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Deciphering Enzyme Activity, Bacterial Diversity, Microbial Biomass and Organic Content in Soil along a Latitudinal Gradient in North-Central India: Exploring Ways for Increased Crop Productivity

Kanchan Vishwakarma [1], Vivek Kumar [2], Nitin Kumar [1], Durgesh K Tripathi [3], Shivesh Sharma [1,3]*

[1] Department of Biotechnology, Motilal Nehru National Institute of Technology Allahabad, Allahabad, Uttar Pradesh, India
[2] Himalayan School of Biosciences, Swami Rama Himalayan University, Jollygrant, Dehradun, India
[3] Centre for Medical Diagnostics and Research, MNNIT Allahabad, Uttar Pradesh, India

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Abstract: The soil on Earth's surface is characterized by the organic and mineral content in them. The activity of enzymes and microbial biomass present in it has significant impact on nutrient cycling which is directly related to fertility and crop yield in that area. In our study, samples of top soil were studied from four different locations in Nothern part of India, namely, Solan (Himachal Pradesh), Jagatpura (Punjab), Allahabad (Uttar Pradesh) and Satna (Madhya Pradesh), with respect to four pre-dominant soil enzymes *viz*. Dehydrogenase, Polyphenol oxidase, Urease and Catalase. The studies regarding spatial variation of activity of these four soil enzyme along with the microbial biomass content along the latitudinal gradient in North-central India are unclear in context with the national status. The present study facilitates the above mentioned assessment with respect to the effect of climatic variations, enzyme activity and biomass on nutrient cycling (organic carbon, available nitrogen and phosphorous) in respective agro-ecosystems. Along the increasing latitude, it was observed that organic carbon decreases whereas available phosphorous and available nitrogen increases ($P<0.05$). Further, it was seen that the amount of microbial biomass carbon (MBC) declined and microbial biomass nitrogen (MBN) increased ($P<0.05$). Polyphenol activity and urease activity was found to decrease and dehydrogenase activity showed an increasing trend. In addition, catalase activity showed no significant relation with latitude ($P<0.05$). In accordance with the present study, it can be inferred that the distribution of enzyme activities and microbial biomass may be the consequence of alterations in temperature and moisture of soil because of which soil properties like organic carbon, nitrogen, phosphorous change along the latitudinal transect.

Keywords: Latitude gradient, microbial biomass, moisture, soil organic carbon, temperature.

Introduction

The conjugated effects of climatic alteration, biological invaders and straight-forward human moderations in the atmosphere have resulted in a drastic transformation with respect to routine disturbances. Such modifications have significant influence on soil micro-flora [39]. Since the release of soil enzymes is directly dependent on the condition of the soil microorganisms, the above mentioned disturbances will affect soil health and quality. This leads to reduced crop yield and productivity [23]. Soil biodiversity and linked services are significantly affected by global climatic changes. These effects can be related directly or indirectly

*Corresponding author (Shivesh Sharma)
E-mail: < shiveshs@mnnit.ac.in; ssnvsharma@gmail.com >

to the changes in atmospheric parameters i.e. temperature and moisture.

Soil organic matter (SOM) is primarily composed of amides, stable material called humus, soil microbes and other organic molecules. The microorganisms present in the soil indicate the soil microbial activity. These microbes are the source of soil enzymes that have significant part in deposition of organic matter present in soil and nutrient cycling [32]. Soil is an active pool of carbon, nitrogen, phosphorus and other minerals and a variable but significant pattern to this pool is contributed by microbial biomass C and N [28].

The enzymes released by soil organisms play as biological catalysts to various reactions and metabolic processes in order to splinter organic pollutants which leads to production of essential compounds both for microbes and plants [9]. As compared to the animals and plants, the soil enzyme activities are considered to be the most sensitive bio-indicator for the disturbances caused naturally and anthropogenetically [12]. Furthermore, the assays to estimate soil enzyme activities are cost-effective and simple. Hence, they can be employed to measure the level of pollution in soil and to understand the characteristic structure of microbial community [5].

Soil enzyme activity has been seen to be influenced by soil temperature and moisture which are regulated by climatic changes. These climate changes, which have a dominant impact on enzyme activity in the soil, occur as one move away from the equator. This shift can be attributed to latitude gradient. Numerous ecological factors like plant community structure, SOM, soil moisture and temperature affect microbial biomass and activities [33, 20].

Parkinson and Coleman [26], Steinberger *et al.* [30] and Li and Sarah [21, 22] have elucidated that in arid and semi-arid atmospheres, nutrient cycles of terrestrial eco-system are affected dominantly by climate changes. Similarly, in a study carried out by Frey *et al.* [11], temperature and moisture are found to affect the enzyme activities indirectly by fomenting microbes and leading to the availability of substrates. If we consider the influence of atmospheric alterations on soil nutrient cycle, it is necessary to check the spatial variation of soil microbial biomass and enzyme activities, along with the understanding of association between soil enzymes, biotic and abiotic parameters [21]. As such, in national scenario, the area of this study is unclear, so it is necessary to understand soil activities with respect to a pronounced relationship between the biological and chemical properties with the latitude transect.

Materials and methods

Site description

North-central latitude in India was considered to perform the study. The regions taken under the latitude include Solan (Himachal Pradesh) in north, Jagatpura (Punjab), Allahabad (Uttar Pradesh) and Satna (Madhya Pradesh) towards central region, located between 24°- 31°N and 76°- 81°E. The sites cover north and north-central region of India. These areas come under the major maize producing states which is thought to be one of the main marketable grains in India. The season of maize growth (*Zea mays* L.) is Kharif i.e. from July to October each year using conventional tillage. Sites were located by the means of Global Positioning System (GPS). The mean temperature ranged from 28° to 31°C and soil moisture content from 46 % to 50 % (Table 1).

Soil sampling

Soil samples were collected from maize fields

Table 1. Climatic parameters of selected four locations along the latitude gradient in north-central India

Location	Latitude N	Longitude E	Temperature (°C)	Moisture (%)
Solan	31.008274	77.080309	28.6	48.27
Jagatpura	30.682275	76.761620	30.2	46.23
Allahabad	25.6744478	81.630580	30.77	49.30
Satna	24.698960	81.013537	31.4	47.93

in respective sites just before the harvesting period i.e. September 2014. Top rhizospheric soils (0-20cm) were taken from each location. The samples were collected by taking the presumption that this time would permit us to know better spatial variation of enzyme activities and microbial biomass as the degradable crop residues are on the verge of complete degradation and there were lesser chances of extreme conditions. The different agricultural management practices were reduced to lowest level.

From every site, three places were randomly selected and soil samples were collected using coring techniques. Hence, there were three samples (three replicates) from each site. The Microprocessor Moisture Meter was taken along so as to rapidly monitor the temperature and moisture of the respective samples. The soils were immediately kept in polythene bags just after sampling. To measure different enzyme activities and soil microbial biomass, the soils were air-dried and sieved through < 2mm sieves and analysed within two weeks.

Isolation and purification of microorganisms

Total culturable bacterial population density was determined on the basis of serial dilution method by spread plate technique in triplicates (1 g soil) using appropriate dilutions [15]. The isolates were purified by streaking the loop-full of culture onto the nutrient agar plates [35].

Morphological and Biochemical Characterization

Various biochemical and morphological tests were performed. They included Gram staining, endospore staining, capsule staining, gelatinase test, IMViC, starch hydrolysis, oxidase test and catalase test. All the above listed assays were performed as mentioned by Kreig and Holf [17].

Enzyme activity, microbial biomass and soil chemical properties

The activity of dehydrogenase was estimated based on the procedure mentioned by Lenhard [19]. Polyphenol oxidase and Catalase activity were determined according to the method of Zhou [35], whereas urease activity was calculated using the method listed by McGarity and Myers [24]. These activities were estimated in duplicates for each sample with control taken for each which was expressed in terms of dry weight of soil.

Soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) estimations were performed using chloroform fumigation and extraction method as described by Ladd and Amato [18]. Similarly, soil organic carbon was estimated using partial oxidation method [36] available nitrogen was measured by alkaline potassium-permanganate method [37] and available phosphorous was estimated using Bray method [38].

Statistical analysis

Analysis of soil biological and chemical properties was done using single factor-ANOVA. Correlation was established for enzyme activity and microbial biomass along the latitudinal gradient. Linear regression was carried out in which enzyme activity and microbial biomass was considered as the dependent variables and latitude as independent variable. Moreover, Pearson's correlation between biological and physico-chemical parameters of soil was calculated using OPSTAT software (Haryana Agricultural University, Hisar-India).

Results

Soil chemical properties along latitude gradient

The site effects upon the chemical properties of soil were found to be significant. Soil organic carbon (SOC) ranged from 0.098 % to 1.011 %. SOC was highest in central region i.e. Satna and lowest in northern region i.e. Solan (Table 2). In contrast, available nitrogen seems to be high with lowest value of 166.20 kg/ ha in central region i.e. Satna and highest value of 292.69 kg/ ha in north i.e. Solan (Table 2). Available phosphorous increased around 7 times from Satna (central site) to Solan (north site) (Table 2). This difference in available P from north to south had sounding effect.

Biochemical and diversity analysis

The results of Gram staining enunciate that bacterial diversity mainly incorporates Gram nega-

Table 2. Chemical properties of soil in four locations along the latitude gradient in north-central India

Location	Total Organic Carbon (%)	Available Nitrogen (kg/ha)	Available Phosphorous (kg/ha)
Solan	0.0983	292.69	51.45
Jagatpura	0.7706	220.56	26.05
Allahabad	0.727	191.29	19.62
Satna	1.011	166.2	6.89

Organic C %		Available nitrogen (Kg/ha)		Available phosphorous (Kg/ha)	
Low	< 0.4 %	Low	< 22.5	Low	< 22.5
Medium =	0.4 %	Medium =	22.5-56.0	Medium =	22.5-56.0
High	> 0.75 %	High	> 56.0	High	> 56.0

tive bacterial isolates (71 %) as compared to Gram positive isolates (29 %) (Fig. 1). Solan and Satna i.e. the sites within the latitude transect showed abundance of Gram negative bacterial isolates whereas Allahabad and Jagatpura showed predominantly Gram positive bacteria. The recognition of bacteria entails the understanding of their biochemical, morphological and physiological characterisation. The details of identifying the isolates biochemically are listed in Table 3.

Soil enzyme activities along the latitude gradient

The spatial variation of soil enzymes in regions along the latitudinal transect showed varying nature with the kind of enzyme being studied (Fig. 2). In general, dehydrogenase (DHA) activities reduced from north (Solan) to central (Satna) region along the latitude transect. It had shown significant correlation with latitude gradient ($P<0.05$). There was around two-fold variation in dehydrogenase activity between north and central region (Fig. 2c). The overall average of the DHA activity was 0.4104 $mg.g^{-1}$ $soil.24h^{-1}$. Urease activity increased from north to central region with the latitude, averaging about 2.614 $mg.g^{-1}$ $soil.24h^{-1}$. Also, it was found to be significantly correlated with the latitude gradient ($P<0.05$) (Fig. 2d). Similarly, polyphenol oxidase (PPO) activity enhanced from north to central site along the latitude gradient, averaging about 0.3704 $mg.g^{-1}$ $soil.24h^{-1}$. Again, it was also significantly correlated with latitude transect ($P<0.05$) (Fig. 2b). The variation of urease and PPO activity between north and

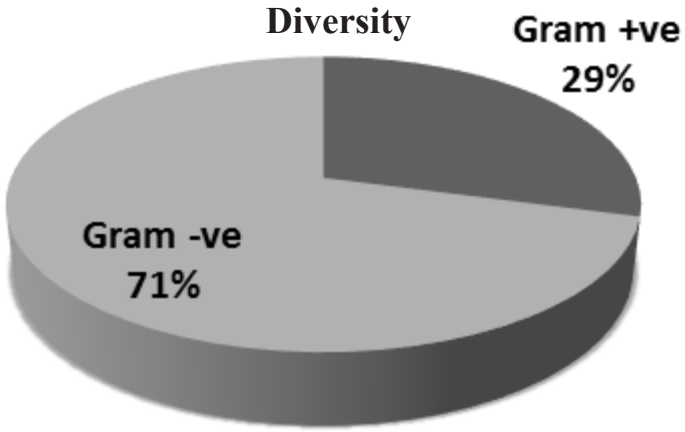

Fig. 1. Percentage of Gram positive and Gram negative bacteria over the latitude

Table 3. Biochemical characterization of various isolates in the sites of interest within the latitude

Sites	Isolates	Catalase	Oxidase	Indole	MR	VP	Citrate	Amylase	Gelatinase
Solan	SLN1	+	+	-	+	-	+	-	-
	SLN2	+	-	-	+	-	+	-	-
	SLN3	+	-	-	+	-	+	-	-
	SLN4	+	+	+	+	-	-	+	+
	SLN5	+	-	-	+	-	-	+	+
	SLN6	-	+	+	+	-	+	-	-
Jagatpura	JGP1	+	+	-	-	+	-	+	-
	JGP2	+	-	+	+	-	-	+	-
	JGP3	+	+	-	+	-	+	+	-
	JGP4	+	-	-	-	+	+	+	-
	JGP5	-	-	-	-	+	-	+	-
Allahabad	ALD1	-	-	-	+	-	-	+	-
	ALD2	+	-	-	-	+	+	+	-
	ALD3	-	-	-	+	-	+	+	-
	ALD4	+	-	-	+	-	+	-	-
	ALD5	+	-	-	-	+	+	+	-
	ALD6	+	-	-	+	-	+	+	-
	ALD7	+	-	-	+	-	-	-	-
Satna	STN1	+	+	-	+	-	+	-	-
	STN2	+	+	-	+	-	+	-	-
	STN3	+	+	-	+	-	+	-	-
	STN4	+	+	-	+	-	+	-	-
	STN5	+	-	-	+	-	-	+	-
	STN6	+	-	-	+	-	+	-	-

central region were not so pronounced as that of DHA activity (i.e. two-fold increase).

However, catalase activity showed no dependency on latitude gradient, averaging about 0.1918 $mg.g^{-1}$ soil.$24h^{-1}$. The catalase activity was contemplated to be highest at Allahabad (near central sampling site) and lowest at Jagatpura and Solan (towards north site) (Fig. 2a).

Soil microbial biomass along the latitude gradient

There was significant reduction in soil microbial biomass nitrogen (MBN) from north i.e. sampling sites in Solan to central region i.e. sampling sites towards Satna and was found to be significantly correlated with latitude gradient ($P<0.05$) (Fig. 3b). Soil microbial biomass carbon (MBC), in general, increased from northern sampling regions to central sampling sites. MBC ranged from 37.33 to 423.67 kg/ha; and there was around 11-fold difference between the largest and smallest MBC (Fig. 3a). It had also shown significant correlation with latitude gradient ($P<0.05$). Similarly, MBC/MBN ratio was also significantly correlated with latitude transect ($P<0.05$). The smallest ratio was at Satna in central region and largest at Solan in north region (Fig. 3c).

Correlation between soil microbiological and chemical properties

Enzyme activity and microbial biomass in soil have vivid correlations with soil's chemical prop-

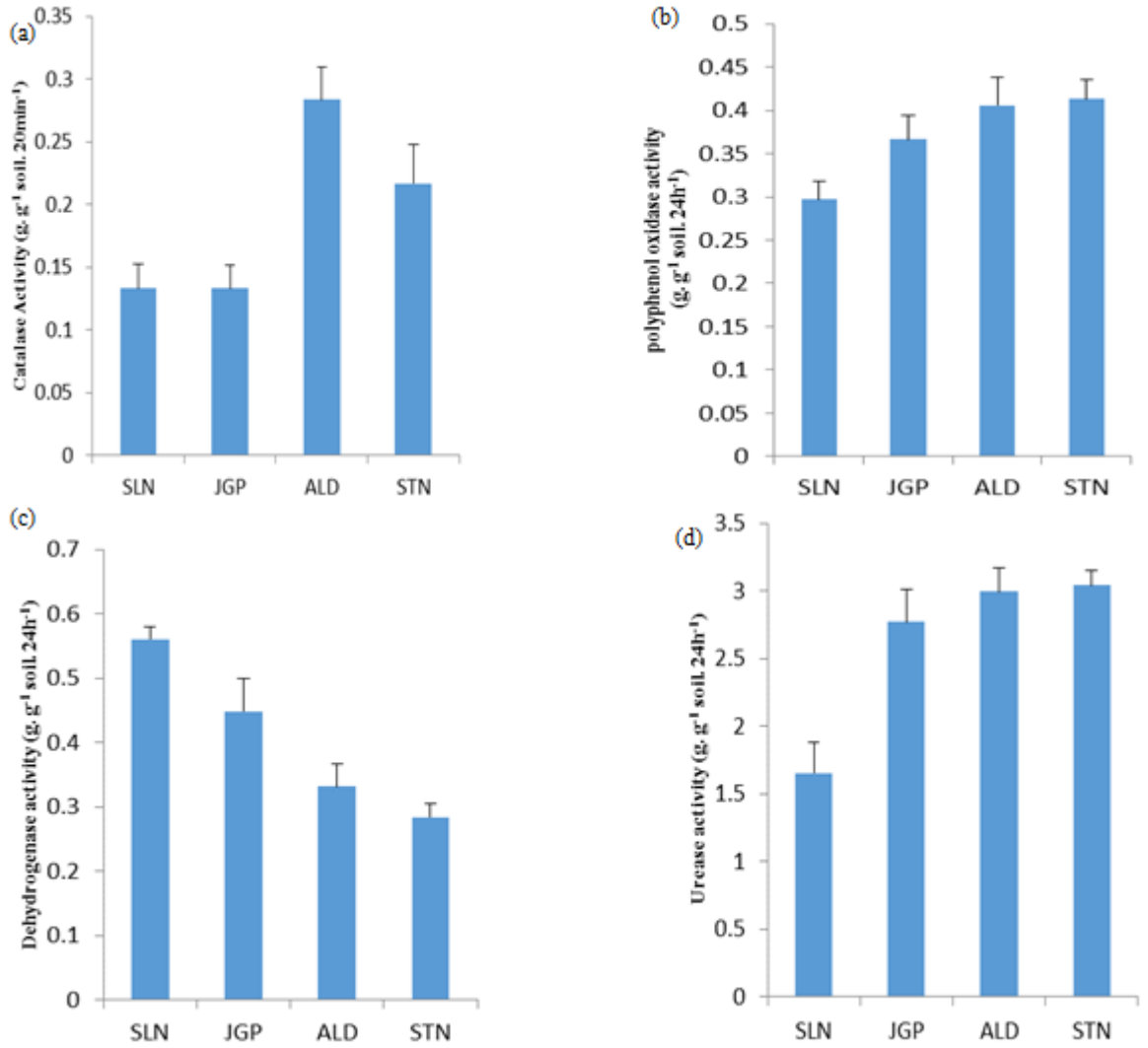

Fig. 2 (a, b, c, d). Soil enzyme activities of four sampling sites along latitude transect in north-central India. Error bars represent the standard deviation. (SLN- Solan; JGP-Jagatpura; ALD-Allahabad; STN- Satna)

erties. Soil catalase activity was positively correlated with organic carbon and negatively correlated with available N and available P ($P<0.05$) (Table 4). Soil dehydrogenase activity was negatively correlated with organic carbon and positively correlated with available N and available P ($P<0.05$). Polyphenol activity and urease activity were perfectly negatively correlated with available N and available P and positively correlated with organic carbon ($P<0.05$).

Soil microbial biomass carbon (MBC) and ratio MBC/MBN were positively correlated with organic carbon and negatively correlated with available nitrogen and available phosphorous ($P<0.05$). However, soil microbial N was positively correlated with available N and available P and negatively correlated with organic carbon ($P<0.05$). MBC and MBN also show significant inter-correlation ($P<0.05$). Furthermore, significant positive and negative correlations were noticed between soil enzyme activities and microbial biomass, for example, negative correlation of catalase, urease and PPO activity with MBN; positive correlation with MBC ($P<0.05$).

Discussion

The principle objective of our study was to check the climate changes on nutrient cycle of soils in

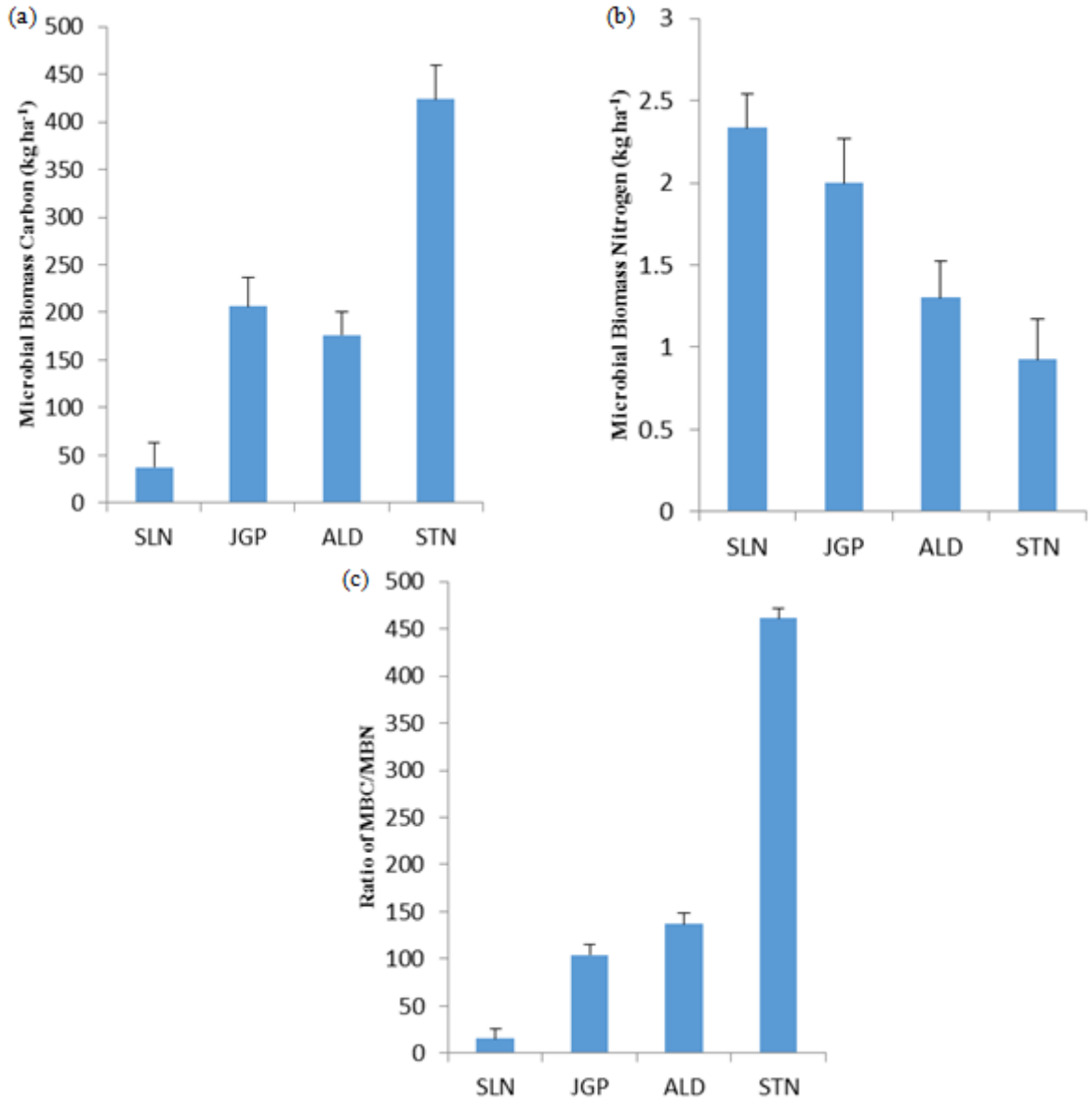

Fig. 3. Microbial biomass carbon, nitrogen and their ratio in four sampling sites along latitude transect in north-central India. Error bars represent the standard deviation. (SLN- Solan; JGP-Jagatpura; ALD- Allahabad; STN- Satna)

Table 4. Correlation coefficients between soil microbial and chemical properties

	Catalase	DHA	PPO	Urease	MBC	MBN	MBC/MBN
Organic C	0.485*	-0.910	0.936	0.961	0.907	-0.829	0.788
Available N	-0.691	0.987	-0.991	-0.969	-0.879	0.937	-0.808
Available P	-0.624	0.973	-0.974	-0.956	-0.919	0.927	-0.847
MBC	0.359*	-0.849	0.809	0.779	1	-0.852	0.966
MBN	-0.788	0.976	-0.925	-0.831	-0.852	1	-0.873

* Non-significant value, and rest of the coefficients give significant correlation at $P<0.05$ level of probability

the north-central India. It was evident from the results that when latitude is increased, the edaphological parameters viz. temperature, moisture, nutrient availability varied magnanimously. As a result, there were huge differences among soil microbial biomass and enzyme activity along the latitude transect, which may further lead to break down of organic matter and affects nutrient cycle in soil.

In our study, the obvious changes in soil temperature and moisture were observed along the latitudinal gradient (Table 1). We noticed that the chemical attributes of soil varied greatly along the latitudinal gradient i.e. soil organic carbon (SOC) increased from north to central region, whereas available nitrogen available phosphorous declined from north to central site. The reason may be attributed to the fact that there are changes in climate from north to central regions as well as also changes in site reclamation takes place which can be correlated to temperature. Since, temperature is more in central site so the duration for reclamation is prevailing there than in north site [27,34]. We also know that higher temperature facilitates more degradation rate of soil organic matter i.e. organic carbon.

Soil urease and polyphenol oxidase activities were seem to be significantly correlated with latitude transect i.e. as we increase latitude, urease and PPO activity decreases. This outcome demonstrated that hydrolytic enzymes of urease and PPO were more actively participating in hydrolysis in central region than north region. Such results indicated faster turnover rate of nitrogen cycle, carbon cycle and more biomass and nutrients in soil by hydrolyzing lignin. The reason might be because of spatial variation of organic matter in soil along the latitude transect. Urease activity was more in central region. It may be due to the reason that Satna has more land holding capacity which has been supplied with urea fertilisers which was degraded by urease to give away NH_3 and CO_2 as N and C sources [2,8], whereas, Solan (northern sampling site) has lesser land-holding capacity indicating the lesser use of urea fertiliser. Urease enzyme also results in rapid nitrogen loss to the environment via ammonia volatilisation [29], which can also be seen from the results that available nitrogen is less at high urease activity region. Also, the study is in coordination with Tabatabai [31] that urease activity increases with increase in temperature. Similarly PPO activity was also more in central region which indicates more lignin hydrolysis in this region as compared to north site.

Dehydrogenase (DHA) activity in soil was found to be more in northern regions as compared to central and was significantly correlated with the latitude transect. Such results can be attributed to the colony forming units which showed similar pattern distribution to DHA activity along the latitude gradient. It happened because DHA activity only reflects the activity of viable cells and not the stabilised soil complexes [7]. DHA activity was also found to be correlated with temperature and moisture ($P<0.05$) because moisture and temperature of soil indirectly influences its activity by affecting soil redox reactions [6]. It also inferred that, in northern sampling site (Solan), low dosage of pesticide was given (high DHA activity) as compared to central site (low DHA activity) [4].

However, catalase activity in soil was found to be independent of the latitude gradient. It was highest in Allahabad (towards centre region) and lowest in north site. High catalase activity gives indication of more contamination of that soil. The lacuna in correlation between catalase activity and latitude may be affected by the soil properties.

Soil temperature and moisture, in general, were increasing from north to central region and significantly correlated with latitude because with changing latitude, the climatic parameters changes which further lead to changes in soil properties [23]. Also, the enzyme activities like that of urease and DHA correlates and have the similar trend with temperature and moisture i.e. urease activity increases with increasing temperature and DHA activity get influenced with changing temperature and moisture.

Microbial biomass is supposed to be the liable store of soil's organic content. Hence, it is contemplated to be the source of available nutrition for plants and microbes [16,25]. Our observation states that MBC and MBC/MBN ratio were positively correlated with latitude whereas MBN was, in general, negatively correlated with latitude.

Such outcomes pinpointed that there were high immobilized levels of labile carbon and low level

of immobilized nitrogen in microbial biomass in central site than in northern site. This can be explained by their intimate relationship with organic carbon and nitrogen levels. Soil microbes get substrates from SOC to act upon; hence, giving speedier turnover rate of soil microbial biomass. The ratio of MBC/MBN can be utilized to give an indication of the relative proportion of fungus to bacterium [1]. According to the findings of Balota *et al*.[3], higher MBC/MBN ratio will give more proportion of fungal biomass in comparison to bacterial biomass through soil management practices. Hence, we hypothesised that spatial distribution of MBC/MBN showed more fungi in central regions and bacteria in north region along the latitude gradient. The combined results of all parameters highlights that effects of climatic changes i.e. temperature and moisture on soil chemical and organic matter in north-central India is complicated, and it is further influenced by union of soil atmosphere and different anthropological practices.

Conclusion

The spatial variation of soil microbe biomass and hydrolytic activities interwined into soil's carbon, nitrogen and phosphorous cycle was assessed in north-central India along the latitudinal transect. The activities of PPO, urease, MBC and MBC/MBN ratio were significantly correlated ($P<0.05$) with the latitude i.e. decreased with increasing latitude. The established distribution among the results was dependent upon the properties of soil which includes soil organic content along the gradient. The more level of biomass and enzyme activities of urease and dehydrogenase towards northern region than central region indicated towards fast turnover rate of nutrient cycles taking place in soil. The present study will go a long way in helping incorporating methodologies by which the crop production and yield can be enhanced keeping in mind the aforementioned results that favor the faster break-down of soil organic matter and involvement of soil enzyme activities.

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References

1. **Anderson, J.P.E. and Domsch, K.H. (1980).** Quantities of plant nutrients in the microbial biomass of selected soils. Soil Sci. 130: 211-216.
2. **Andrews, R.K., Blakeley, R.L. and Zerner, B. (1989).** Urease: a Ni (II) metalloenzyme. In: Lancaster JR (ed) The bioinorganic chemistry of nickel. VCH, New York, pp 141-166.
3. **Balota, E.L., Colozzi-Filho, A., Andrade, D.S. and Dick, R.P. (2003).** Microbial biomass in soils under different tillage and crop rotation systems. Biol. Fertil. Soils. 38: 15-20.
4. **Baruah, M. and Mishra, R.R. (1986).** Effect of herbicides butachlor, 2,4-d and oxyfluorfen on enzyme activities and CO2 evolution in submerged paddy field soil. Plant Soil. 96: 287-291.
5. **Baum, C., Linweber, P. and Schlichting, A. (2003).** Effects of chemical conditions in rewetted peats temporal variation in microbial biomass and acid phosphatase activity within the growing season. Appl Soil Ecol. 22: 167-174.
6. **Brzezinska, M., Stepniewska, Z. and Stepniewski, W. (1998).** Soil oxygen status and dehydrogenase activity. Soil Biol Biochem. 30: 1783-1790.
7. **Burns, R.G. (1978).** Enzyme activity in soil: some theoretical and practical considerations. In: Bums RG (ed) Soil enzymes. Academic, London, pp 295-340.
8. **Byrnes, B.H. and Amberger, A. (1989).** Fate of broadcast urea in a flooded soil when treated with N-(nbutyl) thiophospheric triamide, a urease inhibitor. Fertil Res. 18: 221-231.
9. **Das, S. and Varma, A. (2011).** Role of Enzymes in Maintaining Soil Health. Soil Enzymology, Soil Biology. 22: 25-42.
10. **Espinoza, L., Norman, R., Slaton, N. and Daniels, M. (2005).** The Nitrogen and Phosphorous Cycle in Soils. Agriculture and Natural Resources.

11. **Frey, S.D., Elliott, E.T. and Paustian, K. (1999).** Bacterial and fungal abundance and biomass in conventional and no-tillage agroecosystems along two climatic gradients. Soil Biol. Biochem. 31: 573-585.
12. **Hinojosa, M.B., Carreira, J.A., Garcla-Rulz, R. *et al.* (2004).** Soil moisture pre-treatment effects on enzyme activities as indicators of heavy metal-contaminated and reclaimed soils [J]. Soil Biol Biochem. 36: 1559-1568.
13. **Brito-Vega, H., Espinosa-Victoria, D., Salaya-Domínguez, J.M. and Gómez-Méndez, E. (2013).** The soil biota: importance in agroforestry and agricultural systems. Tropical and Subtropical Agroecosystems. 16: 445-453.
14. **Jenkins, M., Scherr, S.J. and Inbar, M. (2004).** Markets for biodiversity services: potential roles and challenges. Environment. 46: 32-42.
15. **Johnson, L.F. and Curl, E.A. (1972).** Methods for Research on the Ecology of Soil-Borne Plant Pathogens. Burgess Publishing Company.
16. **Kandeler, E., Palli, S., Stemmer, M. and Gerzabek, M.H. (1999).** Tillage changes microbial biomass and enzyme activities in particle-size fractions of a Haplic Chernozem. Soil Biol. Biochem. 31: 1253-1264.
17. **Kreig, N.R. and Holf, J.G. (1984).** Bergeys manual of systematic bacteriology. William and Wilkins, Baltimore, USA.
18. **Ladd, J.N. and Amato, M. (1989).** Relationship between microbial biomass carbon in soils and absorbance of extracts of fumigated soils. Soil boil biochem. 21: 457-459.
19. **Lenhard, G. (1956).** The dehydrogenase activity in soil as a measure of the activity of soil microorganisms, Z. Pflanzenernaehr. Dueng. Bodenkd. 73: 1-11.
20. **Li, X.Z. and Chen, Z.Z. (2004).** Soil microbial biomass C and N along a climatic transect in the Mongolian steppe. Biol. Fertil. Soils. 39: 344-351.
21. **Li, X.Z. and Sarah, P. (2003a).** Arylsulfatase activity of soil microbial biomass along a Mediterranean-arid transect. Soil Biol. Biochem. 35: 925-934.
22. **Li, X.Z. and Sarah, P. (2003b).** Enzyme activities along a climatic transect in the Judean Desert. Catena. 53: 349-363.
23. **Mandal, A. and Neenu, S. (2012).** Imapct of climate change on soil biodiversity - A Review. Agri. Reviews. 33(4): 283-92.
24. **McGarity, J.W. and Myers, M.G. (1967).** A survey of urease activity in soils of northern New South Wales. Plant and Soil. 27: 217-238.
25. **Omay, A.B., Rice, C.W., Maddux, L.D. and Gordon, W.B. (1997).** Changes in soil microbial and chemical properties under longterm crop rotation and fertilization. Soil Sci. Soc. Am. J. 61: 1672–1678.
26. **Parkinson, D. and Coleman, D.C. (1991).** Methods for assessing soil microbial populations, activity and biomass. Agric. Ecosyst. Environ. 34: 3-33.
27. **Piao, H.C., Liu, G.S., Wu, Y.Y. and Xu, W.B. (2001).** Relationships of soil microbial biomass carbon and organic carbon with environmental parameters in mountainous soils of southwest China. Biol. Fertil. Soils. 33: 347-350.
28. **Sicardi, M., García-Préchac, F., Frioni, L. (2004).** Soil microbial indicators sensitive to land use conversion from pastures to commercial Eucalyptus grandis (Hill ex Maiden) plantations in Uruguay. Appl Soil Ecol. 27: 125-133
29. **Simpson, J.R., Freney, J.R., Wetselaar, R., Muirhead, W.A., Leuning, R. and Denmead, O.T. (1984).** Transformations and losses of urea nitrogen after application to flooded rice. Aust J Agric Res. 35: 189-200.
30. **Steinberger, Y., Zelles, L., Bai, Q.Y., von Ltzow, M. and Munch, J.C. (1999).** Phospholipid fatty acid profiles as indicators for the microbial community structure in soils along a climatic

transect in the Judean Desert. Biol. Fertil. Soils. 28: 292-300.

31. **Tabatabai, M.A. (1977).** Effect of trace elements on urease activity in soils. Soil Biol Biochem 9: 9-13.
32. **Waldrop, M.P., Zak, D.R., Sinsabaugh, R.L., Gallo, M. and Lauber, C. (2004).** Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. Ecol Appl 14: 1172-1177.
33. **Wardle, D.A. (1992).** A comparative assessment of factors which influence microbial biomass carbon and nitrogen levels in soil. Biol. Rev. Cambridge Philos. Soc. 67: 321-358.
34. **Yu, W.T., Zhao, S.H., Zhang, L., Shen, S.M. and Ma, Q. (2005).** The relation between the content of organic phosphorus and latitude in Northeast China phaeozem. Biol. Fertil. Soils. 42: 159-162.
35. **Zhou, L.K. (1987).** The Science of Soil Enzyme. The Science Press, Beijing, China. pp 267-270.
36. **Walkley, A. and Black, I.A. (1934).** An examination of Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Sci. 37: 29-37.
37. **Keeny, D.R., and Bremer, J.M. (1966).** Chemical index of soil nitrogen availability. Nature. 211: 892-893.
38. **Bray, R. and Kurtz, L.T. (1966).** Determination of total, organic and available forms of phosphorus in soil. Soil Sci. 59: 39-45.
39. **Vishwakarma, K., Sharma, S., Kumar, N., Upadhyay, N., Devi, S., Tiwari, A. (2016).** Contribution of microbial inoculants to soil carbon sequestration and sustainable agriculture. In: Microbial Inoculants in Sustainable Agricultural Productivity. Springer, New Delhi. pp. 101-113