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Clay amended sandy soil – influence of clay concentration and particle size on nutrient availability and organic carbon content after plant residue addition

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Dedicated to my family

Table of Contents

CHAPTER I

CHAPTER 2 27

CHAPTER 3 71

CHAPTER 4 124

CHAPTER 5 162

CHAPTER 6 184

CHAPTER 7 206

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ABSTRACT

Sandy soils have low water and nutrient holding capacity which limit crop growth. In the short-term, these constraints can be overcome by increased fertiliser application or irrigation. However, long-term solutions are needed to improve farm productivity and sustainability. Clay added to sandy soils may be such a solution. Compared to sandy soils, clay soils have smaller pores and higher cation exchange capacity and therefore greater water and nutrient holding capacity. Clay can also bind organic matter via cation bridges and thereby reduce its accessibility to decomposing microbes. In sandy soils with clay subsoil, the clay can be mixed into the sandy top soil by delving or spading. However, the clay subsoil is not uniformly distributed in the sandy top soil. It forms clay peds of varying size which creates a highly non-uniform soil environment with patches of sandy soil with clay peds next to sandy soil with little or no clay. The clay-rich patches can hold more water and nutrients compared to the surrounding sandy soil that could influence nutrient availability and organic C binding. Little is known about the influence of ped size and rate of clay added to sandy soil on nutrient availability and organic C binding after residue addition. The aims of the study were i) to determine the effect of clay addition rate and ped size in residue amended sandy soil on soil respiration, nutrient availability and organic C retention ii) to assess the effect of clay soil particle size and clay soil properties on nutrient availability and organic C binding after addition of residues with low or high C/N ratio iii) to determine the effect of clay addition rate and ped size on nutrient leaching after mineral fertilizer addition.

A series of incubation experiments were carried out to assess the effect of clay addition rate and ped size on nutrient availability and organic C retention on < 53 µm fraction after mixing with low and high C/N ratio residue.

In the first study, clay peds of 1, 2 or 3 mm size derived from a clay-rich Vertosol (73% clay) were added to a sandy soil (3% clay) at clay addition rates of 10% and 20% w/w. After addition of ground mature faba bean residue (C/N 37) at 10 g kg^{-1}, the soils were incubated for 45 days at 80% of water holding capacity. Clay addition to sandy soil influenced nutrient availability after plant residue addition, particularly when small peds are added at higher rates. Sandy soil with clay peds had a greater maximum NH_4 and P sorption capacity than sandy soil alone, sorption capacity was higher at 20% compared to 10% clay addition and greater with 1 mm than 3 mm peds. Retrieval of clay peds at the end of the experiment showed ped breakdown during the experiment but also formation of larger peds. Compared to the < 53 µm fraction added at the start of the experiment, total organic carbon (TOC) content of the < 53 µm fraction was up to two-fold higher, particularly in the smaller peds (1 and 2 mm). The study confirmed that claying can increase organic C sequestration, but also showed that organic C sequestration is likely to be greatest when the added clay peds are small.

The capacity to bind organic C and nutrients may depend on clay soil properties such as mineralogy, clay concentration and exchangeable Fe and Al. A 45-day experiment was carried out to investigate the effect of clay type on nutrient availability and organic carbon retention with residues differing in C/N ratio (20 or 47). Two clay soils with smectite as a dominant mineral were used. They differed in smectite percentage [high (40%) or low (5-10%)], clay content (73 or 42%) and exchangeable Fe and Al concentration (low or high). The clay soils were added to sandy soil at rate of 20% w/w either finely ground or as 2 mm peds. Over 45 days, available N and P, microbial biomass N and P concentrations and cumulative respiration were greater with low C/N than high C/N residue. With low C/N residue, compared to sandy soil alone clay addition increased available N concentration and initial microbial biomass C and N, but decreased cumulative respiration and P availability. This study showed that addition of clay soil to sandy soil influences nutrient availability, but there were no clear differences between clay soils or sizes. The lack of differences between high and low smectite clay soil suggests that a high concentration of Fe and Al oxides can compensate for a lower clay concentration and proportion of smectite with respect to binding of organic matter and nutrients.

In the previous studies, we found that clay addition had no consistent effect on cumulative respiration and ped size effect was variable. Secondly, low C/N ratio residue had stronger effect on nutrient availability due to its high decomposition rate and nutrient release compared to high C/N ratio residue. The third experiment was conducted to investigate that 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. The aims of this experiment 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 the < 53 µm fraction of the peds. Clay soil addition to sandy soil amended with plant residue reduced respiration rate and available P concentration. Ped size had little effect on respiration and nutrient availability. Clay soil addition increased soil organic carbon retention compared to sandy soil alone. With respect to ped size, the experiment showed substantial ped breakdown and but also formation of larger peds over 45 days.

The first three experiments were conducted over 45 days. But longer term studies are needed to better evaluate the effect of claying in the field. To investigate the effect of repeated addition of residue (finely ground wheat mature shoots added every 2 months) in clay amended sandy soil, a longer term (8 months) study was conducted with clay soil

added as finely ground soil, 1 and 3 mm peds. The organic C content of the whole soil increased during the experiment with a greater increase in clay amended soils. The organic C content of the > 53 µm fraction was very low and changed little over time. With finely ground clay soil and 1 mm peds, the organic C content of the < 53 µm fraction increased mainly in the first 2 months while in 3 mm peds it increased over 6 months to reach similar concentrations as with finely ground clay soil and 1 mm peds.

Excessive use of fertilizer in sandy soils can cause leaching of nutrient elements N and P into water ways and cause eutrophication. In the fifth experiment, clay soil was added in sandy soil at 10% or 20% clay soil w/w finely ground or as 2 and 5 mm peds with and without N and P fertiliser (27 mg N kg^{-1} and 7 mg P kg^{-1}). The clay sand mixture (30 g) was placed in cores with nylon mesh at the bottom. The soils were incubated at 80% water holding capacity and leachate was collected weekly for 50 days. Clay addition significantly reduced leaching of N and P as compared to sandy soil alone. In sandy soil alone, the highest N (68%) leaching occurred after the first week whereas the highest amount (41%) of P was leached after two weeks.

It can be concluded that clay addition to sandy soil can reduce the risk of nutrient leaching and enhance carbon sequestration in sandy soils by decreasing C loss via respiration and leaching. This effect will be greatest with finely ground clay soil or small peds.

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I certify that no part of this work will, in future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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LIST OF PUBLICATIONS

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CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

Introduction

Globally, 900 million hectares are covered by sandy soils (FAO 1995). In Australia, sandy soils with weakly developed soil profile and predominantly siliceous sands are found in 45% of total land area (Anonymous 1994). These soils are not used for cultivation. About 17% of the area in Australia is covered by soils with sandy to sandy loam A horizon and clayey B horizon (Isbell 2002). Large proportion of such shallow sandy soils is cultivated, about 18 million ha in South-western Australia (Gardner et al. 1992) and 1 million hectares in Victoria (McGuinness 1991).

Water and nutrient availability in soil are greatly influenced by soil properties including soil texture, pore size distribution, cation exchange capacity (CEC), pH, soil organic matter content (SOM) and hydraulic conductivity. Soil texture is the relative proportion of sand, silt and clay in a mineral soil and strongly influences soil properties. Sandy soils have inherent low fertility (Xie and Steinberger 2005) due to low organic matter content, cation exchange capacity (CEC), and low water holding capacity (Zotarelli et al. 2007). This makes nutrient and irrigation management difficult for crop production. Intensive use of mineral fertilizers and irrigation during dry periods to increase yields on sandy soils can lead to loss of nutrients via leaching (Hagmann 1994). This causes not only economic loss but can also lead to soil acidification and ground-water contamination that are contributing in environmental pollution.

There are various strategies to overcome the low nutrient and water holding capacity of sandy soils. For example, furrow sowing (Blackwell et al. 1994) and use of slow release fertilisers to enhance nutrient use efficiency (Michelsen and Franco 1994). Another solution for sandy soils is to add organic materials, such as rice and wheat straw, composted leaves, grass, or wood bark. Addition of these organic materials may improve the structure and porosity and enhance both water and nutrient retention. However, adding organic materials like rice straw and wheat straw may also increase the nitrogen fertilisation requirement, to avoid N deficiency caused by immobilisation of N by soil microbes. Addition of compost, particularly in sandy soils may lead to nutrient lost via leaching (Basso and Ritchie 2005; Mamo et al. 1999; Shepherd and Bennett 1998). Addition of clay soil to sandy soil along with compost could increase nutrient binding and reduce nutrient leaching (Naguyen and Marschner 2013).

In contrast to sandy soils, clay soils have high water and nutrient retention capacity and often high organic matter content. Clay minerals have a high cation exchange capacity and can therefore bind and release nutrients (Hamarashid et al. 2010). Clay particles are small and can pack tightly; therefore, clay soils have a large proportion of small pores that can hold water. Therefore, management of heavy clay soils is difficult because they can become anaerobic when wet and hard when they dry (Jayawardane and Meyer 1985).

The addition of clay to sandy soil can enhance soil water storage , plant available water and ultimately crop productivity (Tennant et al. 1992). It results in significant changes in pH, CEC, extractable macro- and micro-elements (Fogden 2010; Hall et al. 2010; Rebbeck et al. 2007) and root growth. In sandy soils with clay subsoil, the clay can be mixed into the sandy top soil by delving or spading. However, the clay subsoil is not uniformly distributed in the sandy top soil. It forms clay peds of varying size which creates a highly non-uniform soil environment is created with patches of sandy soil with clay peds next to sandy soil without or little clay. The clay-rich patches can hold more water and nutrients compared to the surrounding soil.

In this literature review, an overview of nutrient availability and organic matter will be presented, followed by a comparison of sandy and clay soil properties which leads to a discussion about the benefits of claying and lastly the research gaps addressed in the PhD project.

1. Nutrient availability

Plants need inorganic nutrients e.g. N, P and K for growth and development. In the absence of these essential nutrients physiological processes cannot be performed and plants cannot complete their life cycle and (Parikh and James 2012). Plants take up nutrients via roots, mycorrhizas, and leaves in various forms. Generally, soils contain all nutrients in sufficient quantity, but only a small proportion of total nutrient content is available to plants which is in ionic form and present in soil solution for example HPO_4^{2-} $H_2PO_4^-$, NH_4^+, NO_3^-, Fe^{2+} (Marschner 2012). Weathering of soil minerals, runoff and decomposition of organic matter release soluble ions (Osman 2012) which can be sorbed or desorbed on organic or inorganic soil colloids. These soluble and exchangeable forms are considered to be plant available (Marschner, 2012). Some ions can react with minerals and form precipitates for example phosphorus reacts with carbonate, aluminium and iron which strongly reduces its availability to plants (Tinker and Nye 2000). Nutrient availability in the soil is dependent on the form and chemical nature of the element, soil pH, CEC, organic matter, microbial activity, moisture content and interactions with soil colloids (organic and inorganic).

1.2 Soil factors influencing soil nutrient availability

1.2.1 Cation exchange capacity

Cation exchange capacity (CEC) is the total capacity of a soil to hold exchangeable cations. It is an important property of soil influencing nutrient and water availability, buffering capacity and structural stability. Mineral cations adsorb on the negatively charged surface of inorganic and organic colloids. The inorganic soil colloids usually found in soil include silicate clays, iron and aluminium hydroxides. Non-crystalline clay minerals such as allophanes have high surface area, highly variable and pH dependent charge (Powers and Schlesinger 2002). Surface area and CEC are low in non-expanding crystalline clays such as kaolinite (1:1) but high in smectite and other 2:1 clays (Six et al. 2000). Therefore, the amount and type of clay minerals determine the quantity of negative charge contributed by the clay-sized fraction. Compared to sandy soils, clay soils have a large surface area and large number of negative charges; they can attract and hold positively charged ions. The negative charge of clay minerals is due to isomorphic substitution and therefore independent of pH (Foth and Ellis 1988). Nutrients bound to cation exchange sites can become plant available because the bound nutrients are in equilibrium with the soil solution. Binding of nutrients to colloids also reduces nutrient leaching. This is an important property of nutrients because many positively charged ions are plant nutrients, such as NH_4^+, Ca^{2+}, Mg^{2+} and K^+ etc. (Hamarashid et al. 2010). Of all soil types, sandy soils have the lowest CEC and the highest proportion of large pores and therefore hold few nutrients and lose them easily by leaching (Walpola and Arunakumara 2010). Organic matter plays a key role in nutrient and water retention particularly in sandy soils. The CEC contributed by organic matter is different from CEC of clay minerals. The negative charge on organic matter is pH dependent because the chemical groups, e.g. organic acids are deprotonated (positively charged) only in a certain pH range. Below that range, they are protonated and therefore have neutral charge.

1.2.2 Soil pH

Nutrient availability is dependent on soil pH because pH affects the solubility of nutrients, binding to soil colloids, microbial activity and chemical transformations (Marschner 2012). The availability of most nutrients is highest at pH 6-7. In alkaline soils (pH ≥8), the concentration of most micronutrients (Fe, Zn, B, Cu) in the soil solution is low while in acidic soils (pH ≤ 4) Mn and Al can reach toxic concentrations in the soil solution. Phosphorus availability is low in alkaline and acidic soils. The most dominant form of P in the soil solution is HPO_4^{2-} and PO_4^{3-} in alkaline soil and $H_2PO_4^-$ in acidic soils. At high pH (≥ 8.0) phosphate ions are precipitated with calcium and form calcium phosphate (Hinsinger et al. 2009) and at low pH phosphate reacts with aluminium and iron and becomes unavailable (Zheng 2010). Iron (Fe) is a micronutrient and required for plants in small amounts but its availability is also dependent on pH. At high pH (≥ 8.0) in calcareous soils iron solubility is low that can cause iron deficiency in plants. In addition, increasing concentration of phosphorus in soil solution may reduce the availability of iron due to formation of iron phosphates especially under neutral or alkaline conditions (Norvell 1972). At low pH (< 4) or under reduced conditions iron solubility increases which can result in Fe toxicity.

1.2.3 Organic Matter

Organic matter acts as reservoir of plant nutrients (Schnitzer 2005) because it supplies nutrients via mineralization and desorption while binding of nutrients can minimize leaching of nutrients. In addition, organic matter can improve soil water retention (Olness and Archer 2005), infiltration (Pachepsky and Rawls 2003) and aggregation (Franzluebbers 2002) and reduces compaction, crusting, runoff and erosion (Lal 2004a). Therefore, organic amendments are important soil conditioners to improve soil physical properties (Teklay et al. 2006). Soil organic matter (SOM) decomposition and accumulation are influenced by factors such as texture (Krull et al. 2001), clay mineralogy, pH, aeration, temperature, water content and microbial activity through their effect on plant growth (input) and decomposition (Coleman et al. 2004). Further, decomposition is influenced by the chemical composition of the organic amendments (Tian et al. 1992).

2. Factors affecting organic matter decomposition

2.1 Soil Texture

With similar input clay soils have a higher organic matter content than sandy soils due to strong binding to clay minerals (Tisdall and Oades; Von Lutzow et al. 2006) and formation of macroaggregates which reduce organic matter accessibility to decomposers and therefore decomposition rate (Rice 2002; Kaiser and Zech, 2000).

Sandy and clay soils can also differ in aeration which in turn influences microbial activity and organic matter decomposition rate (Cosentino et al. 2006). Clay particles are < 2 μm which results in dominance of micro pores in clay soils (Dixon 1991). This increases water retention but can also lead to anaerobic conditions due to poor gas exchange (Franzluebbers et al. 1996). Diffusion rate of gases is 10,000 times higher in air than in water (Kirkham 2004). Organic matter decomposition rate is lower in anaerobic than aerobic conditions because energy yield of the former is lower. Macro pores drain quickly and are therefore often air filled which promote rapid gas exchange. Consequently, coarse textured soils (sandy soil) have more large air filled pores than clay soils (Mullins et al. 1990) and have very few SOM binding sites which leads to faster decomposition of organic matter (Strong et al. 2004) together with the poor plant growth and thus input, this results in lower organic matter content.

2.2 Mineralogy

Clay mineralogy plays an important role in sorption reactions, carbon sequestration and turnover. For example, kaolinite has lower SSA and CEC than smectite (Six et al. 2000, 2002). Saidy et al. (2012) reported greater organic carbon mineralisation in sandy soil with kaolinitic than smectitic clay. And Feng et al. (2005) found higher sorption of aromatic compounds and proteins on montmorillonite than kaolinite. However, some studies contradict the concept that high specific surface area and CEC of clay minerals increases organic carbon stabilization. Wattel-Koekkoek et al. (2001) found that clay minerals differed in the type of organic compounds bound. Kaolinite associated SOM was dominated in polysaccharide products while smectitic SOM was in aromatic compounds. But the amount of organic carbon in total clay sized fraction did not differ between smectitic and kaolinitic clay. Brunn et al. (2010) compared tropical clay soils with contrasting mineralogy and found higher SOC lability in kaolinitic soils compared to smectitic. They suggested that presence of Fe and Al in clay soils also affects the SOC stabilization. Organic matter is bound to clay surfaces mostly via cation bridges such as Ca^{2+}, Fe^{3+} or Al^{3+} and to Fe and Al oxides by ligand exchange (Von Lützow et al. 2006).

Lal et al. 1997 categorised mechanisms of carbon stabilisation in soil into i) physical stabilisation- physical protection of organic matter that is inaccessible to microbes, ii) chemical stabilisation-formation of organo-mineral complexes iii) biochemical recalcitrance for example lignin derivatives or melanines produced by various microbes. Organo-mineral complexes result from bonding of organic matter with soil minerals through ligand exchange, cation bridges, van der Waals interactions and hydrophobic bonding depending on the properties of both mineral surface and organic matter. Feng et al (2013) found that organic carbon associated with fine particles (< 20 µm) has long turnover time. The amount of carbon associated with < 53 µm fraction is affected by texture (Six et al. 2002), mineralogy (Feng et al. 2005), amount and type of oxides and hydroxides of Iron (Fe) and Aluminium (Al) and type of organic carbon inputs (land use and residue addition).

Several studies have shown the importance of Fe and Al oxides for sorption of dissolved organic C (DOC). According to Kubicki et al. (1999) DOC sorption to clay minerals was lower than to Fe and Al oxides. Other studies confirmed that presence of Fe/Al oxides can increase the bonding of organic C to clay surfaces by providing loci for adsorption and/or complexation of organic matter (Kleber et al. 2007; Saidy et al. 2012; Barré et al. 2014). Esterhues et al. (2005) found that removal of hydrous Al and Fe oxides reduced DOC sorption in soils. Recently, Curtin et al. (2016) showed a positive relationship between pyrophosphate extractable Fe and Al concentration of the clay and silt fractions and their OC content. Therefore, Fe and Al oxides e.g. on clay surfaces may compensate for low OC binding capacity of clay minerals.

2.3 Residue properties (C/N ratio and organic carbon composition)

Plant residue properties such as C/N ratio, water soluble C (Tian et al. 1992; Vanlauwe et al. 1996) and lignin concentration or particle size affect decomposition rates. The C/N ratio is particularly important because microorganisms have a low C/nutrient ratio (typically C/N < 20). Thus, organic amendments with low C/N ratio can satisfy the nutrient demand of microbes, are rapidly decomposed and result in early net nutrient mineralisation (Hadas et al. 2004; Janssen, 1996). Organic amendments with high C/nutrient ratio on the other hand, are decomposed slowly and can result in, at least temporary, net immobilisation of nutrients in the microbial biomass (Moritsuka et al. 2004).

After addition of plant residue in soil, the simple compounds such as sugars, amino acids and low molecular weight phenolics decomposed quickly while complex compounds like lignin, cellulose and hemi-cellulose decomposed slowly (Berg and McClaugherty 2003). Further, organic carbon compounds such as humus, phenolics, cellulose, hemicellulose and lignin (Baldock 2007).

3. Sandy soil and clay soil

As outlined above many soil properties are influenced by soil texture. Sandy soils have > 85% of sand and have particle size 0.05-2 mm and specific surface area of 0.01-0.2 m^2 g^{-1}. These are siliceous sands with silica as a dominant mineral which is usually present in the form of quartz that is resistant to weathering. Sandy soils have very low cation exchange capacity and have no charge on the surface which results in reduced formation of aggregates.

Clay soils on the other hand have > 40% of clay, high water and nutrient retention capacity and often high organic matter content (Kaiser and Zech 2000). Clay minerals have a high cation exchange capacity and can therefore bind positively charged nutrients, e.g. $NH4^+$, Ca^{2+}, Mg^{2+} and K^+ (Hamarshid et al. 2010). Negatively charged ions such as $HPO_4{}^{2-}$ can be bound via multivalent cations such as Ca^{2+} or Fe^{3+} (Frossard et al. 1995; Yaghi and Hartikainen 2014). Clay can also bind organic matter through ligand exchange, cation bridges (Von Lützow et al. 2006) and weak interactions such as van der Waals forces, H-bonding (Arnarson and Keil 2000). Binding to clay protects organic matter from decomposition by reducing accessibility to decomposers (Baldock 2007, Von Lützow et al. 2006).

Soil pores can be classified by size. Micro pores, are less than 0.08 mm diameter (Brady and Weil 2002). Micropores are usually much smaller than the size of root but provide sites for water storage (Whalley et al. 2012). Macro pores are > 0.08 mm diameter, they can be penetrated by roots and provide habitat for microorganisms. Water is held more tightly in micro- than in macro-pores, therefore, water drains more freely by gravity through macro pores and allows air and water movement in soil. As soil dries, hydraulic conductivity decreases which increases resistance for water movement and the remaining moisture held more tightly to the soil particles (Ilstedt et al. 2000). The higher (more negative) the water potential is, the more energy has to be spend to remove it, thus the lower the water availability becomes. There is a unique relationship between water content (θ) and water potential (ψ) for each soil which is described by the water retention curve or soil moisture characteristic curve (Barbour 1998). At saturation, clayey soils contain more total water than the sandy soils due to the greater number of pores. When water is drained as a result of gravitational potential and if there is no evaporation loss, the amount of water retained in soil is called field capacity (FC, pF 2). At this water potential water is drained from macro pores and they are filled with air; the soil retains water in micro pores and on particle surfaces. It is the upper limit of the amount of water available to plants.

Due to large particle size, sandy soils have large pore spaces which improves drainage/aeration and root penetration but increases and leaching of nutrients (Shephered and Bennett 1998). In Florida, Chen et al. (2006) indicated that a typical sandy soil of Florida applied with water-soluble P fertilizer resulted in leaching loss of added P by 96.6%. Other studies, on sandy soils in Zimbabwe (Hagmann 1994), indicated that up to 54% of applied fertilizer was leached out of the top 0.5 m of soil when heavy rains followed N fertilizer application.

Clay soils on the other hand are dominated by micropores which can hold water. Therefore, management of heavy clay soils is difficult because they can become anaerobic when wet and hard when they dry (Jayawardane and Meyer 1985). Clay soils have a large amount of plant available water while it is low in sandy soils. For example, Johnson et al. (2009) found that sandy soils contain 25-100 mm m^{-1} and clay soils contain 175-250 mm m^{-1} available water.

4. Improving sandy soils

The productivity of sandy soils is limited by high infiltration rate, high evaporation, low nutrient and water-holding capacity and low organic matter content. For example, addition of synthetic soil conditioner, (Choudhary et al. 1998; Al-Omran et al. 1997; Al-Harbi et al. 1999) however this is expensive and improvement is only temporary. Slow-release N fertilizers are characterised by consistent release of nutrients over a long period which reduces nutrient leaching (Mitchelson and Franco 1994). But slow-release fertilizers tend to be more expensive, will not produce a rapid growth response, and release may be difficult to predict. Furrow sowing has been successfully used on sandy soils in Western Australia (Blackwell et al. 2014). Use of drip irrigation in sandy soils improves water supply and crop water availability which is critical to reduce potential crop water stress and leaching losses in sandy soils (Zotarelli et al. 2009).

Addition of compost in sandy soils increases water holding capacity, structural stability and amount of available nutrients (Tejada et al. 2009; Curtis and Claassen 2005). However, compost addition can increase nutrient leaching (Basso and Ritchie, 2005). Amendment with plant residues like wheat straw, rice straw could improve the chemical and physical properties of sandy soils (Al-Omran et al. 2002, 2004a). But due to lack of binding sites for organic matter they have only a short-term effect. Strategies must be introduced to keep the nutrients and water available for plant uptake.

5. Addition of clay rich soil to sandy soil

Addition of clay rich subsoil to sandy soil can result in long term improvement of crop production on sandy soils (Davenport et al. 2006). To enhance water and nutrient holding capacity of sandy soils, understanding the physico-chemical properties and depth of the clay-rich subsoil is important. These two parameters determine the most appropriate method for farmers to improve the sandy soil using clay. Deep sands can be ameliorated by spreading clay-rich material from a nearby source (e.g. clay pit or clay-rich soil) followed by incorporation. When clay soil is present at up to 60 cm depth (e.g. in Chromosols or Sodosols), it can be mixed into the sandy top soil by delving or spading (Davenport et al. 2011). Compared to addition of organic matter which has to be done at regular intervals because the material is decomposed, addition of clay may be a modification that permanently changes the soil properties. A careful consideration of the feasibility of these modification methods is important as they are expensive. Also important are the properties of the clay soil which vary with degree of weathering and parent material; for example, highly weathered soils have low CEC. In some areas subsoil clay is unsuitable because it is calcareous, saline or sodic which may aggravate nutrient deficiency in sandy soils (Davenport et al. 2011).

Addition of clay rich soil to sandy soil changes the texture of sandy soil, which can alter both the nutrient status of the soil and ability of the plant to take up nutrients. Furthermore, clay soil addition reduces the water infiltration and leaching of nutrients. Clay soil amendment to sandy soil can increase organic carbon binding by protecting organic matter from decomposition (Baldock and Skjemstad 2000). It has been recently reported by Roychand and Marschner (2013); Shi and Marschner (2012) that clay addition to sandy soil reduces cumulative respiration when crop residue is added. As discussed previously, physical and chemical stabilization is based on adsorption and chemical binding of SOC on to mineral surfaces. Soil organic matter mineralization reduced to 20-30% by sorption of soluble organic matter to clay surfaces compared with mineralization in soil solution (Kalbitz et al. 2005). As a result of the greater physical and clay soils have lower N transformation rates than sandy soils due to greater physical protection of added residue (Chivenge et al. 2011a).

Clay minerals can adsorb NH_4^+-N; Chantigny et al. (2004) reported that highest fixation of NH_4^+-N by clay minerals occurred within the first day and was greater in clay soil (34% of applied $^{15}NH_4^+$-N) than sandy soil (11%). In addition, NH_4^+ fixation was 25% lower in moist (60% of maximum WHC) than in dry soil (Osborne 1976b) due to expansion in clay minerals under wet conditions.

Sandy soils are inherently low in fertility. Sandy soils are characterised by low organic matter content, low CEC, and high risk of leaching (Blanchart et al. 2007; Noble et al. 2000; Weaver et al. 1988). Sitthaphanit et al. (2010) reported that bentonite addition to sandy soil can delay NH_4 leaching by 15 days after fertilizer addition compared to sandy soil alone.

The literature review showed that many properties that constrain crop growth on sandy soils can be alleviated by addition of clay soils. However, several knowledge gaps remain, some of which were addressed in this study. Firstly, most incubation studies on the effect of clay addition to sandy soils were carried out with finely ground clay. However, in the field, the added clay is present in peds ranging in size from millimetres to several centimetres which reduces the clay surface area available for binding compared to finely ground clay and may therefore influence the effect of clay addition. Further, the peds vary in size which can also influence surface area. To better understand the effect of clay addition to sandy soils, incubation studies with clay peds should be carried out. Secondly, most studies with clay amended sandy soils involved addition of inorganic fertilisers. However, nutrients could also be added in the form of organic materials e.g. harvest residues. This allows studying not only binding of inorganic nutrients but also organic carbon to clay. Thirdly, the effect of mineralogy of the added clay on nutrient and organic carbon binding needs further investigation.

The aims of the study were

i) To determine the effect of clay addition rate and ped size in residue amended sandy soil on soil respiration, nutrient availability and organic C retention (Chapter 2, 4 and 5).

ii) To assess the effect of clay soil particle size (finely ground or 2 mm peds) and clay soil properties on nutrient availability and organic C binding in sandy soil after addition of residues with low or high C/N ratio (Chapter 3).

iii) To determine the effect of clay addition rate and ped size to reduce leaching of nitrogen and phosphorus in sandy soil (Chapter 6).

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CHAPTER 2

CLAY AMENDMENT TO SANDY SOIL - EFFECT OF CLAY CONCENTRATION AND PED SIZE ON NUTRIENT DYNAMICS AFTER RESIDUE ADDITION

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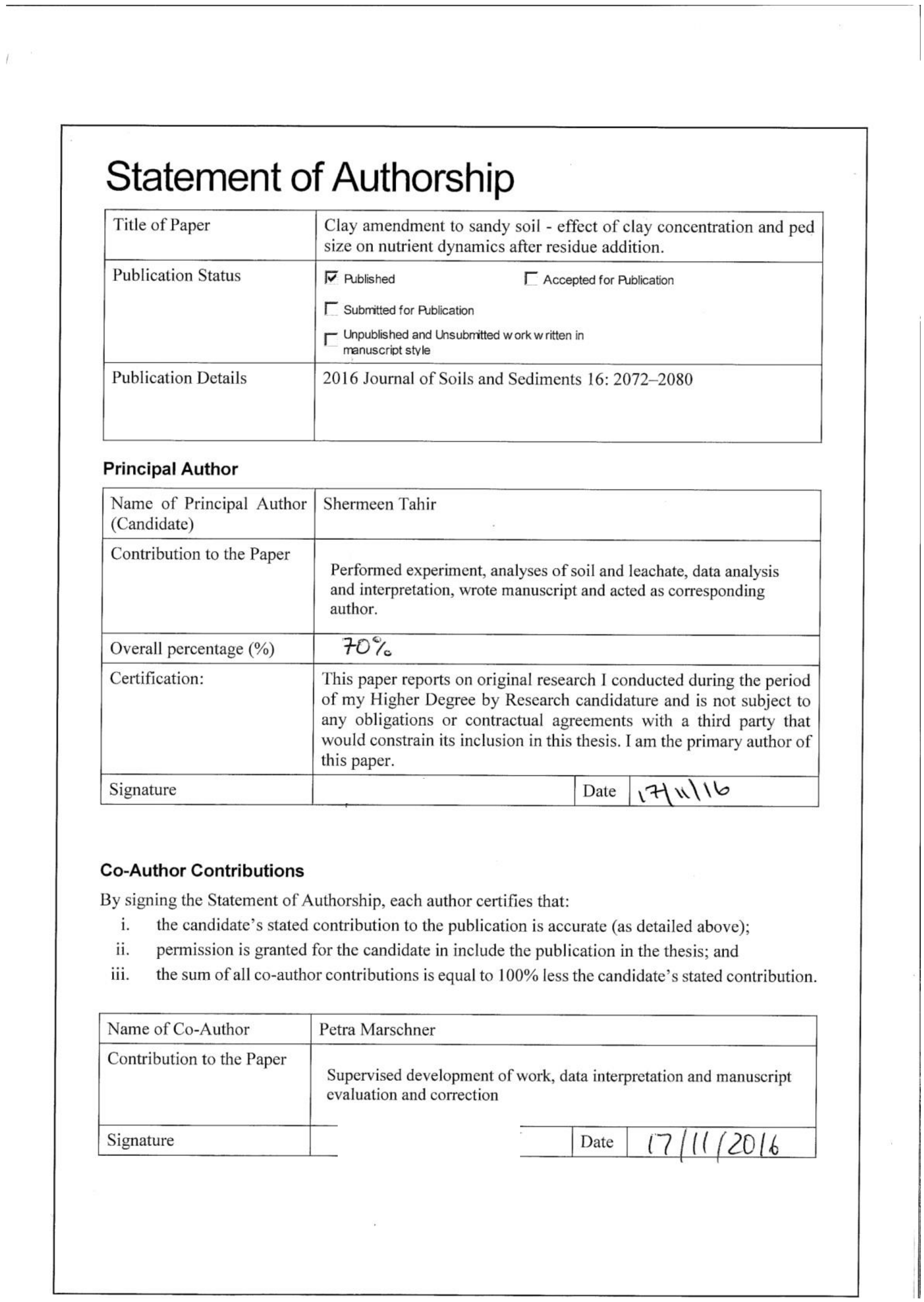

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Contribution to the Paper	Performed experiment, analyses of soil and leachate, data analysis and interpretation, wrote manuscript and acted as corresponding author.		
Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
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iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Petra Marschner		
Contribution to the Paper	Supervised development of work, data interpretation and manuscript evaluation and correction		
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Clay amendment to sandy soil - effect of clay concentration and ped size on nutrient dynamics after residue addition

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Abstract

Purpose: Crop growth on sandy soils can be increased by claying. In modified sandy soils, the added clay is in the form of peds ranging in size from mm to cm creating a highly non-uniform matrix where ped size could influence nutrient availability and organic C binding. The aims of the study were to determine the effect of clay addition rate and ped size in residue amended sandy soil on soil respiration, nutrient availability and organic C retention.

Materials and methods: In this study, clay peds of 1, 2 or 3 mm size derived from a clay-rich Vertosol (73% clay) were added to a sandy soil (3% clay) at clay addition rates of 10% and 20% w/w. After addition of ground faba bean residue (C/N 37) at 10 g kg^{-1}, the soils were incubated for 45 days at 80% of water holding capacity.

Results and discussion: Clay addition had no consistent effect on cumulative respiration, but reduced NH_4^+ availability with a greater reduction at 20% compared to 10% clay and with 1 and 2 mm compared to 3 mm peds. Sandy soil with clay peds had a greater maximum NH_4^+ and P sorption capacity than sandy soil alone, sorption capacity was higher at 20% compared to 10% clay addition and greater with 1 mm compared to 3 mm peds. Retrieval of clay peds at the end of the experiment showed ped breakdown during the experiment but also formation of larger peds. Compared to the < 53 µm fraction added at the start of the experiment, the total organic carbon (TOC) content of the < 53 µm fraction was increased up to nearly two-fold, particularly in the smaller peds (1 and 2 mm).

Conclusions: When sandy soils are amended with clay, N availability and organic C binding depend on both clay addition rate and ped size.

Keywords: Ammonium sorption • Clay peds • Claying • Organic C binding

1 Introduction

Sandy soils have inherent low fertility due to low organic matter content, cation exchange capacity and water holding capacity (Xie and Steinberger 2005). Various strategies have been proposed to overcome the low nutrient and water holding capacity of sandy soils. For example, furrow sowing (Blackwell et al. 1994), slow release fertilisers to enhance nutrient use efficiency or addition of organic materials to improve water and nutrient retention (Mitchelsen and Franco 1994; Shepherd and Bennett 1998) but these management options improve soil fertility only temporarily. Fertilisers have to be added repeatedly because nutrients are rapidly leached out of the root zone. Addition of organic materials such as plant residues can increase microbial activity and organic carbon sequestration as well as provide nutrients for plants (Lal 2005). But due to the lack of binding sites, nutrients are easily leached from sandy soils and the added organic material is rapidly decomposed (Walpola and Arunakumara 2010).

In contrast to sandy soils, clay soils have high water and nutrient retention capacity and often high organic matter content (Kaiser and Zech 2000). Clay minerals have a high cation exchange capacity and can therefore bind positively charged nutrients, e.g. NH_4^+, Ca^{2+}, Mg^{2+} and K^+ (Hamarshid et al. 2010). Negatively charged ions such as HPO_4^{2-} can be bound to the negatively charged clay via multivalent cations such as Ca^{2+} or Fe^{3+} (Frossard et al. 1995; Yaghi and Hartikainen 2014). Clay can also bind organic matter through ligand exchange, cation bridges, e.g., Al, Fe, Ca and Mg (Von Lützow et al. 2006) and weak interactions such as Van der Waals forces, H-bonding (Arnarson and Keil 2000), depending on the properties of both mineral surface and organic matter. Binding to clay protects organic matter from decomposition by reducing accessibility to decomposers (Baldock 2007, Von Lüetzow et al. 2006).

Amelioration of sandy soil by clay rich soil has been shown to increase crop production across Southern Australia (Gardner et al. 1992; Cann 2000; Davenport et al. 2006; Hall et al. 2010). Clay soil can be derived from nearby areas or, in texture-contrast soils with a sandy to sandy loam A horizon and clayey B horizon, subsoil clay can be brought up by delving or spading (Hall et al. 2010; Ismail and Ozawa 2007). In Australia, such soils are found in about 17% of the area (Isbell 2002). Clay addition by spreading or delving is expensive costing AU$250-300 per hectare, but can improve yields for about 10-15 years (GRDC 2015). The use of such technique can produce environmental benefits included increased organic C sequestration and reduced nutrient leaching into the ground water and soil erosion.

Previous studies have shown that addition of ground and sieved clay soil (< 2 mm) to sandy soil amended with plant residues reduced soil respiration and changed the response of soil respiration to drying and rewetting compared to sandy soil alone (Roychand and Marschner 2013, 2014; Shi and Marschner 2014). This can be attributed to binding of organic matter to clay particles and water retention by clay. However, in sandy soils amended with clay, the clay soil mainly occurs as peds of various sizes ranging from mm to cm. The influence of clay peds on nutrient availability and organic C retention could be different to that of finely distributed clay and may depend on clay concentration as well as ped size. This information is important for management of clayed sandy soils.

The aims of the study were to (i) determine the effect of clay addition rate and ped size in residue amended sandy soil on soil respiration, nutrient availability, and (ii) assess ped dynamics and organic C retention. We hypothesised that: (1) clay addition to sandy soil with residue amendment will reduce nutrient availability because of reduced decomposition rate (residues bound to clay) and binding of NH_4^+ and inorganic P, and (2) the clay effect will increase with clay concentration and will be greater with small peds than larger peds because of their greater surface area/volume ratio and therefore greater binding capacity.

2 Materials and methods

2.1 Soils

Clay soil was collected from Waite Campus (latitude 34.97˚S, 138.63˚E). The soil is classified as black Vertosol in the Australian classification (Isbell 2002) or Vertisol in the World Reference Base, with smectite as dominant clay mineral (Table 1). The soil was air-dried, crushed and then sieved through different sieves to achieve peds of different sizes. Three mm peds were collected on a 2-mm sieve after sieving through a 3.35 mm (size range 2-3.35 mm). The soil that passed through the 2-mm sieve was then sieved through a 1 mm sieve. The peds retained on the 1 mm sieve are referred to 2 mm peds (size range 1-2 mm). The < 1 mm material was passed through a 0.5 mm sieve; the peds on the 0.5 mm sieve are referred to 1 mm peds (size range 0.5-1 mm) (Fig. S1). These ped sizes were chosen because in the field a large proportion of clay peds are in this range, particularly after clay spreading (Schapel, personal communication). Fine sandy soil (classified as Arenosol in the World Reference Base) collected in Penola (latitude 37.37˚S, 140.83˚E) which is in an area where claying is used to enhance yield on sandy soils. The sandy soil was air-dried and sieved to < 2 mm (Table 1). The clay peds were added to the sandy soil at clay addition rates of 10% and 20% w/w.

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. Particle size distribution was measured by the hydrometer method (Bouyoucos, 1936) and organic carbon and nitrogen were determined by CHN analyser. 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) (Table 2).

2.2 Main experiment

Mature faba bean (*Vicia faba* L.) residue [C/N 37, pH in 1:20 (residue: RO water) 5.4] was ground, sieved to particle sizes < 2 mm and added a rate of 10 g kg^{-1} to the soil mixes. After thorough mixing of sand, clay peds 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 (0.75 µm, Australian Filter Specialist) base. The soil was packed to a bulk density of 1.5 g cm^{-3} by adjusting the height of the soil mixes in the core. Water content was maintained gravimetrically at 80% of maximal water holding capacity (WHC). This water content was selected on the basis of a preliminary experiment where the sandy soil-clay ped mixtures were maintained at different percentage of WHC (40, 50, 60, and 80% of maximum WHC). Glucose was added as an organic carbon source and soil respiration was measured for one week. Soil respiration was highest at 80% of WHC in all treatments.

Three destructive harvests were carried out, on days 15, 30 and 45 with 4 replicates per treatment and harvest time. The cores to be sampled on day 15 were placed in 1 L jars with gas-tight lids equipped with septa to allow quantification of headspace CO_2 concentration as described below. The remaining cores were placed in a plastic tray covered loosely with a lid. On day 15, the cores in the jars were removed 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.

Due to the upper detection limit of the gas analyser (2% 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) and available N (NH_4 and NO_3) were measured. Available P was determined on day 45.

Microbial biomass carbon 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 with 0.033 M acidified $(NH_4)_2$ $Fe(SO_4)_2.6H_2O$ after dichromate oxidation (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).

Available N was extracted by shaking soil with 2 M KCl solution at a soil:solution ratio of 1:5 for one hour at 200-300 rpm. The suspension was filtered through Whatman filter paper No. 42. Ammonium was measured colorimetrically at 685 nm as described by Willis et al. (1996). Nitrate in the 2 M KCl extracts was determined colorimetrically at 540 nm using a modification of Miranda et al. (2001) as described in Cavagnaro et al. (2006). Available P was measured by the anion exchange resin method (Kouno et al. 1995).

Clay peds were retrieved at the end of experiment (day 45) by sieving from the treatments with 20% clay addition. The soil in each core was sieved through 2, 1 and 0.5 mm sieves to separate peds of different sizes (3, 2 and 1 mm). However, the total weight of the retrieved peds was greater than of added peds because sand particles adhered to the peds and could not be removed manually. To separate coarse and finer particles, the retrieved peds were dispersed by shaking with 3% sodium hexametaphosphate at a ped:solution ratio of 1:13 for two hours at 200-300 rpm. The suspension was sieved through 250 and 53 µm sieves and dried overnight at 70˚C in the oven. This separation was also carried out with the initial 1, 2, and 3 mm peds. The > 53 µm (material retained on 250 and 53 µm sieves) and the < 53 µm fractions were dried, weighed and then ground and analysed for total organic carbon by wet digestion (Walkley and Black 1934).

2.3 Maximum ammonium and phosphorus sorption by clay peds

In separate experiments, maximum ammonium-N and phosphorus sorption were determined. Sandy soil alone and sandy soil with clay added at 10 and 20% as 1 and 3 mm peds were shaken for 30 minutes at 200-300 rpm at a 1:10 soil:solution ratio with solutions containing 0-45 mg NH_4^+-N L^{-1} or 0-25 mg H_2PO_4 L^{-1} P. After shaking, the NH_4^+ and P concentration in the filtered supernatant was determined. Sorption was calculated by subtracting the final concentration from the initial concentration.

2.4 Effect of clay peds and calcium carbonate on pH and respiration

The clay soil had a high pH and contained carbonate (Churchman, personal communication). The pH increase induced by clay addition to sandy soil and dissolution of carbonates could influence CO_2 release. Therefore, a separate experiment was carried out to test the effect of $CaCO_3$ and clay addition to sandy soil on pH and soil with and without 10 g kg^{-1} faba bean residues. Finely ground calcium carbonate was added to sandy soil at the rate of 10 g kg^{-1} soil to achieve a similar pH as clay addition to sandy soil with residues (pH 8.7). This rate was selected by testing the pH of sandy soil after addition of calcium carbonate rates in the range of 10-100 g of $CaCO_3$ kg^{-1} soil. After filling the soil mixes into cores, water was added to achieve 80% of water holding capacity and the bulk density was adjusted to 1.5 g cm^{-3}. The water content was maintained as in the main experiment. Respiration was measured daily for 15 days as described above and the pH (1:5) was measured at the end of experiment.

2.5 Statistical analyses

The data were analysed by one way ANOVA for each sampling time separately using Genstat 15th edition (VSN Int. Ltd, UK). Tukey's multiple comparison test at 95% confidence interval was used to determine significant differences among treatments.

3 Results

Addition of clay peds increased maximum water holding capacity compared to sandy soil alone three to six-fold with a greater increase at 20% compared to 10% clay added (Table 2). At a given clay concentration, the increase in water holding capacity was greatest with 1 mm peds and smallest with 3 mm peds.

Total cumulative respiration (0-45 days) compared to the sandy soil alone was significantly lower with 2 mm peds at 10% clay addition (reduced by 25%), but significantly higher with 3 mm peds (increased by 20%) (Fig. 1). Clay addition at 20% as 1 and 2 mm peds significantly increased cumulative respiration compared to sandy soil alone by 12 and 10%. The microbial biomass C concentration ranged between 100 and 200 µg g^{-1} soil and did not differ among clay treatments or sampling times (data not shown).

The NH_4^+-N concentration was higher on day 15 than day 45 (Fig. 2). On day 15, compared to sandy soil alone, clay addition decreased the NH_4^+-N concentration except for 1 mm peds at 20% clay addition where the NH_4^+-N concentration did not differ significantly from sandy soil alone (Fig. 2a, Table S1). The decrease in NH_4^+-N concentration compared to sandy soil alone was greater with 2 and 3 mm (by 60-70%) than with 1 mm peds (by 10-40%) at both clay addition rates. On days 30 and 45, all clay treatments significantly reduced the NH_4^+-N concentration compared to sandy soil alone. On day 30, for 1 and 2 mm peds the decrease was greater at 10% clay addition (by 60%) than at 20% (by 40%, Fig. 2b, Table S1). The decrease was similar at the two clay addition rates with 3 mm peds (by 50%). On day 45, the decrease in NH_4^+-N concentration compared to sandy soil alone was greater at 20% clay addition rate (by 50-70%) than with 10% (by 30 to 50%) for all ped sizes (Fig. 2c, Table S1). At a given clay concentration, the decrease was greater with 1 and 2 mm than with 3 mm peds. At all sampling times, NO_3^--N concentrations were at or below detection limit (data not shown).

Maximum NH_4^+-N sorption was lowest in sandy soil alone (7 µg NH_4^+-N g^{-1} soil) (Table 3). Clay addition increased maximum NH_4^+-N sorption 8 to 20-fold with a greater increase at 20% compared to 10% clay added and a greater increase with 1 compared to 3 mm peds. For example, with 1 mm peds, maximum sorption was 60% higher at 20% than at 10% clay addition. At 10% clay addition, maximum NH_4^+-N sorption was 60% higher with 1 than with 3 mm peds (Table 3).

Clay addition at 10% increased the available P concentration on day 45 compared to the sandy soil alone (Table S2). This increase was greater with 1 and 2 mm peds (by 30%) than with 3 mm peds (by 20%). At 20% clay addition compared to sandy soil alone, the available P concentration was increased only with 1 mm peds (by 20%). At a given ped size, the available P concentration was greater at 10 than at 20% clay added. Maximum P-sorption was two-fold higher at 20 than at 10% clay addition (Table S3). At a given clay addition rate, maximum P sorption was greater with 1 than with 3 mm peds.

The pH was lower in sandy soil alone (pH 7.4-7.9) than in clay amended treatments (8.1-8.6, Table 4). In sandy soil with clay, the pH did not differ among treatments and was similar on days 15 and 30. On day 45, the pH differed among clay treatments, being lower with 3 mm than with 1 and 2 mm peds.

In the additional experiment with sandy soil amended with $CaCO_3$ or clay peds, the pH was lowest in sandy soil alone (Table 5). Addition of faba bean residues and $CaCO_3$ increased the pH by two to three units, with the greatest increase in the treatment where both residues and $CaCO_3$ were added. Addition of 20% clay as 2 mm peds increased the pH by about two units with and without residue addition. In the treatments without residue addition, cumulative respiration was lowest in sandy soil alone. Cumulative CO_2 release was increased by 30% by addition of $CaCO_3$, but increased nearly two-fold with 20% clay added. Addition of faba bean residues increased cumulative CO_2 release 10-20-fold compared to treatments without residue addition. Compared to sandy soil with residues, cumulative CO_2 release was decreased by 6-8% by $CaCO_3$ and clay addition.

In the peds added at the start of the experiment, the TOC content was 2.9 g kg^{-1} in the > 53 µm fraction and 5.3 g kg^{-1} in the < 53 µm fraction. The TOC content did not differ among ped sizes.

At the end of the experiment peds were retrieved in the treatments with 20% clay addition. Peds of 1, 2 and 3 mm size were retrieved irrespective of the size of peds added (Table 6). But the weight of the retrieved peds was greater than the weight of peds added because sand particles adhered to the peds and could not be removed manually. Therefore, the retrieved peds were separated into > 53 and < 53 µm fractions by sieving. When 1 mm peds had been added, the greatest amount of the < 53 µm fraction was retrieved in the 2-mm clod size, but when 2 and 3 mm peds were added, the greatest amount of the < 53 µm fraction was retrieved in the added ped size, that is in 2 and 3 mm peds, respectively (Table 6). Between 74 and 77% of the clay added was retrieved at the end of the experiment. The TOC content of the < 53 µm fraction ranged from 4.2 to 9.2 g kg^{-1} and was highest in the ped size in which it was added, e.g., in 1 mm retrieved peds when 1 mm peds were added. The TOC content per core calculated for each size of retrieved peds (weight of the < 53 µm fraction of retrieved peds x TOC content of this fraction) and the percentage of total TOC in a given retrieved clod size was highest in the ped size in which the clay had been added for the 2 and 3 mm ped addition treatments, but in the 2-mm fraction of retrieved peds when 1 mm peds were added (Table 6). The total TOC content per core decreased with size of peds added.

4 Discussion

This short-term study showed that addition of clay peds to sandy soil influences N availability and organic C retention, the effect depending on both clay addition rate and ped size. Further, it showed that over a 45-day period, peds broke down but also aggregated to form larger peds. The results only partially confirm our first hypothesis [clay addition to sandy soil with residue amendment will reduce nutrient availability because of reduced decomposition rate (residues bound to clay) and binding of NH_4^+ and inorganic P], but confirm the second hypothesis (the clay effect will increase with clay concentration and will be greater with small peds than larger peds because of their greater surface area/volume ratio and therefore greater binding capacity).

Addition of clay peds had no consistent effect on cumulative respiration neither with respect to ped size nor clay addition rate (Fig. 1). For example, compared to the sandy soil alone, cumulative respiration with 2 mm peds was increased at 20% clay, but decreased at 10% clay. This inconsistent effect of clay addition as peds is in contrast to our previous studies where ground and sieved clay (< 2 mm without separation into peds) was added to sandy soil (Roychand and Marschner 2013, 2014; Shi and Marschner 2014). In these studies, clay addition reduced cumulative respiration compared to sandy soil alone. This can be explained by reduced accessibility of organic matter to microbes due to binding to clay surfaces (Baldock 2007). But the effect of clay added as peds on cumulative respiration was different, likely because peds provide less surface area per gram and are less uniformly distributed than finely ground clay. Thus, a smaller proportion of the sandy matrix and the added residue are in contact with the peds.

Clay addition increased soil pH by 0.4 to 1.2 units (Table 4) which indicates that it contains carbonates which could influence CO_2 release through pH effects on microbial activity (Andersson and Nilsson 2001) and CO_2 release from carbonates (Bertrand et al. 2007). The experiment where sandy soil was amended with $CaCO_3$ to increase the pH to similar values as the clay-amended soil showed the $CaCO_3$ addition had no significant effect on CO_2 release compared to sandy soil alone in presence or absence of faba bean residues (Table 5). Therefore, we can conclude that in this experiment, the addition of $CaCO_3$ with clay peds has negligible effects on soil respiration.

Sandy soils have lower water holding capacity than finer textured soil because they contain mainly large pores. On the other hand, the majority of pores in clay soils are small leading to high water-holding capacity (Lund 1959; Papendick and Campbell 1981). Consequently, addition of clay peds to sandy soil increased water-holding capacity in the present study with a greater increase with 20% than with 10% clay addition (Table 2). Among clay ped sizes, water-holding capacity was greatest with 1 mm peds. This can be explained by the more even distribution of the small peds in the sandy soil matrix compared to the larger peds which would be surrounded by wider zones of sandy soil. Therefore, small pores introduced by the clay would be more evenly distributed in the soil with 1 mm peds compared to addition of larger peds where there are distinct zones with mainly small pores (peds) and those dominated by larger pores (sandy soil). All soils were incubated at 80% of their respective maximum water-holding capacity; therefore, their water content differed, which could influence microbial activity. However, in this experiment, clay addition had no consistent effect on respiration (Fig. 1).

Addition of 20% clay increased the available NH_4^+ concentration compared to sandy soil alone (Table 2). But after addition of faba bean residues, the available NH_4^+ concentration was lower in clay-amended soil than in sandy soil except with 1 mm peds at 20% clay (Fig. 2). A clear effect of clay concentration and ped size only became apparent on day 45 when the NH_4^+ concentration was lower with 20% than with 10% clay added and lower with 1 and 2 mm compared to 3 mm peds. The results of the batch sorption experiment suggest that this decrease in available NH_4^+ concentration with clay addition can, at least in part, be explained by NH_4^+ sorption to the peds (Table 3). Clay minerals have a higher cation exchange capacity than sand and can therefore bind cations such as NH_4^+ (Sowden et al. 1978; Beauchamp and Drury 1991; Nieder et al. 2011). Maximum sorption was about 20-fold higher in clay-amended soil than in sandy soil alone. The higher maximum sorption capacity at 20% than at 10% clay addition and with 1 mm than with 3 mm peds can be explained by the greater surface area at higher clay concentration and the higher surface area to volume ratio of the smaller peds which is in agreement with our second hypothesis.

Clay can bind phosphorus, via multivalent cations such as Ca^{2+} or Fe^{3+} (Frossard et al. 1995; Yaghi and Hartikainen 2014). This was confirmed in this study as clay ped addition increased maximum P sorption capacity compared to sandy soil alone (Table S3). The finding that 10% clay addition increased P availability compared to sandy soil alone (Table S2) suggests that P sorbed to the clay peds may be readily available and in equilibrium with the soil solution. However, P availability was low in all treatments.

During incubation in moist conditions (80% of WHC) sand particles adhered to the peds therefore the weight of the retrieved peds was greater than that of the added peds. To better understand changes in ped properties, we isolated the < 53 µm fraction from the retrieved peds (Table 6). Peds of 1, 2 and 3 mm size were retrieved irrespective of the size of the added peds. This suggests aggregation of smaller peds (1 mm to 2 and 3 mm, 2 mm to 3 mm), but also breakdown of peds (2 and 3 mm to 1 mm, 3 mm to 2 mm). Some peds will have broken down to particles < 1 mm as only 74-77% of the added < 53 µm fraction was retrieved as peds at the end of the experiment. The breakdown may be due to drying and rewetting events. Although the water content of the soil was maintained at

80% of WHC, the peds may have dried out slightly. The moist incubation may have also favoured binding of peds to form larger aggregates. When larger peds were added, the greatest proportion of retrieved < 53 µm fraction was in the ped size added initially, that is 2 and 3 mm, respectively. But when 1 mm peds were added, more of < 53 µm fraction was retrieved from 2 mm than 1 mm peds, suggesting aggregation of the small peds.

Clay surfaces can bind organic matter via cation bridges (Wattel-Koekkoek et al. 2001; Tombacz et al. 2004; Gregory and Nortcliff 2013). In most cases, the TOC content of the < 53 µm fraction was higher at end of the experiment than initially (5.3 g kg^{-1}). In a given clay treatment, the increase in TOC concentration of the < 53 µm fraction compared to the initial value and the proportion of TOC per core were highest in the ped size added. This is most likely due to the length of time peds were exposed to the added plant residue which would be longest in the peds added initially. On the other hand, peds formed during the experiment by breakdown or aggregation would have a shorter period of exposure.

The decreasing TOC content per core with increasing ped size added can be explained by the greater surface area-to-volume ratio of the smaller peds; the smaller peds have a greater surface to bind organic matter than large peds. Although this was only a 45-day study, this finding suggests that particularly addition of small peds will increase organic C sequestration in sandy soils.

5 Conclusions

This study showed that clay addition to sandy soil influences nutrient dynamics after plant residue addition, particularly when small peds are added at higher rates. The increased NH_4^+ binding may have to be compensated by higher fertiliser addition rates, but will also improve N retention in the soil. The study confirmed that claying can increase organic C sequestration but also showed that organic C sequestration is likely to be greatest when the added clay peds are small. In the field, clay peds will range from mm to cm in size. If large peds (several cm) represent a high proportion of peds, the effect of claying on nutrient dynamics is likely to be small.

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Table 1. pH, electrical conductivity (1:5), particle size, organic C and total N content of clay soil and sandy soil.

	pH (1:5)	EC (1:5)	Particle size			Organic C	Total N
			Sand	Silt	Clay		
		µS cm^{-1}		(%)		g kg^{-1}	
Clay soil	8.03	621	12	15	73	12.7	1.0
Sandy soil	5.36	12.2	96	1	3	1.0	nd

nd = not detectable

Table 2. Maximum water holding capacity, ammonium and available (resin) 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*≤ 0.05).

	ped size (mm)	water holding capacity (g water g^{-1} soil)	NH_4-N µg g^{-1} soil	available P µg g^{-1} soil
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
	2	0.07 de	4.1 bc	8.0 bc
	3	0.05 e	4.0 bc	8.9 ab
Sandy soil +20% clay	1	0.13 a	5.4 a	7.5 c
	2	0.11 b	4.6 b	7.4 c
	3	0.10 bc	4.3 bc	7.6 c

Table 3. Maximum ammonium sorption of sandy soil alone or with 10 and 20% clay as 1 and 3 mm peds.

	ped size (mm)	Maximum NH_4-N sorption ($\mu g\ g^{-1}$ soil)
Sandy soil alone	none	7
Sandy soil + 10 % clay	1	84
	3	55
Sandy soil + 20 % clay	1	138
	3	113

Table 4. pH (1:5) on days 15, 30 and 45 of sandy soil amended with faba bean residue alone or with 10 and 20% clay as 1, 2 and 3 mm peds (n=4). Values within a column followed by different letters are significantly different (*P*≤ 0.05).

Treatments	ped size (mm)	pH (1:5) Days 15	30	45
Sandy soil alone	none	7.5 e	7.9 c	7.4 d
Sandy soil + 10 % clay	1	8.2 cd	8.6 a	8.6 a
	2	8.3 bc	8.5 ab	8.4 b
	3	8.1 d	8.3 b	8.3 c
Sandy soil + 20 % clay	1	8.4 a	8.6 a	8.6 a
	2	8.4 a	8.5 ab	8.5 ab
	3	8.3 ab	8.5 ab	8.4 b

Table 5. pH (1:5) and cumulative respiration after 15 days in sandy soil or sandy soil amended with $CaCO_3$, faba bean residues and 20% clay as 2 mm peds (n=3). Values in a column followed by different letters are significantly different ($P \leq 0.05$).

Treatments	Faba bean addition	pH (1:5)	Cumulative CO_2 (mg CO_2-C g^{-1} soil)
sandy soil alone	none	6.5 d	0.1 b
	+	8.1 c	1.9 a
sandy soil + $CaCO_3$	-	8.7 b	0.1 b
	+	9.2 a	1.8 a
sandy soil + 20% clay 2 mm	-	8.7 b	0.2 b
	+	8.7 b	1.8 a

Table 6. Weight, proportion and total organic C content of the < 53 µm fraction and proportion of total organic C content in retrieved peds after 45 days with 20% clay as 1, 2 or 3 mm peds (n=4). Values in a column followed by different letters are significantly different (*P*≤ 0.05).

Treatments with 20% clay added as peds of	Size of retrieved peds	Retrieved < 53 µm fraction	Retrieved < 53 µm fraction	TOC in < 53 µm fraction	TOC per core	Proportion of total TOC in peds
	mm	g core^{-1}	% of added clay	g kg^{-1}	mg core^{-1}	%
1 mm	1	1.38 b		9.2 a	12.7	43
	2	1.61 b		8.8 a	14.2	48
	3	0.63 cd		4.2 cd	2.7	9
	Sum	**3.62**	**77**		**29.6**	
2 mm	1	0.66 cd		5.7 bcd	3.8	16
	2	2.02 a		7.9 ab	16.0	69
	3	0.85 cd		4.2 cd	3.6	15
	Sum	**3.53**	**75**		**23.4**	
3 mm	1	0.62 d		3.6 d	2.2	11
	2	0.94 c		5.7 bcd	5.4	26
	3	2.01 a		6.6 abc	13.3	64
	Sum	**3.57**	**74**		**20.9**	

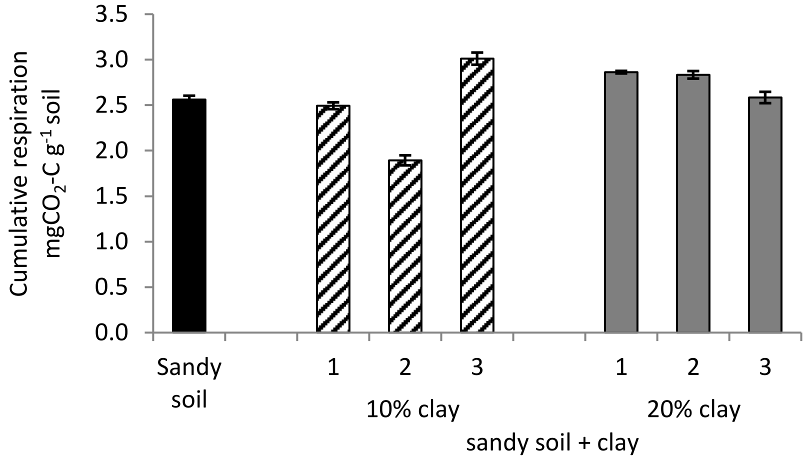

Fig. 1. Cumulative respiration over 45 days in sandy soil amended with faba bean residue alone or 10 and 20% clay as 1, 2 and 3 mm peds (vertical lines indicate standard error, n=4)

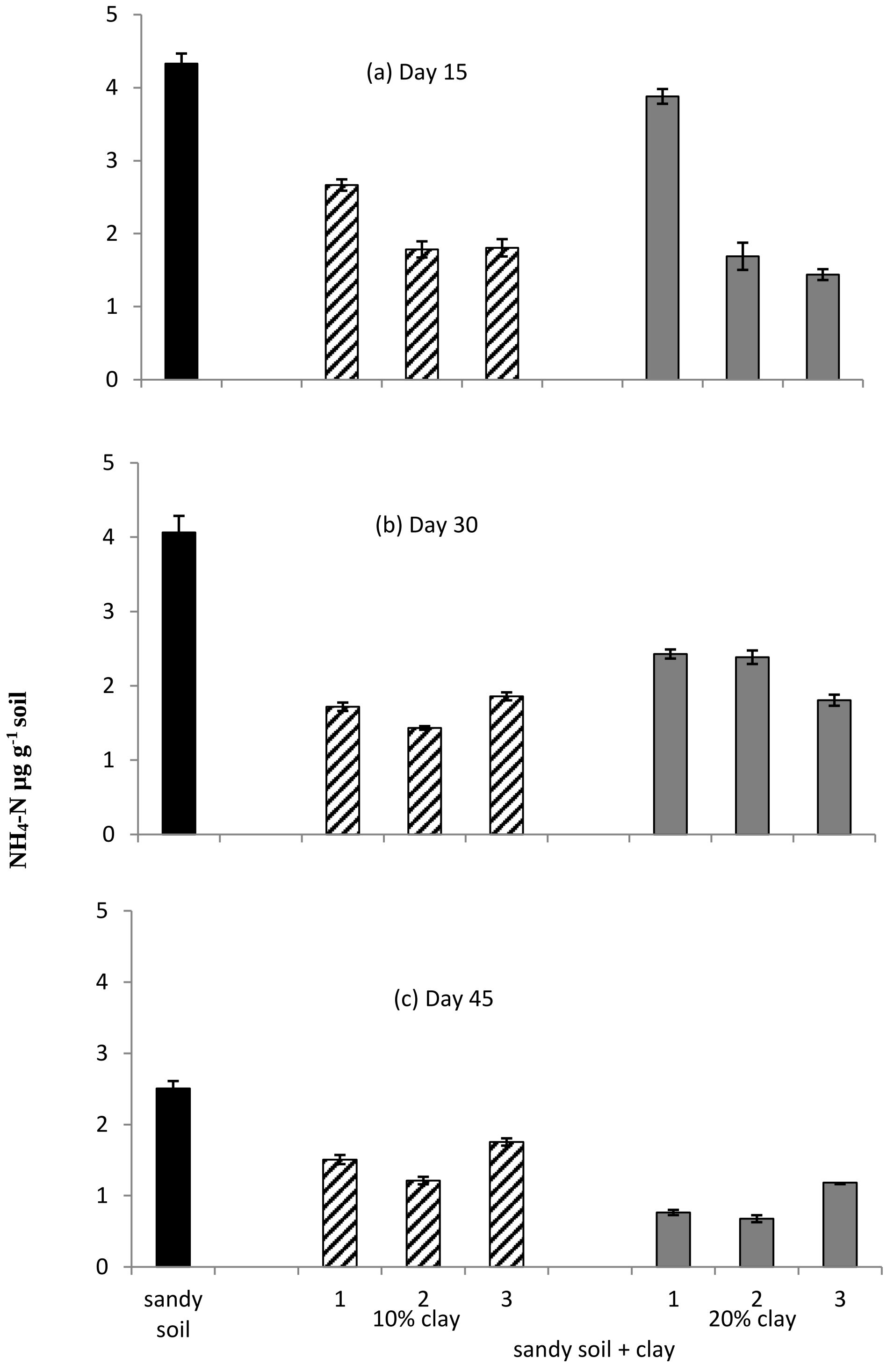

Fig. 2. Ammonium N concentration in sandy soil amended with faba bean residue alone or with 10 and 20% clay as 1, 2 and 3 mm peds on days 15 (a), 30 (b) and 45 (c) (vertical lines indicate standard error, n=4)

APPENDICES

Table S1. Ammonium N (µg N g^{-1} soil) on days 15, 30 and 45 in sandy soil amended with faba bean residue alone or with 10 and 20% clay as 1, 2 and 3 mm peds (n=4). Values within a column followed by different letters are significantly different (*P*≤ 0.05).

Treatments	ped size (mm)	NH_4-N (µg g^{-1} soil)		
		Days		
		15	30	45
Sandy soil alone	-	4.3 a	4.1 a	2.5 a
Sandy soil + 10 % clay	1	2.7 b	1.7 c	1.5 b
	2	1.8 c	1.4 c	1.2 c
	3	1.8 c	1.9 c	1.7 b
Sandy soil + 20 % clay	1	3.9 a	2.4 b	0.8 d
	2	1.7 c	2.4 b	0.7 d
	3	1.4 c	1.8 c	1.2 c

Table S2. Available (resin) P concentration on day 45 in sandy soil amended with faba bean residue alone or with 10 and 20% clay as 1, 2 and 3 mm peds (n=4). Values followed by different letters are significantly different (*P*≤ 0.05).

Treatments	ped size (mm)	µg P g^{-1} soil
Sandy soil alone	-	5.1 d
Sandy soil +10% clay	1	7.3 ab
	2	7.8 a
	3	6.5 bc
Sandy soil + 20% clay	1	6.6 ab
	2	5.3 cd
	3	4.9 d

Table S3. Maximum P sorption of sandy soil alone and or 10 and 20% clay addition as 1 and 3 mm peds.

	ped size (mm)	Maximum P-sorption ($\mu g\ g^{-1}$ soil)
Sandy soil	-	nd
Sandy soil +10% clay	1	33
	3	24
Sandy soil +20% clay	1	58
	3	48

nd is not detectable

1 mm 2 mm 3 mm

Fig. S1 Clay peds of different sizes

APPENDICES

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SOILS, SEC 2 • GLOBAL CHANGE, ENVIRON RISK ASSESS, SUSTAINABLE LAND USE • RESEARCH ARTICLE

Clay amendment to sandy soil—effect of clay concentration and ped size on nutrient dynamics after residue addition

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Abstract

Purpose Crop growth on sandy soils can be increased by claying. In modified sandy soils, the added clay is in the form of peds ranging in size from millimetres to centimetres creating a highly non-uniform matrix where ped size could influence nutrient availability and organic C binding. The aim of the study was to determine the effect of clay addition rate and ped size in residue amended sandy soil on soil respiration, nutrient availability and organic C retention.

Materials and methods In this study, clay peds of 1, 2 or 3 mm size derived from a clay-rich Vertosol (73 % clay) were added to a sandy soil (3 % clay) at clay addition rates of 10 and 20 % *w*/*w*. After the addition of ground faba bean residue (C/N 37) at 10 g kg^{-1}, the soils were incubated for 45 days at 80 % of water-holding capacity.

Results and discussion Clay addition had no consistent effect on cumulative respiration, but reduced NH_4^+ availability with a greater reduction at 20 % compared to 10 % clay and with 1 and 2 mm compared to 3 mm peds. Sandy soil with clay peds had a greater maximum NH_4^+ and P sorption capacity than sandy soil alone, and sorption capacity was higher at 20 % compared to 10 % clay addition and greater with 1 mm compared to 3 mm peds. Retrieval of clay peds at the end of the experiment showed ped breakdown during the experiment but also the formation of larger peds. Compared to the <53 μm fraction added at the start of the experiment, the total organic carbon (TOC) content of the <53 μm fraction was increased up to nearly two fold, particularly in the smaller peds (1 and 2 mm).

Conclusions When sandy soils are amended with clay, N availability and organic C binding depend on both clay addition rate and ped size.

Keywords Ammonium sorption · Clay peds · Claying · Organic C binding

1 Introduction

Sandy soils have inherent low fertility due to low organic matter content, cation exchange capacity and water-holding capacity (Xie and Steinberger 2005). Various strategies have been proposed to overcome the low nutrient and water-holding capacity of sandy soils. For example, furrow sowing (Blackwell et al. 1994) slowly releases fertilisers to enhance nutrient use efficiency or addition of organic materials to improve water and nutrient retention (Michelsen and Franco 1994; Shepherd and Bennett 1998) but these management options improve soil fertility only temporarily. Fertilisers have to be added repeatedly because nutrients are rapidly leached out of the root zone. Addition of organic materials such as plant residues can increase microbial activity and organic carbon sequestration as well as provide nutrients for plants (Lal 2005). But due to the lack of binding sites, nutrients are easily leached from sandy soils and the added organic material is rapidly decomposed (Walpola and Arunakumara 2010).

In contrast to sandy soils, clay soils have high water and nutrient retention capacity and often high organic matter

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content (Kaiser and Zech 2000). Clay minerals have a high cation exchange capacity and can therefore bind positively charged nutrients, e.g. NH_4^+, Ca^{2+}, Mg^{2+} and K^+ (Hamarashid et al. 2010). Negatively charged ions such as HPO_4^{2-} can be bound to the negatively charged clay via multivalent cations such as Ca^{2+} or Fe^{3+} (Frossard et al. 1995; Yaghi and Hartikainen 2014). Clay can also bind organic matter through ligand exchange, cation bridges, e.g. Al, Fe, Ca and Mg (Von Lutzow et al. 2006) and weak interactions such as Van der Waals forces, H-bonding (Arnarson and Keil 2000), depending on the properties of both mineral surface and organic matter. Binding to clay protects organic matter from decomposition by reducing accessibility to decomposers (Von Lutzow et al. 2006; Baldock 2007).

Amelioration of sandy soil by clay-rich soil has been shown to increase crop production across Southern Australia (Gardner et al. 1992; Cann 2000; Davenport et al. 2006; Hall et al. 2010). Clay soil can be derived from nearby areas or, in texture-contrast soils with a sandy to sandy loam A horizon and clayey B horizon, subsoil clay can be brought up by delving or spading (Hall et al. 2010; Ismail and Ozawa 2007). In Australia, such soils are found in about 17 % of the area (Isbell 2002). Clay addition by spreading or delving is expensive costing AU$250–300 per hectare, but can improve yields for about 10–15 years (GRDC 2015). The use of such technique can produce environmental benefits including increased organic C sequestration and reduced nutrient leaching into the ground water and soil erosion.

Previous studies have shown that the addition of ground and sieved clay soil (<2 mm) to sandy soil amended with plant residues reduced soil respiration and changed the response of soil respiration to drying and rewetting compared to sandy soil alone (Roychand and Marschner 2013, 2014; Shi and Marschner 2014). This can be attributed to binding of organic matter to clay particles and water retention by clay. However, in sandy soils amended with clay, the clay soil mainly occurs as peds of various sizes ranging from millimetres to centimetres. The influence of clay peds on nutrient availability and organic C retention could be different to that of finely distributed clay and may depend on clay concentration as well as ped size. This information is important for the management of clayed sandy soils.

The aims of the study were to (i) determine the effect of clay addition rate and ped size in residue amended sandy soil on soil respiration, nutrient availability and (ii) assess ped dynamics and organic C retention. We hypothesised that (1) clay addition to sandy soil with residue amendment will reduce nutrient availability because of reduced decomposition rate (residues bound to clay) and binding of NH_4^+ and inorganic P, and (2) the clay effect will increase with clay concentration and will be greater with small peds than larger peds because of their greater surface area/volume ratio and therefore greater binding capacity.

2 Materials and methods

2.1 Soils

Clay soil was collected from Waite Campus (latitude 34.97° S, 138.63° E). The soil is classified as black Vertosol in the Australian classification (Isbell 2002) or Vertisol in the World Reference Base, with smectite as dominant clay mineral (Table 1). The soil was air-dried, crushed and then sieved through different sieves to achieve peds of different sizes. Three-millimetre peds were collected on a 2-mm sieve after sieving through a 3.35 mm (size range 2–3.35 mm). The soil that passed through the 2-mm sieve was then sieved through a 1-mm sieve. The peds retained on the 1-mm sieve are referred to 2-mm peds (size range 1–2 mm). The <1-mm material was passed through a 0.5-mm sieve; the peds on the 0.5-mm sieve are referred to 1-mm peds (size range 0.5–1 mm) (Fig. S1, Electronic supplementary material). These ped sizes were chosen because in the field, a large proportion of clay peds are in this range, particularly after clay spreading (Schapel, personal communication). Fine sandy soil (classified as Arenosol in the World Reference Base) is collected in Penola (latitude 37.37° S, 140.83° E) which is in an area where claying is used to enhance yield on sandy soils. The sandy soil was air-dried and sieved to <2 mm (Table 1). The clay peds were added to the sandy soil at clay addition rates of 10 and 20 % *w/w*.

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), and organic carbon and nitrogen were determined by CHN analyser. The maximum water-holding capacity (WHC) of the soils was measured by using a sintered glass funnel connected to a 1-m water column ($\Psi_m = -10$ kPa) (Klute 1986) (Table 2).

2.2 Main experiment

Mature faba bean (*Vicia faba* L.) residue [C:N 37, pH in 1:20 (residue/RO water) 5.4] was ground, sieved to particle sizes <2 mm and added a rate of 10 g kg^{-1} to the soil mixes. After thorough mixing of sand, clay peds and residues, 30 g dry

Table 1 pH, electrical conductivity (1:5), particle size, organic C and total N content of clay soil and sandy soil

	pH (1:5)	EC (1:5)	Particle size			Organic C	Total N
			Sand	Silt	Clay		
		µS cm^{-1}	(%)			g kg^{-1}	
Clay soil	8.03	621	12	15	73	12.7	1.0
Sandy soil	5.36	12.2	96	1	3	1.0	ND

ND not detectable

Table 2 Maximum water-holding capacity, ammonium and available (resin) P concentrations in sandy soil alone or with 10 and 20 % clay as peds of 1, 2 and 3 mm ($n = 3$)

	Ped size (mm)	Water-holding capacity (g water g^{-1} soil)	NH_4-N µg g^{-1} soil	Available P µg g^{-1} soil
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
	2	0.07 de	4.1 bc	8.0 bc
	3	0.05 e	4.0 bc	8.9 ab
Sandy soil + 20 % clay	1	0.13 a	5.4 a	7.5 c
	2	0.11 b	4.6 b	7.4 c
	3	0.10 bc	4.3 bc	7.6 c

Values within a column followed by different letters are significantly different ($P \leq 0.05$)

weight equivalent of the mixture was placed in PVC cores (radius 1.85 cm and height 5 cm) with a nylon mesh (0.75 µm, Australian Filter Specialist) base. The soil was packed to a bulk density of 1.5 g cm^{-3} by adjusting the height of the soil mixes in the core. Water content was maintained gravimetrically at 80 % of maximal WHC. This water content was selected on the basis of a preliminary experiment where the sandy soil-clay ped mixtures were maintained at different percentage of WHC (40, 50, 60 and 80 % of maximum WHC). Glucose was added as an organic carbon source, and soil respiration was measured for 1 week. Soil 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 jars with gas-tight lids equipped with septa to allow quantification of headspace CO_2 concentration as described below. The remaining cores were placed in a plastic tray covered loosely with a lid. On day 15, the cores in the jars were removed 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.

Due to the upper detection limit of the gas analyser (2 % 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 3 days until end of the experiment using a Servomex 1450 infrared analyser as described by 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) and available N (NH_4 and NO_3) were measured. Available P was determined on day 45.

Microbial biomass carbon was measured by fumigation extraction as described by 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 with 0.033 M acidified $(NH_4)_2 \cdot Fe(SO_4)_2 \cdot 6H_2O$ after dichromate oxidation (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).

Available N was extracted by shaking soil with 2 M KCl solution at a soil/solution ratio of 1:5 for 1 h at 200–300 rpm. The suspension was filtered through Whatman filter paper no. 42. Ammonium was measured colorimetrically at 685 nm as described by Willis et al. (1996). Nitrate in the 2 M KCl extracts was determined colorimetrically at 540 nm using a modification of Miranda et al. (2001) as described by Cavagnaro et al. (2006). Available P was measured by the anion exchange resin method (Kouno et al. 1995).

Clay peds were retrieved at the end of experiment (day 45) by sieving from the treatments with 20 % clay addition. The soil in each core was sieved through 2, 1 and 0.5 mm sieves to separate peds of different sizes (3, 2 and 1 mm). However, the total weight of the retrieved peds was greater than of added peds because sand particles adhered to the peds and could not be removed manually. To separate coarse and finer particles, the retrieved peds were dispersed by shaking with 3 % sodium hexametaphosphate at a ped/solution ratio of 1:13 for 2 h at 200–300 rpm. The suspension was sieved through 250 and 53 µm sieves and dried overnight at 70 °C in the oven. This separation was also carried out with the initial 1, 2, and 3 mm peds. The >53 µm (material retained on 250 and 53 µm sieves) and the <53 µm fractions were dried, weighed and then ground and analysed for total organic carbon by wet digestion (Walkley and Black 1934).

2.3 Maximum ammonium and phosphorus sorption by clay peds

In separate experiments, maximum ammonium-N and phosphorus sorption was determined. Sandy soil alone and sandy soil with clay added at 10 and 20 % as 1 and 3 mm peds were shaken for 30 min at 200–300 rpm at a 1:10 soil/solution ratio with solutions containing 0–45 mg NH_4^+–N L^{-1} or 0–25 mg $H_2PO_4 L^{-1}$ P. After shaking, the NH_4^+ and P concentration in the filtered supernatant was determined. Sorption was calculated by subtracting the final concentration from the initial concentration.

2.4 Effect of clay peds and calcium carbonate on pH and respiration

The clay soil had a high pH and contained carbonate (Churchman, personal communication). The pH increase induced by clay addition to sandy soil and dissolution of carbonates could influence CO_2 release. Therefore, a separate experiment was carried out to test the effect of $CaCO_3$ and clay addition to sandy soil on pH and soil with and without 10 g kg^{-1} faba bean residues. Finely ground calcium carbonate was added to sandy soil at the rate of 10 g kg^{-1} soil to achieve a similar pH as clay addition to sandy soil with residues (pH 8.7). This rate was selected by testing the pH of sandy soil after addition of calcium carbonate rates in the range of 10–100 g of $CaCO_3$ kg^{-1} soil. After filling the soil mixes into the cores, water was added to achieve 80 % of water-holding capacity and the bulk density was adjusted to 1.5 g cm^{-3}. The water content was maintained as in the main experiment. Respiration was measured daily for 15 days as described above and the pH (1:5) was measured at the end of experiment.

2.5 Statistical analyses

The data were analysed by one-way ANOVA for each sampling time separately using Genstat 15th edition (VSN Int. Ltd, UK). Tukey's multiple comparison test at 95 % confidence interval was used to determine significant differences among treatments.

3 Results

Addition of clay peds increased maximum water-holding capacity compared to sandy soil alone three- to six fold with a greater increase at 20 % compared to 10 % clay added (Table 2). At a given clay concentration, the increase in water-holding capacity was greatest with 1 mm peds and smallest with 3 mm peds.

Total cumulative respiration (0–45 days) compared to the sandy soil alone was significantly lower with 2 mm peds at 10 % clay addition (reduced by 25 %), but significantly higher with 3 mm peds (increased by 20 %) (Fig. 1). Clay addition at 20 % as 1 and 2 mm peds significantly increased cumulative respiration compared to sandy soil alone by 12 and 10 %. The microbial biomass C concentration ranged between 100 and 200 µg g^{-1} soil and did not differ among clay treatments or sampling times (data not shown).

The NH_4^+–N concentration was higher on day 15 than day 45 (Fig. 2). On day 15, compared to sandy soil alone, clay addition decreased the NH_4^+–N concentration except for 1 mm peds at 20 % clay addition where the NH_4^+–N concentration did not differ significantly from sandy soil alone (Fig. 2a, Table S1, Electronic supplementary material). The decrease in NH_4^+–N concentration compared to sandy soil

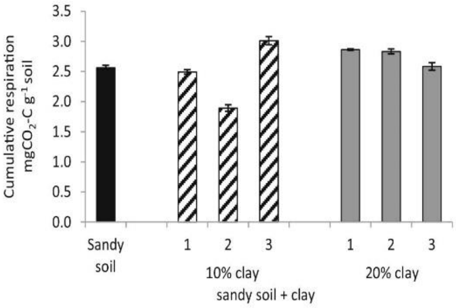

Fig. 1 Cumulative respiration over 45 days in sandy soil amended with faba bean residue alone or 10 and 20 % clay as 1, 2 and 3 mm peds (*vertical lines* indicate standard error, $n = 4$)

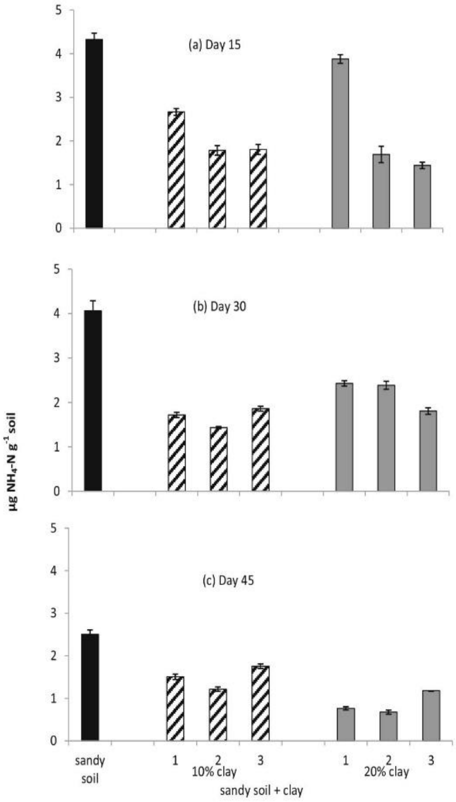

Fig. 2 Ammonium N concentration in sandy soil amended with faba bean residue alone or with 10 and 20 % clay as 1, 2 and 3 mm peds on days 15 (**a**), 30 (**b**) and 45 (**c**) (*vertical lines* indicate standard error, $n = 4$)

Table 3 Maximum ammonium sorption of sandy soil alone or with 10 and 20 % clay as 1 and 3 mm peds

	Ped size (mm)	Maximum NH_4-N sorption (μg g^{-1} soil)
Sandy soil alone	None	7
Sandy soil + 10 % clay	1	84
	3	55
Sandy soil + 20 % clay	1	138
	3	113

alone was greater with 2 and 3 mm (by 60–70 %) than with 1 mm peds (by 10–40 %) at both clay addition rates. On days 30 and 45, all clay treatments significantly reduced the NH_4^+–N concentration compared to sandy soil alone. On day 30, for 1 and 2 mm peds, the decrease was greater at 10 % clay addition (by 60 %) than at 20 % (by 40 %, Fig. 2b, Table S1, Electronic supplementary material). The decrease was similar at the two clay addition rates with 3 mm peds (by 50 %). On day 45, the decrease in NH_4^+–N concentration compared to sandy soil alone was greater at 20 % clay addition rate (by 50–70 %) than with 10 % (by 30 to 50 %) for all ped sizes (Fig. 2c, Table S1, Electronic supplementary material). At a given clay concentration, the decrease was greater with 1 and 2 mm than with 3 mm peds. At all sampling times, NO_3^-–N concentrations were at or below detection limit (data not shown).

Maximum NH_4^+-N sorption was lowest in sandy soil alone (7 μg NH_4^+-N g^{-1} soil) (Table 3). Clay addition increased maximum NH_4^+-N sorption 8- to 20-fold with a greater increase at 20 % compared to 10 % clay added and a greater increase with 1 compared to 3 mm peds. For example, with 1 mm peds, maximum sorption was 60 % higher at 20 % than at 10 % clay addition. At 10 % clay addition, maximum NH_4^+-N sorption was 60 % higher with 1 than with 3 mm peds (Table 3).

Table 4 pH (1:5) on days 15, 30 and 45 of sandy soil amended with faba bean residue alone or with 10 and 20 % clay as 1, 2 and 3 mm peds ($n = 4$)

Treatments	Ped size (mm)	pH (1:5)		
		Days		
		15	30	45
Sandy soil alone	None	7.5 e	7.9 c	7.4 d
Sandy soil + 10 % clay	1	8.2 cd	8.6 a	8.6 a
	2	8.3 bc	8.5 ab	8.4 b
	3	8.1 d	8.3 b	8.3 c
Sandy soil + 20 % clay	1	8.4 a	8.6 a	8.6 a
	2	8.4 a	8.5 ab	8.5 ab
	3	8.3 ab	8.5 ab	8.4 b

Values within a column followed by different letters are significantly different ($P \leq 0.05$)

Clay addition at 10 % increased the available P concentration on day 45 compared to the sandy soil alone (Table S2, Electronic supplementary material). This increase was greater with 1 and 2 mm peds (by 30 %) than with 3 mm peds (by 20 %). At 20 % clay addition compared to sandy soil alone, the available P concentration was increased only with 1 mm peds (by 20 %). At a given ped size, the available P concentration was greater at 10 than at 20 % clay added. Maximum P sorption was two fold higher at 20 than at 10 % clay addition (Table S3, Electronic supplementary material). At a given clay addition rate, maximum P sorption was greater with 1 than with 3 mm peds.

The pH was lower in sandy soil alone (pH 7.4–7.9) than in clay-amended treatments (8.1–8.6, Table 4). In sandy soil with clay, the pH did not differ among treatments and was similar on days 15 and 30. On day 45, the pH differed among clay treatments, being lower with 3 mm than with 1 and 2 mm peds.

In the additional experiment with sandy soil amended with $CaCO_3$ or clay peds, the pH was lowest in sandy soil alone (Table 5). Addition of faba bean residues and $CaCO_3$ increased the pH by two to three units, with the greatest increase in the treatment where both residues and $CaCO_3$ were added. Addition of 20 % clay as 2 mm peds increased the pH by about two units with and without residue addition. In the treatments without residue addition, cumulative respiration was lowest in sandy soil alone. Cumulative CO_2 release was increased by 30 % by addition of $CaCO_3$, but increased nearly two fold with 20 % clay added. Addition of faba bean residues increased cumulative CO_2 release 10–20-fold compared to treatments without residue addition. Compared to sandy soil with residues, cumulative CO_2 release was decreased by 6–8 % by $CaCO_3$ and clay addition.

In the peds added at the start of the experiment, the TOC content was 2.9 g kg^{-1} in the >53 μm fraction and 5.3 g kg^{-1} in the <53 μm fraction. The TOC content did not differ among ped sizes.

At the end of the experiment, peds were retrieved in the treatments with 20 % clay addition. Peds of 1, 2 and 3 mm size were retrieved irrespective of the size of peds added (Table 6). But the weight of the retrieved peds was greater than the weight of peds added because sand particles adhered to the peds and could not be removed manually.

Table 5 pH (1:5) and cumulative respiration after 15 days in sandy soil or sandy soil amended with $CaCO_3$, faba bean residues and 20 % clay as 2 mm peds ($n = 3$)

Treatments	Faba bean addition	pH (1:5)	Cumulative CO_2 (mg CO_2-C g^{-1} soil)
Sandy soil alone	−	6.5 d	0.1 b
	+	8.1 c	1.9 a
Sandy soil + $CaCO_3$	−	8.7 b	0.1 b
	+	9.2 a	1.8 a
Sandy soil + 20 % clay 2 mm	−	8.7 b	0.2 b
	+	8.7 b	1.8 a

Values in a column followed by different letters are significantly different ($P \leq 0.05$)

Therefore, the retrieved peds were separated into >53 and <53 μm fractions by sieving. When 1 mm peds had been added, the greatest amount of the <53 μm fraction was retrieved in the 2-mm ped size, but when 2 and 3 mm peds were added, the greatest amount of the <53 μm fraction was retrieved in the added ped size, which is in 2 and 3 mm peds, respectively (Table 6). Between 74 and 77 % of the clay added was retrieved at the end of the experiment. The TOC content of the <53 μm fraction ranged from 4.2 to 9.2 g kg^{-1} and was highest in the ped size in which it was added, e.g. in 1 mm retrieved peds when 1 mm peds were added. The TOC content per core calculated for each size of retrieved peds (weight of the <53 μm fraction of retrieved peds × TOC content of this fraction), and the percentage of total TOC in a given retrieved ped size was highest in the ped size in which the clay had been added for the 2 and 3 mm ped addition treatments, but in the 2 mm fraction of retrieved peds when 1-mm peds were added (Table 6). The total TOC content per core decreased with size of peds added.

4 Discussion

This short-term study showed that the addition of clay peds to sandy soil influences N availability and organic C retention, the effect depending on both clay addition rate and ped size. Further, it showed that over a 45-day period, peds broke down but also aggregated to form larger peds. The results only partially confirm our first hypothesis [clay addition to sandy soil with residue amendment will reduce nutrient availability because of reduced decomposition rate (residues bound to clay) and binding of NH_4^+ and inorganic P], but confirm the second hypothesis (the clay effect will increase with clay concentration and will be greater with small peds than larger peds because of their greater surface area/volume ratio and therefore greater binding capacity).

Addition of clay peds had no consistent effect on cumulative respiration neither with respect to ped size nor clay addition rate (Fig. 1). For example, compared to the sandy soil alone, cumulative respiration with 2 mm peds was increased at 20 % clay, but decreased at 10 % clay. This inconsistent

Table 6 Weight, proportion and total organic C content of the <53 μm fraction and proportion of total organic C content in retrieved peds after 45 days with 20 % clay as 1, 2 or 3 mm peds ($n = 4$)

Treatments with 20 % clay added as peds of	Size of retrieved peds (mm)	Retrieved <53 μm fraction (g $core^{-1}$)	Retrieved <53 μm fraction (% of added clay)	TOC in <53 μm fraction (g kg^{-1})	TOC per core (mg $core^{-1}$)	Proportion of total TOC in peds (%)
1 mm	1	1.38 b		9.2 a	12.7	43
	2	1.61 b		8.8 a	14.2	48
	3	0.63 cd		4.2 cd	2.7	9
	Sum	3.62	77		29.6	
2 mm	1	0.66 cd		5.7 bcd	3.8	16
	2	2.02 a		7.9 ab	16.0	69
	3	0.85 cd		4.2 cd	3.6	15
	Sum	3.53	75		23.4	
3 mm	1	0.62 d		3.6 d	2.2	11
	2	0.94 c		5.7 bcd	5.4	26
	3	2.01 a		6.6 abc	13.3	64
	Sum	3.57	74		20.9	

Values in a column followed by different letters are significantly different ($P \leq 0.05$)

effect of clay addition as peds is in contrast to our previous studies where ground and sieved clay (<2 mm without separation into peds) was added to sandy soil (Roychand and Marschner 2013, 2014; Shi and Marschner 2014). In these studies, clay addition reduced cumulative respiration compared to sandy soil alone. This can be explained by reduced accessibility of organic matter to microbes due to binding to clay surfaces (Baldock 2007). But the effect of clay added as peds on cumulative respiration was different, likely because peds provide less surface area per gramme and are less uniformly distributed than finely ground clay. Thus, a smaller proportion of the sandy matrix and the added residue are in contact with the peds.

Clay addition increased soil pH by 0.4 to 1.2 units (Table 4) which indicates that it contains carbonates which could influence CO_2 release through pH effects on microbial activity (Andersson and Nilsson 2001) and CO_2 release from carbonates (Bertrand et al. 2007). The experiment where sandy soil was amended with $CaCO_3$ to increase the pH to similar values as the clay-amended soil showed that the $CaCO_3$ addition had no significant effect on CO_2 release compared to sandy soil alone in presence or absence of faba bean residues (Table 5). Therefore, we can conclude that in this experiment, the addition of $CaCO_3$ with clay peds has negligible effects on soil respiration.

Sandy soils have lower water-holding capacity than finer textured soil because they contain mainly large pores. On the other hand, the majority of pores in clay soils are small leading to high water-holding capacity (Papendick and Camprell 1981; Lund 1959). Consequently, the addition of clay peds to sandy soil increased water-holding capacity in the present study with a greater increase with 20 % than with 10 % clay addition (Table 2). Among clay ped sizes, water-holding capacity was greatest with 1 mm peds. This can be explained by the more even distribution of the small peds in the sandy soil matrix compared to the larger peds which would be surrounded by wider zones of sandy soil. Therefore, small pores introduced by the clay would be more evenly distributed in the soil with 1 mm peds compared to the addition of larger peds where there are distinct zones with mainly small pores (peds) and those dominated by larger pores (sandy soil). All soils were incubated at 80 % of their respective maximum water-holding capacity; therefore, their water content differed, which could influence microbial activity. However, in this experiment, clay addition had no consistent effect on respiration (Fig. 1).

Addition of 20 % clay increased the available NH_4^+ concentration compared to sandy soil alone (Table 2). But after the addition of faba bean residues, the available NH_4^+ concentration was lower in clay-amended soil than in sandy soil except with 1 mm peds at 20 % clay (Fig. 2). A clear effect of clay concentration and ped size only became apparent on day 45 when the NH_4^+ concentration was lower with 20 % than with 10 % clay added and lower with 1 and 2 mm compared to 3 mm peds. The results of the batch sorption experiment suggest that this decrease in available NH_4^+ concentration with clay addition can, at least in part, be explained by NH_4^+ sorption to the peds (Table 3). Clay minerals have a higher cation exchange capacity than sand and can therefore bind cations such as NH_4^+ (Sowden et al. 1978; Beauchamp and Drury 1991; Nieder et al. 2011). Maximum sorption was about 20-fold higher in clay-amended soil than in sandy soil alone. The higher maximum sorption capacity at 20 % than at 10 % clay addition and with 1 mm than with 3 mm peds can be explained by the greater surface area at higher clay concentration and the higher surface area to volume ratio of the smaller peds which is in agreement with our second hypothesis.

Clay can bind phosphorus, via multivalent cations such as Ca^{2+} or Fe^{3+} (Frossard et al. 1995; Yaghi and Hartikainen 2014). This was confirmed in this study as clay ped addition increased maximum P sorption capacity compared to sandy soil alone (Table S3, Electronic supplementary material). The finding that 10 % clay addition increased P availability compared to sandy soil alone (Table S2, Electronic supplementary material) suggests that P sorbed to the clay peds may be readily available and in equilibrium with the soil solution. However, P availability was low in all treatments.

During incubation in moist conditions (80 % of WHC), sand particles adhered to the peds; therefore, the weight of the retrieved peds was greater than that of the added peds. To better understand changes in ped properties, we isolated the <53 μm fraction from the retrieved peds (Table 6). Peds of 1, 2 and 3 mm size were retrieved irrespective of the size of the added peds. This suggests aggregation of smaller peds (1 to 2 and 3 mm, 2 to 3 mm), but also breakdown of peds (2 and 3 to 1 mm, 3 to 2 mm). Some peds will have broken down to particles <1 mm as only 74–77 % of the added <53 μm fraction was retrieved as peds at the end of the experiment. The breakdown may be due to drying and rewetting events. Although the water content of the soil was maintained at 80 % of WHC, the peds may have dried out slightly. The moist incubation may have also favoured binding of peds to form larger aggregates. When larger peds were added, the greatest proportion of retrieved <53 μm fraction was in the ped size added initially, which is 2 and 3 mm, respectively. But when 1-mm peds were added, more of <53 μm fraction was retrieved from 2 than 1 mm peds, suggesting aggregation of the small peds.

Clay surfaces can bind organic matter via cation bridges (Wattel-Koekkoek et al. 2001; Tombacz et al. 2004; Gregory and Nortcliff 2013). In most cases, the TOC content of the <53 μm fraction was higher at end of the experiment than initially (5.3 g kg^{-1}). In a given clay treatment, the increase in TOC concentration of the <53 μm fraction compared to the initial value and the proportion of TOC per core were highest in the ped size added. This is most likely due to the length of time peds were exposed to the added plant residue which would be longest in the peds added initially. On the other hand, peds formed during the experiment by breakdown or aggregation would have a shorter period of exposure.

The decreasing TOC content per core with increasing ped size added can be explained by the greater surface area-to-volume ratio of the smaller peds; the smaller peds have a greater surface to bind organic matter than large peds. Although this was only a 45-day study, this finding suggests that, particularly, the addition of small peds will increase organic C sequestration in sandy soils.

5 Conclusions

This study showed that clay addition to sandy soil influences nutrient dynamics after plant residue addition, particularly when small peds are added at higher rates. The increased NH_4^+ binding may have to be compensated by higher fertiliser addition rates, but will also improve N retention in the soil. The study confirmed that claying can increase organic C sequestration but also showed that organic C sequestration is likely to be greatest when the added clay peds are small. In the field, clay peds will range from millimetres to centimetres in size. If large peds (several cm) represent a high proportion of peds, the effect of claying on nutrient dynamics is likely to be small.

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CHAPTER 3

CLAY ADDITION TO SANDY SOIL—INFLUENCE OF CLAY TYPE AND SIZE ON NUTRIENT AVAILABILITY IN SANDY SOILS AMENDED WITH RESIDUES DIFFERING IN C/N RATIO

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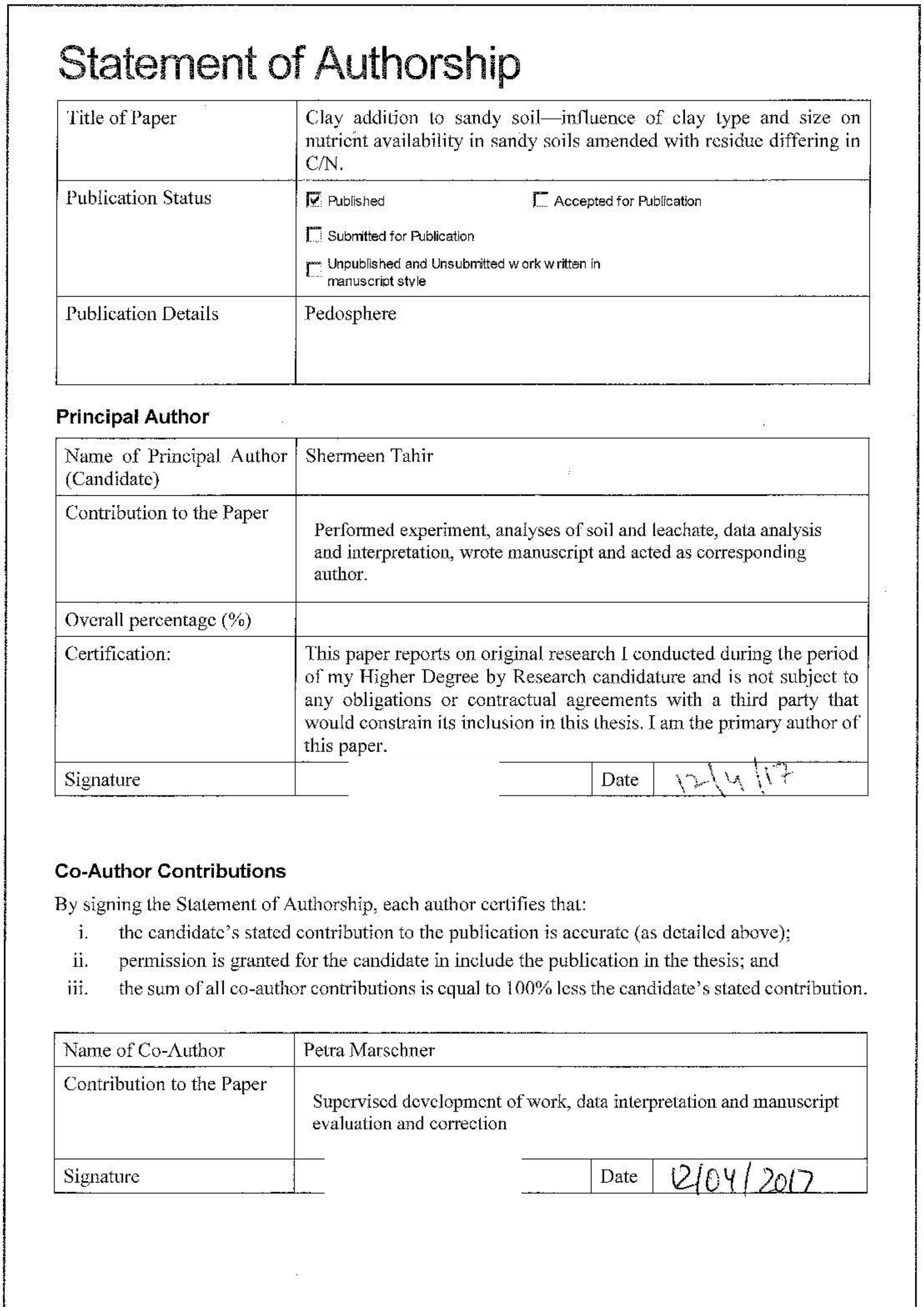

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Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Clay addition to sandy soil—influence of clay type and size on nutrient availability in sandy soils amended with residues differing in C/N ratio

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ABSTRACT

Addition of clay rich subsoil to sandy soil results in heterogeneous soil with clay peds of varying size as well as finely distributed clay which may affect the nutrient availability. The aim of this experiment was to assess the effect of clay soil particle size (finely ground or 2 mm peds) and clay soil properties on nutrient availability and organic C binding in sandy soil after addition of residues with low (20, young kikuyu) or high (47, mature faba bean) C/N ratio. Two clay soils with smectite as a dominant mineral were used: smectite percentage [high (40%) or low (5-10%)], clay (73 or 42%) and exchangeable Fe and Al (low or high) concentration. The clay soils were added to sandy soil at rate of 20% w/w either finely ground or crushed to 2 mm peds. Over 45 days, available N and P, microbial biomass N and P concentrations and cumulative respiration were greater with kikuyu than with faba bean. With kikuyu, compared to sandy soil alone clay addition increased available N concentration and initial microbial biomass C and N, but decreased cumulative respiration and P availability. Differences between clay types and size were inconsistent and varied with time except the increase in TOC in the < 53 µm fraction during the experiment which was greater for finely ground clay than for peds. We conclude that the high exchangeable Fe and Al concentration in the low smectite clay soil can compensate a lower clay concentration and proportion of smectite with respect to binding of organic matter and nutrients.

Key words: clay peds, iron and aluminium, mineralogy, organic carbon binding

INTRODUCTION

Sandy soils have low nutrient retention capacity due to low organic matter content, cation exchange capacity (CEC) (Walpola and Arunakumara, 2010) and low water holding capacity, which makes nutrient and irrigation management difficult. Addition of clay-rich subsoil to sandy soil is an option to increase crop production on sandy soils (Hall *et al*., 2010; Davenport *et al*., 2006) which is mainly attributed to improved water and nutrient holding capacity (Ismail and Ozawa, 2007). Clay addition by delving and spading may create heterogeneous soil with clay peds of varying size that may influence water and nutrient availability.

Organic matter acts as reservoir of plant nutrients (Schnitzer 2005) because it supplies nutrients via mineralization and desorption while binding can reduce leaching of nutrients. Organic matter (SOM) decomposition and accumulation are influenced by soil texture (Krull *et al*., 2001) clay mineralogy (Saidy *et al*., 2012), pH (Von Lützow *et al*., 2006), aeration, temperature, water content and microbial activity (Coleman *et al*., 2004). Clay minerals can bind organic matter (Tisdall and Oades, 1982) which can protect SOM from decomposition through physical protection by formation of macroaggregates (Von Lützow *et al*., 2006) and formation of organo-mineral complexes (Krull *et al*., 2003). Sandy soils on the other hand, have very few SOM binding sites and therefore faster SOM decomposition compared to clayey soils (Strong *et al*., 2004).

Net mineralisation-immobilisation patterns in soil amended with organic materials are influenced by environmental conditions (Saccone *et al*., 2013), and the chemical composition of the organic amendments (Tian *et al*., 1992). The C/N ratio is particularly important because microorganisms have a low C/nutrient ratio (typically C/N < 20). Thus, organic amendments with low C/N ratio can satisfy the nutrient demand of microbes, are rapidly decomposed and result in early net nutrient mineralisation (Hadas *et al.*, 2004; Janssen, 1996). Organic amendments with high C/nutrient ratio on the other hand, are decomposed slowly and can result in, at least temporary, net immobilisation of nutrients in the microbial biomass (Moritsuka *et al.*, 2004).

Clay mineralogy plays a central role in sorption reactions, carbon sequestration and turnover and therefore affects binding and release of nutrients (Hamarashid *et al.*, 2010). This is mainly attributed to specific surface area (SSA) and CEC of clay minerals. For example, kaolinite has lower SSA and CEC than smectite (Six *et al.*, 2000, 2002). Saidy *et al.* (2012) reported greater organic carbon mineralisation in sandy soil with kaolinitic than smectitic clay. And Feng *et al.* (2005) found higher sorption of aromatic compounds and proteins on montmorillonite compared to kaolinite. However, some studies contradict the concept that high specific surface area and CEC of clay minerals increases organic carbon stabilization. Wattel-Koekkoek *et al.* (2001) found that clay minerals used different mechanisms to bind SOM, but the amount of organic carbon in total clay sized fraction did not differ between smectitic and kaolinitic clay. Brunn *et al.* (2010) compared samples of tropical clay soils with contrasting mineralogy and found higher SOC lability in kaolinitic soils compared to smectitic. They suggested that presence of Fe and Al in clay soils also affects the SOC stabilization. Organic matter is bound to clay surfaces mostly through cation bridges such as Ca^{2+}, Fe^{3+} or Al^{3+} and to Fe and Al oxides by ligand exchange (Von Lützow *et al*., 2006). Several studies have shown the importance of Fe and Al oxides for sorption of dissolved organic C (DOC). According to Kubicki *et al.* (1999) DOC sorption to clay minerals was lower than to Fe and Al oxides. Eusterheus *et al.* (2005) found that removal of hydrous Al and Fe oxides reduced DOC sorption in soils. Recently, Curtin *et al*. (2016) showed a positive relationship between pyrophosphate extractable Fe and Al concentration of the clay and silt fractions and their OC content. Therefore, Fe and Al oxides e.g. on clay surfaces may compensate for low OC binding capacity of clay minerals.

In clayed sandy soils, the clay soil may not only be in the form of peds, but also finely distributed. Based on the surface area to volume ratio, one would assume that binding of nutrients is greater in finely distributed clay soil than to clay peds. However, to our knowledge this has not been investigated. Further it is unclear how nutrient binding to peds and finely distributed clay is influenced by clay concentration and mineralogy, exchangeable Fe and Al concentration and residue type.

The aim of this experiment was to assess the effect of clay soil particle size (finely ground to < 2 mm or 2 mm peds) and clay soil properties (clay mineralogy and concentration and exchangeable Fe and Al concentration) on nutrient dynamics in sandy soil after addition of residues with low or high C/N ratio. We hypothesised that (i) compared to sandy soil alone, finely ground clay will bind nutrients and reduce nutrient availability more strongly than clay peds, (ii) nutrient binding will be greater to clay soil with higher clay concentration and proportion of smectite, and (iii) the effect of clay addition on nutrient availability will be greater with low C/N than with high C/N residue.

MATERIALS AND METHODS

Soils

Clay soil with high proportion of smectite was collected at the Waite Campus, South Australia (34.97˚S, 138.63˚E) from 50-70 cm depth, this soil is classified as Black Vertosol in the Australian classification (Isbell 2002) or Vertisol in the World Reference Base. Low smectite clay soil was collected near Furner in the South East of South Australia (-37.38˚S, 140.34˚E) from 30-40 cm depth. The soil is classified as Duplex soil in the Australian classification and as Arenosol in the World Reference Base. The clay soils were air-dried, crushed and then sieved to separate into 2 mm peds and finely ground soil (< 2 mm). Fine sandy top soil (classified as Duplex soil in the Australian classification and as Arenosol in the World Reference Base) was collected from Penola (37.37˚S, 140.83˚E) which is in an area where claying is used to enhance yield on sandy soils. The sandy soil was air-dried and sieved to < 2 mm to remove organic material/roots.

Residues

Residues with different C/N ratio were used. Shoots of mature faba bean (*Vicia faba* L.) (C/N 47) and young kikuyu grass (*Pennisetum clandestinum*) (C/N 20) were dried in a fan forced oven at 40˚C, ground, sieved to particle sizes < 2 mm and added a rate of 10 g kg^{-1} to the soil mixes. Total carbon in residues was determined by wet digestion and titration (Walkley and Black 1934). For total N and P residues were digested with H_2SO_4 and HNO_3 respectively. Total N was determined by a modified Kjeldahl method (Vanlauwe *et al.*, 1996) and total P was measured by phosphovanadomolybdate method (Hanson, 1950).

Preliminary experiment to determine optimal water content

A preliminary experiment was carried out to determine the water content at which microbial activity (measured as soil respiration) was maximal. The soil treatments (sand alone and 20% clay soil w/w as finely ground soil and 2 mm peds) were maintained at different percentage of maximum water holding capacity (WHC, 40, 50, 60, and 80%). Glucose was added as an organic carbon source at 2.5 g C kg^{-1} soil and soil respiration was measured for one week. Cumulative respiration was highest at 80% of WHC in all treatments.

Experimental design of main experiment

Sandy soil was used alone or mixed with clay soils (low and high smectite) finely ground (FG) or 2 mm peds at a rate of 20% w/w and residues (200 g 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 (0.75 µm, Australian Filter Specialist) base. The soil was packed to a bulk density of 1.5 g cm^{-3} which is the bulk density of the sandy soil in the field. Bulk density was adjusted by compacting the soil to a certain volume, which is height in the cores. Water content was maintained gravimetrically at 80% of WHC by checking the weight of the cores and adding reverse osmosis (RO) water if necessary. 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 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.

Analyses

Soil pH, EC and exchangeable cations 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 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 of the soils was measured by wet oxidation and titration (Walkley and Black, 1934). Clay mineralogy of clay soils was determined by X-ray diffraction (XRD) (Brindly *et al.*, 1980) and specific surface area (SSA) by water sorption method (Newman, 1983).

Due to the upper detection limit of the infrared gas analyser (2% 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 (NH_4 and NO_3) and P were measured. Microbial biomass C was measured by chloroform-fumigation extraction as described in Vance *et al.* (1987). Fumigated and un-fumigated samples were extracted with 0.5 M K_2SO_4 at a 1:4 soil to extractant ratio followed by filtration through Whatman filter paper No. 42. The filtrate was used to determine microbial biomass C, N and P. For microbial biomass C (MBC), the filtrate was subjected to dichromate oxidation (Anderson and Ingram, 1993) after which the organic C concentration was determined by titration with 0.033 M acidified $(NH_4)_2$ Fe $(SO_4)_2$. $6H_2O$. 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 (MBN) in the 0.5 M K_2SO_4 extracts was measured colorimetrically at 685 nm as described in Willis *et al.* (1996). To calculate MBN the difference fumigated from un-fumigated samples was divided by 0.57 (Moore *et al.,* 2000). Microbial biomass P (MBP) in the 0.5 M K_2SO_4 was measured colorimetrically at 712 nm according to Murphy and Riley (1962). Microbial biomass P was calculated by multiplying the difference between fumigated and un-fumigated extracts by 2.5 (Brookes *et al.,* 1984).

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 as described by Willis *et al*. (1996). Nitrate in the 2 M KCl extracts was determined colorimetrically at 540 nm using a modification of Miranda *et al.* (2001) as described in Cavagnaro *et al.* (2006). Available P was extracted by the anion exchange resin method (Kouno *et al.*, 1995) and determined colorimetrically at 712 nm as described in Murphy and Riley (1962).

At the end of the experiment the soil mixes were separated into > 53 µm and < 53 µm fractions by wet sieving (Christensen, 2001). Organic C in the < 53 µm fraction is referred to as mineral-associated organic C (Kögel-Knabner, 2000). 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 µm sieves and dried overnight at 70˚C. The initial clay peds were also separated into < 53 µm and > 53 µm. The < 53 µm fraction was ground and analysed for total organic carbon by wet digestion (Walkley and Black, 1934).

Maximum ammonium and phosphorus sorption by clay

In separate experiments, maximum ammonium-N and phosphorus sorption were determined. Sandy soil alone and sandy soil with clay added at 20% as 2 mm peds or finely ground clay soil were shaken for 30 minutes at 200-300 rpm in a 1:10 soil:solution ratio with solutions containing 0-45 mg NH_4-N L^{-1} or 0-25 mg H_2PO_4 L^{-1} P. After shaking, the NH_4 concentration in the filtered supernatant was determined at 685 nm as described in Willis *et al*. (1996) and P concentration was determined at 712 nm (Murphy and Riley 1962). Sorption was calculated by subtracting the final concentration from the initial concentration.

Statistical analyses

The data were analysed by two-way ANOVA (clay x residue) for each sampling time separately using Genstat 15th edition (VSN Int. Ltd, UK). Tukey's multiple comparison test at 95% confidence interval was used to determine significant differences among treatments.

RESULTS

The clay content of the sandy soil was only 3%, the sand content 96% (Table I). Compared to the low smectite clay soil, the high smectite clay soil had a 70% higher clay concentration, two-fold higher CEC, higher pH and three-fold higher available N concentration, but the available P concentration was only 25% of that in low smectite clay (Table I). High smectite clay soil also had a 70% greater surface area, four-fold higher percentage smectite, 50-100% higher concentration of exchangeable Ca and Mg and 67% higher TOC than low smectite clay soil (Table II). However, exchangeable Fe and Al concentrations were more than 10-fold higher in low smectite compared to high smectite clay soil.

Total carbon content was 341 and 405 g kg^{-1} in kikuyu and faba bean residue. Low C/N residue (kikuyu shoots) had higher total N and P (17.6 and 4.5 g kg^{-1}) concentrations than high C/N residue (faba bean, 8.8 g N and 1 g P kg^{-1}). Of the total organic C in the soil-residue mix, 100% was from the residues in the sandy soil alone, whereas with high and low smectite clay it was about 70% and 88% (Table S1). Of the total N in the soil-residue mix, about 96% was from the residues in sandy soil alone, with high and low smectite clay it was about 35% and 75%. Of the total P in the soil-residue mix, 57% (kikuyu) and 22% (faba bean) was from the residues in sandy soil alone and in the clay-sandy soil mixes it was about 43% with kikuyu and 12% with faba bean.

The available N (NH_4 + NO_3) concentration varied little over time and was higher with kikuyu than with faba bean (Fig. 1 and Table S2). With faba bean, the available N concentration remained low throughout the experiment and did not differ among soil treatments. The available N concentration was up to 10-fold higher with kikuyu than with faba bean. With kikuyu compared to sandy soil alone, the available N concentration was greater in soil treatments with clay except for finely ground high smectite clay which did not differ from sandy soil alone on days 15 and 45. The available N concentration was higher in soil mixes with low smectite clay than in sandy soil alone or with high smectite clay. In treatments with high smectite clay, the available N concentration was lower with finely ground soil than with peds, particularly on day 45. However, with low smectite clay, available N concentrations did not differ between clay size classes except on day 15 when it was higher with finely ground clay compared to peds. Nitrate concentrations were two to three-fold lower than NH_4 concentrations (Table S2).

The available P concentration was up to 10-fold higher with kikuyu than with faba bean (Fig. 2). With faba bean, treatments differed little in available P and concentrations remained low throughout the experiment. With kikuyu, the available P concentration was highest on day 30 and always higher in sandy soil alone compared to sandy soil with clay. Clay type and size did not influence available P concentration.

Maximum NH_4 sorption was 10 to 20-fold higher in clay amended soil compared to sandy soil alone (Table IV). It was greater with high smectite clay compared to low smectite clay and higher with finely ground clay than with clay peds. Sandy soil alone had no detectable P sorption (Table IV). Maximum P sorption was greater with high than low smectite clay and higher with finely ground clay compared to peds.

The MBC concentration was greatest on day 15 and decreased with time (Fig. 3). On day 15, the MBC concentration was lower in sandy soil alone than with clay and in most treatments higher with kikuyu than with faba bean. With kikuyu, the MBC concentration on day 15 was greatest with peds of low smectite clay. With low smectite clay, the MBC concentration was greater with peds than with finely ground clay, but the reverse was true with high smectite clay. With faba bean on day 15, the MBC concentration was higher with low smectite than high smectite clay and higher with finely ground clay than with peds. On day 30 with faba bean, the MBC concentration did not differ among soil treatments. With kikuyu, the MBC concentration was greater with finely ground clay than with peds in both clay types. There were no significant differences in MBC concentration among soil or residue treatments on day 45.

The MBN concentration was lower in sandy soil alone than in treatments with clay (Fig. 4). Among clay amended soils, the MBN concentration was greater with kikuyu than with faba bean. On days 15 and 30 with faba bean, the MBN concentration was greater with high than with low smectite clay while with kikuyu this was true only for day 15. On days 15 and 30, the MBN concentration with kikuyu was lower with high smectite clay as peds compared to finely ground clay, but generally, the size of the added clay had little influence on MBN concentration.

The MBP concentration was greater in sandy soil alone than in clay amended soils and higher with kikuyu than with faba bean (Fig. 5). With faba bean, the MBP concentration was very low in clay amended soils and higher with high smectite clay as peds than in the other clay treatments. With kikuyu, the MBP concentration was greater with high than with low smectite clay, but not influenced by clay soil size.

Clay addition increased the pH compared to the sandy soil alone (Table S3). MBP and available P were positively correlated (correlation coefficient 0.95). In clay amended soils, the pH was lower with low than with high smectite clay whereas residue type had little effect on pH. The size of the added clay had no consistent effect on pH.

Cumulative respiration on day 45 was greater with kikuyu than with faba bean and higher in sandy soil alone than in clay amended soils (Fig. 6). There was no consistent effect of clay type or size on cumulative respiration.

In the first five days, respiration rates were higher with kikuyu than with faba bean (Fig. S1 and S2). On day 1, respiration rate was greater with high than with low smectite clay or sandy soil alone, but later respiration rates did not differ between soil treatments. Clay size did not influence respiration rate.

The initial TOC concentration of the < 53 µm fraction was greater in low than in high smectite clay (Fig. 7). Addition of faba bean residues did not increase TOC concentration at the end of the experiment compared to the initial concentrations except for the peds of high smectite clay where it increased by about 11%. With kikuyu, the TOC concentration at the end of the experiment was higher than at the start in both size classes with low smectite clay, but with high smectite clay only in the finely ground clay. With finely ground clay, the TOC concentration in low and high smectite clay was about 15% higher at the end of the experiment compared to the start.

DISCUSSION

In this experiment, the measured parameters were mainly influenced by residue type and less by clay treatment. There were marked differences between sandy soil alone and sandy soil with clay, however the differences between clay types and clay size were small and, in most cases, inconsistent. Based on the results we can only confirm the third hypothesis because the effect of clay addition on nutrient availability was greater with low C/N kikuyu than with high C/N faba bean. This can be explained by the higher decomposition and net nutrient mineralisation rate of kikuyu compared to faba bean residues (Fig. 1 and 2) (Hadas *et al.,* 2004). Even with low C/N kikuyu, differences among clay treatments were quite small. With faba bean, nutrient concentrations were generally low, thus differences among clay treatments were even smaller. In the following, the discussion will focus on the treatments with kikuyu.

Compared to sandy soil alone, cumulative respiration was lower whereas MBC and MBN were higher in clay amended soils (Fig. 3, 4 and 6). The lower cumulative respiration in clay amended soils can be explained by binding of organic C to clay which is also shown in the increase in TOC in the < 53 um fraction during the experiment. Binding to clay surfaces which occurs via bridges of cations such as Ca^{2+}, Fe^{3+} and Al^{3+} reduces accessibility of organic C to microbes (Baldock, 2007; Von Lützow *et al.,* 2006).

The lower cumulative respiration in combination with higher MBC and MBN in clay amended soils suggests that a greater proportion of residue C was used for microbial growth in soils amended with clay. Clay amended soils contained more water and the clay surface may provide more favourable habitats for microbes. Formation of biofilms and binding of residue particles on the clay surface could improve effectiveness of enzymes in breaking down the residues (Vogel *et al.*, 2015). Apparently, N release from residues was greater than microbial demand because despite higher MBN concentrations and capacity to bind NH_4 to clay surfaces compared to sandy soil alone (Table IV and Fig. 4), available N concentrations were higher in clay amended soils.

In contrast to MBN and available N, MBP and available P were higher in sandy soil alone than in clay amended soils (Fig. 2 and 5). The higher MBP concentration suggests a shift in microbial C/N/P ratio towards lower values in clay amended soils. In sandy soil alone, microbes took up P but were apparently limited in their C and N uptake.

The differences in measured parameters were small and inconsistent between clay soils and clay size. Therefore, we cannot confirm the first two hypotheses [(i) compared to sandy soil alone, finely ground clay will bind nutrients and reduce nutrient availability more strongly than clay peds, (ii) nutrient binding will be greater to clay soil with higher clay concentration and proportion of smectite]. The lack of effect of clay size may be because the size classes (2 mm peds and < 2 mm for finely ground clay soil) were not sufficiently different. The < 2 mm fraction may contain a proportion of particles only slightly smaller than 2 mm and some of the 2 mm peds may have broken down during the experiment. Although clay concentration, percentage smectite, CEC and surface area of high smectite clay soil were greater than of low smectite clay soil there were only small differences between two clay soils in the measured properties in this study. In the short-term, binding to high smectite clay soil appears to be greater than to low smectite clay soil as NH_4 and P sorption (Tables III and IV) was greater in the former and available N, MBC and MBN with kikuyu on day 15 were higher with high smectite clay (Fig. 3 and

4). However, available N, MBC and MBN on days 30 and 45, cumulative respiration (Fig. 6) and the increase in TOC of the < 53 µm fraction (Fig. 7) did not differ between high and low smectite soil. Thus, in the longer term (>15-45 days), differences in clay content, surface area, CEC and percentage smectite between the two-clay soil had no effect on binding of organic matter or nutrients. This is in agreement with previous studies. For example, Vogel *et al.* (2015) found that SSA is of minor importance for C and N sequestration. Wattel-Koekkoek *et al.* (2001) and Pronk *et al.* (2013) showed that clay mineralogy had little effect on decomposition rate and organic C binding. The lack of differences in binding of organic matter or nutrients between the two clay soils may be due to the 10-fold higher exchangeable Fe and Al concentration in the low compared to the high smectite clay soil. This can influence both nutrient and organic C binding. Sorption of P to soil particles or organic matter occurs cation bridges (Gerke and Hermann, 1993). Presence of Fe/Al oxides on clay surfaces can increase organic C binding (Kleber *et al.,* 2007; Saidy *et al.*, 2012; Curtin *et al.*, 2016). Although we did not measure the concentration of Fe and Al oxides, it is reasonable to assume that the higher exchangeable Fe and Al concentrations in the low smectite soil are, at least partly, due to higher concentrations of Fe and Al-oxides. The higher initial TOC concentration in the < 53 µm fraction of the low smectite clay soil indicates a high organic C binding capacity, but could also be because the low smectite clay soil was collected from shallower soil depth than the high smectite soil (30-40 cm compared to > 50 cm). In general, TOC content decreases with soil depth because the organic matter input is greatest in the top soil (Lawrence *et al.,* 2015).

The TOC content of the whole soils (Table I) differed from those of the initial < 53 µm fraction (Fig. 7). In the high smectite soil, the TOC content of the whole soil was greater than that in the < 53 µm. This is probably due to removal of particulate organic matter

(POM) during extraction of the < 53 µm fraction which suggests that a large proportion of the organic carbon in the high smectite soil is in the form of POC. In the low smectite soil on the other hand, the TOC content of the whole soil was lower than that in the < 53 µm. This is likely due to the lower clay content of this soil and suggests that in this soil most organic C is bound to the < 53 µm and the proportion as POC is small.

CONCLUSION

This study showed that addition of clay soil to sandy soil influences nutrient availability, but there were no clear differences between clay soils or sizes. The lack of differences between high and low smectite clay soil suggests that a high concentration of Fe and Al oxides can compensate a lower clay concentration and proportion of smectite with respect to binding of organic matter and nutrients. Longer term studies are required to assess the fate of the bound nutrients and OM to better understand the consequences of claying on nutrient availability to plants and organic C sequestration.

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TABLE I Selected physico-chemical properties of sandy soil, high and low smectite clay soils used in experiment (n=3 except texture where n=1). Values followed by different letters are significantly different ($P \leq 0.05$).

Soils	pH	EC	Particle size (%)			TOC*	WHC	inorganic N	available P
		µS cm^{-1}	Sand	Silt	Clay	g kg^{-1}	g g^{-1} soil	mg kg^{-1}	mg kg^{-1}
sandy	5.4 c	12 c	96	1	3	nd	0.02 c	4.4 c	1.4 b
High smectitic clay	8.0 a	621 a	12	15	73	7.4 a	0.45 a	21 a	0.9 b
Low smectitic clay	5.7 b	39 c	53	5	42	2.4 b	0.36 b	7.8 b	3.8 a

(nd= non-detectable)

[*] TOC content of whole soil

TABLE II Selected properties of high and low smectitic clay soil: specific surface area, Total organic carbon, mineralogy, exchangeable calcium, magnesium, sodium, potassium iron and aluminium concentration, cation exchange capacity (CEC) and exchangeable sodium percentage (ESP) (n=3 except mineralogy where n=1). Values followed by different letters are significantly different (*P*≤ 0.05).

Properties	**High smectite clay**	**Low smectite clay**
Specific surface area (m^{2} kg^{-1})	424 a	247 b
TOC (g kg^{-1})	7.4 a	2.4 b
Mineralogy (% wt)	11 Kaolinite	<3 Kaolinite
	5 Illite	2-5 Hematite
	40 Smectite	5-10 Smectite
Exchangeable cations cmol$_{c}$kg^{-1}		
Ca^{2+}	11.8 a	8.0 a
Mg^{2+}	9.2 a	4.4 b
Na^{+}	0.6 a	0.8 a
K^{+}	0.83 b	3.9 a
CEC (cmol$_{c}$kg^{-1})	35.8 a	17.1 b
ESP %	2.5 b	4.4 a
Fe ^{mg} kg^{-1}	38.6 b	345 a
Al mg kg^{-1}	55.1 b	650 a

TABLE III Selected physico-chemical properties of initial sandy soil with high (HS) or low smectite (LS) clay soil added as peds or finely ground (FG) (n=3). Values followed by different letters are significantly different ($P \leq 0.05$).

Sandy soil with		pH	EC $\mu S\ cm^{-1}$	WHC $g\ g^{-1}$ soil	inorganic N $mg\ kg^{-1}$	available P $mg\ kg^{-1}$
HS	peds	8.1 a	157 b	0.10 b	8.8 b	0.9 b
	FG	8.1 a	173 b	0.12 a	8.7 b	1.3 b
LS	peds	5.7 b	17 c	0.09 b	5.3 c	1.4 b
	FG	5.8 b	15 c	0.11 ab	5.6 c	1.5 b

TABLE IV Maximum ammonium sorption in sandy soil without (none) or with high (HS) or low smectite (LS) clay soil added as peds or finely ground (FG) (n=1).

Soil mixes	Clay types		Maximum NH_4 sorption (mg kg^{-1}soil)
sandy soil	HS	None	7.4
		Peds	133
		FG	139
	LS	peds	84
		FG	99

TABLE V Maximum phosphorus sorption in sandy soil without (none) or with high (HS) or low smectite (LS) clay soil added as peds or finely ground (FG) (n=1).

Soil mixes	Clay types		Maximum P sorption (mg kg^{-1}soil)
sandy soil		none	nd
	HS	peds	39
		FG	46
	LS	peds	21
		FG	36

(nd= non-detectable)

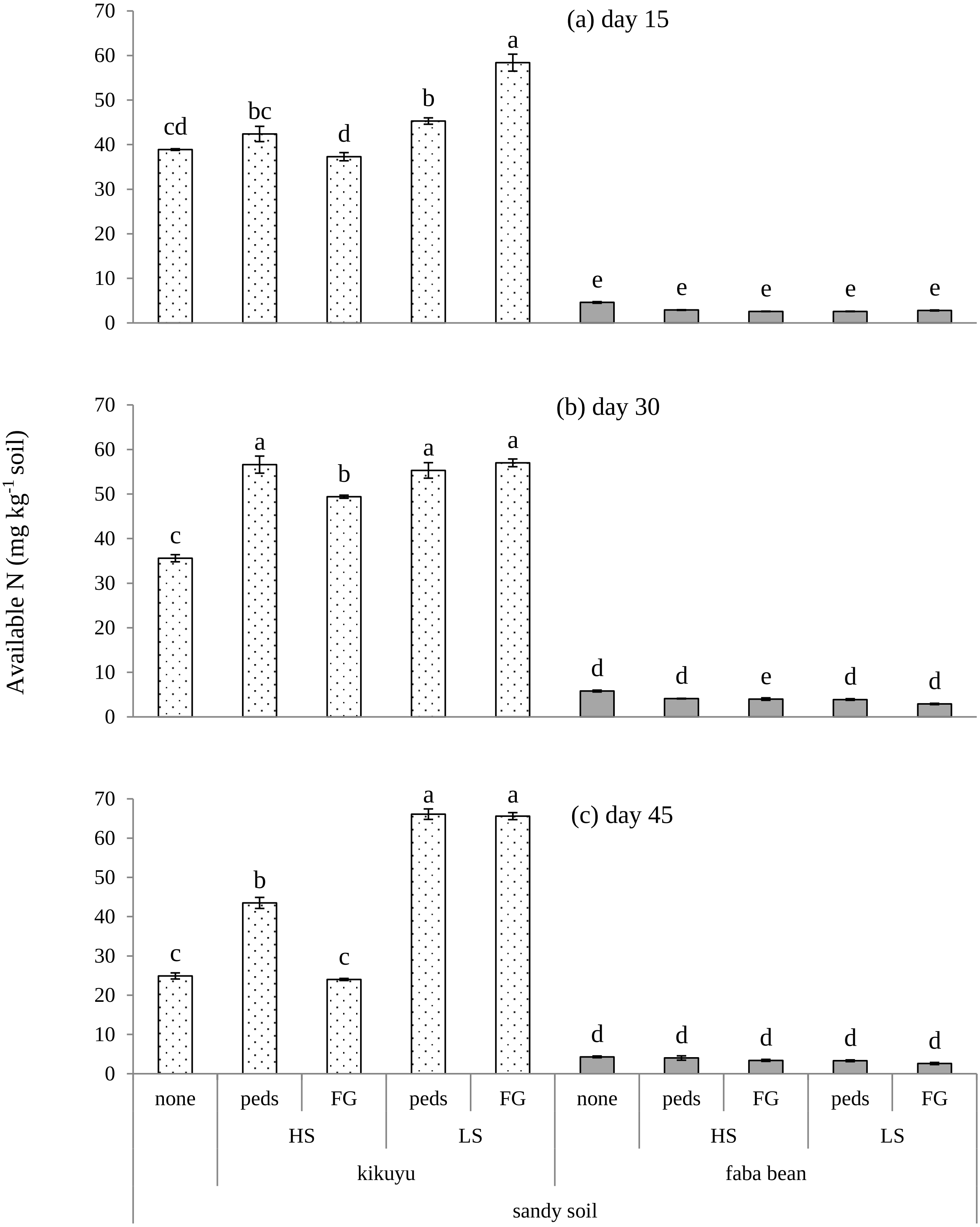

Fig. 1 Available-N (mg kg^{-1} soil) in sandy soil without (none) or with high (HS) or low smectite (LS) clay soil added as peds or finely ground (FG) amended with kikuyu or faba bean on days (a) 15, (b) 30 and (c) 45 (vertical lines indicate standard error, n=4). At a given sampling time columns with different letters are significantly different (*P*≤0.05, for clay x residue interaction)

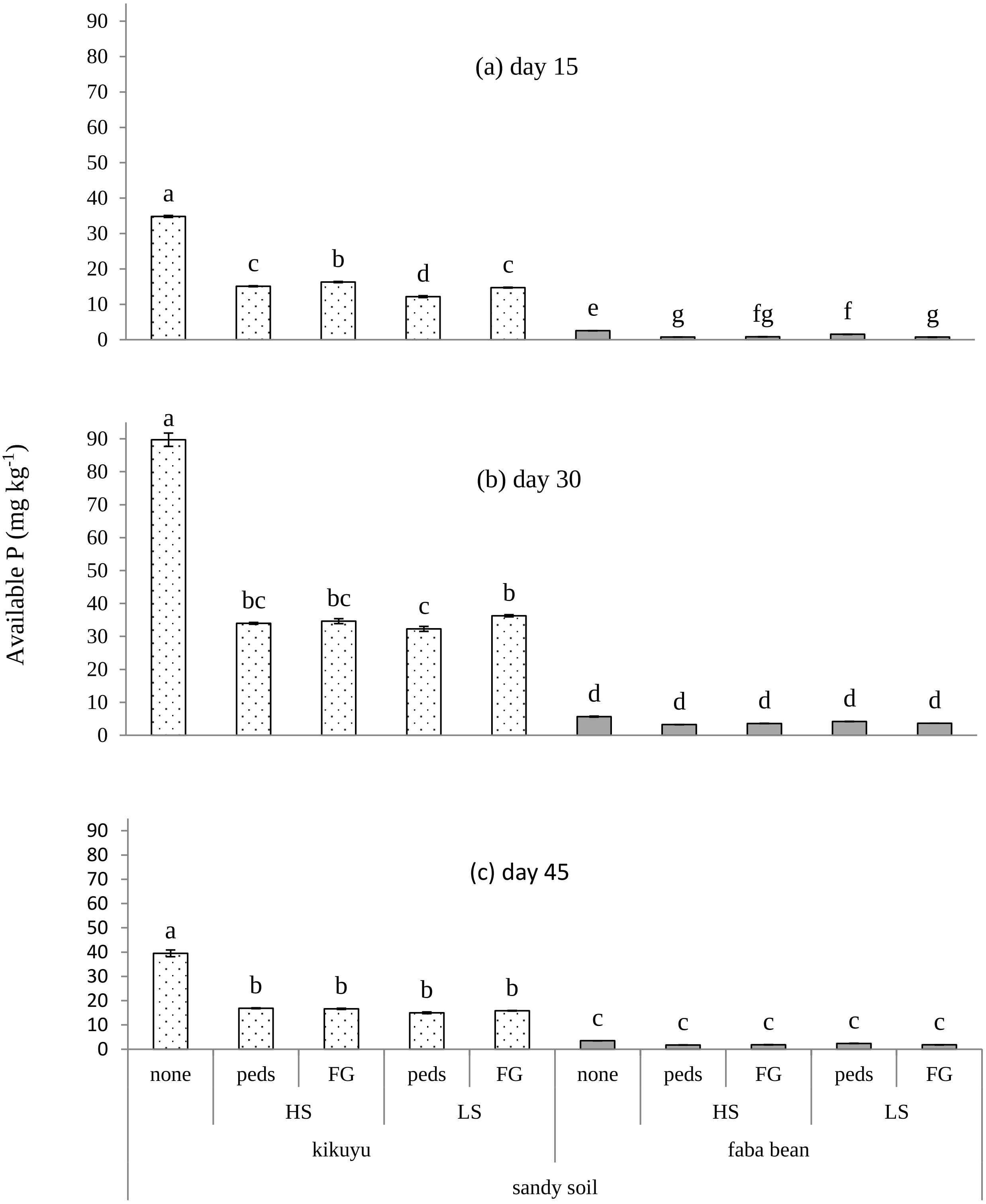

Fig. 2 Available P (mg kg^{-1} soil) in sandy soil without (none) or high (HS) or low smectite (LS) clay soil added as peds or finely ground (FG) amended with kikuyu or faba bean on days (a) 15, (b) 30 and (c) 45 (vertical lines indicate standard error, n=4). At a given sampling time columns with different letters are significantly different ($P \leq 0.05$, for clay x residue interaction)

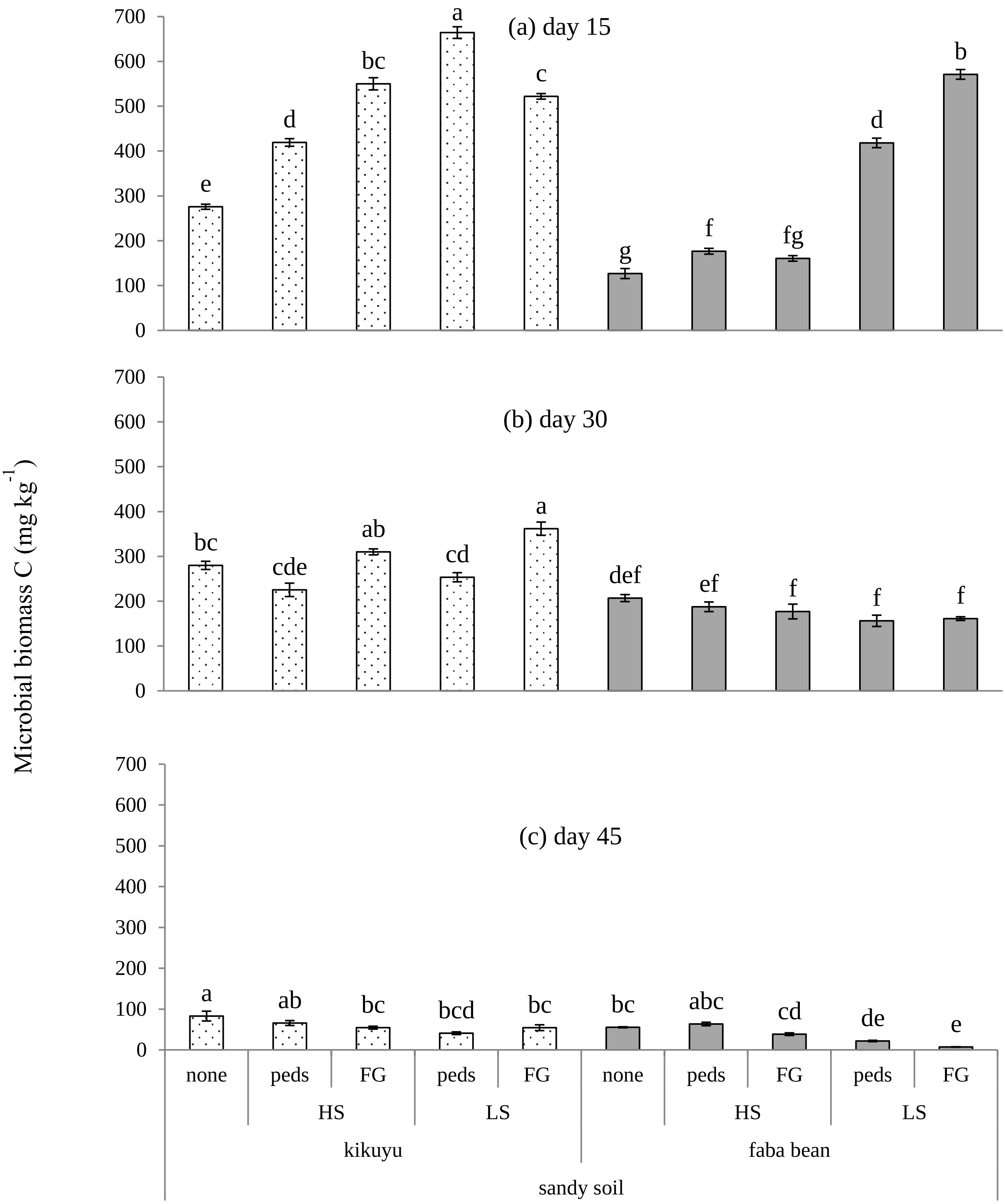

Fig. 3 Microbial biomass C (mg kg^{-1} soil) in sandy soil without (none) or high (HS) or low smectite (LS) clay soil added as peds or finely ground (FG) amended with kikuyu or faba bean on days (a) 15, (b) 30 and (c) 45 (vertical lines indicate standard error, n=4). At a given sampling time columns with different letters are significantly different ($P \leq 0.05$, for clay x residue interaction)

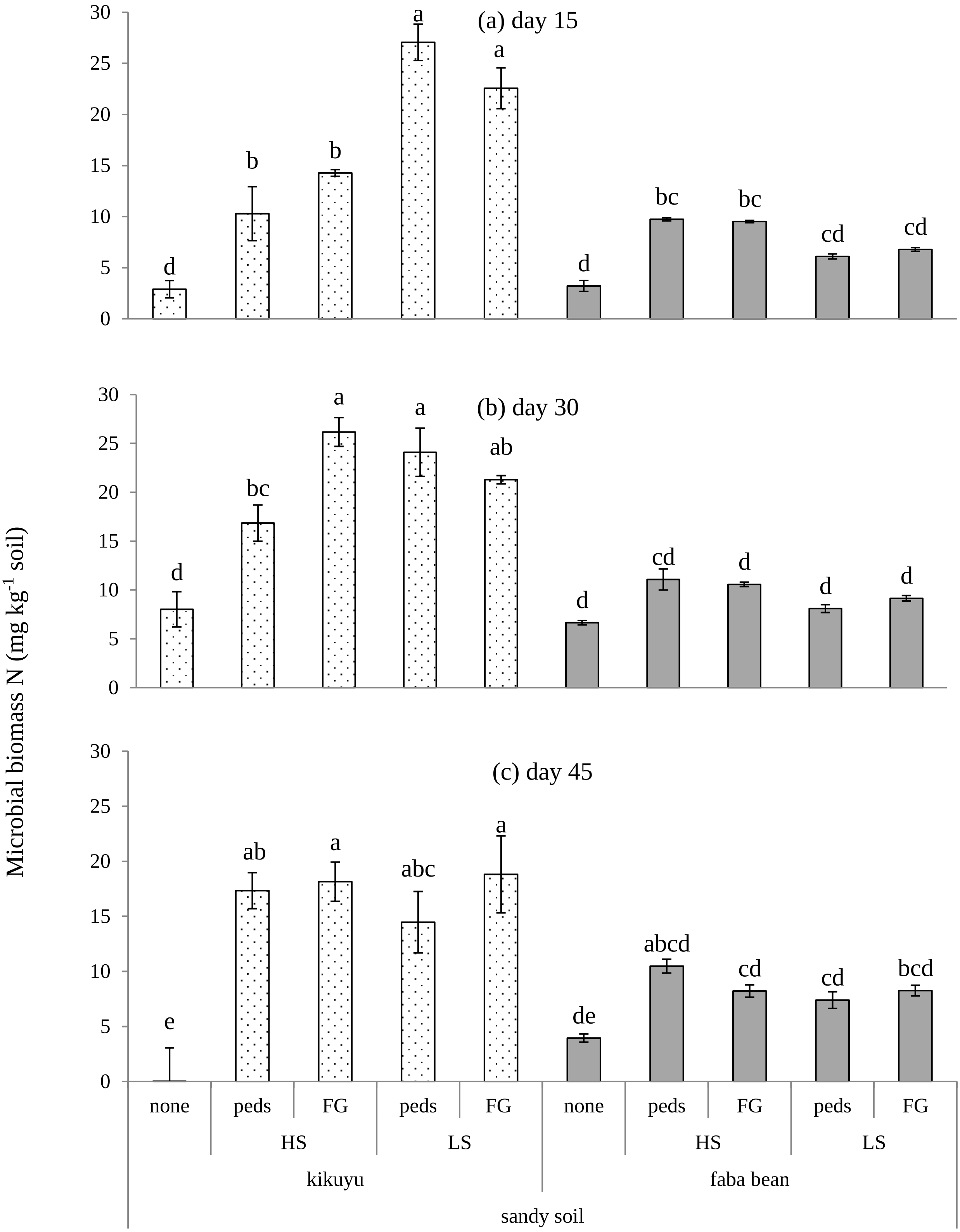

Fig. 4 Microbial biomass N (mg kg^{-1} soil) in sandy soil without (none) or high (HS) or low smectite (LS) clay soil added as peds or finely ground (FG) amended with kikuyu or faba bean on days (a) 15, (b) 30 and (c) 45 (vertical lines indicate standard error, n=4). At a given sampling time columns with different letters are significantly different (*P*≤0.05, for clay x residue interaction)

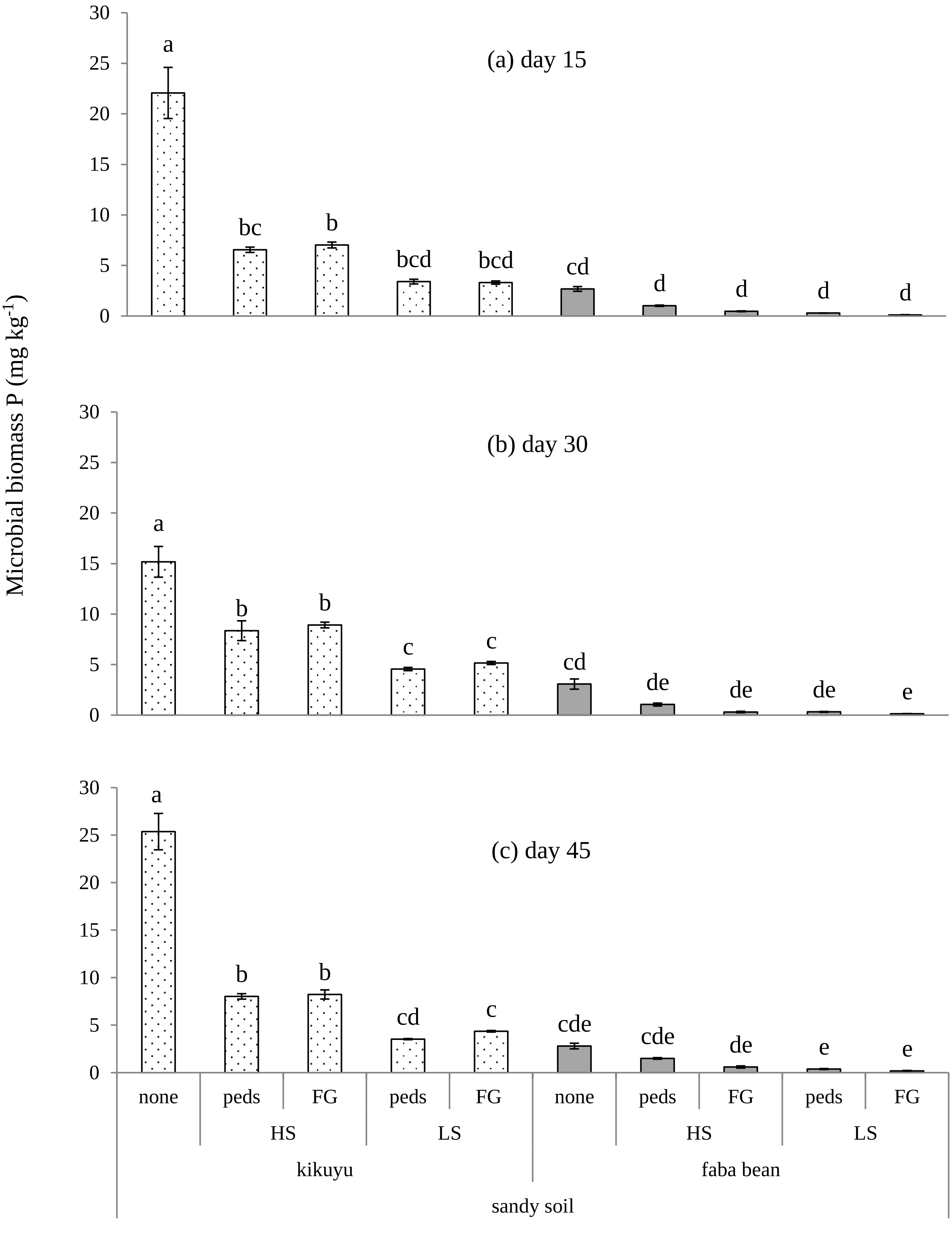

Fig. 5 Microbial biomass P (mg kg^{-1} soil) in sandy soil without (none) or high (HS) or low smectite (LS) clay soil added as peds or finely ground (FG) amended with kikuyu or faba bean on days (a) 15, (b) 30 and (c) 45 (vertical lines indicate standard error, n=4). Columns with different letters are significantly different ($P \leq 0.05$, for clay x residue interaction)

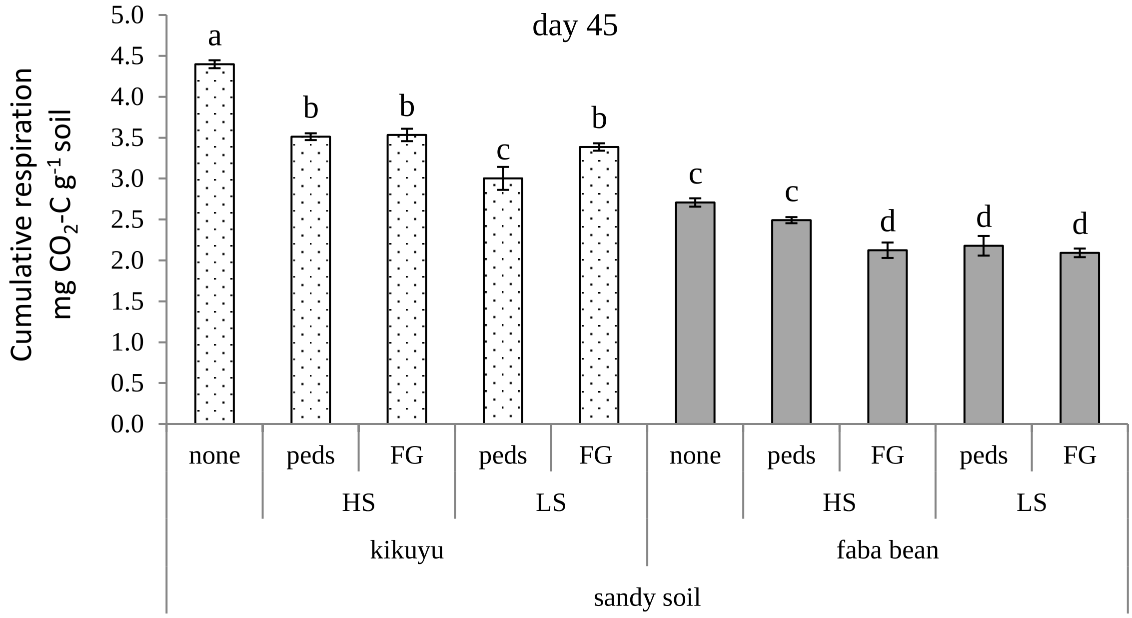

Fig. 6 Cummulative respiration (mg CO_2-C g^{-1} soil) after 45 days in sandy soil without (none) or with high (HS) or low smectite (LS) clay soil added as peds or finely ground (FG) amended with kikuyu or faba bean (vertical lines indicate standard error, n=4). Columns with different letters are significantly different (*P*≤0.05, for clay x residue interaction)

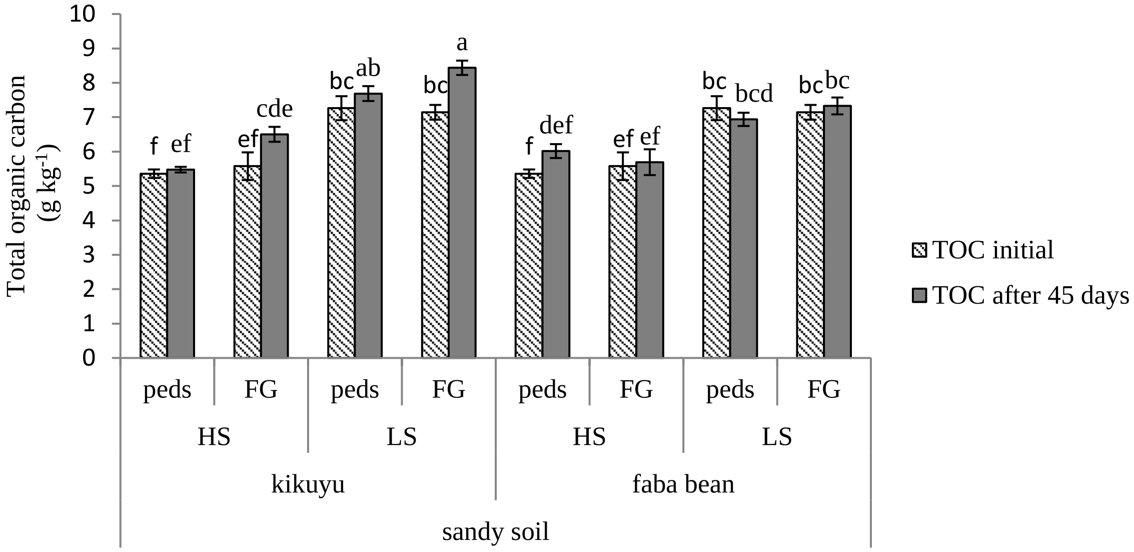

Fig. 7 Total organic carbon (mg kg^{-1}) in the < 53 µm fraction of soil with high (HS) or low smectite (LS) clay soil added as peds or finely ground (FG) amended with kikuyu or faba bean, initially and after 45 days (vertical lines indicate standard error, n=4). Columns with different letters are significantly different ($P \leq 0.05$, for clay x residue interaction)

APPENDIX

Table S1 Percentage of total organic C, N and P added with residues in soil-residue mixes with sandy soil without (none) or with high (HS) or low smectite (LS) clay soil added as peds or finely ground (FG) amended with kikuyu or faba bean.

Residue	Clay treatment	C	N	P
		% of total from residues		
Kikuyu	none	100	98	57
	HS	70	46	33
	LS	88	80	43
Faba bean	none	100	95	22
	HS	73	30	10
	LS	90	66	14

TABLE S2 Ammonium and nitrate concentration (mg kg^{-1} soil) in sandy soil without (none) or with high (HS) or low smectite (LS) clay soil added as peds or finely ground (FG) amended with kikuyu or faba bean on days 15, 30 and 45 days. Values in a column followed by different letters are significantly different (n=4, *P*≤ 0.05).

Treatments				Available N (mg kg^{-1} soil)					
	Residue type	Clay type		15 days		30 days		45 days	
				NH_4	NO_3	NH_4	NO_3	NH_4	NO_3
sandy soil	kikuyu		none	37.8 cd	1.05 bc	35.5 c	0.1 de	24.5 c	0.37 d
		HS	peds	41.3 bc	1.09 bc	44.6 b	12.0 b	38.4 b	5.09 a
			FG	35.4 d	1.87 a	33.8 c	15.5 a	19.0 d	5.07 a
		LS	peds	44.4 b	0.83 cd	52.6 a	2.7 c	63.8 a	2.29 c
			FG	57.7 a	0.71 d	54.0 a	3.0c	62.2 a	3.41 b
	faba bean		None	3.43 e	1.14 b	5.3 d	0.5 d	4.3 e	0.02 e
		HS	peds	2.6 e	0.31 e	4.0 d	0.04 e	3.9 e	0.11 e
			FG	2.4 e	0.27 e	3.9 d	0.04 e	3.3 e	0.13 e
		LS	peds	2.4 e	0.16 e	3.8 d	0.02 e	3.3 e	0.03 e
			FG	2.6 e	0.20 e	2.9 d	0.03 e	2.6 e	0.04 e

TABLE S3 pH of sandy soil without (none) or with high (HS) or low smectite (LS) clay soil added as peds or finely ground (FG) amended with kikuyu or faba bean on days 0, 15, 30 and 45 days (n=4). Values in one column followed by different letters are significantly different (*P*≤ 0.05).

Treatments				pH			
	Residue type	Clay type		Day 0	Day 15	Day 30	Day 45
sandy soil	kikuyu		none	5.4 c	7.3 d	8.2 d	8.3 d
		HS	peds	8.1 a	8.3 bc	8.7 c	8.5 b
			FG	8.1 a	8.2 c	8.7 c	8.4 c
		LS	peds	5.7 b	6.6 f	6.9 h	6.9 gh
			FG	5.8 b	6.9 e	6.9 gh	7.2 f
	faba bean		none	5.4 c	7.2 d	7.5 e	7.8 e
		HS	peds	8.1 a	8.5 ab	8.8 b	8.8 a
			FG	8.1 a	8.6 a	8.9 a	8.8 a
		LS	peds	5.7 b	6.4 g	7.0 g	6.8 h
			FG	5.8 b	6.3 g	7.3 f	6.9 g

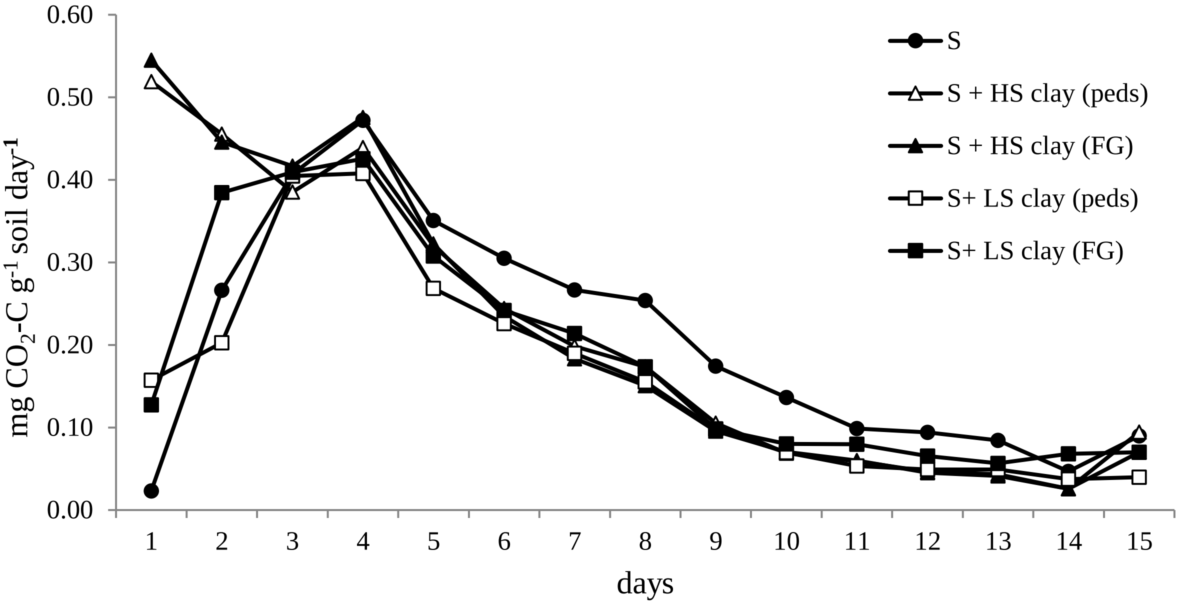

Fig. S1 Respiration rate (mg CO_2-C g^{-1} soil day^{-1}) from day 1 to 15 in sandy soil (S) without or with high (HS) or low smectite (LS) clay soil added as peds or finely ground (FG) amended with kikuyu (n=4)

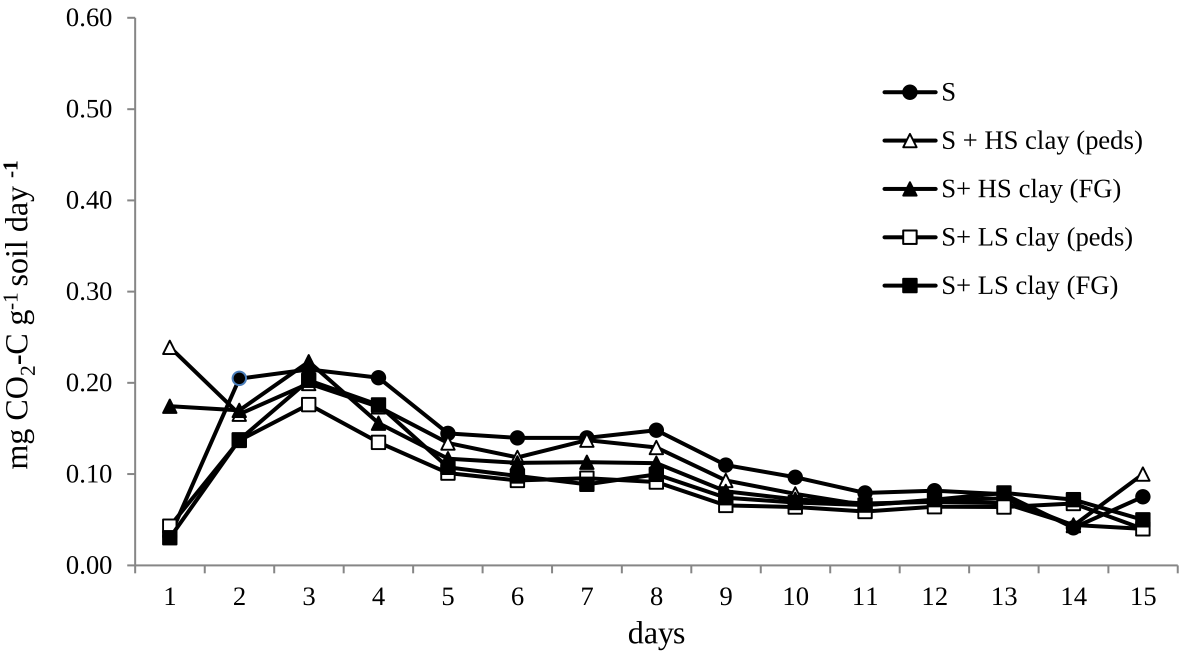

Fig. S2 Respiration rate (mg CO_2-C g^{-1} soil day^{-1}) from day 1 to 15 in sandy soil (S) without or with high (HS) or low smectite (LS) clay soil added as peds or finely ground (FG) amended with faba bean (n=4)

APPENDICES

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Clay Addition to Sandy Soil—Influence of Clay Type and Size on Nutrient Availability in Sandy Soils Amended with Residues Differing in C/N ratio

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ABSTRACT

Addition of clay-rich subsoil to sandy soil results in heterogeneous soil with clay peds (2-mm) or finely ground (< 2 mm) clay soil (FG), which may affect the nutrient availability. The aim of this study was to assess the effect of clay soil particle size (FG or peds) and properties on nutrient availability and organic C binding in sandy soil after addition of residues with low (young kikuyu grass, KG) or high (faba bean, FB) C/N ratio. Two clay soils with high and low smectite percentage, clay and exchangeable Fe and Al were added to a sandy soil at a rate of 20% (weight/weight) either as FG or peds. Over 45 d, available N and P as well as microbial biomass N and P concentrations and cumulative respiration were greater in soils with residues of KG than FB. For soils with KG residues, clay addition increased available N and initial microbial biomass C and N concentrations, but decreased cumulative respiration and P availability compared to sandy soil without clay. Differences in measured parameters between clay type and size were inconsistent and varied with time except the increase in total organic C in the < 53 μm fraction during the experiment, which was greater for soils with FG than with peds. We concluded that the high exchangeable Fe and Al concentrations in the low-smectite clay soil can compensate a lower clay concentration and proportion of smectite with respect to binding of organic matter and nutrients.

Key Words: Fe and Al oxides, finely ground clay soil, mineralogy, organic C binding, peds, smectite

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INTRODUCTION

Sandy soils have low nutrient retention capacity due to low organic matter content, cation exchange capacity (CEC) (Walpola and Arunakumara, 2010) and water-holding capacity (WHC), which makes nutrient and irrigation management difficult. Addition of clay-rich subsoil to sandy soil is an option to increase crop production in sandy soils (May, 2006; Hall *et al*., 2010), which is mainly attributed to improved water- and nutrient-holding capacity (Ismail and Ozawa, 2007). Clay addition by delving and spading may create heterogeneous soil with clay peds of varying sizes that may influence water and nutrient availability.

Organic matter acts as reservoir of plant nutrients (Schnitzer, 2005) because it supplies nutrients *via* mineralisation and desorption while binding can reduce leaching of nutrients. Soil organic matter (SOM) decomposition and accumulation are influenced by soil texture (Krull *et al*., 2001), clay mineralogy (Saidy *et al*., 2012), pH (Lützow *et al*., 2006), aeration, temperature, water content and microbial activity (Coleman *et al*., 2004). Clay minerals can bind organic matter (Tisdall and Oades, 1982), which can protect SOM from decomposition through physical protection by formation of macroaggregates (Lützow *et al*., 2006) and organo-mineral complexes (Krull *et al*., 2003). Sandy soils on the other hand, have very few SOM-binding sites and therefore faster SOM decomposition compared to clayey soils (Strong *et al*., 2004).

Net mineralisation-immobilisation patterns in soil amended with organic materials are influenced by environmental conditions (Saccone *et al*., 2013) and the chemical composition of the organic amendments (Tian *et al*., 1992). The C/N ratio is particularly important because microorganisms have a low C/nutrient ratio (typically C/N ratio < 20). Thus, organic amendments with low C/N ratio can satisfy the nutrient demand of microbes, are rapidly decomposed and result in early net nutrient mineralisation (Janssen, 1996; Hadas *et al*., 2004). Organic amendments with high C/nutrient ratio, on the other hand, are decomposed slowly and can result in, at least temporary, net immobilisation of nutrients in the microbial biomass (Mori-

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tsuka *et al.*, 2004).

Clay mineralogy plays a central role in sorption reactions, C sequestration and turnover, and therefore affects binding and release of nutrients (Hamarashid *et al.*, 2010). This is mainly attributed to specific surface area (SSA) and CEC of clay minerals. For example, kaolinite has lower SSA and CEC than smectite (Six *et al.*, 2000, 2002). Saidy *et al.* (2012) reported greater organic C mineralisation in sandy soil with kaolinitic than smectite clay. Feng *et al.* (2005) found higher sorption of aromatic compounds and proteins on montmorillonite compared to kaolinite. However, some studies contradict the concept that high SSA and CEC of clay minerals increase organic C stabilization. Wattel-Koekkoek *et al.* (2001) found that clay minerals used different mechanisms to bind SOM, but the amount of organic C in total clay-sized fraction did not differ between smectitic and kaolinitic clay. Brunn *et al.* (2010) compared samples of tropical clay soils with contrasting mineralogy and found higher SOC lability in kaolinitic soils compared to smectitic soils. They suggested that the presence of Fe and Al in clay soils also affects the SOC stabilization. Organic matter is bound to clay surfaces mostly through cation bridges such as Ca^{2+}, Fe^{3+} or Al^{3+} and to Fe and Al oxides by ligand exchange (Lützow *et al.*, 2006). Several studies have shown the importance of Fe and Al oxides for sorption of dissolved organic C (DOC). According to Kubicki *et al.* (1999), DOC sorption to clay minerals was lower than to Fe and Al oxides. Eusterhues *et al.* (2005) found that removal of hydrous Al and Fe oxides reduced DOC sorption in soils. Recently, Curtin *et al.* (2016) showed a positive relationship between pyrophosphate extractable Fe and Al concentrations of the clay and silt fractions and their organic C content. Therefore, Fe and Al oxides, *e.g.*, on clay surfaces, may compensate for low organic C binding capacity of clay minerals.

In clay-amended sandy soils, the clay soil may not only be in the form of peds, but also finely distributed. Based on the ratio of SSA to volume, one would assume that binding of nutrients is greater in finely ground clay soil (FG) than to clay peds. However, to our knowledge, this has not been investigated. Furthermore, it is unclear how nutrient binding to peds and FG is influenced by clay concentration and mineralogy, exchangeable Fe and Al concentrations and residue type.

The aim of this experiment was to assess the effects of clay soil particle size (< 2 mm FG or 2-mm peds) and clay soil properties (clay mineralogy and concentration and exchangeable Fe and Al concentrations) on nutrient dynamics in sandy soil after addition of residues with low or high C/N ratio. We hypothesised that: i) FG will bind nutrients and reduce nutrient availability more strongly than peds compared to sandy soil alone, ii) nutrient binding will be greater to clay soil with higher clay concentration and proportion of smectite, and iii) the effect of clay addition on nutrient availability will be greater for residues with low C/N ratio than with high C/N ratio.

MATERIALS AND METHODS

Soils and residues

Clay soil with high proportion of smectite was collected at the Waite Campus, South Australia (34.97° S, 138.63° E) from 50–70 cm depth. This soil is classified as Black Vertosol according to the Australian classification (Isbell, 2002) or Vertisol in the World Reference Base. Low-smectite clay soil was collected near Furner in the South East of South Australia (37.38° S, 140.34° E) from 30–40 cm depth. The soil is classified as Duplex soil according to the Australian classification and as Arenosol in the World Reference Base. The clay soils were air-dried, crushed and then sieved to separate into 2-mm peds and < 2 mm FG. Fine sandy top soil (classified as Duplex soil according to the Australian classification and as Arenosol in the World Reference Base) was collected from Penola, South Australia (37.37° S, 140.83° E), where claying is used to enhance yield on sandy soils. The sandy soil was air-dried and sieved to < 2 mm to remove organic materials/roots.

Residues used had different C/N ratios. Shoots of mature faba bean (*Vicia faba* L.) (FB, C/N ratio of 47) and young kikuyu grass (*Pennisetum clandestinum*) (KG, C/N ration of 20) were dried in a fan-forced oven at 40 °C, ground, sieved to particle sizes < 2 mm and added to the soil mixes at a rate of 10 g kg^{-1}. Total C in the residues was determined by wet digestion and titration (Walkley and Black, 1934). Total N was determined by a modified Kjeldahl method (Vanlauwe *et al.*, 1996), and total P was measured by phosphovanadomolybdate method (Hanson, 1950) after digestion with H_2SO_4 and HNO_3, respectively.

Preliminary experiment to determine optimal water content

A preliminary experiment was carried out to determine the water content at which microbial activity (measured as soil respiration) was maximal. Sandy soil alone and that amended with both high- and low-smectite clay soils as FG (HFG and LFG, respectively) or 2-mm peds (HP and LP, respectively) (20%, weight/weight), were maintained at 40%, 50%, 60% and 80% of maximum WHC. Glucose was added as an organic C source at 2.5 g C kg^{-1} soil, and soil respiration was measured for one week. Cumulative respiration was the highest at 80% WHC in all treatments.

Experimental design of main experiment

Residues of KG and FB (10 g kg^{-1}) were added to sandy soil mixed without or with FG (HFG and LFG) or 2-mm peds (HP and LP) at a rate of 20% (weight/weight, equivalent to 200 g kg^{-1}). After thorough mixing of sandy soil, clay soils and residues, 30 g dry weight equivalent of the mixture was placed in PVC cores (1.85-cm radius and 5-cm height) with a nylon mesh base (0.75 µm, Australian Filter Specialist Pty Ltd., Huntingwood, Australia). The soil was packed to a bulk density of 1.5 g cm^{-3} which is the same as that of the sandy soil in the field. Bulk density was adjusted by compacting the soil to a certain volume, which is the height in the cores. Water content was maintained gravimetrically at 80% WHC by checking the weight of the cores and adding reverse osmosis water if necessary. 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 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.

Measurements

Soil pH, electrical conductivity (EC) and exchangeable cations were determined in a 1:5 soil:water (weight:volume) suspension after shaking on an end-over-end shaker at room temperature for 1 h (Setia *et al.*, 2013). Particle size distribution was measured by the hydrometer method (Bouyoucos, 1936). The maximum WHC of the soils was measured by using a sintered glass funnel connected to a 1-m water column (matric potential of water = −10 kPa) (Klute, 1986). Total organic C (TOC) of the soils was measured by wet oxidation and titration (Walkley and Black, 1934). Clay mineralogy and SSA of clay soils were determined by X-ray diffraction (XRD) (Brindley *et al.*, 1980) and water sorption method (Newman, 1983), respectively.

Due to the upper detection limit of the infrared gas analyser (2% CO_2) and the decrease in respiration rate over time after residue addition, soil respiration was measured daily for the first 15 d, every second day until day 30 and then every 3 d until end of the experiment using a Servomex 1450 infra-red analyser (Servomex Group, Crowborough, UK) 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) and N (MBN) and available N (NH_4^+-N and NO_3^--N) and P were measured. Microbial biomass C was measured by chloroform-fumigation extraction as described in Vance *et al.* (1987). Fumigated and un-fumigated samples were extracted with 0.5 mol L^{-1} K_2SO_4 at a 1:4 soil to extractant ratio (weight:volume) followed by filtration through Whatman filter paper No. 42. The filtrate was used to determine MBC, MBN and MBP. For MBC, the filtrate was subjected to dichromate oxidation (Anderson and Ingram, 1993), after which the organic C concentration was determined by titration with 0.033 mol L^{-1} acidified $(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$. 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 mol L^{-1} K_2SO_4 extracts was measured colorimetrically at 685 nm as described in Willis *et al.* (1996). To calculate MBN, the difference between fumigated and un-fumigated samples was divided by 0.57 (Moore *et al.*, 2000). Microbial biomass P in the 0.5 mol L^{-1} K_2SO_4 was measured colorimetrically at 712 nm according to Murphy and Riley (1962) and calculated by multiplying the difference between fumigated and un-fumigated extracts by 2.5 (Brookes *et al.*, 1984).

Available N was extracted by shaking soil with 2 mol L^{-1} KCl solution at a soil:solution ratio of 1:5 (weight:volume) for 1 h at 200–300 r min^{-1}. The suspension was filtered through Whatman filter paper No. 42. NH_4^+-N was measured colorimetrically at 685 nm as described by Willis *et al.* (1996). NO_3^--N in the 2 mol L^{-1} KCl extracts was determined colorimetrically at 540 nm using a modification of Miranda *et al.* (2001) as described in Cavagnaro *et al.* (2006). Available P was extracted by the anion exchange resin method (Kouno *et al.*, 1995) and determined colorimetrically at 712 nm as described in Murphy and Riley (1962).

At the end of the experiment, the soil mixes were separated into > 53 µm and < 53 µm fractions by wet sieving (Christensen, 2001). Organic C in the < 53 µm fraction is referred to as mineral-associated organic C (Kögel-Knabner, 2000). The sand-clay mixture was dispersed by shaking with 3% sodium hexa-metaphosphate at a soil:solution ratio of 1:13 (weight:volume) for 2 h at 200–300 r min^{-1}. The suspension was sieved through 250- and 53-µm sieves and dried overnight at 70 °C. The initial clay peds were also separated into < 53 µm and > 53 µm. The < 53 µm fraction was ground and analysed for TOC by wet digestion (Walkley and Black, 1934).

Maximum NH_4^+-N and P sorption by clay

Maximum NH_4^+-N and P sorption were determined in separate experiments. Sandy soil alone and that amended with clay soil at 20% (weight/weight) as 2-mm peds or FG were shaken for 30 min at 200–300 r min^{-1} in a 1:10 soil:solution ratio (weight:volume) with solutions containing 0–45 mg NH_4^+-N L^{-1} or 0–25 mg H_2PO_4 L^{-1} P. After shaking, the NH_4^+-N concentration in the filtered supernatant was determined at 685 nm as described in Willis *et al.* (1996) and P concentration was determined at 712 nm (Murphy and Riley 1962). Sorption was calculated by subtracting the final concentration from the initial concentration.

Statistical analyses

The data were analysed by two way analysis of variance (ANOVA) (clay × residue) for each sampling time separately using Genstat (15th edition, VSN International Ltd., Oxford, UK). Tukey's multiple comparison test at 95% confidence interval was used to determine significant differences among treatments.

RESULTS

The sandy soil intially contained 3% clay and 96% sand (Table I). Compared to the low-smectite clay soil, the high-smectite clay soil had a 74% higher clay content, two-fold higher CEC, higher pH and three-fold higher available N concentration, but the available P concentration was only 24% of that in low-smectite clay soil (Tables I and II). High-smectite clay soil also had a 70% greater SSA, 4-fold higher smectite percentage, 50%–100% higher concentrations of exchangeable Ca and Mg and 67% higher TOC than low-smectite clay soil (Table II). However, exchangeable Fe and Al concentrations were more than 10-fold higher in low-smectite clay soil compared to high-smectite clay soil. In the preliminary experiment, compared to sandy soils with high-smectite clay soil, pH, EC and available N were lower in those with low-smectite clay soil. There was no significant difference in properties between peds and FG in sandy soils with low-smectite clay soil, but with high-smectite clay soil, WHC, available P and EC were higher with FG than with peds (Table III).

The available N (NH_4^+-N and NO_3^--N) concentration varied little over time and was up to 10-fold higher in soils amended with KG than with FB residues (Fig. 1). The available N concentration remained low throughout the experiment in soils with FB residues, and did not differ among soil treatments. For soils amended with KG residues, the available N concentration was greater in soils with clay soils compared to those without clay soils, except for those with HP on day 15 and with HFG on days 15 and 45, which did not differ from sandy soil without clay. The available N concentration was generally higher in soils with low-smectite clay soil than in sandy soil without clay or with high-smectite clay soil. In treatments with high-smectite clay soil, the available N concentration was lower with FG than with peds, particularly on day 45. However, in those with low-smectite clay soil, available N concentrations did not differ between clay-size classes except on day 15, when it was higher with FG compared to peds. The NO_3^--N concentrations were 2- to 3-fold lower than NH_4^+-N concentrations (data not shown).

The available P concentration was up to 10-fold hi-

TABLE I

Selected initial physico-chemical properties[a)] of sandy soil and high- and low-smectite clay soils used in this study

Soil	pH	EC	Particle size			TOC	WHC	Available N	Available P
			Sand	Silt	Clay				
		µS cm^{-1}		%		g kg^{-1}	g g^{-1}	mg kg^{-1} soil	
Sandy soil	5.4[b)]c[c)]	12c	96	1	3	ND[d)]	0.02c	4.4c	1.4b
High-smectite clay soil	8.0a	621a	12	15	73	7.4a	0.45a	21.0a	0.9b
Low-smectite clay soil	5.7b	39c	53	5	42	2.4b	0.36b	7.8b	3.8a

[a)] EC = electrical conductivity; TOC = total organic C; WHC = water-holding capacity.

[b)] Data are means ($n = 3$ except texture, for which $n = 1$).

[c)] Means followed by the same letter in a column are not significantly different ($P \leq 0.05$).

[d)] Non-detectable.

gher with KG residues than with FB residues, for which available P concentrations differed little and remained low throughout the main experiment (Fig. 2). In soils amended with KG residues, the available P concentration was the highest on day 30 and always higher in sandy soil without clay compared to those with clay soil. Clay type and size did not influence available P concentration.

TABLE II

Selected properties[a] of high- and low-smectite clay soils used in this study

Property	High-smectite clay soil	Low-smectite clay soil
SSA (m^2 kg^{-1})	424[b]a[c]	247b
TOC (g kg^{-1})	7.4a	2.4b
Mineralogy (%, weight/weight)		
Kaolinite	11	< 3
Illite	5	–
Hematite	–	2–5
Smectite	40	5–10
Exchangeable cation (cmol$_c$ kg^{-1})		
Ca^{2+}	11.8a	8.0a
Mg^{2+}	9.2a	4.4b
Na^+	0.6a	0.8a
K^+	0.83b	3.90a
CEC (cmol$_c$ kg^{-1})	35.8a	17.1b
ESP (%)	2.5b	4.4a
Fe (mg kg^{-1})	39b	345a
Al (mg kg^{-1})	55b	650a

[a] SSA = specific surface area; TOC = total organic carbon; CEC = cation exchange capacity; ESP = exchangeable sodium percentage.

[b] Data are means ($n = 3$ except mineralogy, for which $n = 1$).

[c] Means followed by the same letter in a row are not significantly different ($P \leq 0.05$).

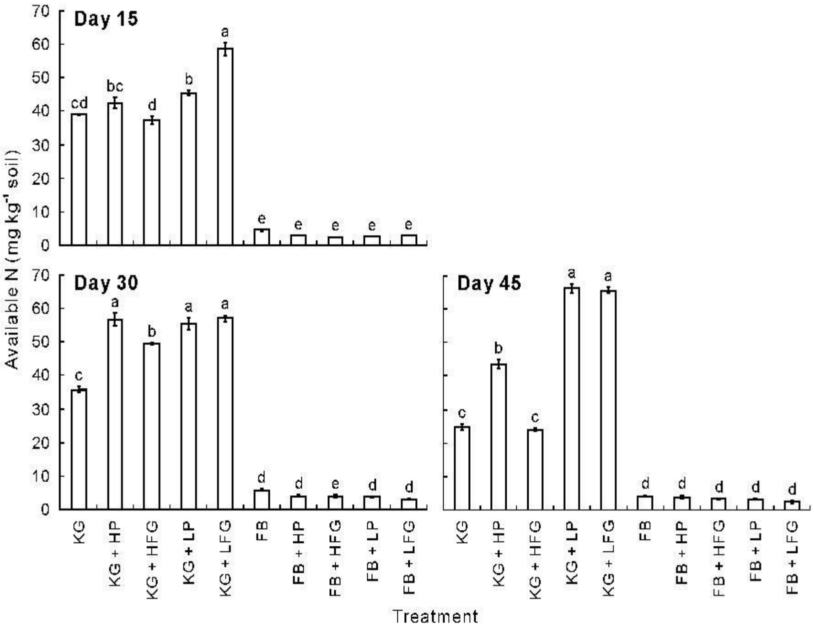

Fig. 1 Available N concentrations on days 15, 30 and 45 in sandy soils amended with residues of kikuyu grass (KG) or faba bean (FB) (10 g kg^{-1}) along with or without high- and low-smectite clay soils as 2-mm peds (HP and LP, respectively) or finely ground (< 2 mm) clay soil (HFG and LFG, respectively) at a rate of 20% (weight/weight). Vertical bars indicate standard errors of the means ($n = 4$). For a given sampling day, bars with different letters are significantly different at $P \leq 0.05$ for clay and residue interaction.

TABLE III

Selected physico-chemical properties[a] of sandy soil amended with high- or low-smectite clay soils added as 2-mm peds or finely ground (< 2 mm) clay soil (FG)

Amendment	pH	EC	WHC	Inorganic N	Available P
	µS cm^{-1}	g g^{-1}	mg kg^{-1} soil	mg kg^{-1} soil	
High-smectite clay soil					
Peds	8.1[b]a[c]	157b	0.10b	8.8a	0.9b
FG	8.1a	173a	0.12a	8.7a	1.3a
Low-smectite clay soil					
Peds	5.7b	17c	0.09b	5.3b	1.4a
FG	5.8b	15c	0.11ab	5.6b	1.5a

[a] EC = electrical conductivity; WHC = water-holding capacity.

[b] Data are means ($n = 3$).

[c] Means followed by the same letter(s) in a column are not significantly different ($P \leq 0.05$).

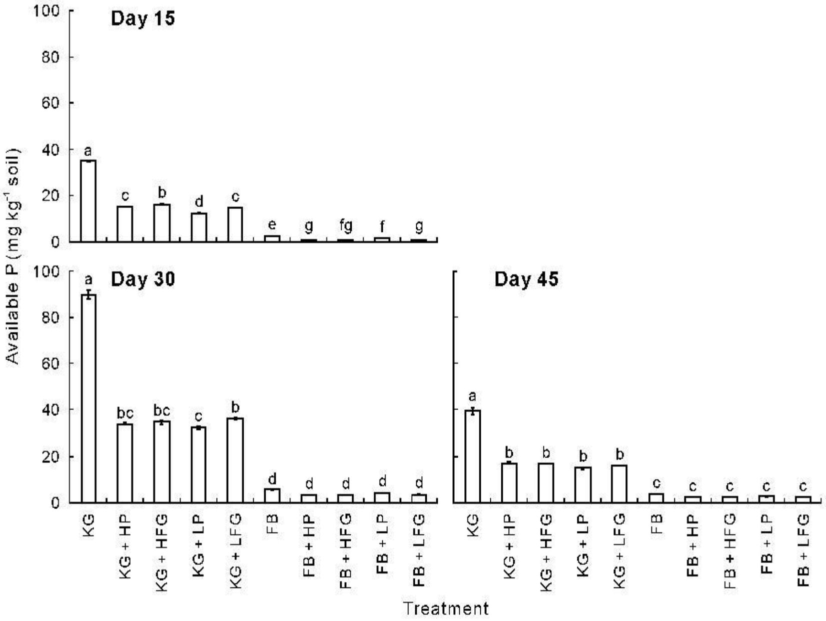

Fig. 2 Available P concentrations on days 15, 30 and 45 in sandy soils amended with residues of kikuyu grass (KG) or faba bean (FB) (10 g kg^{-1}) along with or without high- and low-smectite clay soils as 2-mm peds (HP and LP, respectively) or finely ground (< 2 mm) clay soil (HFG and LFG, respectively) at a rate of 20% (weight/weight). Vertical bars indicate standard errors of the means ($n = 4$). For a given sampling day, bars with different letters are significantly different at $P \leq 0.05$ for clay and residue interaction.

Maximum NH_4^+-N sorption was 10- to 20-fold higher in clay amended soil compared to sandy soil alone (Table IV). It was greater with high smectite clay soil compared to low smectite clay soil and higher with FG than with peds. Sandy soil alone had no detectable P sorption (Table IV). Maximum P sorption was greater with high than low smectite clay soil and higher with FG compared to peds.

The MBC concentration was the greatest on day 15 and decreased with time (Fig. 3). On day 15, the MBC concentration was lower in sandy soil without clay than with clay soils and higher with residues of KG than FB in most treatments. On day 15 with KG residue, the MBC concentration was the greatest with LP. On day 15 with low-smectite clay soil, the MBC concentration was greater with peds than with FG, but the reverse was true with high-smectite clay soil. On day 15 with FB residues, the MBC concentration was higher with low smectite clay soil than with high-smectite clay soil and higher with HFG than HP. On day 30 with FB

TABLE IV

Maximum NH_4^+-N and P sorption in sandy soil without or with high- or low-smectite clay soils as 2-mm peds or finely ground (< 2 mm) (FG) ($n = 1$)

Treatments	Maximum sorption	
	NH_4^+-N	P
	mg kg^{-1} soil	
No clay	7	ND[a)]
High-smectite clay soil		
Peds	133	39
FG	139	46
Low-smectite clay soil		
Peds	84	21
FG	99	36

[a)] Non-detectable.

residues, the MBC concentration did not differ among soil treatments. On day 30 with KG residues, the MBC concentration was greater with FG than with peds for both clay types. There were no significant differences in MBC concentration among soil or residue treatments on day 45.

The MBN concentration was lower in sandy soil without than with clay soils (Fig. 4). Among clay-amended soils, the MBN concentration was greater with KG residues than with FB residues. On day 30, the MBN concentration with KG residues was lower with HP compared to HFG, but generally, the size of the added clay had little influence on MBN concentration.

The MBP concentration was greater in sandy soil without clay than in clay-amended soils and generally higher with residues of KG than FB (Fig. 5). For soils with FB residues, the MBP concentration was very low in clay-amended soils compared to soils with KG residues. For soils with KG residues, the MBP concentration was greater with high- than low-smectite clay on days 30 and 45, but it was not influenced by clay soil size.

The MBP and available P concentrations were positively correlated (correlation coefficient = 0.95). Clay addition increased the pH compared to the sandy soil without clay by up to three units on day 0, one unit on day 15 and 0.5 units on days 30 and 45. In clay-amended soils, the pH was lower with low- than high-smectite clay soils, whereas residue type had little effect on pH. The size of the added clay had no consistent effect on pH (data not shown).

Cumulative respiration on day 45 was greater with

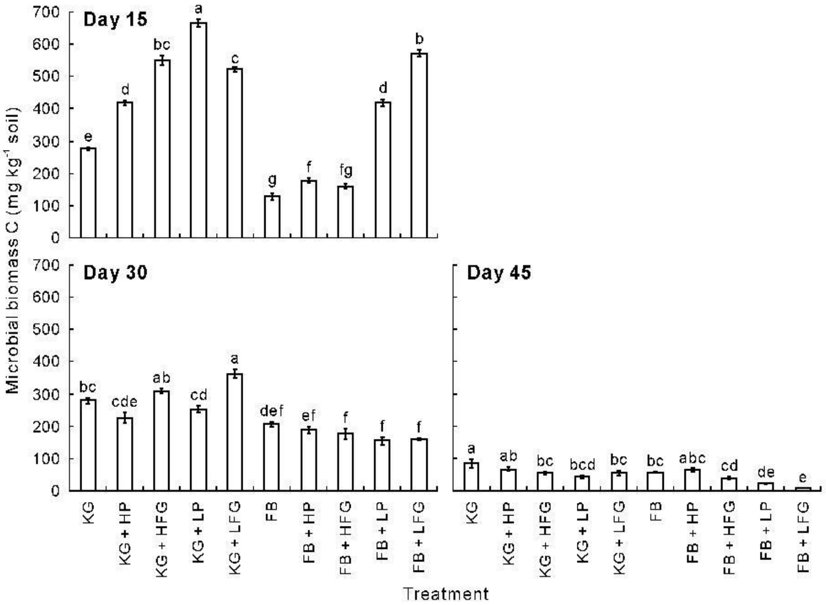

Fig. 3 Microbial biomass C on days 15, 30 and 45 in sandy soil amended with residues of kikuyu grass (KG) or faba bean (FB) (10 g kg^{-1}) along with or without high- and low-smectite clay soils as 2-mm peds (HP and LP, respectively) or finely ground (< 2 mm) clay soil (HFG and LFG, respectively) at a rate of 20% (weight/weight). Vertical bars indicate standard errors of the means ($n = 4$). For a given sampling day, bars with different letters are significantly different at $P \leq 0.05$ for clay and residue interaction.

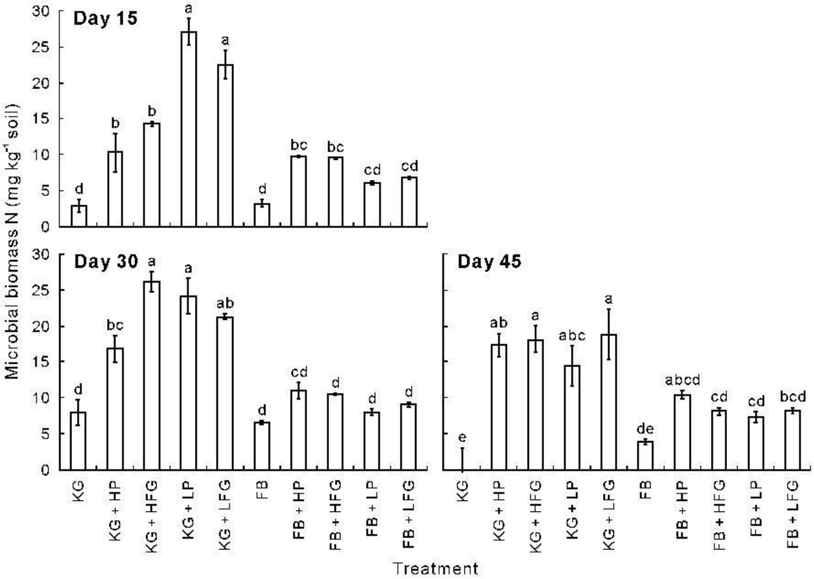

Fig. 4 Microbial biomass N on days 15, 30 and 45 in sandy soil amended with residues of kikuyu grass (KG) or faba bean (FB) (10 g kg^{-1}) along with or without high- and low-smectite clay soils as 2-mm peds (HP and LP, respectively) or finely ground (< 2 mm) clay soil (HFG and LFG, respectively) at a rate of 20% (weight/weight). Vertical bars indicate standard errors of the means ($n = 4$). For a given sampling day, bars with different letters are significantly different at $P \leq 0.05$ for clay and residue interaction.

KG than FB residues and higher in sandy soil without clay than in clay-amended soils (Fig. 6). There was no consistent effect of clay type or size on cumulative respiration.

During the first five days, respiration rates were higher with KG than FB residues (Figs. 7 and 8). On day 1, respiration rate was greater with high- than low-smectite clay soil or without clay, but later, respiration rates did not differ among soil treatments. Clay size did not influence respiration rate.

The initial TOC concentration on day 0 of the < 53 μm fraction was greater in low- than high-smectite clay-amended soils (Fig. 9). Addition of FB residues did not increase TOC concentration at the end of the experiment compared to the initial concentrations except for the soil with high peds, for which TOC concentration increased by about 11%. With KG residues, the TOC concentration at the end of the experiment was higher than the initial TOC concentration in soil with low-smectite clay soil as FG.

DISCUSSION

In this study, the measured parameters were mainly influenced by residue type and less by clay treatment. There were marked differences between sandy soil with and without clay; however, the differences between clay type and clay size were small and, in most cases, inconsistent. Based on the results we can only confirm the third hypothesis because the effect of clay addition on nutrient availability was greater with low C/N ratio KG residues than with high C/N ratio FB residues. This can be explained by the higher decomposition and net nutrient mineralisation rate of KG compared to FB residues (Figs. 1 and 2) (Hadas *et al.*, 2004). Even with low C/N ratio KG residues, differences among clay treatments were quite small. With FB residues, nutrient concentrations were generally low; thus, differences among clay treatments were even smaller. The discussion below will focus on the treatments with KG residues.

Compared to sandy soil without clay, cumulative respiration was lower, whereas MBC and MBN were higher in clay-amended soils (Figs. 3, 4 and 6). The lower cumulative respiration in clay-amended soils can be explained by the binding of organic C to clay which was also shown in the increase in TOC in the < 53 μm fraction during the main experiment. Binding to clay surfaces which occurs *via* bridges of cations such as

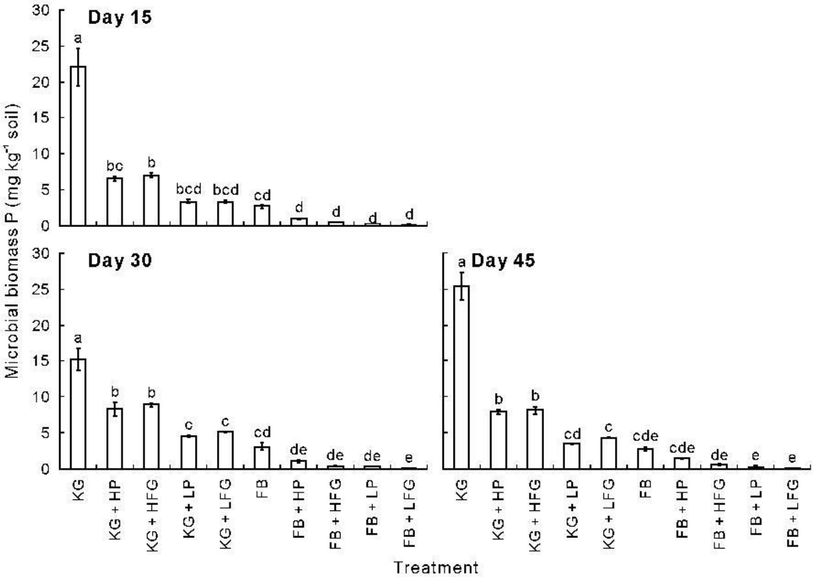

Fig. 5 Microbial biomass P on days 15, 30 and 45 in sandy soil amended with residues of kikuyu grass (KG) or faba bean (FB) (10 g kg^{-1}) along with or without high- and low-smectite clay soils as 2-mm peds (HP and LP, respectively) or finely ground (< 2 mm) clay soil (HFG and LFG, respectively) at a rate of 20% (weight/weight). Vertical bars indicate standard errors of the means ($n = 4$). For a given sampling day, bars with different letters are significantly different at $P \leq 0.05$ for clay and residue interaction.

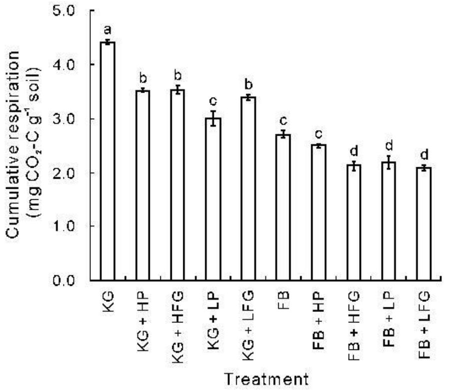

Fig. 6 Cummulative respiration after 45 d in sandy soil amended with residues of kikuyu grass (KG) or faba bean (FB) (10 g kg^{-1}) along with or without high- and low-smectite clay soils as 2-mm peds (HP and LP, respectively) or finely ground (< 2 mm) clay soil (HFG and LFG, respectively) at a rate of 20% (weight/weight). Vertical bars indicate standard errors of the means ($n = 4$). Bars with different letters are significantly different at $P \leq 0.05$ for clay and residue interaction.

Ca^{2+}, Fe^{3+} and Al^{3+} reduces accessibility of organic C to microbes (Baldock, 2007; Lützow *et al.*, 2006). The lower cumulative respiration in combination with higher MBC and MBN concentrations in clay-amended soils suggests that a greater proportion of residue C was used for microbial growth in soils amended with clay soil. Clay-amended soils contained more water, and the clay surface may provide more favourable habitats for microbes. Formation of biofilms and binding of residue particles on the clay surface could improve effectiveness of enzymes in breaking down the residues (Vogel *et al.*, 2015). Apparently, N release from residues was greater than microbial demand because despite the higher MBN concentrations and capacity to bind NH_4^+-N to clay surfaces compared to sandy soil without clay (Table IV, Fig. 4), available N concentrations were higher in clay-amended soils.

In contrast to MBN and available N concentrations, MBP and available P concentrations were higher in sandy soil without clay than in clay-amended soils (Figs. 2 and 5). The higher MBP concentration suggests a shift in microbial C/N/P ratio towards lower values in clay-amended soils. In sandy soil without clay, microbes took up P but were apparently limited in their C and N uptake.

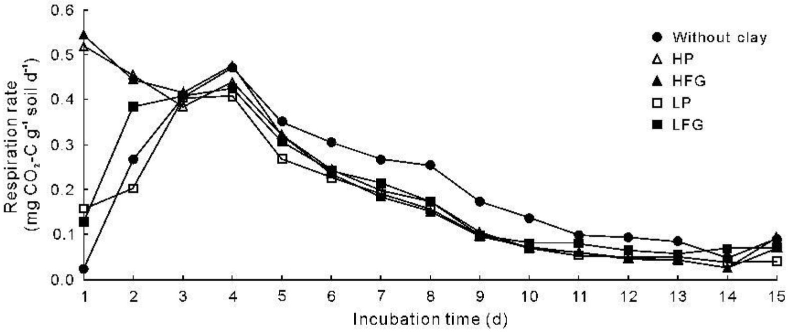

Fig. 7 Respiration rate from days 1 to 15 in sandy soil amended with kikuyu grass residues (200 g kg^{-1}) along with or without high- and low-smectite clay soils as 2-mm peds (HP and LP, respectively) or finely ground (< 2 mm) clay soil (HFG and LFG, respectively) at a rate of 20% (weight/weight).

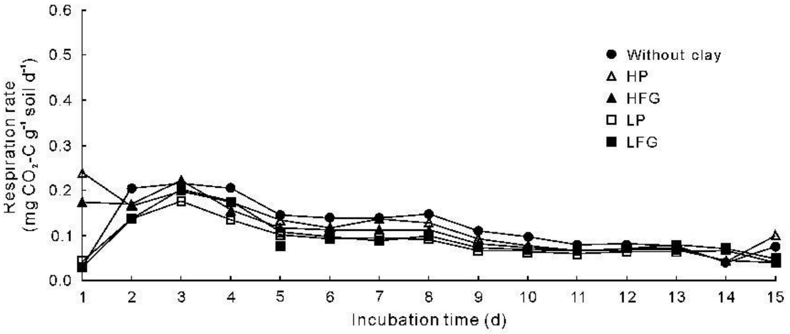

Fig. 8 Respiration rate from days 1 to 15 in sandy soil amended with faba bean residues (200 g kg^{-1}) along with or without high- and low-smectite clay soils as 2-mm peds (HP and LP, respectively) or finely ground (< 2 mm) clay soil (HFG and LFG, respectively) at a rate of 20% (weight/weight).

The differences in measured parameters were small and inconsistent between clay type and size. Therefore, we can not confirm the first two hypotheses. The lack of effect of clay size may be because the size classes (2-mm peds and < 2 mm FG) were not sufficiently different. The < 2 mm fraction may contain a proportion of particles only slightly smaller than 2 mm and some of the 2-mm peds may have broken down during the experiment. Although clay concentration, smectite percentage, CEC and SSA of high-smectite clay soil were greater than those of low-smectite clay soil, there were only small differences between two clay soils in the measured properties in this study. In the short term, binding to soil with high-smectite clay soil appears to be greater than that to low-smectite clay soil as NH_4^+-N and P sorptions were greater in the former (Table IV) and MBC and MBN concentrations with KG residues on day 15 were higher with high-smectite clay soil (Figs. 3 and 4). However, available N, MBC and MBN concentrations on days 30 and 45, cumulative respiration (Fig. 6) and the increase in TOC of the < 53 μm fraction (Fig. 9) did not differ between soils amended with high- and low-smectite clay soil. Thus, in the longer term (> 15–45 d), differences in clay content, SSA, CEC and smectite percentage between the two clay soils had no effect on binding of organic matter or nutrients. This is in agreement with previous studies. For example, Vogel *et al.* (2015) found that SSA is of minor importance for C and N sequestration. Wattel-Koekkoek *et al.* (2001) and Pronk *et al.* (2013) showed that clay mineralogy had little effect on decomposition rate and organic C binding. The lack of differences in binding of organic matter or nutrients between the two clay soils may be due to the 10-fold higher exchangeable Fe and Al concentrations in the low- compared to high-smectite clay soil. This can in-

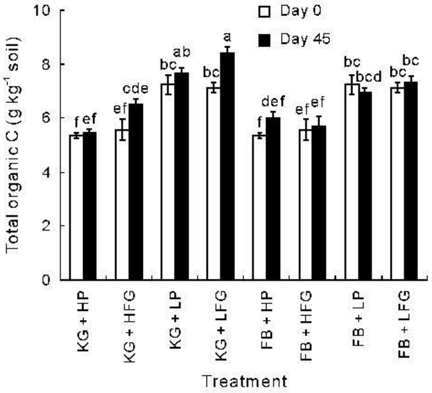

Fig. 9 Total organic C at the beginning (day 0) and the end (day 45) of the incubation in the < 53 μm fraction of sandy soil amended with residues of kikuyu grass (KG) or faba bean (FB) (200 g kg^{-1}) along with high- and low-smectite clay soils as 2-mm peds (HP and LP, respectively) or finely ground (< 2 mm) clay soil (HFG and LFG, respectively) at a rate of 20% (weight/weight). Vertical bars indicate standard errors of the means ($n = 4$). Bars with different letters are significantly different at $P \leq 0.05$ for clay and residue interaction.

fluence both nutrient and organic C binding. Sorption of P to soil particles or organic matter occurs by cation bridges (Gerke and Hermann, 1992). Presence of Fe/Al oxides on clay surfaces can increase organic C binding (Kleber *et al.*, 2007; Saidy *et al.*, 2012; Curtin *et al.*, 2016). Although we did not measure the concentrations of Fe and Al oxides, it is reasonable to assume that the higher exchangeable Fe and Al concentrations in the low-smectite clay soil are, at least partly, due to higher concentrations of Fe and Al oxides. The higher initial TOC concentration in the < 53 μm fraction of the soil with low-smectite clay soil indicates a high organic C-binding capacity, but could also be because the low-smectite clay soil was collected from shallower soil depth than the high-smectite clay soil (30–40 cm compared to > 50 cm). In general, TOC concentration decreases with soil depth because the organic matter input is the greatest in the top soil (Lawrence *et al.*, 2015).

The TOC concentration of the whole soils (Table I) differed from those of the initial < 53 μm fraction (Fig. 7). In the high-smectite clay soil, the TOC concentration of the whole soil was greater than that in the < 53 μm fraction. This is probably due to removal of particulate organic matter (POM) during extraction of the < 53 μm fraction which suggests that a large proportion of the organic C in the high-smectite clay soil is in the form of particulate organic C (POC). In the low-smectite clay soil on the other hand, the TOC concentration of the whole soil was lower than that in the < 53 μm fraction. This is likely due to the lower clay content of this soil and suggests that in this soil most organic C is bound to the < 53 μm fraction and the proportion as POC is small.

CONCLUSIONS

Addition of clay soil to sandy soil influenced nutrient availability, but there were no clear differences between clay types or sizes. The lack of differences between high- and low-smectite clay soils suggests that high concentrations of Fe and Al oxides can compensate a lower clay concentration and proportion of smectite with respect to binding of organic matter and nutrients. Longer-term studies are required to assess the fate of the bound nutrients and organic matter to better understand the consequences of claying on nutrient availability to plants and organic C sequestration.

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CHAPTER 4

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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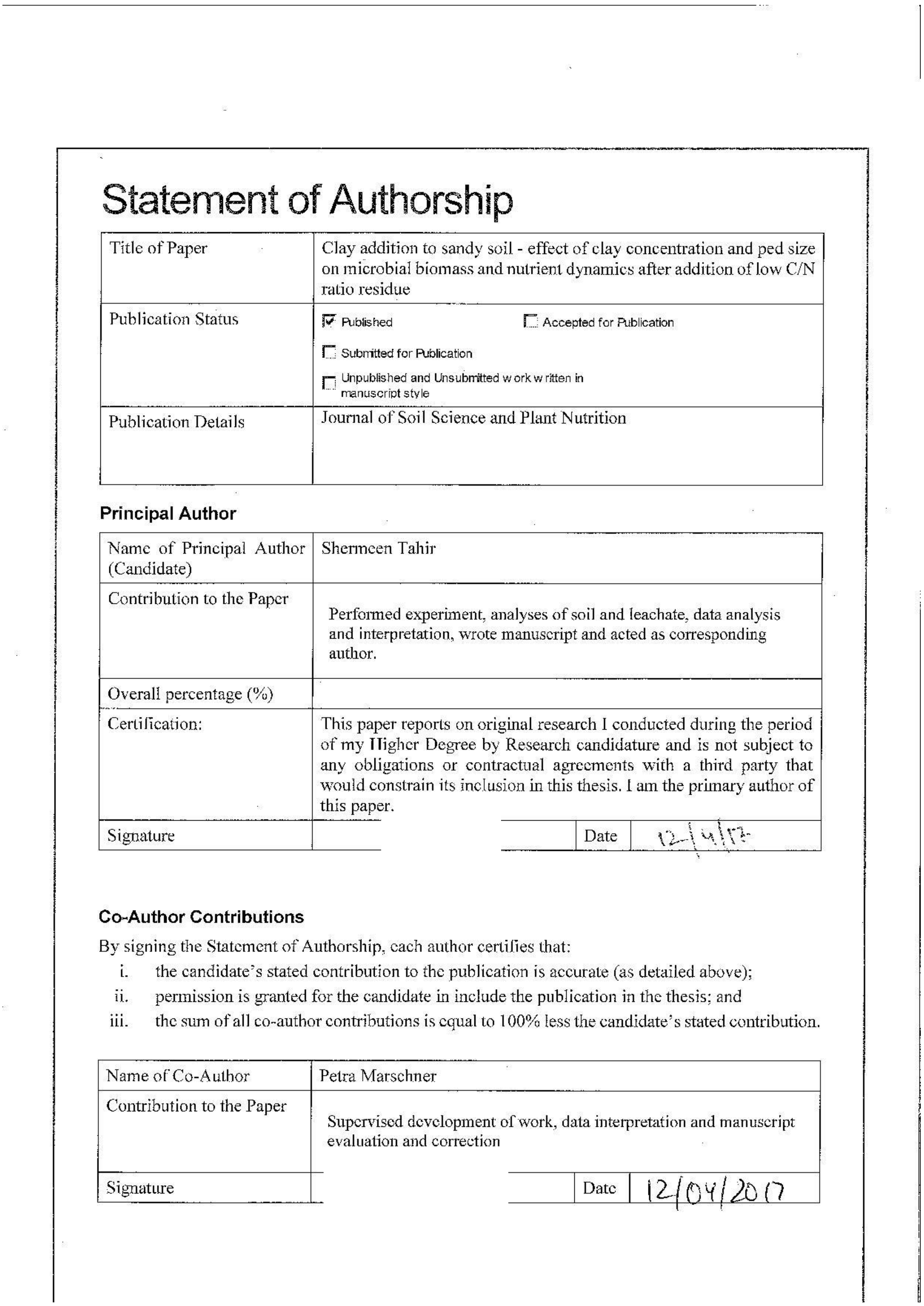

Statement of Authorship

Title of Paper	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
Publication Status	☑ Published ☐ Accepted for Publication ☐ Submitted for Publication ☐ Unpublished and Unsubmitted work written in manuscript style
Publication Details	Journal of Soil Science and Plant Nutrition

Principal Author

Name of Principal Author (Candidate)	Shermeen Tahir		
Contribution to the Paper	Performed experiment, analyses of soil and leachate, data analysis and interpretation, wrote manuscript and acted as corresponding author.		
Overall percentage (%)			
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Petra Marschner		
Contribution to the Paper	Supervised development of work, data interpretation and manuscript evaluation and correction		
Signature		Date	12/04/2017

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

Running title: Clay addition to sandy soil

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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 centimetres. 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 10 g 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.

Key words: 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 subsoil 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 centimetres 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 2016a) 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 2017) 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 µm 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 4.5 g 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 maximum WHC (40, 50, 60, and 80%). Glucose was added as an organic carbon source at 2.5 g C kg^{-1} soil and soil respiration was 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 H_2SO_4 and HNO_3 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% 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 (NH_4 and NO_3) 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 K_2SO_4 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 $(NH_4)_2$ $Fe (SO_4)_2$. $6H_2O$. 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 K_2SO_4 extracts was measured as NH_4-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 0.5 mm 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 µm and < 53 µm fractions by wet sieving (Christensen 2001). Organic C in the < 53 µm 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 µm sieves and dried overnight at 70˚C. The initial clay peds were also separated into < 53 µm and > 53 µm. The < 53 µm 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 times was 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 mg kg^{-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.

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.

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.

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.

Cumulative respiration over 45 days was significantly higher in sandy soil alone than in treatments with clay added at either 10% or 20% (Fig 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.

In the treatments with 20% clay addition, peds were retrieved on day 45 by sieving. Between 75 and 77% of the added < 53 µm 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 µm fraction was greater with 3 and 5 mm peds added than with 1 mm peds. The greatest proportion of the < 53 µm particle 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 µm particle size in the other fractions ranged between 38% and 48%. The TOC of < 53 µm 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 µm 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.

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 µm 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 2017) 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; Banerjee *et 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 µm fraction. The greatest proportion of peds was in the initially added ped size, but between 38 and 48% of the < 53 µm 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 µm 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 µm 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 µm 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 µm 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.

5. 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 as formation 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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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*≤ 0.05).

	pH (1:5)	EC (1:5)	Particle size			Organic C	Total N	MBC
			Sand	Silt	Clay			
		μS 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

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 ($P \leq 0.05$).

	Ped size (mm)	Water holding capacity (g water g^{-1} soil)	NH_4-N µg g^{-1} soil	Available P µg g^{-1} soil
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

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, $P \leq 0.05$).

	Clay concentration	ped size (mm)	MBC (mg kg^{-1} soil)		
			days		
			15	30	45
Sandy soil	none	none	335 a	282 ab	94 f
	10% clay	1	255 bc	241 bcd	204 cde
		3	227 cd	252 bc	216 cde
		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 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, $P \leq 0.05$).

	Clay concentration	ped size (mm)	Available N (mg kg^{-1} soil)		
			days		
			15	30	45
Sandy soil	none	none	34.5 h	34.7 h	34.5 h
	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

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*≤ 0.05).

	Clay concentration	ped size (mm)	Available P (mg kg^{-1} soil)		
			days		
			15	30	45
Sandy soil	none	none	39.4 a	35.0 ab	33.7 b
	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

Table 6. Properties of the < 53 μm 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*≤ 0.05).

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

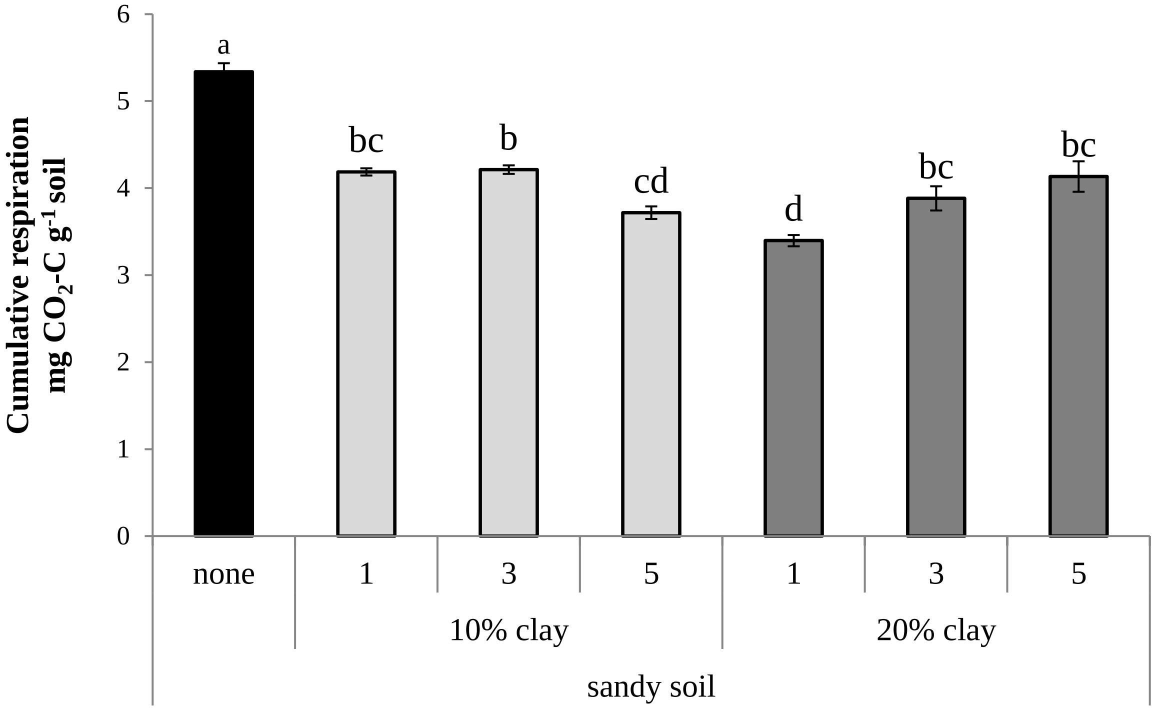

Figure 1. Cumulative respiration over 45 days (mg CO_2-C g^{-1} soil) in sandy soil without (none) or with clay soil added as 1, 3 and 5 mm peds amended with kikuyu (vertical lines indicate standard error, n=4). Columns with different letters are significantly different ($P \leq 0.05$).

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RESEARCH ARTICLE

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 thisexperiment 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 subsoil 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 μm 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 H_2SO_4 and HNO_3 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% 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 (NH_4 and NO_3) 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 K_2SO_4 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 $(NH_4)_2$ Fe $(SO_4)_2$. $6H_2O$. 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 K_2SO_4 extracts was measured as NH_4-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 µm and <53 µm fractions by wet sieving (Christensen 2001). Organic C in the < 53 µm 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 µm sieves and dried overnight at 70°C. The initial clay peds were also separated into < 53 µm and > 53 µm. The < 53 µm 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.

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 ($P \leq 0.05$).

	pH	EC	Particle size			Organic C	Total N	MBC
	(1:5)	(1:5)	Sand	Silt	Clay			
		µS 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

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 ($P \leq 0.05$).

	Ped size (mm)	Water holding capacity (g water g^{-1} soil)	NH_4-N µg g^{-1} soil	Available P µg g^{-1} soil
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.

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, $P \leq 0.05$).

	Clay concentration	ped size (mm)	MBC (mg kg^{-1} soil)		
			days		
			15	30	45
Sandy soil	none	none	335 a	282 ab	94 f
	10% clay	1	255 bc	241 bcd	204 cde
		3	227 cd	252 bc	216 cde
		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

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, $P \leq 0.05$).

	Clay concentration	ped size (mm)	Available N (mg kg^{-1} soil)		
			days		
			15	30	45
Sandy soil	none	none	34.5 h	34.7 h	34.5 h
	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, $P \leq 0.05$).

	Clay concentration	ped size (mm)	Available P (mg kg^{-1} soil)		
			days		
			15	30	45
Sandy soil	none	none	39.4 a	35.0 ab	33.7 b
	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.

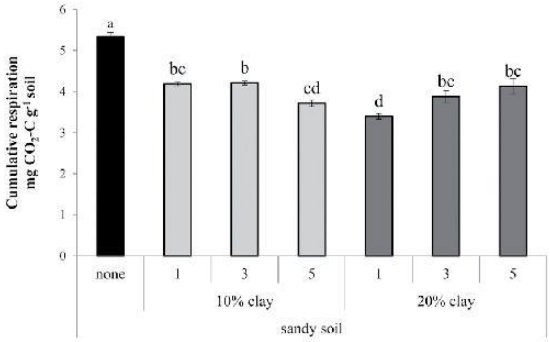

Figure 1. Cumulative respiration over 45 days (mg CO_2-C g^{-1} soil) in sandy soil without (none) or with clay soil added as 1, 3 and 5 mm peds amended with kikuyu (vertical lines indicate standard error, n=4). Columns with different letters are significantly different ($P \leq 0.05$).

In the treatments with 20% clay addition, peds were retrieved on day 45 by sieving. Between 75 and 77% of the added < 53 µm 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 µm fraction was greater with 3 and 5 mm peds added than with 1 mm peds. The greatest proportion of the < 53 µmparticle 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 µm particle size in the other fractions ranged between 38% and 48%. The TOC of < 53 µm 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 µm 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 µm 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≤ 0.05).

	peds added (mm)	retrieved peds (mm)	< 53 µm fraction (g core^{-1})	% TOC of <53 µm	Total TOC mg core^{-1}	% of total TOC
sandy soil + 20% clay	1	>1	1.76 b	0.40 bcd	7.04	33
		<1->0.5	2.84 a	0.50 a	14.2	67
			4.6		21.2	
	3	3-2	3.11 a	0.47 ab	14.6	65
		2-1	1.28 c	0.42 bc	5.4	24
		<1->0.5	0.89 d	0.28 e	2.5	11
			5.28		22.5	
	5	5-3	2.89 a	0.42 bc	12.1	54
		3-2	1.28 c	0.34 de	4.4	19
		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 µm 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; Banerjee*et 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 µm fraction. The greatest proportion of peds was in the initially added ped size, but between 38 and 48% of the < 53 µm 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 µm 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 µm 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 µm 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 µm 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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CHAPTER 5

CLAY AMENDMENT TO SANDY SOIL - EFFECT OF REPEATED ADDITION OF WHEAT RESIDUE ON ORGANIC CARBON CONTENT OF DIFFERENT SOIL FRACTIONS OVER 8 MONTHS

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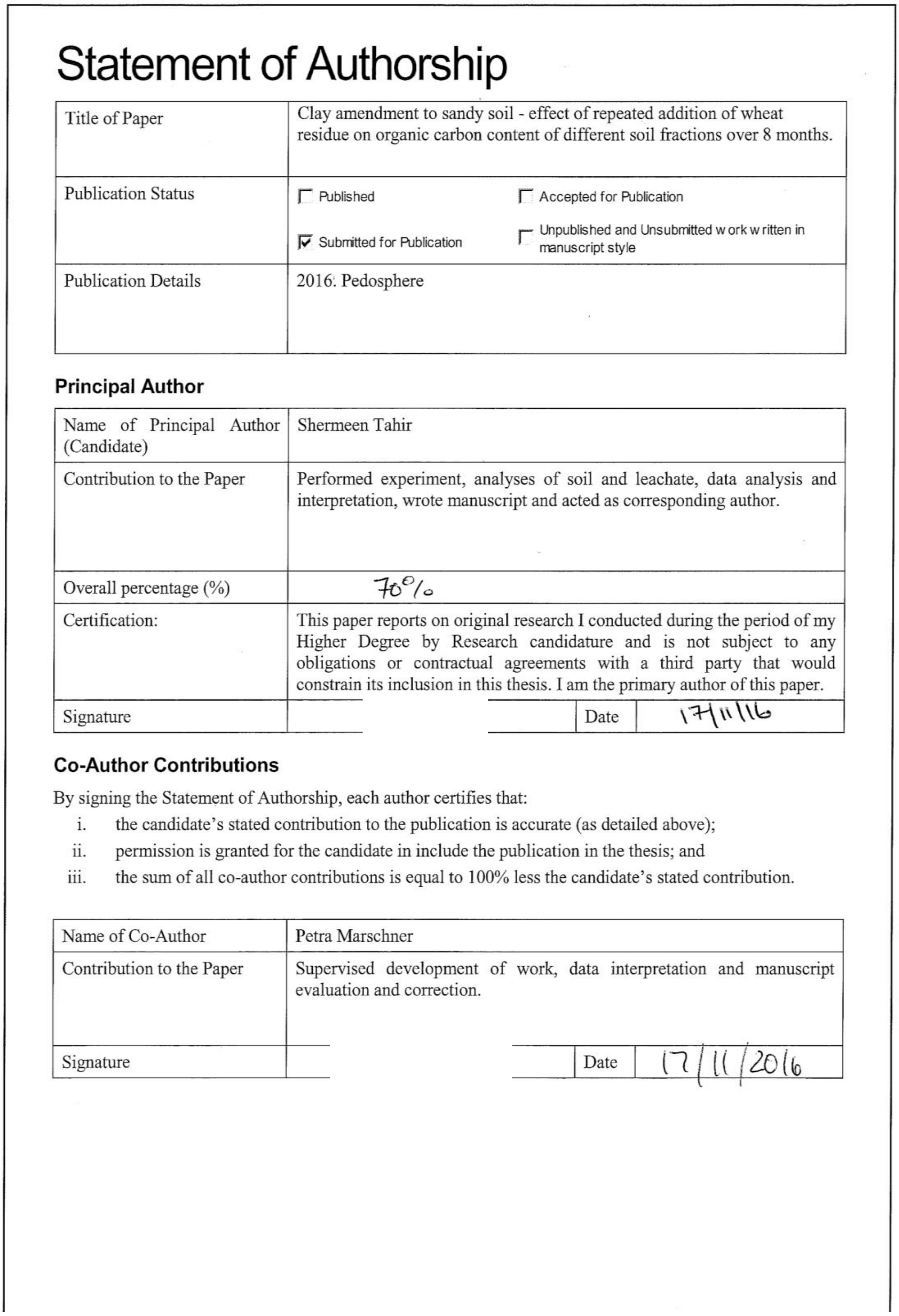

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Clay amendment to sandy soil - effect of repeated addition of wheat residue on organic carbon content of different soil fractions over 8 months

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ABSTRACT

Addition of clay soil has been shown to increase organic C retention, but little is known about how organic C retention changes over the first months. In this study, sandy soil was amended with clay soil at 200 g kg^{-1}. The clay soil was added finely ground or as 1 or 3 mm peds. Finely ground mature wheat straw (C/N 90) was mixed into the soils at the start of the experiment and again after 2, 4 and 6 months. Soils were sampled before adding residues at 2 and 4 months and at the end of the experiment (8 months). After 2 months, the organic C content of the whole soil was lower in sandy soil alone than in clay amended treatments although the initial increase was greater in sandy soil alone than with clay. The organic C of the whole soil continued to increase during the experiment with a greater increase in clay amended soils. The organic C content of the > 53 µm fraction was very low and changed little over time. With finely ground soil and 1 mm peds, the organic C content of the < 53 µm fraction increased mainly in the first 2 months. With 3 mm peds on the other hand, the organic C content did not increase in the first 2 months, but then increased in the following 6 months to reach similar concentrations as with finely ground clay soil and 1 mm peds.

Key words: claying, nutrient availability, organic carbon retention, wheat residue

INTRODUCTION

Sandy soils are characterized by low cation exchange capacity (CEC), low organic carbon and low clay content which leads to low nutrient and water holding capacity (Xie and Steinberger, 2005). Various strategies can be used to alleviate these constraints, for example synthetic soil conditioners (Choudhary *et al.*, 1998) or slow release N fertilizers (Mitchelson and Franco, 1994), but they are expensive and last only for few months. Another option is organic amendments, such as rice and wheat straw and compost. Organic amendments may improve soil structure and porosity and enhance both water and nutrient retention (Al-Omran *et al.*, 2002, 2004a). But due to a lack of binding sites for organic matter in sandy soil, decomposition rates and nutrient loss via leaching are high.

Addition of clay to sandy soil can enhance soil water storage, plant available water and crop growth (Tennant *et al.*, 1992). In sandy soils with clay subsoils, clay can be mixed into the sandy top soil by delving or spading. In the modified soils, the clay subsoil is not uniformly distributed creating a highly non-uniform soil environment with patches of clay-rich sandy soil next to sandy soil without or little clay. Clay-rich patches can hold more water and nutrients compared to the surrounding sandy soil which can alter both the nutrient availability and ability of the plant to take up nutrients. Nutrient availability is altered because in contrast to sand particles, clay minerals have a high cation exchange capacity and can therefore bind positively charged nutrients, e.g. NH_4^+, Ca^{2+}, Mg^{2+} and K^+ (Hamarshid *et al.*, 2010).

The association of organic matter and mineral surfaces is considered to be an important process because it protects organic matter against microbial decomposition (Mikutta *et al*., 2006). Fine particles are effective in binding soil organic matter because of their high surface area to volume ratio. Clay minerals bind organic matter through ligand exchange, cation bridges (Von Lützow *et al*., 2006) and weak interactions such as Van der Waals forces and H-bonding (Arnarson and Keil, 2000). Iron and aluminium oxides coated on clay particles can provide additional organic matter binding sites (Beare *et al*., 2014). The amount of carbon associated with < 53 µm fraction (silt and clay particles) is affected by texture (Six *et al*., 2002), mineralogy (Feng *et al*., 2005), amount and type of oxides and hydroxides of iron (Fe) and aluminium (Al) and type of organic carbon inputs (land use and residue addition).

In short term experiments (45 days), we showed that the effect of clay peds on nutrient availability and organic C retention differs from that of finely distributed clay and depends on clay ped size (Tahir and Marschner, 2016b). However, the effect of clay size on organic matter binding in the field may be different because clay will interact with organic matter over several months and plant residues may be added repeatedly, e.g. through root turnover and leaf litter fall.

The aims of the eight-month study with repeated residue addition were to (i) determine the effect of clay addition as finely ground soil or 1 and 3 mm peds to sandy soil on organic C retention and nutrient availability, and (ii) assess organic C retention in the < 53 µm fraction. We hypothesised that: i) organic C retention will be greater in sandy soil with clay than sandy soil alone, particularly in the first four months, (ii) organic C binding to the < 53 µm fraction will be greater with finely ground clay soil and 1 mm peds than with 3 mm peds, and iii) clay addition to sandy soil with residue amendment will reduce nutrient availability because of reduced decomposition rate (residues bound to clay) and binding of NH_4^+ and inorganic P to clay.

MATERIALS AND METHODS

Clay soil was collected from Waite Campus (latitude 34.97˚S, 138.63˚E) at 50-70 cm depth and air-dried. The soil is classified as black Vertosol in the Australian classification (Isbell, 2002) or Vertisol in the World Reference Base with smectite as dominant clay mineral (Table I). For finely ground soil, the soil was ground and passed through a 2-mm sieve. To generate peds of varied sizes, the soil was crushed and then sieved through different sieves. Three mm peds were collected on a 2-mm sieve after sieving through a 3.35 mm (size range 2-3.35 mm). The < 1 mm material was passed through a 0.5 mm sieve; the peds on the 0.5 mm sieve are referred to 1 mm peds (size range 0.5-1 mm). These ped sizes were chosen because in the field a substantial proportion of clay peds are in this range, particularly after clay spreading (Schapel, personal communication). Fine sandy soil (classified as Arenosol in the World Reference Base) collected in Penola (latitude 37.37˚S, 140.83˚E) which is in an area where claying is used to enhance yield on sandy soils. The sandy soil was air-dried and sieved to < 2 mm (Table I). The clay soil was added to the sandy soil at a rate of 200 g kg^{-1}.

Mature wheat straw (C/N 90) was ground, sieved to particle size < 2 mm and added at rate of 2.5 g kg^{-1} at the start of the experiment and again after 2, 4 and 6 months to give a total amount of 10 g kg^{-1}. After each addition sand, clay peds and residues were mixed thoroughly and 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 (0.75 µm, Australian Filter Specialist) base. The soil was packed to a bulk density of 1.5 g cm^{-3} by adjusting the height of the soil mixes in the core. Soil water content was maintained gravimetrically at 80% of maximum water holding capacity (WHC). This water content was selected based on a preliminary experiment where the sandy soil-clay ped mixtures were maintained at different percentage of WHC (40, 50, 60, and 80% of maximum WHC). Glucose was added as an organic carbon source and soil respiration was measured for one week. Soil respiration was highest at 80% of WHC in all treatments.

Three destructive harvests were carried out after 2, 4 (before the next residue addition) and 8 months with four replicates per treatment and harvest time. Soil was not sampled after 6 months because we planned for the experiment to go over 6 months with three sampling times (12 cores per treatment). But due to the slight change in TOC in the first 4 months, we decided to extend the experiment to 8 months with the last residue addition after 6 months. Samples were analysed for available N and P (resin method), TOC of whole soil, TOC of > 53 µm and < 53 µm fraction.

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. Particle size distribution was measured by the hydrometer method (Bouyoucos, 1936) and organic C was measured according to Walkley and Black, 1934. The maximum water holding capacity (WHC) of the soils and soil mixes was measured by using a sintered glass funnel connected to a 1 m water column (Ψ_m= -10 kPa) (Klute, 1986).

Microbial biomass C was measured by fumigation extraction as described in Vance *et al*. (1987). However, MBC concentrations were very low and therefore not reported.

Available N was extracted by shaking soil with 2 M KCl solution at a soil:solution ratio of 1:5 for one hour at 200-300 rpm. The suspension was filtered through Whatman No. 42 filter paper. Ammonium was measured colorimetrically at 685 nm as described by

Willis *et al.* (1996). Nitrate in the 2 M KCl extracts was determined colorimetrically at 540 nm as described in Cavagnaro *et al*. (2006). Available P was measured by the anion exchange resin method (Kouno *et al.,* 1995).

The sampled soil was analysed for total organic C by wet digestion (Walkley and Black, 1934) and the clay mixes were separated into > 53 µm and < 53 µm fractions by wet sieving (Christensen, 2001). Organic C in the < 53 µm fraction is referred to as mineral-associated organic C (Kögel-Knabner, 2000). 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 µm sieves and dried overnight at 70˚C. The initial clay peds were also separated into < 53 µm and > 53 µm fractions. The < 53 µm and > 53 µm fraction were ground and analysed for organic C 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 times was 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.

RESULTS

The clay soil (73% clay) was alkaline (pH 8.0) whereas the sandy soil (96% sand) was acidic (pH 5.4) (Table I). Organic C and total N were very low in the sandy soil. Compared to sandy soil, total P concentration was eight-fold higher, available N was five-fold and P concentration two-fold higher in clay soil. Clay soil addition to sandy soil increased maximum water holding capacity about five-fold and doubled ammonium concentration but decreased available P concentration compared to sandy soil alone (Table II).

In the first 2 months, the TOC content of the whole soil increased by about 1 g kg^{-1} in sandy soil alone, but only by 0.5 g kg^{-1} in clay amended sand. Nevertheless after 2 months, the TOC content of the whole soil was about 50% lower in sandy soil alone than in clay amended soil, but later it was only about 25% lower (Fig. 1a). Among clay amended soils, the TOC content was higher with finely ground soil than with 3 mm peds after 2 and 8 months. The TOC content of the whole soil increased in all treatments in the first 4 months by 20-30%. In the following 4 months, it increased again by about 20% in clay amended soils, but remained unchanged in sandy soil alone.

In the first 2 months, the TOC content of the < 53 µm fraction increased by about 1 g kg^{-1} (about 20%) in finely ground clay and 1 mm peds, but was similar as the original soil in 3 mm peds (Fig. 1b). The TOC content of the < 53 µm fraction was higher in the finely ground clay and 1 mm peds than 3 mm peds at 2 and 4 months and at 8 months it was higher in 1 than 3 mm peds. In 3 mm peds, the TOC content of the < 53 µm fraction increased by about 20% from 2 to 4 months. The TOC content of the < 53 µm fraction remained unchanged in the following 4 months in finely ground clay and 1 mm peds. But in 3 mm peds it increased by 20%.

The TOC content of the > 53 µm fraction was only about 10% of that of the < 53 µm fraction, was similar in all clay treatments and did not change significantly over time (Fig. 1c).

The ammonium concentration did not differ among treatments (Table III). Nitrate was not detectable. Compared to the initial soils, available P concentration increased in the first two months by about 25% in sand soil alone, four-fold in finely ground clay and 1 mm peds and doubled in 3 mm peds (Table IV). Available P concentration increased from 2 to 4 months in sandy soil alone and with finely ground clay, but remained unchanged with 1 and 3 mm peds. After 4 months, the available P concentration was highest with finely ground clay. The available P concentration decreased in the following 4 months with a greater decrease in clay amended soil than sandy soil alone. Among clay treatments, the decrease was greatest with finely ground clay. Thus after 8 months, soil treatments did not differ in available P concentration.

DISCUSSION

This study confirmed that organic C retention is increased when clay soil is added to sandy soil and that in clay amended soils organic C is mainly bound to the < 53 µm fraction. However, the study also indicated that clay amended sandy soils have a maximum binding capacity for organic C which is reached earlier in finely ground clay than in peds.

We can only partly confirm the first hypothesis because organic C retention was greater in sandy soil with clay than sandy soil alone, but on a whole soil basis the increase was greater from 4 to 8 months than earlier. We had assumed that the increase would be greater in the first four months because a greater proportion of potential TOC binding sites would be free. However, the results suggest that this was not the case. The sandy soil used here had a very low TOC content, thus addition of clay soil to sandy soil increased TOC content even without residue addition as seen in the initial data. The increase in TOC content of the whole soil in the first 2 months was greater in sandy soil alone than in clay amended soils which could be due to the higher maximum WHC of clay amended soils. This could provide a better habitat for microbes and therefore higher decomposition rates of added wheat straw than in sandy soil where water may be limiting microbial activity (Roychand and Marschner, 2013).

In general, we can confirm the second hypothesis (organic C binding to < 53 µm fraction will be greater in FG soil and 1 mm peds than 3 mm peds), but the temporal pattern of OC binding to the < 53 µm fraction depended on the size of the clay soil particles. Although a proportion of the added wheat straw was decomposed, the TOC content of the < 53 µm fraction increased in the first 2 months by about 1 g kg^{-1} with finely ground clay and 1 mm peds which is approximately the amount of C added with the residues. The TOC content of the 3 mm peds did not increase compared to the initial content in the first 2 months, likely because of the smaller surface area to volume ratio compared to 1 mm peds and finely ground clay soil. Thus, the higher TOC content of the whole soil amended with finely ground clay and 1 mm peds compared to sandy soil alone was mainly due to organic C bound to the < 53 µm fraction. The TOC content of the < 53 µm fraction did not increase further from 2 to 4 months with finely ground clay and 1 mm peds suggesting that the binding capacity had been reached. However, the TOC content of the < 53 µm fraction increased from 2 to 4 and again to 8 months with 3 mm peds and was higher after 8 than after 2 months with 1 mm peds. This could be due to breakdown of peds into smaller particles. In a previous study with the same clay soil over 45 days, we showed that about 40-60% of 1 and 3 mm peds had broken down to smaller particles (Tahir and Marschner, 2016a). This would increase the surface area to volume ratio and thus capacity of the < 53 µm fraction to bind organic C. The increase in TOC content of the whole soil from 4 to 8 months was greater than the increase in TOC content of the < 53 µm fraction. This suggests accumulation of largely undecomposed wheat straw that is not bound to the < 53 µm fraction. Decomposition of the high C/N wheat straw in the later stages of the experiment may have been limited by N and P as available ammonium and P concentrations were lower after 8 than after 4 months.

The third hypothesis [clay addition to sandy soil with residue amendment will reduce nutrient availability because of reduced decomposition rate (residues bound to clay) and binding of NH_4^+ and inorganic P to clay] has to be declined because ammonium concentration was similar in all treatments and because in the first 4 months, P availability was greater in clay amended soils than with sandy soil alone. This hypothesis was based on our earlier shorter term study with faba bean residue (C/N 37) which showed that sandy soil alone had a lower ammonium and P sorption capacity than clay amended soil and the ammonium concentration after 45 days was lower in clay amended soil.

The ammonium concentration after 2 months was about half of that in the initial soils which can be explained by immobilisation of N by microbes decomposing the high C/N ratio wheat straw. The following increase of available ammonium from 2 to 4 months may be due to turnover of the microbial biomass. The decline from 4 to 8 months may be due to binding of ammonium to clay (Nieder *et al*., 2011; Tahir and Marschner, 2016a) in clay amended treatments and denitrification in all treatments (Rengel, 2007; Miller *et al*., 2008). The lack of difference in available ammonium in this study is in contrast to the lower ammonium concentration after 45 days in our earlier study (Tahir and Marschner 2016a). This suggests that longer term studies are needed to better evaluate the effect of clay addition on nutrient availability.

Initially, the available P concentration was lower in clay-amended soils than sandy soil alone which may be due to the fact that the clay soil was taken at depth whereas the sandy soil was mainly top soil and therefore more likely to receive P fertilisation (Menzies, 2009; Yan *et al*., 2013). Further, P can be bound to clay (Frossard *et al*., 1995; Yaghi and Hartikainen, 2014). The increase in available P concentration in the first 2 months in all treatments, but particularly in clay amended soil could be due to exchange of bound P by organic acid anions generated during decomposition of the wheat straw (Gerke *et al*., 2000). The available P concentration further increased from 2 to 4 months in most treatments, but the increase was significant only in finely ground clay. Finely ground clay would have the greatest surface area among the treatments and thus the largest number of exposed P binding sites where P can be exchanged by organic acid anions. The higher available P concentration in clay amended soil in the first 4 months is in agreement with our earlier study (Tahir and Marschner, 2016a). The decrease in available P concentration from 4 to 8 months may be due to microbial P immobilisation or binding of P to undecomposed wheat straw.

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TABLE I. Selected physico-chemical properties of sandy and clay soil used in experiment (n=3 except texture where n=1).

Soils	pH	EC	Particle size (%)			TOC*	WHC	Total		Available	
								N	P	N	P
		µS cm^{-1}	Sand	Silt	Clay	g kg^{-1}	g g^{-1} soil	g kg^{-1}		mg kg^{-1}	
Sandy soil	5.4	12	96	1	3	nd	0.02	nd	0.04	4.4	1.6
Clay soil	8.0	621	12	15	73	12.7	0.45	1.0	0.32	21	0.9

(nd= not detectable)

[*] TOC content of whole soil

TABLE II. Maximum water holding capacity, ammonium and available P concentrations in sandy soil alone or with 20% clay soil finely ground (FG) soil or peds of 1 and 3 mm (n=3). Values within a column followed by different letters are significantly different (*P*≤ 0.05).

Clay soil (20% w/w)	water holding capacity (g water g^{-1} soil)	TOC g kg^{-1}	TOC < 53 µm fraction g kg^{-1}	NH_4-N µg g^{-1} soil	available P µg g^{-1} soil
none	0.020 c	nd	-	3.8 b	1.6 a
FG	0.119 a	2.5 a	5.2 a	6.6 a	1.34 b
1	0.107 a	2.5 a	5.1 a	5.4 ab	1.44 b
3	0.105 b	2.5 a	5.6 a	4.3 b	1.48 ab

TABLE III. Ammonium N (mg kg^{-1}) concentration in sandy soil alone and with clay added finely ground soil (FG) and peds of 1 and 3 mm amended with wheat residue after 2, 4 and 8 months. Values followed by different letters are significantly different (n=4, $P \leq 0.05$).

Clay soil (20% w/w)	Months		
	2	4	8
	Ammonium N (mg kg^{-1} soil)		
none	1.59 d	5.25 a	3.29 b
FG	2.49 bc	5.16 a	3.02 b
1 mm	2.15 cd	5.78 a	3.27 b
3 mm	1.96 cd	5.38 a	3.18 b

TABLE IV. Available P (mg kg^{-1}) concentration in sandy soil alone and with clay added finely ground soil (FG) and peds of 1 and 3 mm amended with wheat residue after 2, 4 and 8 months. Values followed by different letters are significantly different (n=4, $P \leq 0.05$).

Clay soil (20% w/w)	Months		
	2	4	8
	Available P (mg kg^{-1} soil)		
none	1.96 efg	2.56 def	1.16 fg
FG	5.53 b	9.83 a	0.59 g
1 mm	5.43 bc	4.15 bcd	1.14 fg
3 mm	3.55 cde	3.85 bcde	0.75 fg

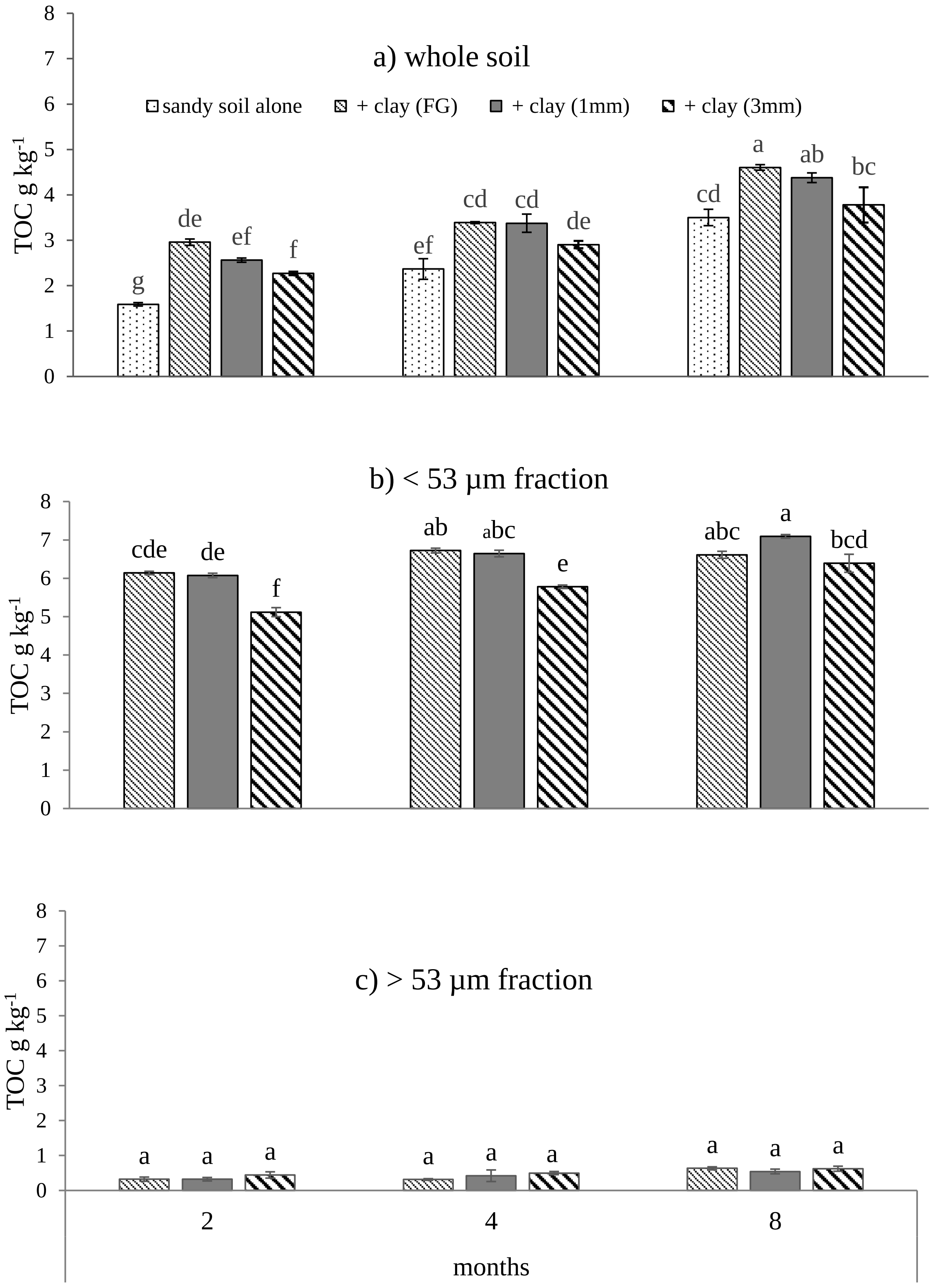

Fig. 1. Total organic carbon (g kg^{-1}) of a) whole soil, b) < 53 µm fraction and c) > 53 µm fraction of sandy soil alone or with clay added as finely ground soil (FG) and peds of 1 and 3 mm amended with wheat residue after 2, 4 and 8 months. Vertical lines indicate standard error (n=4). Columns with different letters are significantly different ($P \leq 0.05$).

CHAPTER 6

CLAY ADDITION TO SANDY SOIL REDUCES NUTRIENT LEACHING - EFFECT OF CLAY CONCENTRATION AND PED SIZE

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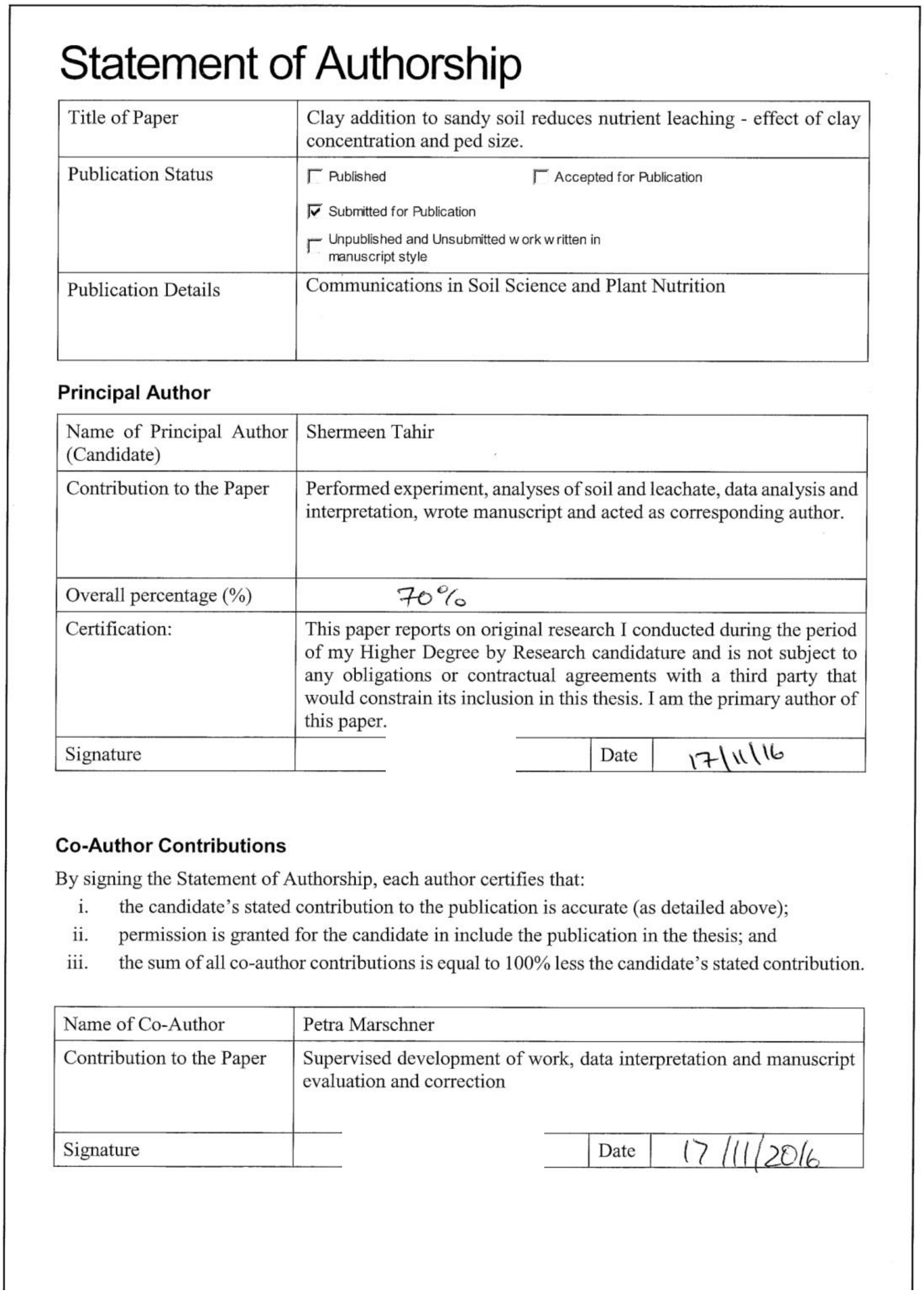

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Overall percentage (%)	70%		
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);
ii. permission is granted for the candidate in include the publication in the thesis; and
iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Petra Marschner		
Contribution to the Paper	Supervised development of work, data interpretation and manuscript evaluation and correction		
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Clay addition to sandy soil reduces nutrient leaching - effect of clay concentration and ped size

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Abstract

Leaching of nutrients, particularly in sandy soil with low nutrient and water holding capacity, is a major threat to marine and fresh water pollution. Addition of clay soil to sandy soil could be an option to increase water and nutrient holding capacity of sandy soils, but the effect of clay soil addition may depend on addition rate and the form in which it is added (particles or peds). Clay soil was added to sandy soil at rate of 10 or 20% clay soil w/w finely ground or as 2 and 5 mm peds size with and without N and P fertilizer equivalent to 15 kg P ha^{-1} 60 kg N ha^{-1}. The clay sand mixture for each treatment was weighed (30 g) in cores with nylon mesh at the bottom. The soils were incubated at 80% water holding capacity for 7 weeks. To obtain leachate, 20 mL RO water was added every week to each core. Leachate was analysed for inorganic N, P and pH. Soil was analysed for N, P and pH before and after the leaching. Clay addition significantly reduced the leaching of N and P compared to sandy soil alone, with greatest reduction by finely ground clay soil and least with 5 mm peds. Compared to sandy soil alone, 83%

more N was retained in clay-amended soil and P retention was doubled. This study showed that addition of finely ground clay soil can substantially reduce N and P leaching and thereby increase fertiliser retention compared to sandy soil alone.

Key words: clay soil addition, nutrient retention, N and P leaching

Introduction

Leaching of nutrients and agro-chemicals such as herbicides is a major problem in many agricultural areas, particularly those with light-textured soils. Leaching increases production costs for farmers and can cause soil acidification (Goulding 2000; Weaver et al. 1988), but also poses a risk for the environment (Laird et al. 2010). High nutrient levels in surface and/or groundwater can promote eutrophication and thus freshwater and marine pollution (Karaca et al. 2004). For example, coral bleaching in the Great Barrier Reef in Australia is thought to be in part due to increased nutrient input (D'Angelo and Wiedenmann 2014; De'ath and Fabricius 2010).

In Florida, Chen et al., (2006) showed that 97% of P added as water-soluble fertilizer was leached from a sandy soil. In sandy soils in Zimbabwe, Hagmann (1994) found that up to 54% of applied fertilizer was leached out of the top 0.5 m of soil when heavy rains followed N fertilizer application. Factors which affect rate and total amount of leached ions include soil texture, cation/anion exchange capacity of soil, total amount of rainfall, rate and form of fertilizer, solubility and adsorption of ions and presence of accompanying ions (Havlin et al. 1999). Sandy soils have low water and nutrient holding capacity and are therefore prone to leaching (Shepherd and Bennett 1998). To increase water and nutrient holding capacity of sandy soils, strategies must be developed to keep applied nutrients within the root zone. One strategy may be addition of clay soil to sandy soil because clay soils are dominated by micropores which can hold water. Further, clay surfaces have high cation exchange capacity and can also bind P (Froassard et al. 1995; Yaghi and Hartikainen 2014). The effect of clay soil addition is likely to depend on its surface area available for nutrient binding, and thus addition rate and size of clay soil particles/peds.

The aim of this experiment was to assess the effect of clay soil addition rate (10% and 20% w/w) and size (finely ground soil or peds of 2 or 5 mm) on leaching of N and P from unfertilised or fertilised sandy soil. We hypothesised that (i) clay addition will reduce N and P leaching particularly in fertilised soil, and (ii) the reduction of N and P leaching will be greater with finely ground clay soil than with peds and greater at 20% than 10% clay soil addition rate.

Materials and methods

Experimental design

Clay soil (73% clay, total organic C, total N and P 12.7, 1 and 0.32 g kg^{-1}, pH 8.0) was collected from Waite Campus (34.97˚S, 138.63˚E). The soil is classified as black Vertosol in the Australian classification (Isbell 2002) or Vertisol in the World Reference Base, with smectite as dominant clay mineral. After air-drying, the soil was either finely ground or crushed and sieved through different sieves to achieve 3 and 5 mm peds. 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). Sandy soil (96% sand, total organic C, total N not detectable, total P 0.04 g kg^{-1}, pH 5.4) 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 soil addition to improve crop growth on sandy soils. The clay soil was added to the sandy soil at 10 and 20% w/w as finely ground soil (FG), 3 and 5 mm peds with and without fertilizer. After thorough mixing of sand and clay soils, 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}.

The fertilizer rates were selected on the basis of a preliminary experiment where different fertilizer rates for P (0, 5, 10, 15, 20, 30, 40 and 50 kg ha^{-1}) as KH_2PO_4 and N (0, 40, 60, 80, 100, 150 and 200 kg ha^{-1}) as NH_4Cl were applied to sandy soil alone and leachate was collected every week for three weeks and analysed for pH, N and P concentration. Fertilizer was applied at 15 kg P ha^{-1} (7 mg P kg^{-1}) and 60 kg N ha^{-1} (27 mg N kg^{-1}) as KH_2PO_4 and NH_4Cl. These rates were selected because N and P concentrations in leachate were neither very high nor very low. In treatments with fertilizer, fertilizer solutions were added while in control without fertilizer only reverse osmosis (RO) water was added. Solutions or water were added to achieve a water content of 80% of maximal water holding capacity (WHC) which was maintained throughout the experiment. This water content was selected based on our previous studies with the clay-sandy soil mixes (Tahir and Marschner 2016a). Microbial activity was maximal at 80% of WHC indicating that the soil was well aerated, but also contained sufficient water.

The cores were incubated at 25˚C in the dark for seven weeks and leached with 20 ml RO water weekly. The 20-ml volume of RO water was used to have sufficient leachate (10 ml) for analysis. At each leaching event, the cores were placed in plastic funnels lined with filter paper to avoid clay particles in the leachate. After every leaching event, the cores were placed in a fan-forced oven at 40˚C for 2-3 h, until the soil dried to

80% of WHC. Between leaching events, the cores were kept in the dark at 25˚C. Soil was analysed for pH, available N and (resin P) approximately 4 h after fertiliser application and adjustment to 80% of WHC, before the first leaching event and again after the last leaching event.

The leachate was analysed for inorganic N and P and pH. Leachate data was expressed as N or P µg g^{-1} [(concentration in leachate x leachate volume)/g soil] per leaching event or total (sum of seven leaching events). Nitrogen N or P leached from the applied fertiliser was calculated as follows:

[(Concentration of element (inorganic N or P) in leachate of amended soil per ml x amount of leachate in ml) ÷ 30 g soil] – [(Concentration of element (inorganic N or P) in leachate of unamended soil per ml x amount of leachate in ml) ÷ 30 g soil] = total concentration of inorganic N or P leached µg g^{-1}

Fertiliser retention per g was calculated as:

Total concentration of inorganic N or P (µg g^{-1}*) applied – total concentration of inorganic N or P leached µg g*$^{-1}$ *= retained inorganic N or P (µg g*$^{-1}$*)*

Soil analysis

Soil pH was 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 and mixtures was measured by using a sintered glass funnel connected to a 1 m water column (Ψ_m= -10 kPa) (Klute 1986).

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). Inorganic N in the leachate was determined as described for KCl extract. 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). Inorganic P concentration in leachate was determined by the Murphy and Riley method.

The data were analysed by one way ANOVA for each sampling time or leaching event separately using Genstat 15th edition (VSN Int. Ltd, UK). Tukey's multiple comparison test at 95% confidence interval was used to determine significant differences among treatments. There were large differences in N and P concentrations between fertilised and unfertilised soil, obscuring differences among the clay soil treatments. Therefore, the one-way ANOVAs were carried out separately for fertilised und unfertilised soils.

Results

The pH of sandy soil alone or clay amended soil did not change during the experiment (data not shown). The pH of the leachate collected at each leaching event did not differ among leaching events but it was lower in sandy soil alone (pH 4.1-4.3) than in clay amended sandy soil (6.2-7.5).

Soil available N and P concentration before first leaching and after last leaching

Soil available N concentration was about 60% higher with fertilizer than without fertilizer (Fig. 1). Before the first leaching, the available N concentration was about 20% greater in clay amended soil than in sandy soil alone, except in fertilised soil with 2 mm clods where it was similar to sandy soil alone. Without fertilizer addition, the available N concentration in clay-amended soil changed little between before the first and after the last leaching, but it decreased by about 75% in sandy soil alone. After the last leaching, the available N concentration was four to eight-fold higher in clay amended soil than in sandy soil alone. With fertiliser addition, the available N concentration after the last leaching was between 20 and 60% lower than before the first leaching. At both clay soil addition rates, the relative decrease was smallest with 5 mm peds. After the last leaching, the available N concentration was lowest in sandy soil alone. Among clay soil treatments, it was higher at 20% than 10% addition rate and highest with 5 mm peds.

Before the first leaching, the soil available P concentration in the fertilised treatment was about three-fold higher in sandy soil alone or with finely ground clay soil than without fertiliser (Fig. 2). With 2 or 5 mm peds, the available P concentration was very low in unfertilised soil and ranged between 4 and 6 µg g^{-1} with fertiliser. After the last leaching, available P concentrations differed little between soil with and without fertiliser. In unfertilised soil before and after leaching, the available P concentration was very low with 2 and 5 mm peds (Fig 2). Compared to sandy soil alone, available P concentration before and after leaching was two-fold higher with finely ground clay soil at 10% addition rate and three-fold higher with 20% clay soil. After the last leaching in sandy soil alone or with finely ground clay soil the available P concentration was about three times lower than before the first leaching.

In fertilised soil, the available P concentration before the first leaching decreased in the following order: with finely ground clay soil at 20% > finely ground at 10% > sandy soil alone > with 2 or 5 mm peds. The available P concentration compared to sandy soil alone was about two-fold higher with finely ground clay soil at 10% and three-fold higher with finely ground clay soil at 20%. After the last leaching, the available P concentration was six to eight-fold lower than before the first leaching. It was lowest in sandy soil alone and highest with finely ground clay soil where it was about two-fold higher than with 2 and 5 mm peds.

Inorganic N and P concentration in leachate

Without fertiliser, leachate inorganic N concentration fluctuated among leaching events. It was lower in the second than the first leaching but then increased again to the third leaching after which it remained low (Table 1). About 70% of total inorganic N was leached in the first four leaching events. In general, leachate inorganic N concentration was lower in clay amended soil than sandy soil alone in the first four leaching events except for 5 mm peds at 10% clay soil in the first leaching event where it was similar as in sandy soil alone. Neither clay soil concentration nor size influenced leachate N concentration.

With fertiliser, leachate N concentration was highest in the first leaching event. It was about three-fold lower in the second leaching after which it decreased more slowly (Table 1). In the first four leaching events, the inorganic N concentration was three to four-fold higher in sandy soil alone than in clay amended soil. The decrease in leachate N concentration was greater with 20% clay soil than with 10%. At 10% clay soil, inorganic N concentration in the first four leaching events was highest with 5 mm peds, but there was no difference among clay soil sizes at 20% clay soil. Cumulative N leached was greater in sandy soil alone than clay amended soil with greater differences in fertilised soil (Table 3). With fertiliser, more N was leached with 10% clay soil than with 20%. At 10% clay soil, more N was leached with 5 mm peds than with finely ground soil or 2 mm peds. In sandy soil alone, 68% of total inorganic N was leached in the first event whereas it was only between 40 and 53% in clay amended soil. In all soils, about 80% of total inorganic N was leached in the first four events.

In both unfertilised and fertilised soil, the inorganic P concentration in the first five leaching events was up to six-fold lower in clay amended soil than sandy soil alone (Table 2). The inorganic P concentration was highest in the second leaching. In fertilised clay amended soil, the inorganic P concentration in the first four leachates was highest with 5 mm peds and lowest with finely ground clay soil.

Cumulative inorganic P leached was three to five-fold higher in sandy soil alone than clay amended soils (Table 3). Among clay amended soils, cumulative inorganic P leached was higher with 5 mm peds than finely ground clay soil and 2 mm peds and higher with 10% clay soil than 20%. The greatest proportion of total inorganic P was leached in the second event, ranging between 41 and 80%. This proportion was higher in clay amended soil than sandy soil alone. Less than 10% of total inorganic P was leached after the third leaching event.

In fertilised soil compared to sandy soil alone, more than three-fold more N was retained in clay-amended soils (Table 4). All P applied with the fertiliser was leached from sandy soil alone. In clay amended soils, more N and P was retained at 20% clay soil than at 10% and least with 5 mm peds.

Discussion

This study showed that clay soil addition to sandy soil can reduce leaching of N and P both without and with fertiliser, confirming the first hypothesis. It is well-known that clay soils have a greater capacity to bind cations such as NH_4^+ and anions than sandy soil (Yang et al. 2007; He et al. 1992; Chantigny et al. 2004) which is also in agreement with our earlier study with this clay soil (Tahir and Marschner 2016). However, to our knowledge this has not been studied in clay amended sandy soils.

Initially, the available N concentration was higher in clay amended than sandy soil alone, for available P this was only true with finely ground clay soil. The higher available N and P concentration can be explained by the higher total N and P concentration in the clay soil compared to the sandy soil. The effect is greater with finely ground clay soil because its surface area to volume ratio and thus exposed area is higher than in peds.

Without fertiliser, the inorganic N concentration was higher in the first and third leachate than in the later leaching events. This suggests that initially available N was released with the first leaching. Then it took about two weeks until N had been mineralised or released by desorption to result in high leachate concentrations. The inorganic P concentration was highest in the second leachate. This may be because only very small amounts of P were available initially and the first leaching reduced the P concentration in the soil solution and thereby triggered desorption of P from binding sites.

With fertiliser, the inorganic N concentration was highest in the first leachate, likely because N was added in soluble form and could therefore be easily leached. On the other hand, inorganic P concentration was highest in the second leachate although soluble P was added. Likely the time between fertiliser addition and the first leaching was sufficient for P to precipitate or bind to particle surfaces (McLaughlin et al. 2011). The first leaching then reduced the P concentration in the soil solution sufficiently to stimulate desorption of bound P (Atalay 2001).

We cannot unequivocally confirm the second hypothesis (the reduction of N and P leaching will be greater with finely ground clay soil than with peds and greater at 20% than 10% clay soil addition rate) because the importance of clay soil size differed between N and P leaching. Inorganic N concentration was more strongly reduced by finely ground clay soil than 5 mm peds at 10% clay soil addition, but did not differ between these sizes at 20% clay soil. This suggests that with 20% clay soil, even the 5 mm peds provided sufficient binding sites to reduce inorganic N compared to sandy soil alone. For P, the reduction of leachate concentration compared to sandy soil alone was greater for finely ground clay soil than with 5 mm peds at both clay soil addition rates. This indicates that the 5 mm peds provided limited P binding sites even at 20% clay soil addition.

Conclusion

This study showed that particularly addition of finely ground clay soil can substantially reduce N and P leaching and thereby increase fertiliser retention compared to sandy soil alone. After the last leaching event, available N and P concentrations were higher in clay amended soil than sandy soil alone. It is likely that a proportion of the retained N and P is not available to a crop, but may become available over time. Importantly, clay soil addition may reduce fertiliser cost and substantially reduces the risk of eutrophication. In this study, 10% clay soil addition was nearly as effective in reducing N and P leaching as 20% clay soil. Adding only 10% clay soil to a sandy soil is likely to be less expensive than 20%. However, it will not be possible in the field to add the clay soil homogenously and as very small particles to sandy soil. Further, the clay soil used here had a very high clay content (73%). If the clay content of the added clay soil is lower, the reduction in N and P leaching is likely to be smaller. For example, Atalay (2001) found that P sorption was affected by soil type and particle size. Nevertheless, addition of clay soil may be option to reduce N and P leaching in particularly sensitive ecosystems or areas with high rain fall.

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Table 1. Inorganic N (μg g^{-1}) concentration of leachate of sandy soil alone or clay amended sandy soil with and without fertilizer addition per leaching event. Clay soil was added finely ground or as 2 or 5 m peds at 10 or 20% w/w. For each fertiliser treatment, and leaching event, values followed by different letters are significantly different (n=4, $P \leq 0.05$).

Clay soil addition		Leaching events						
		1	2	3	4	5	6	7
		Without fertilizer						
	none	1.40 a	0.27 a	2.1 a	1.60 a	0.48 ab	0.31 a	0.20 b
10%	FG	0.87 b	0.05 bc	0.53 b	0.43 c	0.29 b	0.27 a	0.21 b
	2 mm	0.66 bc	0.07 bc	0.61 b	0.52 bc	0.30 b	0.22 a	0.22 b
	5 mm	1.37 a	0.09 b	0.64 b	0.90 b	0.50 ab	0.20 a	0.44 a
20%	FG	0.87 b	0.04 bc	0.70 b	0.68 bc	0.59 a	0.36 a	0.32 ab
	2 mm	0.54 c	0.03 c	0.58 b	0.69 bc	0.37 ab	0.24 a	0.22 b
	5 mm	0.71 bc	0.04 bc	0.49 b	0.55 bc	0.32 b	0.29 a	0.21 b
		With fertilizer						
	none	19.72 a	4.42 a	2.23 a	1.66 a	0.48 ab	0.35 b	0.28 c
10%	FG	4.29 bc	1.25 c	0.67 c	0.88 b	0.38 ab	0.33 b	0.26 c
	2 mm	4.13 bc	1.36 c	0.71 c	0.77 b	0.36 b	0.30 b	0.29 bc
	5 mm	7.06 b	2.54 b	1.31 b	1.17 ab	0.54 ab	0.32 b	0.34 abc
20%	FG	3.27 c	1.33 c	0.82 c	0.85 b	0.59 a	0.35 b	0.24 c
	2 mm	2.62 c	1.27 c	0.72 c	0.81 b	0.47 ab	0.42 ab	0.46 ab
	5 mm	3.81 c	1.22 c	0.73 c	1.12 ab	0.50 ab	0.58 a	0.46 a

Table 2. Inorganic P (μg g^{-1}) concentration of leachate of sandy soil alone or clay amended sandy soil with and without fertilizer addition per leaching event. Clay soil was added finely ground or as 2 or 5 m peds at 10 or 20% w/w. For each fertiliser treatment, and leaching event, values followed by different letters are significantly different (n=4, *P*≤ 0.05).

Clay soil addition		Leaching events						
		1	2	3	4	5	6	7
		Without fertilizer						
	none	0.44 a	1.82 a	0.48 a	0.12 a	0.08 a	0.18 a	0.16 a
10%	FG	0.06 cd	0.68 bc	0.05 b	0.06 ab	0.02 b	0.10 a	0.04 b
	2 mm	0.11 bc	0.54 c	0.04 b	0.04 b	0.02 b	0.05 a	0.05 b
	5 mm	0.15 b	1.02 b	0.11 b	0.04 b	0.03 b	0.12 a	0.06 b
20%	FG	0.03 d	0.63 c	0.03 b	0.05 b	0.02 b	0.06 a	0.04 b
	2 mm	0.03 d	0.66 bc	0.02 b	0.03 b	0.02 b	0.05 a	0.04 b
	5 mm	0.04 cd	0.58 c	0.20 ab	0.04 b	0.02 b	0.06 a	0.06 b
		With fertilizer						
	none	1.80 a	4.41 a	2.47 a	0.82 a	0.33 a	0.58 a	0.43 a
10%	FG	0.21 d	3.08 abc	0.12 c	0.12 b	0.06 cd	0.16 bc	0.11 b
	2 mm	0.58 c	3.61 ab	0.23 bc	0.17 b	0.10 bc	0.20 bc	0.12 b
	5 mm	1.08 b	3.86 a	0.39 b	0.21 b	0.13 b	0.25 b	0.12 b
20%	FG	0.06 d	1.80 c	0.09 c	0.06 b	0.05 d	0.13 c	0.09 b
	2 mm	0.12 d	1.87 bc	0.10 c	0.05 b	0.05 d	0.09 c	0.05 b
	5 mm	0.30 cd	2.01 bc	0.21 bc	0.08 b	0.06 cd	0.10 c	0.07 b

Table 3. Cumulative inorganic N and P leached from sandy soil alone or clay amended sandy soil with and without fertilizer addition. Clay soil was added finely ground or as 2 or 5 m peds at 10 or 20% w/w. For each fertiliser treatment values followed by different letters are significantly different ($P \leq 0.05$).

Fertilizer	Clay soil addition		Cumulative inorganic N leached μg g^{-1}	Cumulative inorganic P leached μg g^{-1}
without	10% clay	none	6.32 cde	3.29 cde
		FG	2.66 f	1.01 fg
		2 mm	2.61 f	0.85 g
		5 mm	4.14 def	1.53 efg
	20% clay	FG	3.56 ef	0.87 g
		2 mm	2.67 f	0.85 g
		5 mm	2.62 f	0.99 fg
with	10% clay	none	29.14 a	10.63 a
		FG	8.07 c	3.85 cd
		2 mm	7.93 c	5.00 bc
		5 mm	13.29 b	6.06 b
	20% clay	FG	7.45 c	2.27 defg
		2 mm	6.77 cd	2.33 defg
		5 mm	8.42 c	2.82 def

Table 4. Inorganic N and P retained in sandy soil alone or clay amended sandy soil with fertilizer addition per g soil and in percentage of N and P applied. Clay soil was added finely ground or as 2 or 5 m peds at 10 or 20% w/w. For each fertiliser treatment values followed by different letters are significantly different ($P \leq 0.05$).

	Clay soil addition		inorganic N retained µg g^{-}	% of retained N to the applied N fertilizer	inorganic P retained µg g^{-1}	% of retained P to the applied P fertilizer
Sandy soil		None	3.8 c	14	0.0 e	0
	10% clay soil	FG	21.2 ab	80	4.0 bc	59
		2 mm	21.3 ab	80	2.7 cd	40
		5 mm	17.5 b	66	2.3 d	34
	20% clay soil	FG	22.7 a	85	5.4 a	79
		2 mm	22.5 a	85	5.3 a	78
		5 mm	20.8 ab	78	5.0 ab	74

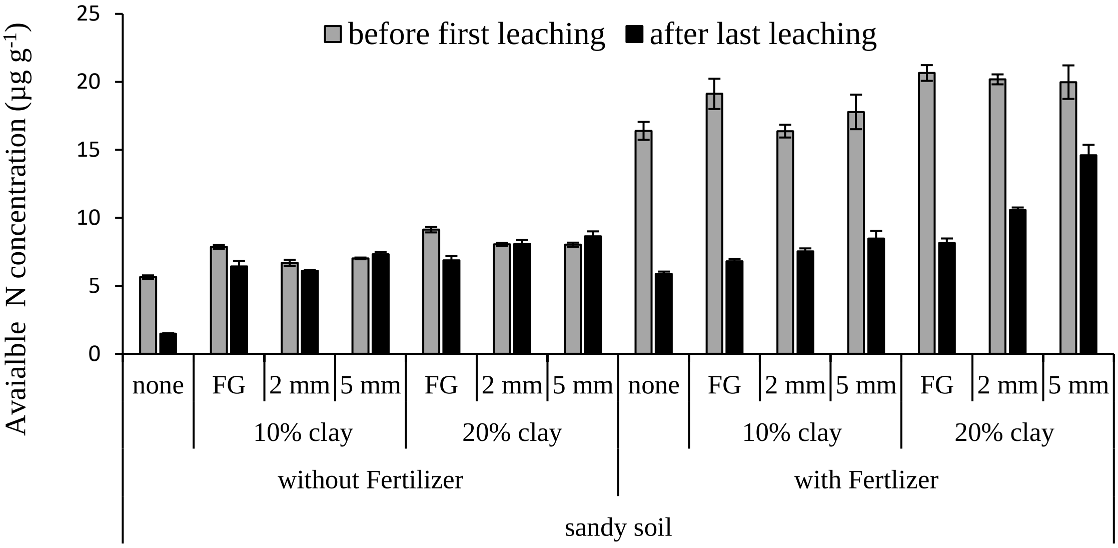

Fig 1. Soil available N concentration before the first leaching and after the last leaching in sandy soil alone or clay amended sandy soil with and without fertilizer addition. Clay soil was added as finely ground or 2 or 5 m peds at 10 or 20% w/w. (Vertical lines indicate standard error, n=4)

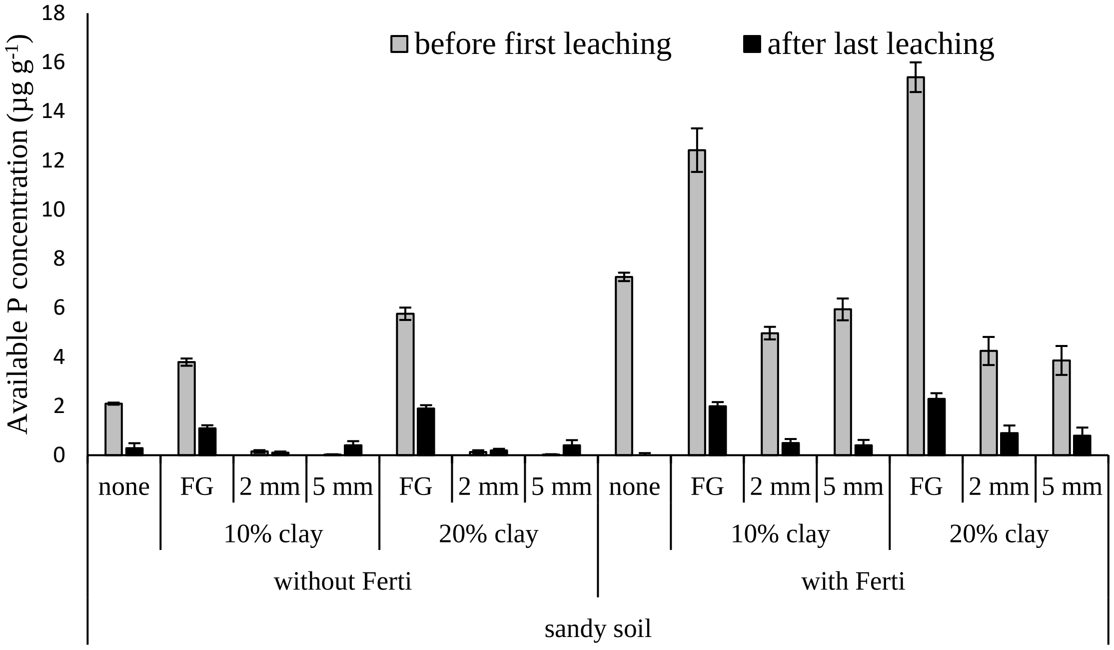

Fig. 2 Available P before the first leaching and after the last leaching in sandy soil alone or clay amended sandy soil with and without fertilizer addition. Clay soil was added as finely ground or 2 or 5 mm peds at 10 or 20% w/w (Vertical lines indicate standard error, n=4)

CHAPTER 7

CONCLUSIONS AND FUTURE RESEARCH

The productivity of sandy soils is limited by high infiltration rate, high evaporation, low nutrient and water-holding capacity, and low organic matter content. Management strategies to improve crop growth on sandy soils include addition of synthetic soil conditioners (Choudhary et al. 1998), slow-release fertilizers (Mitchelson and Franco 1994), composts (Tejada et al., 2009) and drip irrigation (Blackwell et al. 2014). While effective, most of these approaches have only short-term effects or are expensive. Addition of clay rich subsoil to sandy soil may permanently change soil properties because clay soil has predominantly small pores, high water and nutrient holding capacity. Thus, clay amended sandy soils are characterised by a heterogeneous matrix with patches of clay soil and sandy soil which influence nutrient availability and organic C binding.

Recently Roychand and Marschner (2013) and Shi and Marschner (2012) reported that clay addition to sandy soil reduces cumulative respiration when crop residues were added. Many incubation studies on the effect of clay addition to sandy soils have been carried out finely ground clay soil (Roychand and Marschner 2013; Shi and Marschner, 2012). However, in the field, the added clay soil is present in peds of varying size. Compared to finely ground clay soil, peds have a smaller specific surface area which may influence the effect of clay addition. To better understand the effect of clay soil addition to sandy soils on nutrient availability and organic C binding, incubation studies with finely ground clay soil and peds were carried out. The aims of this study were to i) determine the effect of clay soil addition rate and ped size in residue amended sandy soil on soil respiration, nutrient availability and organic C retention, and ii) assess the effect of clay soil addition rate and ped size on nutrient leaching after mineral fertilizer addition.

The incubation experiments described in Chapters 2, 3, 4 and 5 showed that clay soil addition to sandy soil influenced nutrient availability after plant residue addition, particularly when small peds were added at higher rates. Sandy soil with clay peds had greater maximum NH_4^+ and P sorption capacity than sandy soil alone, and sorption capacity was higher at 20% compared to 10% clay soil addition and greater with 1 mm than 3 mm peds. With low C/N ratio residue, compared to sandy soil alone clay soil addition increased available N concentration and initial microbial biomass C and N, but decreased cumulative respiration and P availability. In contrast, clay soil addition had negligible effect on nutrient availability with mature faba bean residue which had a C/N ratio 37. Clay type, rate and ped size (1-5 mm) had little and variable effect on respiration and nutrient availability. This indicates that within the addition rates (10 and 20% clay soil) and ped sizes, the effect of clay soil addition to sandy soil amended with residues may be difficult to predict. The lack of differences between high and low smectite clay soil (Chapter 3) suggests that with respect to binding of organic matter and nutrients, a high concentration of Fe- and Al-oxides can compensate a lower clay concentration and proportion of smectite.

Organic C retention by the fine fraction of the clay soil was assessed by measuring TOC in the < 53 µm fraction at the start and the end of the experiments. In the 45-day incubation experiments compared to the < 53 µm fraction added at the start of the experiment, total organic carbon (TOC) content of the < 53 µm fraction was up to two-fold higher, particularly in the smaller peds (1 and 2 mm). Retrieval of clay peds at the end of the experiments showed that larger peds broke down during the experiment whereas with smaller peds, aggregation of peds dominated (Chapter 2 and 4). The TOC content was greatest in the ped size added initially, likely because they had a longer time of contact with the added residues than peds formed during the experiment.

In the eight months study with repeated addition of high C/N wheat straw (Chapter 5), organic C content of the whole soil increased over time with a greater increase in clay amended soils. The organic C content of the > 53 µm fraction was very low and changed little over time. With finely ground soil and 1 mm peds, the organic C content of the < 53 µm fraction increased mainly in the first two months. On the other hand, with 3 mm peds the organic C content did not increase in the first two months, but then increased in the following six months to reach similar concentrations as with finely ground clay soil and 1 mm peds. Thus, larger ped size delays organic C retention likely due to the smaller surface area to volume ratio.

In the experiment described in Chapter 6, sandy soil alone or with clay soil was amended with N and P fertilisers and then leached several times. Clay addition significantly reduced leaching of N and P compared to sandy soil alone. Compared to sandy soil alone, 83% more N was retained in clay-amended soil and P retention was doubled. In clay amended treatments, retention was highest with finely ground clay soil and smallest with 5 mm peds. This study showed that addition of finely ground clay soil can substantially reduce N and P leaching and thereby increase fertiliser retention compared to sandy soil alone.

It can be concluded that clay soil addition to sandy soil can reduce the risk of nutrient leaching and enhance carbon sequestration in sandy soils. This effect will be greatest with finely ground clay soil or small peds. Large peds as they occur in the field may only have longer term effects after break-down into smaller peds.

This project provided novel information about nutrient availability and retention in clay-amended sandy soil, but also revealed knowledge gaps that could be addressed in future research.

Future Research

To better understand short and longer term fate of organic C in clay amended sandy soils, ^{13}C labelled residue could be added either once or several times. The ^{13}C in CO_2, microbial biomass and the < 53 µm fraction could be determined. If these measurements were continued over longer periods after the last residue addition, the fate of retained C could be studied.

In the field, organic C retention is difficult to assess because clay addition often increases crop productivity and thus C input into the soil. Therefore, a higher organic C content in clay amended soil may be mainly due to higher C input rather than reduced decomposition or greater retention. To assess the effect of clay addition more accurately, field experiments could be set up with different rates of residue addition. The same rates would then also be added to sandy soil alone. Organic C in the whole soil, peds and < 53 µm fraction would be determined after 2, 4, 6 and 8 months. During this period, any plants growing on the soil would have to be removed.

In this study, the two clay soils (high or low smectite content) differed little in effect on respiration and nutrient availability. This may be because the difference in properties of the two clay soils was too small or because the experiment was too short. A wider range of clay soils could be tested that differ with respect to clay mineralogy, clay content, pH and sodicity. The experiment could be similar as Experiment 4, with repeated residue addition and measurement of the TOC content of < 53 µm fraction after 2, 4 and 8 months.

Such a screening could use multivariate analysis to identify clay soil properties that have the greatest effect on TOC binding.

To assess N leaching in the field, lysimeters could be installed and ^{15}N labelled fertiliser added. Leaching could either depend on natural rainfall or by adding known amounts of water. Suction cups installed at different depths could then be used to track the movement of ^{15}N in the soil profile. The leachate could also be analysed for organic C and P to assess its eutrophication potential.

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