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ORIGINAL RESEARCH

Soil extracellular oxidases mediated nitrogen fertilization effects on soil organic carbon sequestration in bioenergy croplands

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Abstract

Nitrogen (N) fertilization significantly affects soil extracellular oxidases, agents responsible for decomposition of slow turnover and recalcitrant soil organic carbon (SOC; e.g., lignin), and consequently influences soil carbon sequestration capacity. However, it remains unclear how soil oxidases mediate SOC sequestration under N fertilization, and whether these effects co-vary with plant type (e.g., bioenergy crop species). Using a spatially explicit design and intensive soil sampling strategy under three fertilization treatments in switchgrass (SG: *Panicum virgatum* L.) and gamagrass (GG: *Tripsacum dactyloides* L.) croplands, we quantified the activities of polyphenolic oxidase (*PHO*), peroxidase (*PER*), and their sum associated with recalcitrant C acquisition (*OX*). The fertilization treatments included no N fertilizer input (NN), low N input (LN: 84 kg N ha^{-1} $year^{-1}$ in urea), and high N input (HN: 168 kg N ha^{-1} $year^{-1}$ in urea). Besides correlations between soil oxidases and SOC (formerly published), both descriptive and geostatistical approaches were applied to evaluate the effects of N fertilization and crop type on soil oxidases activities and their spatial distributions. Results showed significantly negative correlations between soil oxidase activities and SOC across all treatments. The negative relationship of soil oxidases and SOC was also evident under N fertilization. First, LN significantly depressed oxidases in both mean activities and spatial heterogeneity, which corresponded to increased SOC in SG (though by 5.4%). LN slightly influenced oxidases activities and their spatial heterogeneity, consistent with insignificant changes of SOC in GG. Second, HN showed trends of decrease in soil oxidase activities, which aligned with the significantly enhanced

Jianjun Duan and Min Yuan contributed equally to the work.

SOC in both croplands. Overall, this study demonstrated that soil oxidase activities acted as sensitive and negative mediators of SOC sequestration in bioenergy croplands and optimizing fertilizer use particularly in switchgrass cropland can improve for both carbon sequestration and environmental benefit.

KEYWORDS

bioenergy cropland, extracellular oxidase, nitrogen fertilization, soil carbon sequestration, spatial heterogeneity, switchgrass

1 | INTRODUCTION

Nitrogen (N) fertilizer amendment is projected to increase worldwide this century (Gallo et al., 2004). N fertilization has the potential to promote soil organic carbon (SOC) sequestration (Jian et al., 2016), although some studies show a negative or no effect of N fertilization on SOC sequestration (Aref & Wander, 1997; Brown et al., 2014; Khan et al., 2007). The contrasting effects of N fertilization on SOC stock partly lie in the differential responses of mineralization of slow turnover SOC fractions (e.g., lignin or litterfall; Dou et al., 2016), which are depolymerized by extracellular oxidative enzymes (oxidases) excreted by soil bacteria and fungi (Gallo et al., 2004; Sharma et al., 2016). As either plant inputs or indigenous SOC are chemically recalcitrant (Trumbore, 2000) and must be processed via microbial decomposition and oxidative catalyzation, the net changes of plant inputs and decomposition loss, that is, SOC accumulation, are likely represented by the dynamics of oxidases. For instance, the elevated SOC with N fertilization was accompanied by depressed oxidases activities (Chen, Luo, Garcia-Palacios, et al., 2018; Jian et al., 2016), which implied reduced decomposition of slow turnover SOC. However, in many studies, the responses of oxidases were rarely reported.

Oxidases may also mediate the response of the microbial respiration and SOC storage to climate change factors. For instance, warming induced soil respiration was found correlated with ligninase rather than cellulase (Chen, Luo, Garcia-Palacios, et al., 2018) and increased precipitation significantly enhanced soil oxidative enzyme activities but had no effects on hydrolytic enzyme activities (Ren et al., 2017). In general, soil extracellular oxidases play important roles in indexing soil health, microbial metabolism, and nutrient status (Askin & Kizilkaya, 2006; Caldwell, 2005; Stott, 2019). To optimize N fertilizer use for the sake of soil carbon sequestration while minimizing greenhouse gas emission (Zhu & Chen, 2002), it is imperative to understand how N fertilization affects the controls on SOC storage, hence on oxidases regulating SOC decomposition, especially long-lived carbon forms in various plant–soil ecosystems.

Spatial variation of soil properties and structure can affect the local distribution and abundance of plant species and the performance of individual plants and microorganisms (Abbott et al., 2015) and therefore have consequences for both community structure and ecosystem-level processes (Robertson & Gross, 1994; Schlesinger et al., 1996; Tilman, 1988; Van Veen & Kuikman, 1990). Intensive N fertilizer inputs substantially restructure spatial heterogeneity of soil biogeochemical and microbial features at a variety of spatial scales (Fraterrigo et al., 2005; Guo et al., 2007; Heinze et al., 2010; Li et al., 2010b). This inevitably affects the activity and spatial heterogeneity of extracellular oxidases in soils. Knowledge of spatiotemporal variations of soil enzymes will further our understanding of soil changes and help develop best management practices with respect to soil quality and carbon sequestration, crop production sustainability, and climate change mitigation under rapid global change.

The energy crops such as switchgrass (SG: *Panicum virgatum* L.) and gamagrass (GG: *Tripsacum dactyloides* L.) are key for supplying plant biomass for biofuel production (Gelfand et al., 2013; Kering et al., 2012) and to reduce fossil fuel consumption (Monti et al., 2012). Consistent with the enhanced bioenergy crop yields by N fertilizers (Owens et al., 2013), Li, Jian, Lane, Lu, et al. (2020) reported higher SOC content but less pronounced SOC enhancement in GG than in SG, which is partly attributed to the contribution of more structurally complex dissolved organic matter in GG root. This suggested that soil C sequestration potential varied with bioenergy crop species, but the underlying microbial functions (e.g., soil extracellular enzyme activities) that catalyze the decomposition of labile and recalcitrant SOC in these cropland are rarely examined. Only one recent study revealed greater activities of soil glycosidases in GG than SG but no significant changes of soil glycosidases in response to N fertilization in both bioenergy croplands (Yuan et al., 2020). N fertilization with a high amendment rate increased SOC in both SG and GG with a greater effect in SG (Li, Jian, Lane, Lu, et al., 2020); however, the role of soil extracellular oxidases in SOC responses to N fertilization however has not been examined. This gap hinders our capacity to elucidate the microbial control of soil carbon sequestration in bioenergy crop species under varying fertilization rates. Understanding effects of fertilization on soil extracellular oxidase functionality activities and spatial structures in various

bioenergy croplands may enhance our ability to manipulate nutrient cycling in situ especially with respect to soil carbon sequestration.

Extracellular oxidases are generally more versatile than hydrolytic enzymes and uncorrelated with hydrolytic enzymes in the environment (Bandick & Dick, 1999; Bell & Henry, 2011; Martín-Lammerding et al., 2015; Sinsabaugh, 2010a). Among extracellular oxidases, polyphenolic oxidase (*PHO*) and peroxidase (*PER*) are two major oxidases in soil that are produced and excreted by microbes to depolymerize long-chain and high molecular weight carbon substrates such as lignin, which is a secondary compound in the plant cell wall, and in slow turnover organic carbon (Klose & Tabatabai, 2002; Sinsabaugh et al., 2008). *PHO* is a group of Cu-containing enzymes that catalyze the oxidation of several phenols to *o*-quinones (Marusek et al., 2006; VanGelder et al., 1997). In turn, *o*-quinones are highly reactive molecules that can undergo non-enzymatic secondary reactions to form brown complex polymers such as melanins and cross-linked polymers with protein functional groups (Rolff et al., 2011; Taranto et al., 2017). In contrast, *PER* are lignolytic enzymes that catalyze oxidation reactions via the reduction of hydrogen peroxide (H_2O_2) to degrade molecules without a precisely repeated structure (Wong, 2009). These *PHO* and *PER* enzymes are expressed for ontogeny, defense, and the acquisition of carbon and nitrogen (Sinsabaugh, 2010a). Through excretion or lysis, these enzymes enter the environment where their aggregate activity mediates key ecosystem functions of lignin degradation, humification, carbon mineralization, and dissolved organic carbon export (Kellner et al., 2014; Sinsabaugh, 2010a). Their different enzyme structures and associated reactions may potentially lead to varied responses of *PHO* and *PER* to N fertilization and consequently differential impacts on soil carbon sequestration.

N fertilization can induce significant impacts on the expression, excretion, and function of soil extracellular oxidases (Matocha et al., 2004; Zhang et al., 2020). N fertilizer markedly enhanced potential activities of *PER* in a 10-year field experiment in a wheat–corn rotation system (Zhang et al., 2020). However, N fertilization suppressed soil *PHO* activity in no-tillage cropland systems (Matocha et al., 2004). Relative to low and high N fertilization rates, a medium rate of N fertilization increased *PHO* in tobacco roots (Sheen et al., 1969). The differential responses of the two oxidases are likely related to variations of soil pH, electrolytic conductivity, and water content under N fertilization (Garbuz et al., 2016; Martínez-Olivas et al., 2019; Sinsabaugh, 2010a). The opposing effects of N fertilization on soil *PER* and *PHO* therefore likely contributed to insignificant changes of their summed activity (i.e., *OX*) across various croplands (Jian et al., 2016; Xiao et al., 2018). At the global scale, N amendment of basidiomycete-dominated soils of temperate and boreal forest ecosystems often leads to losses of oxidative enzyme activity while activities in grassland soils dominated by glomeromycota and ascomycetes show little net response (Sinsabaugh, 2010a). Nevertheless, the negative effects of N addition on soil lignin-modifying enzymes (e.g., oxidases) appeared more important than other environmental and edaphic factors in explaining the variation of SOC in response to N addition (Chen, Luo, van Groenigen, et al., 2018).

Nitrogen fertilization increased spatial heterogeneity of both microbial biomass and soil glycosidases in bioenergy croplands (Li et al., 2018; Yuan et al., 2020). The effects of N fertilization on spatial patterns of oxidases, however, could not be readily derived due to weak correlations of oxidases with either microbial biomass or glycosidases (Boeddinghaus et al., 2015; Kim et al., 2019; Sinsabaugh, 2010a). A study with focus on both glycosidase and oxidase also revealed that the specific patterns of spatial variation were unique to each enzyme (Decker et al., 1999). Despite lacking direct observations of N fertilization on spatial distribution of oxidases in soils, past studies suggested that the spatial pattern of soil oxidases under N fertilization may vary with indigenous site fertility (Gravuer et al., 2020a), soil depth and substrate concentrations (e.g., phenolics; Pind et al., 1994), soil water content (Bartkowiak et al., 2020; Minick et al., 2019), and fungal or bacterial biomass spatial distribution with a ranges of dependence at <1 cm to >10 m (Mills & Franklin, 2007). For instance, in a California grassland, fertilization with N plus other macronutrients and micronutrients diminished spatial variability of fungal composition due to increases in antagonistic fungi, whereas bacterial and archaeal community composition showed little change in their spatial variability (Gravuer et al., 2020b). In a semi-arid Mediterranean shrubland, high N deposition (50 kg N ha^{-1} $year^{-1}$) resulted in significant losses of the spatial pattern of soil oxidase activities that was attributed to the presence of well-developed biocrust communities mediating the effects of high N deposition on soil oxidases (Benvenutto-Vargas & Ochoa-Hueso, 2019). Nevertheless, microbial spatial footprint associated with higher enzyme activities and microscale pores promoted soil carbon stabilization (Kravchenko et al., 2019). Therefore, identifying both changes in oxidase activities and their spatial heterogeneity will help better understand the mediation of these enzymes on spatiotemporal variation of soil carbon sequestration.

A 3-year N fertilization experiment was established in SG and GG croplands with management history of no-tillage and minor mechanical disturbance at the campus farm of Tennessee State University, Nashville TN. N fertilizer input was the dominant management practice in these crops. This study aims to examine how N fertilization affects plot-level mean and spatial distribution of *PHO* and *PER* and their sum (i.e., *OX*), and how their changes relate to SOC sequestration in both bioenergy croplands. We hypothesized that N fertilization would depress *PHO*, *PER*, and *OX* activities and

their spatial heterogeneity in both bioenergy croplands. We also hypothesized that there was a significantly negative correlation between soil oxidase and SOC content so that the N fertilization effects on soil oxidases would explain the SOC changes under different fertilization rates in these croplands. Third, we hypothesized that the N fertilization effects would vary with enzyme type due to the different oxidase enzyme structures and associated reactions they catalyzed.

2 | MATERIALS AND METHODS

2.1 | Study site description and experimental design

The bioenergy crop field fertilization experiment was established in 2011 at the Tennessee State University (TSU) Main Campus Agriculture Research and Education Center (AREC) in Nashville, TN, USA (Latitude: 36.12°N, Longitude: 36.98°W, elevation 127.6 m above sea level). For several decades prior to the establishment of switchgrass and gamagrass croplands, the land was a mowed grassland. No fertilizers were applied during the prior land use. Climate in the region is a warm humid temperate climate with an average annual temperature of 15.1°C, and total annual precipitation of 1200 mm (Deng et al., 2017). The crop type and N fertilization treatments were applied in a randomized block design (Dzantor et al., 2015; Li et al., 2018; Li, Jian, Lane, Guo, et al., 2020; Li, Jian, Lane, Lu, et al., 2020). The two crop types were *Alamo* SG (*Panicum virgatum* L.) and *Highlander* GG (*Tripsacum dactyloides* L.). The three N levels included no N fertilizer input (NN), low N fertilizer input (LN: 84 kg N ha^{-1} $year^{-1}$ as urea), and high N fertilizer input (HN: 168 kg N ha^{-1} $year^{-1}$ as urea), and each treatment had four replicated plots with a dimension of 3 m × 6 m. The low N fertilization rate was determined as the optimum N rate to maximize cellulosic ethanol production in established northern latitude grasslands (Jungers et al., 2015). The high N rate doubled the low rate to create appreciable gap and detectable effect between the two levels. The fertilizer was manually applied in June or July each year after the second cut in the two-cut per year system. The soil series for the plots is *Armour* silt loam soil (fine-silty, mixed, thermic Ultic *Hapludalfs*) with acidic soil pH (i.e., 5.97) and intermediate organic matter content of 2.4% (Li et al., 2018; Yu et al., 2016).

2.2 | Soil collection and laboratory assay

After the grass cut on June 6th, 2015, soil samples (0–15 cm) were collected using soil auger (Thermo Fisher Scientific) from two of the four replicated plots in each treatment (2 crop × 3 N × 2 replicates = 12 plots). Within each plot of 3 m × 6 m, a sampling area of 2.75 m × 5.5 m (i.e., 15.1 m^2) was determined and the southwestern corner point was identified as the origin (Figure 1). Each sampling area was divided into two square subplots, and within each subplot, four centroids were identified. Three cores were collected at a random direction and distance relative to each centroid (Figure 1). Unique *x*, *y* coordinates were assigned to each sampling point. In all, 24 cores were collected from each plot yielding 288 soil cores in 12 plots. Soil samples were stored in coolers filled with ice packs and immediately transported to the TSU laboratory. Prior to chemical analysis, the visible roots and rocks were removed from soil cores by passing through a 2 mm soil sieve. The subsamples used for this study were stored at −20°C for enzymatic

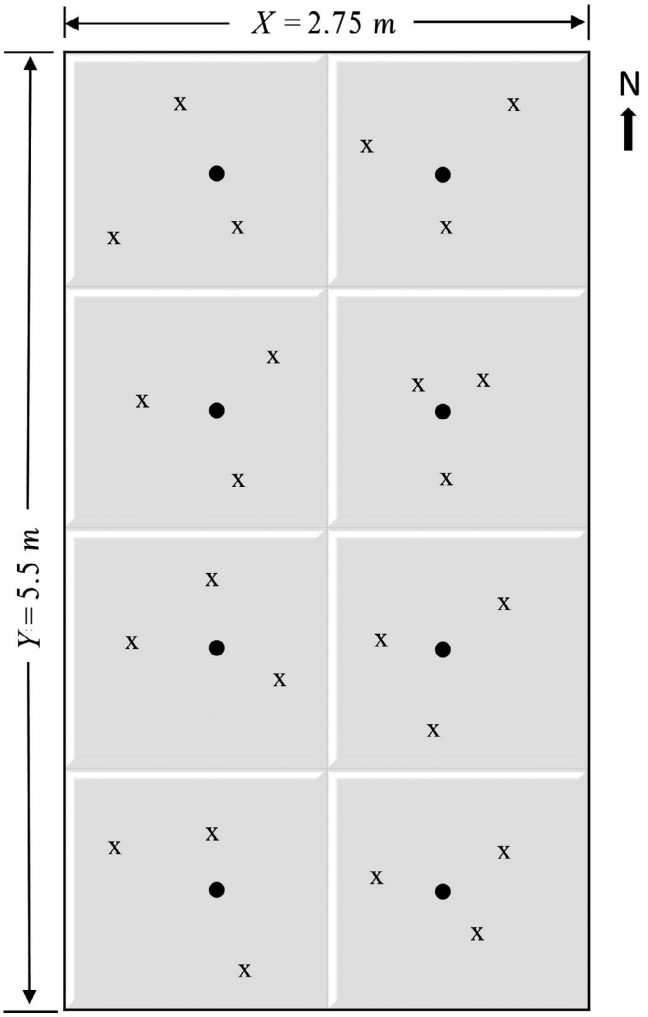

FIGURE 1 Illustration of an efficient clustered random sampling design within a plot (2.75 m × 5.5 m). The plot was divided into eight subplots (grey zone) and there was a centroid (dark solid circle) in each subplot (1.375 m × 1.375 m), where three soil sampling points (×) were determined from random directions and distances from a centroid in each sampling region (grey area). The extent of an interpolation map was thus determined by the minimum and maximum values at horizontal and vertical axes, and each map can attain its extent less than or equivalent to a plot area

assay. Other details about soil collections and chemical analysis can be found in Li et al. (2018).

Soil oxidase activities were quantified by soil colorimetric enzymatic assay methods. Each core sample was assayed for polyphenol oxidase (*PHO*) and peroxidase (*PER*) using 25 mM l-3,4-dihydroxyphenylalanine (L-DOPA) as substrate following published protocols (Saiya-Cork, Sinsabaugh, & Zak, 2002; Sinsabaugh et al., 2003). Sample suspensions were prepared by placing 1.0 g soil in a 125 ml Nalgene bottle. Acetate buffer (50 mM, pH 5) was added to the bottle and the resulting suspension was homogenized using a Brinkmann Polytron for approximately 1 min. Additional buffer was added to the bottle to bring the final suspension volume to 125 ml.

The assay of oxidase activities was conducted on clear 96-well microtiter plates. The plates were placed in an Echotherm incubator at 20°C for 18–24 h depending on the enzyme. The assay design included eight wells of blank control (250 μl buffer), 8 wells of substrate control (200 μl buffer + 50 μl L-DOPA), eight wells of sample control (200 μl soil slurry + 50 μl buffer), and 16 wells of assay (200 μl soil slurry + 50 μl L-DOPA) for each soil sample. Peroxidase assays received an additional 10 μl of 0.3% H_2O_2 in all wells. Activity was quantified by measuring absorbance at 450 nm using a FilterMaxF5 Multi-Mode Microplate Reader (Molecular Devices). All enzyme activities were calculated as μmol activity h^{-1} g $soil^{-1}$. A total of 576 enzyme activity readings were collected for 288 soil samples and two enzymes. Laboratory tests were conducted and specific protocols were optimized to secure sufficient soil mixing by sealing soil samples in a plastic Ziplock bag, repeatedly pressing, shaking, and mixing with hands. As a result, the variation of each measurement (i.e., coefficient of variation) in multiple tests ranged from 2% to 8%.

2.3 | Statistical analysis

We used descriptive statistics, analysis of variance (ANOVA), and geospatial analytical methods to illustrate the central tendency and spatial heterogeneity of enzyme activities assayed. Mean, frequency distribution, plot-level variance, and within-plot coefficient of variation (CV) were estimated to describe central tendencies and variations for enzyme activities in each plot. Two-way ANOVA was used to test whether N fertilization, crop species, and their interaction significantly affected each enzyme. The post-hoc Tukey test was used when ANOVA reported significant main or interactive effects. The significance level was set at $p < 0.05$. The Pearson moment correlation coefficients were derived for oxidases, SOC, total nitrogen (TN), microbial biomass carbon (MBC), microbial biomass nitrogen (MBN), and glycosidases. Except oxidases, these variables were formerly measured on these soil cores as part of previous studies (Li et al., 2018; Li, Jian, Lane, Guo, et al., 2020; Yuan et al., 2020).

Cochran's C test was performed to test the assumption of variance homogeneity. The test statistic is a ratio that relates the largest empirical variance of a particular treatment to the sum of the variances of the remaining treatments. The theoretical distribution with the corresponding critical values can be specified. Soil properties that exhibited non-normal distributions were log-transformed to better conform to the normality assumption of the Cochran's C test (Cochran, 1941; Underwood, 1997).

The sample size required in a research plot can be determined quantitatively from the within-plot coefficient of variation (CV) under given desired sampling error (Li, 2019). That is, under a desired sampling error, the sample sizes derived can be used equivalently as CV to evaluate the plot-level variations between different research plots. A larger required sample size indicates greater plot-level variation. Comparing the sample size required enables evaluation of the plot-level variations between different treatments. In this study, the sample size requirement (N) in each plot was derived given specified relative error (γ), which was defined as the ratio of error term ($t_{0.975} \times \frac{s}{\sqrt{n}}$) over plot mean ($\overline{X}$) with a range of 0%–100% (Equations 1–3). To evaluate how sample size requirement varied with N fertilization or crop types at certain relative error, the average of sample size (N) in two plots was derived and plotted. Under a relative error of 10%, the sample sizes were also derived from each plot and compared between different plots. For comparison, the higher sample size the greater plot-level variation under the same relative error.

$$\mathrm{CI} = \overline{X} \pm t_{0.975} \times \frac{s}{\sqrt{n}}, \tag{1}$$

$$\gamma = \frac{t_{0.975} \times \frac{s}{\sqrt{n}}}{\overline{X}} = t_{0.975} \times \frac{\mathrm{CV}}{\sqrt{N}}, \tag{2}$$

$$\ln(N) = 2 \times \ln(t_{0.975} \times \mathrm{CV}) - 2 \times (\gamma), \tag{3}$$

where CI, $\overline{X}$, s, n, N, CV, and γ denote confidence interval, plot means, plot standard deviation, sample number in each plot ($n = 24$), sample size requirement, coefficient of variation, and relative error, respectively. $t_{0.975} = 1.96$. The log-transformed sample size requirement (N) has a negative linear relationship (i.e., slope = 2) with the log-transformed relative error (γ).

2.4 | Geostatistical analysis

Three different geostatistical tools were applied to describe the spatial structure of soil exoenzyme activities within and among plots. The methods were briefly

described below and more details could be found in Li et al. (2010a). First, relationships between soil properties and x and y coordinates of their measurement location within the sampling plots are estimated by trend surface analysis (TSA), which fits a model that accounts for the linear trends and nonlinear structures and variations of an attribute (Equation 4):

$$\text{Soil property value} = \beta_0 + \beta_1 x + \beta_2 y + \beta_3 xy + \beta_4 x^2 + \beta_5 y^2. \quad (4)$$

The presence of a trend in the data was determined by the significance of any of the parameters β_1 to β_5 while the β_0 was the intercept (Gittins, 1968; Legendre & Legendre, 1998). Linear gradients in x- or y-directions were indicated by the significance of β_1 or β_2. A significant β_3 indicated a significant diagonal trend across a plot. Significant β_4 and β_5 parameters indicated a more complex, nonlinear spatial structure such as substantial humps or depressions. Trend surface regressions were estimated using *R* 3.5.0 (R Development Core Team., 2019). Model parameters were determined to be significant at a level of $p < 0.05$.

Second, residuals from the trend surface regressions were saved for subsequent spatial analysis using Moran's *I* index (Legendre et al., 2012). The Moran's *I* analysis (Cressie, 1992; Legendre & Fortin, 1989; Moran, 1950) was used to quantify the degree of spatial autocorrelation that existed among all soil cores taken from each plot. The resulting local Moran's *I* statistic is in the range from –1 to 1. Positive Moran's *I* values indicate similar values (either high or low) are spatially clustered. Negative Moran's *I* values indicate neighboring values are dissimilar. Moran's *I* values of 0 indicate no spatial autocorrelation or spatial randomness. A significant autocorrelation is determined if the observed Moran's *I* value is beyond the projected 95% confidence interval at a certain distance. Correlograms for local Moran indices were estimated for each soil variable in each plot in a range of 0–5.5 m with 0.25 m incremental interval.

The inverse distance weighting (IDW) interpolation was applied to compare the spatial distributions of the soil properties among the plots. IDW was chosen because it can be applied with a smaller sample size than ordinary kriging. The IDW maps were formerly used to distinguish the effects of different land uses on spatial distributions of soil biogeochemical features in South Carolina, USA (Li et al., 2010a). Briefly, the weights for each observation were inversely proportional to the power of its distance from the location being estimated. Exponents between 1 and 3 were typically used for IDW. Tests with different IDW exponents indicated the exponent of 2.0 showed the best fit between estimated values and actual data in cross-validation tests (Gotway et al., 1996). ArcGIS 10.6 (ESRI) was used to generate the IDW maps and perform cross-validations.

3 | RESULTS

3.1 | Central tendencies and correlation coefficients

There were significant main and interactive effects of N fertilization and crop species on *PER* and *OX* activities, but none on *PHO* activity (Table 1). The post-hoc tests showed that relative to unfertilized treatment (NN) and high fertilization treatment (HN), low N fertilization treatments (LN) significantly diminished *PER* activity by 13%−16% in SG and 2.1%−2.5% in GG (Table 2). *OX* activity showed similar responses to fertilization as *PER* activity, and the corresponding reductions under LN, as compared to NN and HN, were 18%−19% in SG and 2.2% in GG (Table 2). Across all fertilization treatments, SG showed significantly higher *PER* and *OX* activities than GG by 4.6% and 7.4%, respectively. The post-hoc tests of significant interaction of N fertilization and crop species showed significantly lower *PER* and *OX* activities under LN than NN and HN only in SG (Table 2). There were significantly negative correlations between oxidases (e.g., *PER*, *PHO*) and SOC and TN (Table 3). On the other hand, there were generally no significant correlations between oxidases (e.g., *PER*, *PHO*) and MBC, and there were significantly positive or negative correlations between one oxidase and MBN or some glycosidases (Table 3).

3.2 | Within-plot variances

The frequency diagrams of two oxidase activities and *OX* showed nearly normal distributions under all treatments in two croplands (Figure S1). The frequency distributions contrasted substantially among different N fertilization treatments for SG (e.g., LN vs. NN; LN vs. HN), whereas the frequency distributions of different N fertilization treatments showed relatively close ranges for GG (Figure S1). Remarkably, for LN in SG, *PER* and *OX* appeared to have the highest frequency in lower values, whereas for NN and HN, *PER* and *OX* had the highest frequency in higher values. These frequency distributions were consistent with a significantly

TABLE 1 *P*-values of two-way ANOVA tests for the main and interactive effects of N fertilization and crop species on *PHO*, *PER*, and *OX* (μmol g^{-1} soil h^{-1})

Enzyme type	Fertilization	Crop	Fertilization × Crop
PHO	0.28	0.16	0.28
PER	**0.01**	**0.03**	**0.022**
OX	**0.01**	**0.02**	**0.017**

Note: Bold numbers denote significant treatment effects at $p < 0.05$. *PHO*, phenolic oxidase; *PER*, peroxidase; *OX*, the sum of *PHO* and *PER*.

TABLE 2 Means (± SE) of *PHO, PER*, and *OX* (μmol g^{-1} $soilh^{-1}$) under three N fertilization treatments (NN, LN and HN) in two bioenergy croplands (SG and GG)

		PHO		*PER*		*OX*	
Crop	**Fertilization**	**Mean ± SE (%)**	**CV (%)**	**Mean ± SE (‰)**	**CV (%)**	**Mean ± SE (%)**	**CV (%)**
SG	NN	83.2 ± 26.6^{a}	45.3	641.3 ± 1.1^{a}	0.24	724.5 ± 25.6^{a}	4.99
	LN	45.5 ± 5.8^{a}	18.0	540.4 ± 23.0^{c}	6.02	585.9 ± 17.2^{b}	4.15
	HN	96.7 ± 18.9^{a}	27.6	620.6 ± 11.4^{ab}	2.59	717.3 ± 7.5^{a}	1.48
GG	NN	56.7 ± 6.2^{a}	15.4	577.5 ± 2.2^{abc}	0.54	634.2 ± 4.0^{ab}	0.88
	LN	55.1 ± 12.1^{a}	31.0	565.2 ± 12.3^{bc}	3.08	620.3 ± 24.4^{b}	5.55
	HN	54.7 ± 7.9^{a}	20.4	579.7 ± 2.1^{abc}	0.50	634.4 ± 9.9^{ab}	2.22

Note: In each column, different lowercase letters denote significant difference between fertilization treatments at $p < 0.05$ ($N = 48$). GG, gammagrass; HN, High nitrogen (168 kg N ha^{-1} yr^{-1} in urea); LN, low nitrogen (84 kg N ha^{-1} yr^{-1} in urea); NN, No nitrogen fertilizer input; SG, switchgrass.

TABLE 3 Pearson correlation coefficients between SOC, TN, C/N, MBC, MBN, MBC/MBN, hydrolase activities (i.e., *AG*, *BG*, *BX*, *CBH*), and oxidative activities (i.e., *PHO*, *PER*) under three N fertilization treatments (NN, LN and HN) in two bioenergy croplands (SG and GG)

	SOC	**TN**	**C/N**	**MBC**	**MBN**	**MBC/MBN**	***AG***	***BG***	***BX***	***CBH***	***PPO***	***PER***
SOC	1.0											
TN	0.92***	1.0										
C/N	0.28***	−0.12	1.0									
MBC	0.20***	0.21***	−0.01	1.0								
MBN	0.18**	0.21***	−0.05	0.36***	1.0							
MBC/MBN	−0.04	−0.04	0.001	0.41***	−0.64	1.0						
AG	−0.05	−0.09	0.11	−0.04	−0.08	0.03	1.0					
BG	0.19***	0.23***	−0.067	0.05	0.13*	−0.11	0.47***	1.0				
BX	0.09	0.07	0.07	0.01	0.10	−0.10	0.54***	0.54***	1.0			
CBH	0.21***	0.267***	−0.12	−0.02	0.08	−0.10	0.53***	0.69***	0.53***	1.0		
PPO	−0.16**	−0.29***	0.31***	−0.02	−0.15*	0.11	0.19*	−0.1	−0.07	−0.07	1.0	
PER	−0.13*	−0.16**	0.09	−0.05	−0.08	0.04	0.07	−0.11	−0.12*	−0.06	0.62***	1.0

Note: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Except the results of correlations associated with oxidases, they were adapted from the published table in Yuan et al. (2020). AG, α-glucosidase; BG, β-glucosidase; BX, β-xylosidase; CBH, cellobiohydrolase; C/N, the ratio of SOC over TN; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; *PER*, peroxidase; *PHO*, polyphenolic oxidase; SOC, soil organic carbon; TN, total nitrogen.

diminished *PER* and *OX* activities in LN compared to NN and HN (Table 2). On the other hand, the Cochran's C tests showed that N fertilization changed plot-level variation for *PER* and *OX* activities in both bioenergy croplands, and that LN induced the highest plot-level variances for *PER* and *OX* in SG (P2; Table S1).

The within-plot CVs of *PER*, *PER* and *OX* ranged from 8% to 58% in all treatments (Figure S2) and were similar between SG and GG although the largest CVs appeared consistently in SG (P2; Figure S2). In 12 plots, the number of plots with CVs larger than 40% for *PHO*, *PER*, and *OX* were 2, 0, and 0 in SG, and 4, 0, and 0 in GG, respectively; Accordingly, the number of plots with CVs less than 20% were 0, 3, and 5 in SG, and 0, 5, and 5 in GG, respectively. The within-plot CVs were generally much larger for *PHO* than those for *PER* and *OX* (Table 2; Figure S2).

The sample size required for all enzymes varied largely between different fertilization treatments except *PHO* in GG (Figure S3). In general, a larger number of samples were required under one LN plot than all other plots for all oxidases in SG (Figure S3). Given the same desired sampling error of 10%, the sample size required for each oxidase was always 1.5–2 times larger in SG than that in GG particularly under LN (Table S2).

3.3 | Surface trend, autocorrelation and spatial map

Trend surface analysis results showed very few significant linear or nonlinear trends in oxidase activity in most plots with no significant trends detected in NN plots in both

croplands (Table 4; Table S3). In SG, one or two significant linear or nonlinear trends were identified in the LN plot (e.g., P2) for each enzyme and only one significant nonlinear trend was evident in the HN plot (e.g., P1) for *PHO*. In GG, one or two significant linear or nonlinear trends were identified in the HN plot (e.g., P2) for *PER* and *OX*. Relative to NN plots, there were more significant linear or nonlinear surface trends of oxidases in LN or HN plots (Table 4).

The number and distance of significant spatial autocorrelations varied with N fertilization treatments, bioenergy croplands, and among oxidases (Table 5). Significant spatial autocorrelations were identified more frequently in SG than in GG particularly for *PER* and *OX* across three N fertilization treatments. In SG, LN treatment possessed a lower number of significant spatial autocorrelations than NN and HN treatments for all three oxidases. In GG, NN treatment (particularly P1) possessed many more significant autocorrelations than LN and HN treatments for *PER* and *OX*. The distance of significant spatial autocorrelation appeared to be positive or negative in any plot with more than one significant autocorrelation. The distances in which the significant spatial autocorrelations appeared from −5 to 4 m across all enzymes (Table 5). For *PHO*, LN showed fewer significant spatial autocorrelations than NN and HN in SG and GG (Figure S4).

With the same scale for each enzyme in two crops, the IDW maps of all enzymes exhibited relatively higher activities (e.g., darker color) in GG than those in SG, and this was true in NN and HN treatments (Figures 2 and 3). In SG, all IDW maps exhibited higher activities (e.g., darker colors) and heterogeneity (e.g., the number of scattered spots or mosaic) in NN and HN plots than in LN plots for all three oxidases (Figure 2). In GG, the IDW maps exhibited different patterns from those in SG. The IDW maps exhibited more pronounced heterogeneity (e.g., the number of scattered spots

TABLE 4 The number of significant regression coefficients of trend-surface analysis for *PHO*, *PER*, and *OX* (μmol g^{-1} soil h^{-1}) under three N fertilization treatments (NN, LN and HN) in two bioenergy croplands (SG and GG)

Crop type	Enzyme	NN	LN	HN
SG	*PHO*	0	1	1
	PER	0	2	0
	OX	0	2	0
GG	*PHO*	0	0	0
	PER	0	0	2
	OX	0	0	1

Note: Values represent the sum of significant regression coefficients in two replicated plots under each treatment. The regression coefficients denote parameters to in Eq. 4. The significant coefficients of trend-surface analysis for each plot was presented in Table S1. The abbreviations are referred to Table 1 and Table 2.

TABLE 5 Summary of significant distance for spatial dependence based on Moran's I values for *PHO*, *PER*, and *OX* (μmol g^{-1}soil h^{-1}) under three N fertilization treatments (NN, LN and HN) in two bioenergy croplands (SG and GG)

Crop	Fertilization	Plot	*PHO*	*PER*	*OX*
SG	NN	P1	−2.75	0.5, 0.75, −3.75	0.5, 0.75, 1, −3.25, −3.75
		P2	−2.75	−3.25, −3.5, 4	−3, −3.25, −3.5, 4
	LN	P1		1.75, −3.75	1.75, −3.75
		P2		0.75, −2, −2.5	0.75, −2.5
	HN	P1	−3.5, 5	0.5, 0.75, 1, −3.5, −5	0.5, 0.75, −3.5, −5
		P2	0.75, 2.25, −4.25, −4.5, −4.75	0.75, 1.25, 2, −4, −4.25, −4.5, −4.75	0.75, 1.25, 2, −3, −4, −4.25, −4.5, −4.75
GG	NN	P1	1, 1.5, −4, −4.5	0.5, 0.75, 1, 1.5, 1.75, 2, 2.25, −2.75, −3, −3.25, −3.75, −4, −4.25, −4.75, −5	0.5, 0.75, 1, 1.5, 1.75, 2, 2.25, −2.75, −3, −3.25, −3.75, −4, −4.25, −4.75
		P2	0.75, 2, −3, −4.75		−3
	LN	P1		0.5, 1, −3.75, −4, −4.25, −5	0.5, −3.75, −4, −4.25, −5
		P2	0.75		3.5, −4.75
	HN	P1	0.75, 2, −3, −3.25	0.75	0.75
		P2	−3.5	2.5, −4.5, −5	2.5, −3.5, −4.5, −5

Note: The unit of the distance for spatial dependence is meter. The abbreviations are referred to Table 1 and Table 2.

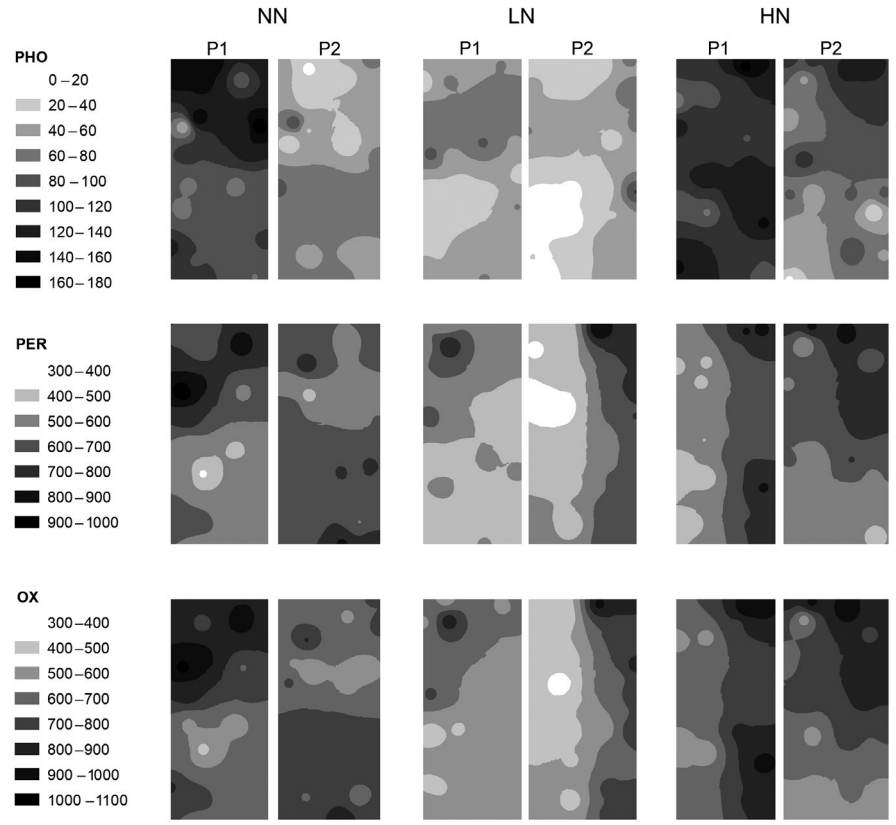

FIGURE 2 Spatial distributions of PHO, PER, and OX activity (μmol g^{-1} soil h^{-1}) in soils under three N fertilization treatments (i.e. NN, LN and HN) in SG. The interpolation maps were produced by inverse distance weighting (IDW) method. The abbreviations are referred to Tables 1 and 2

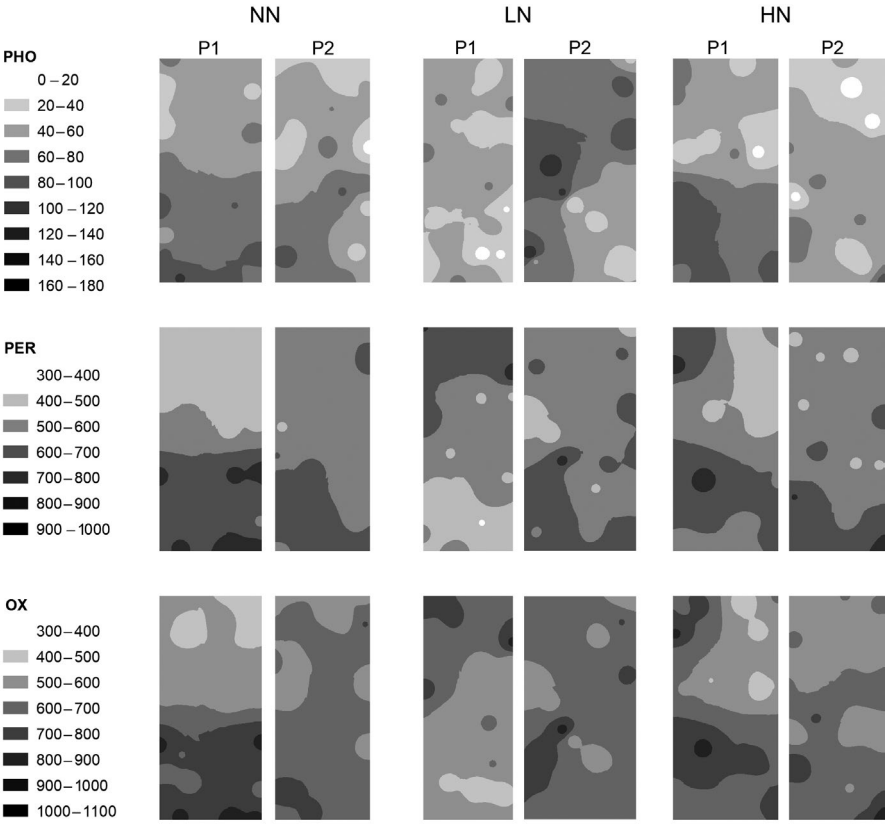

FIGURE 3 Spatial distributions of PHO, PER, and OX activity (μmol g^{-1} soil h^{-1}) in soils under three N fertilization treatments (i.e. NN, LN and HN) in GG. The interpolation maps were produced by inverse distance weighting (IDW) method. The abbreviations are referred to Tables 1 and 2

or mosaic) in LN and HN plots than those in NN plots for all three oxidases (Figure 3). However, the overall color regimes were comparable among all plots for each enzyme (Figure 3).

4 | DISCUSSION

4.1 | Low N fertilization depressed oxidase activity in switchgrass cropland soils

The first hypothesis was supported by the depressed *PER* and *OX* under low N fertilization (84 kg N ha^{-1} $year^{-1}$) in SG soil. This result is consistent with the generally diminished *PER*, *PHO*, and *OX* synthesized across various soil and ecosystems (Jian et al., 2016; Xiao et al., 2018). As found in forest and grassland soils, N fertilization increased the enzymatic acquisition of labile substrates so that the hydrolytic enzymes were stimulated and this resulted in less enzymatic acquisition of recalcitrant substrates catalyzed by oxidases (Jian et al., 2016). This study corroborated the general oxidase enzyme responses to N fertilization and the likely underlying mechanisms in bioenergy croplands.

Our results however also revealed insignificant high fertilization (168 kg N ha^{-1} $year^{-1}$) effects on oxidases activities in both croplands. This specific effect echoed the insignificant high fertilization effect (>150 kg N ha^{-1} $year^{-1}$) based on a synthesis (Jian et al., 2016) and in an upland cropland soil in wheat with intensive fertilization rates (182 and 225 kg N ha^{-1} $year^{-1}$; Ullah et al., 2019). When considering a large range of fertilization rates across various sites, a very low or intermediate fertilization rate (<50 or 100–150 kg N ha^{-1} $year^{-1}$) depressed soil oxidase activities but a low or high fertilization rate (50–100 or >150 kg N ha^{-1} $year^{-1}$) showed little influence (Jian et al., 2016). This suggested a strong nonlinear impact of N fertilization intensity on soil oxidase activities which may be driven by site-specific microbiomes regulating the expression and function of oxidases. Our analysis indicated that a more sophisticated accounting for N budget and optimizing N fertilizer use in cultivation is imperative to meet the global rise in competing demands for crop productivity and environmental protection (Zhang et al., 2013). This finding also reinforces the development of precision agriculture approaches to match nitrogen fertilizer inputs to spatial variability in fertility in crop fields (Dahal et al., 2020; Shaw et al., 2016; Wang et al., 2021).

Contrary to our first hypothesis, there was no significant effect of N fertilization on soil oxidase activities in GG, which contrasted with the negative responses in SG. This suggests differential underlying mechanisms likely regulate the oxidase response to N fertilization in SG and GG. The larger GG root structure and consequently more C and nutrients inputs to soil likely not only contributed to a larger soil C stock (Li, Jian, Lane, Lu, et al., 2020) but also rendered the microbial communities to capitalize on the hydrolases (Yuan et al., 2020), rather than on the oxidases which are energetically expensive for microbes to produce (Burns et al., 2013; Sinsabaugh, 2010b). In fact, GG soil showed higher SOC and total nitrogen contents (Li, Jian, Lane, Lu, et al., 2020) as well as glycosidase activities (Yuan et al., 2020) than SG soil.

Contrasting root chemistry of SG and GG may also induce different strategy to compete with soil microbes for nutrients (Kaye & Hart, 1997). Relative to GG root, SG root showed lower molecular weight and less structural complexity of dissolved organic matter (DOM), higher percent tyrosine-like DOM, and lower percent tryptophan-like DOM (Li, Jian, Lane, Lu, et al., 2020). Given the more structurally complex nature of GG root than SG root, the lignin compound of GG root is likely a more important C source to microbes. Lignin decomposition is however slow and catalyzed by ligninase, a type of oxidase (Gomes et al., 2009). Given the readily available N from fertilizer amendment (e.g., ammonium or nitrate), hydrolase production rather than oxidase production is thus favored for microbial nutrient acquisition, which is consistent with increased hydrolase activities in GG (Yuan et al., 2020).

The microbial community and genetic-level responses may also contribute to the observed insignificant effects of N fertilization on soil oxidases in GG. N fertilization affected microbial community composition and structure (Nemergut et al., 2008; Yevdokimov et al., 2008) and also altered the abundance and diversity of laccase genes, which are responsible for biosynthesis and secretion of phenolic oxidases (Blackwood et al., 2007; Giardina et al., 2010; Luis et al., 2005). The more structurally complex and recalcitrant lignin compound of GG root may increase the relative abundance of fungal groups that prefer slow turnover substrates (Li et al., 2012a; Ziegler et al., 2013). At the genomic level, N deposition could increase the abundance while decreasing the diversity of bacterial laccase genes (Freedman & Zak, 2014), and could also induce significant correlations between basidiomycete laccase gene abundance and phenol oxidase activity (Hofmockel et al., 2007). These studies suggest that N fertilization effects on oxidases are likely regulated at the functional gene level. Basidiomycete laccase gene abundance can be 5–10 times greater in high-lignin soils than in low-lignin forest soils (Blackwood et al., 2007), implying that GG soil may experience stronger genetic-level response and consequently higher oxidase responses than SG soils. However, the bacterial laccase gene abundance may be diminished under N fertilization so that a net effect of N fertilization on laccase gene abundance and oxidase activities may be insignificant. These speculations need to be tested in both SG and GG croplands in our sites.

The first hypothesis was also supported by the diminished spatial heterogeneity of oxidative activities under low N fertilization (84 kg N ha^{-1} $year^{-1}$) in SG soil. Similar to other

important agricultural practices (i.e., plowing, mechanical disturbance), N fertilization is generally regarded to homogenize the spatial distribution of soil biogeochemical features in long-term cultivated lands particularly when compared with forests (Li et al., 2010b). This effect of low N fertilizer input may also attribute to the abated mean soil oxidative activities that could have been caused by a widespread plot-level depression of soil oxidases. Nevertheless, spatial heterogeneity in SG was less evident than in GG plots with no N fertilizer input. Interestingly, this indicated that the indigenously low spatial variability of soil oxidases could be further homogenized with a reasonable amount of N fertilization.

However, spatial heterogeneity of soil oxidases under low N fertilization in GG was little influenced and the spatial patterns of soil oxidases under high N fertilization in both SG and GG were highly variable so that no consistent N effect can be derived. These suggested that both fertilization rate and crop species could moderate the effect of N fertilization on spatial distribution of soil oxidases. This corroborated that for edaphically similar sites, spatial patterns and the scale of soil variability could differ markedly and these differences were conditioned by intensity and duration of fertilizations (Basaran et al., 2008; Fraterrigo et al., 2005; Li et al., 2010b) and bioenergy crop species (Röver & Kaiser, 1999). In particular, among switchgrass and gamagrass, the latter possessed larger root biomass and volume (Clark et al., 1998) thus likely favoring nutrients scavenging and microbial activities and contributing to long-term spatial heterogeneity of soil microbial biomass (Dockersmith et al., 1999; Gross et al., 1995; Li et al., 2018; Rhoades, 1997; Wang et al., 2009) and glycosidases (Yuan et al., 2020). Despite very few studies examining the effects of N fertilization on soil oxidases, it is presumable that the high responsiveness of soil microbial features with disturbance could potentially override the general prediction of homogenization under N fertilizations (Allison & Martiny, 2008; Liang & Balser, 2012). This suggested a more dynamic spatial structure and pattern of oxidative enzyme activities than expected.

4.2 | Soil oxidases mediated the effects of N fertilization on SOC content in two bioenergy croplands

Consistent with our second hypothesis, there was a significantly negative correlation between soil oxidases (e.g., *PHO*, *PER*) and SOC content across all treatments in two bioenergy croplands. This was consistent with a global synthesis that showed the response ratio of ligninase, not cellulase, negatively correlated with SOC content (Chen, Luo, van Groenigen, et al., 2018). This is likely caused by the overall recalcitrance of SOC compounds and its stimulating effect on oxidases activities over the long term. The majority of soil organic carbon (SOC) is characterized as slow turnover and complex organic compounds (Trumbore, 2000) and may act as a major positive feedback of terrestrial biosphere to climate warming due to its high intrinsic temperature sensitivity (Davidson & Janssens, 2006; Friedlingstein, 2015; Li et al., 2012b). Although the biochemically recalcitrant SOM fractions are enriched with alkyl carbon (C) structures and resist decomposition due to intrinsic molecular properties (Lorenz et al., 2007), the large quantity of slow turnover substrate pool could stimulate the production and expression of oxidases and cause loss of SOC. This SOC loss can exceed the overall external C input thus leading to lowered SOC content. On the other hand, although soil organic matter content is not correlated with mean potential phenol oxidase and peroxidase activities, Sinsabaugh (2010a) pointed out that high *in situ* oxidative activities limit soil organic matter accumulation and low in situ oxidative activities promote soil organic matter storage. Furthermore, the potential phenol oxidase activity, in addition to other environmental and edaphic factors accounted for 37% of variation of SOC content, and peroxidase activity describes 32% of SOM variance (Sinsabaugh, 2010a).

Our study further demonstrated that under N fertilization, the negative relationship between soil oxidase activities and SOC content held true under different fertilization rates in SG and GG bioenergy croplands. Low fertilization rate significantly decreased soil oxidases activities, which corresponds to increased SOC in SG, though only a minor change of 5.4%. This suggested that low N fertilizer rate lowers oxidase enzyme activities, which should lessen decomposition of slow carbon forms, lengthening their stay in the soil and so benefitting carbon sequestration in SG. Meanwhile, low fertilization rate also decreased spatial heterogeneity of oxidase activities in SG so that the lower spatial variation in oxidase enzyme activity with fertilization would imply more spatially homogeneous enzyme activity and benefit to C storage. This holds true especially for switchgrass compared to gamagrass because low fertilization rate had little influence on oxidase activities and their spatial heterogeneity, which were consistent with insignificant changes of SOC in GG.

High fertilization rate also showed decreasing trends in soil oxidase activities (though not statistically significant), which aligned with the significantly enhanced SOC in both croplands. In particular, the observed greater SOC enhancement at the high fertilizer rate corresponded to a relatively greater reduction of oxidase activities in SG. Though low and high fertilization tended to increase spatial heterogeneity of soil oxidase activities in GG, the elevated SOC content suggested less control by spatial variation of oxidases activities in regulating SOC decomposition in GG. Overall, our analysis supported that fertilizer amendment was a powerful mediator of sequestration in switchgrass and optimizing fertilizer management in switchgrass may favor soil carbon

sequestration and environmental benefit. However, fertilizer management may play a less important role for C sequestration in gamagrass though, GG still acted as an important carbon sink due to its high SOC content. This corroborated the role of precision agriculture in promoting specific management practice and strategy given different plant and crop species (Miao et al., 2011).

4.3 | N fertilization effects on oxidases varied with enzyme type

This study supported our third hypothesis in that the N fertilization effects on mean activity and spatial heterogeneity of oxidases varied with enzyme type. As compared with *PER*, *PHO* showed no or less responses to N fertilization in mean activity, surface trend, and spatial heterogeneity. The lower response of *PHO* to N fertilization may lie in its special characteristics. The *PHO* catalyzed reactions result in intermediate compound such as *o*-quinones (Marusek et al., 2006; VanGelder et al., 1997), which are largely regulated by non-enzymatic reactions (Rolff et al., 2011; Taranto et al., 2017) and thus likely influenced by more interactions with edaphic characteristics rather than those typically impacting enzyme optimum (e.g., pH; Sinsabaugh, 2010a). Relative to *PHO*, *PER* catalyzes oxidation reactions in the presence of H_2O_2 representing a simpler reaction (Wong, 2009). Due to the complexity of reactions catalyzed by *PHO*, detergents may induce modifications of the enzyme structure and properties (Nicolas & Rouet-Mayer, 2003), and therefore the non-enzymatic reaction step may be inhibited due to *o*-quinones binding with other molecules thus resulting in the overall slowed enzymatic reaction (Rouet-Mayer et al., 1990).

Besides the unique chemical characteristics of each enzyme, the indigenous variation of each enzyme in a plot may regulate the effect of N fertilization. Relative to glycosidases with CVs more than 40% (Yuan et al., 2020), *PHO* exhibited similar CVs. Whereas *PER* and *OX* showed CVs that were generally smaller than 40%, similar to variation in soil moisture, total pore space, pH, SOC, TN, $\delta^{13}C$, and $\delta^{15}N$ (Baldrian, 2014; Li, Jian, Lane, Guo, et al., 2020; Röver & Kaiser, 1999) and substantially lower than that for extractable soil Fe and Mn (Li et al., 2010a). The substantially different CVs between *PHO* and *PER* found in this study demonstrated their contrasting plot-level variations, which led to a difference of sample size required up to an order of magnitude (94 vs. 9; Table 4). Given the same number of soil samples collected in each plot (i.e., 24), it unavoidably induced differential error terms for different enzymes that have contributed to the relatively larger variation in each plot and consequently insignificant treatment effects for *PHO*.

In contrast with the weaker response of *PHO*, *PER* showed pronounced responses to both N fertilization and crop type revealing a suite of high-order interaction of these enzymes with plant and soil, mediated by microbial community strategy. This suggested that the N fertilization effects on central tendency and spatial distribution of oxidases depended on the nature of the oxidase. However, the consistently negative correlations between oxidases (e.g., *PER*, *PHO*) and soil organic carbon and total nitrogen and an absence of correlation with microbial biomass and some glycosidases were also identified (Table 3), which corroborated the synergistic behavior of the two oxidases involved in soil organic carbon cycling across treatments and crop species (Hassan et al., 2013; Sinsabaugh, 2010a; Zhang et al., 2020).

5 | CONCLUSIONS

Our study demonstrated that soil extracellular oxidases mediated the N fertilization effects on SOC such that a low fertilization rate depressed oxidase activities and their spatial heterogeneity, which favored SOC accretion in SG. On the other hand, a low fertilization rate had little influence on oxidase activities and their spatial heterogeneity, consistent with insignificant changes of SOC in GG. However, high fertilization rate showed decreasing trends in soil oxidases activities, which aligned with the significantly enhanced SOC in both croplands. In particular, the observed greater SOC enhancement with high fertilizer use corresponded to a relatively greater reduction of oxidases activities in SG. Although high fertilization tended to increase spatial heterogeneity of soil oxidases activities in GG, the elevated SOC content suggested less control of spatial variation of oxidases activities in regulating SOC decomposition in GG. Overall, optimizing fertilizer management in switchgrass may favor soil carbon sequestration and environmental benefit, but less so in gamagrass. Nevertheless, N fertilization effects varied with enzyme types due to the unique enzyme characteristics. This informed the importance of accounting for multiple factors for studying oxidative activities and their role in soil carbon sequestration in bioenergy croplands. More broadly, the current study in bioenergy croplands highlights the role of mechanistic exploration of oxidases in elucidating N fertilization effects on SOC changes among forest, grassland, and cropland.

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CONFLICT OF INTEREST

The authors declare no competing interests.

AUTHOR CONTRIBUTION

J. L. designed the study; J. L., S. J., J. D., L. G., and M. P. conducted the field and laboratory analyses; J. D., M. Y., and S. J. conducted the data analysis; J. D. and M. Y. wrote the paper; all authors analyzed the result and revised the paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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