Received: 2 February 2024 | Accepted: 7 June 2024 | Published online: 16 July 2024

DOI: 10.1002/saj2.20731

Soil Science Society of America Journal

ORIGINAL ARTICLE

Fundamental Soil Science

Clay soil amendment suppressed microbial enzymatic activities while increasing nitrogen availability in sandy soils

Pratima Poudel[1,2] | **Rongzhong Ye**[1,2] | **Binaya Parajuli**[1,2]

[1]Plant and Environmental Sciences, Clemson University, Clemson, South Carolina, USA

[2]Pee Dee Research and Education Center, Clemson University, Florence, South Carolina, USA

Correspondence
Rongzhong Ye, Plant and Environmental Sciences, Clemson University, Clemson, SC 29634, USA.
Email: rongzho@clemson.edu.

Assigned to Associate Editor Priyanka Kushwaha.

Abstract

Conservation management practices often produced positive but limited desirable outcomes in US Southeast sandy soils, likely due to their intrinsically low clay contents that constrain the soil's capacity to preserve organic carbon (C) and nutrients. In the field, we tested the effectiveness of a novel approach, that is, clay soil amendment, to improve sandy soils. In October 2017, clay-rich soils (25% clay) were spread at 25 metric tons ha^{-1} and tilled onto a sandy soil (1.9% clay) in the field, which was further mixed by light tillage at 0- to 15-cm depth, followed by planting winter cover crop mixtures (cereal rye, crimson clover, and winter pea). The crop rotation was cotton and corn with cover crop mixtures planted in the winter fallow season. Soils (0–15 cm) were collected in August 2021 and subjected to physio-biochemical analyses. Clay amendment increased soil clay content to 3.4%, which improved nitrogen (N) availability by 51% but inhibited the activities of C (β-D-cellubiosidase [CB]; β-xylosidase [BX]; *N*-acetyl-β-glucosaminidase [NAG]) and N (leucine aminopeptidase [LAP]) cycling enzymes, resulting in up to 78% reduction in microbial respiration. A follow-up kinetic study on BG and LAP enzymes suggested that clay addition can have different impacts on enzymes with diverse biological origins through distinct mechanisms. Clay addition can potentially improve sandy soils by stabilizing the organic inputs in soils. However, more research is required to understand its long-term impacts making this approach practical.

1 | INTRODUCTION

Sandy soils are often characterized by low cation exchange capacity, poor nutrient and water holding capacity, meager fertility, and low soil organic carbon (SOC) content (Huang & Hartemink, 2020; Yost & Hartemink, 2019). Increasing SOC is widely recommended to enhance the chemical, physical, and biological properties of the managed sandy soils (Brust, 2019; Johnston et al., 2009). However, the intrinsically low clay contents of the sandy soils can limit their capacities to preserve and stabilize the organic inputs (Stewart et al., 2012), which has already been demonstrated in many studies including long-term experiments (Franzluebbers, 2020; Nash et al., 2018; Novak et al., 2007). Despite these findings, the current management approach to improving sandy soils largely overlooks this existing "barrier" (i.e., low clay content in surface soils) to improving soil C retention. New strategies to

Abbreviations: BX, β-xylosidase; CB, β-D-cellubiosidase; DI, deionized; EC, electrical conductivity; EE, extracellular enzymes; LAP, leucine aminopeptidase; MBC, microbial biomass carbon; NAG, *N*-acetyl-β-glucosaminidase; PMN, potential mineralizable N; SOC, soil organic carbon; TC, total carbon; TN, total nitrogen.

promote the stabilization of the introduced C in sandy soils are needed and are crucial for long-term soil sustainability and crop productivity.

Soil clay and silt contents are widely considered the "priori" limit for soil C sequestration (Feng et al., 2014; Stockmann et al., 2013; Van De Vreken et al., 2016). It is believed that clay minerals can stabilize and protect SOC through organo-mineral interactions (Baldock & Skjemstad, 2000). In addition, soil aggregates formed by clay and other primary particles can physically protect SOC by entrapment making it inaccessible to soil microorganisms and their degradative enzymes (Dungait et al., 2012; Schmidt et al., 2011). Ameliorating sandy soils with clay-rich soils has been demonstrated in Australia to increase nutrient retention (Tahir & Marschner, 2016), SOC concentration (Schapel et al., 2018), microbial biomass C (Riaz & Marschner, 2020), and crop yield (Hall et al., 2010). It is therefore not surprising that clay addition has been proposed as a new management strategy to improve sandy soils (Button et al., 2022; Tahir & Marschner, 2016; Ye et al., 2019). However, the evidence is still limited, and the practicality and long-term impacts of clay addition on soil physio-biogeochemical properties remain unknown (Button et al., 2022).

Coastal Plain soils of the southeastern United States have high sand contents throughout the soil profile with a clay content as low as 1% in the plow layer, largely resulting from extensive weathering of clay minerals and clay eluviation (Markewich et al., 1990). A recent study revealed that additions of clay-rich subsoils to sandy Coastal Plain soils at 0- to 15-cm depth improved soil aggregations, suppressed C-cycling enzyme activities, and promoted the preservation of labile organic C 1 year after the addition (Ye et al., 2019). A similar study also demonstrated the reduction of microbial respiration and increased C stability after the addition of clay-rich subsoil (Nguyen & Marschner, 2014). It is clear that clay addition has the potential to influence SOC dynamics via its impact on soil structure and microbial activities. However, the long-term responses remain elusive as a microbial adaptation to clay addition may occur (Olagoke et al., 2019), especially under continuous organic inputs by crop residue incorporation.

Direct organic inputs are known to either stimulate or suppress the activities of extracellular enzymes (EEs), influencing the transformation, decomposition, and stabilization of the SOC (Chen et al., 2014; Mason-Jones et al., 2018; Shahbaz et al., 2017). It is generally assumed that the EEs' associations with soil minerals can protect them against degradation but may also cause enzyme deformation (Leprince & Quiquampoix, 1996; Quiquampoix, 1987; Secundo, 2013; Zimmerman & Ahn, 2010). Previous studies have reported both stimulatory and inhibitory effects of clay on EEs activities, making it difficult to understand how clay-mineral interaction influences SOC turnover in soils (Sheng et al., 2022). In the present study, we collected soil samples from an ongoing field experiment to evaluate the impacts of clay addition on soil properties 4 years after the implementation (Ye et al., 2019). The objectives were to understand whether clay amendment poses consistent impacts on soil properties and microbial activities. Enzyme kinetics were also investigated to understand how the interactions of clay minerals and EEs affect their catalytic activities. It was hypothesized that clay soil amendment improves soil C retention and N availability with the suppression of degradative soil enzyme activities. This study will lead to a foundation for precisely managing and improving the overall soil health of the sandy soils with clay addition and the links to sustainable agriculture.

Core Ideas

- Clay soil amendment reduced the enzymatic activities.
- Clay soil addition inhibited carbon and nitrogen enzyme kinetics with distinct mechanisms.
- Nitrogen availability was improved with clay amendment.

2 | MATERIALS AND METHODS

2.1 | Site description, experimental setup, and soil sampling

The experiment was carried out in the Pee Dee Research & Education Center of Clemson University, Florence, SC (34°18′ N, 79°44′ W). The soil was Ultisols (loamy, siliceous, semiactive, thermic Grossarenic Paleudults) (USDA classification) (USDA, 1999) with 2% clay and 89% sand content. The average annual precipitation (2006–2020) for this area is 1272 mm with average annual high and low air temperatures of 24°C and 11°C, respectively (NOAA, data available online at https://www.ncei.noaa.gov/access/us-climate-normals/).

In October 2017, eight experimental plots (6 × 4 m) were established, where clay treatment was randomly imposed on half of the plots by applying clay-rich soils excavated from subsoils (B horizon) of adjacent fields (<0.3 km). The clay-rich soils were disposed of with a manure spreader at 25 metric tons ha^{-1}, followed by mixing the soil (0–15 cm) with a disc cultivator. Using clay-rich subsoils instead of pure clay was considered to be cost-effective. On top of that the clay-rich subsoils were evacuated from a nearby field (<3 km in distance) with the same origins that constitute a mixture of natural soil particles, organic matter (OM), and clay minerals. The control plots were set up in parallel, except that no

clay-rich soils were applied. The applied clay-rich soils had a pH of 5.3 ± 0.1, 25 ± 0.6% clay, and 74 ± 0.5% sand, 0.9 ± 0.1 mg kg^{-1} inorganic N, 0.07 ± 0.03 g kg^{-1} total N, and 0.66 ± 0.00% g kg^{-1} total C. Three days after the clay amendment, cover crop mixtures of cereal rye (*Secale cereale*), crimson clover (*Trifolium incarnatum*), and winter pea (*Pisum sativum*) were drill seeded at 67, 17, and 39 kg ha^{-1}, respectively. Since its establishment in 2017, the field has been rotated with cotton (*Gossypium* spp.) and corn (*Zea mays*) with the same cover crop mixtures planted in the fallow season. The field was strip-tilled during the planting of cotton and corn and no irrigation was applied. Fertilization and field management were implemented according to local guidelines (M. A. Jones et al., 2021; Plumblee, 2022).

Soil samples were collected before the corn harvest in August 2021. Note that 8–10 soil cores were randomly collected from each plot with a soil probe (AMS) (2.5 cm in diameter, 15 cm in depth), composited, and transported on ice to the nearby laboratory, where the samples were sieved (2 mm) and stored at 4°C until used.

2.2 | Soil physio-biogeochemical properties

Soil moisture content was determined as mass loss after drying the soil at 60°C until a constant weight was achieved. Soil pH and electrical conductivity (EC) were measured with an Orion Star A325 pH/conductivity meter (Thermo-scientific) in deionized (DI) water (1:2 and 1:1 w/v ratio, respectively) after being equilibrated for 30 min. Total soil C (TC) and N (TN) were determined with a Carlo-Erba NA 1500 CNS analyzer (Haak-Buchler Instruments). Extractable inorganic N (sum of NH_4^+ and NO_3^-) was determined after extracting the soils with 1 M KCl for 1 h, followed by filtration and the colorimetrical analysis of NH_4^+ and NO_3^- (Doane & Horwáth, 2003; Verdouw et al., 1978).

Microbial respiration was measured by incubating 30 g of fresh field soil (dry equivalent) in a closed mason jar at room temperature (20 ± 1°C) in the dark for 24 h, followed by the analysis of headspace CO_2 production with a gas chromatograph (Shimadzu). The EEs activities associated with C (β-D-cellubiosidase [CB]; β-glucosidase [BG]; β-xylosidase [BX]; and *N*-acetyl-β-glucosaminidase [NAG]) and N (leucine aminopeptidase [LAP]) cycling were measured using the fluorescence method (Ye et al., 2019). In brief, 5-g field soil samples (dry equivalent) were mixed with 20 mL of DI water, shaken for 20 min in a reciprocal shaker, and settled down for 10 min in a refrigerated centrifuge (Sorvall ST 16 R, Thermo Fisher Scientific). For the assay, nearly 200 µL of samples were mixed with 50 µL of respective substrates and incubated at room temperature in the dark for 24 h. The incubation was triplicated along with the controls (substrates were replaced with DI water). The EEs activity was calculated as described (Ye et al., 2019). Potential mineralizable N (PMN) was estimated with the anaerobic incubation of fresh soil samples at 30°C in the dark for 7 days, followed by 1 M KCl extraction and subsequent colorimetric analysis of NH_4^+ (Cadisch et al., 1996). Microbial biomass C (MBC) was estimated using the chloroform fumigation-incubation method (Jenkinson & Powlson, 1976). The MBC was calculated as follows:

$$\mathrm{MBC} = \mathrm{F} - \mathrm{CO_2}/\mathrm{kc}$$

where F-CO_2 is the CO_2-C evolved from the fumigated soil during the incubation minus the CO_2-C evolved from the non-fumigated soils, while the kc is the conversion factor of 0.37.

2.3 | Enzyme kinetics

The C-cycling enzyme BG and N-cycling enzyme LAP were selected for the follow-up kinetic study. Approximately, 5-g of fresh field soil samples (dry equivalent weight) was mixed with 20 mL of DI water, 200 µL of which was incubated with 50 µL of 25, 75, 200, 500, 700, 1000, 1200, and 1500 µM substrates in 96-well microplates in dark at room temperature, respectively. For the BG, the incubation was carried out for 4 h, whereas it was 24 h for the LAP due to its low activity. Changes in fluorescence intensity were monitored at an interval of 10 and 30 min for BG and LAP, respectively.

The Michaelis Menten constant (K_m) and maximum velocity (V_{max}) were determined with the Michaelis–Menten equation:

$$V = \frac{V\mathrm{max}\ [\mathrm{S}]}{K\mathrm{m} + [\mathrm{S}]}$$

where V is the reaction velocity, [S] is the substrate concentration, Km is the substrate concentration at half-maximal velocity, and Vmax is the maximal velocity of the reaction.

2.4 | Cover crop biomass and crop yield

In 2020, after 3 years of clay amendment, cover crop biomass was not collected due to the circumstances related to the COVID-19 pandemic. Cotton yield was estimated by harvesting the middle two rows of each plot. In 2021, before the termination of the cover crop, biomass was estimated by collecting aboveground plant tissues with two 0.5 m by 0.5 m quadrants. The biomass was dried at 60°C until a constant weight was achieved. Dried samples were ground and analyzed for TC and TN at the Agricultural Service Laboratory of Clemson University. Similarly, corn yield was also estimated by harvesting the two rows of each plot in 2021.

TABLE 1 Analysis of variance of clay amendment impacts on measured soil parameters.

Variables	*p*-value
Clay content (%)	0.0069*
Soil pH	0.9513
Inorganic N	0.0293*
NH_4^+-N	0.7602
NO_3^--N	0.0149*
TC	0.7023
TN	0.3548
Respiration (CO_2-C)	0.0278*
β-D-Cellubiosidase	0.0053*
β-Glucosidase	0.0952
β-Xylosidase	0.0011*
N-Acetyl-β-glucosaminidase	0.0330*
Leucine aminopeptidase	0.0014*
PMN	0.6409
MBC	0.5255

Abbreviations: MBC microbial biomass carbon; PMN, potential mineralizable nitrogen; TC, total soil carbon; TN, total soil nitrogen.
*Significance at $\alpha = 0.05$.

2.5 | Statistical analysis

One-way analysis of variance was used to determine the effect of clay amendment on the measured variables at $\alpha = 0.05$. Pairwise correlation analysis was carried out to show the correlation between measured soil variables and cover crop biomass. All analyses were performed with JMP Pro 14 (SAS Institute), except that the enzyme kinetics were analyzed with Sigma Plot 13.0 (Systat Software Inc).

3 | RESULTS

3.1 | Soil physio-biogeochemical properties

The clay amendment increased soil clay content by 80% (Tables 1 and 2). However, the amendment did not change soil pH, EC, TC, and TN 4 years after the application (Tables 1 and 2). Soil inorganic N (NH_4^+ plus NO_3^-) was dominated by NO_3-N (Tables 1 and 2). Its concentration was greater in the clay-amended soil than in the control soil (Tables 1 and 2).

The clay amendment suppressed the activities of CB, BX, NAG, and LAP enzymes with a similar marginal impact on the BG enzymes (Table 1; Figure 1). Similarly, soil microbial respiration was reduced by 78% when the clay was amended (Table 1; Figure 2A). In contrast, no such inhibitory impacts were found for PMN and MBC (Figure 2B,C).

TABLE 2 Selected soil physio-chemical properties (0–15 cm) 4 years after the application of clay-rich soils.

	Clay content (%)	Soil pH	EC (μS cm^{-1})	TC (g kg^{-1})	TN (g kg^{-1})	Inorganic N (mg kg^{-1})	NH_4-N (mg kg^{-1})	NO_3-N (mg kg^{-1})
Clay-amended	3.4 ± 0.4a	4.79 ± 0.1a	189 ± 32a	5.59 ± 0.6a	0.34 ± 0.1a	4.6 ± 0.2a	0.8 ± 0.3	3.8 ± 0.2a
Control	1.9 ± 0.1b	4.80 ± 0.1a	155 ± 31a	5.27 ± 0.5a	0.43 ± 0.1a	3.0 ± 0.5b	0.7 ± 0.2	2.3 ± 0.4b

Note: Values denote means ± one standard error ($n = 4$). Different letters indicate significant differences at $\alpha = 0.05$.
Abbreviations: EC, electrical conductivity; TC, total carbon; TN, total nitrogen.

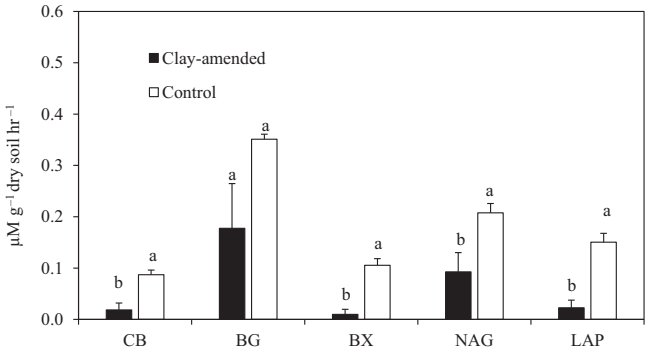

FIGURE 1 Extracellular enzyme activities in soils with (Clay-amended) and without (Control) the amendment of clay-rich soils. Bars indicate the mean ± SE. CB, β-D-cellubiosidase; BG, β-glucosidase; BX, β-xylosidase; NAG, *N*-acetyl-β-glucosaminidase; LAP, leucine aminopeptidase. Different letters indicate a significant difference ($p < 0.05$).

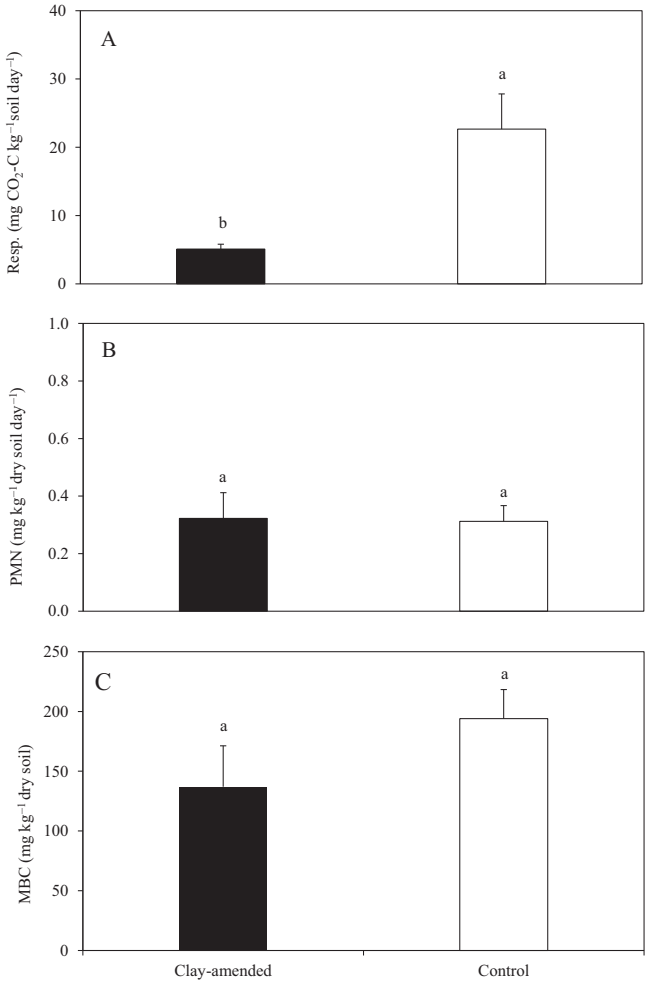

FIGURE 2 Microbial respiration (A), organic nitrogen mineralization potential (PMN) (B), and microbial biomass carbon (MBC) (C) in soils (0–15 cm) with (clay-amended) and without (control) the amendment of clay-rich soils. Bars indicate the mean ± SE. Different letters indicate a significant difference ($p < 0.05$).

3.2 | Enzyme kinetics

The kinetics study demonstrated that the saturation kinetics fitted well with the Michaelis–Menten equation (Figure 3; Table 3). The coefficient of determination (R^2; used to explain the relationship between an independent and dependent variable) for the LAP for the clay-amended soils and control was 0.98 and 0.99, respectively, whereas for the BG for the clay-amended soils and control, it was 0.79 and 0.97, respectively. The data estimated that the *V*max for BG decreased from 0.66 to 0.17 μM g^{-1} h^{-1} when the clay was amended (Table 3). Similarly, the *V*max for LAP decreased by 80% when the clay was amended. The Michaelis constant *K*m of the BG was also decreased by the clay amendment, whereas the constant *K*m of the LAP increased after the amendment (Table 3).

3.3 | Cover crop biomass and crop yield

An increase in cover crop aboveground biomass production was observed after the clay-amended treatment in 2021 (Table 4). Total N in biomass was also higher with clay-amendment soils (Table 4). There were no clay amendment impacts on crop yields (Table 4).

3.4 | Relation between measured variables

Microbial respiration was positively correlated to CBH, LAP, and BX (Table 5). A similar positive correlation was also observed between respiration and the BG-*V*max and LAP-*V*max, respectively. However, none of the *K*m was correlated to respiration. The pH, PMN, and TC had no correlation with any of the measured variables. The NO_3^--N was found only negatively correlated with CBH. The clay % was negatively correlated with the measured C-cycling enzymes CBH, BG, and BX but was not correlated with the BG-*V*max and BG-*K*m. In addition, the clay % did not correlate with the NAG and LAP but was correlated with LAP-*V*max and LAP-*K*m. Positive correlations were also observed between cover crop biomass and clay %, inorganic N and NO_3^--N concentrations, respectively (Table 5).

4 | DISCUSSION

Recent studies have demonstrated the proof-of-concept of clay addition to improve sandy soils (Cann, 2000; Schapel et al., 2018; Ye et al., 2019). However, relevant studies are limited, and long-term impacts remain elusive. In the present study, we continued to monitor the impacts of clay addition on soil biogeochemical properties in a typical sandy Ultisol 4 years after the application. The data indicated consistent inhibitory effects of clay addition to enzyme activities

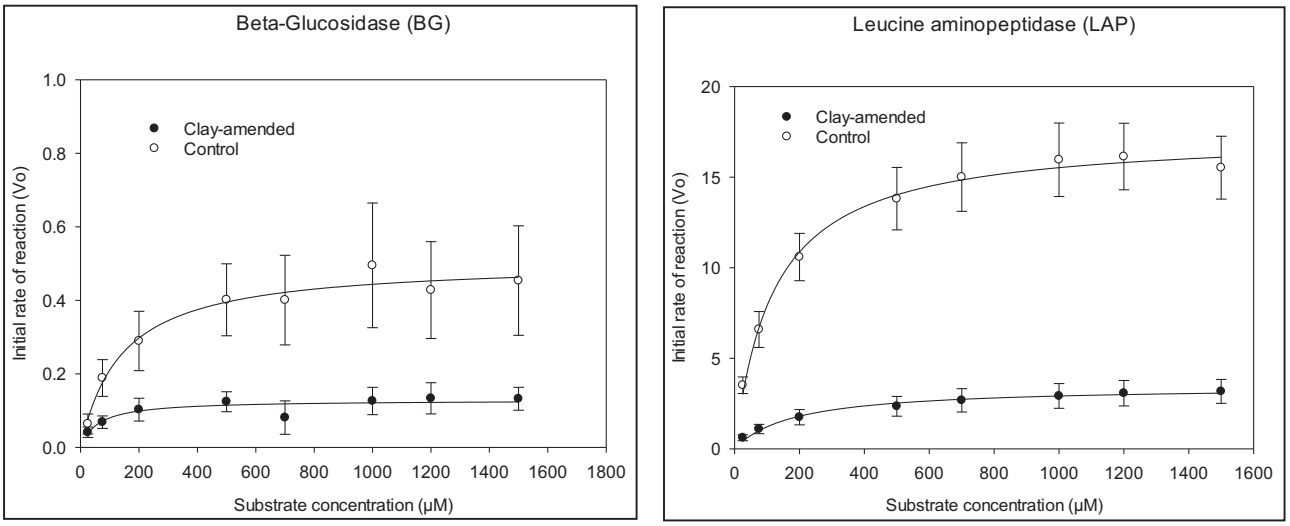

FIGURE 3 Enzyme activity (*Vo*) as a function of substrate concentration (*S*) in soils with (clay-amended) and without (control) the amendment of clay-rich soils.

TABLE 3 Kinetic parameters and the coefficient of determination (R^2) for the fitting model (Michaelis–Menten equation).

	β-Glucosidase			Leucine aminopeptidase		
	*V*max ($\mu Mg^{-1}h^{-1}$)	*K*m (μM)	R^2	*V*max ($\mu Mg^{-1}h^{-1}$)	*K*m (μM)	R^2
Clay-amended	0.17b	68b	0.79	1.48b	199a	0.98
Control	0.66a	158a	0.97	7.34a	123b	0.99

Note: Different letters indicate significant differences at $\alpha = 0.05$.
Abbreviation: *V*max, maximum velocity.

TABLE 4 Crop yield and cover crop biomass production in fields with and without clay-amendment.

	Cotton lint yield (2020)	Corn yield (2021)	Cover crop biomass (2020) ($kg\ ha^{-1}$)	Cover crop biomass (2021) ($kg\ ha^{-1}$)	Cover crop C (%)	Cover crop N (%)
Clay-amended	668 ± 82a	6965 ± 988a	N/A	1505 ± 160a	39.31 ± 0.44a	2.97 ± 0.26a
Control	609 ± 99a	7860 ± 875a	N/A	855 ± 129b	38.68 ± 0.83a	2.03 ± 0.23b

Note: Values are means and standard errors ($n = 4$). Different small letters indicate significant differences at $\alpha = 0.05$. N/A, Data not available due to the COVID-19 pandemic impacts.

(Figure 1) resulted in suppressed microbial respiration but not organic N mineralization potential (Figure 2A,B) (supporting the hypothesis). Enzyme kinetics in the present study further suggested that the clay addition can impact enzymes via different mechanisms.

4.1 | Enzyme activities

Extracellular enzymes are excreted by microorganisms to soils for sequestrating energy and nutrients (Burns et al., 2013; Luo et al., 2017). The activities of those enzymes are critical for the degradation of SOC and plant detritus and are regulated by substrate availability and other environmental factors (Allison & Treseder, 2008; Burns et al., 2013; Fierer et al., 2003; Finzi et al., 2006; Henry et al., 2005). In the present study, the measured C- and N-cycling enzymes were all suppressed by clay amendment, except the BG, which demonstrated marginal impacts ($p = 0.095$). Similar clay suppression on the C- and N-cycling enzymatic activities have been reported (Olagoke et al., 2019; Rakhsh & Golchin, 2018; Ye et al., 2019). Apparently, the inhibitory impacts were not mediated through the measured soil chemical properties, as evidenced by their insignificant pair-wise correlations (Table 5). It is generally assumed that such reduction was due to the adsorption of enzymes onto clay minerals, which subsequently blocks enzyme active sites or causes enzyme deformation. However, the direction and magnitudes of clay

TABLE 5 Pairwise correlation of the measured variables.

	CBH	NAG	LAP	BG	BX	PMN	CO_2-C	MBC	Inorganic N	NH_4^+-N	NO_3^--N	clay %	TC	TN	pH	EC	BG-*V*max	BG-*K*m	LAP-*V*max	LAP-*K*m
NAG	0.88**																			
LAP	0.80*	0.85**																		
BG	0.83*	0.89**	0.61																	
BX	0.78*	0.77*	0.91**	0.65																
PMN	0.38	0.46	0.06	0.61	0.01															
CO_2-C	0.85*	0.77	0.94**	0.54	0.88*	0.13														
MBC	0.70	0.38	0.21	0.58	0.41	0.38	0.20													
Inorganic N	−0.65	−0.36	−0.46	−0.43	−0.60	0.27	−0.25	−0.69												
NH_4^+-N	0.09	0.43	0.16	0.4	0.02	0.66	−0.01	−0.26	0.50											
NO_3^--N	−0.79*	−0.61	−0.60	−0.69	−0.69	0.02	−0.34	−0.67	0.92**	0.12										
clay %	−0.91**	−0.70	−0.68	−0.79*	−0.77*	−0.28	−0.69	−0.80*	0.80*	0.01	0.90**									
TC	0.01	0.37	0.17	0.08	−0.08	0.26	0.25	−0.46	0.53	0.49	0.39	0.39								
TN	0.50	0.80*	0.60	0.71	0.61	0.50	0.62	0.08	0.10	0.63	−0.17	−0.30	0.54							
pH	0.14	−0.15	−0.02	−0.16	0.02	0.23	0.07	0.36	0.01	−0.10	0.06	−0.21	−0.36	−0.24						
EC	−0.22	0.24	0.03	0.20	−0.06	0.42	0.11	−0.54	0.70	0.81*	0.44	0.37	0.6	0.66	−0.42					
BG-*V*max	0.71*	0.76*	0.82*	0.57	0.70	0.47	0.93**	0.21	−0.07	0.36	−0.24	−0.54	0.26	0.65	0.26	0.2				
BG-*K*m	0.43	0.53	0.71	0.39	0.54	−0.08	0.66	−0.12	−0.28	0.07	−0.35	−0.36	0.12	0.31	−0.44	0.17	0.57			
LAP-*V*max	0.8**	0.82*	0.94**	0.70	0.93**	0.22	0.98**	0.43	−0.51	0.11	−0.63	−0.81*	−0.02	0.59	0.12	−0.03	0.88**	0.66		
LAP-*K*m	−0.74*	−0.50	−0.56	−0.63*	−0.71	−0.31	−0.72	−0.75*	0.62	−0.01	0.72*	0.92**	0.56	−0.22	−0.47	0.3	−0.57	−0.23	−0.76*	
Cover crop biomass	−0.68	−0.41	−0.56	−0.49	−0.56	0.01	−0.48	−0.55	0.78*	0.30	0.75*	0.80*	0.49	0.07	−0.01	0.48	−0.40	−0.66	−0.68	0.71*

Abbreviations: BG, β-glucosidase; BX, β-xylosidase; EC, electrical conductivity; *K*m, Michaelis Menten constant; LAP, leucine aminopeptidase; MBC microbial biomass carbon; NAG, *N*-acetyl-β-glucosaminidase; PMN, potential mineralizable nitrogen; TC, total soil carbon; TN, total soil nitrogen; *V*max, maximum velocity.

*$p < 0.05$; **$p < 0.01$.

impact on EEs may depend on the binding capacities of the clay and the chemical structure of the enzymes (Olagoke et al., 2019, 2020; Sheng et al., 2022). In the present study, the added clay-rich soils (25% of clay) are dominated by kaolinite, a 1:1 clay mineral. However, the inhibitory impacts of the clay on EEs still existed 4 years after the amendment, except for the BG (Ye et al., 2019). Enzymes can respond differently to clay because of their differences in chemical structure (Olagoke et al., 2019; Sheng et al., 2022). In addition, clay adsorption can stabilize the enzymes' structure allowing the EEs to retain or enhance their catalytic activities, which may at least partially explain why the BG acted differently against other enzymes upon clay amendment (Figure 1; Table 1).

The kinetic parameter *V*max reflects the maximum rate of activity when all enzymes are substrate saturated, while the *K*m indicates the affinity of the enzyme for a substrate (Marxa et al., 2005). A reduction in the *K*m value implies an enhanced affinity of the enzyme toward the substrate. In the present study, both BG's *V*max and *K*m were reduced in the clay-amended soils (Table 3), suggesting that the clay was an uncompetitive inhibitor for the BG (Olagoke et al., 2019). It is plausible that the binding increased its catalytic activities resulting in the observed marginal clay impacts on the BG activities (i.e., no reduction in overall activities).

The LAP activity is linked with protein degradation (N-cycle) and is a sensitive indicator of SOC decomposition (Allison & Jastrow, 2006; Babaev & Orujova, 2009). However, in contrast to the BG, the kinetic parameters *V*max of LAP decreased upon clay amendment while the LAP-*K*m increased (Table 3), suggesting that the clay is a mixed non-competitive inhibitor of the LAP. According to the inhibition kinetics theory, a mixed type of inhibition involves both competitive and non-competitive processes in which the inhibitor may either bind to the active site of the enzyme preventing the enzyme from binding to the substrate, or bind to the enzyme at a separate site together with or without the substrate (Shirvani et al., 2020). The decreased *V*max with increased *K*m often suggests enzyme immobilization, showing an increase in stability and a decrease in enzyme activity (Datta et al., 2017; Sarkar & Burns, 1984). It also revealed that microorganisms may have to spend more energy on enzyme production to maintain a similar level of activities to degrade the target substrates upon clay amendment. However, the long-term impacts of such microbial adjustment remain unknown.

4.2 | Microbial respiration

In addition to suppressing the EEs activities, clay amendment reduced microbial respiratory production significantly by 78% (Figure 2A). However, the reduction was not accompanied by a decreased microbial biomass (Figure 2C), suggesting that the impacts of clay amendment did not reduce microbial growth but decreased microbial metabolic quotient indicated by the lower ratio of CO_2 production to MBC growth and nutrient uptake by microbes (Pal & Marschner, 2016). The decrease in metabolic quotient with the increase in soil clay content was expected to be more noticeable at the clay contents <25% (Müller & Höper, 2004). However, it is also plausible that the addition of soil clays reduced SOC accessibility (i.e., substrate availability) to soil microbes and their degradative enzymes by providing extra physiochemical protections through the interactions of SOC and clay minerals (Baldock & Skjemstad, 2000) and entrapments of SOC in soil aggregates (Dungait et al., 2012; Schmidt et al., 2011). After clay amendment, improved soil aggregates have already been demonstrated in the tested soils (Ye et al., 2019). The data highlighted the concept that clay addition can affect soil microbial functions through various mechanisms, further indicating the potential of clay amendment to improve the preservation and stability of organic C in these soils.

4.3 | Soil C and N

The stabilization of SOC largely depends on its spatial accessibility to microbes and their associated degradative enzymes, which is influenced by its interaction with mineral surfaces, and entrapment into soil aggregates (Dungait et al., 2012; Kleber, 2010). Intriguingly, the consistently suppressed enzyme activities and microbial respiration did not lead to increased TC concentrations at 0- to 15-cm depth 4 years after the clay addition (Tables 1 and 2), likely because most of the organic inputs (i.e., crop residues) were largely left on the ground but not incorporated into the entire soil profile. In addition, it has been frequently reported that organic C accumulations were mostly observed in topsoil (0–5 cm) but not in bulk soils in systems with conservation tillage and residue returns (Badagliacca et al., 2018; Balkcom et al., 2013; Hubbard et al., 2013; Luo et al., 2010; Sun et al., 2020). It is therefore possible that the lack of C inputs to the subsurface soil limited the accumulation of SOC in bulk soils in the short term. Long-term studies on the SOC dynamics at different soil depths are therefore warranted.

Nitrogen is essential for crop production whose availability is affected by various biotic and abiotic factors. In the present study, organic N mineralization appeared to be the main N source since no fertilization was applied during the entire cover crop season. No changes were observed for PMN 4 years after the addition of clay-rich soils (Figure 2B), consistently in line with previous observations (Ye et al., 2019). However, there were higher concentrations of NO_3^- and inorganic N (NO_3^- plus NH_4^+) in clay-amended soils (Table 2). Despite lacking NO_3^- retention capacity, clay was found to decrease NO_3^- leaching by increasing the soils' inherent water-holding capacity (Dempster et al., 2012). It was therefore plausible

that the additions of clay-rich soils reduced N leaching from the soils, which was further supported by the insignificant correlations of inorganic N with PMN and enzyme activities, respectively, but a significant correlation between inorganic N and clay % in soils (Table 5). The increased N availability may also explain the higher cover crop biomass production and N content in the clay-amended soils (Table 5).

4.4 | Clay amendment as a novel soil management strategy

The proof-of-concept study of clay amendment to improve sandy soils has been well demonstrated by our pilot study and other similar research (Button et al., 2022; Tahir & Marschner, 2016; Ye et al., 2019). In the present study, we further demonstrated that clay amendment had consistent impacts on suppressing soil microbial decomposition but increased N availability 4 years after the amendment, suggesting its potential as a novel practice to restore SOC in sandy soils. However, limited relevant studies are jeopardizing the adoption of such practices (Button et al., 2022). One potential challenge is the minimal amendment rates and time that are required to improve soil physical, chemical, or biological properties. In addition to application rates, the mineralogy and ped size of the clay may also affect the outcomes. Excess clay additions have been found to promote the formation of soil hard pan (Harper & Gilkes, 2004) and the destructure of the soil structure (Dixon, 1991; Ogunniyi, J.E., 2017), both of which affected plant growth. Despite the uncertainties, our field pilot study indicated positive soil impacts within a year after the application of clay-rich soils at 25 metric tons ha^{-1} (Ye et al., 2019), which also effectively increased the biomass production and N content of the winter cover crops after 4 years of the amendment (Table 4). However, no effects on main crop yields were observed likely due to the confounding impacts of fertilization in the spring (Table 4). Meanwhile, clay-rich soils are abundant and commercially available at low prices in the US Southeast, making the clay amendment attractive and practical in the context of improving sandy soils. However, more research (e.g., application rates and methods and cost-effectiveness) is needed to investigate its long-term effects on soil physio-biochemical processes and the productivity and sustainability of the crop systems.

5 | CONCLUSION

Clay addition continued to pose inhibitory impacts on soil microbial activities 4 years after the additions. The suppressed activities of the CB, BX, LAP, and NAG enzymes, along with the neutral response of the BG activities, indicated that clay additions can deactivate the enzymes binding sites to reduce their activities but can also stabilize the enzymes to maintain or enhance their activities. The kinetics of the BG and LAP further indicated that clay addition can have different impacts on enzymes with diverse biological origins through distinct mechanisms. Higher inorganic N concentrations were observed in soils with clay additions at 0- to 15-cm depth, likely resulting from reduced NO_3^- leaching. It is apparent that clay addition has the potential to improve sandy Coastal Plain soils by increasing SOC stability and nutrient availability. However, more research is needed to understand the long-term impacts on microbial communities, SOC stability, and N dynamics while making this approach practical.

AUTHOR CONTRIBUTIONS

Pratima Poudel: Methodology; visualization; writing—original draft; writing—review and editing. **Rongzhong Ye**: Conceptualization; methodology; supervision; visualization; writing—original draft; writing—review and editing. **Binaya Parajuli**: Methodology; writing—review and editing.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

ORCID

Pratima Poudel https://orcid.org/0000-0002-8115-2297
Rongzhong Ye https://orcid.org/0000-0003-3366-317X

REFERENCES

Allison, S. D., & Jastrow, J. D. (2006). Activities of extracellular enzymes in physically isolated fractions of restored grassland soils. *Soil Biology & Biochemistry*, *11*, 3245–3256. https://doi.org/10.1016/j.soilbio.2006.04.011

Allison, S. D., & Treseder, K. K. (2008). Warming and drying suppress microbial activity and carbon cycling in boreal forest soils. *Global Change Biology*, *14*, 2898–2909. https://doi.org/10.1111/j.1365-2486.2008.01716.x

Babaev, M. P., & Orujova, N. I. (2009). Assessment of the biological activity of soils in the subtropical zone of Azerbaijan. *Eurasian Soil Science*, *42*, 1163–1169. https://doi.org/10.1134/S1064229309100111

Badagliacca, G., Benítez, E., Amato, G., Badalucco, L., Giambalvo, D., Laudicina, V. A., & Ruisi, P. (2018). Long-term effects of contrasting tillage on soil organic carbon, nitrous oxide and ammonia emissions in a Mediterranean Vertisol under different crop sequences. *Science of the Total Environment*, *619–620*, 18–27. https://doi.org/10.1016/j.scitotenv.2017.11.116

Baldock, J. A., & Skjemstad, J. O (2000). Role of the soil matrix and minerals in protecting natural organic materials against biological attack. *Organic Geochemistry*, *31*, 697–710. https://doi.org/10.1016/S0146-6380(00)00049-8

Balkcom, K. S., Arriaga, F. J., & van Santen, E. (2013). Conservation systems to enhance soil carbon sequestration in the southeast U.S. Coastal Plain. *Soil Science Society of America Journal*, *77*, 1774–1783. https://doi.org/10.2136/sssaj2013.01.0034

Brust, G. E. (2019). Management strategies for organic vegetable fertility. *Safety and Practice for Organic Food*, 193–212. https://doi.org/10.1016/B978-0-12-812060-6.00009-X

Burns, R. G., Deforest, J. L., Marxsen, J., Sinsabaugh, R. L., Stromberger, M. E., Wallenstein, M. D., Weintraub, M. N., & Zoppini, A. (2013). Soil enzymes in a changing environment: Current knowledge and future directions. *Soil Biology & Biochemistry*, *58*, 216–234. https://doi.org/10.1016/j.soilbio.2012.11.009

Button, E. S., Pett-Ridge, J., Murphy, D. V., Kuzyakov, Y., Chadwick, D. R., & Jones, D. L. (2022). Deep-C storage: Biological, chemical and physical strategies to enhance carbon stocks in agricultural subsoils. *Soil Biology & Biochemistry*, *170*, 108697. https://doi.org/10.1016/j.soilbio.2022.108697

Cadisch, G., Imhof, H., Urquiaga, S., Boddey, R. M., & Giller, K. E. (1996). Carbon turnover (δ13C) and nitrogen mineralization potential of particulate light soil organic matter after rainforest clearing. *Soil Biology & Biochemistry*, *28*(12), 1555–1567. https://doi.org/10.1016/S0038-0717(96)00264-7

Cann, M. A (2000). Clay spreading on water repellent sands in the south east of South Australia promoting sustainable agriculture. *Journal of Hydrology*, *231–232*, 333–341. https://doi.org/10.1016/S0022-1694(00)00205-5

Chen, B., Liu, E., Tian, Q., Yan, C., & Zhang, Y. (2014). Soil nitrogen dynamics and crop residues. A review. *Agronomy for Sustainable Development*, *34*(2), 429–442. https://doi.org/10.1007/s13593-014-0207-8

Datta, R., Anand, S., Moulick, A., Baraniya, D., Pathan, S. I., Rejsek, K., Vranova, V., Sharma, M., Sharma, D., Kelkar, A., & Formanek, P. (2017). How enzymes are adsorbed on soil solid phase and factors limiting its activity: A Review. *International Agrophysics*, *31*(2), 287–302. https://doi.org/10.1515/intag-2016-0049

Dempster, D. N., Jones, D. L., & Murphy, D. V. (2012). Clay and biochar amendments decreased inorganic but not dissolved organic nitrogen leaching in soil. *Soil Research*, *50*, 216–221. https://doi.org/10.1071/SR11316

Dixon, J. B. (1991). Roles of clays in soils. *Applied Clay Science*, *5*(5–6), 489–503. https://doi.org/10.1016/0169-1317(91)90019-6

Doane, T. A., & Horwáth, W. R. (2003). Spectrophotometric determination of nitrate with a single reagent. *Analytical Letters*, *36*(12), 79–82. https://doi.org/10.1081/AL-120024647

Dungait, J. A. J., Hopkins, D. W., Gregory, A. S., & Whitmore, A. P. (2012). Soil organic matter turnover is governed by accessibility not recalcitrance. *Global Change Biology*, *18*, 1781–1796. https://doi.org/10.1111/j.1365-2486.2012.02665.x

Feng, W., Xu, M., Fan, M., Malhi, S. S., Schoenau, J. J., Six, J., & Plante, A. F. (2014). Testing for soil carbon saturation behavior in agricultural soils receiving long-term manure amendments. *Canadian Journal of Soil Science*, *94*(3), 281–294. https://doi.org/10.4141/CJSS2013-012

Fierer, N., Schimel, J. P., & Holden, P. A. (2003). Influence of drying-rewetting frequency on soil bacterial community structure. *Microbial Ecology*, *45*(1), 63–71. https://doi.org/10.1007/s00248-002-1007-2

Finzi, A. C., Sinsabaugh, R. L., Long, T. M., & Osgood, M. P. (2006). Microbial community responses to atmospheric carbon dioxide enrichment in a warm-temperate forest. *Ecosystems*, *9*, 215–226. https://doi.org/10.1007/s10021-005-0078-6

Franzluebbers, A. J. (2020). Soil-test biological activity with the flush of CO2: V. Validation of nitrogen prediction for corn production. *Agronomy Journal*, *112*, 2188–2204. https://doi.org/10.1002/agj2.20094

Hall, D. J. M., Jones, H. R., Crabtree, W. L., & Daniels, T. L. (2010). Claying and deep ripping can increase crop yields and profits on water repellent sands with marginal fertility in southern Western Australia. *Australian Journal of Soil Research*, *48*, 178–187. https://doi.org/10.1071/SR09078

Harper, R. J., & Gilkes, R. J. (2004). The effects of clay and sand additions on the strength of sandy topsoils. *Australian Journal of Soil Research*, *42*(1), 39–44. https://doi.org/10.1071/SR03063

Henry, H. A. L., Juarez, J. D., Field, C. B., & Vitousek, P. M. (2005). Interactive effects of elevated CO2, N deposition and climate change on extracellular enzyme activity and soil density fractionation in a California annual grassland. *Global Change Biology*, *11*, 1808–1815. https://doi.org/10.1111/j.1365-2486.2005.001007.x

Huang, J., & Hartemink, A. E. (2020). Soil and environmental issues in sandy soils. *Earth-Science Reviews*, *208*, 103295. https://doi.org/10.1016/j.earscirev.2020.103295

Hubbard, R. K., Strickland, T. C., & Phatak, S. (2013). Effects of cover crop systems on soil physical properties and carbon/nitrogen relationships in the coastal plain of southeastern USA. *Soil and Tillage Research*, *126*, 276–283. https://doi.org/10.1016/j.still.2012.07.009

Jenkinson, D. S., & Powlson, D. S. (1976). The effects of biocidal treatments on metabolism in soil-V. A method for measuring soil biomass. *Soil Biology & Biochemistry*, *8*, 209–213. https://doi.org/10.1016/0038-0717(76)90005-5

Johnston, A. E., Poulton, P. R., & Coleman, K. (2009). Chapter 1 soil organic matter: Its importance in sustainable agriculture and carbon dioxide fluxes. *Advances in Agronomy*, *101*, 1–57. https://doi.org/10.1016/S0065-2113(08)00801-8

Jones, M. A., Farmaha, B. S., Greene, J. K., Marshall, M., & Mueller, J. D. (2021). *South Carolina cotton growers' guide*. Clemson University Cooperative Extension.

Kleber, M. (2010). What is recalcitrant soil organic matter? *Environmental Chemistry*, *7*, 320–332. https://doi.org/10.1071/EN10006

Leprince, F., & Quiquampoix, H. (1996). Extracellular enzyme activity in soil: Effect of pH and ionic strength on the interaction with montmorillonite of two acid phosphatases secreted by the ectomycorrhizal fungus *Hebeloma cylindrosporum*. *European Journal of Soil Science*, *47*, 511–522. https://doi.org/10.1111/j.1365-2389.1996.tb01851.x

Luo, L., Meng, H., & Gu, J.-D. (2017). Microbial extracellular enzymes in biogeochemical cycling of ecosystems. *Journal of Environmental Management*, *197*, 539–549. https://doi.org/10.1016/j.jenvman.2017.04.023

Luo, Z., Wang, E., & Sun, O. J. (2010). Can no-tillage stimulate carbon sequestration in agricultural soils? A meta-analysis of paired experiments. *Agriculture Ecosystems and Environment*, *139*, 224–231. https://doi.org/10.1016/j.agee.2010.08.006

Markewich, H. W., Pavich, M. J., & Buell, G. R. (1990). Contrasting soils and landscapes of the Piedmont and Coastal Plain, eastern United States. *Geomorphology*, *3*, 417–447. https://doi.org/10.1016/0169-555X(90)90015-I

Marx, M.-C., Kandeler, E., Wood, M., Wermbter, N., & Jarvis, S. C. (2005). Exploring the enzymatic landscape: Distribution and kinetics of hydrolytic enzymes in soil particle-size fractions. *Soil Biology & Biochemistry*, *37*(1), 35–48. https://doi.org/10.1016/j.soilbio.2004.05.024

Mason-Jones, K., Schmücker, N., & Kuzyakov, Y. (2018). Contrasting effects of organic and mineral nitrogen challenge the N-Mining Hypothesis for soil organic matter priming. *Soil Biology & Biochemistry*, *124*, 38–46. https://doi.org/10.1016/j.soilbio.2018.05.024

Müller, T., & Höper, H. (2004). Soil organic matter turnover as a function of the soil clay content: Consequences for model applications. *Soil Biology & Biochemistry*, *36*(6), 877–888. https://doi.org/10.1016/j.soilbio.2003.12.015

Nash, P. R., Gollany, H. T., Novak, J. M., Bauer, P. J., Hunt, P. G., & Karlen, D. L. (2018). Simulated soil organic carbon response to tillage, yield, and climate change in the southeastern Coastal Plains. *Journal of Environmental Quality*, *47*(4), 663–673. https://doi.org/10.2134/jeq2017.05.0190

Nguye, T.-T., & Marschner, P. (2014). Respiration in mixes of sandy and clay soils: Influence of clay type and addition rate. *Journal of Soil Science and Plant Nutrition*, *14*(4), 881–887. https://doi.org/10.4067/s0718-95162014005000070

Novak, J. M., Bauer, P. J., & Hunt, P. G. (2007). Carbon dynamics under long-term conservation and disk tillage management in a Norfolk loamy sand. *Soil Science Society of America Journal*, *71*(2), 453–456. https://doi.org/10.2136/sssaj2005.0284n

Ogunniyi, J. E. (2017). *Effects of clay and organic matter amendments on water and nutrient retention of sandy soils* [Doctoral dissertation, University of Warwick].

Olagoke, F. K., Kaiser, K., Mikutta, R., Kalbitz, K., & Vogel, C. (2020). Persistent activities of extracellular enzymes adsorbed to soil minerals. *Microorganisms*, *8*(11), 1796. https://doi.org/10.3390/microorganisms8111796

Olagoke, F. K., Kalbitz, K., & Vogel, C. (2019). Control of soil extracellular enzyme activities by clay minerals—Perspectives on microbial responses. *Soil Systems*, *3*(4), 64. https://doi.org/10.3390/soilsystems3040064

Pal, S., & Marschner, P. (2016). Influence of clay concentration, residue C/N and particle size on microbial activity and nutrient availability in clay-amended sandy soil. *Journal of Soil Science and Plant Nutrition*, *16*(2), 350–361. https://doi.org/10.4067/S0718-95162016005000033

Plumblee, M. (2022). *South Carolina corn production guide*. https://www.clemson.edu/extension/agronomy/_files/corn-production-guide.pdf

Quiquampoix, H. (1987). A stepwise approach to the understanding of extracellular enzyme activity in soil I. Effect of electrostatic interactions on the conformation of a β-D-glucosidase adsorbed on different mineral surfaces. *Biochimie*, *69*(6-7), 753–763. https://doi.org/10.1016/0300-9084(87)90196-9

Rakhsh, F., & Golchin, A. (2018). Carbohydrate concentrations and enzyme activities as influenced by exchangeable cations, mineralogy and clay content. *Applied Clay Science*, *163*, 214–226. https://doi.org/10.1016/j.clay.2018.07.031

Riaz, M., & Marschner, P. (2020). Sandy soil amended with clay soil: Effect of clay soil properties on soil respiration, microbial biomass, and water extractable organic C. *Journal of Soil Science and Plant Nutrition*, *20*, 2465–2470. https://doi.org/10.1007/s42729-020-00312-z

Sarkar, J. M., & Burns, R. G. (1984). Synthesis and properties of β-D-glucosidasephenolic copolymers as analogues of soil humic-enzyme complexes. *Soil Biology and Biochemistry*, *16*(6), 619–625. https://doi.org/10.1016/0038-0717(84)90082-8

Schapel, A., Marschner, P., & Churchman, J. (2018). Clay amount and distribution influence organic carbon content in sand with subsoil clay addition. *Soil and Tillage Research*, *184*, 253–260. https://doi.org/10.1016/j.still.2018.08.001

Schmidt, M. W. I., Torn, M. S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens, I. A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D. A. C., Nannipieri, P., Rasse, D. P., Weiner, S., & Trumbore, S. E. (2011). Persistence of soil organic matter as an ecosystem property. *Nature*, *478*(7367), 49–56. https://doi.org/10.1038/nature10386

Secundo, F. (2013). Conformational changes of enzymes upon immobilisation. *Chemical Society Reviews*, *42*(15), 6250–6261. https://doi.org/10.1039/c3cs35495d

Shahbaz, M., Kuzyakov, Y., & Heitkamp, F. (2017). Decrease of soil organic matter stabilization with increasing inputs: Mechanisms and controls. *Geoderma*, *304*, 76–82. https://doi.org/10.1016/j.geoderma.2016.05.019

Sheng, Y., Dong, H., Coffin, E., Myrold, D., & Kleber, M. (2022). The important role of enzyme adsorbing capacity of soil minerals in regulating β-glucosidase activity. *Geophysical Research Letters*, *49*, e2021GL097556. https://doi.org/10.1029/2021GL097556

Shirvani, M., Khalili, B., Kalbasi, M., Shariatmadari, H., & Nourbakhsh, F. (2020). Adsorption of alkaline phosphates on palygorskite and sepiolite: A tradeoff between enzyme protection and inhibition. *Clays and Clay Minerals*, *68*, 287–295. https://doi.org/10.1007/s42860-020-00066-w

Stewart, C. E., Follett, R. F., Wallace, J., & Pruessner, E. G. (2012). Impact of biosolids and tillage on soil organic matter fractions: Implications of carbon saturation for conservation management in the virginia coastal plain. *Soil Science Society of America Journal*, *76*(4), 1257–1267. https://doi.org/10.2136/sssaj2011.0165

Stockmann, U., Adams, M. A., Crawford, J. W., Field, D. J., Henakaarchchi, N., Jenkins, M., Minasny, B., Mcbratney, A. B., Courcelles, V. D. R. D., Singh, K., Wheeler, I., Abbott, L., Angers, D. A., Baldock, J., Bird, M., Brookes, P. C., Chenu, C., Jastrow, J. D., Lal, R., … Zimmermann, M. (2013). The knowns, known unknowns and unknowns of sequestration of soil organic carbon. *Agriculture Ecosystems and Environment*, *164*, 80–99. https://doi.org/10.1016/j.agee.2012.10.001

Sun, W., Canadell, J. G., Yu, L., Yu, L., Zhang, W., Smith, P., Fischer, T., & Huang, Y. (2020). Climate drives global soil carbon sequestration and crop yield changes under conservation agriculture. *Global Change Biology*, *26*(6), 3325–3335. https://doi.org/10.1111/gcb.15001

Tahir, S., & Marschner, P. (2016). Clay amendment to sandy soil—Effect of clay concentration and ped size on nutrient dynamics after residue addition. *Journal of Soils and Sediments*, *16*, 2072–2080. https://doi.org/10.1007/s11368-016-1406-5

USDA. (1999). *Soil taxonomy: A basic system of soil classification for making and interpreting soil surveys* (2nd ed.). U.S. Government Printing Office. https://www.nrcs.usda.gov/sites/default/files/2022-06/Soil%20Taxonomy.pdf

Van De Vreken, P., Gobin, A., Baken, S., Van Holm, L., Verhasselt, A., Smolders, E., & Merckx, R. (2016). Crop residue management and oxalate-extractable iron and aluminium explain long-term soil organic carbon sequestration and dynamics. *European Journal of Soil Science*, *67*, 332–340. https://doi.org/10.1111/ejss.12343

Verdouw, H., Van Echteld, C. J. A., & Dekkers, E. M. J. (1978). Ammonia determination based on indophenol formation with sodium salicylate. *Water Research*, *12*(6), 399–402. https://doi.org/10.1016/0043-1354(78)90107-0

Ye, R., Parajuli, B., & Sigua, G. (2019). Subsurface clay soil application improved aggregate stability, nitrogen availability, and organic carbon preservation in degraded ultisols with cover crop mixtures. *Soil*

Science Society of America Journal, *83*(3), 597–604. https://doi.org/10.2136/sssaj2018.12.0496

Yost, J. L., & Hartemink, A. E. (2019). Soil organic carbon in sandy soils: A review. *Advances in Agronomy*, *158*, 217–310. https://doi.org/10.1016/bs.agron.2019.07.004

Zimmerman, A. R., & Ahn, M.-Y. (2010). Organo-mineral–enzyme interaction and soil enzyme activity. In G. Shukla & A. Varma (Eds.), *Soil enzymology* (pp. 271–292). Springer.

How to cite this article: Poudel, P., Ye, R., & Parajuli, B. (2024). Clay soil amendment suppressed microbial enzymatic activities while increasing nitrogen availability in sandy soils. *Soil Science Society of America Journal*, *88*, 1647–1658. https://doi.org/10.1002/saj2.20731