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Clay addition to sandy soil - effect of clay concentration and ped size on microbial biomass and nutrient dynamics after addition of low C/N ratio residue

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Abstract

Addition of clay-rich subsoil to sandy soil has been shown to increase crop production on sandy soils. The added clay is present as peds ranging in size from a millimetre to several centimetre. In this experiment clay soil (73% clay) was added to sandy soil (3% clay) at 10 and 20% clay w/w as 1, 3 and 5 mm peds. Shoots of young Kikuyu grass (C/N 20) were ground and added at 10g kg⁻¹, and soils were incubated for 45 days at 80% of water holding capacity. The study confirmed that clay addition to sandy soil increased soil organic carbon retention but decreased cumulative respiration and available P compared to sandy soil alone. Ped size had little effect on respiration and nutrient availability. Over the course of 45 days peds broke down and organic C was bound to the < 53 µm fraction. The greatest proportion of peds and total organic carbon (54-67%) was in the initially added ped size. The TOC content of < 53 µm fraction of initially added peds was 0.38% and at the end of the experiment (after 45 days) the TOC had increased by 24, 19 and 10% in 1, 3 and 5 mm peds respectively.

Keywords: Claying, organic carbon retention, clay peds, sandy soils

1. Introduction

Sandy soils have low organic matter content, cation exchange capacity (CEC) and therefore low nutrient retention capacity (Walpola and Arunakumara 2010) and low water holding capacity resulting in low yield. Clay-rich soils on the other hand, have high CEC as well as high water and nutrient retention capacity (Hamarashid *et al.* 2010). Addition of clay-rich sub-

soil to sandy soil has been shown to increase crop production on sandy soils (Davenport *et al.* 2006; Hall *et al.* 2010) which is mainly attributed to improved water and nutrient holding capacity (Ismail and Ozawa 2007). The added clay soil may be taken from nearby areas and spread on the surface of the sandy soil. Another option is available on so-called duplex soils which have a sandy to sandy loam A horizon and clayey B horizon (Isbell 2002). In these soils, subsoil clay can be mixed into the sandy topsoil by spading or delving. The added clay is present as peds ranging in size from a millimetre to several centimetre thereby creating a heterogeneous soil with patches of clay-rich soil surrounded by sandy soil.

Clay soil addition to sandy soil may increase nutrient availability if the clay soil is nutrient-rich. However, clay subsoils often have low nutrient content (Jobbágy and Jackson 2001; Lawrence et al. 2015). Therefore nutrients have to be added after delving or spading to maximise the benefits of claying. Nutrient addition can be in the form of inorganic fertilisers or organic amendments such as plant residues, manures or compost. Organic amendments have the additional advantage that they can increase soil organic matter content which further improves water and nutrient retention. Decomposition and nutrient release from organic amendments depends on their properties such as C/N ratio and particle size. Low C/N ratio residue can satisfy the microbial N demand which results in fast decomposition, early net mineralization and increased microbial biomass (Hoyle and Murphy 2011; Yani et al. 2011). Decomposition rate also depends on accessibility of the organic amendment to soil microbes. Sandy soils have few organic matter binding sites (Strong et al. 2004). Therefore organic amendments are decomposed rapidly. Decomposition rate is lower in clay soils because binding of organic matter to clay surfaces or occlusion within aggregates can reduce organic matter accessibility to microbes (Baldock 2007; Pal and Marschner, 2016).

In a previous study (Tahir and Marschner 2016) with sandy soil amended with 1, 2 or 3 mm clay peds and faba bean residues (C/N 37), we found that clay addition reduced N availability but had no consistent effect on cumulative respiration. Ped size had no effect. The experiment described here was conducted to investigate if clay addition has a different effect on respiration and nutrient availability when added as peds with a greater range of sizes (1, 3 and 5 mm) in presence of plant residue with lower C/N ratio (C/N 20). The low C/N residue was used because the effect of clay addition was stronger with this residue in our previous study (Tahir and Marschner 2017b) due to its high decomposition rate and nutrient release compared to the added faba bean residue.

The aims of this study were to (i) determine the effect of clay addition rate and ped size in residue amended sandy soil on nutrient availability, and (ii) assess breakdown of peds during the experiment and organic C retention by $< 53 \mu m$ fraction of the peds.

We hypothesised that (i) the smallest peds (1 mm) will have a greater effect on soil respiration rate and nutrient availability than the 5 mm peds, and (ii) the larger peds will break down during the experiment into smaller peds with each resulting ped size binding organic carbon.

2. Materials and Methods

Clay soil was collected from Waite Campus (34.97°S, 138.63°E), air-dried, crushed and then sieved through different sieves to achieve peds of 1, 3 and 5 mm. Five mm peds were collected on a 3.35 mm sieve after sieving through a 5 mm (size range 3.35-5 mm). The soil that passed through the 3.35 mm sieve was then sieved through a 2 mm sieve; the peds on 2 mm sieve are considered 3 mm peds (size range 2-3.35 mm). The peds passed through 1 mm sieve and collected on 0.5 mm sieve are referred to 1 mm peds (size range 0.5-1 mm). Sandy soil from Penola (37.37°S, 140.83°E) was air-dried and sieved (2 mm sieve) to remove organic material/roots. In this area, many farmers have used clay addition to improve crop growth on sandy soils. The clay soil was added to the sandy soil at 10 and 20% w/w as 1, 3 and 5 mm peds. Shoots of young Kikuyu grass (*Pennisetum clandestinum* L.) (C/N 20) were dried in a fan forced oven, ground, sieved to particle sizes < 2 mm and added a rate of 10 g kg⁻¹ to the sandy soil alone and the soil mixes. Total carbon content in young kikuyu shoots was 341 g kg⁻¹, total N and P were 17.6 and 4.5g kg⁻¹.

After thorough mixing of sand, clay and residues, 30 g dry weight equivalent of the mixture was placed in PVC cores (radius 1.85 cm and height 5 cm) with a nylon mesh (7.5 µm, Australian Filter Specialist) base. The soil was packed to a bulk density of 1.5 g cm⁻³. Water content was maintained gravimetrically at 80% of maximal water holding capacity (WHC) by checking the weight of the cores and adding reverse osmosis (RO) water if necessary. This water content was selected on the basis of a preliminary experiment in which the soil treatments were maintained at different percentage of maximumWHC (40, 50, 60, and 80%). Glucose was added as an organic carbon source at 2.5 g C kg⁻¹ soil and soil respirationwas measured for one week. Cumulative respiration was highest at 80% of WHC in all treatments.

Three destructive harvests were carried out on days 15, 30 and 45, with four replicates per treatment and harvest time. The cores to be sampled on day 15 were placed in 1 L glass jars with gas-tight lids equipped with septa for quantification of soil respiration. The remaining cores were placed in a plastic tray covered loosely with a lid. On day 15, the cores in the jars were removed for analysis and replaced by the cores to be harvested on day 30. This procedure was repeated on day 30. The glass jars and plastic trays were incubated at 23°C in the dark.

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 one hour (Setia *et al.* 2013). Particle size distribution was measured by the hydrometer method (Bouyoucos 1936). The maximum water holding capacity (WHC) of the soils was measured by using a sintered glass funnel connected to a 1 m water column (Ψ_m = -10 kPa) (Klute 1986). Total organic carbon in residue was determined by wet digestion and titration (Walkley and Black 1934). For total N and P, residue was digested with H₂SO₄ and HNO₃ respectively. Total N was determined by a modified Kjeldahl method (Vanlauwe *et al.*1996) and total P was measured by phosphovanadomolybdate method (Hanson 1950).

Due to the upper detection limit of the infrared gas analyser $(2\% \text{ CO}_2)$ and the decrease in respiration rate over time after residue addition, soil respiration was measured daily for the first 15 days, every second day until day 30 and then every three days until end of the experiment using a Servomex 1450 infra-red analyser as described in (Setia *et al.*2011). After every measurement the jars were flushed with air using fan, resealed and then remained closed until the next measurement.

At the three destructive samplings soil pH, microbial biomass C (MBC), P (MBP), N (MBN), available N (NH₄ and NO₃) and P were measured. Microbial biomass C and N were measured by chloroform fumigation extraction as described in (Vance et al. 1987). Fumigated and un-fumigated samples were extracted with 0.5 M K₂SO₄ at a 1:4 soil to extractant ratio. After filtering through Whatman filter paper No. 42, the filtrate was used to determine microbial biomass C and N. For Microbial biomass C, the filtrate was subjected to dichromate oxidation (Anderson and Ingram 1993) followed by determination of the organic C concentration by titration with 0.033 M acidified (NH₄), Fe (SO₄), 6H₂O. Microbial biomass C 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). Microbial biomass N in the 0.5 M K₂SO₄ extracts was measured as NH₄-N colorimetrically at 685 nm as described in Willis et al. (1996). To calculate microbial biomass N the difference between fumigated and un-fumigated samples was divided by 0.57 (Moore *et al.* 2000). Microbial biomass P and available P were determined by the anion exchange resin method Kouno *et al.* (1995) and the P concentration measured colorimetrically at 712 nm following Murphy and Riley (1962). For fumigated samples 1 ml hexanol was added along with water before overnight shaking and samples without hexanol were considered as un-fumigated samples, representing available P.

Available N was extracted by shaking soil with 2 M KCl solution at a soil:solution ratio of 1:5 for 1 hour at 200-300 rpm. The suspension was filtered through Whatman filter paper No. 42. Ammonium was measured colorimetrically at 685 nm following Willis *et al.* (1996). Nitrate in the 2 M KCl extracts was determined colorimetrically at 540 nm as described in Cavagnaro *et al.* (2006).

At the end of the experiment the soil mixes were dried and separated through various sieves to retrieve the peds of different sizes; for example where 1 mm peds were added the soil mixes were passed through a 1 mm and collected on the 0.5 mm sieve. The soil with 3 mm peds was passed successively through 3, 2, 1 mm and collected on the 0.5mm sieve and soil with 5 mm peds was passed successively through 5, 3, 2, 1 mm and collected on the -0.5 mm sieve. The retrieved peds of different sizes were separated into $> 53 \mu m$ and $<53 \mu m$ fractions by wet sieving (Christensen 2001). Organic C in the $< 53 \mu m$ fraction is referred to as mineral-associated organic C (Kögel-Knabner 2000). For fractionation, the soil was dispersed by shaking with 3% sodium hexa-metaphosphate at a soil:solution ratio of 1:13 for two hours at 200-300 rpm. The suspension was sieved through 250 and 53 μ m sieves and dried overnight at 70°C. The initial clay peds were also separated into < 53 μ m and > 53 μ m. The < 53 μ m fraction was ground and analysed for total organic carbon by wet digestion (Walkley and Black 1934).

The data measured once (initial soil properties, properties of retrieved peds) was analysed by one-way ANOVA. The data measured at different sampling timeswas analysed by repeated measures ANOVA using Genstat 15th edition (VSN Int. Ltd, UK). This showed that the interaction time x treatment was significant. Tukey's multiple comparison test at 95% confidence interval was carried out for the time x treatment interaction.

3. Results

The clay soil (73% clay) was alkaline (pH 8.0) whereas the sandy soil (96% sand) was acidic (pH 5.4) (Table 1). Organic C and total N were very low in the sandy soil. The available N and P concentration in clay soil was 21 mg kg⁻¹ and 0.9 mg kg⁻¹ while in sandy soil it was 4.4 mgkg⁻¹ and 1.4 mg kg-1 soil. Clay soil addition to sandy soil increased maximum water holding capacity two to six-fold with a greater increase at 20% than at 10% and with 1 mm peds compared to the larger peds (Table 2). Clay soil addition had little effect on initial ammonium concentration, but 20% clay addition reduced available P concentration compared to sandy soil alone (Table 2). On day 15, the pH was lower in sandy soil alone than the sand-clay mixtures (pH 8.3 and 8.7). In all treatments, the pH was about 0.4 units lower on day 45 than day 15.

	pН	EC	Particle size				MDC	
	(1:5)	(1:5)	Sand	Silt	Clay	Organic C	Total N	MDC
		$\mu S \text{ cm}^{-1}$		(%)	-	g kg ⁻¹		mg kg ⁻¹
Clay soil	8.0 a	621 a	12	15	73	12.7 a	1.0	384 a
Sandy soil	5.4 b	12 b	96	1	3	1.0 b	nd	12 b

Table 1. pH, electrical conductivity (1:5), particle size, organic C and total N content of clay soil and sandy soil. Values followed by different letters are significantly different ($P \le 0.05$).

nd = not detectable

Table 2. Maximum water holding capacity, ammonium N and available P concentrations in sandy soil alone or with 10 and 20% clay as peds of 1, 2 and 3 mm (n=3). Values within a column followed by different letters are significantly different ($P \le 0.05$).

	Ped Water holding size capacity		NH4-N	Available P
	(mm)	(g water g ⁻¹ soil)	µg g son	µg g son
Sandy soil	none	0.02 f	3.8 c	9.9 a
Sandy soil + 10% clay	1	0.08 cd	4.1 bc	8.7 b
	3	0.05 e	4.0 bc	8.9 ab
	5	0.04 ef	4.2 bc	8.9 ab
Sandy soil +20% clay	1	0.13 a	5.4 a	7.5 c
	3	0.10 bc	4.3 bc	7.6 c
	5	0.09 cd	4.2 bc	7.5 c

Clay soil addition had no consistent effect on MBC concentration on day 15, but on day 30, the MBC concentration was lower with 5 mm peds than in sandy soil alone (Table 3). In contrast on day 45, clay soil addition increased MBC concentration compared to sandy soil alone, except 5 mm peds

at 20% clay addition. In the sandy soil alone, the MBC concentration decreased significantly from day 30 to day 45 whereas it did not change over time with 10% clay. With 20% clay, the MBC concentration was greater on day 15 than on day 45.

	~	ped size	MBC (mg kg ⁻¹ soil) days			
	Clay concentration	(mm)				
			15	30	45	
	none	none	335 a	282 ab	94 f	
	10% clay	1	255 bc	241 bcd	204 cde	
		3	227 cd	252 bc	216 cde	
Sandy soil		5	227 cd	231 cd	207 cde	
	20% clay	1	338 a	249 bcd	204 cde	
		3	334 a	209 cde	178 de	
		5	274 abc	153 ef	150 ef	

Table 3. Microbial biomass C on days 15, 30 and 45 in sandy soil alone or with 10 and 20% clay as 1, 3 and 5 mm peds amended with kikuyu residue (n=4). Values followed by different letters are significantly different (treatment x time interaction, $P \le 0.05$).

Neither time nor clay soil addition had consistent effect on MBN concentration (data not shown). Microbial biomass P was also not consistently influenced by clay soil addition, but whereas the MBP concentration did not change in sandy soil alone, it was 50-70% lower on day 45 than day 15 in clay amended treatments (data not shown).

Compared to sandy soil alone, clay soil addition did not affect available N concentration on day 15, but increased it on days 30 and 45 (Table 4). On day 30 in clay amended treatments, the available N concentration was lower with 5 mm peds than with the smaller peds. Available N concentration did not change over time in sandy soil alone, but was generally highest on day 30 in clay amended treatments.

Table 4. Available N (mg kg⁻¹ soil) on days 15, 30 and 45 of sandy soil alone or with 10 and 20% clay as 1, 3 and 5 mm peds (amended with kikuyu residue (n=4). Values followed by different letters are significantly different (treatment x time interaction, $P \le 0.05$).

	Class	ped size	Available N (mg kg ⁻¹ soil)			
	concentration	(mm)	days			
			15	30	45	
	none	none	34.5 h	34.7 h	34.5 h	
Sandy soil	10% clay	1	45.4 defgh	55.1 abcd	44.8 defgh	
		3	38.5 fgh	60.6 abc	48.8 cdefg	
		5	47.6 defg	43.6 defgh	62.1 ab	
	20% clay	1	41.2 efgh	61.0 ab	47.8 defg	
		3	38.0 gh	66.0 a	50.1 bcdef	
		5	45.0 defgh	52.3 bcde	52.4 bcde	

Clay soil addition reduced available P concentrations compared to sandy soil alone at all sampling times by 24-50% with a greater reduction at 20% clay addition compared to 10% (Table 5). In sandy soil alone, available P concentration was higher on day 15 than day 45, but it did not change over time in clay amended treatments.

Table 5. Available P (mg kg⁻¹ soil) on days 15, 30 and 45 of sandy soil alone or with 10 and 20% clay as 1, 3 and 5 mm peds amended with kikuyu residue (n=4). Values followed by different letters are significantly different (treatment x time interaction, $P \le 0.05$).

	C1	ped size	Available P (mg kg ⁻¹ soil)			
	Clay concentration	(mm)	days			
			15	30	45	
	none	none	39.4 a	35.0 ab	33.7 b	
Sandy soil	10% clay	1 24.2 c		25.0 c	22.7 cde	
		3	24.3 c	24.5 c	25.6 c	
		5	24.6 c	25.3 c	22.9 cd	
	20% clay	1	18.0 def	18.2 def	17.0 f	
		3	16.6 f	17.8 def	17.5 f	
		5	16.0 f	18.3 def	16.1 f	

Cumulative respiration over 45 days was significantly higher in sandy soil alone than in treatments with clay added at either 10% or 20% (Figure 1). Clay concentration and ped size had no consistent effect on cumulative respiration but it was lowest with 20% clay as 1 mm peds where it was 36% lower than sandy soil alone.



Figure 1. Cumulative respiration over 45 days (mg CO₂-C g⁻¹ soil) in sandy soil without (none) or with clay soil added as 1, 3 and 5 mm peds amended with kikuyu (vertical lines indicate standard error, n=4). Columns with different letters are significantly different ($P \le 0.05$).

In the treatments with 20% clay addition, peds were retrieved on day 45 by sieving. Between 75 and 77% of the added < 53 μ m particle size was retrieved in peds on day 45. The retrieved peds ranged in size (Table 6). Peds greater than 1 mm were found in the treatment where 0.5-1 mm peds had been added, indicating aggregation of peds. On the other hand, in treatments with 3 and 5 mm peds added, smaller peds were also retrieved. The total weight of the retrieved < 53 μ m fraction was greater with 3 and 5 mm peds added ed than with 1 mm peds. The greatest proportion of the < 53 μ mparticle size was in the ped size in which

they had been added (0.5-1 mm with 1 mm peds, 3-2 mm with 3 mm peds and 5-3 mm with 5 mm peds). But the sum of weight of the < 53 μ m particle size in the other fractions ranged between 38% and 48%. The TOC of < 53 μ m fraction of initially added peds was 0.38% and at the end of the experiment (after 45 days) the increase was 24, 19 and 10% in 1, 3 and 5 mm peds respectively. The TOC concentration of the < 53 μ m fraction was highest in the ped size in which the peds had been added. This is also the case when TOC is expressed as percentage of TOC in peds per core.

Table 6. Properties of the $< 53 \mu m$ fraction after 45 days with 20% clay as 1, 3 or 5 mm peds, weight, proportion and total organic C content and proportion of total organic C content in retrieved peds (n=4). Values in a column followed by different letters are significantly different (P \le 0.05).

	peds added	retrieved peds	< 53 µm fraction	% TOC of	Total TOC	% of total
	(mm)	mm) (mm) (g core ⁻¹		<53 µm	mg core ⁻¹	TOC
		>1	1.76 b	0.40 bcd	7.04	33
	1	<1->0.5	2.84 a	0.50 a	14.2	67
			4.6		21.2	
sandy soil + 20% clay		3-2	3.11 a	0.47 ab	14.6	65
	2	2-1	1.28 c	0.42 bc	5.4	24
	3	<1->0.5	0.89 d	0.28 e	2.5	11
			5.28		22.5	
		5-3	2.89 a	0.42 bc	12.1	54
		3-2	1.28 c	0.34 de	4.4	19
	5	2-1	0.97 cd	0.35 cde	3.4	15
		<1->0.5	0.84 d	0.31 e	2.6	12
			5.98		22.5	

4. Discussion

This study showed that clay addition to sandy soil amended with plant residue reduces respiration rate and available P concentration. It also showed that over 45 days, peds can breakdown, but small peds can also be formed. Over the course of the experiment, organic C was bound to the $< 53 \mu m$ fraction particularly in the ped sizes that were added initially. Ped size had little effect on respiration and nutrient availability. Therefore the first hypothesis (the smallest peds (1 mm) will have a greater effect on soil respiration rate

and nutrient availability than the 5 mm peds) has to be declined. This is in agreement with our previous study with 1-3 mm peds (Marschner and Tahir 2016) and suggests that the difference in surface area and number between 1 mm and 5 mm peds was not large enough to influence decomposability and binding of residues. Further the present study showed that this is the case even when decomposability and nutrient release are higher than in our previous study. The second hypothesis (the larger peds will break down during the experiment into smaller peds with each resulting ped size binding organic matter) can be accepted. On the first day, the respiration rate was lower in sandy soil alone than in clay amended soil which may be due to a low initial microbial biomass in the sandy soil (Table 1). This is probably due to the low TOC content of this soil because microbial biomass is positively correlated with TOC content (Arunachalam 1999; Banerjeeet al. 2006). However, by day 2, the respiration rate was similar in all soils indicating that the small initial microbial biomass in the sandy soil was stimulated by residue addition. From day 6 to day 14, respiration rates were about two-fold higher in the sandy soil than in clay amended soils which is most likely due to binding of residue particles to the clay peds. Binding to clay reduces organic matter accessibility to microbes (Boldock 2007; Chenu and Plante 2006; Nguyen and Marschner, 2014; Pal and Marschner, 2016). Binding to clay may also explain the lower available P concentration in clay amended soils compared to sandy soil alone (He et al. 1991; Scalenghe et al. 2007).

The retrieval of the peds at the end of the experiment showed that over the course of 45 days peds broke down and organic C was bound to the $< 53 \mu m$ fraction. The greatest proportion of peds was in the initially added ped size, but between 38 and 48% of the $< 53 \mu m$ fraction was in other ped sizes which indicates high turnover rate of the peds. The soil was

maintained at 80% of WHC throughout the 45 days, but slight drying followed by rewetting when the water content was adjusted cannot be ruled out. Drying as well as rewetting can cause breakdown of aggregates (Denef et al. 2001b). It could be expected that the TOC content is greater in small compared to larger peds because of their higher surface area to volume ratio (Mayer 1994). However, this was not the case in this experiment. The TOC content was greatest in the ped size added initially which had a longer time of contact with the added residues than peds formed during the experiment. This suggests that in this experiment, time of contact may be more important than the surface area to volume ratio with respect to TOC binding to the $< 53 \mu m$ fraction. The surface area to volume ratio may become more important later when the added residue has been decomposed.

The finding that when 1 mm peds were added about 38% of the $< 53 \mu$ m fraction was retrieved in the > 1 mm size class suggests that there was aggregation of smaller peds during the experiment. It cannot be ruled out that aggregation also occurred in the treatments where 3 and 5 mm peds were added. But in these treatments, ped breakdown dominated ped formation. A smaller amount of the $< 53 \mu$ m fraction was retrieved at the end of the experiment in the treatment with 1 mm than in those with 3 and 5 mm peds. This indicates that with 1 mm peds added, more of the $< 53 \mu$ m fraction was in particle sizes < 0.5 mm and therefore not considered in this experiment.

Breakdown and formation of peds may explain why soil respiration and nutrient availability were not influenced by the size of initially added peds. Ped size may have a greater effect if the range of ped sizes added is greater as it is after clay addition in the field where ped diameters can range from a few mm to cm. However, adding larger peds would not have been possible in our experimental setup.

Conclusion

The study confirmed that clay addition to sandy soil increases soil organic carbon retention compared to sandy soil alone. It also showed substantial ped breakdown as well asformation of larger peds over 45 days. The newly formed peds can also influence nutrient cycling and bind organic C which may explain why ped size did not have a consistent effect on soil respiration and nutrient availability. Field experiments are needed to assess ped breakdown and organic C binding in relation to ped size over longer periods of time.

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