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# Contrasting carbon and nitrogen responses to tillage at different soil depths: An observation after 40-year of tillage management

Binaya Parajuli<sup>1,2</sup>  | Rongzhong Ye<sup>1,2</sup>  | Min Luo<sup>3</sup> | Thomas F. Ducey<sup>4</sup>  |  
Dara Park<sup>1</sup> | Matthew Smith<sup>2</sup> | Gilbert Sigua<sup>4</sup> 

<sup>1</sup> Plant and Environmental Sciences,  
Clemson Univ., Clemson, SC 29634, USA

<sup>2</sup> Pee Dee Research and Education Center,  
Clemson Univ., Florence, SC 29506, USA

<sup>3</sup> School of Environment and Resource,  
Fuzhou Univ., Fuzhou 350116, China

<sup>4</sup> USDA-ARS, Coastal Plains Soil, Water, &  
Plant Research Center, Florence, SC 29501,  
USA

## Correspondence

Rongzhong Ye, Plant and Environmental Sci-  
ences and Pee Dee Research and Education  
Center, Clemson Univ., Florence, SC 29506,  
USA.

Email: [rongzho@clemson.edu](mailto:rongzho@clemson.edu)

Assigned to Associate Editor Shannon  
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## Abstract

Conservation tillage (CS) is a major component of sustainable soil management. The objective of the study was to investigate soil C and N pools and the associated microbial activities in sandy Ultisols after 40 yr of CS and conventional tillage (CT). Soil samples were collected from fields under continuous CS and CT for 40 yr (1979–2018) and subjected to a range of physio-biogeochemical analyses. When compared with CT, CS increased total C, total N, and active C by 35, 45, and 44% at 0-to-5-cm depth, respectively, but not at 5-to-15-cm depth. In contrast, CT had 128 and 121% higher inorganic N and dissolved organic N at 5-to-15-cm depth, which was not observed at 0-to-5-cm depth. Respiratory CO<sub>2</sub> production and organic N mineralization were found to be higher in CS soils than in CT soils at 0–5 cm, but both were higher in CT than CS at 5–15 cm. Concurrently with increased active C concentrations, potential activities of C-cycling enzymes were higher in CS soils than CT soils at 0–5 cm, which was not observed at 5–15 cm. The increased labile C supply stimulated microbial activities in CS soils at 0–5 cm, but at 5–15 cm, the higher N availability increased microbial biomass N and organic N mineralization potentials in CT than CS soils. The contrasting CS and CT impacts on C and N at different soil depths likely reflected the decouple of C and N cycling in the tested soils.

## 1 | INTRODUCTION

Continuous intensive management of agricultural soils, including tillage, changes every perspective of soil characters, resulting in losses of soil biodiversity (Álvaro-Fuentes et al., 2013; Feng et al., 2003), degraded soil structure (Abdollahi

& Munkholm, 2014), decreased soil fertility (Edwards et al., 1992), and rapid decomposition of soil organic C (SOC) (Lal, 2004; Novak et al., 2007), all of which lead to degraded soil health (Ghimire et al., 2015; Lal et al., 2007), impairing the sustainability and productivity of agricultural soils. Conservation agriculture has therefore been widely recommended and adopted to reverse such degradation by improving soil physical (Indoria et al., 2017; Patra et al., 2019), chemical (Ligowe et al., 2017; Parihar et al., 2018; Ranaivoson et al., 2017), and biological properties (Lienhard et al., 2013; Souza et al., 2018).

Conservation tillage (CS) is a key component of conservation agriculture (Pittelkow et al., 2015; Thierfelder et al.,

**Abbreviations:** BD, bulk density; BG,  $\beta$ -glucosidase; BX,  $\beta$ -xylosidase; CBH,  $\beta$ -D-cellubiosidase; CS, conservation tillage; CT, conventional tillage; DON, dissolved organic nitrogen; LAP, leucine aminopeptidase; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; MWD, mean weight diameter; NAG, N-acetyl- $\beta$ -glucoaminidase; Nin, inorganic nitrogen; Nmin, nitrogen mineralization; POXC, permanganate oxidizable carbon; SOC, soil organic carbon; TCtotal carbon; TN, total carbon; TNtotal nitrogen.



2018). When compared with conventional tillage (CT), CS minimizes soil disturbance while maintaining soil structure, providing physical protections for SOC through soil aggregations (Ayoubi et al., 2012; Bronick & Lal, 2005; Hobbs et al., 2008; Pretty & Bharucha, 2014). It also increased SOC stocks and N availability (Franzluebbers, 2010; Jacobs et al., 2009; Sombero & de Benito, 2010) and enhanced microbial biomass (Kabiri et al., 2016; Mbuthia et al., 2015; van Groenigen et al., 2010; Zhang et al., 2012). However, similar tillage research also reported no difference between CS and CT in their influence on soil total C (TC) and N (TN) (Paul et al., 2013), microbial biomass (Govaerts et al., 2007), and organic N mineralization potential (Cookson et al., 2008; Dalal, Allen, et al., 2011; Dalal, Wang, et al., 2011). For instance, Hernanz et al. (2009) found that no-till for 11 yr resulted in 14% higher C sequestration than CT, whereas Halvorson et al. (2002) reported no such difference after a decade of CS and CT management.

In addition to the abovementioned discrepancies, CS often caused C accumulation only in topsoil, promoting C stratification in bulk soils (Cookson et al., 2008; Jacobs et al., 2009; Novak et al., 2007, 2020; Sombero & de Benito, 2010). For instance, Novak et al. (2007, 2020) demonstrated significant SOC increase after adopting reduced-tillage and residue incorporation for 36 yr, which was only observed in the 0-to-5-cm layer. Parajuli et al. (2021) reported an increase of SOC in soil aggregates at 0–5 cm after 40 yr of CS management. In contrast, Al-Kaisi et al. (2005) reported increased SOC and TN at 0-to-15-cm depths after 7 yr of no tillage when compared with chisel plow in Iowa soils. Chivenge et al. (2007) further suggested that, regardless of depth effects, CS practices (i.e., ripping tillage) are more likely to increase SOC in clay soils than in sandy soils.

These observed discrepancies of tillage effects (CS vs. CT) were likely attributable to difference in soil texture (sandy vs. clayey), implementing duration (short-term vs. long-term), sampling depths (topsoil vs. subsurface soils), and management history (Franzluebbers, 2010), necessitating site-specific research. A better understanding of how tillage influences soil C and N dynamics will help to improve SOC and soil health management supporting sustainable agricultural production. In the present study, we utilized an existed long-term (~40 yr) field research aimed to investigate the tillage impacts on SOC dynamics in typical sandy Coastal Plain soils. The research was established and maintained by Coastal Plain Soil, Water, & Plant research Center, USDA-ARS, Florence, SC. A recent study has demonstrated SOC accumulation only at 0-to-5-cm soil depth (Novak et al., 2007, 2020). We therefore extended the study with an objective to quantify how such accumulation affects active C and N pools, microbial biomass and activities, and the potential of SOC decomposition and organic N mineralization at two soil depths (0–5 and 5–15 cm) within the tillage layer (0–15 cm). It was hypothesized that

### Core Ideas

- Conservation tillage increased soil active C concentrations only at 0-to-5-cm depth
- Increased active C stimulated microbial activities at 0–5 cm.
- Conventional tillage improved N availability in soils at 5–15 cm.
- Increased N availability at 5–15 cm induced higher microbial activities.
- Long-term tillage managements resulted in contrasting C and N response at different soil depth.

(a) CS increases microbial biomass and activities (described by enzyme activities and respiration) as a result of increased active C at least at 0-to-5-cm depth, and (b) N availability and mineralization follow the same responsive patterns.

## 2 | MATERIALS AND METHODS

### 2.1 | Site description and soil sampling

The experimental site is located at Pee Dee Research and Education Center of Clemson University at Florence, SC, USA (34°18' N, 79°44' W). The site was established in mid-1979 with an objective to compare the effects of two tillage practices on SOC dynamics—namely, CS (in-row subsoiling to 42-cm-depth soils to remove the hardpan within the E-horizon once before spring cropping) and CT (disking surface 15-cm-depth soils two to three times to incorporate all the residues after in-row subsoiling to 42-cm-depth soils to remove the hardpan within E-horizon once before spring cropping). Annual average temperature for 40 yr (1979–2018) was 17.4 °C with maximum average in July (32.9 °C) and minimum average in December (0.8 °C), and annual mean precipitation for 40 yr was 1,223 mm, respectively (NOAA <https://www.ncdc.noaa.gov>). In 2015, a cover crop (with and without) study was introduced as a split plot design within each tillage treatment. Cover crop treatments included a mixture of rye (*Secale cereale* L.), radish (*Raphanus sativus* L.), and crimson clover (*Trifolium incarnatum* L.) sown after the harvest of main crop. Since the 1980s, the main crop was corn (*Zea mays* L.) rotated with either cotton (*Gossypium* spp.) or soybean [*Glycine max* (L.) Merr.]. The experimental soil is primarily Norfolk loamy sand (fine-loamy, siliceous, thermic Typic Kandiodults) (Novak et al., 2007, 2020; Ye et al., 2020) with 7.6 ± 0.2% clay and 14.1 ± 1.5% silt. Fertilizers and lime were applied according to soil testing results conducted by Agricultural Service Laboratory of Clemson University and

recommendation guidelines provided by Clemson University extension services (Clemson University, 1982). Further detail about sites description, climate, crop rotation, cover cropping, and management practices can be found in Bauer et al. (1997, 2006), Campbell et al. (1984), Hunt et al. (2004), Nash et al. (2018), and Novak et al. (2007, 2020).

Soil samples were collected once in May 2018 after cover crop termination but prior to cash crop planting. Eight cores (0–15 cm) of bulk soil were randomly collected with an AMS core sampler (5-cm diam.) from four replicated tillage main plots covering subplots with and without winter cover crops, despite that the imbedded cover cropping for 2 yr did not change SOC in bulk soils and soil aggregates (Parajuli et al., 2021). Those four plots had corn–soybean rotation for the past few years, and soybean was the main crop in 2017. Collected soil cores were cut into two sections (0–5 and 5–15 cm), sieved (2 mm), and stored at 4 °C until use.

## 2.2 | Soil physiochemical properties

During soil sampling, two subsamples from each plot were also taken for bulk density (BD) using the AMS soil core sampler (5 cm in diameter, 15-cm depth). Soils were sectioned at 0–5 and 5–15 cm and oven dried at 60 °C until constant weight was attained. The BD was calculated as dried soil mass per unit volume ( $\text{g cm}^{-3}$ ). Soil particle size distributions were estimated via micropipette method (Miller & Miller, 1987). Moisture content was analyzed as loss of mass after oven drying at 60 °C until constant weight was attained. Wet soil aggregate stability was measured via wet sieving (Márquez et al., 2004; Six, Elliott, & Paustian, 2000; Six, Paustian, et al., 2000). Mean weight diameter (MWD) was calculated after correcting for sand content (Márquez et al., 2004). Soil pH was analyzed with an Orion 8107 pH probe (Thermo Scientific) with deionized water (1:1 ratio) after equivalent for 30 min. Soil TC and TN were analyzed using oven-dried grounded samples with a Carlo-Erba NA 1500 CNS analyzer (Haak-Buchler Instruments). The soil is acidic ( $\text{pH} < 6.0$ ) and has low  $\text{CaCO}_3$  contents (Novak et al., 2007); hence, TC was considered SOC in this study. Extractable N was estimated by colorimetric method after extracting soils with 1 M KCl for 1 h and centrifuged (1,007g, 10 min) followed by filtration from Whatman 42 filter paper. Filtrates were analyzed for  $\text{NH}_4^+$  (Verdouw et al., 1978) and  $\text{NO}_3^-$  (Doane & Horwáth, 2003). Permanganate oxidizable C (POXC), the active C, was measured as mentioned by Culman et al. (2012) and Lucas and Weil (2012).

## 2.3 | Soil biological properties

Microbial biomass C (MBC) and N (MBN) were measured by the fumigation method (Voroney et al., 2008). The MBC was

the difference in 0.5 M  $\text{K}_2\text{SO}_4$  extractable C between fumigated and unfumigated samples using a conversion factor of 0.37, whereas MBN was calculated similarly using a conversion factor of 0.54. Dissolved organic N (DON) was measured as the difference between in  $\text{NO}_3^-$ -N between the digested and undigested unfumigated samples (Voroney et al., 2008). Microbial respiration was measured as 24 h  $\text{CO}_2$  production in mason jar incubated with 30 g of field soil at room temperature (Bridgham & Ye, 2013). The headspace  $\text{CO}_2$  concentration was measured at the end of incubation with a gas chromatograph (Shimadzu). The  $\text{CO}_2$  production was expressed as function of soil over time ( $\text{mg CO}_2\text{-C kg}^{-1}$  dry soil  $\text{d}^{-1}$ ). Organic N mineralization (Nmin) was determined based on a 7-d incubation followed by extraction with 1 M KCl and analysis for  $\text{NH}_4^+$  (Cadisch et al., 1996). The C-cycling ( $\beta$ -D-cellubiosidase [CBH],  $\beta$ -glucosidase [BG], N-acetyl- $\beta$ -glucoaminidase [NAG], and  $\beta$ -xylosidase [BX]) and N-cycling (leucine aminopeptidase [LAP] and NAG) enzymes were measured as described by Ye et al. (2019). Enzymatic activity was determined by calculating the mean fluorescence reading change over 24 h with a calibration curve.

## 2.4 | Data analysis

The data were examined for heterogeneity and normality with residue plots, and log-transformed when such transformation improved the normality (including MBC, MBN, extracellular enzymatic activities, respiration, and Nmin). Two-way ANOVA was used to determine the main effect of tillage, depth, and their interaction at  $\alpha = .05$ . Post-hoc analysis using Student's *t* test was further performed to detect significant difference at various treatment levels. Pairwise correlation analysis was done to study the associations between measured variables. All the statistical analysis was performed in JMP Pro 14.3 (SAS Institute).

# 3 | RESULTS

## 3.1 | Soil physiochemical properties

No-tillage effects were observed in BD ( $1.30\text{--}1.52 \text{ g cm}^{-3}$ ), which was affected by depth with higher BD at 5–15 cm ( $1.48 \text{ g cm}^{-3}$ ) than at 0–5 cm ( $1.36 \text{ g cm}^{-3}$ ) (Tables 1 and 2). Similarly, only depth effects were observed in MWD (Tables 1 and 2). No-tillage and depth effects were found in soil pH (Tables 1 and 2). The interaction of tillage and depth affected TC, TN, POXC, DON,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , and inorganic N (Nin,  $\text{NO}_3^-$  plus  $\text{NH}_4^+$ ) (Table 1). At 0–5 cm, CS had higher TC, TN, and POXC than CT, whereas such affects were not observed at 5–15 cm (Table 2, Figure 1). In contrast, at 5–15 cm, Nin and DON were higher in CT than CS soils, but such difference was not found at 0–5 cm (Table 2, Figure 1).

**TABLE 1** The ANOVA results of tillage and depth effects and their interaction on measured soil variables

Variable	p value		
	Tillage	Depth	Tillage × depth
<b>Physiochemical</b>			
Bulk density, g cm <sup>-3</sup>	.8941	<b>.0053*</b>	.3485
Moisture, %	.3185	<b>.0452*</b>	.0509
Mean weight diameter, mm	.1411	<b>.0039*</b>	.1102
pH	.6639	.0712	.0560
Total C, g kg <sup>-1</sup>	.1842	<b>&lt;.0001*</b>	<b>.0228*</b>
Total N, g kg <sup>-1</sup>	<b>.0253*</b>	<b>&lt;.0001*</b>	<b>&lt;.0001*</b>
Permanganate oxidizable C, mg kg <sup>-1</sup>	.5022	<b>&lt;.0001*</b>	<b>.0032*</b>
Dissolved organic N, mg kg <sup>-1</sup>	<b>.0006*</b>	<b>&lt;.0001*</b>	<b>.0002*</b>
Ammonium ion, mg kg <sup>-1</sup>	.9879	.1842	<b>.0224*</b>
Nitrate ion, mg kg <sup>-1</sup>	<b>&lt;.0001*</b>	<b>&lt;.0001*</b>	<b>&lt;.0001*</b>
Inorganic N, mg kg <sup>-1</sup>	<b>&lt;.0001*</b>	<b>&lt;.0001*</b>	<b>&lt;.0001*</b>
<b>Biological</b>			
Microbial biomass C, mg kg <sup>-1</sup>	.6663	<b>&lt;.0001*</b>	.1323
Microbial biomass N, mg kg <sup>-1</sup>	.5020	.3439	<b>.0003*</b>
Respiration, mg C g <sup>-1</sup> d <sup>-1</sup>	.7251	<b>&lt;.0001*</b>	<b>.0012*</b>
Organic N min., mg N g <sup>-1</sup> d <sup>-1</sup>	.2130	<b>&lt;.0001*</b>	<b>.0001*</b>
β-D-cellubiosidase, mg MUB g <sup>-1</sup> h <sup>-1</sup>	.1110	<b>&lt;.0001*</b>	.1054
N- acetyl-β-glucoaminidase, mg MUB g <sup>-1</sup> h <sup>-1</sup>	.8672	<b>&lt;.0001*</b>	<b>.0004*</b>
Leucine aminopeptidase, mg MUC g <sup>-1</sup> h <sup>-1</sup>	.5164	<b>&lt;.0001*</b>	.1288
β-glucosidase, mg MUB g <sup>-1</sup> h <sup>-1</sup>	.0699	<b>&lt;.0001*</b>	<b>.0127*</b>
β-xylosidase, mg MUB g <sup>-1</sup> h <sup>-1</sup>	.3001	<b>&lt;.0001*</b>	.1733

Note. Inorganic N, nitrate plus ammonium; MUB, 4-methylumbeliferone; MUC, 7-amino-4-methylcoumarin.

\*Significant at the .05 probability level.

**TABLE 2** Soil physiochemical properties in a Norfolk Ultisols under 40-yr conservation (CS) and conventional tillage (CT) at 0-to-5-cm and 5-to-15-cm depths

Variable	0–5 cm		5–15 cm	
	CS	CT	CS	CT
BD, g cm <sup>-3</sup>	1.34 ± 0.04(b)	1.38 ± 0.03(b)	1.50 ± 0.04(a)	1.46 ± 0.04(a)
MWD, mm	0.28 ± 0.03(b)	0.28 ± 0.02(b)	0.33 ± 0.04(b)	0.42 ± 0.04(a)
pH	5.6 ± 0.10(b)	5.8 ± 0.05(ab)	5.9 ± 0.07(a)	5.8 ± 0.06(ab)
TC, g kg <sup>-1</sup>	13.6 ± 1.2(a)	10.1 ± 0.6(b)	6.57 ± 1.2(c)	7.54 ± 0.7(bc)
TN, g kg <sup>-1</sup>	1.18 ± 0.09(a)	0.82 ± 0.03(b)	0.46 ± 0.04(c)	0.59 ± 0.02(c)
Nin, g kg <sup>-1</sup>	11.1 ± 1.0(b)	11.6 ± 0.7(b)	10.5 ± 0.9(b)	23.5 ± 1.6(a)

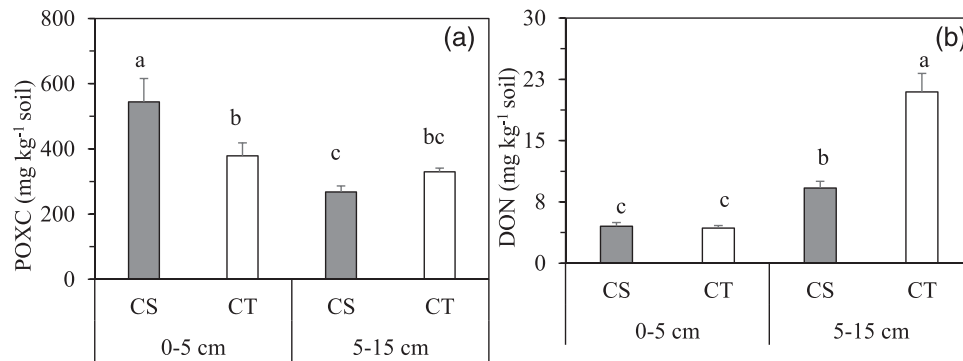
Note. BD, bulk density; MWD, mean weight diameter; TC, total C; TN, total N; Nin, inorganic N (NO<sub>3</sub><sup>-</sup> plus NH<sub>4</sub><sup>+</sup>). Values are means (n = 4) with one standard error. Different letters in parentheses indicate significant difference at α = .05 within a given measured variable.

### 3.2 | Soil biological properties

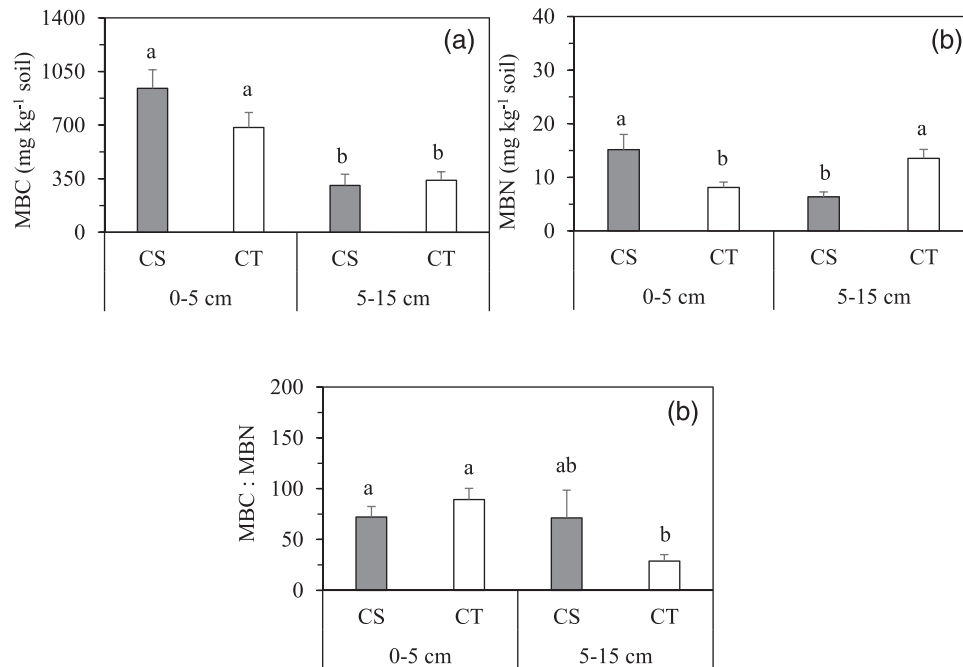
The MBC concentrations were no different between CS and CT soils at both depths, but they were higher at 0-to-5-cm depth than 5-to-15-cm depth (Table 1, Figure 2). Interaction of tillage and depth effects were significant for MBN, respiration, and Nmin (Table 1). At 0–5 cm, CS soils had

higher MBN than CT soils, but it was higher in CT than in CS soils at the depth of 5–15 cm (Figure 2). At 0–5 cm, both CO<sub>2</sub> production and Nmin were higher in CS than CT soils, but at 5–15 cm, CT soils had higher values than CS soils (Figure 3).

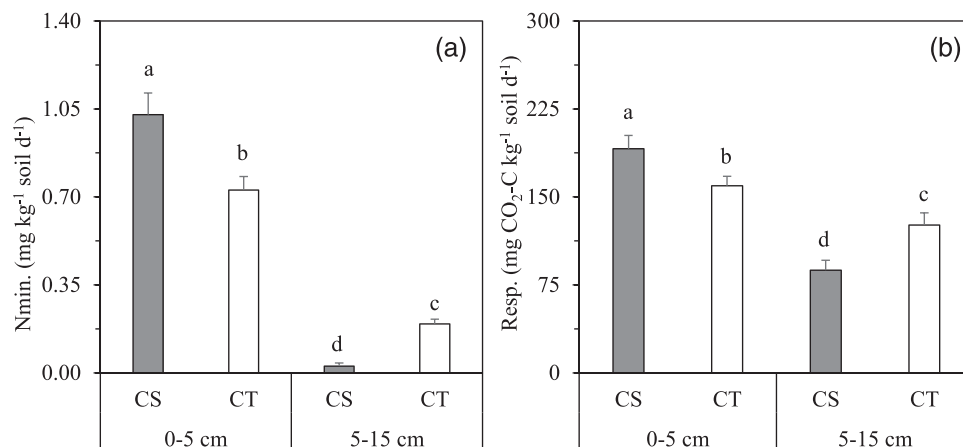
Significant interactions of the two main factors were observed for BG and NAG potentials (Table 1). Regardless of



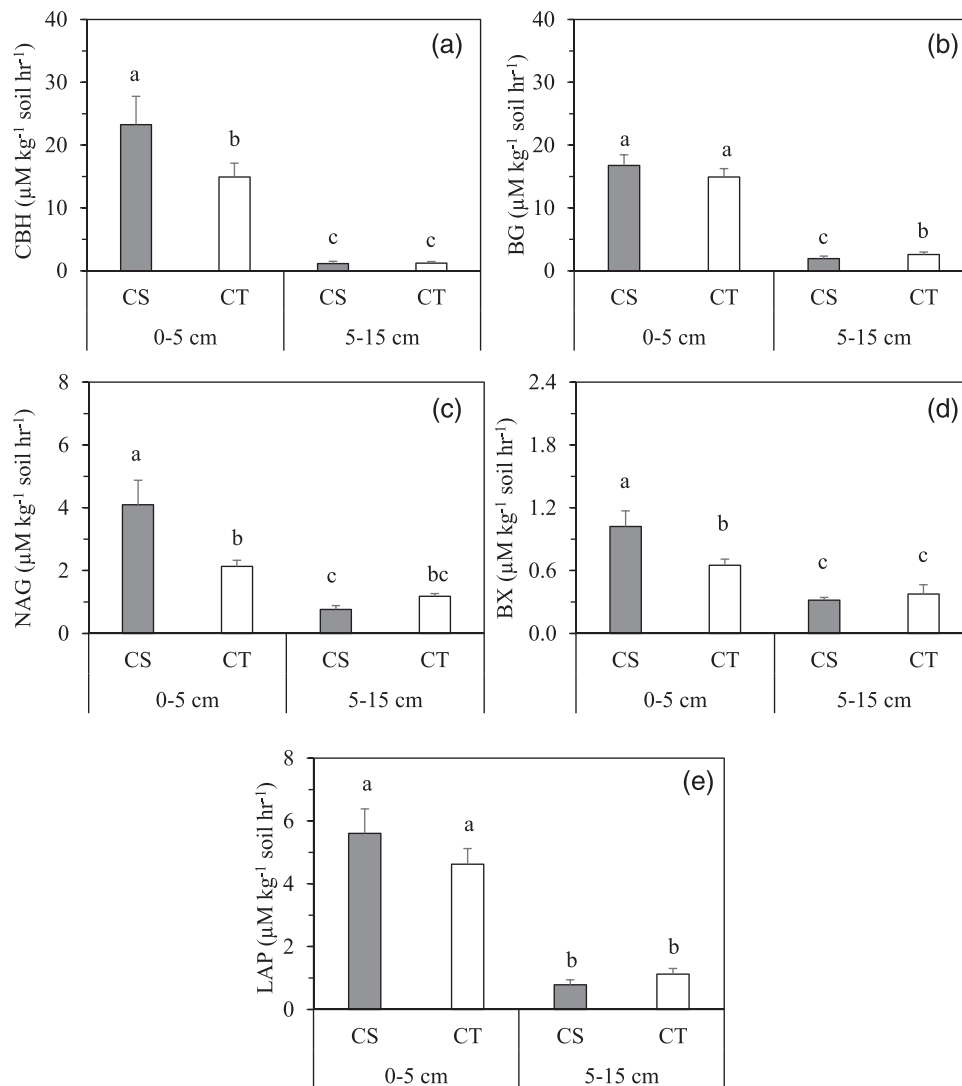
**FIGURE 1** (a) Permanganate oxidizable C (POXC) and (b) dissolved organic N (DON) in soils (0–5 and 5–15 cm) under 40-yr conservation (CS) and conventional tillage (CT). Different letters represent significant difference at  $\alpha = .05$



**FIGURE 2** (a) Soil microbial biomass C (MBC), (b) N (MBN), and (c) MBC/MBN in soils (0–5 and 5–15 cm) under 40-yr conservation (CS) and conventional tillage (CT). Different letters represent significant difference at  $\alpha = .05$



**FIGURE 3** (a) Organic N mineralization potentials (Nmin) and (b) respiratory CO<sub>2</sub> production (Resp.) in soils (0–5 and 5–15 cm) under 40-yr conservation (CS) and conventional tillage (CT). Different letters represent significant difference at  $\alpha = .05$



**FIGURE 4** (a) Potential activities of  $\beta$ -D-cellobiosidase (CBH), (b)  $\beta$ -glucosidase (BG), (c) N- acetyl- $\beta$ -glucoaminidase (NAG), (d)  $\beta$ -xylosidase (BX), and (e) leucine aminopeptidase (LAP) in soils (0–5 and 5–15 cm) under 40-yr conservation (CS) and conventional tillage (CT). Different letters represent significant difference at  $\alpha = .05$

the tillage type, all enzyme activities were found to be higher at 0–5 cm than at 5–15 cm (Figure 4). At 0–5 cm, all C-cycling enzymes, except BG, exhibited higher activities in CS soils than CT soils, which was not found at 5–15 cm.

### 3.3 | Correlations between measured variables

At 0–5 cm, POXC was positively correlated with TC, TN, MBC, MBN, Nmin, and C-cycling (CBH, BG, NAG, BX) enzymes (Table 3). The DON was positively correlated with CBH activity only (Table 3). At 5–15 cm, POXC was positively correlated with TC, TN, MBN, Nin, DON, Nmin, respiration, and N-cycling enzyme (LAP) activity, whereas no correlations with C-cycling (CBH, BG, NAG, BX) enzymatic

activity were observed (Table 4). The DON was positively associated with TC, TN, POXC, Nin, MBN, respiration, and Nmin (Table 4).

## 4 | DISCUSSION

Increasing SOC and nutrient availability is a major focus of sustainable soil managements (Lal, 2016). In the present study we quantified C and N processes in a typical Coastal Plain Ultisols that was under continuous CS and CT management for nearly 40 yr (Novak et al., 2020). The results demonstrated that when compared with CT, CS increased active C (i.e., POXC) concentrations and microbial activities (i.e., enzymatic activities and respiration) at 0-to-5 cm soils, concurrently with increase TC (Novak et al., 2020) (supporting

**TABLE 3** Pairwise correlation of the measured variables in the 0-to-5-cm soil layer. Values are correlation coefficient *R* with designated significant values

Variable	TN	POXC	Nin	MBC	MBN	DON	Nmin.	Resp.	CBH	NAG	LAP	BG	BX
TC	.94**	.75**	.40	.82**	.64**	.33	.76**	.37	.52*	.66**	.38	.39	.69**
TN		.74**	.33	.70**	.74**	.16	.84**	.51*	.49	.62**	.39	.38	.73**
POXC			.09	.50*	.49*	.38	.68**	.32	.85**	.76**	.23	.58**	.66**
Nin				.35	.28	.04	.08	-.20	-.02	.11	-.16	.07	.26
MBC					.59*	.42	.62**	.43	.27	.33	.63**	.23	.50*
MBN						-.10	.84**	.72**	.21	.36	.70**	.14	.59*
DON							.16	-.04	.52*	.34	.10	.35	.38
Nmin								.71**	.50*	.57*	.63**	.42	.73**
Resp.									.19	.30	.82**	.07	.57*
CBH										.84**	.06	.82**	.60*
NAG											.11	.61**	.61**
LAP												.03	.48
BG													.54*

Note. TC, total C; TN, total N; POXC, permanganate oxidizable C; Nin, inorganic N; MBC, microbial biomass C; MBN, microbial biomass N; DON, dissolved organic N; Nmin, organic N mineralization; Resp., respiration; CBH,  $\beta$ -D-cellulobiosidase; BG,  $\beta$ -glucosidase; NAG, N- acetyl- $\beta$ -glucoaminidase; BX,  $\beta$ -xylosidase; LAP, leucine aminopeptidase.

\*Significant at the .05 probability level.

\*\*Significant at the .01 probability level.

**TABLE 4** Pairwise correlation of the measured variables in the 5-to-15-cm soil layer. Values are correlation coefficient *R* with designated significant values

Variable	TN	POXC	Nin	MBC	MBN	DON	Nmin.	Resp.	CBH	NAG	LAP	BG	BX
TC	.83**	.69**	.46	.23	.02	.51*	.22	.58*	-.38	-.18	.32	-.34	-.27
TN		.89**	.73**	.14	.36	.73**	.64**	.85**	-.16	.16	.53*	.00	-.17
POXC			.76**	.03	.49*	.79**	.68**	.89**	-.21	.08	.69**	.06	-.01
Nin				.22	.64**	.96**	.85**	.72**	-.26	.37	.37	.00	-.09
MBC					-.12	.18	.14	-.00	.22	-.02	-.45	.19	-.50*
MBN						.64**	.73**	.42	-.02	.40	.22	.13	.34
DON							.83**	.78**	-.34	.30	.45	-.06	-.14
Nmin								.76**	.11	.47	.43	.36	-.00
Resp.									-.10	.20	.79**	.21	-.16
CBH										.41	-.20	.88**	.28
NAG											.07	.39	.41
LAP												.13	.09
BG													.16

Note. TC, total C; TN, total N; POXC, permanganate oxidizable C; Nin, inorganic N; MBC, microbial biomass C; MBN, microbial biomass N; DON, dissolved organic N; Nmin, organic N mineralization; Resp., respiration; CBH,  $\beta$ -D-cellulobiosidase; BG,  $\beta$ -glucosidase; NAG, N- acetyl- $\beta$ -glucoaminidase; BX,  $\beta$ -xylosidase; LAP, leucine aminopeptidase.

\*Significant at the .05 probability level.

\*\*Significant at the .01 probability level.

the Hypothesis 1). However, such increases were not observed at 5-to-15-cm (not supporting the Hypothesis 2). Instead, CS had lower N availability (both Nin and DON), Nmin, and microbial respiration than CT at 5-to-15-cm soils, suggesting potential decouple of C and N cycling caused by long-term tillage managements.

#### 4.1 | Tillage impacts on soil physical processes

Intensive tillage destroys soil structure breaking soil aggregates (Pretty & Bharucha, 2014). Many researches have demonstrated that conservation management improves soil



structure and MWD (Ayoubi et al., 2012; Sithole et al., 2019; Singh et al., 2020). However, in the present study, 40-yr CS did not improve aggregate stability and BD, when compared with CT (Table 2). The intrinsically low clay and silt content of the tested soils likely resulted in this insignificant result, which supports the concept that CS has little to no effect in improving soil structure, protecting, or building SOC in sandy soils (Chivenge et al., 2007). The experimental site is located at coastal region with hot and humid climate favoring rapid decomposition of residues (i.e., losses of organic materials) impeding the formation of aggregates (Six et al., 2002). In addition, local climate is also characterized by frequent heavy rainfalls along with strong winds and seasonal hurricanes, which have been suggested to destabilize the poorly aggregated sandy soil (Chivenge et al., 2007; McIntyre, 1958).

## 4.2 | Tillage impacts on soil C and N pools

Conservation tillage has often resulted in C accumulations in topsoils (i.e., C stratification) (Cookson et al., 2008; Jacobs et al., 2009; Novak et al., 2007, 2020; Sombrero & de Benito, 2010), which has also been observed in soils used in the present study (Novak et al., 2007, 2020). It is therefore not surprising to observe similar accumulation pattern of POXC in the present study (Figure 1). The POXC is a readily available C pool for microbial biomass and sensitive to management (Culman et al., 2012; Tirol-Padre & Ladha, 2004). It is widely considered as a good indicator for soil health (Fine et al., 2017). We observed that increased POXC induced microbial activities (Figures 2, 3, and 4), which has also been reported in other studies (Hurisso et al., 2016; Plaza-Bonilla et al., 2014). Our findings were further supported by the association of POXC with MBC, Nmin, and enzymatic activities at 0-to-5-cm depth soils (Table 3).

Nitrogen is an integral component of soil organic matter, and its dynamics often couples with that of SOC (Lal, 2014). Positive correlation between TN and TC has been widely documented (Tong et al., 2009), which was also observed in the present study (Tables 3 and 4). A few studies have reported increase in TN under CS when compared with CT (Anders et al., 2012; Halpern et al., 2010; Van Eerd et al., 2014), whereas Dalal, Allen, et al. (2011) and Dalal, Wang, et al. (2011) found no such difference. In the present study, soils in the 0-to-5-cm layer had higher TN and POXC than those at 5–15 cm, whereas both DON and Nin were higher at 5–15 cm than at 0–5 cm (Table 1; Figure 1). Increased Nin and DON at subsurface soil have been reported (Halvorson et al., 2001; Hu et al., 2019; Matthews et al., 2000). Walmsley et al. (2018) explained this phenomenon as N leaching from surface to subsurface. Sandy soils with poor structure, like the tested soils in the present study, often have low nutrient holding capacity (i.e., high leaching potentials) leading to accumulation of N

in subsurface soils. The higher inorganic and organic N in CT soils at 5–15 cm (Table 2, Figure 1) were also likely a result of plant residue incorporation by disk tillage.

## 4.3 | Tillage impacts on microbial activities

Microbial biomass and their activities are often regulated by C substrate and nutrient availability (Galloway et al., 2008). It is therefore not surprising that the increased POXC at the 0–5 cm was accompanied with increased respiration, activities of C-cycling enzymes, and Nmin rates in CS soils when compared with CT soils, which was not observed at 5–15 cm (Figures 2, 3, and 4). Nonetheless, insignificant impacts of CS on microbial biomass and activities have also been documented suggesting possible confounding effects of other factors including nutrient availability. Sun et al. (2016) reported no difference in MBC between no-till and CT prior to spring crop planting (April), but the difference was significant during crop growing season (June) and the harvest (September). Similarly, fertilization increased microbial biomass in surface soil (Hao et al., 2008). In the present study, the MBC to MBN ratio, ranging from 28 to 89 (Figure 2), suggested concurrent limitations of N on microbial communities in the tested soil (Curtis et al., 2004; Ostrowska & Porębska, 2015; Zhang et al., 2015). The higher MBN, Nmin, and microbial respiration rates in the CT soils at 5–15 cm were likely due to their higher N availability when compared with CS soils at the same soil depth (Table 1, Figures 2 and 3). The observed different impacts of CS and CT at different soil depths reinforce the concept that tillage can influence the pool size and transformation of C and N in soils through its impacts on microbial biomass and vice versa (Martín-Lammerding et al., 2015; Vazquez et al., 2017).

Hydrolytic enzymes catalyze nutrient regeneration and C transformation in soils (Jin et al., 2009). These enzymes are sensitive to change in soil properties (Caravaca et al., 2002) and useful indicator of soil management (Bandick & Dick, 1999). In the present study, like POXC concentrations, potential activities of the C-cycling enzymes were higher in CS than CT at 0–5 cm, concurrently with microbial biomass and respiration rates (Table 3, Figures 1, 2, 3 and 4). Similar results were also reported by other studies (Zuber & Villamil, 2016). Enzymatic activities under CS management were regulated either by the substrate availability (Cotrufo et al., 2013; Nivellet et al., 2016) or reduced disturbance on microbial metabolic activities (Ciccolini et al., 2015; Huang et al., 2013). Minimal soil disturbance and residue returns at surface soil were believed to induce the enzymatic activities on topsoils (Chen et al., 2019; Piazza et al., 2020). Nonetheless, when accounting for MBC concentration (i.e., the specific enzymatic activities), no difference was observed between the CS and CT at both soil depths (data not shown),

suggesting that the observed increased activities were largely due to increased microbial biomass at 0–5 cm, but not substrate use efficiency (Bonner et al., 2018; Ye et al., 2009).

#### 4.4 | Contrasting tillage impacts on C and N cycling at different soil depths

Both C and N are integral component of soil organic matter (Gårdenäs et al., 2011). It is therefore often expected that organic N mineralization is closely coupled with SOC decomposition in terrestrial ecosystems (Thornton et al., 2007). However, decoupled C and N dynamics in soils have also been documented, which was largely explained by contrasting bioavailability of C substrate and nutrient (Bimüller et al., 2014; Tian et al., 2016). In the present study, CS increased POXC concentrations in top 5-cm soil layer, resulting in higher microbial activity (Tables 3 and 4; Figures 3 and 4), whereas higher N availability in CT soil in the 5-to-15-cm soil layer led to increased MBN, Nmin, and respiration (Tables 3 and 4; Figures 2 and 3), demonstrating distinct CS and CT impacts at different soil depths. Distinct microbial responses to increased labile C and N pools at different soil depths supported varied disruptions of C and N cycling caused by different tillage managements. Higher C and N mineralization under CS as compared with CT have been often reported (Anders et al., 2012; Halpern et al., 2010; Van Eerd et al., 2014). Vazquez et al. (2019) found higher C and N mineralization under no-till in the top 5-cm soil layer compared with CT, whereas both were higher in CT in the 5-to-10-cm soil layer than no-till in Ultisols of pasture fields. The results also reinforced that C and nutrient availability regulate microbial biomass and activities affecting C and N cycling (Galloway et al., 2008; Schlesinger, 2009).

## 5 | CONCLUSIONS

The 40-yr CS management, when compared with CT, increased TC and TN only in topsoil (0–5 cm), which was accompanied by increased active C (i.e., POXC) concentrations, resulting in higher C-cycling enzymes activities, microbial respiration, and organic N mineralization potentials. In contrast, 40-yr CT management increased both organic and inorganic N concentrations at 5–15 cm when compared with CS. The increased N availability in CT soils at 5–15 cm resulted in higher MBN, respiration rates, and organic N mineralization potentials. The contrasting impacts of CS and CT on soil C and N pools and the associated microbial activities at different depths suggested potential decouples of C and N cycling in the tested sandy soils after long-term soil management distinguished by tillage types.

## AUTHOR CONTRIBUTIONS

Binaya Parajuli: Data curation; Formal analysis; Investigation; Methodology; Software; Visualization; Writing-original draft. Rongzhong Ye: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Visualization; Writing-review & editing. Min Luo: Software; Supervision; Writing-review & editing. Thomas F. Ducey: Project administration; Resources; Supervision; Visualization; Writing-review & editing. Dara Park: Supervision; Writing-review & editing. Matthew Smith: Project administration; Supervision; Writing-review & editing. Gilbert Sigua: Formal analysis; Resources; Writing-review & editing.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ORCID

Binaya Parajuli  <https://orcid.org/0000-0002-1067-5947>  
 Rongzhong Ye  <https://orcid.org/0000-0003-3366-317X>  
 Thomas F. Ducey  <https://orcid.org/0000-0001-8199-0584>  
 Gilbert Sigua  <https://orcid.org/0000-0001-5187-1883>

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**How to cite this article:** Parajuli B, Ye R, Luo M, Ducey TF, Park D, Smith M, & Sigua G. Contrasting carbon and nitrogen responses to tillage at different soil depths: An observation after 40-year of tillage management. *Soil Sci Soc Am J*. 2021;85:1256–1268. <https://doi.org/10.1002/saj2.20277>