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Long-term residue removal under tillage decreases *amoA*-nitrifiers and stimulates *nirS*-denitrifier groups in the soil

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ABSTRACT

No-till in continuous corn (*Zea mays* L.) production helps to keep an important volume of residues on the soil surface, creating management challenges that could be alleviated by residue removal for bioenergy or animal use. Crop residues, however, are essential to stimulate microbial nutrient cycling in agroecosystems. Thus, both residue removal and tillage options need to be fully evaluated for their impacts on ecosystem services related to soil health, including microbial N cycling. We explored the main steps of the microbial N cycle in relation to soil properties by using targeted gene abundance as a proxy following over a decade of residue removal in continuous corn systems either under no-till or chisel tillage. We used real-time quantitative polymerase chain reaction (qPCR) for the quantification of phylogenetic groups and functional gene screening of the soil microbial communities, including genes encoding critical enzymes of the microbial N cycle: *nifH* (N_2 fixation), *amoA* (nitrification – ammonia oxidation), *nirK* and *nirS* (denitrification – nitrite reduction), and *nosZ* (denitrification – nitrous oxide reduction). Our results showed that long-term residue removal and tillage decreased soil organic matter (SOM), water aggregate stability (WAS), and the relative abundance (RA) of ammonia-oxidizing bacteria (AOB) carrying nitrifying *amoA* genes. Denitrifiers carrying *nirS* genes decreased under no-till as crop residue was removed. In addition, our results evidenced strong correlations among soil properties and phylogenetic groups of bacteria, archaea, and fungi. Overall, this study demonstrated limited but definite impacts of residue management and tillage on the soil environment, which could be exacerbated under less resilient conditions.

1. Introduction

Soil degradation from anthropogenic sources, including conventional intensive agriculture, threatens food production around the globe (FAO and ITPS, 2015). Soil chemical imbalance from excessive N fertilizer input is one source of soil degradation (Belay et al., 2002; Khan et al., 2007; Schroder et al., 2011). Excessive fertilizer input also imposes environmental and societal costs through eutrophication of water sources (Selman et al., 2008), elevated nitrate pollution in drinking water (Pennino et al., 2017; Zhang et al., 2018), and higher greenhouse gas (GHG) emissions (Fowler et al., 2013). A notable example of such impacts in the US is the seasonal hypoxic zone that forms in the Gulf of Mexico due in large part to excess N from the agricultural inputs that ultimately drain into the Mississippi River Basin (EPA, 2017). In the US, the Midwest region is one of the most intensively cultivated areas and is the country's top producer of corn and soybean. Nitrogen fertilizers are applied at high rates in this region, but this excess nitrogen is underutilized by crops (Mulholland et al., 2008). Not surprisingly, the Midwest region is a major contributor of N leaching into the Gulf of Mexico (White et al., 2014).

Excess N and its leaching are largely regulated by the soil N cycle and crop N utilization efficiency (Hirsch and Mauchline, 2015), and thus mitigating excess N requires a detailed understanding of these processes. As important is the question of how agricultural operations, including timing and applications of fertilizer and tillage practices, affect soil N cycling. While directly measuring the products of each step in the soil biological N cycle may provide clues to gauge overall impacts of a particular agricultural practice, investigating soil microbial functional genes known to drive N cycling processes is necessary to understand these impacts on a mechanistic level (Hirsch and Mauchline, 2015).

Maintaining crop residue provides various benefits for soil health, such as increased soil organic C (Lemke et al., 2010; Stetson et al.,

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2012), physical protection from erosion (Beniston et al., 2015), and enhanced soil aggregate stability (Karlen et al., 1994). Research by Henderson et al. (2010), Németh et al. (2014), and Bent et al. (2016) assessed the effects of residue management on soil health and found that the microbial abundance, richness, and community structure differed significantly between residue removal and retention, where residue retention promoted more microbial abundance. Meanwhile, tillage is a central part of residue management because it determines the amount and condition of the crop residue. For example, crop residue remains aboveground with intact physical structures under no-till management, while some tillage methods incorporate crop residue into the soil. Residue retention and conservation tillage together enhance soil properties such as soil organic C (Beniston et al., 2015; Dendooven et al., 2012; Sindelar et al., 2015), soil microbial community diversity and richness (Ceja-Navarro et al., 2010), and soil microbial biomass C and N (Govaerts et al., 2007; Kushwaha et al., 2000). Moreover, studies from Németh et al. (2014) and Segal et al. (2017) found significant residue removal and tillage effects on some soil N cycling functional genes, thereby alluding the need to evaluate these practices in terms of important soil N cycle functional genes.

To our knowledge, there has not been a comprehensive long-term study that investigated the effects of both residue removal and tillage on the soil properties and the critical soil N cycling functional genes. Our goal, therefore, was to fill that knowledge gap to improve our understanding of microbial N processes in terms of soil health within a highly relevant U.S. agroecosystem. We used a data reduction technique in combination with linear mixed models and correlation analyses to assess the effects of residue removal, tillage, and their interaction on soil properties and the soil microbiome in a system that spans a decade of treatments under continuous corn production. We identified soil properties and microbial functional genes that responded sensitively to these practices and found significant correlations between them. Ultimately, we sought to understand the implications of these correlations on the soil microbial N cycle. Our results will advance understanding how residue removal and tillage affect soil health and inform best practices specific to continuous corn production systems in high fertility soils of temperate regions.

2. Materials and methods

2.1. Experimental site description

Field experiments were established in the fall of 2005 following uniformly cropped corn at the University of Illinois Crop Sciences Research Center located at Urbana, IL (40°6′ N, 88°12′ W). The site was part of a multilocation study to evaluate levels of residue removal, N fertilization, and tillage practices on crop yields (Coulter and Nafziger, 2008) and soil properties (Villamil et al., 2015; Villamil and Nafziger, 2015) under continuous rainfed corn production. The experimental site was on Flanagan silt loam (fine, smectitic, mesic Aquic Argiudolls) soil series, a deep, somewhat poorly drained soil that formed in loess on nearly flat terrain (Soil Survey Staff, 2019). Mean annual cumulative precipitation amounts to 1051 mm yr^{-1}, with a mean annual temperature of 10.9 °C (30-yr averages 1981–2010).

2.2. Treatments and cultural practices

Four of the original treatments at the site were selected for the current study resulting in a split-plot arrangement of residue removal (RR) and tillage (T) treatments in a randomized complete block design with four replications. Main plots consisted of one of two levels of corn residue removal (RR: full and none) in early November after grain harvest, with full removal accomplished by chopping stalks and raking them off the plots. Split plots of 24 x13m consisted of two tillage systems (T: chisel tilled and no-till). Primary tillage was conducted with a chisel plow to a depth of 25 cm during the fall after crop harvest each year. A detailed description of treatment implementation and plot management is available in Coulter and Nafziger (2008). Corn was planted in April or May each year in 76 cm rows at a rate of 75 to 85,000 seeds ha^{-1}. Nitrogen fertilization of corn occurred in the spring either at planting or before the crop reached the 5-leaf stage as incorporated urea ammonium nitrate solution (UAN 28%) at a rate of 202 kg N ha^{-1}. Nitrogen fertilization on no-till plots occurred at planting. Additional P and K fertilizer and lime were applied occasionally to the entire experimental area as necessary based on soil test results and did not differ based on residue removal or tillage option. Fertilizer and pest management decisions for all plots were based on best management practices for the site according to the Illinois Agronomy Handbook (Nafziger, 2009).

2.3. Soil sampling and determinations

Sampling protocols and determinations generally followed Huang et al. (2019) which reported results from one of three long-term experiments we are currently exploring to assess the soil microbiome responses to agronomic practices. Briefly, soil samples were taken after harvest in October 2015 and 2016, following 10 and 11 years respectively since the start of the experiment. Three composited subsamples (~500 g each) per plot to a depth of 10 cm were taken with an Eijelkamp grass plot sampler (Eijkelkamp Agrisearch Equipment, Netherlands) for the analyses of soil properties and soil DNA. Field moist subsamples were analyzed for available N ($NO3^-$ and $NH4^+$ in mg kg^{-1}) using KCl extraction (1:5 soil to solution) followed by flow injection analysis with a SmartChem 200 (Westco Scientific Instruments, Inc., Danbury, CN, USA). Samples were air-dried and sieved, and three subsamples of the 1- to 2-mm soil fraction were used to determine WAS (%) with an Eijkelkamp wet sieving apparatus using the procedure developed by Kemper and C (1986). Remaining soil was sent to a commercial laboratory (Brookside Laboratories, Inc., New Bremen, OH) to determine soil organic matter (SOM, %) by loss on ignition; soil pH (1:1 soil:water) via potentiometry; available phosphorus (P, mg kg^{-1}) with Bray I extraction; macronutrients (K, Ca, Mg, S) and micronutrients (B, Fe, Mn, Cu, Zn), as well as Na and Al levels, via Mehlich III extraction (expressed in mg kg^{-1}); and cation exchange capacity (CEC, cmol kg^{-1}) by the summation method of exchangeable cations (Ca, Mg, K, Na, and H). Results from laboratory analyses and determinations were averaged to get one value per plot per block.

2.4. Soil DNA extraction and real time quantitative PCR analysis

A detailed description of soil DNA extraction procedures and quantitative PCR analyses are provided in Huang et al. (2019). Soil DNA was extracted using PowerSoil® DNA isolation kits (MoBio Inc., Carlsbad, CA) following the manufacturer's instructions. The purity and quantity of the extracted DNA were checked with a Nanodrop1000 Spectrophotometer (Thermo Fisher Scientific, USA) and with electrophoresis using a 1.5% agarose gel. Soil DNA concentrations were between 10 and 40 ng/μl with A260/A280 values between 1.7 and 2.0. DNA samples were stored at −20 °C immediately after extraction. Quantification of marker gene abundance was obtained via a Fluidigm BioMark HD™ System at the Roy Carver Biotechnology Center for Functional Genomics lab at the University of Illinois at Urbana-Champaign using primers published in a previous study (Huang et al., 2019; Table S1). Standardization curves for each target gene were created using dilution series (representing 102–108 copies each target gene/μl) of fluidigm-prepared amplicons from 225 pooled soil samples as detailed in Huang et al. (2019). All standards, samples, and no-template controls were run in six replicates on six plates. Amplicon specificity was monitored by performing a melting curve analysis after the final qPCR cycle. Amplification efficiencies were within 86%–96% for all the primer-pairs. Copy numbers of the target genes in the samples were then calculated from standard curves of known concentrations.

Table 1
Principal component analysis of 18 soil variables for 0–10 cm soil depth with eigenvalues and cumulative proportion of the data set variability explained by the five principal components (PC) extracted with eigenvalues > 1. Component correlation scores (eigenvectors) with loadings greater than |0.5| are bolded. Probability values for the analysis of variance (ANOVA) and degrees of freedom (df) available for the effects of Residue Removal (RR), Tillage (T), and their interaction are shown for each extracted PCs along with the mean separation procedures those PCs that showed a statistically significant response to RR x T effect (PC2), and to the main effects of T (PC3). Within a column, means followed by the same letter are not statistically different (α = 0.10).

		PC1	PC2	PC3	PC4	PC5
Eigenvalue		6.44	2.94	2.21	1.85	1.10
Cum. proportion		0.36	0.52	0.64	0.75	0.81
Soil variable		**Component correlation scores**				
pH		**−0.55**	−0.26	**0.51**	0.47	0.13
CEC		**0.92**	−0.28	0.00	−0.18	−0.05
SOM		0.41	**−0.50**	−0.39	0.25	0.20
WAS		0.05	0.13	**−0.63**	0.03	−0.47
NO_3^-		0.18	**0.75**	0.04	−0.28	0.13
NH_4^+		−0.03	0.00	−0.49	−0.26	**0.66**
P		0.47	0.19	−0.42	**0.62**	−0.06
S		0.46	0.39	0.35	**0.62**	−0.03
K		**0.71**	0.39	0.11	−0.15	0.23
Ca		**0.76**	−0.47	0.31	0.13	−0.04
Mg		**0.51**	**−0.60**	0.45	−0.07	0.11
Na		**0.60**	0.25	0.34	−0.34	−0.31
Al		**0.70**	0.28	0.29	−0.43	−0.05
Fe		**0.80**	0.25	−0.44	−0.02	0.01
B		**0.57**	**0.52**	0.12	0.37	−0.07
Zn		**0.80**	−0.09	−0.21	0.31	0.13
Cu		**0.89**	−0.36	0.07	−0.05	0.18
Mn		−0.27	**0.69**	0.30	0.19	0.37
Factor	**df**	**Probability values**				
RR	1	0.3822	0.5134	0.8483	0.8077	0.9997
T	1	0.8461	0.8353	*0.0174*	0.9499	0.7215
Tilled		–	–	0.54 a	–	–
No-till		–	–	−0.54 b	–	–
RR x T	1	0.2126	*0.0337*	0.4889	0.7944	0.8135
Full - tilled		–	−0.23 b	–	–	–
Full - no-till		–	0.39 a	–	–	–
None - tilled		–	0.18 ab	–	–	–
None – no-till		–	−0.34 b	–	–	–

Probability values lower than the statistical significance threshold of each test are italicized.

2.5. Statistical analyses

The statistical methodology deployed for this study followed the general steps thoroughly explained in Huang et al. (2019). Briefly, we used principal component analyses (PCA) in SAS software version 9.4 (SAS Institute, Cary, NC) as a data reduction technique on the soil data set (Table S2). PCA was implemented by PROC FACTOR with priors = 1 option (default). Five principal components (PCs) with eigenvalues ≥1, were extracted from the soil data set, hereby called PC1 to PC5. Soil variable loadings greater than |0.5| were considered in the interpretation of each PC (Table 1). Linear mixed models were then fit to each PCs extracted using PROC GLIMMIX in SAS to evaluate the effect of residue removal, and tillage and their interaction effects on the soil health parameters now summarized within our PCs. Blocks and years were considered random effects, and residue removal, tillage, and their interaction, as fixed effects. Therefore, blocks and years are considered spatial and temporal replications, respectively. Similarly, linear mixed models were fit to each of the soil microbial parameters, e.g. the copy numbers of bacteria *16S*, *ITS*, *Archaea*, and of *nifH,* AOB, AOA, *nirK, nirS,* and *nosZ*, expressed as counts per microgram of DNA (Table S3). Model residuals for each of these parameters were not normally distributed, so a lognormal distribution link function (dist = logn) was used within the model statement and with a Kenward-Rogers adjustment to the degrees of freedom (ddfm = kr) to account for model complexity (Gbur et al., 2012). When appropriate, lsmeans were separated using the lines option of the lsmeans statement, setting the probability of Type I error (α) at 0.10. To assess relationships among the soil properties and microbial parameters, we used Pearson's correlation coefficients obtained with the CORR procedure. All plots were created within the R environment, version 3.6.1 (R Core Team, 2019) using the package ggplot2 (Wickham, 2016).

3. Results

3.1. Residue removal and tillage effects on surface soil properties

The PCA conducted on soil properties rendered a set of five uncorrelated PCs (PC1 to PC5) with eigenvalues larger than 1 that together explained 81% of the variability on the soil data set (Table 1). PC1 had the largest eigenvalue (6.44) and explained 36% of the soil variability and its eigenvector included positive loadings (> 0.50) for CEC, K, Ca, Mg, Na, Al, Fe, B, Zn and Cu, and a single negative loading for soil pH. PC2 showed an eigenvalue of 2.94 and explained an additional 16% of the soil data variability with positive loadings for NO_3^-, B, and Mn, and negative loadings for SOM and Mg. The eigenvalue for PC3 was 2.21 with an additional 12% explanation of the total variability, showing a positive dominant loading for pH and a single negative loading for WAS. The eigenvalue of PC4 was 1.85 explaining an additional 11% of the variability. PC4 showed two dominant positive loadings for P and S. Lastly, PC5 eigenvalue was 1.10 and explained an additional 6% of the variability, mainly related to levels of NH_4^+, the only dominant positive loading for this specific component (Table 1). The probability values and degrees of freedom associated with the ANOVA for the effects of residue removal, tillage, and their interaction are shown in the lower portion of Table 1 for each extracted PC. Results showed a statistically significant interaction effect (RR x T, Table 1) for PC2 ($p < 0.0337$). This interaction effect indicated that the soil variables represented by PC2 responded differently to residue removal depending on the tillage practices. Specifically, full residue removal in continuous corn under no-till had the highest PC2 values associated with the highest NO_3^- and the lowest Mg levels compared to the other treatment combinations (Table S2). Residue removal decreased the Mg levels under no-till compared to tilled plots (458 mg kg^{-1}), yet the Mg level did not differ by tillage under residue retention (Table S2). On the other hand, residue retention under no-till management had the highest levels of SOM (3.9%).

PC3 scores had a statistically significant response to the tillage main effect ($p < 0.0174$), with lower values measured under no-till management compared to chisel tilled plots (Table 1). We found higher WAS (74%) and lower pH (5.9) under no-till management compared to tilled plots regardless of residue management (WAS 64%, pH 6.1). PC1, PC4, and PC5 did not have a statistically significant response to residue removal, tillage, and interaction effects (Table 1).

3.2. Residue removal and tillage effects on the abundance of microbial communities and functional genes

The copy number of fungal *ITS* sequences ranged from 1.7×10^8 to 2.0×10^8 copies/μg DNA on average. The copy number of bacterial *16S* ranged from 2.9×10^7 to 3.3×10^7 copies/μg DNA, while the copy number of archaeal *16S* ranged from 7.0×10^6 to 8.7×10^6 copies/μg DNA on average (Table S3). No statistically significant differences in *ITS* abundance, nor bacterial and archaeal *16S* gene abundance were detected within residue removal and tillage practices (*data not shown*). The copy numbers of the *nifH* gene, encoding nitrogenase reductase, was used as a proxy for the abundance of N_2 fixation communities in the soil. The copy number of *nifH* in the soil samples collected from our study ranged from 3.1×10^5 to 3.3×10^6 copies/μg DNA on average (Table S3). The copy number of *amoA* gene encoding ammonia monooxygenase in bacteria (AOB), and archaea (AOA), was used as a proxy

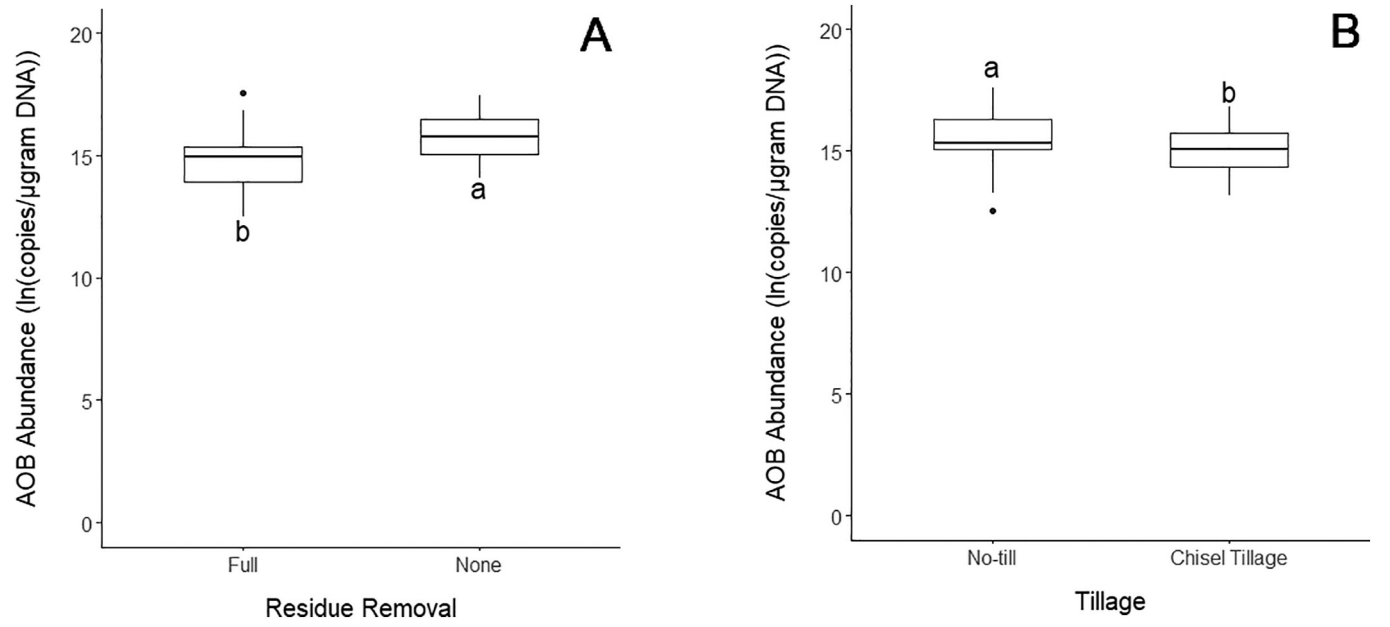

Fig. 1. Distribution of gene copy numbers of bacterial *amoA* (AOB) functional genes in the soils following long-term management practices of A) residue removal, and B) tillage treatments. Within a given factor, boxplots with the same letter are not statistically different ($\alpha = 0.10$).

for the community abundance of ammonia oxidizers. The copy number of *amoA* genes (AOB and AOA) ranged from 4.6×10^6 to 2.0×10^7 copies/µg DNA (Table S3). The copy number of AOB showed a statistically significant main effect of residue removal ($p < 0.0325$) and a marginally significant effect of tillage ($p < 0.0572$). Thus, across tillage options, residue retention had a statistically higher AOB abundance than full removal (Fig. 1A). No-till also had higher AOB abundance compared to chisel tilled plots regardless of residue management (Fig. 1B).

The copy numbers of three genes *nirK, nirS*, and *nosZ* quantified the abundance of denitrifying groups in the soil. The copy number of *nirK* ranged from 4.4×10^6 to 5.6×10^6 copies/µg DNA on average. The abundance of *nirS* ranged from 3.9×10^4 to 7.9×10^4 copies/µg DNA, whereas *nosZ* copy numbers ranged from 2.3×10^6 to 3.2×10^6 copies/µg DNA on average (Table S3). Abundances of the denitrifying groups carrying *nirK* or *nosZ* genes did not differ statistically in response to residue removal and tillage effects. We did however detect a statistically significant interaction effect of residue removal and tillage ($p < 0.0156$) for the least abundant of the denitrifier groups, the *nirS*-carrying denitrifiers. Accordingly, *nirS* abundance was the highest with full residue removal within tilled plots and the lowest with full removal of residues under no-till (Fig. 2). With residue retention, *nirS* abundance showed intermediate values regardless of tillage.

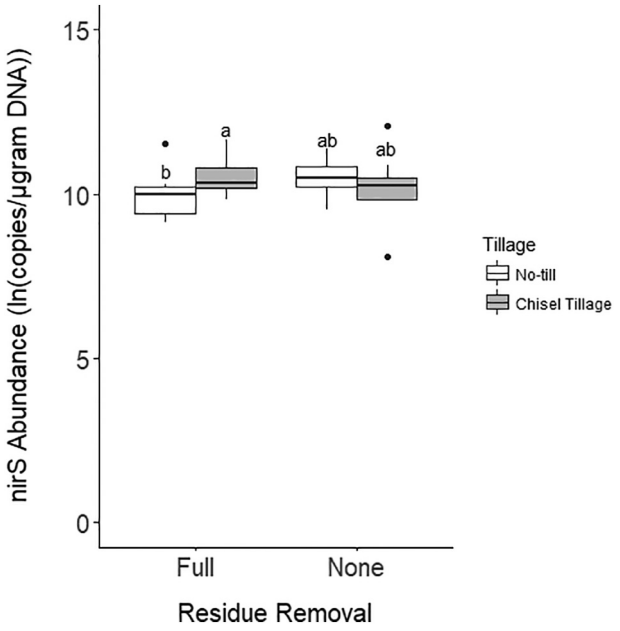

Fig. 2. Distribution of copy numbers of *nirS* functional genes in the soils following long-term management practices of residue removal and tillage treatments. Boxplots with the same letter are not statistically different ($\alpha = 0.10$).

3.3. Correlation among soil microbial parameters and soil properties

Table 2 shows the Pearson's correlation coefficients among all five PCs summarizing the soil properties data set, and the soil microbial parameters estimating community abundance of fungi, bacteria, and archaea, as well as N functional genes. Most statistically significant correlations found fell within the 'moderate' (|0.4–0.6|) association range with only one correlation within the 'weak' (|0.2–0.4|) association range. Fungal *ITS* copies were moderately and positively associated with PC2 values ($r = 0.54, p < 0.0001$). Bacterial *16S* counts (Bacteria Table 2) associate weakly and positively with PC1 scores ($r = 0.36$, $p < 0.0001$), while archaea *16S* copy numbers associated moderately and positively with PC4 scores ($r = 0.53$, $p < 0.0001$). Regarding functional gene quantifications, *nifH* counts associated moderately and positively with PC4 ($r = 0.53$, $p < 0.0001$). AOB abundance associated moderately and positively with PC2 scores ($r = 0.47$, $p = 0.005$). We did not find an association with AOA and any of the PCs summarizing the soil properties in the study. Both *nirK* and *nosZ* showed a moderate and positive association with PC2 scores, ($r = 0.46$, $p < 0.001$), while *nirS* showed a moderate and positive relationship to PC1 and PC4 scores ($r = 0.41$, $p < 0.0001$). Correlations among soil microbial parameters in Table 2 showed that many correlation coefficients fell within the strong (|0.6–0.8|) association range, with only a selected few falling within the very strong (> |0.8|) category. Among these, the associations between *nifH* and bacteria and Archaeal *16S* counts were moderate ($r = 0.63$ and $r = 0.69$ respectively). In addition, all denitrifiers were strongly and very strongly associated among themselves, and to bacteria *16S* and fungal *ITS* abundances (Table 2).

Table 2
Matrix of Pearson' correlation coefficients among soil properties (PCs) and soil microbial parameters describing the abundance of fungi (copy numbers of ITS region), and prokaryotes (copy numbers of bacteria 16S, Bact; and archaea 16S, Archaea), and functional genes of the microbial N cycle (*nifH,* AOB, AOA, *nirK, nirS,* and *nosZ*). Bolded correlation coefficients indicate statistical significance at $\alpha = 0.05$.

	PC1	PC2	PC3	PC4	PC5	ITS	Bact	Arch	*nifH*	AOB	AOA	*nirK*	*nirS*	*nosZ*
PC1	1.00													
PC2	0.00	1.00												
PC3	0.00	0.00	1.00											
PC4	0.00	0.00	0.00	1.00										
PC5	0.00	0.00	0.00	0.00	1.00									
ITS	0.30	**0.54**	−0.08	0.10	0.04	1.00								
Bact	**0.36**	0.26	0.10	0.22	0.05	**0.80**	1.00							
Archaea	0.11	−0.07	0.13	**0.53**	−0.30	0.20	**0.49**	1.00						
nifH	0.16	0.09	−0.03	**0.53**	−0.18	**0.57**	**0.63**	**0.69**	1.00					
AOB	−0.13	**0.47**	−0.06	0.05	−0.06	**0.72**	**0.55**	0.20	**0.46**	1.00				
AOA	0.24	−0.02	−0.01	0.23	−0.19	0.11	0.13	**0.41**	0.19	0.00	1.00			
nirK	0.25	**0.46**	−0.03	0.22	−0.07	**0.91**	**0.77**	0.25	**0.67**	**0.79**	0.11	1.00		
nirS	**0.41**	0.23	0.06	**0.41**	−0.17	**0.77**	**0.71**	**0.46**	**0.78**	**0.52**	**0.35**	**0.87**	1.00	
nosZ	0.15	**0.46**	−0.06	0.16	−0.06	**0.92**	**0.72**	0.28	**0.69**	**0.85**	0.04	**0.94**	**0.79**	1.00

4. Discussion

Our study assessed the accumulated long-term effects of residue removal and tillage practices on the soil N cycle following over a decade since treatment establishment. Although seasonal variations in soil biological activity and composition are expected, its evaluation was outside the scope of our work. The long-term nature of our experiment, along with the selected time of sampling events after harvest each year, ensured a sufficient level of biological stability to investigate accumulated residue and tillage management effects on the soil.

Overall, our study showed that long-term tillage and residue removal treatments significantly altered certain soil chemical and physical properties and soil microbial parameters. We examined the results on these parameters first individually and then sought potential connections between them to explain how residue and tillage practices affected the soil microbial N cycle.

4.1. Tillage and residue removal affected soil properties

Our results generally agreed with past studies on tillage and residue management effects on soil properties. Notably, we found significant residue and tillage interaction effects on PC2 including SOM, and significant tillage main effects on PC3 including water aggregate stability (WAS). These results translated into greatest SOM under residue retention with no-till, and greater WAS under no-till, regardless of residue removal. For PC2 and its SOM component, studies have reported higher SOM, or its soil organic C (SOC) component, with residue retention (Hammerbeck et al., 2012; Stetson et al., 2012), no-till (Santiago et al., 2019), or their combination (Dalzell et al., 2013; Salinas-Garcia et al., 2001; Villamil et al., 2015). This result is expected as retained crop residues decompose into either readily available or stabilized SOM (Turmel et al., 2015). As for the tillage effects, Salinas-Garcia et al. (2001) explained that crop residues under no-till have less contact with the soil microbiome and decompose slower, thereby accumulating more SOM and SOC. Also, retained crop residues provide physical structures that prevent SOM-rich topsoil from eroding (Beniston et al., 2015).

For PC3 and its WAS component, higher WAS under no-till and residue retention reinforced results from previous studies on the effects of residue removal, tillage, and N fertilization practices on soil properties across several Illinois sites, including the site of our present study (Villamil and Nafziger, 2015; Villamil et al., 2015). Additional research supports the increase in WAS under no-tillage practices (Blanco-Canqui and Lal, 2009; Mikha and Rice, 2004; Rasmussen, 1999), as no-till enhances soil microbial activities that form soil aggregates and encapsulate SOM from microbial consumption (Kasper et al., 2009; Rampazzo et al., 1995; Edwards and Bremner, 1967; Villamil et al., 2015). Villamil et al. (2015) reported that both SOC and WAS increased with residue retention and no-till, which is consistent with our results measured 7–8 years after this initial report. Lastly, retained crop residues may enhance WAS by providing physical protection against soil erosion (Turmel et al., 2015). Overall, clear increases in SOM and WAS under long-term no-till and residue retention suggest that these management strategies enhance soil health (Stott, 2019). Accordingly, these results may reciprocate with the soil microbiome to affect the soil microbial N cycle as discussed below.

4.2. Tillage and residue removal effects on soil microbiome

Both chisel tillage and residue removal decreased AOB abundance in our study, which agrees with the previous findings (Badagliacca et al., 2018; Li et al., 2015; Liu et al., 2017; Munroe et al., 2016; Németh et al., 2014; Segal et al., 2017). Prior studies speculated that soil pH and the *amoA* substrate ammonia (NH_3) availability could be the key factors behind AOB abundance (Segal et al., 2017; Yao et al., 2013). Indeed, our study found higher soil NH_4^+ and almost doubled AOB abundance under no-till plots. This result is consistent with Ouyang et al. (2017) where AOB abundance increased with NH_4^+. Although residue management alone did not affect NH_4^+ level in our study, past studies found greater NH_4^+ level in soils with crop residues retained (Bent et al., 2016; Henderson et al., 2010; Hirsch and Mauchline, 2015; Wessén et al., 2011). Likewise, Németh et al. (2014) found that residue retention increased both NH_4^+ and *amoA* abundance.

Compared to the abundance of other denitrifying functional genes, *nirS* abundance was two orders of magnitude smaller. Similar studies on Hapludalfs (Ontario series) (Németh et al., 2014; Thompson et al., 2018) and other less fertile soils reported narrower differences between abundances of *nirS* and other denitrifying genes (Čuhel et al., 2010; Liu et al., 2018; Tang et al., 2016). Interestingly, Németh et al. (2014) reported *nirK* abundance below the detection level. These comparisons suggest that different soils can have drastically different N cycling functional gene pool compositions.

Under residue removal, chisel tillage significantly increased the *nirS* gene abundance. In contrast, Kaurin et al. (2015) showed that *nirS* abundance decreased under moldboard plow tillage on a more complex crop rotation, while a recent study by Wang et al. (2019a) reported higher *nirS* abundance under conventional tillage and no difference between no-till and chisel tillage. Also, unlike how our results did not show significant residue effects, Németh et al. (2014) reported decreases in *nirS* and *nosZ* abundances with residue removal. These studies contradict our findings, yet the disparity seems to arise from different experimental conditions. In summary, responses of N cycling

functional genes to residue removal and tillage are subtle and require further studies to resolve the apparent contradictions with previous reports.

4.3. Tillage and residue removal effects on soil biogeochemical N cycling

Differences found in AOB and *nirS* gene abundances may imply residue management and tillage effects on both nitrification and denitrification steps of the microbial N cycle (Hirsch and Mauchline, 2015). For example, levels of SOM and NH_4^+ were highest under residue retention and no-till. Possibly, SOM from residue retention and less aeration from no-till increased net ammonia (NH_3) mineralization, which increased AOB abundance while the unused NH_3 protonated into NH_4^+ (Hirsch and Mauchline, 2015; Osterholz et al., 2017). Moreover, research on the same experimental site by Yuan et al. (2018) reported reduced nitrous oxide (N_2O) emissions under no-till management compared to that of chisel tillage, regardless of residue management. As no-till plots had more AOB, which also harbors the *hao* genes responsible for completing the ammonia oxidation, no-till plots could have had a higher rate of completed nitrification and less spontaneous decomposition of hydroxylamine into N_2O (Hirsch and Mauchline, 2015). Moreover, decreased *nirS* may also lead to less nitrite (NO_2) reduction into nitric oxide (NO) and eventually N_2O. While NO_3^- levels did not differ significantly between treatments in our study, previous research suggests that increased AOB abundance does not necessarily translate into increased nitrification (Lu et al., 2015; Rudisill et al., 2016). Yet, considering that our study compared no-till to less intrusive chisel-tillage on a very fertile soil, a greater impact on the microbial N cycle may be found in more sensitive soils or with more aggressive tillage practices. Indeed, a meta-analysis by Zuber and Villamil (2016) observed greater differences in microbial biomass and activity between no-till and more intrusive tillage methods. Therefore, expanding this question to other regions and systems will be worthwhile to evaluate the effects of residue removal and tillage on the microbial N cycle.

4.4. Correlation between soil properties and abundance of main microbial groups and functional N genes

Correlations between principal components summarizing the soil properties and the soil microbial parameters demonstrated that while soil microbial parameters associated significantly between themselves, they did so moderately at best with soil properties (Table 2). These correlations between parameters were consistent with past findings or provided new insights into relationships between soil properties and microbial parameters. For example, PC1 including soil pH, CEC, and several soil nutrients showed moderate correlations with bacterial abundance and denitrifier *nirS* gene carrier groups. Past findings also reported moderate correlations between *nirS* abundances, soil pH, and CEC (Huang et al., 2019; Ouyang et al., 2018), and *nirS* and bacteria abundance significantly differed by soil pH (Čuhel et al., 2010; Rousk et al., 2009) and CEC (Liu et al., 2018). PC2 including SOM, NO_3^-, Mg, B, and Mn had moderate correlations with abundances of fungi, AOB, and denitrifier *nirK* and *nosZ* genes. Similarly, a meta-analysis by Ouyang et al. (2018) demonstrated significant correlations between SOC and these functional genes, and Huang et al. (2019) showed a moderate correlation between SOM and *nirK* and *nirS* genes. The two denitrifier genes likely associated with NO_3- because denitrifier microbes respond to NO_3^- availability by producing NO_3^- reductase (*nirK*) and N_2O reductase (*nosZ*). Similarly, nitrifier AOB associates with NO_3^- as this microbial group initiates nitrification by oxidizing NH_3 (Hirsch and Mauchline, 2015). Considering that the relationships among these N cycling genes and NO_3^- are well-established knowledge, their moderate correlations in our result indicate the credibility of this method.

PC4 including soil phosphorus (P) and sulfur (S) contents had moderate correlations with abundances of archaea, *nifH* gene, and *nirS* gene copies. Past studies have shown that *nirS* and *nifH* abundances associate with soil P level (Huang et al., 2019; Tang et al., 2016; Wang et al., 2019b; Wei et al., 2017; Xun et al., 2018). The correlation between S and *nirS* can perhaps be attributed to the presence of S in the *nirS* cd1 cytochrome structure (Ferguson and Fulop, 2000).

Soil microbial parameters displayed significant correlations among themselves. Specifically, fungi, bacteria, and functional genes correlated significantly with each other. Functional genes of the microbial N cycle expectedly showed high associations with the respective microbial groups that harbor them. For instance, both fungi and bacteria had a high association with denitrifier genes (Hirsch and Mauchline, 2015; Marco, 2014; Xu et al., 2019a; Xu et al., 2019b), whereas bacteria and archaea highly associated with their respective *amoA* gene. Considering that long-term residue removal and tillage decreased AOB abundance, the significant associations between AOB abundance and N cycling functional genes imply consequences of these practices on the soil microbial N cycling.

Unlike other microbial groups, however, archaea displayed distinct patterns by only having a moderate correlation with bacteria, N-fixing *nifH*, and denitrifier *nirS*, while AOA correlated significantly to archaea only. This result is perplexing because some archaeal groups harbor *nirK* and *nosZ*, and AOA has *nirK* (Bartossek et al., 2010; Helen et al., 2016; Lehtovirta-Morley, 2018; Long et al., 2015). Moreover, Huang et al. (2019) reported opposite results with high associations between archaea and other microbial parameters. Ouyang et al. (2018) also reported significant correlations between AOB and AOA and other N cycling functional genes. Since these two studies focused on N fertilizer effects, their contradictions to our results suggest that archaea and AOA respond differently to tillage and residue management than N fertilization. Indeed, Segal et al. (2017) found that while both AOB and AOA abundances did not respond to N fertilizer rates, only AOB responded significantly to tillage. Also, Kaurin et al. (2015) reported that tillage did not affect archaeal community composition unlike those of bacteria and fungi.

5. Conclusion

This study explored the long-term effects of tillage and residue removal on soil properties and the soil microbiome, especially regarding soil microbial N cycle, under continuous corn on high fertility soils. Tillage and residue removal did alter some of the soil properties and components of the soil microbiome. Residue retention and no-till practices enhanced soil health by increasing soil organic matter and water aggregate stability. These treatments also increased AOB abundance and soil NH_4^+ levels, however, this did not necessarily translate into increased greenhouse gas emissions, which can endorse residue retention and no-till as conservation practices if confirmed by further research. Full residue removal under chisel tillage increased the abundance of denitrifier groups carrying the *nirS* gene, potentially accelerating N losses under those conditions. Furthermore, we found significant correlations among soil properties and soil microbial parameters, implying that residue removal and tillage effects on these parameters might impact the soil N cycle. Our results provided new evidence on the importance of keeping the residues in the field and opting for no-till practices to enhance soil health and minimize the environmental impacts of agricultural practices.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.apsoil.2020.103730.

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