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RESEARCH ARTICLE

Effect of nitrogen fertilization on central tendency and spatial heterogeneity of soil moisture, pH and dissolved organic carbon and nitrogen in two bioenergy croplands

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Abstract

Background: Soil moisture, pH, dissolved organic carbon and nitrogen (DOC, DON) are important soil biogeochemical properties in switchgrass (SG) and gamagrass (GG) croplands. Yet their spatiotemporal patterns under nitrogen (N) fertilization have not been studied.

Aims: The objective of this study is to investigate the main and interactive effects of N fertilization and bioenergy crop type on central tendencies and spatial heterogeneity of soil moisture, pH, DOC and DON.

Methods: Based on a 3-year long fertilization experiment in Middle Tennessee, USA, 288 samples of top horizon soils (0–15 cm) under three fertilization treatments in SG and GG croplands were collected. The fertilization treatments were no N input (NN), low N input (LN: 84 kg N ha^{-1} in urea) and high N input (HN: 168 kg N ha^{-1} in urea). Soil moisture, pH, DOC and DON were quantified. And their within-plot variations and spatial distributions were achieved via descriptive and geostatistical methods.

Results: Relative to NN, LN significantly increased DOC content in SG cropland. LN also elevated within-plot spatial heterogeneity of soil moisture, pH, DOC and DON in both croplands though GG showed more evident spatial heterogeneity than SG. Despite the pronounced patterns described above, great plot to plot variations were also revealed in each treatment.

Conclusion: This study informs the generally low sensitivity of spatiotemporal responses in soil biogeochemical features to fertilizer amendments in bioenergy croplands. However, the significantly positive responses of DOC under low fertilizer input informed the best practice of optimizing agricultural nutrient amendment.

KEYWORDS
dissolved organic carbon (DOC), dissolved organic N (DON), gamagrass, nitrogen (N) fertilization, soil moisture, soil pH, spatial heterogeneity, switchgrass

1 | INTRODUCTION

Bioenergy crops such as the perennial switchgrass (SG) (*Panicum virgatum*) and gamagrass (GG) (*Panicum virgatum*) serve as an important alternative technology for sustainable replacement of fossil fuels (Monti et al., 2012; Tulbure et al., 2012) and will provide over 30% of biofuel plant biomass in the future (Gelfand et al., 2013; Kering et al., 2013). Nitrogen (N) fertilizers are widely used to increase yield of bioenergy crops (Behrman et al., 2013; Jung & Lal, 2011; Kiniry et al., 2013; Robertson et al., 2011; Smith et al., 2013), but their impacts on belowground soil physiochemical properties received attentions only in the recent decade. For instance, past studies examined N fertilization effects on SG soil aggregate stability, bulk density, phosphorus and potassium, soil organic carbon (SOC), microbial community abundance and composition (Chen et al., 2019; Krapfl et al., 2014; Lai et al., 2018; Li et al., 2018; Li, Jian, Lane, Guo, et al., 2020; Stewart et al., 2015; Valdez et al., 2017). Soil moisture, pH, dissolved organic carbon (DOC) and dissolved organic nitrogen (DON) are of importance for plant growth, microbial activities, nutrients' turnover and ecosystem health (Ding et al., 2019; Moradizadeh & Srivastava, 2021). However, only a few studies have investigated N fertilization effects on soil pH and moisture content (Krapfl et al., 2014; Lai et al., 2018), and DOC and DON in bioenergy croplands (Hussain et al., 2020). In addition, their spatial distributions and temporal dynamics that likely vary with different bioenergy crop species are largely unknown.

Soil moisture and pH are widely monitored and evaluated in many terrestrial and aquatic ecosystems such as forest, grassland, cropland, rivers and wetlands (Ghazali et al., 2020; Ritsema et al., 1998; Wang et al., 2021). Soil moisture and pH exert substantial influences on soil biogeochemical processes such as nutrient availability, soil microbial activities and crop growth and development (Neina, 2019; Parajuli & Duffy 2013). DOC is generally defined as organic matter that can pass through a filter which removes material between 0.70 and 0.22 mm in size (Hussain et al., 2020). DOC in soils supplies 80%–90% of plant and microbial C uptake and therefore represents a key component of biogeochemical cycle (Leenheer et al., 2000). DON, as the major form of nitrogen in soil water (J. L. Campbell, Hornbeck, et al., 2000), could amount up to 35% to >70% of total nitrogen export (D. H. Campbell, Baron, et al., 2000; Dafner & Wangersky, 2002), indicating the key role of DON in maintaining ecosystem N availability. Relative to SOC and total N (TN) that are monitored to indicate long-term effects of crop management practices (Li, Jian, Lane, Guo, et al., 2020; Li, Jian, Lane, Lu, et al., 2020), DOC and DON may reflect short-term changes and bioavailability in soil carbon and nutrients resulting from management practices (Kalbitz et al., 2000; McDowell, 2003). In bioenergy croplands, the specific patterns and variations of soil moisture, pH, DOC and DON have not been investigated. Elucidating their spatiotemporal variations under N fertilization and in different bioenergy croplands will help improve our management practice in mediating climate change via SOC sequestration and high nutrient use efficiency.

Soil moisture and pH can be affected by N inputs, removal of N in plant and N uptake (Holloway & Dahlgren, 2002). Given the fact that soil volumetric moisture content was little affected by N fertilization (Krapfl et al., 2014), past studies thus have focused on the combined effects of N fertilization and soil moisture regimes on microbes, soil and plant functions (Azizi et al., 2009; Haugland & Froud-Williams, 1999; Ramirez et al., 2010). Long-term N fertilizations led to substantial and widespread soil acidification in Chinese croplands, that is, a lower pH of up to 0.5 pH units (Guo et al., 2010). The decline of soil pH induced by N fertilization was also observed in SG cropland (Krapfl et al., 2014). However, N fertilization rate did not significantly impact soil pH for the first few years of SG establishment (Lai et al., 2018). These results collectively suggested likely major different responses given crop species. Despite very few studies investigated DOC and DON in SG croplands, past studies in other ecosystems showed that N fertilization could increase DON but not DOC (McDowell et al., 1998; Yano et al., 2000), diminish both DOC and DON (Vestgarden et al., 2001) or little affect DOC or DON (Emmett et al., 1998; Gundersen et al., 1998; Raastad & Mulder, 1999; Sjöberg et al., 2003; Stuanes & Kjønaas, 1998).

Collectively, these inconsistent observations could be primarily attributed to the contrasting edaphic characteristics and the fertilization rate and fertilizer form applied in each specific experimental site (Sanchez-Martin et al., 2008; Singh Mavi et al., 2018). Besides N fertilization, other management practices have been reported to affect DOC. For instance, no tillage soil showed 22% higher DOC concentration than conventionally tillage soil on average (Dou et al., 2008). Furthermore, intensified cropping and rotations also increased DOC pools (Dou et al., 2007). However, how DOC vary with bioenergy cropland type remains unknown. Nitrogen fertilization can also potentially affect spatial distribution of soil physiochemical properties. Nitrogen fertilization can generate hotspots of microbial communities, which result in greater soil C and N accumulations (Liang & Balser 2011; Ma et al., 2018; Naveed et al., 2014). In a 3-year N fertilization experiment at bioenergy croplands, N fertilizer inputs enhanced the spatial heterogeneity of microbial biomass C and N (Li et al., 2018).

In general, soil physiochemical properties and their spatial pattern and distribution could vary significantly with different plant species given their positive effects on soil C and N nutrient cycles and development of the soil biological community beneath the plant species (Ushio et al., 2010). These effects occur through alterations to the quantity and quality of root exudates, aboveground and belowground litter and micro-environmental conditions such as temperature and moisture (Zhang et al., 2018). As for bioenergy crops, SG and GG differ substantially in their aboveground conformation (Waramit et al., 2011) and their root morphology and chemistry (Li, Jian, Lane, Lu, et al., 2020), and the resultant soil physiochemical features and available nutrients status may thus contrast substantially between the two crops. As for the impacts of plant species on soils, the effects on spatial distribution were no less than the changes of average trend, that is, central tendency, of soil physiochemical properties (Charley & West, 1975; Boettcher & Kalisz, 1990; Hirose & Tateno, 1984; Matson, 1990; Schlesinger & Pilmanis, 1998). Local spatial patterns of soil properties are affected by the aboveground vegetation cover formed by different plant species. Further, the life-span life-form of

dominant species is associated with the spatial pattern of plant biomass, which decides the spatial scale and magnitude of heterogeneity of soil properties (Hook et al., 1991). Thus, it is expected that these striking differences in plant traits between different plant species may affect soil biogeochemistry and their spatial patterns (Fu et al., 2020; Hirobe et al., 2001).

In turn, the altered spatial variation of soil biogeochemistry is likely to affect the local distribution and abundance of plant species and the performance of individual plants and microorganisms and, therefore, to have consequences for both community structure and ecosystem-level processes (Robertson & Gross, 1994; Schlesinger et al., 1996; Tilman, 1988). Understanding the effects of plant type and management practice such as fertilization on soil spatial variability is important for soil quality management and improvement, sustainable land use and avoiding environmental degradation. Via the explicit demonstration and elucidation of spatial variations of soil biogeochemistry at a specific site (Li, 2019), it also enables a more accurate soil sampling strategy to be adopted in a highly heterogenous field environment, potentially promoting ecosystem management practices in different landscapes with various plant cover and management regimes. Past studies however were rarely done about the effects of bioenergy plant species on soil physiochemical properties, and even more scarcely on their spatial heterogeneity.

A 3-year long N fertilization experiment was initiated in 2011 at Tennessee State University's campus farm in Nashville, Tennessee, USA. Three fertilization rates (i.e., no input, low input and high input) and two bioenergy croplands (SG and GG) were implemented in the experiment using a complete random block design. A range of soil physiochemical features were quantified including soil moisture, pH, DOC and DON. The objective of this study is to investigate the effects of N fertilization and crop type on mean and spatial distribution of soil moisture, pH, DOC and DON. We hypothesized that N fertilization would increase DOC and DON concentrations, little change moisture content and decrease soil pH. Second, there are significant interactions of N fertilization and crop species such that N fertilization effects on soil properties are more pronounced in SG than that in GG given the contrasting plant traits such as root morphology and chemistry. Third, relative to soils that have never been fertilized for years, long-continued N fertilization re-structures spatial patterns of soil moisture, pH, DOC and DON at both croplands.

2 | MATERIALS AND METHODS

2.1 | Study site description and experimental design

In 2011, a bioenergy crop field fertilization experiment was established located at the Tennessee State University (TSU) Main Campus Agriculture Research and Education Center (AREC) in Nashville, TN, USA. Prior to the croplands, the land was mowed grassland for several decades with no amendment of fertilizers. Therefore, the indigenous variations are assumed to be similar before bioenergy croplands were established. The experimental site marks 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 included 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 GG (*Tripsacum dactyloides* L.). The three N levels included no N fertilizer input (NN), low N fertilizer input (LN: 84 kg N ha^{-1} y^{-1} as urea), and high N fertilizer input (HN: 168 kg N ha^{-1} y^{-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 in order to create appreciable gap and detectable effect between the two levels. The fertilizer was manually applied in June or July each year after cutting the grass. 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 analysis

On June 6, 2015, soil cores were collected from 0 to 10 cm depth using soil auger (Thermo Fisher Scientific, Waltham, Massachusetts, USA) from 12 plots (2 crop × 3 N × 2 replicates). Within each plot, we identified a sampling area of 2.75 m × 5.5 m rectangle, and the southwestern corner point was identified as the origin. Each plot was divided into two-square subplots and within each subplot, four centroids were identified and three cores were collected randomly given random direction and distance relative to each centroid (Figure 1). When a soil core was collected, we recorded its location in reference to the origin taken as the southwestern corner, that is, each sampling point had a unique *x*, *y* coordinates. Twenty-four cores were collected from each plot yielding 288 soil cores in 12 plots. All soil samples were transported to TSU lab in cooler filled with ice packs and subsequently stored at 4°C until microbial analysis.

The visible roots and rocks were removed from soil cores by passing through a 2-mm soil sieve prior to microbial and chemical analysis. A composited subsample was produced by combining six soil samples of equivalent dry weight for each treatment. The air-dried subsamples were ground to a fine powder and sent to University of North Carolina at Wilmington Center for Marine Science for analysis of SOC content, nitrogen content (TN), stable carbon and nitrogen isotopic signatures ($\delta^{13}C$, $\delta^{15}N$). Note that 0.5 M K_2SO_4 was used to extract soil DOC and nitrogen from fumigated and unfumigated soil samples. Soil gravimetric moisture content was determined by oven drying subsamples at 105°C for 24 h. And water extractable soil pH was measured given soil:water ratio of 1:5. To minimize the variation likely induced due to unevenly soil mixing, laboratory tests were conducted and specific protocols were created to secure sufficient soil mixing. The variation of each measurement (i.e., coefficient variation) in multiple tests ranged from 2% to 8% based on our protocol.

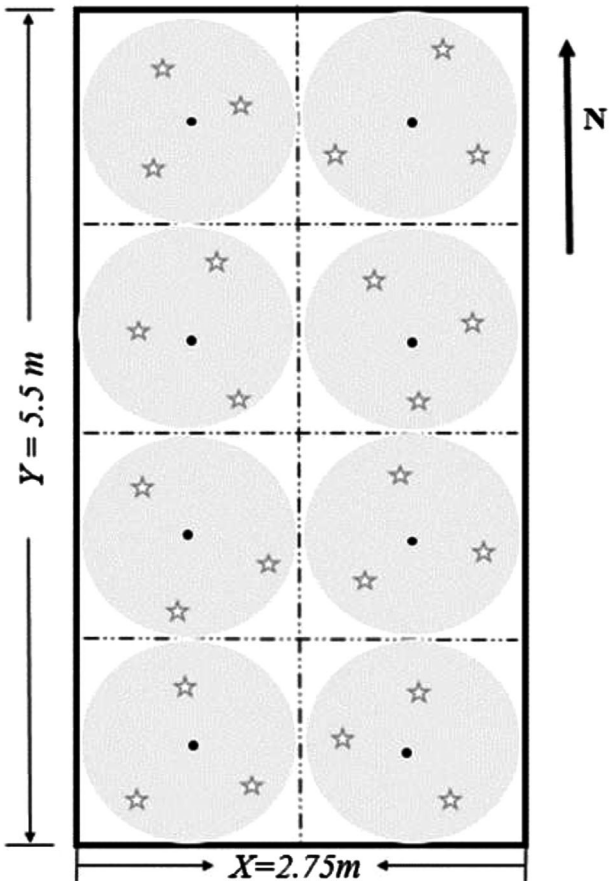

FIGURE 1 Illustration of an efficient clustered random sampling design within a plot (2.75 m × 5.5 m). The plot was divided into eight subplots (grey zone), and there was a centroid (dark solid circle) in each subplot (1.375 m × 1.375 m), where three soil sampling points (★) were determined from random 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

2.3 | Statistical analysis

We use both descriptive and geospatial analytical methods to illustrate the central tendency and spatial heterogeneity of the four soil properties. Mean, frequency distribution, plot-level variance and with-plot coefficient of variation (CV) were estimated to describe central tendencies and variations for enzyme activities in each plot. The two-way analysis of variance (ANOVA) was used to test whether N fertilization, crop species and their interaction significantly affected each property. To avoid the pseudo-replication impacts, the plot means were used in the two-way ANOVA test. The statistically significant level was set at $p < 0.05$.

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 under given desired sampling error (Li, 2019). That is, under a desired sampling error, the sample sizes derived can be used to evaluate the plot-level variations between different research plots. 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 ($\bar{X}$) 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.

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

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

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

where CI, $\bar{X}$, s, n, N, CV, and γ denote confidence interval, plot means, plot standard deviation, sample number ($n = 24$), coefficient of variation, sample size requirement 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 properties within and among plots. The methods were briefly described below and more details could be found in Li et al. (2010). First, trend surface analysis (TSA) is the most common regionalized model in which all sample points fit a model that accounts for the linear and non-linear variation of an attribute. Relationships between soil properties and x and y coordinates of their measurement location within the sampling plots are estimated with the trend surface model (Equation 4):

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

The presence of a trend in the data was determined by the significance of any of the parameters β_1–β_5, while the β_0 was the intercept (Gittins, 1968; Legendre & Legendre, 2012). Linear gradients in x or y directions were indicated by the significance of β_1 or β_5. A significant β_3 indicated a significant diagonal trend across a plot. Significant β_4 and β_5 parameters indicated a more complex, non-linear spatial structure such as substantial humps or depressions. Trend surface regressions

TABLE 1 *p*-Values of two-way analysis of variance (ANOVA) tests for the main and interactive effects of nitrogen (N) fertilization and crop species on soil dissolved organic carbon (DOC), dissolved organic nitrogen (DON), moisture and pH

p-Value	Fertilization	Crop	Fertilization × Crop
DOC	**0.01**	0.56	**0.073**
DON	0.16	0.47	0.378
Moisture	0.23	0.32	**0.053**
pH	0.49	0.40	0.810

Note: Bold numbers denote significant treatment effects at $p < 0.05$, or marginally significant treatment effects at $p < 0.1$.

were estimated using R program (R Core Team, 2014). 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 a Moran's *I* index (Legendre et al., 2012). The Moran's *I* analysis (Cressie, 1994; Legendre & Fortin, 1989; Moran, 1950) was used to quantify the degree of spatial autocorrelation that was present in each plot. The resulting local Moran's *I* statistics is in the range from −1 to 1 with a positive Moran's *I* values indicating similar values (either high or low) are spatially clustered, and a negative Moran's *I* values indicating neighboring values are dissimilar. No spatial autocorrelation or spatial randomness was reached with a Moran's *I* value of 0. Given that the observed Moran's *I* value is beyond the projected 95% confidence interval at a certain distance, this is identified as a significant autocorrelation. In this study, correlograms were produced for soil variables in all plots given a range of 0–5.5 m with 0.25 m incremental interval.

Third, an ordinary kriging method was usually used to produce maps which offered direct and visual assessments from which to compare the spatial distributions of the soil properties among the plots (Hohn, 1991). The ordinary kriging method required a large sample size (i.e., a few hundred or more) in order to achieve reliable interpretation maps (Hohn, 1991). Due to the fine-scale sampling region (1.375 m × 1.375 m) and a relatively small sample size per plot ($n = 24$), inverse distance weighting (IDW) interpolation was used in this study. 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., 2010). 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 that 2 was optimal with data collected in this study, as 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, USA) was used to generate the IDW maps and perform cross-validations.

3 | RESULTS

3.1 | Central tendencies and within-plot variances

There were significant effects of N fertilization on DOC content ($p = 0.01$; Table 1) and marginally significant interaction of crop species and N fertilization on DOC content ($p = 0.073$; Table 1). Post hoc tests showed that relative to NN and HN, LN significantly escalated DOC content in SG (Table 2). Yet there was no significant fertilization effect or crop species effect on soil moisture. And there were no significant effects of N fertilization or interaction of N fertilization and crop species on DON or soil pH (Table 1).

The frequency diagrams of four soil properties showed nearly normal distributions under all treatments in two croplands (Figure 2). The frequency distributions of all soil variables contrasted substantially among different N fertilization treatments for both SG and GG (Figure 2). On the other hand, the Cochran's C tests showed that N fertilization little changed plot-level variation for DOC and DON concentration or moisture in both bioenergy croplands. Yet remarkably, NN induced much higher plot-level variance for pH in both SG and GG plots (Table 3). The within-plot CVs of four soil properties ranged from 1% to 28% in all treatments (Figure 3). The CVs of the four properties were not much different in SG and GG (Figure 3). In 12 plots, the number of plots with CVs larger than 20% for DOC, DON, Moisture and pH were 2, 2, 1 and 0 in SG, and 0, 3, 1 and 0 in GG, respectively. Accordingly, the number of plots with CVs less than 10% were 1, 0, 1, and 6 in SG, and 1, 0, 3, 6 in GG, respectively. Overall, N fertilized plots showed more pronounced variance than control for both SG and GG (Figure 3).

The sample size requirement (SSR) for all properties was generally higher under low fertilizer input treatment (LN) than no fertilizer input (NN) or high fertilizer input (HN) in both croplands for DON and

TABLE 2 Means (± SE) of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), moisture and pH under three N fertilization treatments [no N (NN), low N (LN) and high N (HN)] in two bioenergy croplands [switchgrass (SG) and gamagrass (GG)]

Crop	Fertilization	DOC	DON	Moisture	pH
SG	NN	0.77 ± 0.04^{b}	0.43 ± 0.06^{a}	17.20 ± 0.3^{a}	6.06 ± 0.03^{a}
	LN	1.06 ± 0.04^{a}	0.46 ± 0.006^{a}	17.68 ± 0.51^{a}	5.96 ± 0.04^{a}
	HN	0.77 ± 0.03^{b}	0.37 ± 0.06^{a}	17.41 ± 0.38^{a}	6.02 ± 0.02^{a}
GG	NN	0.81 ± 0.03^{ab}	0.38 ± 0.02^{a}	18.57 ± 0.23^{a}	6.07 ± 0.05^{a}
	LN	0.94 ± 0.06^{ab}	0.53 ± 0.03^{a}	16.65 ± 0.6^{a}	6.04 ± 0.07^{a}
	HN	0.92 ± 0.07^{ab}	0.43 ± 0.07^{a}	18.1 ± 0.15^{a}	6.04 ± 0.08^{a}

Note: In each column, different lowercase letters denote significant difference between fertilization treatments at $p < 0.05$ ($n = 2$).
Abbreviations: NN, no nitrogen fertilizer input; LN, low nitrogen (84 kg N ha^{-1} y^{-1} in urea); HN, high nitrogen (168 kg N ha^{-1} y^{-1} in urea).

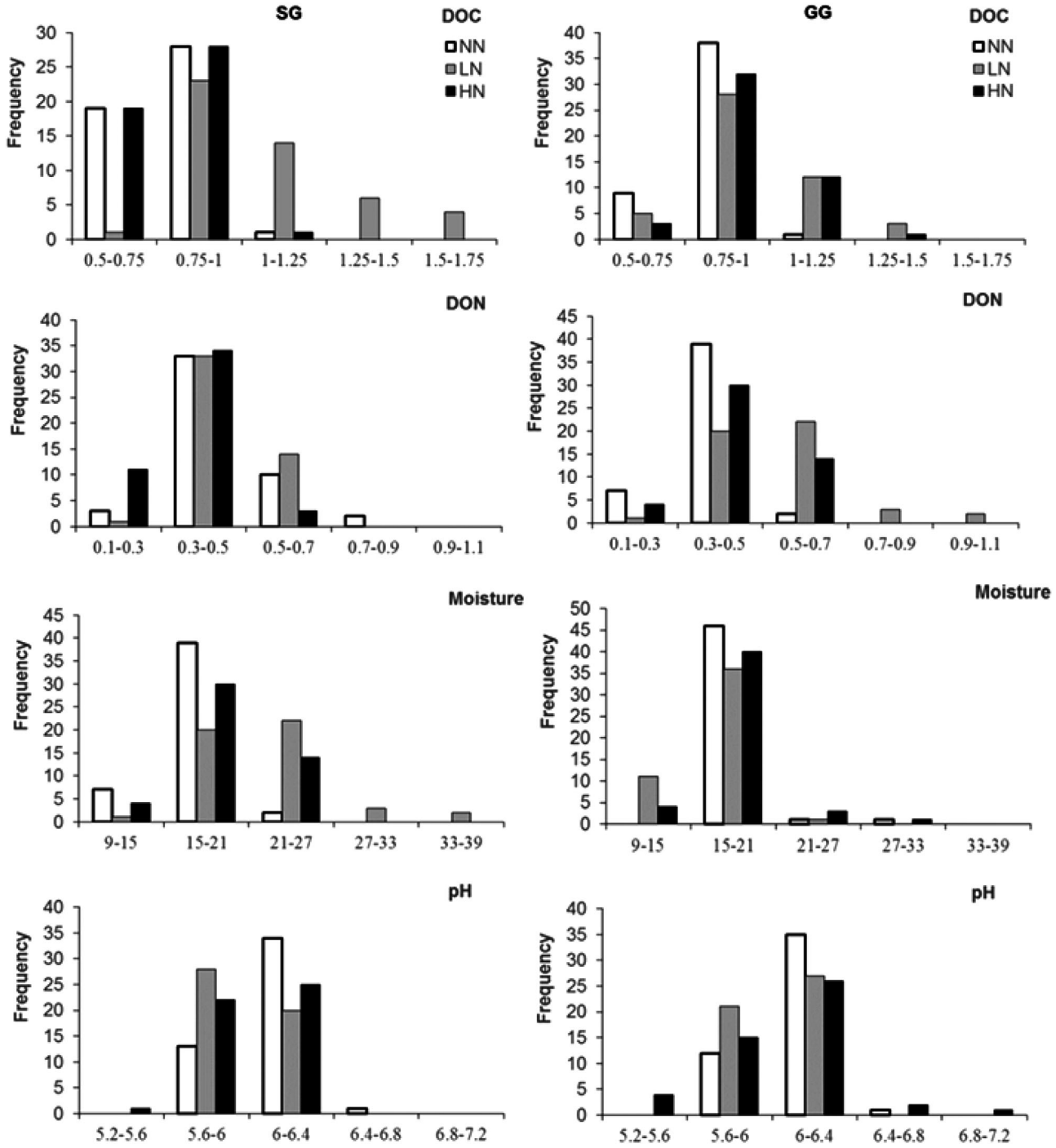

FIGURE 2 Frequency histograms of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), moisture and pH under three N fertilization treatments [no N (NN), low N (LN) and high N (HN)] in two bioenergy croplands [switchgrass (SG) and gamagrass (GG)]

DOC (Table 4). The plotted lines of SSR against relative sampling error departed from one to another in GG in a much wider extent than those in GG for moisture and pH (Figure 4). Under low fertilizer input treatment (LN), a larger number of samples were required in SG than that in GG for all properties under the same desired relative error. Yet under high fertilizer input treatment (HN), a larger number of samples were required in GG than that in SG.

3.2 | Surface trend, autocorrelation and spatial map

Trend surface analysis results showed only a few significant linear or non-linear trends in each plot, and more than half of plots showed no significant linear or non-linear trends (Table 5 and Table S1). SG plot showed much more linear or non-linear trends than GG plots (Table 5

TABLE 3 Comparison of the variances and Cochran's C test results for dissolved organic carbon (DOC), dissolved organic nitrogen (DON), moisture and pH under three N fertilization treatments [no N (NN), low N (LN) and high N (HN)] in two bioenergy croplands [switchgrass (SG) and gamagrass (GG)]

Crop	Fertilization	Plot	DOC	DON	Moisture	pH
SG	NN	P1	0.002	0.009	3.76	0.017
		P2	0.024	0.008	22.07	0.017
	LN	P1	0.070	0.007	8.69	0.007
		P2	0.054	0.011	4.28	0.025
	HN	P1	0.009	0.002	2.38	0.023
		P2	0.007	0.003	6.42	0.031
Cochran's test		*c*-Value	0.42	0.27	0.46	0.26
		p-Value	0.00	0.10	0.00	0.18
GG	NN	P1	0.009	0.005	1.84	0.011
		P2	0.005	0.003	10.16	0.026
	LN	P1	0.025	0.025	2.78	0.015
		P2	0.025	0.017	7.83	0.011
	HN	P1	0.012	0.007	17.09	0.133
		P2	0.019	0.009	2.52	0.014
Cochran's test		*c*-Value	0.27	0.38	0.40	0.63
		p-Value	0.12	0.00	0.00	0.00
Total Cochran's test		*c*-Value	0.27	0.24	0.25	0.40
		p-Value	0.00	0.00	0.00	0.00

TABLE 4 Sample size requirement for dissolved organic carbon (DOC), dissolved organic nitrogen (DON), moisture under the relative error of 10% and pH under the relative error of 2% under three N fertilization treatments [no N (NN), low N (LN) and high N (HN)] in two bioenergy croplands [switchgrass (SG) and gamagrass (GG)]. Each sample size denotes the average of sample size in two plots under the same treatment

Property	Crop type	Relative error (%)	NN	LN	HN
DOC	SG	10	8	21	5
DOC	GG	10	4	11	7
DON	SG	10	19	17	7
DON	GG	10	11	28	16
Moisture	SG	10	17	8	6
Moisture	GG	10	7	8	12
pH	SG	2	2	2	3
pH	GG	2	2	2	4

and Table S1). In SG, there were no significant linear or non-linear trends in any of NN plots except for moisture. Relative to NN and LN plots, there were more significant linear or non-linear surface trends of DOC and DON in HN plots, while the surface trends of moisture and pH were less (Table 5). In GG, there were no significant linear or non-linear trends in any of LN or HN plots for all properties, and there were not any significant surface trends in any plot for DOC or moisture (Table 5). Under the same treatment, the number of significant linear or non-linear trends varied between the two replicated plots (Table S1).

Spatial autocorrelations for moisture and pH were more frequently identified in LN than NN and HN in SG (Table 6). Spatial autocorrelations for DOC were not identified at any distance in HN plots, but showed at different distances in NN and LN plots in GG. Remarkably, DON in LN plots and pH in NN plots showed highly frequent spatial autocorrelations (Table 6). The comparable spatial autocorrelations for DOC and DON were identified in both SG and GG (Table 6; Figure 5 and Figure S1). These significant autocorrelations were either positive or negative, and the lagging distances ranged from 0.25 to 5.25 m. Spatial autocorrelations for DOC, DON, moisture and pH were all generally more frequently identified in GG than SG (Table 6; Figures S1–S3).

With the same scale for four variables in two crops, the IDW maps of all properties exhibited higher levels (e.g., darker color) in GG than those in SG except for DOC under LN treatment. And this was true in both unfertilized and fertilized plots (i.e., NN, LN and HH). In SG, the plots under LN treatment showed obvious higher level than other treatments. Other than that, there was not any trend exhibited by IDW maps from NN to HN treatments plots (Figure 6). In GG, the IDW maps exhibited higher levels of DOC and DON under LN treatment. For pH,

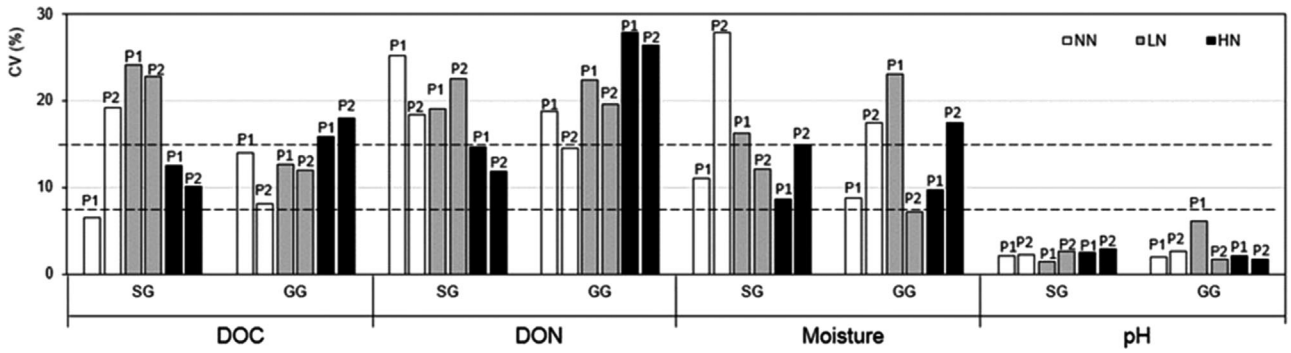

FIGURE 3 Within-plot coefficient of variations (CVs) of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), moisture and pH under three N fertilization treatments [no N [NN], low N (LN) and high N (HN)] in two bioenergy croplands [switchgrass (SG) and gamagrass (GG)]. The dashed lines represent a CV of 10% and 20%

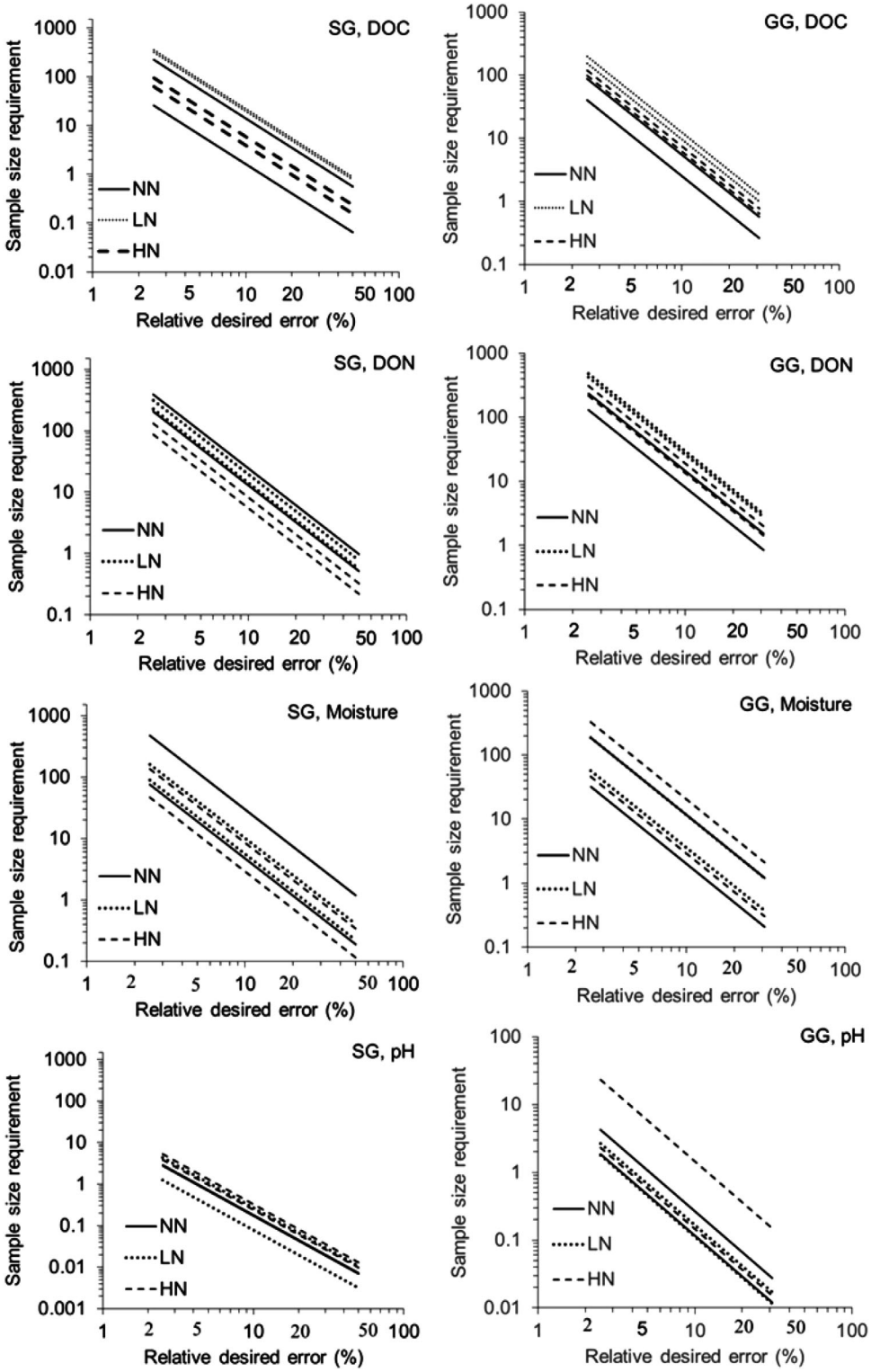

FIGURE 4 Plots of log transformed sample size requirements (SSR) and desired relative errors dissolved organic carbon (DOC), dissolved organic nitrogen (DON), moisture and pH under three N fertilization treatments [no N (NN), low N (LN) and high N (HN)] in two bioenergy croplands [switchgrass (SG) and gamagrass (GG)]. NN, black solid line; LN, black dotted line and HN, black dashed line. The log scale was applied on both axes. SSR denotes two plots in each treatment

TABLE 5 The number of significant regression coefficients of trend-surface analysis for dissolved organic carbon (DOC), dissolved organic nitrogen (DON), moisture and pH under three N fertilization treatments [no N (NN)], low N (LN) and high N (HN)] in two bioenergy croplands [switchgrass (SG) and gamagrass (GG]). Values represent the sum of significant regression coefficients in two replicated plots under each treatment. The regression coefficients denote parameters β_1–β_5 in Equation (4). The significant coefficients of trend-surface analysis for each plot are presented in Table S1

Crop type	Properties	NN	LN	HN
SG	DOC	0	0	1
	DON	0	0	1
	Moisture	2	1	0
	pH	0	4	0
GG	DOC	0	0	0
	DON	1	0	0
	Moisture	0	0	0
	pH	2	0	0

IDW maps exhibited low to high levels (e.g., shallower and gradually darker colors) from NN plots, through LN, to HN plots (Figure S4).

4 | DISCUSSIONS

4.1 | Low nitrogen fertilization elevated DOC content in SG cropland soils

Our current study identified that relative to no N input, low nitrogen input rather than high input significantly elevated DOC content in SG cropland soils, which partly supported our first hypothesis that N fertilization would increase DOC concentrations. Their respective relative increases of DOC (38% vs. ≈ 0%) under low and high N fertilization treatments imparted in the key differences as well. In general, soil DOC concentration increases with nitrogen fertilization rates as revealed by various field experiments (Adams et al., 2005; McTiernan et al., 2001; Oladele & Adetunji, 2021; Shang et al., 2015). The explanation lied in the elevated plant growth and input to soils via litter fall and root that supplied organic acids and substrates for microbial decomposition resulting in soluble C as byproducts (Nakamura et al., 2012). Yet our result is consistent with the findings that DOC leaching was higher in the low N input system and lower in the higher N input system in hill country grazed by sheep in New Zealand (Parfitt et al., 2009). This could be explained by the presence of the more hydrophobic fraction of DOC which is known to readily adsorb to soil mineral surfaces, contributing to an increased DOC concentration in soil (Marschner & Kalbitz, 2003). Another possible biological mechanism is that extra N input created a low C:N environment for microbes in high N input croplands, which could induce more competitive C acquisitions between microbe and plant root. As a result, DOC availability was lower in high N input croplands than low N input croplands.

This study also found that DOC contents were elevated under low N fertilization input in SG croplands but remained unchanged in GG cropland. That is, N fertilization effects on soil DOC were more pronounced in SG than that in GG, which supported our second hypothesis. This crop-specific response can be contributed to the different root traits and aboveground plant biomass between two crops. For instance, SG has a lower specific root length (i.e., root length per unit root biomass) (de Graaff et al., 2013; Dzantor et al., 2015) and GG has larger coarse root biomass and volume (Clark et al., 1998). This suggested a relatively short turnover time for SG root and much longer

TABLE 6 Summary of significant distance for spatial dependence based on Moran's *I* values for dissolved organic carbon (DOC), dissolved organic nitrogen (DON), moisture and pH under three N fertilization treatments [no N (NN), low N (LN) and high N (HN)] in two bioenergy croplands [switchgrass (SG) and gamagrass (GG)]. The unit of the distance for spatial dependence is meter

Crop	Fertilization	Plot	DOC	DON	Moisture	pH
SG	NN	P1	1.5, −3, −3.5, −4		−3.75	−0.5
		P2		1.5, 2.75	−0.5	
	LN	P1			0.5, −3.25, −3.75	0.75, 1, −2, −2.25
		P2	3	−5.25	2.75, −4.5	0.75, 4.5, 5
	HN	P1	0.75	−2.5, 5.25		
		P2	3.75		−0.5	−2.25, −3.75
GG	NN	P1		0.75, −3.5, 4	−3.25, 5	5
		P2	2.75, −4	−3.75	−3.5, 4	2, −4.25, −4.5, −4.75, −5.25
	LN	P1			−5.5	−4.25, 5
		P2	0.25, 1, −3.75	0.75, 1.25, 1.5, 1.75, −3, −3.75, −4, −4.25		−2.25
	HN	P1		0.5	−0.75	1, −2, 3, 3.25, −4.25, −4.75
		P2		1, −4, −4.25		

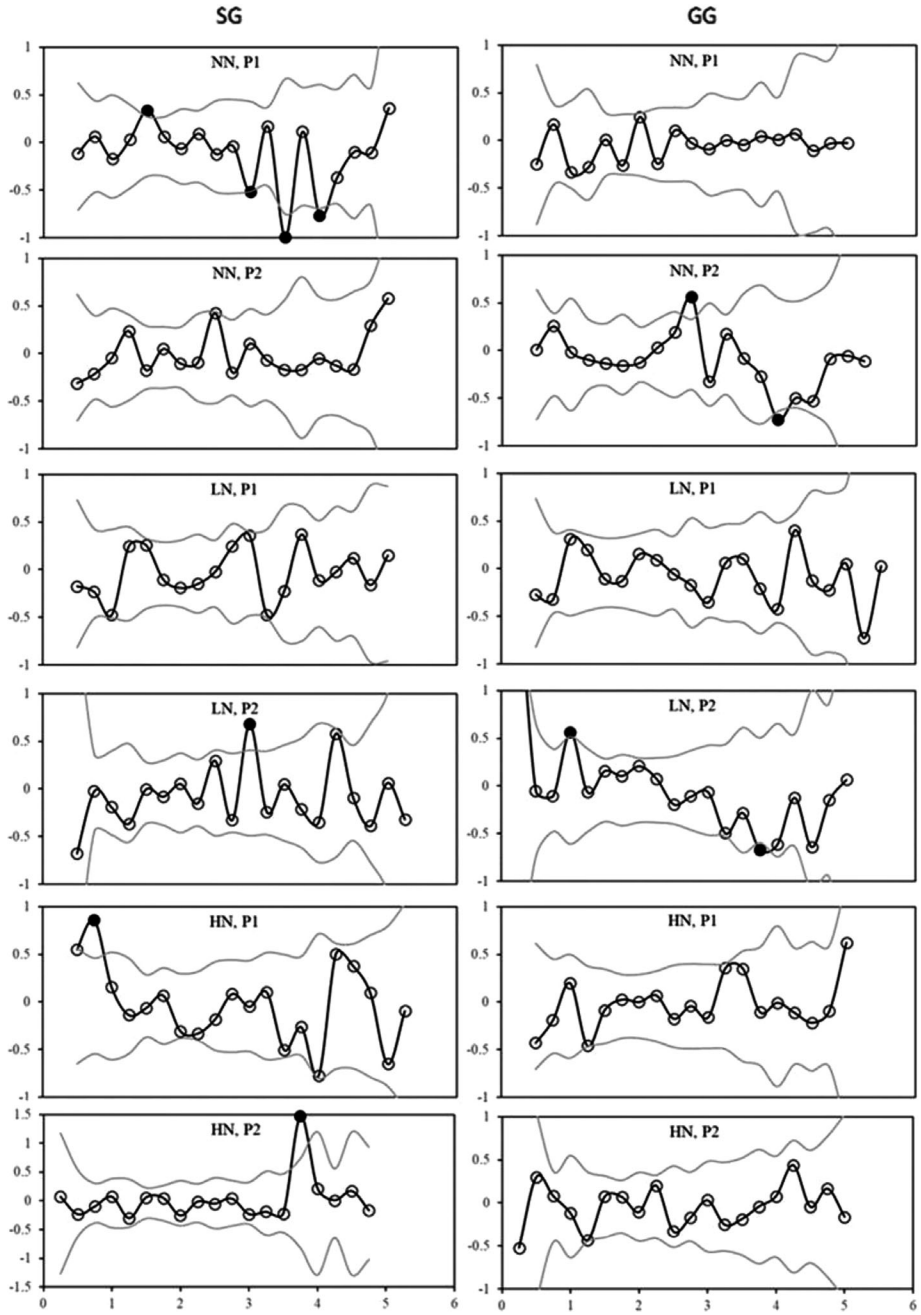

FIGURE 5 Correlograms of Moran's *I* for dissolved organic carbon (DOC) under three nitrogen (N) fertilization treatments [no N (NN), low N (LN) and high N (HN)] in two bioenergy croplands [switchgrass (SG) and gamagrass (GG)]. Filled circles, positioned beyond the upper and lower lines, represent positive or negative Moran's *I* values that exhibited significant autocorrelation. Middle line: observations; lower line: low confident limit and upper line: upper confident limit. On x-axis, unit is meter

turnover time for GG root (Dietzel et al., 2017). In addition to significantly greater aboveground plant biomass in SG than GG (Li, Jian, Lane, Lu, et al., 2020), these contrasting traits may contribute to more pronounced plant input to soils via root and biomass in SG than GG. As plant root and biomass are known as main drivers of soil microbial communities (Eisenhauer et al., 2017), the differences between SG and GG might led to different uptake of DOC by microbes and thus caused the different spatial distributions.

4.2 | Nitrogen fertilization had no significant effects on DON content, soil moisture or pH

DON concentrations were not significantly affected by N inputs or crop type, which did not support our hypothesis that DON concentrations would be increased. N fertilizer generally increased N mineralization (Khan et al., 2007; Mulvaney et al., 2009; Robertson et al., 2013) and consequently elevated DON concentration. This positive effect could

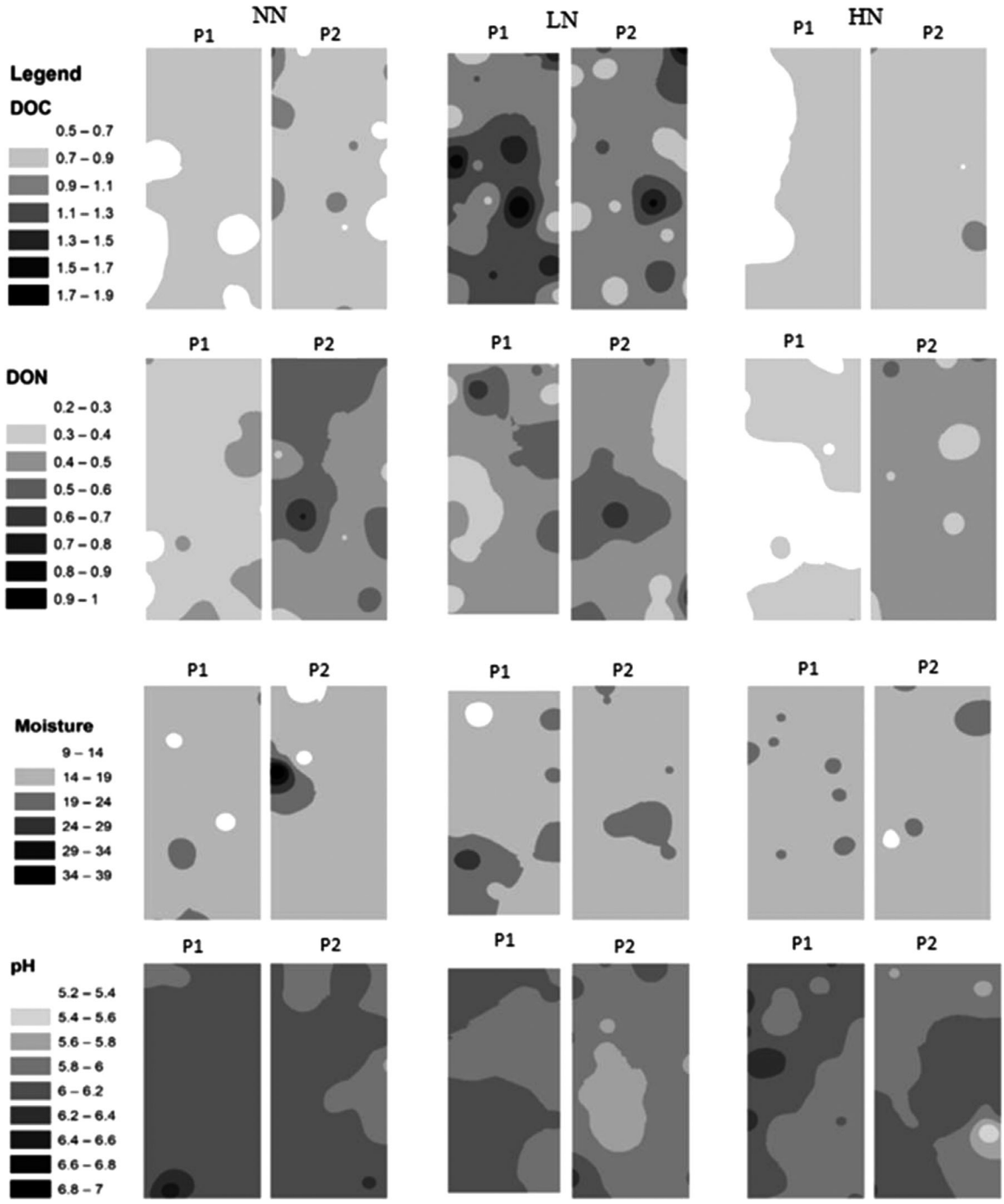

FIGURE 6 Spatial distributions of dissolved organic carbon (DOC), dissolved organic nitrogen (DON), moisture and pH under three N fertilization treatments [i.e., no N (NN), low N (LN) and high N (HN)] in SG. The interpolation maps were produced by inverse distance weighting (IDW) method using ArcGIS software by Esri (version 10.2.1, http://www.esri.com)

be mitigated due to DON uptake by plant roots, specifically amino acids (Streeter et al., 2000). Another possible reason is the increased uptake of DON by soil microorganisms, which is supported by increased microbial C:N in N fertilized croplands (Li et al., 2018). Due to the multiple pathways of transformation and opposing trends of change, the resultant percentile changes under low and high fertilizations varied largely in magnitude and sign, for instance, 7.0% versus −14% for SG, and 40% versus 13% for GG, respectively.

The result that soil moisture was not significantly affected by nitrogen inputs or crop types supported our hypothesis. This result contradicted the finding that soil water content was 18% higher in NPK fertilized crop lands than in control crop lands (Yang et al., 2011). This disparity may be attributed to the facts that NPK fertilizer applied in their experiment (Yang et al., 2011) produced an overall beneficial effect on plant growth and improvement in soil quality, compared with the sole N fertilizer applied in our experiment. Other possible reasons

may lie in the sampling frequency and crop types. The single sample in our experiment may just represent a transient phenomenon associated with specific timing, which could contrast largely with more frequent samplings in another experiment, representing a type of response on average over a longer time period. It may be critical to note that the crops employed in our experiment were SG and GG, two typical bioenergy crops with their generally greater drought tolerance, soil water content may thus have persistently remained low in all treatments.

Soil pH was not significantly affected by N inputs, or crop type is contradictory to our hypothesis that soil pH would be decreased. Despite widespread soil acidification due to N fertilization in croplands (Guo et al., 2010; Kirchmann et al., 1994), soil pH was not significantly affected by N fertilization in SG filed in Dakota, USA (Lai et al., 2018). Yet our results that pH was not significantly affected by N fertilization differed with the research showing that the pH decreased with increasing rates of N fertilization (Geisseler & Scow, 2014). Fertilizer type can also play an important role in determining soil pH change. Ammonium (NH_4^+) fertilizers were found to reduce soil pH, while the application of nitrate (NO_3^-) had little effect on soil pH (Malhi et al., 2000; Volk & Tidmore, 1946; Wolcott et al., 1965). This difference lies in the fact that soil pH decreases due to the release of H^+ in soil solution for charge balance originated from the plant uptake of NH_4^+ and the release of HCO_3^- in soil solution for charge balance originated from the plant uptake of NO_3^-. In our experiment, although urea (NH_2–CO–NH_2) was applied, there was no significant change in soil pH. This can be explained by the fact that although urea can be a direct source of N for plant (Liu et al., 2003), most of it is rapidly hydrolyzed by soil ureases and turned in ammonium (NH_4^+), which can be converted into nitrate (NO_3^-) (Arora & Srivastava, 2013). The acidity produced by nitrification can be neutralized when plants take up more nitrate in exchange of HCO_3^- released in soil solution (Barak et al., 1997).

4.3 | Low N fertilizer input elevated spatial distributions of soil moisture, pH, DOC and DON

N fertilization elevated within-plot variations in both crop plots. And low N fertilizer input generally elevated the spatial variations (i.e., more significant surface trends in various directions and more pronounced spatial autocorrelations) of soil moisture, pH, DOC and DON in both croplands. This partially supported our third hypothesis that N fertilization would re-structure spatial patterns of soil moisture, pH, DOC and DON at both croplands. We speculate that the manual spread of N fertilizers in the field will likely lead to irregularity of nutrient deposit and clusters and consequently favor the formation of hotspots of microbial communities (Kuzyakov & Blagodatskaya, 2015). This may explain the general N fertilizer effects (either low or high rate) that tended to elevate the overall variation or fine-scale spatial heterogeneity. However, fertilizer amendments with a high rate may create fewer hotspots with higher nutrient concentrations than those created under low N input rate. These fewer hotspots could directly contribute to great plot level variations. The different effects revealed under the low and high N inputs may also lie in their impacts on plant growth. That is, more widespread root exploitation and growth underground and greater extent and return of litterfall to surface soil, under high rate, tends to reduce heterogeneity, but low N input might only intensify impacts in some spots or locations, which led to more heterogeneity.

4.4 | GG plots showed more pronounced spatial heterogeneity of soil moisture, pH, DOC and DON

Relative to SG, GG showed greater spatial variations of all four variables by more detectable linear and non-linear surface trends, autocorrelations and hotspots across fertilization treatments. This may reflect the dominating influence of root morphology and its interaction with soil microbes in sustaining spatial distributions of microbial biomass. It is well known that plant root and microbes interact closely as mutualist and thus the large root and deeper root depth can favor clusters of microbial biomass and higher activities.

5 | CONCLUSIONS

Our study demonstrated that low input N fertilization significantly enhanced central tendency of DOC in SG lands yet had no significant effect on central tendencies of soil moisture, pH or DON. N fertilization, particularly in low input, consistently elevated the spatial heterogeneity of soil moisture, pH, DON and DOC in both crop lands, with more within-plot variance and pronounced spatial heterogeneity in GG croplands. We speculate that the enhanced DOC under low N input treatment is likely associated with more competitive C acquisitions between microbe and plant root under intensive N fertilizer input. It is also reasonable to presume that aboveground crop growth and root exploitation have been stimulated and induced return of litterfall to surface soil to greater extent and range in space under high rate of N fertilization. Collectively, these might lead to lower heterogeneity in high N input croplands. This study informs the generally low sensitivity of soil biogeochemical responses to fertilizer amendments in bioenergy croplands. Meanwhile, the detectable responses of dissolved organic carbon and elevated spatial features of various variables at relatively frugal fertilizer input have important implications for agricultural nutrient amendment practice, soil carbon turnover and sequestration. This corroborated the recommendation of abated fertilizer requirement in croplands reached in our former studies, particularly in the wake of soil organic carbon sequestration (Li, Jian, Lane, Lu, et al., 2020) and relevant microbial mediation mechanism through extracellular oxidases (Duan et al., 2021). Future research efforts should strive to optimize N fertilizer input rate required for not only high bioenergy crop yield but also amiable soil health for the sake of best management practice.

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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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