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ORIGINAL RESEARCH

Spatial Assessment of Selected Soil Properties within an Industrial Poultry Production Site

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Abstract: Waste resulting from industrial poultry production systems is becoming an increasingly significant environmental problem in the US, threatening both soil and water quality. The goal of this study was to assess the spatial variability and interactions of selected soil properties (physical, chemical, and biochemical), viz., particle size, pH, enzymatic activity, Soil Organic Carbon (SOC), and Total Nitrogen (TN), across an agricultural landscape used for industrial poultry production. The measured soil properties were separated according to biochemical constituents and soil texture based on the first two principal components, accounting for approximately 60% of the variability across the site. These principal components were then used to generate soil surface maps, indicating areas of possible catalytic activity. Surface maps showed possible increases in biochemical activity around areas of stored poultry litter, suggesting the utility of these methods in determining changes to soil management.

Keywords: geostatistics, kriging, principal components, soil enzymatic activity

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Introduction

More than 65% of US broiler production is concentrated in the Southeastern States as Alabama ranks third in production behind Georgia and Arkansas, accounting for 11% of the total US production.[1] In order to capitalize on this production, industrial poultry producers have focused their efforts on fewer and larger operations, raising environmental and economic concerns associated with the use of the resulting manure and litter.[2] Research has suggested that the agricultural use of manure can alter the physical,[3–5] biochemical,[5,6] and biological[6,7] status of soils. In particular, using poultry litter as an organic fertilizer has been shown to directly alter some soil properties including pH,[8,9] soil organic matter content,[3,6,9] and microbial enzymatic activity[6] among other soil properties. Microbial enzymatic activity has been shown to have a strong response to changes in field management,[10] thus suggesting its potential as part of an integrative indicator of soil quality at the landscape level. Among the soil enzymes studied, phosphatase enzymes have been shown to be the best indicators of soil quality[11,12] due to their sensitivity to land management practices[13,14] and organic matter content.[15,16]

Though there have been studies that have focused on the impact of poultry litter as an organic amendment, the impact of on-site management on soil quality is poorly understood. The consequences of production site handling of poultry litter have similar implications as soil application with respect to environmental quality (ie, leaching of nutrients and carbon retention). Site-specific concerns affecting soil quality in response to management decisions should first be quantified in order to establish the most sustainable management practices which cause the least disturbance.[14]

The inherent complexity of soil makes it particularly difficult to develop an indicator of soil quality. There have recently been attempts to provide mathematical indices of soil quality reflective of algebraic operations[17,18] or multivariate analysis[19] which reduce the variability encountered in the soil. Mathematical techniques that are being employed to reduce variables around redundant measures by soil scientists and ecologists include principal component analysis (PCA)[20] and factorial analysis.[21]

Recent studies assessing spatial effects on biochemical indicators of soil quality using geostatistical techniques have looked at soil microbial biomass within pasture and beech forest soils,[22,23] soil properties of irrigated and dry agricultural soils,[24] soil enzymes in pastured, urban, and agricultural soils,[14,25,26] and soil acidification in forest soils in conjunction with PCA.[27] Additionally, soil properties have also been spatially characterized in response to grazing management, which has been shown to increase the spatial patchiness of soil N,[28] homogenize soil properties through trampling,[29] and degrade soil chemical and physical properties in overgrazed grasslands.[30] As these studies have examined discrete areas of soil under a single management type, it is unclear how soil properties respond spatially to diversely managed agroecosytems. Thus the objectives of this study were to: determine spatial trends, if any, of soil properties with respect to the site's multiple management practices, identify interactions between soil properties across the landscape as principal components of variation, and generate soil surface maps of the site with respect to the principal components generated.

Materials and Methods

Study sites

The study site was Wayne Farms broiler production unit located at 32° 4′ 2.2″ N and 85° 42′ 35.9″ W, on 4 Ha land in Bullock County, Alabama, USA. The soil series of the study area are Alaga (loamy sand, thermic, coated Typic Quartzipsamments) and Conecuh (sandy loam, fine, smectic, thermic Vertic Hapludults). For the past 10 years this land has been used as an industrial broiler production site. During each of those ten years, 5–6 batches (~80,000 broilers per batch) were produced with residual litter being removed annually and stored outside of the poultry houses at a designated site until a market could be established for the litter. In addition, there was approximately 1 Ha of pasture for a herd of 10 horses to graze. This area is only lightly grazed, as the horses are released onto this portion of the site for only 2–3 days a week.

In accordance with Brus and de Gruijter,[31] a stratified random sampling method was chosen to meet the requirements of cost efficiency and statistical utility. Preliminary studies on soil physiochemical properties across the site allowed for the reasonable designation of strata (sampling areas) that captured the spatial variability (Fig. 1). The sampling area "B" was primarily covered with four poultry houses, sampling area

"X" was where used bedding material that has been cleaned out of the houses was temporarily stored, and sampling area "L" was a pasture grazed infrequently by horses.

Field moist soil samples (120 g) were collected from the randomized locations across the three strata on September 28, 2008. Samples were collected from the upper 15 cm of soil at 45 sampling points in the landscape using a soil sampler, while simultaneously geocoding sample sites in decimal degrees using a handheld geographic positioning system (Garmin GPS 12 XL, Olathe, Kansas USA). Samples were preserved in ice and transported to the laboratory. Upon arrival soils were shortly stored at field moist conditions at 4 °C, and prior to soil enzyme assays, soils were air-dried for 48 h and sieved using a 2 mm mesh and mixed thoroughly thereafter.

Soil enzyme analysis

Soil enzyme analysis was performed according to the method proposed by Tabatabai,[32] with slight modification. The artificial substrate, *p*-nitrophenyl (1 mL, 0.05 M), and a pH buffer (pH = 11.0 for alkaline phosphatase [*APA*], 6.5 for acid phosphatase

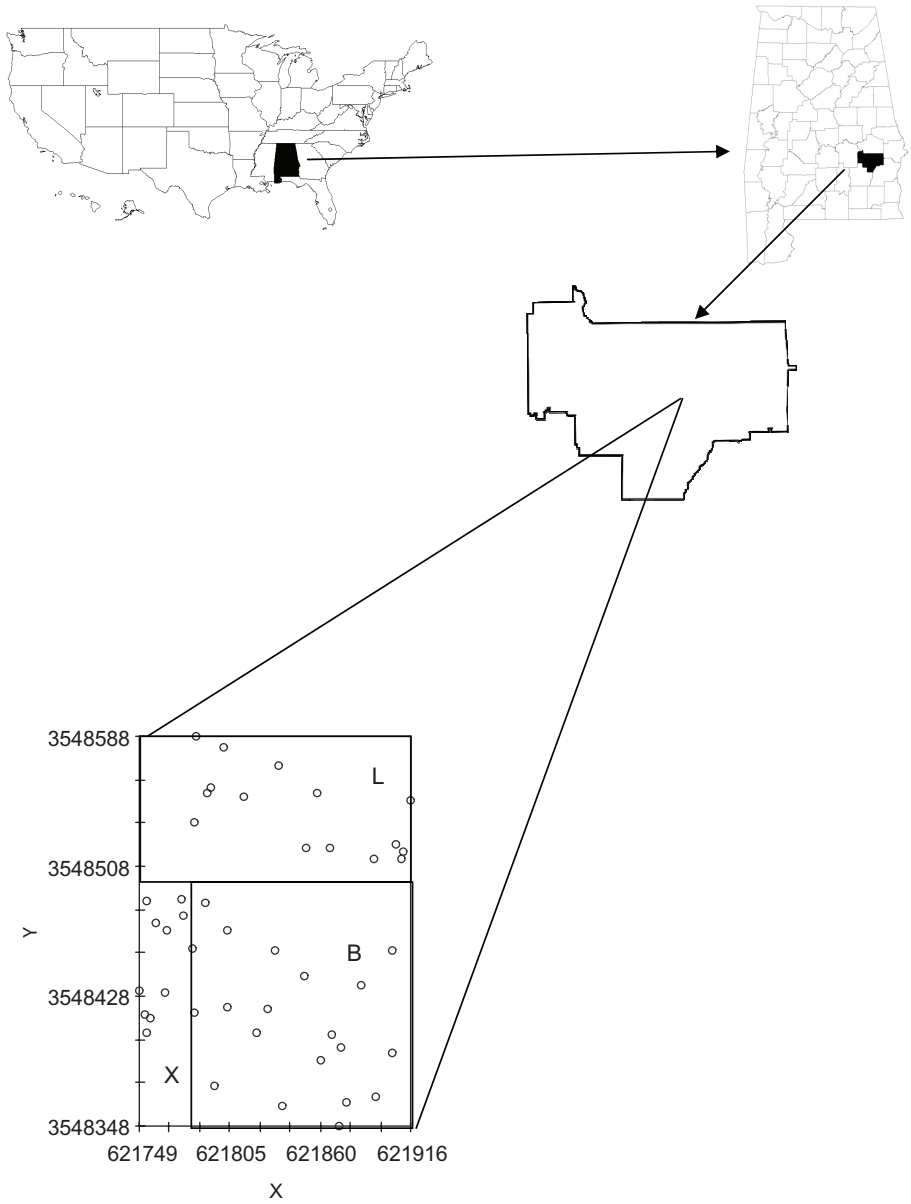

Figure 1. The figure demonstrates the location of the agroecosystem understdy in Bullock County, AL along with the stratified sampling layout. Coordinates are in Universal Transverse Mercator units (meters from meridian).
Abbreviations: L, Pastured area; X, Storage area; B, Broiler house area.

[*ACP*], and 8.0 for phosphodiesterase [*PD*]) were incubated in 25 mL glass flasks and capped at 37 °C for 1 h with 1 g of soil. At the end of incubation, enzyme activity was stopped by addition of 4 mL of 0.5 M NaOH for phosphomonoesterases and 4 mL of 0.5 THAM-NaOH for phosphodiesterase followed by extraction with 1 mL of 0.5 M $CaCl_2$. The mixture was then filtered (Whatman No. 2) and the extract analyzed using a Genesys 10VIS spectrophotometer at (Thermo Fisher Scientific Inc., Waltham MA, USA) at 420 nm. Enzyme activity in filtrates was determined from a standard curve developed using *p*-nitrophenol standards. To account for non-enzymatic hydrolysis, values for controls were subtracted from sample readings. Toluene was not used in accordance with Bandick and Dick[10] and Elsgaard,[33] who showed that with incubation periods fewer than two hours, the absence of toluene was inconsequential to measured enzyme activity. All enzyme activities reported are expressed on a moisture-free basis.

Determination of soil pH, soil organic carbon, and total nitrogen

Samples were analyzed for pH (1:2, soil/water) with a S500 pH Meter (A. Daigger & Co., Vernon Hills, Illinois USA). For soil organic carbon (SOC) and total nitrogen (TN), air-dried soils were sent to Auburn University Soil Testing Laboratory for analysis where dry combustion method on an Elementar Vario Macro NCS Combustion Analyzer (Elementar Americas Inc., Mt. Laurel, New Jersey, USA) was utilized.

Soil textural analysis

Soil textural analysis was determined using the Bouyoucos hydrometer method for 40 g samples after passing the sample through a ≤2 mm diameter sieve. Chemical dispersion of the soil particles was achieved by addition of 100 mL of 0.05 g mL^{-1} Sodium hexametaphosphate (HMP) solution to each sample while mechanical dispersion was achieved by placing the samples in an orbital shaker for 12 h at room temperature.[34] After shaking, the soil solutions were transferred into a 1000 mL sedimentation cylinder. Deionized water was added to bring the total volume in the sedimentation cylinder to 1000 mL. To begin the sedimentation process, the cylinder was agitated by manually shaking the cylinder back and forth for a minimum of 30 s, taking care to ensure that particles did not adhere to the sedimentation cylinder. After agitation the cylinder was placed on the countertop, signifying time zero. Readings were then taken at elapsed times of 0.667, 3, 10, 30, 90, 120, and 720 min using a 152H hydrometer (H-B Instrument Company, Collegeville, Pennsylvania, USA). Temperature of the suspension liquid was recorded simultaneously with hydrometer readings. To calibrate the hydrometer, a blank reading was taken in a solution containing 0.05 g mL^{-1} 100 mL of Sodium HMP solution and 900 mL deionized water, but without soil.

Statistical and geostatistical data analysis

Principal Components Analysis (PCA) and factor analysis (XLStat 7.5, Addinsoft) were used to determine the interaction of soil properties across the field site. Factor analysis was used to group the retained variables into statistical factors based on their correlation structure. To eliminate the effect of different units of variables, factor analysis was done using the correlation matrix on the standardized values of the measured soil properties.[22] Using the correlation matrix, principal components (factors) with eigenvalues >1 were retained and subjected to varimax rotation with Kaiser to estimate the proportion of the variance of each attribute explained by each selected factor loadings.

The geostatistical measure of semivariance for interpolation of unsampled locations was determined using the general equation for semivariograms as presented below:

$$\gamma(h) = 0.5 * \frac{\sum \left[Z(x_i) - Z(x_i + h) \right]^2}{N(h)} \qquad (1)$$

where $\gamma(h)$ is the semivariance at a separation distance h, $N(h)$ is the number of pairs in the lag interval, $Z(x_i)$ and $Z(x_i + h)$ are the observed values of Z at location x_i and $x_i + h$, respectively.

In order to interpolate surface maps of measured soil properties, the data was fitted to theoretical models. Data was fit to Spherical, Exponential, Linear, or Gaussian semivariogram models for the data that was kriged. In this study the following theoretical semivariogram models were used in accordance with our best fit parameters (r^2 and Residual Sum of

Squares [RSS]), with ten lag intervals at a distance of 13.25 m:

Spherical:

$$\gamma(h) = C_o + C\left[1.5\left(\frac{h}{A_o}\right) - 0.5\left(\frac{h}{A_o}\right)^3\right], \quad h \le A_o \quad (2)$$

$$\gamma(h) = C_o + C, \quad h \ge A_o \quad (3)$$

where C_O is the nugget; $C_O + C$ is the sill; A_o is the range.

Gaussian:

$$\gamma(h) = C_o + C\left[1 - \exp\left(-\frac{h^2}{A_o^2}\right)\right] \quad (4)$$

The resulting semivariograms provided values used in the quantification of the spatial effects of measured variables. The range (A_o) is the separation distance beyond which two observations are independent of each other (ie, no autocorrelation). The sill ($C_o + C$) is parameter of the semivariogram that represents a semivariance value at which the variogram levels off. The discontinuity at the origin is called the nugget effect (C_o) and arises from a combination of independent errors, measurement error, and microscale variation.[35] Isotropic semivariogram models were fit to the empirical variograms for soil properties, as there were no trends detected in directional autocorrelation of variables. Theoretical isotropic semivariogram models were calculated and compared on the basis of range of influence (A_o) and proportion of structural variance (PSV) among Spherical, Exponential and Gaussian models. Surface maps were generated using block-kriging (2 × 2) that searched for up to 16 neighbors within a 250 m radius. All geostatistical analysis was performed using GS+ software package (Gamma Design, Plainwell, MI, USA).

Results and Discussion

Relationship between soil properties and enzyme activities

The calculated mean percentages of soil particle sizes (Table 1) were 73% sand, 9% silt, and 16% clay. Soil enzymatic and chemical properties showed non-normal distribution across the site, and prior to statistical analysis were transformed by square root transformation and subsequently back transformed. Also significant heterogeneity was observed across the site with respect to enzyme activities, sand fractions, and pH values between strata (Table 2), making this site a good candidate for soil surface modeling. The results revealed high enzyme activities for the storage area. This can be explained by the soils under the litter storage area being exposed to the abiotic factors of the greater ecosystem (primarily water), which has been shown to have a stimulatory effect on soil microbial and enzymatic activity.[36] Accompanying the highest measured values for enzymatic activity in the storage area was the increase in pH (7.70 ± 0.19). This pH value was close to the optimum for APA activity, and seems to be ideal for PD which prefers non-acidic soils. These findings are consistent with those of other scientist

Table 1. Descriptive statistics of measured soil properties (n = 45).

Soil properties	Min	Max	Mean	Std. dev.	Std. error	Transformation type
APA†	0.10	6.13	2.07	0.40	8.9e-3	Square root
ACP†	0.31	3.76	1.83	1.06	0.024	Square root
PD†	0.05	3.10	0.90	0.40	8.9e-3	Square root
Sand‡	0.54	0.89	0.75	0.07	1.6e-3	None
Silt and Clay‡	0.02	0.21	0.25	0.06	9.6e-3	None
pH (H_2O)	3.79	9.67	6.63	0.11	0.016	None
SOC (%)	0.25	4.89	1.61	1.09	0.024	Square root
TN (%)	0.06	0.74	0.44	0.14	3.1e-3	Square root

Notes: Data was transformed for the variables identified using square root transformation and back transformed prior to geostatistical analysis. †Values for enzyme activity are in units of μmol *p*-nitrophenol g soil^{-1} hr^{-1}. ‡Values for particle size are expressed as a fraction of total soil particles (1.00).
Abbreviations: APA, acid phosphatase; ACP, alkaline phosphatase; PD, phosphodiesterase; SOC, soil organic carbon; TN, total nitrogen.

Table 2. Selected soil properties amongst different land use strata.

Soil property	Broiler housing	Storage	Pastured
APA	2.25a	2.81b	2.52ab
ACP	1.75a	1.91b	1.90b
PD	0.98ab	1.34b	0.71a
pH	6.39a	7.70b	6.55a
SOC	1.34	2.17	1.61
TN	0.22	0.26	0.18
Sand	0.72a	0.73a	0.79b
Silt and Clay	0.28	0.27	0.21

Notes: Different letters denote significant differences between measured variables at $P < 0.05$. †Values for enzyme activity are in units of μmol p-nitrophenol g soil^{-1} hr^{-1}. ‡Values for particle size are expressed as a fraction of total soil particles (1.00).
Abbreviations: APA, acid phosphatase; ACP, alkaline phosphatase; PD, phosphodiesterase; SOC, soil organic carbon; TN, total nitrogen.

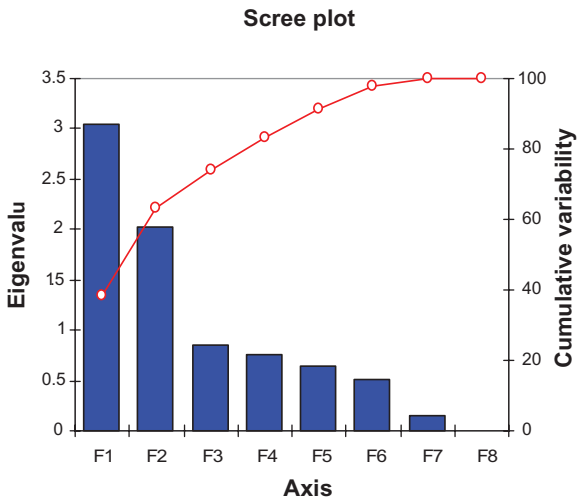

Figure 2. A scree plot showing the relative eigenvalues for the principle components generated for the measured variables.

who have found that poultry litter amendment tends to slightly increase soil pH.[37,38]

Contributions of measured soil properties to principal components

In the PCA applied, the first two principal components were selected (Table 3) since their eigenvalues were both greater than 1. These principal components accounted for ~60% of the total variation. The remaining six components contributed to the residual ~40% of variation as shown in the scree plot (Fig. 2 and Table 3). According to the loading component theory, only factor loadings (correlation values) greater than 0.50 should be considered in explaining the components.[39] The positive loadings on the first component (explaining 32.75% of total variation) were large and positive for *APA* ($r^2 = 0.75$), *PD* ($r^2 = 0.61$), SOC ($r^2 = 0.91$), and *TN* ($r^2 = 0.86$), (Table 3), indicating the influence of SOM and enzymes on the observed variation (also representing the co-occurrence of enzyme and substrate). Organic matter has long been known to contain organic phosphorous, and readily explains the interaction assumed by the inclusion of phosphatase enzymes and organic matter (SOC and TN) on PC 1 as the substrate for the phosphatase enzymes that we used are organic phosphomonoester and phosphodiester bonds which can be found in cellular remnants among other organic material.

These variables are important to soil as extracellular enzymes use organic matter as its substrate to provide intermediary metabolites necessary to the nutrient cycles of the soil ecosystem. The inclusion of SOC and TN on the primary component was expected, as the interaction of soil organic matter to enzymatic activity have previously been demonstrated.[6,40,41] The loadings on the second component (27.58% of total variation) were large and positive for Silt + Clay ($r^2 = 0.97$), and equally negative for Sand ($r^2 = 0.97$) (Table 3). This component is simply referred to as 'soil texture'. Both of these factors have been shown to influence soil microbial and biochemical properties,[42,43] with clay content affecting SOC retention as well.[44,45]

Spatial variability of measured soil properties

Using the PSV model of quantification of spatial dependence (strong ≥ 0.75; moderate <0.75, ≥0.25;

Table 3. Eigenvalues and corresponding values of percentage of variance for each component.

	F1	F2	F3	F4	F5	F6	F7	F8
Eigenvalue	3.052	2.028	0.845	0.755	0.643	0.510	0.158	0.009
% variance	38.153	25.353	10.557	9.437	8.037	6.373	1.975	0.115
Cumulative %	38.153	63.506	74.063	83.500	91.538	97.911	99.885	100.000
Number of removed trivial eigenvalues: 0								

weak < 0.25), it was observed that the models selected for all soil properties demonstrated strong spatial dependence (Table 4). Accordingly, the spatial structure of the soil properties was strong and not interrupted at distances shorter than their range of influence. According to Rover and Kaiser,[46] moderate spatial dependence is due to an equal influence of soil physical, biological, and chemical properties on the measured variable.

The semivariogram model showed a good match between the experimental data and the semivariogram model chosen which had an r^2 value of 0.92 (Table 4). In considering their distribution throughout the study site, PC 1 showed large and positive loadings in the area designated the X strata where poultry litter was stored (Fig. 3B). This particular type of management would be expected to generate high levels of interaction between the variables on this component as poultry litter when applied to soil as nutrient and soil amendment has been shown to increase both SOM and enzymatic activity.[6] A few "hot spots" of PC 1 influence were detected in the northern grazing pasture, which can also be tied to the deposition of animal manure in the pasture.[47] The microbial population and organic matter in manure deposited into the soil randomly (grazing preference) may have given rise to high interaction observed between the SOM and enzymes associated with PC 1.

Areas of low interaction were identified on the map by the cooler colors, and were predominant in the poultry house area (Strata B), and a large part of the grazed pasture. Specifically low spatial interaction of the SOM and enzymes on PC 1 was seen in the poultry housing area. This area had the lowest values for SOC as well as APA along with

Table 4. Factor loading values of the first two principal components after varimax rotation.

	F1	F2
APA	**0.754**	0.016
ACP	0.256	–0.005
PD	**0.614**	0.229
Sand	–0.116	**–0.972**
Silt and Clay	0.111	**0.971**
pH	0.157	0.490
SOC	**0.905**	0.009
TN	**0.860**	0.162
% of total variation	32.75	27.58

Note: Values greater than 0.500 (selection criteria) are shown in bold.

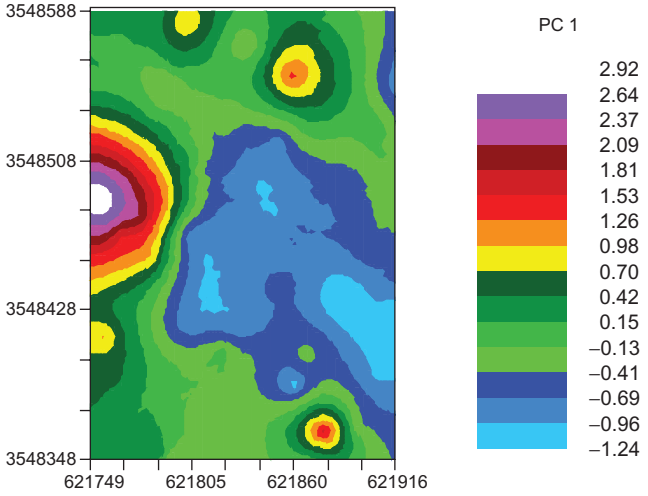

Figure 3. A 2 × 2 block-kriged surface map for PC 1; values upon which the scale is constructed are factor scores.

a marginally acidic pH. Although all of these factors are contributors to the poor performance of PC 1 in this area, it is also suspected that the bedding material of the poultry house floor played a role as well. Bedding material is necessary to provide absorbency,[48] so that organic and inorganic materials resulting from poultry fecal matter and urine are not leached into the soils below. Although there is no reason to assume the absence of catalytic activity below these floors, with low values for PC 1 the area is understood as having a low co-occurrence of soil enzymes and SOM.

As was the case with the semivariogram for PC 1, the semivariogram for PC 2 (Fig. 4A) shows a good match between the experimental and modeled data

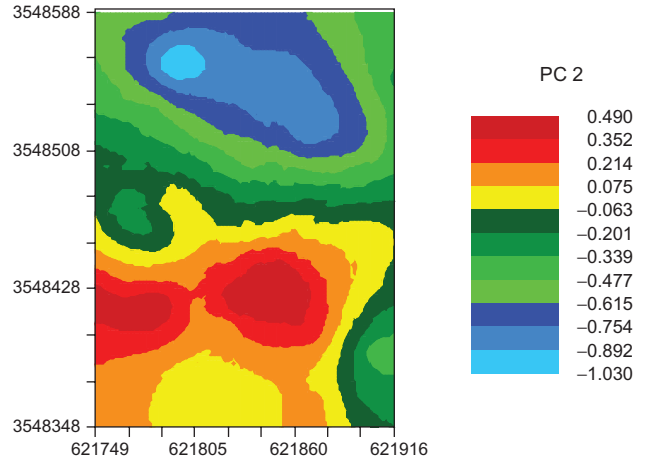

Figure 4. A 2 × 2 block-kriged surface map for PC 2; values upon which the scale is constructed are factor scores.

Table 5. Statistics describing fit of isotropic models to empirical data for principle components.

Principal components	Nugget (C_o)	Sill ($C_o + C$)	Range [m] (A_o)	PSV $C/(C_o + C)$	Model[a]	SD[b]	r^2	RSS[c]
PC 1	0.001	1.14	86.40	0.99	Sp	S	0.92	0.12
PC 2	0.02	0.11	96.13	0.82	G	S	0.81	0.004

Notes: [a]Theoretical semivariogram model; [b]Spatial dependence; [c]Residual sum of squares.
Abbreviations: Sp, Spherical; G, Gaussian; S, Strong.

(r^2 = 0.81). The spatial patterns of PC 2 (Table 5) showed strong spatial dependence at a range of 96.13 m. The map generated by kriging of the PC 2 values is shown in Figure 4B. The negative values represented by the cooler colors (yellow to blue), the entire landscape is under the influence of the sandy aspect of the component. Areas that are hot (orange and red) represent the spatial interaction of silt and clay, and these areas can be seen in the extreme western, southern, and eastern parts of the map. What is of note is the spatial co-occurrence of both components. This co-occurrence can be seen in the litter storage area, as well as a very small area at the southeast part of the maps. These particular areas may interact to provide support for microbial communities as it has been shown that enzyme-mediated catalysis in soils is assisted by the enzyme-clay complex.[49] Clays provide binding surfaces with the ability to buffer enzymes from degradative compounds,[42] as well as facilitate conformational changes in enzyme or substrate to inhibit or activate catalysis.[50] Hope and Burns[51] showed that clays play a major role in the spatial structure of enzymes and microbial communities in soil by limiting diffusion and community growth respectively.

Conclusion

Soil quality is known for its ability to respond to changes in the environment over various periods of time. This study shows the physical confluence of these soil properties spatially, demonstrating the geographic variability of soil activity in relation to SOM. The confluence of the related soil properties has the ability to influence the activity of the microbes and enzymes which support organic matter decomposition and nutrient cycling in soils. Just as soil quality is a confluence of certain biological, chemical, and physical phenomena, so are many of the ecologically important processes in soil. Through multivariate/non-parametric methods, this study was able to condense the provided integrative measures into two salient variables, critical to the maintenance of environmental quality. By minimizing the number of variables, this study provides benefits for policy makers and farmers, helping them to focus on just a few variables that are critical for the maintenance of environmental quality. Hence, this can lead to substantial saving of resources in form of time and money.

As SOC demonstrated a large impact on the soil properties assessed in this study, it underscores the environmental impact of soil carbon management that has been recognized by domestic and international governments. With the ability to assess these properties in space, the ability of site specific management is realized as a benefit for policy makers, land managers, and farmers, among many others. Providing policy that aims at enhancing and protecting soil carbon may prove to be an effective compliment to policies that focus on the control green house gas emissions. Moreover, providing a method for landowners to spatially identify areas across their landscapes may allow for more precise monitoring and planning of management activities that contribute to these carbon stocks as well as the soil properties and activities associated with them. More studies should be conducted to assess the spatial structure of components of soil processes in similar and various soils at varying scales, so that theoretical considerations as to the spatial distribution and integration of soil processes may be further elucidated.

Author Contributions

Conceived and designed the experiments: RS, RA, and RZ. Analysed the data: RS. Wrote the first draft of the manuscript: RS. Contributed to the writing of the manuscript: RS, RA. Agree with manuscript results and conclusions: RS, RA, LG, RZ. Jointly developed the structure and arguments for the paper: RS, RA, RZ. Made critical revisions and approved final

version: RA, LG. All authors reviewed and approved of the final manuscript.

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Disclosures and Ethics

As a requirement of publication author(s) have provided to the publisher signed confirmation of compliance with legal and ethical obligations including but not limited to the following: authorship and contributorship, conflicts of interest, privacy and confidentiality and (where applicable) protection of human and animal research subjects. The authors have read and confirmed their agreement with the ICMJE authorship and conflict of interest criteria. The authors have also confirmed that this article is unique and not under consideration or published in any other publication, and that they have permission from rights holders to reproduce any copyrighted material. Any disclosures are made in this section. The external blind peer reviewers report no conflicts of interest.

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