Estimation of soil enzyme activity with respect to decomposition of leaf litter types

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ABSTRACT

The measurement of enzyme activities give an index of the extent of specific biochemical processes in soil and in many situations acts as an indicator of soil fertility. Historical evidences show that this area was once a thick forest but widespread forest denudation by human settlements form early nineteenth century had converted the entire area barren up to the line of horizon. The objective of the present study was to evaluate the effect of litter types of the above-mentioned four tree species on edaphic properties of tropical deciduous afforested ecosystem in Srisailam reserve forest. The present climatic record of the local meteorological station shows average monthly temperature of 26.10C in 2010 (range 10.90C in January to 38.40 C in May), 26.40C in 2011 (range 12.40C in January to 37.70C in June), total annual rainfall of 1516.7 mm in 2010 and 1381.5 mm in 2011, and mean monthly relative humidity 78.1% in 2010 and 77.8% in 2011. The changes in enzyme activities were clearly evident with respect to leaf litter types and between winter, summer and rainy Seasons. Among different enzymes the activities of invertase, protease and acid phosphatase were higher than that of amylase, cellulase and dehydrogenase in all the sites. The enzyme activity of all the sites, except Shorea showed equally high levels during rainy season.

Keywords: Srisailam, Invertase, Amylase, Cellulase, Enzyme activity.

INTRODUCTION

Nutrient mineralization from fresh plant litter occurs via the enzymatic activities of the microbial communities that become established on the litter surfaces. Soil enzyme activities are the principal "sensors" since they integrate information, on the one hand, from microbial status, and on the other hand, from soil physico-chemical conditions. Plants and Micro-organisms like bacteria and fungi are main producers of the soil enzymes. Some enzyme functions are associated with the microbes themselves, such as dehydrogenase activity, which are mainly localized in the plasma membrane of bacteria or in mitochondrial membranes of fungi. Other enzymes are synthesized and secreted extracellularly by bacteria or fungi (phosphatases, urease, cellulases, pectinases). Microbially secreted enzymes may take part of the soil matrix as extra cellular enzymes, also called abiotic (Dick, 1997; Sinsabaugh, 1994). Production of soil enzymes as a result of microbial metabolism is a sensitive indicator of soil microbial activity, thus factors influencing the latter will certainly exert control over the former. The soil enzymes are representatives of main nutrient cycles (C, N, P) and of microbial biomass. β-Glucosidase is one of the three or more enzymes

involved in the saccharification of cellulose (Tabatabai, 1994). Glucosidases are widely distributed in nature and are found in microorganisms, animals and plants (Eivazi and Tabatabai, 1988; Dick et al., 1996). Their activity reflects soil management effects (Bandick and Dick, 1999). Phosphatases are involved in the transformation of organic and inorganic phosphorus compounds in soil (Spiers and McGill, 1979; Amador et al., 1997).

The cascade of enzyme activities approach has been considered by Nannipieri et al., (2002) as one of the best among those using biochemical properties as indicators of soil quality due to its accurate and focused selection of enzyme activities. The dehydrogenase activity of the soil is considered to be an indicator of the microbial redox system and the oxidative activities of the soil (Trevors, 1984). Like microbial biomass C, this property has been used mainly to assess the influence of management on soil quality and contradictory results have also been obtained. For example, ploughing can both increase and decrease dehydrogenase activity (Bergstrom et al., 1998, 2000) whilst the addition of organic fertilizers, landiff effluents and industrial waste generally increases dehydrogenease activity (Bardgett et al., 1995; Pascual et al., 1999; Langer and Gunther, 2001). In general, this activity is not affected by the presence of heavy metals, unless in very high does (Kandeler et al., 1996; Filip, 2002). Dehydrogenase activity has also been used to evaluate the degree of recovery of degraded soils, being considered as a good indicator, even in soils that have been contaminated by petroleum spillage (Margesin et al., 2000).

Decomposotion is mediated by microorganisms and extracellular enzymes that are constrained by the interaction between climatic conditions and biochemical phenomena (e.g., soil temperature × plant productivity), that influence and are influenced by the supply of accessible nutrients such as nitrogen and phosphorous (Sinsabaugh, 1994; Shackle et al., 2000). Numerous results from field and laboratory experiments confirm that soil decomposition processes increase with physical changes in temperature and aeration (Peterjohn et al., 1994; Katterer et al., 1998). The microbial response to seasonal soil temperature variation may be limited by other chemical and biological factors. The activity of microbially derived extracellular hydrolytic and oxidative enzymes that are responsible for the conversion of organic matter from high to low molecular weight compounds is principally regulated by temperature, hydrology, nutrient availability and vegetation type (Insam, 1990). In particular, the interaction between physicochemical factors such as temperature, changing pH, redox potential, inhibitors (e.g. trace metal ions, phenolics) and activators (e.g) have been proven to be important (Eivazi and

Tabatabai, 1990; Freeman et al., 1996, 2001). A number of studies have shown that remporal variations in soil DOC concentrations are closely linked with climatic seasonality, biological productivity and soil chemistry (Bonnett, 2006).

As soil organic matter accumulates, ecosystem productivity becomes increasingly dependent on mineralization by the microbial community (Parfitt et al., 2005). The rate-limiting step in mineralization is widely believed to be degradation by microbially produced extra-cellular enzymes. (Sinsabaugh et al., 1993; Waldrop and Firestone, 2004), although substrate solubility is also likely to be an important factor (Condron and Tiessen, 2005). At a cellular level, enzyme production is subject to end-product inhibition and repression: as availability of inorganic or organic forms of a nutrient increases, production of the enzyme required for catalysis of that nutrient is down regulated. Although production of enzymes may also be induced by the presence of a substrate, the intermediate and end products of hydrolysis repress activity. Thus induction will not occur if end products are present, leading to an overall negative relationship between nutrient concentrations and enzyme production. High enzyme activity indicates nutrient limitation (Sinsabaugh et al., 1993), and a pattern of increasing enzyme activity with decreasing nutrient availability is sometimes found in soil. For example, phosphatase activity commonly increases as P declines (McGill and Cole, 1981; Allison and Vitousek, 2005), and activity of the N-releasing enzyme chitinase increases as N declines (Olander and Vitousek, 2005). However, negative relationships between nutrient availability and enzyme activity have not been consistently demonstrated.

While total enzyme activity is an important determinant of decomposition rate, controls on enzyme production occur at the level of the cell. Thus, controls on enzyme activity must be assessed on a per microbial biomass basis (Hassett and Zak, 2005) if microbial biomass changes are occurring simultaneously with shifts in enzyme activity. When assessed as efficiency (activity per unit microbial biomass), there are strong patterns of increasing efficiency of P-, and decreasing efficiency of C-, N- and S-hydrolyzing enzymes with site age (Allison et al., 2007). The increase in P-hydrolyzing enzyme efficiency is strongly negatively correlated with P concentrations in both the organic and mineral horizons, while efficiencies of C-, N- and S-hydrolyzing enzymes are negatively correlated with C, N and S concentrations in mineral soil (Allison et al., 2007). Although addition of carbon can in some cases induce enzyme production (a positive relationship between C and C-hydrolyzing enzymes) (Fonaine et al., 2003), this is likely to be due to a stimulation of microbial biomass, or is a transient effect, which does not occur in equilibrium systems due to the accumulation of intermediates. The

negative correlation between enzyme efficiency and nutrient availability suggests either that down-regulation of enzymes occurs when nutrient availability is high, or that the composition of the community has shifted such that the community has inherently lower levels of enzymes for C-, N- and S-hydrolyzing enzymes in late successional sites.

Among the hydrolases, acid phosphor-mono-esterase activity has been most frequently used for estimating changes in soil quality due to either management or the presence of contaminants. It is a good index of the quality and quantity of organic matter in the soil (Jordan et al., 1995; Mullen et al., 1998; Bergstrom et al., 2000) and can be very high in arable soils as long as the levels of organic matter in the soil are maintained (Dick et al., 1994). For this reason, if the organic matter content of degraded soils increases during recovery, the level of this enzyme activity also rises (Gil-Sotres et al., 1992; Garcia et al., 1997a; Vance and Entry, 2000). Additionally, in soils affected by forest fires, phosphomonoesterase activity is clearly influenced by fire intensity (Staddon et al., 1998) and increases after the fire in parallel with the soil recovery (Saa et al., 1993). Different studies have shown that this enzymatic activity increases as a consequence of organic fertilization (Pascual et al., 1999; Chakrabarti et al., 2000). phosphomonoesterase activity is significantly decreased by the presence of lead (Marzadori et al., 1996) and other heavy metals (Kandeler et al., 1996), and the presence of pesticides in the soils only decreases it temporarily (Schaffer, 1993).

Some litter types that decayed faster under fertilization also showed time-dependent increases in carbon-degrading enzyme activities, but other decayed faster independent of enzyme changes. These results suggest that extracellular enzyme activities partially determine litter decomposition rates, but high soluble carbon content may circumvent the requirement for enzyme-catalyzed decomposition. In arid and semiarid ecosystems, where variation in the spatial and temporal availability of water and nutrients is extreme, dominant plants cause changes in soil properties that lead to complex local interactions between vegetation and soil (Wilson and Agnew, 1992). Degradation of the soils from the loss of plant cover, leads to increased erosion and salinization. The presence of vegetation is important since it provides physical protection and contributes organic matter that enhances soil water holding capacity (WHC) and soil fertility characteristics (Garcia et al., 1994). These plants also affect the composition of the soil microbial community. The objective of the present study was to evaluate the effect of litter types of the above-mentioned four tree species on edaphic properties of tropical deciduous afforested ecosystem in Srisailam reserve forest.

Materials and Methods

Site Description:

The site of present investigation is a deciduous tropical forest known as the Srisailam reserve forest, situated in between 23029' to 23045' south latitude and 87035' to 87045' East longitude. It is a part of Mahabubnagar district under the province of Andhra Pradesh in the Southern region of India. Historical evidences show that this area was once a thick forest but widespread forest denudation by human settlements form early nineteenth century had converted the entire area barren up to the line of horizon. These vast eroded wastelands bear an undulating and barren desert like landscape. However, large scale afforestation programmers undertaken in this area during the past 50 years by the Forest Department, Local Government Bodies, and Institutions and Organizations using several native and exotic tree species could bring about some notable improvements in the soil and floral characteristics. In general, the soil of eroded laterite wastelands is yellowish red to brick red in colour, sandy loam in texture, has poor water holding capacity and low electrical conductivity, acidic pH and very poor organic carbon and phosphate status, below average nitrogen content and rich amount of oxides of aluminum and iron (Bhattacharya, 1979). Joy (1983) has noticed that the nature of soil is influenced greatly by the afforestation and agricultural practices with the pH varying between 5.38 and 7.51 and organic carbon content ranging from 0.14% to 0.61% in different local ecosystems. Ray (1986) recorded notable improvements in the soil characteristics (pH 7.5, organic carbon 1.38%) in the local Acacia auriculiformis forest area. A recently conducted comparative study of soil in different natural forests of Mahabubnagar district has recorded slightly acidic to alkaline pH condition (6.11 – 7.75), moderate electrical conductivity (0.43 – 0.68 mmoh), high organic carbon (1.2 – 1.4%), and nitrate nitrogen (47.7 – 69.1 ppm) contents. Bhattacharya (1974) designated the local climate as "dry sub-humid mega-thermal" following the climatic nomenclature of Thornthwaite (1948). However, with the advancement of forest cover and human activities during the past few decades the local climatic conditions have undergone notable improvement.

The present climatic record of the local meteorological station shows average monthly temperature of 26.1^0C in 2010 (range 10.9^0C in January to 38.4^0 C in May), 26.40C in 2011 (range 12.4^0C in January to 37.7^0C in June), total annual rainfall of 1516.7 mm in 2010 and 1381.5 mm in 2011, and mean monthly relative humidity 78.1% in 2010 and 77.8% in 2011. This climatic improvement could result in higher moisture content of the soil and enhanced litter breakdown under forest conditions

and canopy base of trees. The Srisailam reserve forest has very rich in floral diversity with a dense vegetation of trees as well as well-developed under story consisting of shrubs, herbs, grasses and ferns. Afforestation started in this area during 1960's with monoculture stands of trees like Cassia siamea, Shorea robusta, Eucalyptus citriodora, Acacia auriculiformis, Anacardium occidentale, Dalbergia sissoo, etc, and a portion of the forest is now earmarked as a deer sanctuary. However, the ecological suitability of even the most commonly used tree species in restoring the lost properties and in enhancing nutrient status and biodiversity of soil remains underestimated. This background knowledge has led to the formulation of specific objectives of the present study to undertake an ecologically oriented evaluation. Emphasis has been given on the restoration of nutrient status, biological activity and biodiversity in the soil of different afforested monoculture stands, which are potential indices for evaluation of the success of ecofriendly activities such as afforestation for the conservation of ecological equilibrium and functional diversity of soil sub-system.

Results & Discussion

Soil enzyme activity:

Soil enzymes catalyze the reactions that are necessary for organic matter decomposition and the enzyme activities are often used as indices of microbial activity and soil fertility. The objective of the present study was to evaluate the effect of litter types of the above-mentioned four tree species on edaphic properties of tropical deciduous afforested ecosystem in Srisailam reserve forest. A detailed consideration of soil enzyme activity of four tree stands at three different seasons during the year 2011 is given in Table–1.

Table–1 represents a comparison of the changes in soil enzymatic activity in different forest stands during major seasons of the year. The changes in enzyme activities were clearly evident with respect to leaf litter types and between winter, summer and rainy Seasons. Among different enzymes the activities of invertase, protease and acid phosphatase were higher than that of amylase, cellulase and dehydrogenase in all the sites. All the enzymes except invertase registered higher activity values in the soil of Cassia siamea and Dalbergia sissoo trees when compared to the patterns of enzyme activities in the soil of Shorea robusta and Acacia auriculiformis stands. Regarding seasons the amylase and cellulase activities showed progressive increase from winter to summer with highest values during rainy season in all the four study sites. On the other hand, invertase and protease activity showed a reverse trend with lower values during the rainy season. Dehydrogenase activity was very negligible

in the soils of Shorea and Acacia trees, but in the soil of Dalbergia the activity recorded more or less uniformly high values when compared to higher activity only during the winter season in Cassia stand. The acid phosphatase activity in the soil of all the 4 tree species fluctuated without any significant variation between seasons.

Figure–1 demonstrates changes in the amylase activity of soil of Cassia, Shorea, Acacia, and Dalbergia forest sites at three major seasons of the year namely winter, summer and rainy. A clear linear increase in the enzyme activity could be observed in all the sites with lowest value in winter and highest activity during rainy season. The soil of Dalbergia stand had greater activity than Cassia and Acacia. Shorea stand showed lowest activity and increased only during rainy season. The analysis of Two-way ANOVA indicated that amylase activity in soil varied significantly between seasons as well as between the four types of tree stands. Highest F value between intervals indicates the importance of seasons in controlling the soil enzyme activity.

Figure–2 depicts the changes in cellulase activity of soil in different forest sites during winter, summer, and rainy seasons. Cellulase activity was negligible during winter and summer seasons but increased during rainy season due to high rate of decomposition. Among the sites the activity was more in Dalbergia followed by Cassia and Shorea. In case of Acacia stand the cellulase activity did not increase appreciably throughout the year. The difference in cellulase activity between the litter types and between major seasons were statistically highly significant. Here also the higher F value between intervals depicts the seasonal impact on soil enzyme activity.

The trend of variations of invertase activity in the soil of Cassia, Shorea, Acacia and Dalbergia tree stands in three different seasons is elaborated in Figure–3. In contrast to amylase and cellulase activities, the invertase activity was very high and maintained more or less steady level in all the 4 forest sites irrespective of the seasons of the year. Similarly, the invertase activity did not differ so much among the tree species although highest activity was recorded from Dalbergia followed by Shorea, Cassia and Acacia in the descending order. The analysis of 2-Way ANOVA showed that the variation in the invertase activity of soil between tree species and between three seasons of the year were statistically highly significant. Higher F value between the litter types is an indication of the effect of litter sources in regulating the invertase activity.

Figure–4 incorporates a comparison of protease activity in the soil of Cassia, Shorea, Acacia, and Dalbergia forest stands in 3 consecutive seasons. In contrast to the trend of carbohydrases mentioned above, the protease activity was high in the soil of

Table – 1: Seasonal variations in soil enzyme activity at four different forest tree stands.

| Enzyme activity	Seasons	Types of forest stands			
		Cassia	Shorea	Acacia	Dalbergia
Amylase (µg glucose/g dry soil/24 hr)	Winter	15.39 ± 0.89	5.48 ± 0.65	8.66 ± 0.61	22.82 ± 0.84
	summer	29.29 ± 0.84	13.92 ± 0.85	24.76 ± 0.55	47.37 ± 0.84
	Rainy	39.22 ± 1.61	37.04 ± 1.33	28.4 ± 0.62	49.98 ± 2.92
	Average	**27.97**	**18.82**	**20.61**	**40.04**
Cellulase (µg glucose/g dry soil/24 hr)	Winter	8.66 ± 0.49	2.61 ± 0.68	14.98 ± 0.67	3.48 ± 0.92
	summer	41.68 ± 1.96	29.0 ± 1.24	11.05 ± 1.01	46.46 ± 1.29
	Rainy	192.0 ± 1.43	161.8 ± 0.82	73.61 ± 3.06	259.6 ± 2.94
	Average	**80.79**	**64.48**	**33.21**	**103.18**
Invertase (µg glucose/g dry soil/24 hr)	Winter	912.3 ± 80.7	1003.2 ± 4.5	715.34 ± 50.1	1564.1 ± 37.7
	Summer	1219.8 ± 9.9	956.6 ± 11.9	588.9 ± 9.54	1055.9 ± 11.57
	Rainy	709.8 ± 8.58	835.5 ± 12.3	773.5 ± 5.38	903.95 ± 18.6
	Average	**947.3**	**931.77**	**692.57**	**1174.65**
Protease (µg glucose/g dry soil/24hr)	Winter	235.36 ± 4.27	124.4 ± 6.64	117.68 ± 0.56	289.14 ± 2.93
	summer	278.53 ± 2.84	114.33 ± 2.96	137.54 ± 5.61	206.35 ± 7.88
	Rainy	153.76 ± 1.48	128.93 ± 4.16	143.93 ± 2.19	171.16 ± 9.49
	Average	**222.55**	**122.58**	**133.05**	**222.22**
Dehydrogenase (µg TPF/g dry soil/24hr)	Winter	65.23 ± 3.73	3.69 ± 0.23	4.3 ± 0.3	66.3 ± 1.65
	summer	17.26 ± 0.78	0.625 ± 0.08	2.87 ± 0.22	38.94 ± 3.98
	Rainy	19.19 ± 0.55	9.12 ± 0.58	3.76 ± 0.23	49.62 ± 3.09
	Average	**33.89**	**4.48**	**3.64**	**51.61**
Acid phosphatase (µg PNP/g dry soil/24hr)	Winter	168.46 ± 2.19	98.69 ± 6.94	100.76 ± 3.2	179.01 ± 0.8
	summer	171.49 ± 2.33	50.57 ± 2.46	99.19 ± 3.13	188.14 ± 0.7
	Rainy	180.8 ± 2	50.24 ± 1.13	164.45 ± 2.18	201.33 ± 1.76
	Average	**173.59**	**66.5**	**121.47**	**189.49**

Cassia and Dalbergia particularly during the winter and summer seasons and decreased in the rainy season. Protease activity of Shorea and Acacia litter maintained almost similar low level during the course of study. It appears that litter nutrients during the initial stage of decomposition could have favored higher protease activity in the soil of Cassia and Dalbergia forests. Here also the differences in enzyme activity with respect to different forest stands and different seasons were statistically significant. High F value between litter types confirms the role of litter nutrients on protease activity.

The classic example of variation of soil enzyme activity with respect to litter types and seasons is demonstrated in Figure–5, which clearly shows negligible dehydrogenase activity in the soil of Shorea and Acacia stands as against very prominent values in Cassia and Dalbergia litter. In both Cassia and Dalbergia the dehydrogenase activity was uniformly high during winter and then decreased during summer and rainy seasons particularly in the former. On the other hand, the dehydrogenase activity in the soil of Dalbergia forest showed high

levels in 3 consecutive seasons.In Shorea the activity was extremely low during summer whereas Acacia maintained more or less uniform low dehydrogenase activity in all the seasons. Summary of 2- way ANOVA confirmed that the differences in dehydrogenase activity with respect to plant type and seasonal intervals were statistically significant. This was further evident from the higher F values between forest litter types.

Figure–6 represents a comparative idea of the changes in acid phosphatase activity in Cassia. Shorea, Acacia, and Dalbergia forest sites during winter, summer and rainy seasons. The activity was uniformly high in the soil of Dalbergia and Cassia trees measured in all three seasons. A gradual but prominent increasing trend was observed in Acacia stand to reach highest level during rainy season, but in the case of Shorea, the acid phosphatase activity decreased during summer and rainy seasons. The enzyme activity of all the sites, except Shorea showed equally high levels during rainy season. Analysis of 2-way ANOVA showed highly significant

differences in acid phosphatase activity between tree   litter types and between the seasons.

Figure-1. Seasonal changes of amylase activity in soil of different forest sites
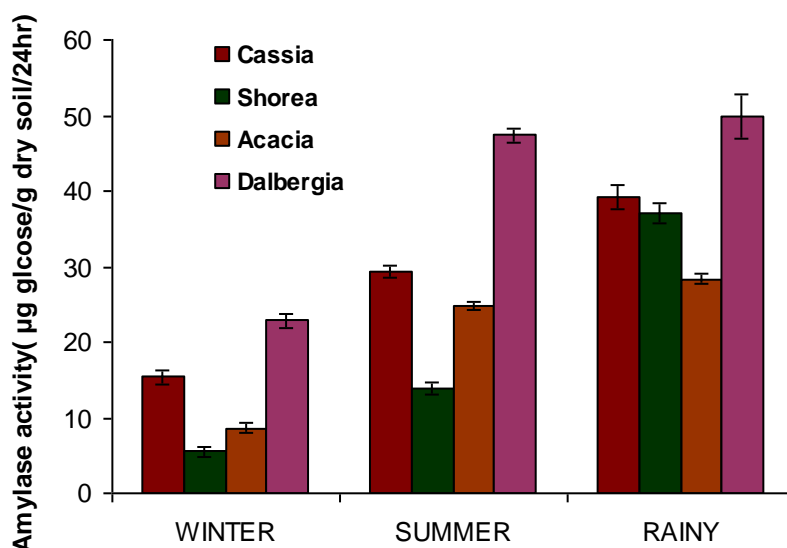
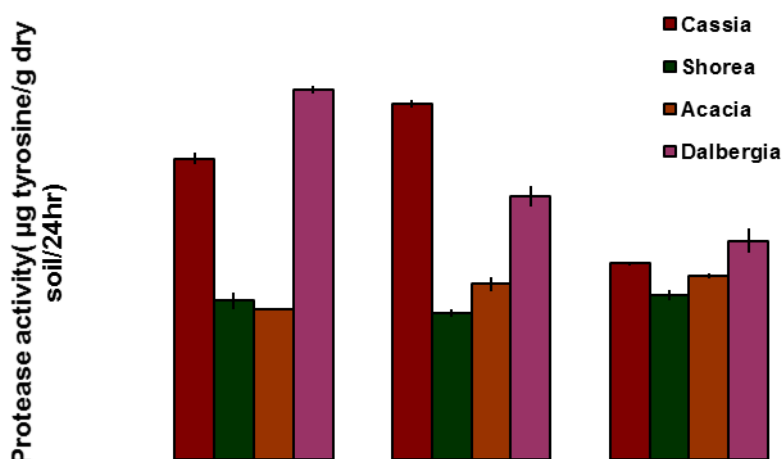## Two-way ANOVA: Amylase activity in soil of four forest sites

Source of variation	df	Sum of sq	Mean sq	F	P
Between forest litter types	3	5074.009	1691.34	188.96	<0.0001
Between seasonal interval	2	7995.33	3997.67	446.62	<0.0001
Interaction	6	973.31	162.22	18.12	<0.001
Residuals	60	537.06	8.95		

Figure-2. Seasonal changes of cellulase activity in soil of different forest sites
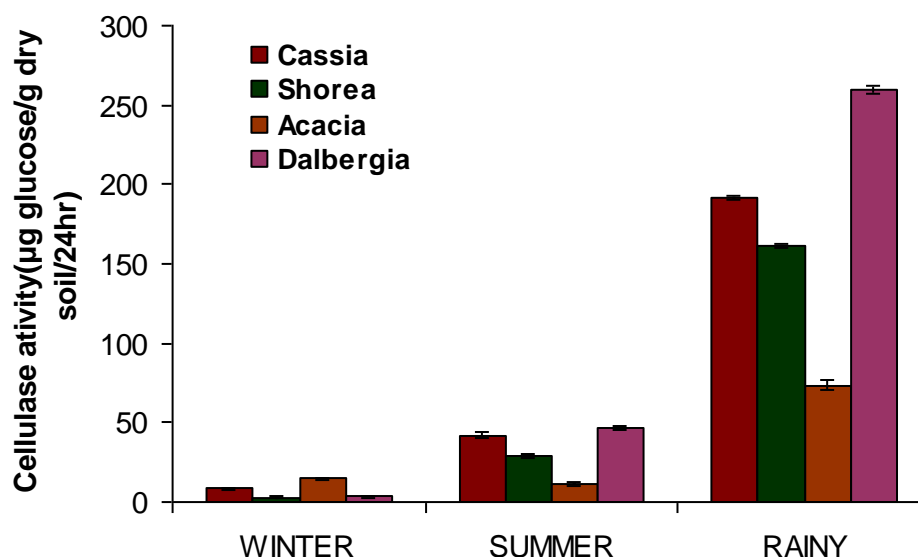
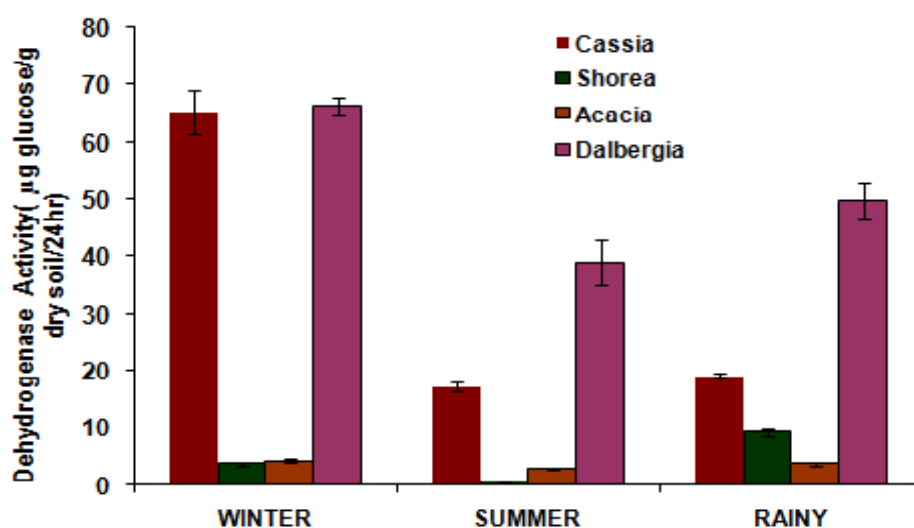## Two-way ANOVA: Cellulase activity in soil of four forest sites

Source of variation	df	Sum of sq	Mean sq	F	P
Between forest litter types	3	46804.7	15601.6	1008.21	<0.0001
Between seasonal interval	2	377072.3	188536.2	12183.71	<0.0001
Interaction	6	65435.5	10905.9	704.77	<0.0001
Residuals	60	928.5	15.5		

Figure-3. Seasonal changes of invertase activity in soil of different forest sites
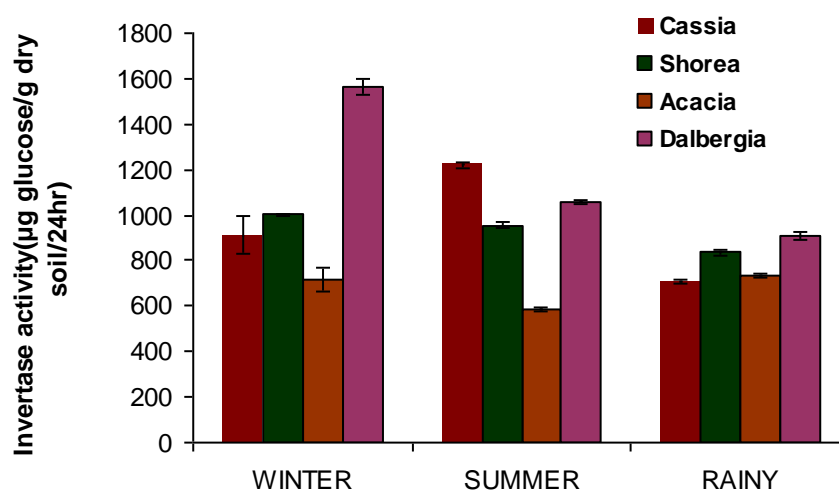
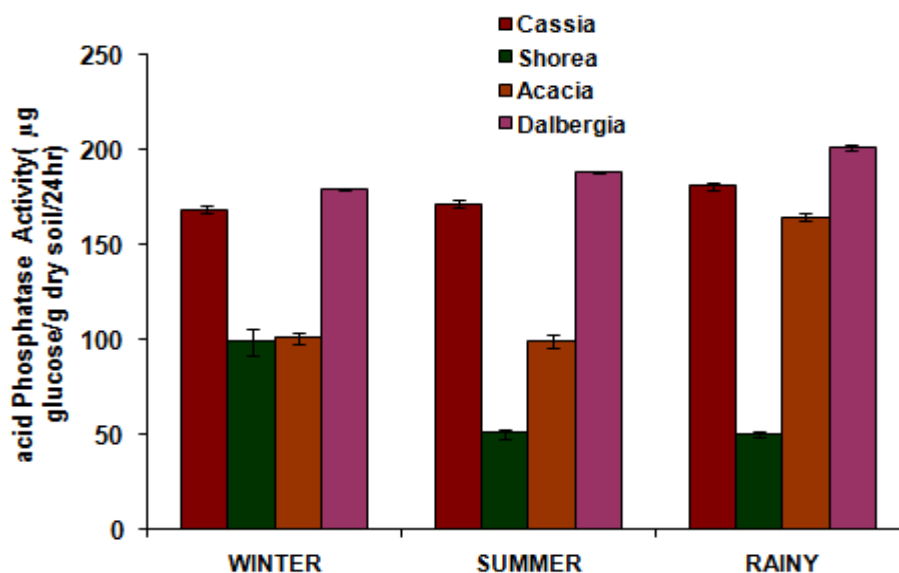### Two-way ANOVA: Invertase activity in soil of four forest sites

Source of variation	DF	Sum of sq	Mean sq	F	P
Between forest litter types	3	2094915	698304.9	120.37	<0.0001
Between seasonal interval	2	714978	357489	61.62	<0.0001
Interaction	6	1695927	282654.4	48.72	<0.0001
Residuals	60	348071	5801.2		

Figure-4. Seasonal changes of protease activity in soil of different forest sites

Two-way ANOVA: Protease activity in soil of four forest sites

Source of variation	dF	Sum of sq	Mean sq	F	P
Between forest litter types	3	80978.23	26992.74	364.49	<0.0001
Between seasonal interval	2	12183.14	6091.57	82.26	<0.0001
Interaction	6	35379.44	5896.57	79.62	<0.0001
Residuals	24	1777.36	74.06		

Figure-5. Seasonal changes of dehydrogenase activity in soil of different forest sites

Two-way ANOVA: Dehydrogenase activity in soil of four forest sites

Source of variation	df	Sum of sq	Mean sq	F	P
Between forest litter types	3	19856.65	6618.88	454.76	<0.0001
Between seasonal interval	2	3400.43	1700.22	116.82	<0.0001
Interaction	6	4172.61	695.43	47.78	<0.0001
Residuals	24	523.97	14.56		

Figure-6. Seasonal changes of acid phosphatase activity in soil of different forest sites

Two-way ANOVA: Acid phosphatase activity in soil of four forest sites

Source of variation	df	Sum of sq	Mean sq	F	P
Between forest litter types	3	111647.3	37215.75	1143.63	<0.0001
Between seasonal interval	2	3849.3	1924.67	59.14	<0.0001
Interaction	6	14799.6	2466.6	75.79	<0.0001
Residuals	24	1171.5	32.54		

Consideration of F values reveals that litter types are mainly responsible for the observed variations of the enzyme activity than different seasons.

Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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