Influence of clay concentration, residue C/N and particle size on microbial activity and nutrient availability in clay-amended sandy soil

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Abstract

The low fertility of sandy soils can be ameliorated by addition of clay-rich soil, but the effect of clay may differ between high and low C/N residues and could be modulated by residue particle size. An incubation experiment was conducted with addition of a clay-rich subsoil (73% clay) to a sandy soil (10% clay). The final clay concentrations were 10, 15, 20 and 30% (w/w). The residues of young kikuyu shoots (C/N 14) and mature wheat shoots (C/N >120) in two particle sizes (0.2-2 and 3-4 mm) were added at 10 g kg⁻¹ soil. Soil respiration, pH, available N, microbial biomass C (MBC), N and P were measured. Cumulative respiration was up to 4-fold higher with kikuyu than with wheat and 30% lower at the highest clay concentration. The MBC concentration was up to 2-fold higher with kikuyu than with wheat. The available N concentration was up to 2-fold higher with kikuyu than with wheat and up to 50% lower at highest clay concentration. Thus, clay addition to sandy soils may reduce nutrient availability by reducing accessibility of plant residues to microbes and binding of nutrients, but this clay effect is not influenced by residue C/N or particle size.

Keywords: C/N ratio, clay, microbial biomass, nutrient availability, plant residue, respiration

1. Introduction

Soil fertility is strongly influenced by soil texture. Most pores in sandy soils are large and drain rapidly and sand particles have low cation exchange capacity; therefore sandy soils have low nutrient and water retention capacity (Zotarelli *et al.* 2007). Due to the low cation exchange capacity, sandy soils also

have a low capacity to store organic C. Water infiltration into sandy soil may be reduced by water repellency (Ismail *et al.* 2007; McKissock *et al.* 2002). Therefore, plant growth and thus carbon input into sandy soils are often low. Clay particles on the other hand have a high cation exchange capacity and

can therefore hold nutrients and organic matter (Don and Schulze, 2008; Kaiser and Zech, 2000). Organic matter accumulates in clay-rich soils because it is poorly accessible to decomposing microbes by binding to clay particles and occlusion within aggregates (Baldock 2002; Roper and Smith, 1991).

Fertility of sandy soils can be increased by addition of clay, either from the subsoil or from the nearby clay-rich top-soils (Hall *et al.* 2010; Ismail *et al.* 2007). Clay addition increases not only soil fertility, but could also protect the incoming organic material (e.g., plant residues, manures) from decomposition (Shi and Marschner, 2013) by binding to clay particles (Nguyen and Marschner, 2014; Zeraatpishe and Khomali 2012).

Decomposition rates of plant residues are influenced by residue properties such as C/N ratio (Partey *et al.* 2014; Tian *et al.* 1992), water soluble C (Perez-Suarez *et al.* 2012), and lignin concentration or particle size (surface area to volume ratio (Jensen, 1994; Vanlauwe *et al.* 1996). Easily decomposable plant residues generally have a low C/N ratio and lignin concentration (Jensen, 1994). Residue particle size influences accessibility to decomposing microbes in two contrasting ways. Firstly, through surface area to volume ratio; per unit volume, the area that can be colonised by microbes is greater in smaller than larger particles. Secondly, smaller particles are more likely to bind to soil particles, which reduce accessibility to microbes (Jensen, 1994). The effect of residue particle size on soil respiration and nutrient availability is not clear with some studies reporting differences between particle sizes (Jensen, 1994), whereas others found no effect (Bending *et al.* 2002; Bhupinderpal *et al.* 2006).

In a previous study we showed that clay soil added to sandy soil in peds ranging in size from 1 to 3 mm had no consistent effect on cumulative respiration in resi-

due amended sandy soil (Tahir and Marschner, 2016). On the other hand, addition of finely ground isolated clay to a pure sand influenced cumulative respiration after residue addition, but the effect differed between residue types. Compared to sand alone, cumulative respiration was increased when high C/N wheat straw was added, but decreased after addition of low C/N pea straw (Roychand and Marschner, 2013). This suggests that the effect of clay addition to sandy soils on cumulative respiration may depend on residue C/N ratio. But that study was conducted in a rather artificial soil medium consisting of isolated clay and pure sand with only one clay addition rate and only soil respiration was measured. Little is known about the effect of clay addition on nutrient availability and uptake into the microbial biomass. Further, it is not known if residue particle size modulates the effect of clay addition on soil respiration and nutrient availability.

The aim of the experiment was to determine if the effect of clay addition to sandy soil on soil respiration, microbial biomass and nutrient availability depends on clay addition rate, residue C/N and residue particle size. We tested the following hypotheses (i) clay addition to sandy soil will have a stronger effect on soil respiration, microbial biomass and N and P availability when low C/N residue is added compared to high C/N residue therefore clay addition will reduce the differences in soil respiration, microbial biomass and nutrient availability between the residues, and (ii) the effect of clay addition will be greater with smaller size residue. The first hypothesis is based on the assumption that decomposition rates and nutrient release will be greater with low compared to high C/N residue. The second hypothesis assumes that smaller residue particles are more likely to be bound to clay than larger residue particles.

2. Materials and Methods

2.1. Soils and residues

Two soils from South Australia were used: a loamy sand from 0-10 cm depth at Monarto (Latitude 35° 05'S and Longitude 139° 06'E, Entisol) and a clay-rich black Vertisol (73% clay) from 30 to 50 depth in Urrbrae (34°58'S and 138°38'E) (Table 1). The clay mineralogy of Vertisol, determined by X-ray diffraction, was dominated by smectite (40%) with smaller proportions of kaolinite (11%) and illite (5%). The sampling sites are

in a semi-arid region and have a Mediterranean climate with cool, wet winters and hot, dry summers interspersed by occasional rainfall events. The soils were air-dried and sieved to 2 mm.

Shoot residues of mature wheat (*Triticum aestivum* L., referred to as W) and young kikuyu grass (*Pennisetum clandestinum* L., referred to as K) were used. The residues were dried in a fan-forced oven at 40 °C, ground and sieved into two particle sizes: 0.25 to 2 mm (referred to as 2 mm) and 3.35-4 mm (referred to as 4 mm). The C/N ratio of kikuyu residues was 13 and 15 and of wheat straw 122 and 163 for 2 and 4 mm particles.

Table 1. Properties of sandy soil (Entisol) and clay-rich soil (Vertisol).

	Sand	Silt	Clay	pH _{1:5}	Organic C	Total N	Total P
	%					g kg ⁻¹	
Sandy soil	83	7	10	8.7	4.0	0.04	0.3
Clay soil	12	15	73	8.3	2.8	0.47	0.5

2.2. Experimental design

The loamy sand (9% clay) was mixed with the clay-rich subsoil to achieve clay concentrations of 15, 22 and 30% w/w, referred to as clay concentrations 10 (loamy sand alone), 15, 20 and 30%. The maximal water holding capacity (WHC) of soil mixes was 57, 115, 164 and 208 g water kg⁻¹ soil for 10, 15, 20 and 30% clay, respectively. The mixes were wetted thoroughly with reverse osmosis (RO) water to 75% of maximum water holding capacity (WHC) of the soil mixes. This percentage of WHC was used because Setia et al. (2011) showed that microbial activity was maximal at this water content in loamy sand soil. Setia et al. (2011) found that the optimal water content for respiration decreased with clay content, but prelimi-

nary experiments showed that soil respiration in the soil mixes used here respiration differed little between 50 and 75% WHC. Residue were added at 10 g kg⁻¹, and mixed thoroughly with the soils. There were four residue treatments at each clay soil addition rate (kikuyu 2 or 4 mm and wheat 2 or 4 mm). Un-amended controls were not included because it was not the aim of this experiment to assess the effect of residue addition on the measured parameters. Further, preliminary experiments had shown that respiration was very low in un-amended sandy-clay soil mixes.

Thirty grams dry weight equivalent of the mixed soils was filled into PVC cores (radius 1.85 cm and height 5 cm) with a nylon mesh base (0.75 µm, Australian Filter Specialist). The cores were packed to a bulk density of 1.5 g cm⁻³. The cores to be sampled on day 15 were

immediately placed into 1 L glass jars (Ball® Half Pint Wide Mouth Jars, Jarden Corporation) and sealed with gas-tight lids equipped with a stainless septum. A vial containing 10 mL reverse osmosis (RO) water was also placed in the jar to maintain the humidity. The rest of the cores were placed in a plastic container with a loose fitting lid. The jars and the container were incubated at 22 °C in the dark for 4 weeks. There were three destructive sampling dates: day 0 (5 h after residue addition) and days 15 and 30, but not all variables were measured on all sampling days because previous studies showed that they change little over two weeks (for details see below). After removing the cores from the jars on day 15 for destructive sampling, the set of cores to be sampled on day 30 was placed in the jars for respiration measurement. The water content of all cores was maintained throughout the experiment gravimetrically.

2.3. Measurements

Soil particle size distribution was measured by the hydrometer method (Ashworth *et al.* 2001), soil pH and EC in a 1:5 soil to water ratio after one hour end-over-end shaking. Maximum WHC of sand-clay mixes were measured by placing the soils in rings in the sintered glass funnel attached to a 100 cm water column ($\Psi_m = -10$ kPa). Then the soil was wetted to saturation and drained for 48 h. The dry soil was weighed after drying at 105 °C for 24 h. Organic carbon and nitrogen were determined by Walkley and Black (1934) and Kjeldahl distillation (Rayment and Higginson, 1992), respectively.

Soil respiration was measured by quantifying the CO₂ concentration in the headspace of jars using a Servomex 1450 infra-red analyser (Servomex Group, Crowborough, UK) as described in Setia *et al.* (2011). Due to the upper detection limit of the gas analyser (2% CO₂) and the decrease in respiration over time after

residue addition, soil respiration was measured daily in the first 12 days, every second day from day 13 to 21, thereafter every 3 days until the end of the experiment. After each measurement, the jars were vented to refresh the headspace using a fan, and then resealed followed by determination of the CO₂ concentration (t₀). The CO₂ evolved from each sample for a given interval was calculated as the difference in CO₂ concentration between t₁ and t₀. Linear regression based on injection of known amounts of CO₂ in the jars was used to define the relationship between CO₂ concentration and detector reading. The clay soil is alkaline and contains carbonates and could therefore release CO₂ from carbonate dissolution. However, other studies in our lab showed that CO₂ release from sandy soil alone and sandy soil with this clay soil added at similar concentration as used in the present study did not differ significantly (Tahir and Marschner, 2016). This shows that CO₂ release from carbonates is negligible. Microbial biomass C and N were determined by chloroform fumigation-extraction with 0.5 M K₂SO₄ at a 1:4 soil to extractant ratio. The organic C concentration was determined by titration with 0.033M acidified (NH₄)₂Fe(SO₄)₂•6H₂O after dichromate oxidation (Anderson and Ingram 1993). Microbial biomass C was calculated as the difference between fumigated and non-fumigated multiplied by 2.64 (Vance *et al.* 1987). Microbial biomass N was determined by measuring Ninhydrin-reactive N in the 0.5 M K₂SO₄ extracts of soil as described in (Joergensen and Brookes, 1990). The difference in N concentration between fumigated and non-fumigated samples was divided by 0.57 to calculate microbial biomass N (Jenkinson, 1988). Available and microbial biomass P (MBP) was determined as described in Kouno *et al.* (1995) except that hexanol was used as the fumigant instead of chloroform and resins were eluted with 0.1 M NaCl/HCl instead of 0.5 M HCl. The P concentration in the eluate was determined according to Murphy and Riley

(1962). Microbial biomass P was calculated as difference between fumigated and non-fumigated extracts. Soil inorganic N was extracted in 2 M KCl at a 1:10 soil/extractant ratio; ammonium and nitrate concentrations were determined using standard colorimetric methods (Murphy and Riley, 1962).

Soil pH and available N were measured on days 0, 15 and 30. Available P was measured only on days 0 and 30, microbial biomass C on days 15 and 30 and microbial biomass N and P on day 30.

2.4 Statistical analysis

Data of cumulative respiration, microbial biomass C, N and P, available N and pH at was analysed separately for each sampling time by two way analysis of variance with clay concentration (10, 15, 20 and 30%) and residue treatment (wheat 2 or 4 mm, kikuyu 2 or 4 mm) as fixed factors (GenStat® for Windows, 11.0, VSN Int. Ltd, UK, 2005). Tukey's multiple comparison test at 95% confident interval was used to determine significant differences among treatments. The interaction between residue type and clay concentration was not significant, therefore results of the multiple comparison test are shown for main effects only (see supplementary table).

3. Results

Cumulative respiration was 2-4 fold higher in the first 15 days than from day 15-30 (Figure 1, Table S1). Residue type (wheat or kikuyu) and clay concentration significantly influenced cumulative respiration in the first 15 days and over the entire experimental period (0-30 days), but had no effect on cumulative respiration from day 15-30. Cumulative respiration in the first 15 days and from day 0 to 30 was two to three-fold higher with kikuyu than with wheat.

Figure 1. Cumulative respiration for periods 0-15 (a) and 15-30 days (b) in sandy soil amended with clay to achieve 10, 15, 20 and 30% clay and kikuyu and wheat residue particles of 0.2-2 and 3-4 mm size, $n=4$, vertical lines indicate error bars. The interaction of clay concentration and residue type shown here was not significant. Average values for the main effects (residue type and clay concentration) are shown in Table S1.

Residue size (2 or 4 mm) did not influence cumulative respiration. In both periods (0-15 and 0-30 days), cumulative respiration was lowest with 30% clay and highest with 5 or 15% clay. It was about 40% lower at the highest than at the lowest clay concentration. On day 15, the microbial biomass C (MBC) concentration was significantly different only for the 4 mm residues where it was about 30% higher with kikuyu than with wheat. On day 30, the MBC concentration was 2 fold higher in soil amended with kikuyu 2 mm than with wheat 4 mm (Figure 2, Table 2).

Table S1. Averages of main effects for cumulative respiration in periods 0-15, 15-30 and 0-30. Within each main effect, values followed by different letters are significantly different ($P=0.05$). Least significant difference (l.s.d.) refers to main effects.

		Cumulative respiration ($\text{mg CO}_2\text{-C g}^{-1}\text{ soil}$)		
Period		0-15	15-30	0-30
Clay concentration	(%)			
	10	3.3 b	0.8 ns	4.1 b
	15	3.0 b	0.8	3.8 b
	20	2.6 ab	0.8	3.4 ab
	30	2.0 a	0.6	2.7 a
Residue	mm			
Wheat	0.2-2	1.5 a	0.6 a	2.2 a
	3-4	1.0 a	0.6 a	1.7 a
Kikuyu	0.2-2	4.4 b	1.0 b	5.4 b
	3-4	3.9 b	0.8 ab	4.6 b
l.s.d.		0.7	0.2	0.7
Clay concentration		**	ns	**
Residue		**	**	**
Clay x Residue		ns	ns	ns

On both dates, the MBC concentration was lowest at the highest clay concentration. On day 30, microbial biomass N and P (MBN and MBP) concentrations were not significantly influenced by clay concentration, but the MBN concentration was 2-fold and the MBP concentration 50% higher with kikuyu than with wheat (Figure 3). The available N concentration was higher in kikuyu amended soil than in wheat amended soil (Figure 4, Table 2). On day 0, the available N concentration was about 2-fold higher in soil

amended with kikuyu 2 mm than in soil with wheat 4 mm. A similar difference occurred on day 15 but between kikuyu 4 mm and wheat 4 mm. On day 30, the available N concentration was two-fold higher in kikuyu amended soil than with wheat irrespective of residue particle size.

On days 15 and 30, the available N concentration was lowest with 30% clay and highest with 5% clay with up to two-fold difference between the two clay concentrations.

The available P concentration was 2 to 7-fold higher on day 0 than on day 30 (Figure 5). On both sampling dates, the available P concentration did not differ among residue treatments. The effect of clay concentration was different on day 0 and day 30. On day 0, the available P concentration increased with clay concentration and was about 2-fold higher at 30% clay compared to 5% clay. On day 30, the available P concentration was higher in the sandy soil alone (5% clay) than in clay-amended soils. Soil pH ranged between 8.4 and 8.9 and did not differ among residue treatments, but was higher at 10 and 15% clay than at the higher clay concentrations.

4. Discussion

This study showed that clay addition to sandy soil reduces microbial activity and nutrient availability. But it also showed that this effect occurs irrespective of residue decomposability and particle size.

Differences between residue types in the measured parameters were of similar magnitude at all clay concentrations. For example, cumulative respiration over 30 days was 2-fold higher with kikuyu at all clay concentrations (Figure 1, Table 2). This suggests that decomposition of the easily decomposable low C/N residue was not more strongly reduced by clay than the difficult decomposable high C/N residue. Further, residue particle size did not influence the measured parameters. Therefore our two hypotheses (i) clay addition to sandy soil will have a stronger effect on soil respiration, microbial biomass and N and P availability when low C/N residue is added compared to high C/N residue therefore clay addition will reduce the differences in soil respiration, microbial biomass and nutrient availability between the residues, and (ii) the effect of clay addition will be greater with smaller size residue have to be rejected.

In contrast to our earlier study, clay addition to sandy soil had a similar effect on cumulative respiration with high and low C/N residues (Figure 1). This may be due to the lower residue addition rate in this study (10 compared to 20 g kg⁻¹ in Roychand and Marschner 2013) and the fact that in that study, isolated clay was added to a pure sand whereas here a sandy soil was amended with clay soil.

Figure 2. Microbial biomass C concentration on days 15 (a) and 30 (b) in sandy soil amended with clay to achieve 10, 15, 20 and 30% clay and kikuyu and wheat residue particles of 0.2-2 and 3-4 mm size, $n=4$, vertical lines indicate error bars. The interaction of clay concentration and residue type shown here was not significant. Average values for the main effects (residue type and clay concentration). Note that microbial biomass C was measured only on days 15 and 30.

Table 2. Averages of main effects for cumulative respiration, microbial biomass C and available N on day 30. Within each main effect, values followed by different letters are significantly different ($P \leq 0.05$). Least significant difference (l.s.d.) refers to main effects.

Clay concentration	(%)	Cumulative respiration	Microbial biomass C		Available N
		(mg CO ₂ -C g ⁻¹ soil)	(mg kg ⁻¹)		
	10	4.1 b	90	ab	30 c
	15	3.8 b	93	ab	26 bc
	20	3.4 ab	117	b	17 ab
	30	2.7 a	67	a	16 a
Residue	mm				
Wheat	0.2-2	2.2 a	64	a	17 a
	3-4	1.7 a	73	a	15 a
Kikuyu	0.2-2	5.4 b	126	b	29 b
	3-4	4.6 b	105	ab	28 b
l.s.d.		0.7	35		8
Clay concentration		**	**		**
Residue		**	**		**
Clay x Residue		ns	ns		ns

ns=not significant

Isolated clay particles are more exposed and therefore more likely to interact with residues than when clay soil is added where clay surfaces may be occluded within aggregates.

Due to the greater surface area-to-volume ratio, smaller residue particles could decompose more quickly than larger particles. On the other hand, smaller residues are more likely to bind to soil particles which would reduce their accessibility (Jensen, 1994). This contrasting effect of residue size on decomposability may explain why some studies reported differences soil respiration and nutrient availability between particle sizes (Jensen, 1994), whereas others found no

effect (Bending *et al.*, 2002; Bhupinderpal *et al.*, 2006). In the present study residue particle size (0.2-2 mm or 3-4 mm) did not significantly influence respiration, microbial biomass or nutrient availability. This could be due to at least two factors.

Firstly, particle size may not have been sufficiently different to influence accessibility to microbes, particularly because the residues were carefully mixed into the soil and the soil was moist to maximise residue-soil contact. Secondly, the residue addition rate was sufficiently high (10 g kg⁻¹) to allow high decomposition rates even of larger particle sizes.

It has been suggested that smaller residues are more likely to be bound to soil particles than larger particles (Bending *et al.* 2002; Bhupinderpal *et al.* 2006), however if smaller residues were bound more strongly to clay, one would have expected a greater difference between residue particles at the higher clay concentration, thus a significant interaction between residue type and clay concentration. However, this was not the case. In agreement with previous studies (Partey *et al.* 2014; Tian *et al.* 1992), cumulative respiration

Figure 3. Microbial biomass N (a) and microbial biomass P (b) on day 30 in sandy soil amended with clay to achieve 10, 15, 20 and 30% clay and kikuyu and wheat residue particles of 0.2-2 and 3-4 mm size, $n=4$, vertical lines indicate error bars. The interaction of clay concentration and residue type shown here was not significant. Average values for the main effects (residue type and clay concentration). Note that microbial biomass N and P were measured only on day 30.

and available N concentrations were greater with low C/N kikuyu than high C/N wheat (Figure 1, Table 2). As expected, the available N concentration was higher with low C/N kikuyu than with high C/N wheat at all sampling times and changed little over time (Figure 4). In contrast, the available P concentration did not differ among residue treatments on days 0 and 30 and was up to 5-fold higher on day 0 than on day 30 (Figure 5). The available N and P concentrations on day 0 are unlikely to only be due to mineralisation of the added residues because there were only a few hours between residue addition and sampling. Inorganic N and P would also be released from vacuoles in the plant material once the dry residues were added to the soil. The high available P concentration on day 0 indicates that P was released from the clay soil because the concentration was higher at the two higher clay addition rates than in sandy soil alone. This P release may have masked differences among residues in available P. Clay addition influenced cumulative respiration and nutrient availability only at 20 and 30% clay addition, not at 15% (Figures 1, 4 and 5, Table 2). The 15% clay treatment did not differ from the sandy soil alone, most likely because of the small difference in clay concentration compared to the sandy soil which had 10% clay. At 30% clay compared to 10 or 15% clay, cumulative respiration in the first 15 days (Figure 1, Table S1), available N on day 15 and 30 and available P on day 30 were lower (Figures 4 and 5). However clay addition did not significantly affect MBN and MBP concentrations on day 30 (Figure 3). The reduction of respiration and N availability can be explained by binding of the added residues to clay surfaces via divalent cations such as Ca^{2+} and occlusion of residue particles by clay (Baldock, 2002). This reduces organic matter accessibility and thus decomposition. The reduced N availability may also be due to binding of NH_4^+ to cation exchange sites of clay particles (Shen *et al.* 1997).

A reduction in available N by higher clay concentrations was also found in a recent study where clay was added as peds to sandy soil (Tahir and Marschner, 2016). The lack of a clear effect of clay concentration

on MBC, MBN and MBP suggests that despite lower decomposition rates and nutrient availability, growth and nutrient uptake by microbes was not reduced by clay.

Figure 4. Available N concentration on days 0 (a), 15 (b) and 30 (c) in sandy soil amended with clay to achieve 10, 15, 20 and 30% clay and kikuyu and wheat residue particles of 0.2-2 and 3-4 mm size, $n=4$, vertical lines indicate error bars. The interaction of clay concentration and residue type shown here was not significant. Average values for the main effects (residue type and clay concentration).

Figure 5 . Available P concentration on days 0 (a) and 30 (b) in sandy soil amended with clay to achieve 10, 15, 20 and 30% clay and kikuyu and wheat residue particles of 0.2-2 and 3-4 mm size, $n=4$, vertical lines indicate error bars. The interaction of clay concentration and residue type shown here was not significant. Average values for the main effects (residue type and clay concentration) Note that available P was measured only on days 0 and 30.

5. Conclusion

This study showed that clay addition to sandy soil can reduce soil respiration as well as N availability in residue amended soil, but the relative effect of clay on these parameters did not differ between high and low C/N residues. Future longer term studies could add ^{13}C and ^{15}N labelled residue to clay amended sandy soil to determine the fate of C and N in the soil-microbe system.

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