers to increase their field SOM.



**Figure B1.** Crop yield in each study site. The error bars represent the standard error. Within an experiment and year, bars with a different letter differ significantly according to Tukey’s test ( $p < 0.05$ ).

**Data availability.** All raw data are available in the SoilCare community in Zenodo repository (<https://doi.org/10.5281/zenodo.7126484>, Panagea, 2022).

**Author contributions.** ISP conceptualized the work, designed the methodology, did the soil sampling, analysis and visualization. ISP, AA and GW wrote the original draft. JP, JD, MT, AE, IP, AB, CS, ZT, HK, JB and PČ wrote, reviewed and edited the manuscript. GW, JD and JP were the supervisors of the project. GW was the project coordinator and provided funding acquisition. All authors have read and agreed to the published version of the manuscript.

**Competing interests.** The contact author has declared that none of the authors has any competing interests.

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