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Microbial biomass and enzyme activity in relation to shifting cultivation and horticultural practices in humid subtropical North-Eastern India

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Abstract

The present study conducted in the Bhandari or lower range of Wokha district of Nagaland in north-eastern India aimed at analyzing the impact of human activities such as shifting agriculture (*'Jhum'*) and horticultural practices on microbial biomass and Fluorescein diacetate hydrolysis (FDA) and dehydrogenase (DHA) activities in soil. Microbial biomass carbon (MBC) and nitrogen (MBN) as well as FDA and DHA activities were significantly greater in the soils of the undisturbed forest than the soils under various land-use practices. The MBC and MBN in the surface soil layer (0ï 25 cm) were highest (99.0 and 20.43 mg kg^{-1}, respectively) in the forest and lowest (21.89 and 6.25 mg kg^{-1}, respectively) in the 1year old jhum fallow, which was subjected to intense human activities. Similarly, FDA (1.33 mg fluorescein h^{-1} kg^{-1}) and DHA (80.62 mg TPF 24 h^{-1} kg^{-1}) were highest in the forest and lowest (0.67 mg fluorescein h^{-1} kg^{-1} and 41.55 mg TPF 24 h^{-1} kg^{-1}, respectively) in the 1 year old jhum fallow. Thus, human activities in the forest ecosystem were responsible for significant reduction in both microbial biomass and enzyme activities. Both these properties showed recovery during regrowth of vegetation on jhum fallows. Microbial biomass and enzyme activities declined with increasing soil depth in all the land-use. Pearson's correlation matrix revealed strongly significant positive correlation of microbial biomass and enzyme activities with selected soil properties. A highly significant positive relation between MBC and MBN was also observed.

Keywords: Dehydrogenase, Fluorescein diacetate, Human activities, Land-use, Microbial biomass, Soil properties

Abbreviations: DHA: Dehydrogenase; **FDA:** Fluorescein diacetate; **MBC:** Microbial biomass carbon; **MBN:** Microbial biomass nitrogen; **TN:** Total nitrogen

Introduction

In the north-eastern India, forest has been overexploited by clearance for agriculture. Shifting cultivation also known as *'Jhum'* cultivation have contributed significantly to the loss of forest in the region (Ramakrishnan, 1985). Shifting cultivation is the most traditional and dominant land-use in this region and on an average 3,869 km^2 area is put under cultivation every year. The destruction of the natural forest and its conversion to cropland can reduce precipitation or increase temperature, reduce soil productivity because of increased erosion, cause decline in fertility, change in soil flora or fauna, reduce soil organic matter and soil biological properties (soil microbial biomass and activity), which plays a crucial role in sustaining soil quality, crop production, and environmental quality (Ralte *et al.,* 2005; Kara and Bolat, 2007).

The soil microbial biomass acts as a labile reservoir of plant available nutrients (Jenkinson and Ladd, 1981). It constitutes a significant part of the potentially mineralizable-N and plays an important role in N cycling due to rapid turnover rate. Garcìa-Gil *et al.* (2000) concluded that microbial biomass is a much more sensitive indicator of changing soil conditions than the total organic matter content. In recent years, studies on microbial biomass and their activity have engaged the attention of many researchers. However, most of these studies are confined to agricultural soils and such studies are rather limited in forest ecosystem. Microbiological indicators have been used by research groups in numerous studies of soil restoration in forest ecosystem (Wang *et al.,* 2012; Bini *et al.,* 2013).

The enzyme activities have often been used as indices of microbial activity and soil fertility (Kennedy and Papendick, 1995). Human activities that minimize the organic matter content of the soil may reduce enzyme activities and could alter the availability of nutrients for plant uptake (Dick *et al.,* 1998). Dehydrogenases (DHA) are active in cells, and their relative activity levels are taken as an indicator of microbial activity (Casida *et al.,* 1964). Fluorescein diacetate (FDA) is hydrolyzed by a number of different enzymes, such as proteases, lipases and esterases to produce fluorescent compound fluorescein and provide comprehensive microbial activity (Lopes *et al.,* 2010; Nath *et al.,* 2011). Therefore, the study of soil microbial biomass and their activity is important for understanding early changes in biological quality of soil following changes in the land management (Palma *et al.,* 2000).

The objective of the present study was to analyze the effect of various human activities on soil biological properties of forest ecosystem in Wokha district, Nagaland, north-eastern India. The study sites that were exposed to different degrees of human activities, such as ‘Jhum’ (shifting cultivation or slash and burn agriculture) fallows of different ages undergoing natural recovery, arecanut plantation and pineapple cultivation as well as the undisturbed natural forest. In order to achieve the aforesaid objective, the soil microbial biomass carbon (MBC) and soil microbial biomass nitrogen (MBN), and soil enzyme [dehydrogenases (DHA) and Fluorescein diacetate (FDA)] activities were measured in the undisturbed natural forest and above mentioned communities that varied in the degree of disturbance and intensity of human intervention.

Materials and Methods

***Study site*:** The study was carried out in the Bhandari range or lower range (latitude 26° 27′N–26°30′N; longitude 94°10′E–94°15′E) situated in the north western side of Wokha district of Nagaland, north-eastern India. The altitude of the study site ranged between 304 and 438 m above mean sea level. The average annual rainfall ranges between 2000 and 2500 mm with maximum rainfall during July–September. The climate is moderately warm during summer but cold in winter. Mean monthly minimum and maximum temperatures were 7 °C and 36 °C, respectively.

Shifting agriculture is extensively practiced in this region. This agricultural practice involves slashing or complete clearing of the vegetation and burning of dried slash during dry winter, followed by pure and mixed cropping for 1–2 years depending upon the availability of land. Thereafter the field is abandoned for natural recovery of soil fertility. Since shifting agriculture is causing large–scale degradation of land, the government agencies have encouraged the establishment of plantation crops during the past 5–7 years, with twin objective of improving the economic condition of the people and checking further land degradation. In order to study the impact of these activities on soil, three abandoned jhum fields with 1, 3 and 6 years old vegetational regrowth, pineapple (*Ananas comosus*) and arecanut (*Areca catechu*) plantation and undisturbed natural forest land-uses were selected. For each, three replicate sites were identified for the sampling purpose.

***Soil sampling and analysis*:** Soil samples were collected from three representative sites of each of the six selected land-uses. Soil sampling was done up to a depth of 50 cm taking two soil layers (0–25 cm and 25–50 cm) following standard procedure. Soil samples were air dried and ground to pass through a 2 mm sieve. A combined glass–calomel electrode was used to determine the pH of aqueous suspensions (1:2.5 soil/solution ratio). Bulk density was determined by soil core method (Blake and Hartge, 1986). Moisture content was determined gravimetrically. Soil organic carbon (OC) was determined using the wet digestion method of Walkley and Black (1934). Available nitrogen (N) was measured by the alkaline permanganate method as described by Subbiah and Asija (1956). Total Kjeldahl nitrogen (TN) was determined by using Kjeltec Kel Plus–Supra LX (VA). Available phosphorus (P) was determined by the Bray II method (Bray and Kurtz 1945). Available micronutrient content [copper (Cu), manganese (Mn), iron (Fe), and zinc (Zn)] were determined by diethylene-triamine penta acetic acid (DTPA) extraction (Lindsay and Norvell, 1978), followed by atomic absorption spectrophotometry.

Microbial biomass carbon (MBC) determinations were made by using chloroform fumigation technique as described by Jenkinson and Powlson (1976) and Jenkinson and Ladd (1981). Microbial biomass nitrogen (MBN) determination was made by using the standard method (Brookes *et al.,* 1985). The dehydrogenase activity (DHA) was measured by using triphenyl tetrazolium chloride (Tabatabai, 1982), which was reduced to triphenyl formazan. Fluorescein diacetate (FDA) hydrolysis activities were carried out following the method described by Adam and Duncan (2001). Soil was incubated with the substrate, FDA, at 25 °C for 1hr. The amount of fluorescein formed was determined colorimetrically (Nano Drop 1000 spectrophotometer) following extraction with an organic solvent mixture (2:1 chloroform: methanol).

***Statistical analysis*:** All the statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, Ill.). One-way analysis of variance was performed to test the effect of land-uses on each soil properties. Fisher's least significant difference (LSD) *t*-test was performed to examine the significant differences between means. The relationship between soil biological properties and selected soil properties were determined by Pearson's correlation matrix.

Results and Discussion

***Soil properties*: ** The soil was silt–clay–loam in the undisturbed natural forest, silt–loam to loam in the jhum fallows, clay–loam in the pineapple and silt–loam in the arecanut (Table 1). The bulk density of soil ranged from 1.3 g cm^{-3} in the surface soil layer (0–25 cm depth) of the 3 years old jhum to 1.8 g cm^{-3} in the sub-surface soil layer (25–50 cm depth) of pineapple cultivation. However, it did not vary significantly (P < 0.05) between the land-uses except 3 years old jhum fallow and pineapple cultivation in the surface soil layer. In the sub-surface soil layer it varied significantly (P < 0.05) between the primary forest, 6 years old jhum fallow and 3 years old jhum fallow. The soil moisture content of soil ranged from 28.1% in the surface soil layer 3 years old jhum fallow to 39.1% in the sub-surface soil layer of pineapple cultivation. However, it did not vary significantly (P < 0.05) between the 1 year old jhum fallow and arecanut plantation in the surface as well as sub-surface soil layers.

Soil pH showed significant influence of land-uses (Table 2). The soil was acidic in all land-uses with pH ranging between 5.0 and 5.7; the 3 and 6 years old jhum soils were acidic (pH 5.0). The pH decreased with the increase in soil depth. The forest showed greater pH than the arable land, which could be attributed to the release of bases and their deposition over a long period of tree growth. Earlier results also revealed that trees have the capacity to moderate the effects of leaching by contributing bases to the soil (Panwar *et al.* 2011). The OC content was greater in forest (2.34%) followed by arecanut plantation (1.95%). The least value for OC (0.62%) was found in deeper soil layers for all the land-uses. As a general trend, the OC decreased with increase in the depth of soil layer.

Available N and P contents were significantly influenced by land-uses at different soil depths (Table 2). Available N was greatest (161.1 mg kg^{-1}) in forest and least (117.9mg kg^{-1}) in the 1 year old jhum fallow. Available N content tended to decrease with increasing soil depth, and N buildup in forest and horticultural land-uses over jhum fallow is attributed to more accumulation of biomass through litter fall and root biomass (Ralte *et al.,* 2005). The greatest P content was 8.6 mg kg^{-1} in the surface layer of forest and the least was 1.2mg kg^{-1} in the 1 year old jhum at a depth of 25–50 cm. On average, forest had the greatest P content and jhum fallows the least. In forest and horticultural land-uses, the greater P content could be due to recycling of P through uptake by the tree species and subsequently recycling by way of surface litter fall. Ralte *et al.* (2005) and Bruun *et al.* (2006) had also reported greater P in forest than jhum fallows. TN declined from the forest to jhum fallows and also surface to sub-surface soils. The decline was most prominant in case of 1 year jhum fallow soil followed by the 3 and 6 years jhum fallows. However, with increase in fallow length TN was increased. Several other studies had also reported that TN content in jhum soils increases with increase in fallow length (Bruun *et al.,* 2006).

***Soil available micronutrients*: ** The effects of land-use on micronutrients such as Cu, Zn, Mn, and Fe were studied in two different soil layers (Table 3). There was a significant effect of different land-use at different soil layer on micronutrient content. The content of Zn was greatest (2.0 mg kg^{-1}) in forest and least (0.4 mg kg^{-1}) in 1 year jhum fallow. Cu content varied from 1.4 mg kg^{-1} in the surface soil layer of arecanut to 0.3 mg kg^{1} in the 25–50 cm soil layer in 6 years jhum fallow. Mn content was greatest (37.6 mg kg^{-1}) in the surface soil layer of arecanut and least (9.1 mg kg^{-1}) in the sub-surface soil layer of forest. Available Fe varied from 85.6 mg kg^{-1} in the surface soil of forest to 42.2 mg kg^{-1} in the sub-surface soil layer of 6 years jhum fallow. The micronutrients did not show any consistent trend both for land use or soil layer. The inconsistency of micronutrients, particularly Cu and Zn, with respect to soil depth had also been reported by Sharma *et al.* (2009).

***Soil microbial biomass carbon (MBC) and nitrogen (MBN)*: ** MBC and MBN varied significantly (P < 0.01) among the land-uses at different soil depths. The MBC and MBN were greatest under surface soil of forest (99.00 mg kg^{-1} and 20.43 mg kg^{-1}, respectively) and least in the sub-surface soil layer under 1 year jhum fallow (21.89 mg kg^{-1} and 6.25 mg kg^{-1}, respectively) (Table 4). The MBC and MBN in the jhum fallows gradually increased with the age of the fallow. Their values declined significantly (P < 0.01) from the 6 to 1 year old jhum fallow. The high concentration of detrital material in the surface soil layer (0–25 cm) in the subtropical forest increases the availability of soil organic matter in the surface layer due to fast turnover rates of litter and fine roots (John *et al.,* 2002). The chief contributory factor for the higher MBC and MBN in the forest soil than the jhum fallows seems to be the greater availability of organic nutrients in the forest due to higher plant cover (Ralte *et al.,* 2005).

Table 1. Effect of land-use on physical properties of soil

Soil physical properties	Depth (cm)	Natural forest	Jhum fallow			Horticultural system	
			6 years old	3 years old	1 year old	Pineapple	Arecanut
Texture	0–25	sicl	l	l	sil	cl	sil
	25–50	sicl	l	l	sil	cl	sil
Bulk density (g cm^{-3})	0–25	1.6±0.06	1.6±0.03	1.3±0.03	1.5±0.01	1.8±0.06	1.6±0.06
	25–50	1.7±0.07	1.5±0.06	1.3±0.03	1.7±0.03	1.8±0.05	1.7±0.03
SMC (%)	0–25	36.2±6.1	30.5±4.1	28.1±2.4	29.9±2.1	38.2±1.9	30.2±3.4
	25–50	36.8±2.4	30.9±2.5	28.6±1.6	29.3±2.2	39.1±1.4	31.1±1.3

Notes. sicl, silty clay loam; sil, silty loam; cl, clay loam; l, loam; SMC, soil moisture content. Values are means (±S.E.)

Table 2. Effect of land-use on chemical properties of soil

Soil physical properties	Depth (cm)	Natural forest	Jhum fallow			Horticultural system	
			6 years old	3 years old	1 year old	Pineapple	Arecanut
pH	0–25	5.6±0.1	5.2±0.2	5.1±0.09	5.1±0.01	5.3±0.09	5.7±0.2
	25–50	5.5±0.1	5.1±0.1	5.0±0.09	5.0±0.07	5.0±0.03	5.5±0.2
Organic carbon (%)	0–25	2.34±0.01	1.39±0.08	1.12±0.1	0.77±0.1	1.61±0.1	1.95±0.06
	25–50	1.60±0.4	0.95±0.09	0.68±0.1	0.62±0.1	1.22±0.09	1.01±0.05
Available nitrogen (mg kg^{-1})	0–25	161.1±1.9	144.7±1.7	134.1±1.2	117.9±2.0	148.8±5.5	158.0±4.8
	25–50	147.3±6.2	134.1±0.5	122.8±1.8	113.0±4.7	126.2±4.6	126.2±2.9
Available phosphorus (mg kg^{-1})	0–25	8.6±0.1	3.6±0.1	3.0±0.2	2.0±0.4	7.6±0.3	7.8±0.3
	25–50	8.2±0.1	3.1±0.3	2.2±0.4	1.2±0.2	7.1±0.8	7.1±0.2
Total nitrogen (%)	0–25	0.80±0.08	0.41±0.01	0.37±0.02	0.30±0.006	0.51±0.01	0.53±0.01
	25–50	0.70±0.07	0.38±0.02	0.34±0.02	0.26±0.01	0.46±0.02	0.46±0.01

Table 3. Effect of land-use on available micronutrients

Available micronutrients (mg kg^{-1})	Depth (cm)	Natural forest	Jhum fallow			Horticultural system	
			6 years old	3 years old	1 year old	Pineapple	Arecanut
Zinc	0–25	2.0±0.9	0.6±0.03	0.5±0.03	0.9±0.2	0.9±0.2	0.9±0.06
	25–50	1.0±0.3	0.4±0.04	0.8±0.3	0.4±0.05	0.5±0.03	0.9±0.2
Copper	0–25	0.5±0.09	0.3±0.03	0.5±0.03	0.5±0.03	0.8±0.1	1.4±0.5
	25–50	0.4±0.1	0.3±0.04	0.6±0.01	0.4±0.01	0.8±0.2	1.0±0.2
Manganese	0–25	14.7±3.0	28.2±1.8	44.6±6.1	23.2±5.0	16.5±5.1	37.6±3.3
	25–50	9.1±4.2	25.3±5.8	47.9±4.4	16.2±2.0	7.1±1.4	31.2±4.9
Iron	0–25	85.6±4.2	57.0±5.0	53.2±3.7	72.7±1.0	75.9±2.7	64.8±8.7
	25–50	72.4±9.4	42.2±0.3	52.7±1.7	53.9±2.1	61.7±4.9	74.5±5.7

Table 4. Effect of land-use on soil biological properties

Soil biological properties	Depth (cm)	Natural forest	Jhum fallow			Horticultural system	
			6 years old	3 years old	1 year old	Pineapple	Arecanut
Microbial biomass	0–25	99.00±0.25	67.84±3.58	53.45±4.12	34.91±1.27	82.26±4.08	74.03±0.03
carbon (mg kg^{-1} soil)	25–50	49.64±25.12	28.80±8.21	32.89±10.89	21.89±3.11	36.91±7.03	37.04±14.25
Microbial biomass	0–25	20.43±2.63	15.53±3.73	12.95±3.28	12.31±3.08	15.62±1.46	15.70±2.57
nitrogen (mg kg^{-1} soil)	25–50	9.82±4.30	9.37±1.69	9.56±3.96	6.25±2.06	10.53±2.37	9.53±3.61
Fluorescein diacetate	0–25	1.33±0.03	1.20±0.01	1.15±0.03	1.03±0.03	1.26±0.01	1.19±0.01
(mg fluorescein h^{-1} kg^{-1} soil)	25–50	1.03±0.19	0.82±0.14	0.71±0.02	0.67±0.05	0.94±0.11	0.76±0.18
Dehydrogenase activity	0–25	80.62±1.01	57.68±1.44	54.46±0.50	43.70±3.31	67.99±0.70	60.31±1.37
(mg TPF 24 h^{-1} kg^{-1})	25–50	77.40±2.35	48.52±3.86	50.76±0.26	41.55±3.52	65.77±1.46	56.70±1.95

Notes. Values are means (±S.E.)

Table 5. Pearson correlation coefficients between soil biological properties and selected soil properties

	pH	OC	N	P	TN	MBC	MBN	FDA	DHA
pH	1.000								
OC	0.670**	1.000							
N	0.591**	0.921**	1.000						
P	0.681**	0.754**	0.719**	1.000					
TN	0.592**	0.835**	0.795**	0.849**	1.000				
MBC	0.417*	0.799**	0.791**	0.569**	0.672**	1.000			
MBN	0.303	0.554**	0.589**	0.348*	0.428**	0.772**	1.00		
FDA	0.391*	0.708**	0.673**	0.469**	0.528**	0.745**	0.534**	1.000	
DHA	0.553**	0.765**	0.697**	0.848**	0.875**	0.625**	0.387*	0.590**	1.000

**Significant at the 0.01 level; *Significant at the 0.05 level

***Fluorescein diacetate (FDA) hydrolysis and dehydrogenase (DHA) activities*:** FDA hydrolysis has been suggested as a measure of the global hydrolytic capacity of soils and a broad spectrum indicator of soil biological activity. The FDA was greatest under surface soil of forest (1.33 mg fluorescein h^{-1} kg^{-1}) and least in the sub-surface soil layer under 1 year jhum fallow (0.67 mg fluorescein h^{-1} kg^{-1}) (Table 4). DHA varied significantly ($P < 0.01$) between the land-use. The forest soil recorded the greatest activity mainly due to high decomposition of residue. In the case of jhum fallows, it increased from the 1 to 6 years old fallows. The soil in arecanut and pineapple had greater activity than the jhum fallows. The activity declined from the surface to the sub-surface soil layer regardless of the land-uses. Since microorganisms are mostly confined to the surface soil layer owing to better aeration and greater nutrient availability, FDA and DHA activities were greater in the surface soil layer (0–25 cm) compared to the sub-surface (25–50 cm) soil layer where the organic matter content and nutrient availability was low and aeration was poor. The FDA and DHA activities were also dependent on the amount of organic carbon in soil as is evident from the Table 2. Besides organic carbon, soil nutrients are the most important factor likely to regulate microbial activity (Tiwari *et al.,* 2002). Repeated cycles of slash and burn agriculture on hill slopes coupled with high rainfall causing loss of soluble organic matter and nutrients of surface soil layer through overland flow of rainwater appear to be responsible for low enzyme activity in the jhum fallows (Ralte *et al.,* 2005). On the other hand, the low input of detrital material and nutrients through litter fall in the orchards resulted in the decreased enzyme activity in the pineapple and arecanut orchards.

***Correlation between soil biological properties and selected soil properties*:** Pearson's correlation matrix (Table 5) revealed strong significant positive correlations between MBC and OC ($r = 0.799$, $P < 0.01$), MBC and N ($r = 0.791$, $P < 0.01$), MBC and P ($r = 0.569$, $P < 0.01$) and MBC and TN ($r = 0.672$, $P < 0.01$). Reza *et al.,* (2011) also observed a close relationship between OC and microbial biomass. A highly significant positive relation between MBC and MBN ($r = 0.772$, $P < 0.01$) shows an intimate link between changes of N in soil and heterotrophic microbes, which use organic C as their energy source (Arunachalam and Pandey, 2003). FDA and DHA were also dependent on the amount of OC in the soil as it evident from a highly significant positive correlation ($r = 0.708$, $P < 0.01$and $r = 0.765$, $P < 0.01$, respectively). The strong positive correlations between MBN and enzymes activity (FDA $r = 0.534$, $P < 0.01$; DHA $r = 0.387$, $P < 0.05$) clearly suggest that MBN could serve as an indicator of the activities of these enzymes in soil.

Conclusions

It is evident from the study that forest soil having higher OC content, available nutrients and microbial activities over the arable land. Further, human activities such as shifting agriculture and horticultural practices in the hilly region with high rainfall cause depletion of the MBC and MBN, and reduction in their activities in soil. Several chemical properties of soil (pH, OC, TN) also affected MBC, MBN and enzyme activities. As the vegetation regrows after abandonment of cultivation on jhum land, MBC, MBN and enzyme activity gradually increased with age of the secondary successional communities on the jhum fallows.

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