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SHORT COMMUNICATION

Sandy Soil Amended with Clay Soil: Effect of Clay Soil Properties on Soil Respiration, Microbial Biomass, and Water Extractable Organic C

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Abstract
The low organic C (OC) sequestration and low water and nutrient holding capacities of sandy soils can be improved by amendment with clay soils. However, the effect of clay soils may depend on clay soil properties, including cation exchange capacity (CEC) and clay and OC contents. Two experiments were carried out. In the first experiment, sandy soil was mixed with two high clay content soils (Sodosol and Vertosol) at 10% and 40% (w/w). The soil was left unamended or amended with wheat straw, to assess if the effect of clay soil addition was influenced by OC addition. In the second experiment, sandy soil was mixed with two low-clay content soils which differed in CEC without wheat straw amendment. In experiment 1, compared with sandy soil alone, amendment with Sodosol (low OC) reduced cumulative respiration more strongly than addition of Vertosol. Without wheat straw addition, both clay soils increased microbial biomass C (MBC), whereas with wheat straw, they reduced microbial biomass C (MBC) compared with sandy soil alone. Sodosol amendment reduced water-extractable OC (WEOC) without wheat straw but increased it with wheat straw. In experiment 2, only the high CEC clay soil reduced cumulative respiration, MBC and WEOC compared with sandy soil alone. Clay soil addition to sandy soil will result in the greatest OC sequestration if the added soil has high clay but low OC content and its CEC is high.

Keywords Clay · Sandy soil · Soil respiration · MBC · WEOC

1 Introduction

Sandy soils often have low organic C (OC) content because of poor plant growth, thus low input and rapid OC decomposition rates. Plants often grow poorly in sandy soils because they are limited by water and nutrient availability (Walpola and Arunakumara 2010). Due to the low cation exchange capacity (CEC), organic C (OC) remains readily available for decomposing microbes. The low plant input and high decomposition rates result in low OC content of sandy soils (Wada 1996; Strong et al. 2004). Clay soils, on the other hand, are characterised by a high proportion of micropores and high CEC, thus high water and nutrient retention and OC binding capacity (Baldock 2007; Hamarashid et al. 2010; Hassink 2016). Addition of clay soils to sandy soils has been shown to increase crop growth and OC content (Davenport et al. 2011; Schapel et al. 2017; Ye et al. 2019). However, the effect of clay soil addition on OC content has been variable. Field studies by Schapel et al. showed that OC content in clay-amended soils was influenced by clay clod size and distribution (Schapel et al. 2018, 2019). To maximise the effect of clay soil addition to sandy soil on OC sequestration, the effect of other factors that could influence the capacity for OC binding such as CEC and clay amendment rate also need to be evaluated.

The aim of this study was to study the effect of addition of clay soil to sandy soil on soil respiration, microbial biomass C and water-extractable OC with respect to clay soil OC content, CEC and amendment rate. Two experiments were carried out: the first, sandy soil was amended at 10% and 40% (*w*/*v*) with two soils with high clay content, and the second, with two soils with low clay content. The soils also differed in OC

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content and CEC. In the first experiment, soil was left unamended or amended with wheat straw, to assess if the effect of clay soil amendment was influenced by OC addition. The soils in the second experiment remained unamended. The first hypothesis was that without wheat straw, clay soil addition will reduce soil respiration, microbial biomass C (MBC) and water-extractable OC (WEOC), but the reduction will depend on OC content and CEC, i.e. less reduction with high OC clay soil, because clay soil OC is decomposed, and stronger reduction with high CEC clay soil because OC is more strongly bound. The second hypothesis was that with wheat straw addition, mixing clay soil with sandy soil will reduce soil respiration, MBC and WEOC because added OC will bind to clay reducing accessibility for microbes. And the third hypothesis was that the effect of clay soil amendment will be greater with higher addition rate.

2 Materials and Methods

2.1 Soils

Sandy soil, used in both experiments, was collected from Penola (37.37° S, 140.83° E) after wheat harvest and was air-dried and sieved (2-mm sieve) to remove organic material/roots. In this area, sandy soils have been amended with clay on several properties. For clayey subsoils for the first experiment, Vertosol was collected under pasture on Waite Campus (34.97° S, 138.63° E), whereas the Sodosol was collected after wheat harvest from Gre Gre, Victoria (36.66° S, 143.14° E). The Vertosol is a grey cracking clay soil; the Sodosol is a brown clay soil (Isbell 2002; Armstrong et al. 2007). In the second experiment, two clayey subsoils with similar clay contents but different cation exchange capacities (CEC) were collected from Chromosols in the southeast region of South Australia after wheat harvest, low CEC at 36.55° S, 140.20° E, high CEC at 37.35° S, 140.42° E. All soils were air-dried and crushed to pass through a 2.0-mm mesh for uniform particle size. For properties of the soils, see Table 1. Compared with the Vertosol, the Sodosol has lower pH, EC, organic C, and CEC.

2.2 Experimental Design

We performed two experiments. In both experiments, clay soils were mixed with sandy soil at 10% and 40% (w/w basis). Then sandy soil alone and mixes with 10% clay were adjusted to 75% and the mixes with 40% clay were adjusted to 45% of maximum water holding capacity (WHC) with reverse osmosis (RO) water. These water contents were selected based on Setia et al. (2011) who found that soil respiration was maximal at these water contents in soils with respective clay contents. The moist soils were incubated for 14 days at 22 °C before the start of the experiments to allow normalisation of soil respiration. These water contents were maintained throughout the experiments by checking the weight every few days and adding water if necessary.

In experiment 1, sandy soil alone and 10% or 40% mixes with Vertosol or Sodosol were left unamended or amended. For the amended treatments, the soil treatments were thoroughly mixed with 2% (w/w) dry and finely ground mature wheat straw (405 g kg^{-1} total C, 5.5 g kg^{-1} total N, 73 C/N ratio ground and sieved to 0.25–2 mm particle size). Treatments were incubated for 48 days. In experiment 2, sandy soil alone or 10% or 40% mixes with low or high CEC clay soil were incubated for 30 days without straw addition.

Thirty grammes of soil (dry weight equivalent) was filled into polyvinyl cores (PVC, 3.7-cm width and 5.0-cm height) with a nylon mesh base (0.75 μm, Australian Filter Specialist Pty Ltd., Huntingwood NSW, Australia) and packed to a bulk density of 1.3 g cm^{-3}. The cores were transferred into 1-L Mason glass jars after which the jars were sealed with gas-tight lids equipped with septa to allow quantification of the CO_2 concentration in the headspace (Setia et al. 2011). The jars were incubated in the dark at 20 to 24 °C. Each treatment was replicated four times and followed a completely randomised experimental design in both experiments.

2.3 Analytical Methods

Soil respiration was measured with an infrared analyser as described (Servomex 1450) by Setia et al. (2011). Due to

Table 1 Physico-chemical properties of soils

Experiment	Soil	Sampling depth (cm)	$pH_{1:5}$ (H_2O)	$EC_{1:5}$ (H_2O) (dS m^{-1})	Total organic C (g kg^{-1})	Total N (g kg^{-1})	Clay content (g kg^{-1})	CEC (cmol kg^{-1})
1 & 2	Sandy soil	0–15	5.5 ± 0.3	0.67 ± 0.2	18.0 ± 3	0.80 ± 0.2	51 ± 20	6.4 ± 0.4
1	Vertosol	30–50	7.5 ± 0.4	2.62 ± 0.5	15.0 ± 2	1.20 ± 0.1	690 ± 50	30.2 ± 2.0
	Sodosol	30–50	5.9 ± 0.2	0.72 ± 0.2	9.8 ± 2	0.68 ± 0.1	729 ± 40	18.5 ± 3.2
2	Low CEC	10–20	6.2 ± 0.3	0.04 ± 0.1	4.0 ± 1	ND	236 ± 15	3.6 ± 0.5
	High CEC	20–30	7.9 ± 0.5	0.32 ± 0.1	2.9 ± 1	ND	281 ± 20	17.7 ± 2.0

Mean values ± standard errors of means ($n = 4$); *ND* not determined the upper detection limit of the gas analyser (2% CO_2) and the decrease in respiration rate over time, soil respiration was measured daily for the first 15 days, every second day until day 30 and every 3 days from day 30 to 48 (in experiment 1). After every measurement, the jars were flushed with air using a fan to refresh headspace air, resealed and then remained closed until the next measurement.

Soil pH and EC were determined in a 1:5 soil/water suspension after shaking on an end-over-end shaker at room temperature for 1 h. Particle size distribution was measured by the hydrometer method (Bouyoucos 1936). Total organic carbon and nitrogen contents in soils and wheat straw were determined by CHN analyser (Carlo-Erba). Cation exchange capacity (CEC) was calculated from the concentration of exchangeable cations (Na^+, K^+, Ca^{2+} and Mg^{2+}) which were determined after removal of the soluble salts by washing 5 g soil with 25 ml of ethanol (60%) as described in Rayment and Lyons (2011). The concentrations of extractable and exchangeable Na^+, K^+, Ca^{2+} and Mg^{2+} in the soil extracts were measured by inductively coupled plasma-atomic emission spectroscopy (ICP-AES, Agilent).

Microbial biomass carbon (MBC) was measured by fumigation extraction as described in Vance et al. (1987). Fumigated and un-fumigated samples were extracted with 0.5 M K_2SO_4 solution at a 1:4 soil to extractant ratio. After filtering through Whatman filter paper no. 42, the organic C concentration of the extracts was determined by titration against 0.033 M acidified ferrous ammonium sulphate after dichromate oxidation (Walkley and Black 1934; Anderson and Ingram 1993). Microbial biomass carbon was calculated by subtracting the organic C content of fumigated from un-fumigated samples and multiplying the difference by 2.64 (Vance et al. 1987).

Water-extractable organic carbon (WEOC) was determined by shaking 5 g moist soil with 20 ml deionised water for 1 h. The extract was centrifuged at 4000 rpm for 10 min and filtered through a Whatman #42 filter paper. The WEOC concentration of the extract was determined as described for MBC.

2.4 Statistical Analysis

Data were tested for the normal distribution to meet the assumptions of analysis of variance (ANOVA) test. After confirmation of normal distribution, the data was analysed by two-way ANOVA with soil treatment × wheat straw addition in experiment 1 and by one-way ANOVA in experiment 2. Tukey's HSD post-hoc test at $P \leq 0.05$ was used to determine which treatments were significantly different. All statistical analyses were performed in SPSS for Windows v. 19.

3 Results and Discussion

3.1 Experiment 1

Cumulative respiration was about sixfold higher with wheat straw addition than that without (Fig. 1a). Without wheat straw, cumulative respiration did not differ between sandy soil alone and sandy soil with Vertosol or sandy soil with Sodosol at 10%. But cumulative respiration in sandy soil with 40% Sodosol was about 70% lower than that in sandy soil alone. With wheat straw, clay soil addition reduced cumulative respiration compared with sandy soil alone; in sandy soil with Vertosol, it was about 10% lower, and with Sodosol, it was about 30% lower with a greater reduction at 40% Vertosol than that at 10%.

Microbial biomass C was two to tenfold higher with wheat straw than that without with the greatest increase in sandy soil alone (Fig. 2a). Without wheat straw addition compared with the sandy soil alone, MBC was about twofold higher with Vertosol and fourfold higher with Sodosol. With wheat straw, MBC was highest in sandy soil alone. It was 10% lower with both clay soils at 10%, about 20% lower with Vertosol at 40% and 40% lower with Sodosol at 40%.

Wheat straw addition increased WEOC, particularly in sandy soil with Sodosol (Fig. 3a). Without wheat straw, addition of Vertosol to sandy soil had little effect on WEOC, but adding Sodosol clay reduced WEOC by about 30% compared with sandy soil alone. With wheat straw, WEOC did not differ between sandy soil alone and sandy soil with 10% clay soils. But the addition of 40% clay soils increased WEOC by 20–30% compared with sandy soil alone, with a greater effect with Sodosol than Vertosol.

The results show that without wheat straw addition, cumulative respiration and WEOC were reduced only by mixing sandy soil with Sodosol. The lack of reduction with Vertosol is likely due to the high OC content of this soil. Decomposition of clay soil OC apparently compensated for reduced accessibility of sandy soil OC and contributed to the release of WEOC. In contrast, Sodosol had a low OC content; thus, binding of sandy soil OC to clay reduced its decomposition. Therefore, the first part of the first hypothesis (without wheat straw, clay soil addition will reduce soil respiration, MBC and WEOC, but there will be less reduction with high OC clay soil, because clay soil OC is decomposed) can only be partially accepted.

However, in contrast to the hypothesis, MBC was higher in sandy soil with clay compared with that in sandy soil alone. This can be explained by the improved microbial habitat. Clay soil addition increased WHC (data not shown) and proportion of water-filled pores which are ideal habitats for microbes when oxygen is supplied through larger pores, in the sandy soil matrix (Roychand and Marschner 2014). In sandy soil alone on the other hand, the predominant large pores would

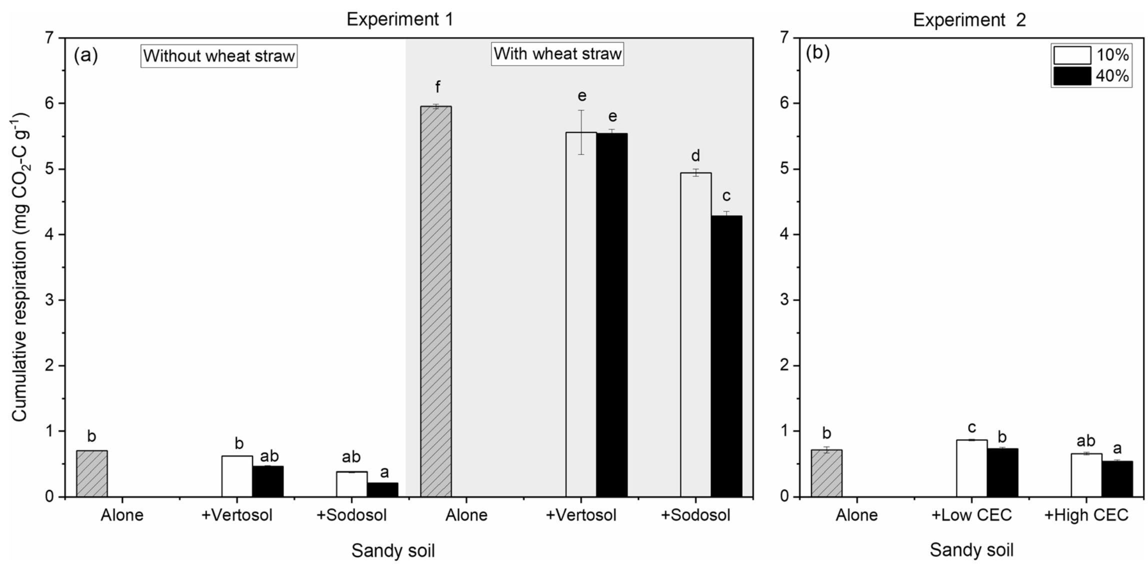

Fig. 1 Cumulative respiration in sandy soil alone or mixed with 10 and 40% of **a** Vertosol or Sodosol clay soils without or with wheat straw in experiment 1 and of **b** low cation exchange capacity (CEC) or high CEC clay soils without wheat straw in experiment 2. Bars represent mean values ± standard errors of means ($n = 4$). In each experiment, bars with different letters indicate significant differences between mean values at $P \leq 0.05$

be air-filled and microbes would be largely confined to water films around sand particles. The greater MBC with Sodosol may be due to the lower CEC of this soil compared with the Vertosol, thus greater OC accessibility. The finding that the measured parameters were little affected by clay soil addition rate indicates that even 10% Sodosol mixed with sandy soil provided sufficient binding sites of sandy soil OC.

With wheat straw, clay soil addition reduced cumulative respiration and MBC but increased WEOC at the 40% addition rate. Therefore, the second hypothesis (with wheat straw addition, mixing clay soil with sandy soil will reduce soil respiration, MBC and WEOC) can be accepted for cumulative respiration and MBC, but not for WEOC. The reduction of cumulative respiration and MBC with clay soil amendment can be explained by binding of added OC to clay which reduced its accessibility to microbes (Vogel et al. 2015; Pal and Marschner 2016; Churchman 2018). The reduction was smaller with Vertosol than that with Sodosol, likely because the Vertosol had a higher OC content. As explained above, microbes would decompose this native OC which can compensate to some extent the reduction of decomposition of OC added with wheat straw. In contrast, amendment with

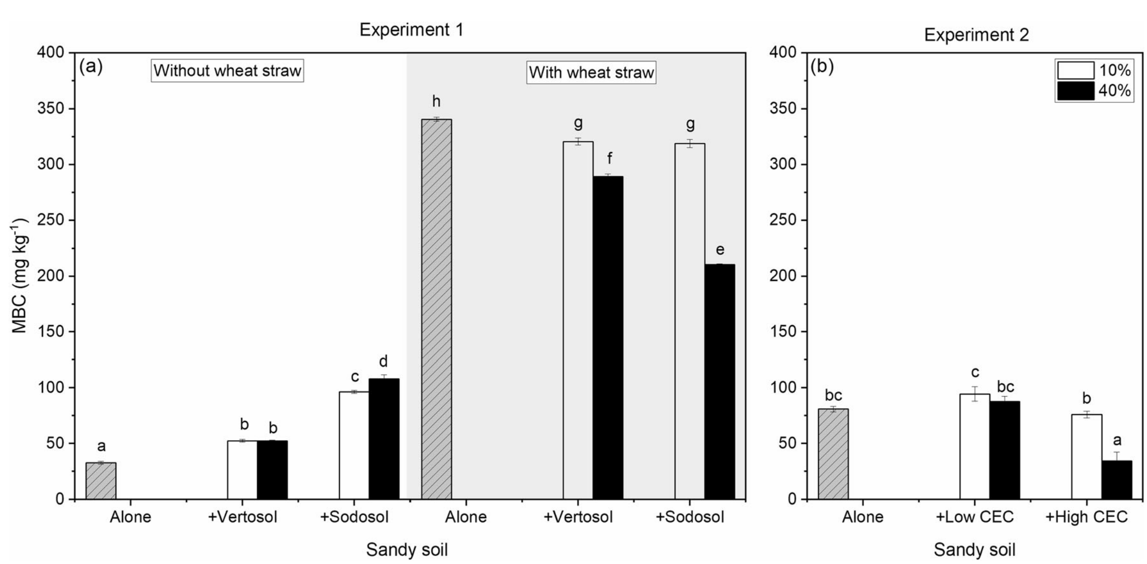

Fig. 2 Microbial biomass C (MBC) in sandy soil alone or mixed with 10 and 40% of **a** Vertosol or Sodosol clay soils without or with wheat straw in Experiment 1 and of **b** low cation exchange capacity (CEC) or high CEC clay soils without wheat straw in experiment 2. Bars represent mean values ± standard errors of means ($n = 4$). In each experiment, bars with different letters indicate significant differences between mean values at $P \leq 0.05$

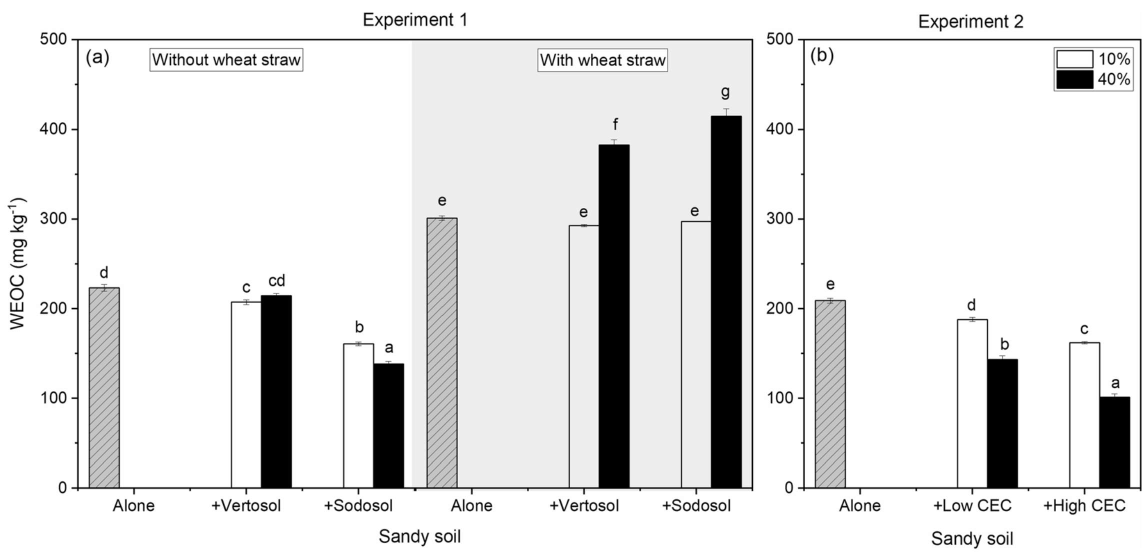

Fig. 3 Water-extractable organic C (WEOC) in sandy soil alone or mixed with 10 and 40% of **a** Vertosol or Sodosol clay soils without or with wheat straw in experiment 1 and of **b** low cation exchange capacity (CEC) or high CEC clay soils without wheat straw in experiment 2. Bars represent mean values ± standard errors of means ($n = 4$). In each experiment, bars with different letters indicate significant differences between mean values at $P \leq 0.05$

Sodosol added little native OC. Thus, the main effect was binding of added OC which strongly reduced respiration and microbial C uptake. The increased WEOC with 40% clay soils compared with sandy soil alone can be explained by the reduced decomposition and microbial uptake which left more of the WEOC released by wheat straw. Furthermore, clay soil addition would increase the abundance of micropores (Roychand and Marschner 2014) where WEOC would be inaccessible to microbes. The higher WEOC with the Sodosol than that with Vertosol may be due to the lower CEC in the former which would reduce the capacity to bind WEOC.

3.2 Experiment 2

Addition of low CEC clay soil or high CEC clay soil at 10% to sandy soil had little effect on cumulative respiration or MBC (Figs. 1b and 2b). But compared with sandy soil alone, addition of high CEC soil at 40% reduced cumulative respiration by about 20% and MBC by 60%. Addition of clay soils reduced WEOC compared with sandy soil alone (Fig. 3b) by 10–25% with low CEC soil and by 15–50% with high CEC soil for both clay soils. The reduction was greater with 40% addition rate than that with 10%.

The second part of the first hypothesis (without wheat straw, clay soil addition will reduce soil respiration, MBC and WEOC and reduction will be smaller with clay soil that has a higher OC content, but greater with high CEC clay soil) can only be partially accepted. Mixing of sandy soil with low CEC clay soil had little effect on cumulative respiration and MBC. As with the Vertosol in experiment 1, this is likely because of the relatively high OC content of this soil. Decomposition of clay soil OC appears to have compensated for the reduction of accessibility of sandy soil OC. Furthermore, binding of OC would be low due to the low CEC of this clay soil. Addition of 40% high CEC soil on the other hand reduced cumulative respiration and MBC compared with sandy soil alone. This can be explained by binding of sandy soil OC to clay and by the low OC content of this soil. This effect was observed only at the 40% addition rate, likely because of the relatively low clay content of this soil (about 280 g kg^{-1}). In contrast to experiment 1 where clay soil addition increased MBC, MBC was lower in sandy soil with 40% high CEC soil than that in sandy soil alone. This discrepancy could be due to the lower clay content of this soil which was half of that of the soils used in experiment 1 (about 700 g kg^{-1}). Mixing of the sandy soil with low clay content soils will have little effect on the microbial habitat because it would increase the abundance of micropores only to a limited extent. The stronger reduction of WEOC in sandy soil mixed with high CEC soil than that when mixed with low CEC soil can be explained by greater binding of OC to the high CEC soil and its lower native OC content.

The third hypothesis (the effect of clay soil addition will be greater at the higher amendment rate) can only be partially accepted as there was little effect of amendment rate on the measured parameters in the first experiment without wheat straw and cumulative respiration was not affected by amendment rate in any of the treatments. However, the effect of clay addition on MBC and WEOC in experiment 1 with wheat straw and in experiment 2 with high CEC soil was greater at the higher amendment rate. The lack of effect of amendment rate on cumulative respiration despite the lower MBC suggests that microbes can compensate for lower biomass by increasing CO_2 release per unit biomass.

This study showed that the effect of clay soil addition to sandy soil on soil respiration, MBC and WEOC depended on clay soil OC content and CEC and whether or not wheat straw had been added. The clay soil addition rate had only relatively small effects. It should be noted that the results of this experiment may not be directly transferable to the field because the added clay soil was finely ground and thoroughly mixed into the sandy soil. In the field, clay soils can be added by spreading of soils from clay pits or, in sandy soils with clay-rich subsoils, by spading or delving (Slattery and Surapaneni 2016). These application methods result in clay soil clods of varying sizes within the sandy soil matrix (Schapel et al. 2019) which will reduce the surface area available for OC binding and result in highly variable pore size distribution.

4 Conclusion

It can be concluded that landholders wishing to maximise organic carbon (OC) sequestration should add a soil that has high clay but low OC content and a high cation exchange capacity. However, the high water-extractable OC in soils amended with wheat straw suggests that clay soil addition may increase the potential for transport of OC within the soil profile.

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