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Spatial variability of soil microbiological properties under different land use systems

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Understanding the spatial variability of soil microbial properties is crucial for assessing their role in nutrient cycling and ecosystem functioning across various land use systems. While many studies emphasize soil physicochemical characteristics, the spatial heterogeneity of microbiological attributes is less explored. This research examined the spatial distribution of microbial biomass carbon (MBC) and dehydrogenase activity (DHA) in surface soils of berseem, rice-wheat, and poplar-wheat cropping systems. Forty-eight georeferenced surface soil samples (0-0.15 m) were collected from each system and analyzed for MBC, DHA, and physicochemical properties. Sensitivity analysis determined the minimum sample size needed for effective sampling. Results showed strong spatial dependence for the berseem and poplar-wheat systems. Principal component analysis (PCA) and discriminant analysis (DA) identified MBC, DHA, and soil organic carbon (SOC) as key variables distinguishing the land use systems. Soil microbiological characteristics exhibited greater variability than chemical properties, with DHA showing more variability than MBC, necessitating larger sample sizes to detect changes. This study highlights the importance of understanding spatial variation in soil microbial properties for designing sampling protocols and implementing sustainable management practices, ultimately enhancing ecosystem functioning and nutrient cycling in diverse agricultural systems.

Key words: Dehydrogenase activity (DHA), discriminant analysis, geo-statistics, microbial biomass carbon (MBC), spatial variability.

INTRODUCTION

Soil microorganisms are essential to terrestrial ecosystems, participating in processes such as organic matter decomposition, nutrient cycling, energy flow, carbon storage, and detoxification of harmful substances (Rashid et al., 2016). The status of soil microbial and biochemical properties serves as a sensitive indicator of environmental pressures and improvements in soil quality within both natural and agricultural settings (Badiane et al., 2001). Microbial biomass and enzyme activities, which are key soil microbial properties, respond more dynamically to management changes across land uses compared to soil organic carbon (SOC) levels. Microbial biomass stores essential nutrients and influences soil fertility by regulating nutrient availability (Cao et al., 2011). Soil enzymes facilitate organic matter breakdown and nutrient cycling, with their activities affected by soil physical and chemical properties, microbial community structure, vegetation type, and ecosystem disturbances and succession (Kourtev et al., 2002). Soil microbial properties exhibit significant spatial and temporal fluctuations (Cavigelli et al., 2005), as soils are among the most diverse microbial habitats on Earth. Various factors influence the spatial organization of microorganisms and their functions, making the assessment of soil microbial properties challenging due to numerous influencing factors and significant temporal variations (Roesch et al., 2007). In managed and homogeneous systems, microbial processes form clusters with distinct activity levels at different spatial scales (Martirosyan et al., 2013). Understanding the spatial arrangement of soil microorganisms and the factors influencing them is crucial in agriculture, ecology, and environmental fields. This knowledge improves predictions related to soil organic matter dynamics, nutrient cycling, soil structure preservation, and biological population regulation (Franklin and Mills, 2009). Determining the operational scale of microbial communities in agricultural soils is essential for developing effective sampling techniques to assess the relationships between microbial communities and soil properties. Soil heterogeneity arises from the interaction of physical, chemical, and biological components that vary across spatial and temporal dimensions (Lan et al., 2018). Inherent disparities in soil attributes, such as parent material, vegetation, and climate, along with anthropogenic factors like tillage, fertilization, and crop rotation, influence soil properties and introduce greater variability.

These practices can alter soil nutrient distribution, affecting soil characteristics and microbial communities (Piotrowska and Długosz, 2012). Existing literature

reveals insufficient emphasis on the variability of microbiological parameters at the field or plot level under different land uses. Spatial analysis of soil microbial properties and their interactions across land uses can enhance the understanding of microbial dynamics in differently managed agroecosystems.

Traditional statistical methods are generally suitable for analyzing spatial heterogeneity when observed characteristics exhibit random fluctuations with minimal spatial correlation. However, substantial spatial correlation can limit conventional statistical approaches, as they fail to account for the association between sample values and their locations (El Sebai et al., 2007). Geostatistics provides a more appropriate method for examining spatial variability, using parameters to assess regionalized variables, verify spatial distributions, and predict unsampled attribute values (Baldrian et al., 2010). Geostatistical techniques, such as correlograms and semi-variograms, are employed to evaluate spatial variability and interactions (Iqbal et al., 2005). These methods have been applied to analyze soil physicochemical properties and their spatial heterogeneity (Aşkın and Kızılkaya, 2006), as well as microbiological and biochemical mechanisms (Kallenbach and Grandy, 2011). Soil scientists use geostatistical techniques to predict spatial heterogeneity at varying scales, from small to large areas (Aşkın and Kızılkaya, 2006). There is an increasing need for accurate assessments of soil microbiology's spatial arrangements at the field level across different land use categories.

Different land uses are associated with specific soil types, each with distinct initial characteristics, such as aeration and water conditions, which influence microbial community responses to management practices. For instance, soils with higher clay content provide better physicochemical protection for microbial biomass reservoirs and are less sensitive to water stress (Yu et al., 2019). Soils resulting from various pedogenesis processes exhibit unique edaphic characteristics and microbial communities (Ashworth et al., 2017). Soil microbial properties demonstrate spatial heterogeneity due to both natural factors and management practices, leading to variations across land use types (Liu et al., 2006). Analysis of variance shows that soil types within different land uses significantly impact the spatial distribution of soil properties. However, the evaluation and comparison of microbial properties' spatial variability across land uses and soil types using geostatistical methods are infrequent. Given the influence of soil type and land use on spatial variability, this study aims to

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differentiate between three land uses and assess their impact on microbial property variability. Multivariate techniques such as principal component analysis (PCA), discriminant analysis (DA), and hierarchical cluster analysis (HCA) can characterize land use systems, identifying a minimum data set (MDS) of relevant parameters for distinguishing between land uses (Garnaik et al., 2020).

The Indo-Gangetic region of northwestern India is highly productive, with a cropping intensity of 205%, making a significant contribution to national food security. The average food grain productivity in this region exceeds 12 t ha⁻¹, a figure unmatched globally in similar expanses. However, this high productivity raises sustainability concerns for natural resources such as soil and water. The extensive use of fertilizers and agrochemicals required to maintain productivity has adversely impacted soil flora and fauna, particularly in rice paddies, creating anoxic conditions that affect microbial communities (Sekhon et al., 2019). Despite considerable focus on soil health, assessments mainly concentrate on soil fertility parameters like macro- and micro-nutrients, with a lack of spatial assessments of microbiological parameters in this region.

Geostatistical analysis of spatial variability can provide insights into the minimum sample size required for accurate characterization of soil microbiological properties. Therefore, this study aims to: 1. Examine the spatial variation of key soil physicochemical properties and two microbiological properties (microbial biomass carbon and dehydrogenase activity) across three land use categories using geostatistics and multivariate statistics; 2. Determine the optimal sample size required for accurately characterizing the spatial variation of these microbiological properties; and 3. Identify a minimum dataset of variables to effectively characterize microbiological parameters in dominant land use systems through PCA and canonical discriminant analysis (CDA).

MATERIALS AND METHODS

Study area

The study was conducted near Ladian village (30.89°N, 75.86°E) in Punjab's Ludhiana district, northwest India, in an intensively cultivated region characterized by conventional tillage practices. This area features three dominant cropping systems: berseem (*Trifolium alexandrinum*), rice-wheat (*Oryza sativa-Triticum aestivum*), and poplar-wheat (*Populus deltoides-Triticum aestivum*). The soil, classified as typical Ustifluvents from Indo-Gangetic Alluvium, is known for its high fertility due to recent alluvial deposits from rivers like the Ganges. Despite sharing the same parent material, the cropping systems exhibit variations in soil physicochemical, biochemical, and microbiological properties. The sites have been cultivated with the same crops and agrotechnical practices for several years. Characterized by a semi-arid to humid subtropical climate, the area receives annual rainfall ranging from 600 to 700 mm, with average temperatures reaching 32°C. 48 georeferenced surface soil samples were collected using a 14 × 7 m grid. The samples, stored at 4°C upon arrival at the lab, were

analyzed within a week for microbial biomass carbon (MBC) and dehydrogenase activity (DHA). Prior to analysis, the samples were sieved through a 2 mm sieve and air-dried at room temperature to examine their physicochemical characteristics.

Laboratory analysis

MBC was determined using the fumigation-extraction method by Vance et al. (1987). Moist soil samples were fumigated with alcohol-free chloroform in a vacuum desiccator for 24 h. The chloroform-labile carbon fraction was calculated as the difference in carbon extracted from fumigated and non-fumigated samples using the chromic acid digestion method with 1 N K₂Cr₂O₇, titrated with 0.5 N ferrous ammonium sulfate (FAS). No conversion factor was used for MBC due to its variability (0.41-0.58). DHA was measured via spectrophotometric analysis, reducing triphenyltetrazolium chloride (TTC) to triphenyl formazan (TPF) after 24 h incubation (Tabatabai, 1994). Soil texture was analyzed using the International Pipette Method (Robinson, 1922). Soil pH and electrical conductivity (EC) were determined in a 1:2 soil-water suspension using a pH meter (Elico LI 127) and a conductivity meter (Elico 304). SOC was measured using the wet chromic acid titration method (Walkley and Black, 1934).

Statistical analysis

Descriptive statistics for various soil physico-chemical and microbiological parameters were determined using SPSS v.15. Variability was quantified using the coefficient of variation (CV = 100 * mean/standard deviation), with CV values indicating low (<15%), medium (15-35%), and high (>35%) variability, following Wilding (Wilding, 1985). PCA was used to create a minimum dataset, identifying important variables by generating uncorrelated principal components (PCs) (Jolliffe and Cadima, 2016). This dataset was then used in CDA, a classification system that identifies significant parameters for distinguishing among land use systems. Data were transformed for normal distribution before analysis. Pearson correlation and regression analyses (using Microsoft Excel 2010) were also performed on the CDA-identified parameters for all land uses.

Sensitivity analysis for determining minimum sample size

A simple statistical methodology based on Fisher's Least Significant Difference (LSD) classical technique (Snedecor, 1980) was utilized to compute a reliable sample size. This technique was adapted to determine the minimum sample size required for characterizing the variability of soil fertility properties (Chaudhuri et al., 2011), as given:

$$N = \frac{S^2 \times 1.96^2}{CD^2} \quad (1)$$

where N = minimum sample size required to detect the desired critical range or critical difference (CD) of the soil parameter, CD = user defined critical limit (for example, the minimum change that can alter the category of a variable, like from low to medium and from medium to high and vice-versa). This categorization is often the case with prevalent schemes of soil fertility classification. Alternatively, sample size can be worked out to detect at least 10% change in a soil property. S² is the sample variance and 1.96 is the constant representing 't_α' for infinite degrees of freedom at the 95% confidence level (α = 0.05). Thus, any sample size smaller than the computed size may not be sensitive enough to capture the required variability.

Geostatistical analysis

Geostatistical analyses were carried out following two distinct steps, that is, spatial continuity analysis and statistical interpolation. The former was done using variography technique and the latter was accomplished through kriging technique. Variography uses semivariograms to characterize the spatial structure and variance of data and kriging uses model variance to estimate the values at the unsampled location. Geostatistical analysis of spatial variability and kriged surface maps of soil microbiological attributes were performed using Arc-GIS (ArcMap 10.4.1, ESRI). A semivariogram is a mathematical model, constructed for each variable of interest that represents semivariance as a function of distance between two sampling points. The semivariance was calculated using the following formula:

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [Z(X_i) - Z(X_i + h)]^2 \quad (2)$$

The experimental semivariance, denoted $\gamma(h)$, is calculated based on the separation distance between two locations, X_i and $X_i + h$; $N(h)$ represents the number of pairs at a given separation distance (h). $Z(X_i)$ and $Z(X_i+h)$ refer to the measured values for the regionalized variables at locations (X_i) and (X_i+h), respectively, which are separated by a vector (h) (Burgess and Webster, 1980). In geostatistics, $Z(X_i)$ is described as regionalized variable. Semivariance estimations may depend on the parameters like lag intervals, number of lags and anisotropy. An experimental semivariogram consists of three fundamental parameters: nugget (C_0), sill ($C_0 + C$), and the range of spatial dependence (a). These parameters reveal spatial structure in the form of the mathematical equation $\gamma(h) = C_0 + C$. Different semivariogram models, such as Gaussian ($\gamma(h) = C_0 + C [1 - e^{-(h^2/a^2)}]$), spherical ($\gamma(h) = C_0 + C [3h/2a - h^3/a^3]$), and exponential ($\gamma(h) = C_0 + C [1 - e^{-(h/a)}]$), were selected for soil fertility parameters based on the lowest RMSE (root mean square error) values.

In these models, C_0 represents the nugget effect, accounting for local variation at scales smaller than the sampling interval, attributed to sampling errors (Mulla and McBratney, 2001). The sum (C_0+C) denotes the sill or maximum variance achievable, reflecting the overall spatial variability. The partial sill C is the difference between nugget and sill, while the range indicates the distance at which spatial correlation ceases beyond the sampled points. Spatial variability was categorized using the criterion by Cambardella and Elliott, 1994, based on the nugget-to-sill ratio. Ratios <25% indicate strong spatial dependence, 25-75% moderate dependence, and 75-100% weak dependence between samples (Cambardella and Elliott, 1994). Selected semivariogram models, chosen for best fit via RMSE, were used for ordinary kriging (OK) to generate estimated surface maps. OK was selected as the preferred kriging technique due to its widespread application in spatial interpolation.

RESULTS

Descriptive statistics

Soil physicochemical and microbiological properties

Descriptive statistical summaries of the soil physicochemical as well as microbiological properties for three different land use systems are presented in Table 1. Soil properties, under different land uses showed considerable variation in the study area. The variability was measured in terms of percent coefficient of variations

(%CV). The mean clay content was higher (>40%) for all the land use types. In terms of %CV, percent silt content exhibited higher variability than sand and clay content in all the three land uses.

The study compared soil properties across three different land use systems in a region of northwest India: rice-wheat, poplar-wheat, and berseem-based systems. Soil pH was highest in the rice-wheat system (mean 8.19), followed by poplar-wheat (7.99) and berseem-based (7.84) systems, with minimal within-field variability. Electrical conductivity (EC) was highest in berseem-based land (0.51 dS m^{-1}), followed closely by rice-wheat (0.47 dS m^{-1}) and poplar-wheat (0.42 dS m^{-1}) systems, indicating non-saline conditions ($\text{EC} < 0.8 \text{ dS m}^{-1}$). However, EC exhibited significant spatial variability (CV 27.6%), particularly in rice-wheat fields. Organic carbon content varied across systems, with berseem-based land showing the highest mean (0.87%) and low variability (CV 13.48%). Rice-wheat had a mean SOC of 0.69% (CV 18.8%), while poplar-wheat showed the highest mean (0.89%) but lower variability (CV 13.4%) than rice-wheat. Silt content and EC exhibited the highest variability among physicochemical parameters (CV > 15%), whereas other parameters showed more homogeneous distributions (CV < 15%). Overall, soils were predominantly clayey, rich in SOC, neutral to alkaline, and low in EC across all land uses. MBC was notably higher in berseem-based systems ($300.13 \mu\text{g g}^{-1}$), followed by poplar-wheat ($260.21 \mu\text{g g}^{-1}$) and rice-wheat ($113.63 \mu\text{g g}^{-1}$) systems. Within-field variability was lowest in berseem-based (CV 15.1%), followed by poplar-wheat (29.4%) and rice-wheat (43.4%) systems. DHA exhibited similar trends to MBC, with higher variability overall compared to physicochemical properties. DHA variability was particularly pronounced in rice-wheat systems (CV > 35%). Both MBC and DHA distributions showed platykurtic patterns (kurtosis < 3), indicating flatter distributions compared to a normal curve.

PCA and DA

PCA analysis was performed to extract most informative soil parameters from each PC on the basis of loading plot (Figure 1a and b). PCA identified two principal components (PCs) with eigenvalues greater than 1 (PC1 = 3.302, PC2 = 1.878), explaining 41.2% and 23.5% of total variation, respectively (Hair et al., 1992). Parameters with factor loadings within 20% of the highest loading in each PC were selected: MBC, SOC, DHA, and sand for PC1; clay and silt for PC2 (Table 2). These variables, highlighted through PCA, were further validated using communality analysis as effective discriminators of different land use systems in subsequent analyses.

A DA was carried out to explore the distinctions between three land use systems by examining the chemical and microbial properties of the soil, as well as identifying key parameters that played an important role

Table 1. Classical statistical descriptions of soil microbiological and other properties for various land uses.

Parameter	Mean	Minimum	Maximum	Median	%CV	Skewness	Kurtosis
Berseem-based							
Sand (%)	33.93	27.82	38.79	33.14	7.40	0.63	0.30
Silt (%)	27.43	17.19	34.74	27.72	15.86	-0.29	-0.66
Clay (%)	41.70	32.60	48.80	42.70	9.25	-0.59	-0.15
pH	7.84	7.65	8.02	7.83	0.90	0.11	0.28
EC (dS m ⁻¹)	0.51	0.32	0.70	0.52	14.09	0.12	0.41
SOC (%)	0.89	0.66	1.17	0.87	13.48	0.27	-0.33
MBC (µg g ⁻¹)	300.13	180.00	370.00	300.13	15.10	-0.85	0.28
DHA (µg TPF ⁻¹ h ⁻¹)	3.46	2.02	4.97	3.41	22.5	-0.13	-0.69
Rice-wheat							
Sand (%)	34.00	28.00	39.00	33.00	7.3	0.64	0.30
Silt (%)	25.00	13.00	35.00	24.00	19.4	-0.21	0.09
Clay (%)	43.00	30.00	49.00	44.00	11.2	-0.88	-0.08
pH	8.19	7.73	8.63	8.21	1.9	-0.43	2.37
EC (dS m ⁻¹)	0.47	0.21	0.75	0.47	27.6	0.21	0.09
SOC (%)	0.69	0.47	1.38	0.69	18.8	2.88	15.82
MBC (µg g ⁻¹)	113.63	30.00	210.00	120.00	43.4	0.12	-1.21
DHA (µg TPF ⁻¹ h ⁻¹)	1.86	0.36	4.21	1.45	53.7	0.67	-0.55
Poplar-wheat							
Sand (%)	37.00	31.00	48.00	37.00	9.8	1.09	1.08
Silt (%)	19.00	9.00	30.00	19.00	22.6	-0.02	0.72
Clay (%)	42.00	35.00	49.00	43.00	9.2	-0.18	-1.03
pH	7.99	7.65	8.47	7.98	1.8	0.39	1.33
EC (dS m ⁻¹)	0.42	0.21	0.58	0.43	19.0	-0.47	0.99
SOC (%)	0.89	0.66	1.17	0.87	13.4	0.27	-0.33
MBC (µg g ⁻¹)	260.21	60.00	390.00	264.50	29.4	-0.81	0.38
DHA (µg TPF ⁻¹ h ⁻¹)	3.29	1.02	4.66	3.31	20.6	-0.56	1.64

Table 2. PCA communality report and associated loading values for PC1 and PC2.

Variable	Rotated Component Matrix		
	Communality Extraction	Component	
		1	2
SOC	0.764	0.807	0.335
MBC	0.823	0.906	0.051
DHA	0.707	0.825	0.161
Sand	0.701	0.806	-0.225
Silt	0.570	-0.105	-0.747
Clay	0.679	0.117	0.816
pH	0.612	-0.670	0.405
EC	0.324	0.138	-0.552

in distinguishing and defining these land use systems. The DA resulted in the creation of two functions (Functions 1 and 2) which respectively represent 87.0% and 64.6% of the total variations:

$$\text{Function 1} = 22.798 - 1.265 \times \text{SOC} + 0.01 \times \text{MBC} + 0.245$$

$$\times \text{DHA} + 0.237 \times \text{Sand} - 0.98 \times \text{Clay};$$

$$\text{Function 2} = -17.119 - 6.305 \times \text{SOC} - 0.007 \times \text{MBC} - 0.055 \times \text{DHA} + 0.234 \times \text{Sand} + 0.180 \times \text{Clay}$$

Overall model fit was assessed using these functions. The coefficients and origin were derived from the

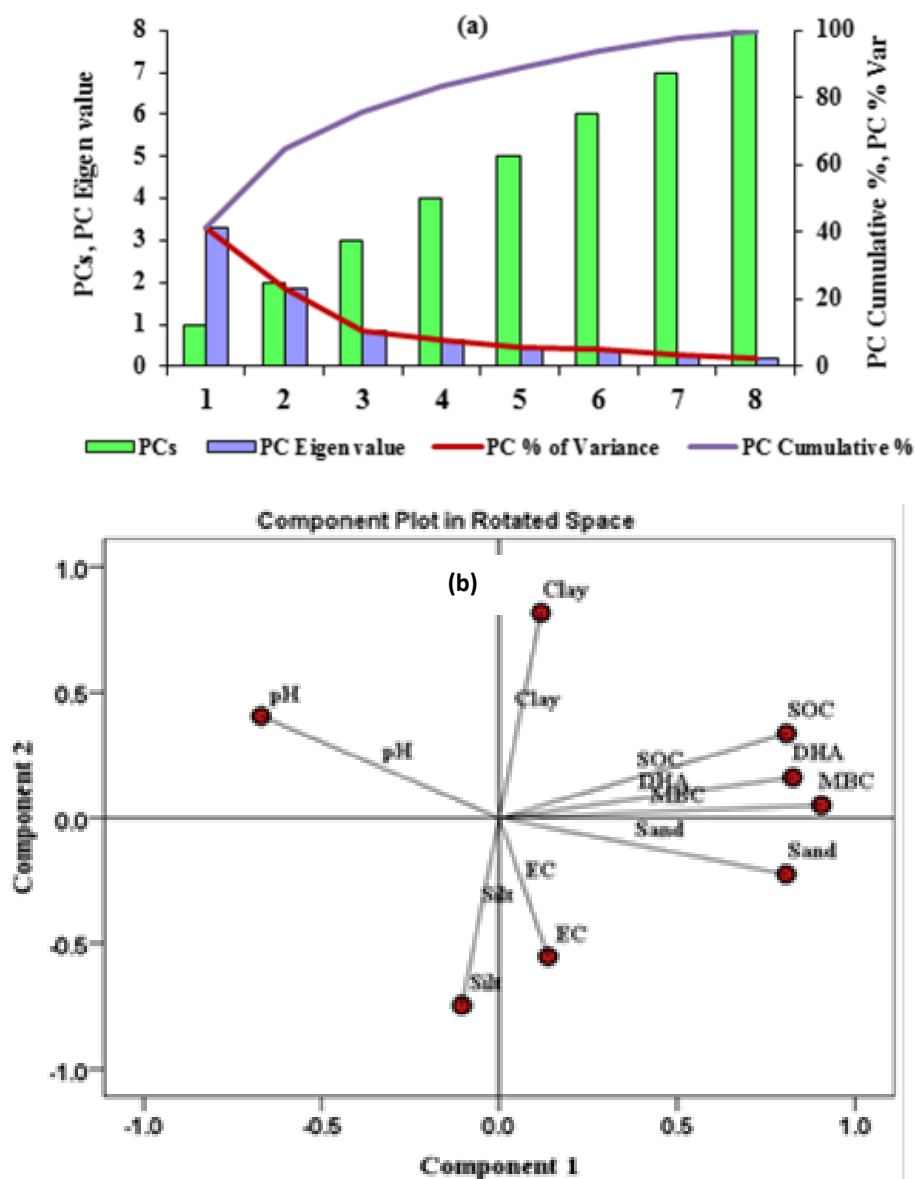


Figure 1. (a) Principal components and eigenvalues related to different soil parameters. (b) PCA bi-plot of different soil parameters for three land use systems.

canonical discriminant function coefficient table, which is not shown here. Canonical correlation represents the multiple correlation between predictors (especially soil-based parameters) and discriminant functions. The square of this canonical correlation indicates the proportion of variance explained. In this particular study, functions 1 and 2 yielded canonical correlations of 0.933 and 0.804, respectively, shown in Table 3.

Figure 2b illustrates the separation of poplar-wheat land use systems from berseem and *rice-wheat* land use systems along the horizontal axis, while the vertical axis distinguishes *rice-wheat* from the other two systems. The soil variables that significantly contributed to the

classification and the two canonical functions are presented in the structural matrix (Table 5), with a score >0.3 . The main soil variables that differentiate poplar-wheat land use systems from berseem and *rice-wheat* land use systems are SOC, sand, MBC, and DHA, which are associated with the first discriminant function. Conversely, clay is the only important parameter to distinguish *rice-wheat* from the other two land use systems, as detected by the second discriminant function. The structural matrix results further validate the loading values of the principal components. The test for equality of group means (Figure 2a) demonstrates that all soil parameters included in the minimum dataset

Table 3. Summary of the discriminant analysis.

Function	Eigenvalue	% of Variance	Cumulative %	Canonical correlation
1	6.726 ^a	78.6	78.6	0.933
2	1.829 ^a	21.4	100	0.804
Wilks' Lambda				
Test of Function(s)	Wilks' Lambda	Chi-square	df	Sig.
1 through 2	0.046	424.113	16	0
2	0.353	142.984	7	0
Box's test of equality of covariance matrices				
196.293	2.506	72	55394.89	0

Table 4. Structural matrix values of different soil parameters obtained from DA.

Variable	Function	
	1	2
SOC	0.589*	0.214
MBC	0.498*	-0.356
DHA	-0.419*	0.030
Sand	0.301*	-0.287
Clay	0.042	0.424*

contribute significantly ($p < 0.0001$) to the discrimination of different land use systems.

Wilks' lambda was utilized to evaluate the effectiveness of each function in differentiating between the land use systems (Table 4). A lower Wilks' lambda value suggests a higher discriminatory ability of the function. Typically, Wilks' lambda ranges from 0 to 1, with values closer to 0 indicating more significant differences in group means. In this analysis, Wilks' lambda was determined to be 0.046 for Functions 1 and 2 (Table 4), indicating a highly significant distinction ($p < 0.000$) between the three land use systems (Figure 2a and b).

The chi-square statistic was used to test the equality of means across the functions between different groups. The chi-square value obtained was 424.1 (Table 4), demonstrating the effectiveness of the discriminant functions in distinguishing among the three land use systems. Eigenvalues provide insight into the variance explained by each discriminant function. Higher eigenvalues indicate greater explanatory power. In this study, Functions 1 and 2 had eigenvalues of 6.726 and 1.829, respectively (Table 4), reflecting their significant explanatory capability.

The centroids represent the discriminant scores for each land use system based on the means of the variables entered into the functions. For berseem, *rice-wheat*, and poplar-wheat land use systems, the centroids for Function 1 were 3.209, -3.072, and -0.137,

respectively. For Function 2, the corresponding values were 0.884, 1.007, and -1.891. These centroid values indicate that the land use systems are significantly distinct from one another. DA also provided insights into the importance of soil parameters in distinguishing the three land use systems. The Box's M test for equality of covariance matrices revealed a significant difference ($p < 0.000$) between the land use systems. The structural matrix further emphasized the significant roles of sand, clay, SOC, MBC, and DHA in differentiating the land use systems (Table 4).

Classification matrix showed that the two discriminant functions were able to classify 96.5% of the three land use systems correctly on the basis of soil chemical and microbial parameters. The classification accuracy values for berseem, rice-wheat, and poplar-wheat land use systems were 100, 97.9 and 91.7%, respectively. For the rice-wheat land use system, 2.1% of the soils had similar characters to poplar-wheat land use system. While 4.2% of the soils showed similarity with each of berseem and rice-wheat land use systems for poplar-wheat land use system. No such mismatching was found for berseem-based land use system.

Regression and correlation analysis

The PCA and DA highlighted the significant roles of SOC, MBC, DHA, and clay content in distinguishing and characterizing the different land use systems. Subsequent regression analyses, illustrated in Figure 4, further examined these relationships.

For the berseem land use system, significant relationships were observed between SOC and MBC ($R^2 = 0.217$, $p < 0.001$), and SOC and DHA ($R^2 = 0.197$, $p < 0.001$). Clay content also showed a significant variation with SOC ($R^2 = 0.135$, $p < 0.01$) in the same land use system. In the *rice-wheat* land use system, SOC demonstrated a significant relationship with MBC ($R^2 = 0.269$, $p < 0.001$). Additionally, SOC significantly explained variations in DHA ($R^2 = 0.187$, $p < 0.01$) and

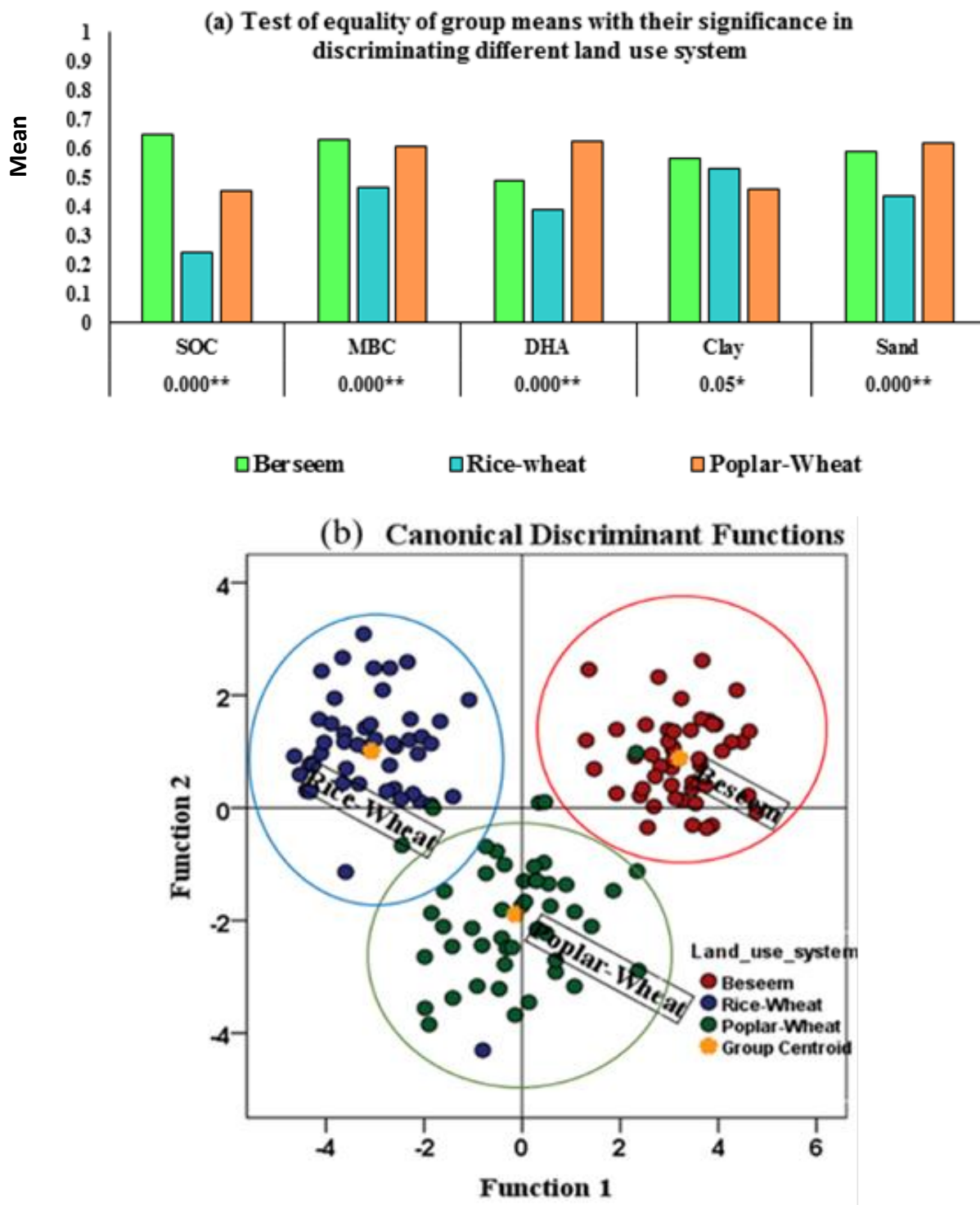


Figure 2. (a) Test of equality of group means with their level of significance in discriminating different land use systems. (b) bi-plot from canonical discriminant function for three land use systems.

clay content ($R^2 = 0.175$, $p < 0.01$).

For the poplar-wheat land use system, SOC was strongly correlated with MBC ($R^2 = 0.315$, $p < 0.001$), DHA ($R^2 = 0.316$, $p < 0.001$), and clay content ($R^2 =$

0.327 , $p < 0.001$), indicating a robust explanatory power of SOC in this system (Figure 4).

Pearson's correlation analysis, presented in Figure 3, further supported these findings. In the berseem land use

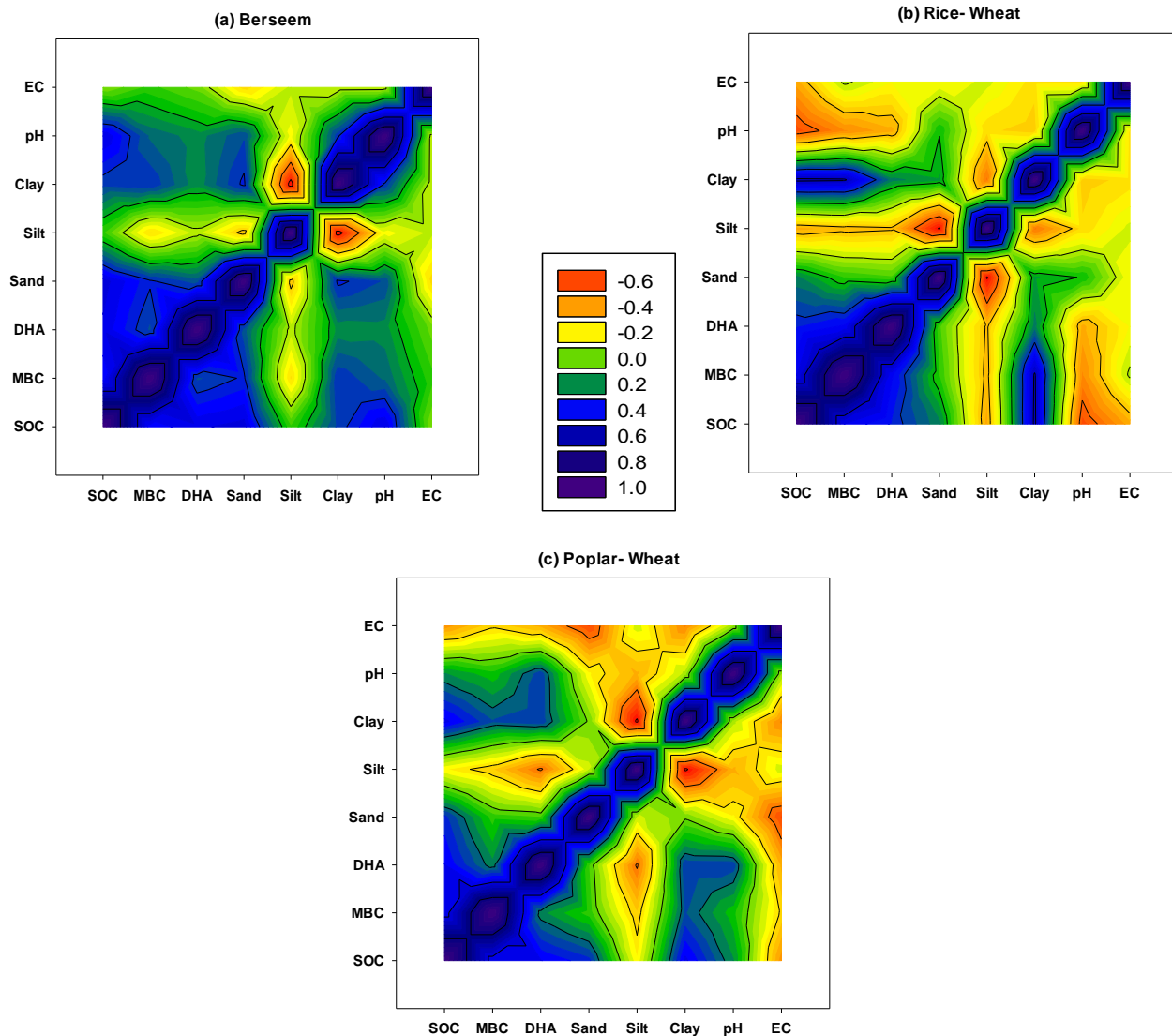


Figure 3. Correlations among various soil parameters for a) Berseem, b) Rice-Wheat, and c) Poplar-Wheat land use system.

system, SOC had highly significant correlations with MBC ($p = 0.0012$), DHA ($p = 0.0002$), and clay content ($p = 0.0093$). MBC also significantly correlated with DHA ($p = 0.0144$) and clay content ($p = 0.0059$). In the *rice-wheat* system, SOC was significantly correlated with MBC ($p = 0.0002$), DHA ($p = 0.0021$), sand ($p = 0.0457$), clay ($p < 0.0001$), and pH ($p = 0.0009$). For the poplar-wheat system, SOC showed highly significant correlations with MBC ($p < 0.0001$), DHA ($p < 0.0001$), and clay content ($p < 0.0001$) (Figure 3).

Sensitivity analysis

A sensitivity analysis was performed to determine minimum sample size for each soil property to compute reliable sample size for detecting desirable changes. This

sensitivity analysis was considerably effective in characterizing the desired soil parameters. Table 5 shows the minimum number of samples that should be analyzed if there was 10% change from the mean value of the kindred soil properties. This analysis revealed that the lowest number of samples was needed for characterizing the soil pH while the highest sample requirement was for proper assessment of DHA across all land use types. For capturing spatial variability in MBC, sample size (number) came out to be considerable.

Geostatistical analysis

Geostatistical techniques analyzed spatial patterns of soil microbiological properties, with key semivariogram parameters presented in Table 6. Minimum RMSE values

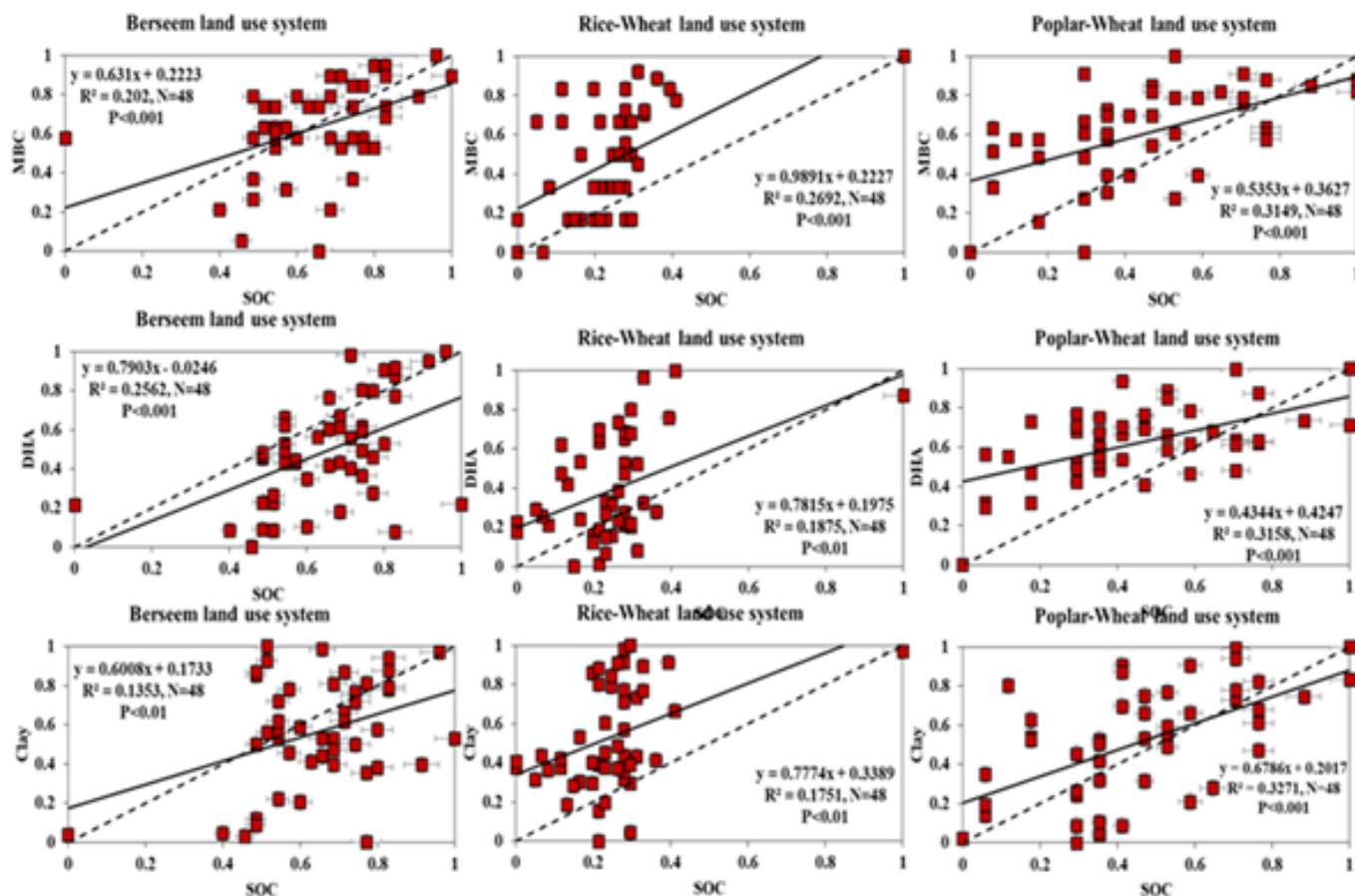


Figure 4. Relationship of MBC, DHA and clay with SOC for three land use systems that is berseem, rice-wheat and poplar-wheat (Regression analysis).

Table 5. Minimum number of samples required to detect 10.00% change from mean in selected soil properties for different land uses at 95% level of confidence ($\alpha = 0.05$).

Variable	Berseem	Rice-wheat	Poplar-wheat
Sand (%)	1	3	4
Silt (%)	22	15	20
Clay (%)	6	5	4
pH	1	1	1
EC (dS m ⁻¹)	10	30	11
SOC (%)	5	14	8
MBC ($\mu\text{g g}^{-1}$)	9	73	34
DHA ($\mu\text{g TPF h}^{-1}$)	20	111	17

determined model selection.

In berseem-based land use, exponential and spherical models characterized MBC and DHA spatial patterns. The spherical model explained both MBC and DHA in rice-wheat, while exponential and spherical models were optimal for MBC and DHA in poplar-wheat. Positive nugget effects indicated inherent variability and sampling

errors.

The nugget-sill ratio determined spatial dependence of soil microbiological properties. Following Cambardella and Elliott (1994), NS ratios <0.25 indicated strong, 0.25-0.75 moderate, and >0.75 weak dependence. In berseem-based land use, clay, MBC, and DHA showed strong spatial dependence (NS = 0.15, 0.14, 0.25). Rice-wheat cropping exhibited moderate dependence for MBC and DHA (NS = 0.62, 0.53). Poplar-wheat showed strong dependence for both MBC and DHA (NS = 0.23, 0.24). Physicochemical properties displayed all types of spatial dependence.

Spatial variability maps generated using kriging (Figure 5) illustrated distinct patterns in MBC and DHA across different land use systems. Berseem-based and poplar-wheat systems exhibited well-defined spatial trends in MBC, contrasting with the less structured pattern observed in rice-wheat fields. In berseem-based land, higher MBC patches were concentrated in the eastern and middle sections, whereas in poplar-wheat, central and western quadrants showed elevated microbial populations. Conversely, MBC in rice-wheat was uniformly lower, with some higher patches in the

Table 6. Semivariogram characteristics of diverse land use systems' soil properties.

Parameter	Model	Nugget (C_0)	Partial sill (C)	Sill (C_0+C)	Nugget:sill (NS ratio)	Spatial dependence	RMSE
Berseem-based							
Sand	Exponential	4.841	0.831	5.672	0.85	Weak	0.946
Silt	Spherical	16.367	2.132	18.499	0.88	Weak	0.987
Clay	Exponential	2.124	12.516	14.640	0.15	Strong	0.941
pH	Exponential	0.012	0.029	0.041	0.29	Moderate	0.970
EC	Exponential	0.016	0.037	0.053	0.30	Moderate	0.961
SOC	Gaussian	0.017	0.025	0.042	0.40	Moderate	0.221
MBC	Exponential	265.434	1620.746	1886.18	0.14	Strong	0.985
DHA	Spherical	0.289	0.845	1.134	0.25	Strong	0.981
Rice-wheat							
Sand	Exponential	2.541	1.652	4.193	0.61	Moderate	1.041
Silt	Gaussian	8.032	5.782	13.814	0.58	Moderate	1.021
Clay	Exponential	12.487	14.417	26.904	0.46	Moderate	1.011
pH	Exponential	0.027	0.019	0.046	0.58	Moderate	1.025
EC	Exponential	0.017	0.001	0.018	0.94	Weak	2.325
SOC	Gaussian	0.019	0.037	0.056	0.33	Moderate	0.057
MBC	Spherical	2003.51	1200.31	3203.82	0.62	Moderate	0.051
DHA	Spherical	0.561	0.487	1.048	0.53	Moderate	0.998
Poplar-wheat							
Sand	Exponential	3.687	4.965	8.652	0.43	Moderate	0.917
Silt	Spherical	7.912	12.534	20.446	0.39	Moderate	0.867
Clay	Exponential	6.452	7.669	14.121	0.46	Moderate	0.945
pH	Exponential	0.031	0.020	0.051	0.61	Moderate	0.987
EC	Gaussian	0.312	0.967	1.279	0.24	Strong	0.765
SOC	Gaussian	0.121	0.357	0.478	0.25	Strong	0.257
MBC	Exponential	1787.05	5923.37	7710.42	0.23	Strong	0.021
DHA	Spherical	0.317	0.986	1.303	0.24	Strong	0.986

northeast. DHA maps similarly highlighted spatial trends: berseem-based areas displayed higher enzymatic activity in the southern and central regions, contrasting with moderate and less structured patterns in rice-wheat fields. Poplar-wheat systems showed higher DHA in eastern, southern, and southwestern areas, indicating robust enzymatic activity across these zones. Overall, these maps underscored the spatial heterogeneity of microbial properties, with implications for understanding and managing soil health across diverse agricultural systems.

DISCUSSION

In summary, the study highlighted significant differences in soil properties among the three land use systems in northwest India. While pH and EC varied moderately across systems, organic carbon content and microbial properties showed more pronounced differences.

Berseem-based systems stood out for their higher microbial biomass and lower variability in both MBC and DHA, suggesting potentially favorable conditions for microbial activity compared to rice-wheat and poplar-wheat systems as pH alone can explain 20% spatial variability of soil microbial community (Bru et al. 2011). These findings underscore the importance of considering land use practices in managing soil health and productivity in agricultural landscapes. Mean soil pH values were highest in the rice-wheat system, followed by the poplar-wheat and berseem-based systems. However, pH decline in rice-wheat soils is due to urea use and cation leaching (Saia et al., 2014). The decline in pH under poplar-wheat and berseem-based systems is attributed to decomposing leaf litter and higher root biomass (Islam et al., 2014). The lower pH in these systems also relates to higher electrical conductivity (Table 2). Soil pH showed the least variability, attributed to high clay and organic carbon content. Variability followed the order: rice-wheat > poplar-wheat >

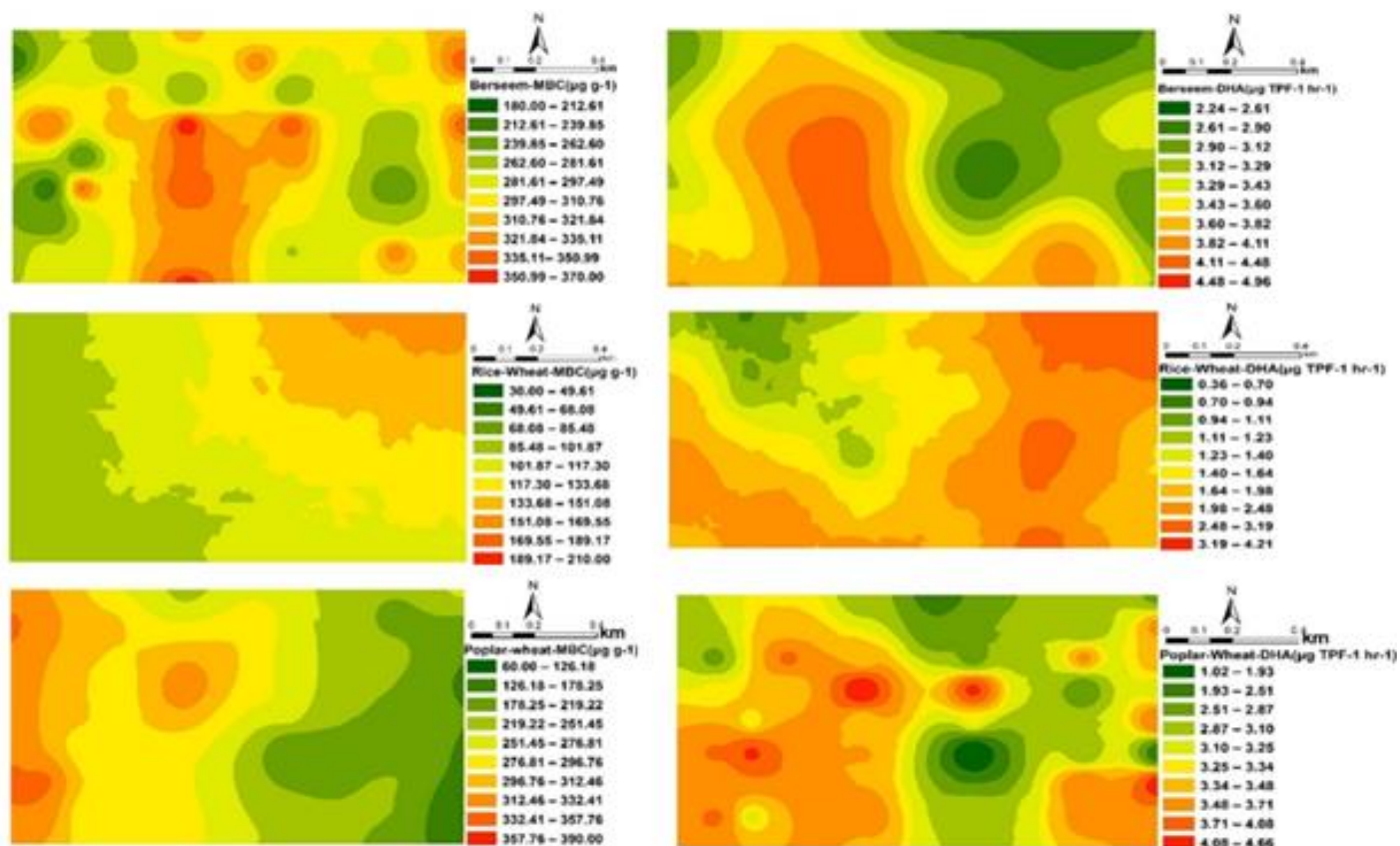


Figure 5. Predicted maps of MBC and DHA (a, b and c =MBC; d, e and f = DHA under berseem, rice-wheat and poplar-wheat system, respectively).

berseem-based, similar to clay content. Mean electrical conductivity (EC) was highest in the berseem-based system, followed by rice-wheat and poplar-wheat systems, reflecting toposequence among land uses. Rice-wheat showed the highest within-field EC variation, due to subsurface layer variation in paddies (Nguyen et al., 2014) and elevation fluctuations (Sung and Jang, 2006). Organic carbon distribution was broader in berseem-based and poplar-wheat systems, while lowest in rice-wheat, supported by higher biomass turnover in the former systems. Rice-wheat showed more within-field variability due to hard pan depth variation and elevation changes (Sung and Jang, 2006). MBC was highest in the berseem-based system, followed by poplar-wheat and rice-wheat, likely due to leguminous berseem's rhizobia population (Sung and Jang, 2006). This pattern aligns with findings by Izaguirre-Mayoral et al. (2002) linking MBC to vegetation type. Within-field MBC variation was highest in rice-wheat, followed by poplar-wheat and berseem-based systems, reflecting SOC's influence (Wallenius et al., 2011). DHA followed the order: berseem-based > poplar-wheat > rice-wheat, paralleling organic carbon and MBC patterns (Järvan et al., 2014). Within-field DHA variability was highest in rice-wheat,

mirroring MBC and organic carbon trends. Higher mean SOC, MBC, and DHA in poplar-wheat than rice-wheat is due to leaf litter addition in agroforestry systems, promoting microbial activities (Miao et al., 2019; Xu et al., 2013). Lower microbial activity in rice-wheat is attributed to its exhaustive nature, supported by Bera et al. (2018).

Changing land uses significantly influenced soil properties, especially microbiological ones. Berseem-based land use had higher MBC and DHA due to higher clay and SOC content (Yang et al., 2018). Clay's significant correlation with MBC and DHA across land uses stems from its ability to stabilize microbial activity (Shahbazi et al., 2013). The findings of this study align with previous studies showing clay's role in extracellular enzyme stabilization (Kallenbac, 2011) and positive DHA-clay content relations. Berseem-based land use's sand content correlated significantly with microbiological parameters, promoting higher microbial activities through better aeration. Environmental factors significantly influence soil microbial communities, with pH being a key determinant (Pietri and Brookes, 2009). In rice-wheat, pH correlated significantly with SOC, MBC, DHA, and clay content, affecting microbial activities (Rousk et al., 2009; Wu et al., 2017). Neutral to slightly

alkaline pH favored bacterial populations, which decrease with soil acidity (Shahbazi et al., 2013). Different land uses showed varying human intervention levels, affecting soil parameters. Microbiological parameters had higher % CV variability than physicochemical ones, consistent with previous arable soil studies (Piotrowska-Długosz et al., 2019). Microbiological properties' higher variability is due to sensitivity to agronomic practices like tillage, crop rotation, and amendments (Ye and Wight, 2010).

Sensitivity analysis confirmed the need for more samples to detect critical changes in microbiological properties compared to physicochemical ones. Using PCA and DA, SOC, MBC, DHA, sand, and clay were identified as significant in separating land use systems. Studies by Bhattacharjya et al. (2017) and Kara and Baykara (2014) support these findings. Correlation among SOC, MBC, and DHA significantly differentiated land uses, aligning with findings by Ge et al. (2013), Liang et al. (2012), Lagomarsino et al. (2011), and Ye and Wright (2010). Classical multivariate methods often ignore spatial dependence, but this study used geostatistics for spatial variability analysis, revealing MBC and DHA's spatial dependence changes with land use (Ceja-Navarro et al., 2010). Isotropic semivariogram analysis indicated DHA followed a spherical model, while MBC fit exponential and spherical models (Shahbazi et al., 2013). Kriged surface maps showed microbial abundance in central and southern parts of the berseem-based system, and well-distributed patterns in poplar-wheat systems due to regular litter input. Root biomass in these systems positively influenced microbial activity, enhancing soil fertility (Jeloudar et al., 2014). Intensive fertilizer uses in rice-wheat reduced microbial population, causing irregular MBC and DHA distribution (Nunan et al., 2002; Muhammad et al., 2006). Kriged maps effectively delineated spatial patterns, highlighting poplar-wheat's superiority in promoting key microbiological properties for soil fertility, recommending its inclusion in farm-level land use plans (Yang et al., 2024).

Conclusions

The study on spatial variability of soil microbial properties in central Punjab, India, revealed that MBC and DHA exhibited greater variability than chemical properties, as confirmed by geostatistical analysis. Among the cropping systems examined, the rice-wheat system had the poorest microbial indicators, while the legume fodder-based berseem system demonstrated the best performance. The poplar-based system performed better than the rice-wheat system but not as well as the berseem system. The analysis highlighted that SOC, MBC, and DHA were key variables for differentiating between land uses. DHA, in particular, showed the highest variability and thus required more samples for accurate detection. Additionally, the berseem-based land use did not display the expected relationship between

MBC and clay content, indicating that enhanced soil aeration in this system might promote better microbial growth. These findings emphasize the importance of understanding spatial variability in microbial soil health parameters and have significant implications for designing effective sampling protocols. The results suggest that specific land use practices, such as those in the berseem and poplar-based systems, could be optimized to improve microbial activity and overall soil health.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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