

Subsurface Clay Soil Application Improved Aggregate Stability, Nitrogen Availability, and Organic Carbon Preservation in Degraded Ultisols with Cover Crop Mixtures

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Coastal Plain soils of the southeastern United States are highly degraded, with low soil organic carbon (C), meager soil fertility, and poor soil structure. We tested the effectiveness of clay soil amendment to promote the formation of soil aggregates, improve organic C stability, and enhance nutrient availability in field. Subsurface clay soils (B horizon, 25% clay) were applied ($\sim 25 \text{ T ha}^{-1}$) to the top of the soil surface, followed by the planting of winter cover crop mixtures. Crop growth and nutrient uptake and relevant soil biogeochemical properties were analyzed at the end of the winter season. Additions of subsurface clay soils prompted the formation of water-stable macroaggregates (250–2000 μm) from 32 to 54% and increased mean weight diameter of the aggregates from 390 to 592 μm . Additions of subsurface clay soils increased clay content from 1.5 to 3.8% but did not change soil texture, pH, water holding capacity, and bulk density. The stock of dissolved organic nitrogen was not affected by the amendment, whereas nitrate leaching from field was reduced by 61% because of clay soil application. Clay soil additions inhibited the activities of C-cycling (N-acetyl- β -glucosaminidase, β -D-cellulobiosidase, β -glucosidase, and β -xylosidase) and N-cycling (leucine aminopeptidase) enzymes but did not modify soil microbial community level substrate utilization patterns, resulting in a 63% reduction in C turnover rate and 19% increases in concentrations of labile organic C. Our results indicated that subsurface clay soil amendment can improve soil structure, nutrient availability, and the stability of labile C in the field, at least in the short term, and may be a promising management alternative to enhance the productivity and sustainability of the degraded Coastal plain soils.

Abbreviations: AWCD, average well color development; CMD, community metabolic diversity; DI, deionized; DOC, dissolved organic carbon; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; SOC, soil organic carbon.

Soil organic carbon (SOC) is essential for soil physical, chemical, and biological functions that are shown to improve crop productivity (Wander, 2004). In the agroecosystem, SOC content maintains soil structure, ensures soil fertility, preserves soil biodiversity, and assures consistent crop production. Increasing SOC in crop lands is therefore widely considered an important and necessary strategy to improve long-term soil productivity and sustainability (Kibblewhite et al., 2008; Lal, 2006).

The stocks of SOC are the balance between inputs from net primary production and outputs, including heterotrophic microbial respiration (i.e., mineralization) (Rousk et al., 2015). However, soils naturally differ in their capacity to stabilize and regulate SOC, largely due to their different intrinsic properties, such as clay content and mineralogy (Hassink, 1997; Six et al., 2002a). It is generally accepted that soil clay plus silt fraction determine the capacity of soils to stabilize SOC and that soil aggregates provide additional variable capacity through additive physical protection that can be modified by soil management (Carter, 2002;

Core Ideas

- Subsurface clay soil amendment improved soil aggregation and its stability.
- Microbial activities were suppressed by clay soil applications.
- Clay soil amendment enhanced labile organic C pools.
- Additions of clay soil promoted ammonium fixation and reduced nitrate leaching.

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Hassink, 1997; Six et al., 2004). Clay minerals stabilize and protect SOC mainly through organo–mineral interactions (Baldock and Skjemstad, 2000). A positive correlation between soil clay and silt content and SOC is frequently reported (Causarano et al., 2008; Six et al., 2002b; Souza et al., 2017). In addition, clay and other primary particles are the basic building blocks for soil aggregates (Six et al., 2004; Tisdall and Oades, 1982), which physically protect SOC by entrapment, making it inaccessible to soil microorganisms and their degradative enzymes (Dungait et al., 2012; Schmidt et al., 2011). It is therefore not surprising that clay and silt content is widely considered the “*priori*” limit for soil carbon (C) sequestration potentials (Feng et al., 2014; Stockmann et al., 2013; Van De Vreken et al., 2016). It is apparent that clay and silt content may influence soil structure, soil fertility, and agricultural production, at least through their effects on the formation of aggregates and the stability of SOC (Bronick and Lal, 2005; Dungait et al., 2012; Feng et al., 2014; Regelink et al., 2015).

Historical intensive cultivation and management of the Coastal Plain soils resulted in losses of SOC and degradation of soil health (Causarano et al., 2006; Novak and Busscher, 2013). To counter these effects, conservation management practices are essential and widely considered promising to restore SOC and improve soil health and its productivity. However, current management practices, including reduced tillage, soil cover (e.g., residue incorporation and cover cropping), and organic amendments, have had limited success due to the high temperatures and humid conditions that favor high microbial activity, leading to increased decomposition of SOC (Busscher et al., 2009; Franzluebbers, 2010; Hubbard et al., 2013; Nash et al., 2018; Novak et al., 2007; Stewart et al., 2012). Moreover, the degraded Coastal Plain soils often have low clay contents (as low as 1%) in the plow layer, resulting from extensive weathering of clay minerals and clay eluviation, which limits the soils’ capacity to preserve SOC and compromises intended management outcomes. For instance, a recent study of 48 farmers’ fields in the Virginia Coastal Plain revealed that some of the soils (containing $<15 \text{ g kg}^{-1}$ SOC) are near or exceeding their silt plus clay protective capacity after implementing no-till combined with organic amendments (Stewart et al., 2012). Similar results were found for other Coastal Plain soils under long-term conservation management practices, in which SOC accumulation was only observed at depths of 0 to 5 cm (mostly having $<15 \text{ g kg}^{-1}$ SOC) and is expected to reach the C saturation point in a decade or two (Franzluebbers, 2010; Nash et al., 2018; Novak et al., 2007). Despite these findings, increasing organic inputs combined with reduced soil disturbance is considered the best management option for the Coastal Plain soils, largely overlooking the existing “*barrier*” (i.e., low clay content in surface soils) against C preservation. New strategies to promote the stabilization of SOC are needed for long-term soil sustainability and crop productivity in the degraded Coastal Plain soils.

The goal of the present study was to improve soil health and agroecosystem services through purposeful additions of clay soil to degraded Coastal Plain soils to support sustainable agricultur-

al production practices. More specifically, the objectives were to explore the proof-of-concept of subsurface clay soil amendment, in combination with minimal tillage and winter cover crop as core management principles, to improve soil structure, nutrient availability, and SOC stability. It was hypothesized that clay soil applications increase soil aggregations (Hypothesis 1), amendments of clay soils reduce soil C turnover (i.e., microbial respiration) by providing extra physical protection reducing the accessibility of labile C pools to degradative enzymes (Hypothesis 2), and additions of clay soils improve nitrogen (N) availability by reducing N leaching (Hypothesis 3).

MATERIALS AND METHODS

Experimental Setup

The experiment was conducted in the Pee Dee Research & Education Center of Clemson University, Florence, SC ($34^{\circ}18' \text{ N}$, $79^{\circ}44' \text{ W}$). The average annual precipitation was 1186 mm, and the average annual high and low air temperatures were 23.8°C and 11.1°C , respectively (US Climate Data, 2018). The field was used for cotton production in 2017, after which it was used for the present study. Soils are Ultisols (loamy, siliceous, semiactive, thermic Grossarenic Paleudults) according to the USDA classification. In October 2017, subsurface clay soils (B horizon) were excavated from an adjacent cotton field ($<0.3 \text{ km}$) and randomly and evenly spread onto four 6 m by 4 m filed plots ($\sim 25 \text{ T ha}^{-1}$), followed by mixing the top soils (0–5 cm) with a cultivator. The applied B horizon soils had a pH of 5.3 ± 0.1 , $25 \pm 0.6\%$ clay and $74 \pm 0.5\%$ sands, $0.9 \pm 0.1 \text{ mg N kg}^{-1}$ inorganic N, $0.07 \pm 0.03 \text{ g kg}^{-1}$ total N, and $0.66 \pm 0.00\%$ g kg^{-1} total C. Four control plots were set up in parallel without the clay soil amendment but with the top soils (0–5 cm) mixed with the cultivator. Three days after the additions of subsurface clay soils, cover crop mixtures, including cereal rye (67 kg ha^{-1}), winter pea (39 kg ha^{-1}), and crimson clover (17 kg ha^{-1}), were drill-seeded at 2 cm depth to all eight plots. No other field managements were applied during the cover crop season (October 2017–April 2018).

Soil Sampling and Physio-Chemical Analyses

Concurrently with plant tissue sampling of the cover crops, soils were collected with a soil core sampler (5 cm in diameter; AMS, American Falls, ID) at 15 cm for the quantification of soil bulk density. Six additional cores (2.5 cm in diameter and 15 cm in depth) were collected and composited from each plot. The soils were sieved (2 mm) in the laboratory after the removal of plant materials and stored at 4°C until use. Moisture content was determined as mass loss after drying at 60°C for 3 d. Soil pH was measured with an Orion 8107 pH probe (Thermo Fisher Scientific, Waltham, MA) in deionized (DI) water (1:3 ratio) after gently mixing soil samples with DI water (1:3 ratio) and equivalent for 30 min. Soil particle-size distribution was estimated with the micro-pipette method (Miller and Miller, 1987). Soil water holding capacity was determined with oven-dried soils (Zhu et al., 2013).

The percentages of macroaggregates (250–2000 mm) and microaggregates (53–250 mm) in bulk soils and their stability was analyzed with wet sieving (Márquez et al., 2004; Six et al., 2000). In brief, 20-g sieved soil samples were placed on a stack of two sieves with decreasing sizes (250 and 53 mm), submerged in DI water for 2 min, and separated by moving the sieves (by hand) up and down 3 cm for 50 strokes in 2 min. All aggregate fractions were collected from respective sieves, dried at 60°C for 3 d, and weighed. Sand contents (>53 mm) of each aggregate fraction were determined by dispersing the dried aggregates with 0.5% sodium hexametaphosphate and used to correct aggregate size distribution and stability (Six et al., 2000). Mean weight diameter (MWD) was calculated as described by Márquez et al. (2004).

Total C (TC) and N (TN) were determined using oven-dried and ground soils with a Carlo-Erba NA 1500 CNS analyzer (Haak-Buchler Instruments, Saddlebrook, NJ). Extractable N was quantified by colorimetric method after extracting the sieved field soils with 1 M KCl for 1 h, followed by centrifugation at 3000 rpm for 15 min and filtration. The filtrates were analyzed for NH_4^+ (Verdouw et al., 1978) and NO_3^- (Doane and Horwath, 2003). Mehlich-3 extractable phosphorus (P) was determined with the molybdate blue–ascorbic acid method after the extraction (Murphy and Riley, 1962). The stock of measured nutrients at 0 to 15 cm depth (kg ha^{-1}) was then calculated with soil bulk density.

Nitrate Leaching Potential

Leaching potential was quantified with Purolite A520E nitrate (NO_3^-) selective resins (Purolite, Bala Cynwyd, PA). Approximately 100 mL of the resins were enclosed in an organza fabric bag and closed with nonmetal staples to a final dimension of 12.7 by 15.2 cm. Total exchange capacity of the A520E is $0.9 \text{ mol}_c \text{ L}^{-1}$, equivalent to $12.6 \text{ g NO}_3^- \text{ N L}^{-1}$. Considering the aerial projection of the resin bags, it in theory could adsorb $651 \text{ kg NO}_3^- \text{ N ha}^{-1}$. The bags (two bags per plot) were randomly buried in the plots at 15 cm depth on the next day of the planting and withdrawn from soils right before the termination of cover crops. In the laboratory, the bags were extracted twice with 400 mL of 3 M KCl. In a pilot testing, 95% of NO_3^- was recovered when extracted twice with 3 M KCl (tested at 25, 50, 75, and 100% of the total capacity; data not shown). Extracted NO_3^- was measured colorimetrically (Doane and Horwath, 2003). The leaching potential was estimated by scaling up the mass of $\text{NO}_3^- \text{ N}$ in the resin bag, with its area and expressed as $\text{kg NO}_3^- \text{ N ha}^{-1}$.

Microbial Analyses

Microbial biomass C (MBC) and microbial biomass N (MBN) were estimated using the fumigation–extraction method. The amount of K_2SO_4 -extracted C (i.e., dissolved organic C [DOC]) was determined with a total organic C analyzer (TOC-5050A; Shimadzu, Norcross, GA). The MBC was calculated as the difference in DOC between the fumigated and unfumigated samples using a conversion factor of 0.37. After digestion (Cabrera and Beare, 1993), the K_2SO_4 extracts were measured for $\text{NO}_3^- \text{ N}$ as described by Doane and Horwath

(2003). The MBN was calculated as the difference in $\text{NO}_3^- \text{ N}$ between fumigated and unfumigated samples using a conversion factor 0.54. Dissolved organic N was calculated as the difference between digested and undigested unfumigated samples.

Mineralization potential of N was quantified with anaerobic incubation of soil samples at 30°C for 7 d, followed by 1 M KCl extraction and colorimetric analysis of NH_4^+ in the extract (Cadisch et al., 1996). Microbial respiration was estimated by incubating 10-g field soil samples in a closed Mason jar (1 L) in the dark at room temperature ($20 \pm 1^\circ\text{C}$) for 7 d. The headspace CO_2 concentration was analyzed at the end of the incubation with a gas chromatograph (Shimadzu, Columbia, MD). Respiration rates were expressed as a function of soil TC over time ($\text{mg CO}_2\text{-C g}^{-1} \text{ soil C d}^{-1}$).

Extracellular enzyme activities associated with C (β -D-cellobiosidase, β -glucosidase, N-acetyl- β -glucosaminidase, β -xylosidase), N (leucine aminopeptidase), and P (phosphatase) were measured using fluorescence. Soil samples (5 g, dry equivalent) were mixed with 30 mL DI water and shaken for 20 min, followed by dilution five times for the assays. In the 96-well microplates, approximately 200- μL samples were incubated with 50 μL of respective substrates at room temperature ($20 \pm 1^\circ\text{C}$) for 24 h, which was conducted in triplicate with controls to assess non-enzymatic production. Enzymatic activity was determined by calculating the mean fluorescence reading change over time with a standard curve and expressed as milligrams of product released per gram soil per hour ($\text{mg}^{-1} \text{ g}^{-1} \text{ h}^{-1}$) (Ye and Wright, 2010).

Community-level physiological profiles were determined with Eco-Plates (BIOLOG Inc., Hayward, CA) (Ye and Wright, 2010). In brief, 5 g moist soil was mixed with 20 mL of DI water and gently shaken for 20 min. The homogenized samples were then diluted 400 times, and soil particles were allowed to settle for 10 min at 4°C. The supernatant (100 μL) of the was dispensed into each well of the Eco-Plates and incubated at room temperature ($20 \pm 1^\circ\text{C}$) for 7 d. Optical densities were measured every 6 or 12 h using a microplate reader (SpectraMax M2; Molecular Devices, San Jose, CA) at 590 nm. Average well color development (AWCD) and community metabolic diversity (CMD) were determined as described by Garland (1996).

Crop Growth and Uptake

Prior to the termination of cover crop mixtures, biomass was estimated by collecting all aboveground plant materials within an area of 1 m by 1 m. The estimation was triplicated in each plot, and the samples were dried at 60°C until a constant weight was reached. Dried samples were ground and analyzed for nutrient contents at the Agricultural Service Laboratory of Clemson University. Nutrient uptake and C assimilation were calculated by multiplying the aboveground biomass (kg ha^{-1}) by the measured C and nutrient contents (%).

Data Analysis

One-way ANOVA was used to determine the effect of subsurface clay soil amendment on measured variables at $\alpha = 0.05$. Pairwise correlation analysis was conducted to reveal cor-

Table 1. Selected physiochemical properties of soils (0–15 cm) with (Treatment) and without (Control) subsurface clay soil amendment. Values are means and standard errors ($n = 4$).

	Silt	Clay	BD†	WHC‡	pH	Macroaggregate	Microaggregate	MWDS
	%		g cm ⁻³	%		%		mm
Treatment	7.1 ± 0.6	3.8 ± 0.5a¶	1.4 ± 0.0	26.5 ± 1.8	5.9 ± 0.1	50.1 ± 3.8a	12.9 ± 1.5	592.4a
Control	7.9 ± 0.6	1.5 ± 0.2b	1.4 ± 0.0	25.6 ± 2.0	6.2 ± 0.0	32.2 ± 5.6b	8.8 ± 1.1	390.4b

† Bulk density.

‡ Water holding capacity.

§ Mean weight diameter.

¶ Lowercase letters indicate significant difference at $p = 0.05$.

relations between different variables. All statistical analyses were performed with JMP pro 14 (SAS Institute, Cary, NC).

RESULTS

Soil Physical Properties

The soils had 1.5% of clay and 7.9% of silt by weight at a depth of 0 to 15 cm (Table 1). Amendments of subsurface clay soils increased the clay content to 3.8% but did not change the silt content and soil texture. Soil bulk density and water holding capacity were not affected by the additions of clay soils (Table 1). In contrast, the percentage of water-stable macroaggregates (250–2000 mm) in bulk soils increased from 32.2 to 50.1%, whereas that of microaggregates (53–250 mm) remained unchanged. Additions of subsurface clay soils increased MWD of the water-stable aggregates by 52% (Table 1).

Soil Chemical Variables

Soil pH was not changed by clay soil amendment (Table 1). Inorganic N (NH_4^+ -N plus NO_3^- -N) in soils was dominated by NH_4^+ , and its concentration was higher in the treated soils than in the control (Table 2). The dissolved organic N stock was not affected by clay soil amendment (range, 5.8–7.4 kg N ha⁻¹), whereas leaching of NO_3^- from the field was reduced by 61% because of clay soil application. However, no significant effects were found for the extractable P, DOC, TC, and TN (Table 2).

Microbial Responses

The additions of subsurface clay soils did not change MBC, MBN, or their ratio (Table 3). Similarly, no effect was observed in N mineralization potentials. Respiratory production of CO_2 was reduced from 1.80 to 0.66 mg CO_2 -C g⁻¹ soil C d⁻¹ by the application of clay soils (Table 3). Both AWCD and CMD followed a sigmoidal curve over time, but neither appeared to be significantly affected by clay soil amendment (Fig. 1). Except for phosphatase, all the measured enzymatic activities were inhibited by the additions of subsurface clay soils, especially for leucine aminopeptidase,

which showed 341% reduction (Fig. 2). Correlation analysis further revealed that the activities of β -D-cellulobiosidase, N-acetyl- β -glucosaminidase, β -xylosidase, and leucine aminopeptidase were negatively correlated with the percentages of macroaggregates, total aggregates, and MWD, whereas β -glucosidase activities and microbial respiration were negatively correlated with soil clay content (Table 4). No significant correlations were found between the measured enzymatic activities and the measured physiochemical properties (data not shown), except that leucine aminopeptidase was correlated to DOC ($r = -0.77$, $p = 0.02$).

Crop Growth and Nutrient Uptake

Aboveground biomass production (dry weight) was 3537 and 2681 kg ha⁻¹ in the fields with and without subsurface clay soil amendment, respectively; the difference was not significant (Table 5). The contents of C, N, P, and K in plant tissues were not affected by clay soil additions, with mean values of 43.7, 1.1, 0.2, and 1.0%, respectively. Similarly, the assimilation (kg ha⁻¹) of C, N, P, and K in biomass was not different between the treated and control soils (Table 5).

DISCUSSION

We tested in the field the ability of subsurface clay soil amendment to enhance and augment the innate capacities of Coastal Plain soils to improve soil structure and C and N retention. Results suggested that such amendment increased the degree of soil aggregation and the stability of soil aggregates (Table 1) (supporting Hypothesis 1), reduced labile organic C turnover by providing more physical protection (Fig. 3) (supporting Hypothesis 2), and enhanced N availability by reducing NO_3^- leaching while encouraging NH_4^+ fixation (Table 2) (supporting Hypothesis 3).

Soil Structure

Soil structure is an essential factor that regulates various soil functions (e.g., water and nutrient movement and retention) and is commonly measured as the arrangement and stability of soil ag-

Table 2. Stocks of inorganic and dissolved organic nitrogen (DON), Mehlich-3 extractable phosphorus, dissolved organic carbon (DOC), total nitrogen (TN), and total carbon (TC) in soils (0–15 cm) with (Treatment) and without (Control) subsurface clay soil amendment. Values are means and standard errors ($n = 4$).

	Leachable NO_3^- -N	NH_4^+ -N	NO_3^- -N	DON	P	DOC	TN	TC
	kg ha ⁻¹							
Treatment	2.1 ± 1.0b†	3.4 ± 0.8a	0.2 ± 0.0	7.4 ± 1.2	17.3 ± 0.8	57.9 ± 4.1	0.8 ± 0.1	9.6 ± 0.7
Control	5.4 ± 0.5a	1.3 ± 0.3b	0.3 ± 0.1	5.8 ± 0.9	18.7 ± 0.8	49.0 ± 2.9	0.8 ± 0.0	9.2 ± 0.4

† Lowercase letters indicate significant difference at $p = 0.05$.

Table 3. Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN), MBC to MBN ratio (C/N), and organic nitrogen (N_{min}) and carbon (respiration) mineralization potentials in soils (0–15 cm) with (Treatment) and without (Control) subsurface clay soils additions. Values are means and standard errors ($n = 4$).

	MBC	MBN	C/N ratio	N_{min}	Respiration
	mg kg ⁻¹			mg N kg ⁻¹ d ⁻¹	mg CO ₂ -C g ⁻¹ soil C d ⁻¹
Treatment	383 ± 27	14.2 ± 2.2	29 ± 4	0.63 ± 0.22	0.66 ± 0.13b†
Control	319 ± 21	17.4 ± 0.3	18 ± 1	0.74 ± 0.05	1.80 ± 0.36a

† Lowercase letters in indicate significant difference at $p = 0.05$.

gregates. Favorable soil structure and a high percentage of stable aggregates are therefore important to maintain or improve soil fertility and agronomic productivity (Bronick and Lal, 2005; Mueller et al., 2010). In the present study, the degree of aggregation and its stability of the tested soil were substantially lower than the soils of other studies that were under similar management practices (i.e., cover cropping and reduced tillage) (Blanco-Canqui et al., 2011; Liu et al., 2005; Martins et al., 2009). The lower MWD and aggregation was likely due to the inherently lower clay content of the tested soils (1.5%; Table 1), which was further supported by the substantial increases of aggregations and MWD after the application of subsurface clay soils (Table 1). These increases were mostly attributable to the formation of macroaggregates, with a marginal effect from microaggregates ($p = 0.07$) (Table 1). According to the aggregate hierarchy theory, soil aggregates are formed in ordered stages with different binding mechanisms (Tisdall and Oades, 1982). Microaggregates are from progressive bindings of free primary particles (i.e., clay and silt) with persistent binding agents (e.g., polyvalent metal cations), whereas macroaggregates are the bindings of microaggregates with temporary or transient binding agents (e.g., microbial- and plant-derived organics) (Six et al., 2004). It is therefore plausible that additions of subsurface clay soils promoted soil aggregations in the present study by providing more building blocks (i.e., clay minerals and silt). Our results demonstrated that clay soil amendment improved soil structures by increasing the degree of aggregation and the stability of aggregates.

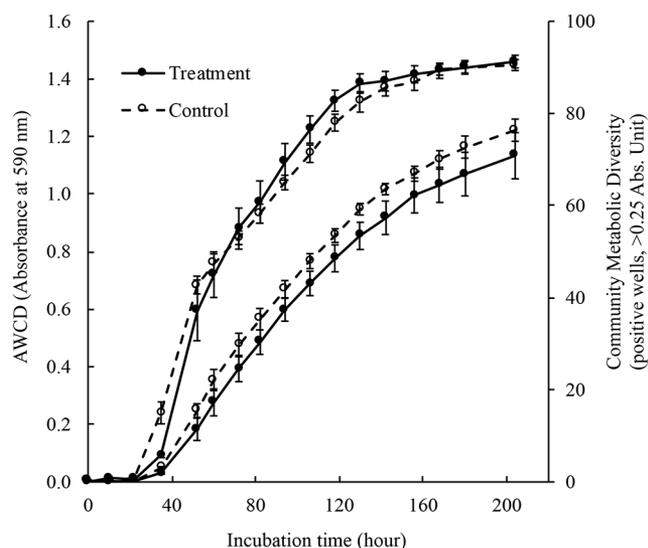


Fig. 1. Microbial community metabolic diversity (top) and average well color development (bottom) of soils with (Treatment) and without (Control) subsurface clay soil addition. Bars indicate mean ± 1 SE ($n = 4$). AWCD, average well color development.

Microbial Activity and Organic C Turnover

The stabilization of SOC depends largely on its spatial accessibility to microorganisms and their degradative enzymes, which is influenced by its interaction with mineral surfaces, metal bridges, and entrapment into soil aggregates (Dungait et al., 2012; Kleber, 2010). It was assumed that the application of subsurface clay soil would provide extra physical protection (i.e., less accessibility) for SOC, leading to its preservation (i.e., low turnover rate). Indeed, concurrently with higher clay content and a greater degree of soil aggregation and aggregate stability (Table 1), higher concentrations of labile organic C (i.e., extractable DOC plus MBC) were found in soils with clay soil additions (Fig. 3). Both DOC and MBC are important components of soil C and play critical roles in various soil biogeochemical processes (e.g., C decomposition and preservation and plant nutrient cycling) (Bolan et al., 2011; Li et al., 2018).

A range of factors is known to affect the retentions of organic C in soils, mainly through their effects on soil microbes (Li et al., 2018; Schmidt et al., 2011). It is often believed that microbial community structure, rather than function, is more sensitive to agricultural managements (Bossio et al., 2005; Ye and Wright, 2010; Zhang et al., 2006), which, however, was not supported by our results. In the present study, clay soil application did not change soil microbial population size (Table 3) or modify the community structure and composition of active microbes, supported by similar AWCD and CMD between the treated and control soils (Fig. 1) (Garland, 1996). In contrast, suppressive enzymatic activities and

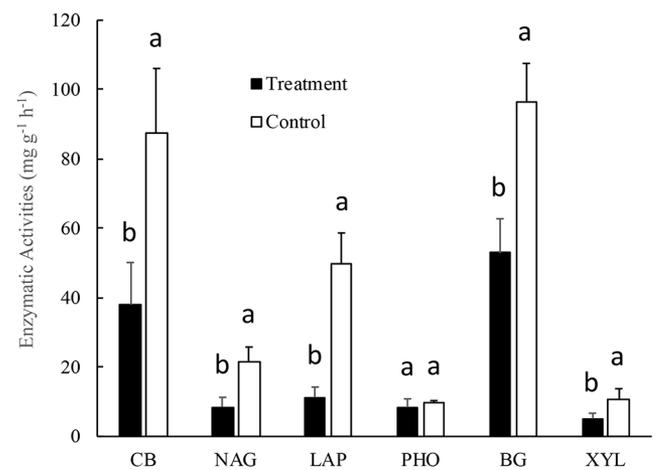


Fig. 2. Extracellular enzyme activities in soils with (Treatment) and without (Control) the amendments of subsurface clay soils. Bars indicate mean ± 1 SE ($n = 4$). BG, β -glucosidase; CB, β -D-cellulobiosidase; LAP, leucine aminopeptidase; NAG, N-acetyl- β -glucosaminidase; PHO, phosphatase; XYL, b-xylosidase. Different letters indicate significant difference ($p < 0.05$).

Table 4. Correlations between measured microbial activities and soil aggregate distribution and wet stability. Values are coefficients for significant correlation at $\alpha = 0.05$.

	Clay content	Microaggregate	Macroaggregate	Total aggregates	MWD†	Respiration
β -Glucosidase	-0.72	NS‡	NS	NS	NS	0.92
β -D-cellubiosidase	NS	-0.75	-0.72	-0.79	-0.72	0.82
N-acetyl- β -glucosaminidase	NS	NS	-0.85	-0.88	-0.85	NS
β -Xylosidase	NS	NS	-0.88	-0.90	-0.88	NS
Leucine aminopeptidase	NS	NS	-0.72	-0.73	-0.73	NS
Phosphatase	NS	NS	NS	NS	NS	NS
Respiration	-0.86	NS	NS	NS	NS	
Microaggregate	0.81		NS	NS	NS	NS
Macroaggregate	NS	NS		0.98	0.97	NS

† Mean weight diameter.

‡ Not significant at $\alpha = 0.05$.

respiratory production were found in soils with clay amendment (Fig. 2; Table 3). Enzymes are released extracellularly by microorganisms and are the main drivers of C and nutrient cycling in various environments (Arnosti et al., 2014). In soils, they are present either in solution or bound to clay and/or soil aggregates (Naidja et al., 2000). The formation of enzyme–clay complex limits the transportation and distribution of enzymes and reduces their reactivities (Naidja et al., 2000; Tietjen and Wetzel, 2003). It is therefore not surprising that the measured reduced enzyme activities in C and N cycles were negatively correlated to either clay content or the degree and stability of soil aggregation (Table 4). Moreover, the decreased activities of the four C-cycling enzymes (Fig. 2) caused lower C turnover rates (i.e., lower respiratory production) in the clay soil–amended soils (Table 3), promoting the preservation of labile organic pools in these soils (Fig. 3).

Nitrogen Availability

Nitrogen is one of the main factors that limit crop production. In the present study, no fertilizers were applied for cover crops, and mineralization was seemingly the main source of N. The rates of N mineralization potentials in the tested soils were low, and no clay soil amendment effects were observed (Table 3), which may explain the observed neutral impacts on cover crop growth and performance (Table 5). However, we did find higher $\text{NH}_4\text{-N}$ concentration in soils with clay soil additions (Table 2). Similar results have been reported (Dempster et al., 2012). Apparently, the additions of clay soils provided more ion retention capacities and hence promoted NH_4^+ fixation (i.e., the adsorption or absorption of NH_4^+ by soil minerals) (Nieder et al., 2011). However, it has been suggested that the fixation is temporary and that the fixed NH_4^+ would be released slowly

Table 5. Aboveground biomass production; biomass carbon to nitrogen ratio; and biomass carbon, nitrogen, phosphorus, and potassium in fields with (Treatment) and without (Control) subsurface clay soil amendments. Values are means and standard errors ($n = 4$).

	Biomass	C/N ratio	C	N	P	K
	kg ha ⁻¹		kg ha ⁻¹			
Treatment	3537 ± 708	45 ± 3	1546 ± 302	34 ± 5	7 ± 1	137 ± 7
Control	2681 ± 354	38 ± 6	1167 ± 148	34 ± 7	6 ± 1	127 ± 3

and would be completely available for plants (Buresh et al., 2008; Nieder et al., 2011), playing an active role in N dynamics during crop growth season (Hall et al., 2010; Nieder et al., 2011). Moreover, the fixation of NH_4^+ may protect it from losses via nitrification (NO_3^- , N_2O) or volatilization processes (NH_3). Indeed, during the entire season, total $\text{NO}_3\text{-N}$ leaching was reduced by 61% as a result of clay soil addition (Table 2).

Implications

Soil organic C in the Coastal Plain soils of the southeastern United States is inherently low mainly due to the warm and wet climate, which enhances microbial decomposition (Franzluebbers, 2010; Novak and Busscher, 2013). Extensive clay mineral weathering and clay eluviation further limit the soils' capacity to stabilize organic C, which cannot be fully addressed by current management practices of combining high residue return and conservation tillage (Nash et al., 2018; Novak et al., 2007). Innovative management strategies are therefore required to stabilize SOC and improve soil fertility and productivity. In the present study, we demonstrated that applications of in situ subsurface clay soils improved soil structure (Table 1), nutrient availability (Table 2), and

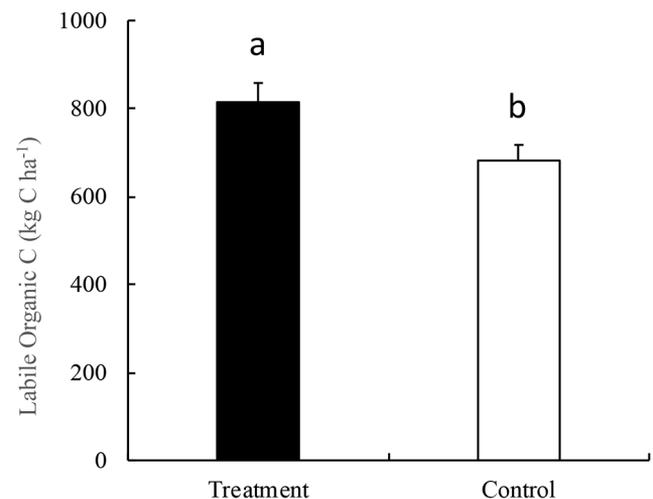


Fig. 3. Carbon retention in microbial biomass and dissolved pools in soils with (Treatment) and without (Control) the amendments of subsurface clay soils. Bars indicate mean ± 1 SE ($n = 4$). Different letters indicate significant difference ($p < 0.05$).

the preservation of labile organic C (Fig. 3) in one cover crop season, indicating its effectiveness in enhancing soil productivity and health in these degraded Ultisols across the southeastern United States, where a quarter of US agricultural products is currently produced (Ruth et al., 2007). The Coastal Plain soils (mostly Ultisols) typically have a clay-enriched B horizon (USDA–NRCS, 1998). Modifying surface soils with the subsurface clay soils, either by amendment or deep plow, to increase clay content is therefore reasonable and applicable but has been barely been explored despite the well-documented importance of clay minerals. The present study explored and demonstrated the proof-of-concept of clay soil amendment to support and augment the sustainable management strategies currently used. More research (e.g., application rates and method and cost-effectiveness) is needed to investigate its long-term effects on soil physical, chemical, and biological processes.

CONCLUSIONS

Reversing the loss of SOC is important to improve long-term soil health, productivity, and sustainability. We demonstrated in field that applications of in situ subsurface clay soils increased soil clay contents, promoting the formation of macroaggregates. Such application did not affect active microbial community structure and composition but suppressed enzymatic activities, resulting in low C turnover rates and the preservation of labile organic C. Clay soil additions increased the fixation of NH_4^+ and reduced NO_3^- leaching substantially, leading to higher N availability. Our results suggested that subsurface clay soil amendment may be an effective soil management strategy to improve soil structure, soil fertility, and SOC content in the degraded sandy Coastal Plain soils of the southeastern United States. More research on its long-term effects on soil processes and crop productivity is needed.

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