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Research Paper

Correlation Analysis Between Soil Enzyme Activities With C, N, S, P In Bhitarkanika Region Of Odisha, India

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Abstract -
Soil microbial community and enzyme activity play pivotal roles in ecosystem processes, including nutrient cycling, organic matter decomposition, and soil fertility. The composition of the microbial community (MC) is significant in sustaining environmental services because the structure and activity also influence nutrient turnover, distribution, and the breakdown rate of soil organic matter. Soil microbial activity is an essential predictor of soil quality alterations, and microbiome responsiveness is imperative in addressing the escalating sustainability concerns in the wetland ecosystem. This study investigates the seasonal variations in soil microbial diversity, physic-chemical analysis and enzyme activity to land conversions in the Bhitarkanika mangrove ecosystem, Odisha, India.. Results indicate significant seasonal fluctuations in microbial community composition and enzyme activity, highlighting the dynamic nature of soil processes in wetland ecosystems. Enzyme activity assays were conducted to assess the functional capacity of soil microbes. Soil enzymes are involved in biogeochemical cycle of soil carbon (C), nitrogen (N) and phosphorus (P), which can be used as early sensitive indicators of soil nutrient changes caused by climate change.
Keywords: *Soil enzyme activity, Physico-chemical properties, Ecosystem, Microbial population*

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I. INTRODUCTION

Soil serves as the backbone of agricultural production and is gaining recognition as a linchpin for environmental sustainability and battling climate change[1], It's like the silent engine powering ecological processes and guiding land use planning strategies[2]. Soil enzymes possess significant catalytic powers, overseeing numerous vital reactions critical for microbial processes, soil structure stabilization, soil organic matter formation (SOM), and nutrient cycling[3][4]. Their reliability as indicators of biological shifts is unparalleled, offering swift insights into even the slightest alterations within the soil environment [5][6]. Yet, their activity dances to the rhythm of seasons, locations, and soil depth, reflecting the dynamic nature of the earth beneath our feet. These enzyme activities serve as windows into essential nutrient cycles and oxidation-reduction processes, aiding in our understanding of how land transitions and management practices shape soil health[7]. Our study aimed to assess how varying seasons affect soil microbial communities, physical-chemical parameters, and enzyme activities within the Bhitarkanika mangrove ecosystem, while also uncovering the intricate interplay among different soil properties and microbial and enzyme dynamics.

II. METHODS AND MATERIALS

Description of study area:

Bhitarkanika, situated along India's eastern coastline in the northeastern part of Odisha's Kendrapara District, is renowned as one of the largest and most biodiverse mangrove ecosystems in the country. Spanning an extensive area within the deltaic region formed by the Brahmani, Dhamra, and Baitarani rivers, this unique wetland habitat comprises dense mangrove forests, intricate estuarine channels, and expansive mudflats. Designated as a Ramsar site of international importance in 2002 and declared a National Park in 1992, Bhitarkanika boasts a rich array of flora and fauna species, including several endangered and endemic ones. Bhitarkanika's ecological significance, coupled with its susceptibility to environmental changes and

anthropogenic pressures, makes it an ideal study area for exploring wetland dynamics, biodiversity conservation, and ecosystem management.

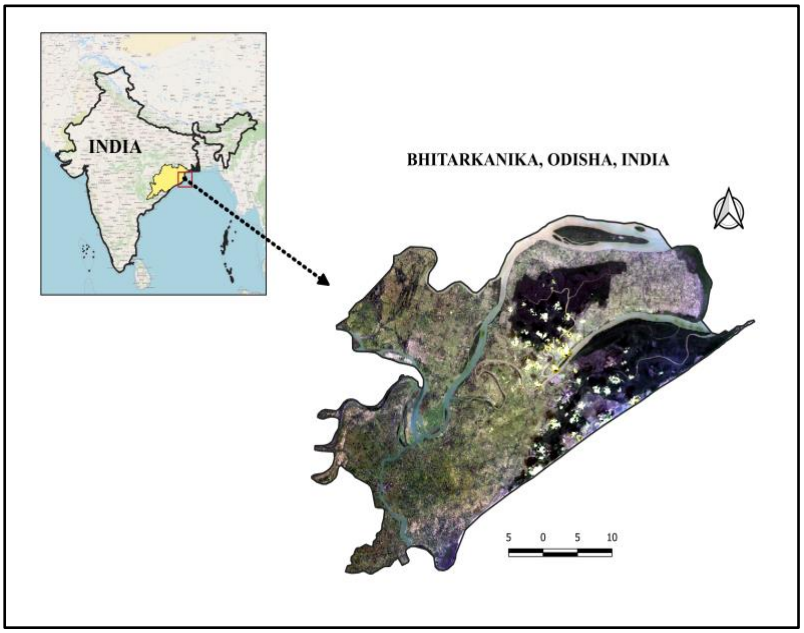


Figure 1: Study map of Bhitarkanika, Odisha, India

Soil sample collection and physic-chemical analysis:

Soil samples were collected on quarterly basis to cover all major seasons of a year pre-monsoon, post-monsoon, winter and summer seasons respectively. The four seasons namely S1, S2, S3, and S4 at depths 0–15 cm. The samples were air dried and sieved with 2 mm sieve for further analysis. Physico-chemical characteristics of soil were estimated and followed as per standard protocol[8], respectively.

Table 1: Physico-chemical analysis of soil sample by following methods:

Physic-chemical parameters	Methods	References
Soil pH	pH meter	J. Forster
Organic Carbon (OC)	Modified Walkley-Black method	Jackson 1958
Total Nitrogen (TN)	Kjeldahl method	Keeney and Nelson 1982
Total Sulphur (TS)	Digestion method	Bardsley and Lancaster., 1962
Total Phosphorous (TS)	Fiske-Subbarow Method	S. H. Yuen and A. G. Pollard., 1951

Soil microbial count and enzyme activity

For viable plate count and microbial analysis soil suspension was serially diluted and from each dilutin of each sample 0.1ml was spread on suitable agar medium. For enumeration of bacteria high plate count; for fungi potato dextrose agar; for Actinomycetes actinomycetes isolation agar were used. Spread plate count were carried out in triplicate. Colonies were counted periodically after incubation and total count were calculated by multiplying colony number with respective dilution factor.

Determination of different enzymes activities which is involved Tannase (K.C. Mondal., 2001), Urease (Tabatabai., 1982; Misra., 1968), L-Asparaginase (Frankenberg., 1991a), L-Glutaminase (Frankenberg., 1991b), Amidase (Frankenberg., 1991c), Phosphatase (Tabatabai and Bremner., 1969) and Cellulase and Amylase (Schinner and Von Mersi., 1990), estimated as per standard protocol[8].

Table 2: Soil enzyme activity determine by following methods:		
Soil enzyme	Reagent and doges	References
Tannase	The reaction mixture consisting of 2g moist soil and substrate tannic acid 0.3 ml was incubated at 60°C for a defined period. The enzymatic reaction was stopped by the addition of 3 ml BSA solution, which also precipitated the remaining tannic acid. The tubes were centrifuged (5000rpm for 3-5 min) and the resultant precipitate was dissolved in 3 ml SDS–triethanolamine solution. Then 1 ml of $FeCl_3$ reagent was added and kept for 15 min for stabilization of the color. The absorbencies were measured at 530 nm, against the blank (i.e., without tannic acid).	Mondal et al., 2001
Urease	For urease determination, 2 g of fresh soil was added to 0.25 ml toluene, 1 ml urea solution (200mM), and 9 ml Tris buffer then incubated at 37° C for 24 h. after incubation add 35 ml of KCL-Ag2SO4 solution, swirl the flask for few seconds, bring content upto 50 ml. determine the release of ammonia by add ninhydrin, the pH was 10 using NaOH solution and the mixing has done at room temperature. The reaction was found to be complete to stand for 20 min forming a red colored product. The absorbance was measured at 400- 800 nm using UV/Vis spectrometer. to perform control, add urea solution after incubation.	Tabatabai and Bremner 1972; Baker and Alzboon 2015
Asparaginase	Asparaginase determination, 2 g of fresh soil was added to 0.25 ml toluene, 1 ml L-asparagin solution (0.5M), and 9 ml Tris buffer then incubated at 37° C for 2 h. after incubation add 35 ml of KCL-Ag2SO4 solution, swirl the flask for few seconds, bring content upto 50 ml. determine the release of ammonia by add ninhydrin, the pH was 10 using NaOH solution and the mixing has done at room temperature. The reaction was found to be complete to stand for 20 min forming a red colored product. The absorbance was measured at 400- 800 nm using UV/Vis spectrometer. to perform control, add L-asparagine solution after incubation.	Frankenberger and Tabatabai 1991a, 1991b; Baker and Alzboon 2015
Glutaminase	For glutaminase determination, 2 g of fresh soil was added to 0.25 ml toluene, 1 ml L-glutamine solution (0.5M), and 9 ml Tris buffer then incubated at 37° C for 2 h. after incubation add 35 ml of KCL-Ag2SO4 solution, swirl the flask for few seconds, bring content upto 50 ml. determine the release of ammonia by add ninhydrin, the pH was 10 using NaOH solution and the mixing has done at room temperature. The reaction was found to be complete to stand for 20 min forming a red colored product. The absorbance was measured at 400- 800 nm using UV/Vis spectrometer. to perform control, add L-glutamine solution after incubation.	Frankenberger and Tabatabai 1991c, 1991d; Baker and Alzboon 2015
Amidase	For Amidase determination, 2 g of fresh soil was added to 0.25 ml toluene, 1 ml amide solution (0.5M), and 9 ml Tris buffer then incubated at 37° C for 24 h (the incubation time varies with the specific substrate added, formamide is hydrolysis rapid and takes 2h whereas, acetamide and propionamide are slower and takes 24h for incubation). after incubation add 35 ml of KCL-Ag2SO4 solution, swirl the flask for few seconds, bring content upto 50 ml. determine the release of ammonia by add ninhydrin, the pH was 10 using NaOH solution and the mixing has done at room temperature. The reaction was found to be complete to stand for 20 min forming a red colored product. The absorbance was measured at 400- 800 nm using UV/Vis spectrometer. to perform control, add urea solution after incubation	Frankenberger and Tabatabai 1980a; Baker and Alzboon 2015
Amylase	To determine amylase activity, sucrose solution and pH 5.5 phosphate buffer were added to 2 g of fresh soil, and a few drops of toluene were added. The solution was shaken and incubated in a 37° C incubator for 24 h. Next, 3, 5- dinitrosalicylic acid was added to determine the glucose produced by a 540nm using uv-vis	Schinner and Von Mersi 1990; khatri et. al., 2020

	spectrophotometry.	
Cellulase	Soil (2g) incubated with 15 ml acetate buffer and 15 ml of CMC solution, incubate for 24 h at 50^0C. after incubation, filter the soil suspension. The control is prepared by adding 15 ml CMC solution after incubation but before filtration. Dilute 1 ml of the filter with 30 ml distilled water. Add 1 ml reagent A and reagent B. placed the tube water bath for 15min after cooling add reagent C. stand at 200C for 60 min for color development. Measure optical density at 690nm. (reagent A: anhydrous sodium carbonate and potassium cyanide in dist. Water; reagent B: potassium ferric hexacyanide in dist.water; reagent C: ferric ammonium sulphate, SDS and conc. H_2SO_4 in dist. Water and bring up to liter.)	Schinner and Von Mersi 1990
Phosphatase	Soil (2g) treated with 0.25 ml of toluene, 4 ml of modified universal buffer (MUB) and 1 ml of p-nitrophenyl phosphate substrate. The content are mixed and incubation for 1 h at 37^0C. after incubation, add 1 ml of $CaCl_2$(0.5M) and 4 ml of NaOH(0.5M). Mix the contents and filter the soil through a whatman filter paper. Measure the absorbance at 400nm. To perform control, add 1 ml of PNP solution after the addition of $CaCl_2$ and NaOH and immediately before filteration of the soil suspension.	Tabatabai and Bremner 1969; Eivazi and Tabatabai 1977

Statically analysis

Descriptive statistical analysis was performed using PAST software (version 4.03). The standard error of the mean was calculated for each parameter. The significance tests were performed among seasons and parameters together at 0.05 levels using ANOVA test. ANOVA help to identify the variability among the group and between the groups. A coefficient of correlation was calculated between physicochemical characteristics, soil microbial counts, and enzyme activities using R studio (version 4.2.1).

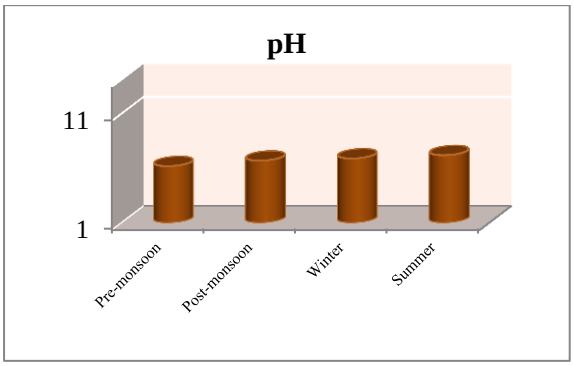
III. RESULTS AND DISCUSSION:

Physical and chemical property of soil:

The pH of soil at all season is slightly acidic in nature. The pH of soil samples in increased from pre-monsoon to summer season. However, soil sample higher pH (7.2) was observed at summer and lower pH (6.2) at pre-monsoon (Figure 2, a). The pH was recorded in the order of pre-monsoon>post-monsoon>winter>summer in soil sample. The pH level of soil serves as a reliable indicator of potential nutrient availability in the land. Soil having pH < 5.6 is usually considered to be acidic in nature, pH from 5.6 to 6.0 ranges is moderately acidic while < 5.5 are strongly acidic in nature[9].

Table 3: Physico-chemical analysis of soil sample

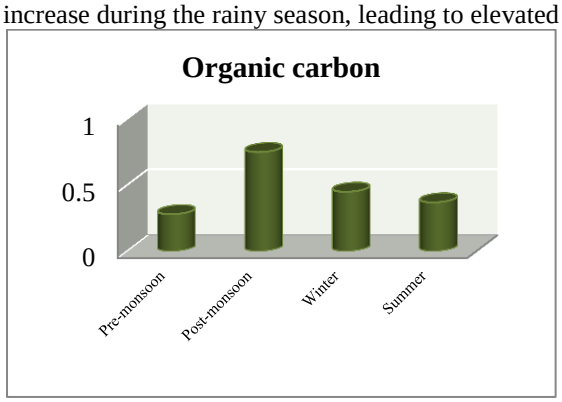
Sr.no	Parameters	Pre-monsoon (S1)	Post-monsoon (S2)	Winter (S3)	Summer (S4)
1	Soil pH	6.2	6.7	6.9	7.2
2	Organic carbon	0.28	0.75	0.45	0.37
3	Total nitrogen	0.068	0.27	0.072	0.0044
4	Total sulphur	0.068	0.37	0.23	0.32
5	Total phosphorous	1.32	1.45	0.45	0.53



(a)

Organic carbon was recorded maximum in post-monsoon in soils while; minimum was recorded in pre-monsoon season (Figure 2, b). Seasonal variations significantly impact organic carbon levels in wetland soils. During the wet season, organic carbon content tends to increase due to higher plant productivity, increased organic matter input from vegetation, and reduced decomposition rates. In contrast, dry season or winter, organic carbon levels may decrease as decomposition accelerates under drier conditions and microbial activity increases. The nutrients release after litter decomposition occurs naturally in the internal biogeochemical cycle of an ecosystem[10]. The findings also demonstrated that the natural forest soil consistently exhibited the highest organic carbon content across all seasons, whereas the grassland consistently showed the lowest. The organic carbon was observed in the order of pre-monsoon>winter>summer>post-monsoon in soils, respectively.

Total nitrogen content was recorded maximum during post-monsoon season followed by summer and further increased from pre-monsoon in soil. During monsoon periods, nitrogen levels may be higher due to increased inputs from atmospheric deposition, runoff, and biological nitrogen fixation by plants and microorganisms. Total nitrogen was observed in the order of post-monsoon>winter>pre-monsoon>summer in soils. Additionally, waterlogged conditions can lead to reduced nitrogen loss through leaching and denitrification, contributing to nitrogen accumulation in the soil. During winter and summer season, nitrogen levels may decrease as biological activity slows down, leading to increased nitrogen uptake by plants and microbial immobilization. Evidence indicates that both biological nitrogen fixation and mineralization rates increase during the rainy season, leading to elevated nitrogen levels during this period. [11].



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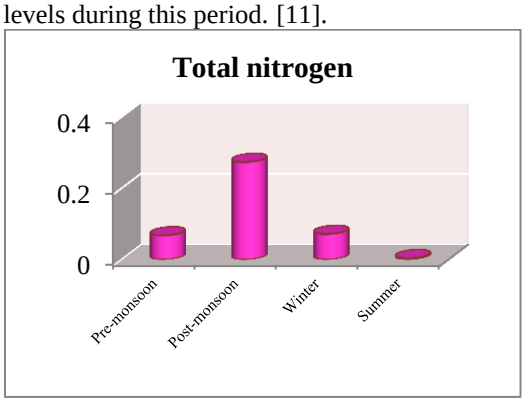

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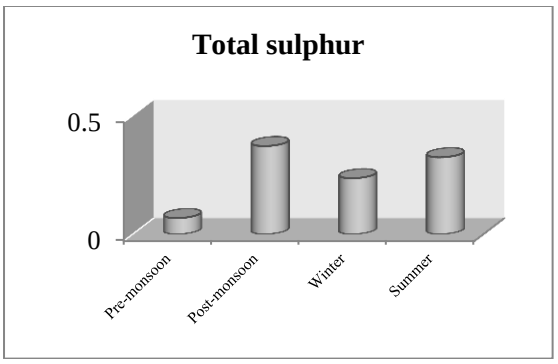

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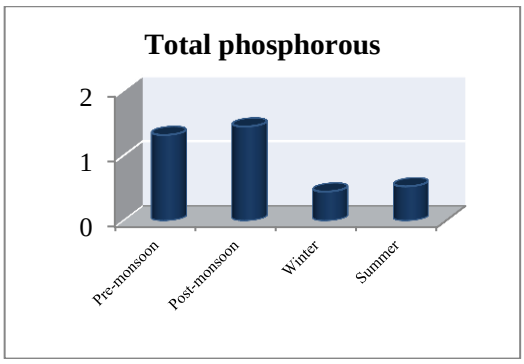


(e)

Figure 2: plot for physical and chemical analysis of soil; (a) pH , (b) total carbon , (c) total nitrogen, (d) total sulphur, (e) total phosphorous

Total sulphur content was high during post-monsoon season followed by winter, then further increased from summer seasons. During the monsoon and post-monsoon periods of high precipitation, sulfur levels may increase due to the influx of sulfur-containing compounds from atmospheric deposition, runoff, and organic matter decomposition. Wetland soils often act as sinks for sulfur, as anaerobic conditions prevalent in waterlogged environments promote the accumulation of sulfides through microbial processes such as sulfate reduction. Conversely, in summer seasons low precipitation, sulfur levels may decrease as organic matter decomposition and microbial activity slow down, reducing the input and transformation of sulfur compounds in the soil. Understanding seasonal variations in sulfur dynamics is essential for managing wetland ecosystems and assessing their role in sulfur cycling and environmental sulfur contamination. Sulphur deposits required by the soil are adequately met by the organic sulphur mineralization and atmosphere [12]. Depending on the type of soil, greater the amount of percolating water, greater will be the net downward movement of sulphate in the soil [13].

Table 4: Significant level of soil physicochemical parameters and soil enzyme

Sr.no	Soil parameters & enzymes	Min.	Max.	Mean±SE	Standard deviation
1	pH	6.2	7.2	6.75±0.21	0.42
2	Organic carbon	0.28	0.75	0.46±0.101	0.203
3	Total nitrogen	0.004	0.27	0.103±0.057	0.115
4	Total sulphur	0.068	1.22	0.68±0.255	0.510
5	Total phosphorous	0.45	1.45	0.93±0.26	0.520
6	Tannase	0.17	0.79	0.45±0.134	0.268
7	Urease	2.58	2.97	2.74±0.082	0.164
8	Asparaginase	0.73	6.5	4.075±1.319	2.63
9	Glutaminase	3.49	6.11	4.5±0.58	1.166
10	Phosphatase	0.1	0.14	0.115±0.009	0.019
11	Amidase	1.03	5.13	2.89±0.86	1.72
12	Amylase	0.21	3.8	1.93±0.75	1.51
13	Cellulase	1.68	6.52	4.09±0.988	1.97

Note: SE, standard error of mean

Total phosphorous in soil samples recorded higher value in pre-monsoon and post-monsoon comparatively low value in winter and summer season. Every aspect of plant life, from growth and respiration to reproduction, hinges on the presence of phosphorus in every plant cell[14]. The rates of weathering also affect the availability of phosphorus to the plants. Phosphorus in turn affects the input level of the plant residue (Brown et al., 1994). The phosphorus content in soil directly influences its suitability for supporting specific plant growth, thereby determining the vegetation type in a given area. Additionally, phosphorus is pivotal in facilitating plant cell division, energy conversion, and a range of metabolic activities[14].

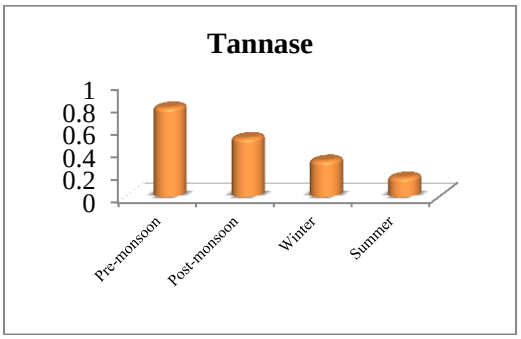
Soil microbial count and enzyme activity

The bacterial count (BC) of soils followed the order: post-monsoon>winter>pre-monsoon>summer. The mean values ranged from 8 CFU × 106 g−1 in pre-monsoon, 13 CFU × 106 g^{-1} in post-monsoon, 8.2 CFU × 106 g^{-1} in winter and 4 CFU × 106 g^{-1} in summer season. The abundance of substrate in forests, along with good vegetation cover, enhances microbial activity[15][16]. In addition, Bacteria in particular, rapidly assimilate easily digestible compounds like simple sugars and amino acids, promoting their growth and activity. Furthermore, in undisturbed ecosystems, the activity of plant roots and a slightly lower pH facilitate bacterial proliferation[17]. Conversely, in cultivated soils lacking in substrate, bacterial activity diminishes accordingly. The fungal count (FC) of the soils followed the order: pre-monsoon>post-monsoon>winter>summer. The mean fungal count in the pre-monsoon is 0.8 CFU × 106 g^{-1}, post-monsoon is 0.4 CFU × 106 g^{-1}, winter is 0.25 CFU × 106 g^{-1} and summer contain 0.22 CFU × 106 g^{-1}. The actinomycete count (AC) of soils in order: post-monsoon>winter>pre-monsoon>summer. The value contain in pre-monsoon 0.79 CFU × 106 g^{-1} , in post-monsoon 2.1 CFU × 106 g^{-1}, in winter 0.53 CFU × 106 g^{-1} and summer 0.22 CFU × 106 g^{-1}.

Table 5: Enzyme activity analysis of soil sample					
Sr.no	Soil enzyme	Pre-monsoon(S1)	Post-monsoon(S2)	Winter(S3)	Summer(S4)
1	Tannase	0.79	0.52	0.32	0.17
2	Urease	2.58	2.7	2.71	2.97
3	Asparaginase	5.84	6.5	0.73	3.23
4	Glutaminase	3.82	6.11	3.49	4.58
5	Phosphatase	0.1	0.14	0.1	0.12
6	Amidase	1.03	5.13	3.16	2.24
7	Amylase	1.38	2.33	3.8	0.21
8	Cellulase	6.52	1.68	4.15	4.04

The tannase are higher in pre-monsoon season and lower during summer season. Seasonal fluctuations in factors like moisture levels, temperature, and substrate availability can influence the activity and abundance of tannase enzyme in wetland soils. During the wet season or periods of high precipitation, increased soil moisture and organic matter inputs create optimal conditions for microbial activity, potentially leading to higher tannase activity as microorganisms break down tannin-rich substrates. Conversely, in drier seasons or periods of low precipitation, reduced soil moisture levels and limited substrate availability may result in decreased tannase activity. Understanding these seasonal dynamics is crucial for comprehending the role of tannase in nutrient cycling and organic matter decomposition within wetland ecosystems.

Urease activity almost equal pattern was recorded in all seasons. Seasonal variations can significantly impact urease activity in wetland soils due to changes in environmental conditions that influence microbial activity and substrate availability. High precipitation, increased soil moisture levels create favorable conditions for microbial growth and activity, potentially leading to higher urease activity. Additionally, higher temperatures during warmer seasons can further stimulate microbial activity, including urease-producing microorganisms. Furthermore, fluctuations in substrate availability, such as the presence of urea from fertilizers or organic matter decomposition, can also influence urease activity throughout the seasons.



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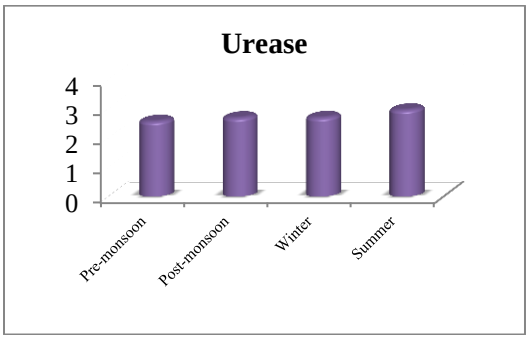

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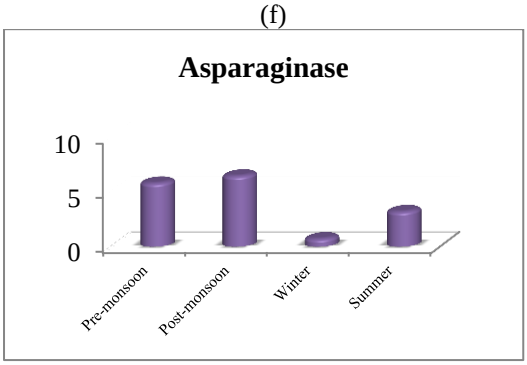

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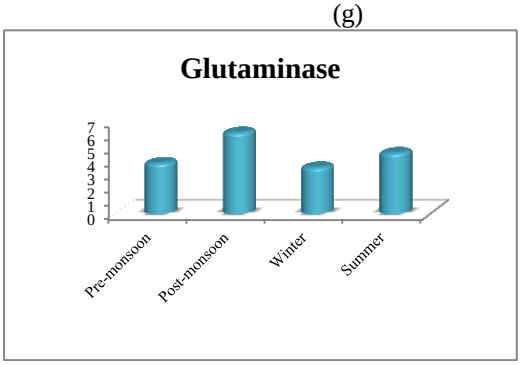
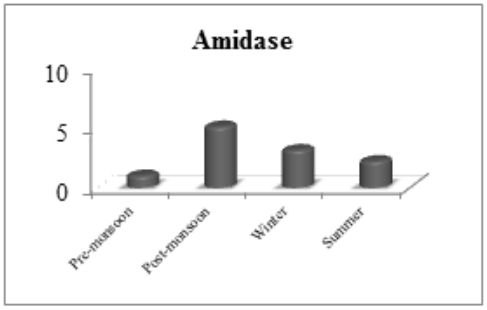


(i)

Asparaginase and Glutaminase activity is increased from summer season to post-monsoon and decreased in winter season. Activity shows highest value during pre-monsoon and low in winter season. Temperature fluctuations affect enzyme activity, with warmer seasons potentially increasing it and colder seasons decreasing it. However, the specific impact of seasonal variations on asparaginase and glutaminase activity in wetland soils may vary depending on factors such as nutrient availability, pH, and the composition of the microbial community. Further research is needed to fully understand the seasonal dynamics of asparaginase activity in wetland ecosystems.

Amidase activity show decreasing patten from post-monsoon to summer season, high value in post-monsoon and low value in pre-monsoon season. During warmer seasons like spring and summer, increased temperatures and soil moisture levels stimulate microbial activity, leading to heightened amidase activity. Microbes thrive in these conditions, accelerating enzymatic processes involved in organic matter decomposition. Conversely, in colder seasons such as fall and winter, microbial activity diminishes due to lower temperatures and reduced substrate availability, resulting in decreased amidase activity.
Phosphatase activity show similar patten through out the all seasons, post-monsoon and summer contain slight fluctuation. This is often associated with heightened organic matter decomposition and nutrient cycling. Additionaly, higher soil moisture levels during these seasons can provide optimal conditions for microbial growth and enzyme activity.



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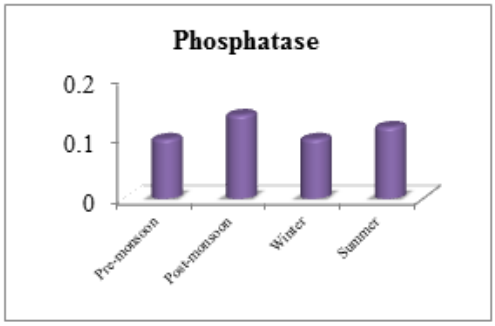

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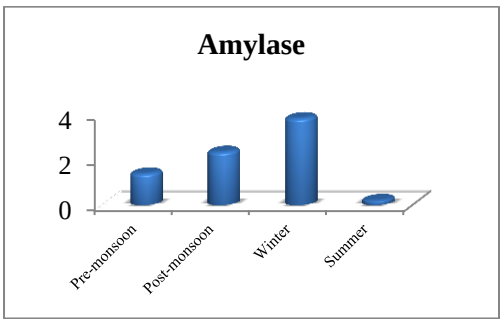

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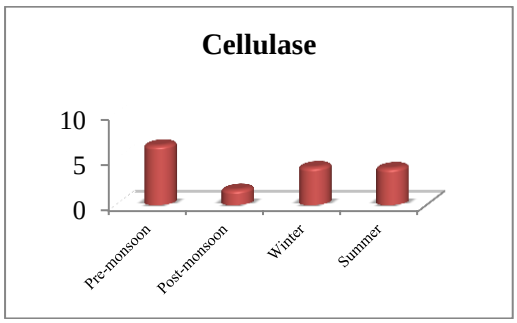

(m)

Figure 3: plot for enzyme determine from soil (shown from f to m)

Amylase acivity increased from pre-monsoon to winter season whereas, decreased in summer seasons. the seasonal variation in amylase activity is crucial for managing wetland ecosystems, as it influences carbon cycling, soil health, and the overall productivity of these vital habitats. Cellulose activity show high activity during pre-monsoon season while decreased in post-monsoon, further initiate increased from winter to summer season. Production of these extracellular enzymes from microbes during litter degradation may be influenced by temperature, moisture, pH, and substrate involvement[18][19]. The activity of amylase showed a significant correlation with the populations of fungi and bacteria, as well as the moisture content of litter. [20]. Changes in amylase activity during litter decomposition were attributed to changes in microbial populations[21].

Relationship Between Soil Microbial Count, Enzyme Activities and Physicochemical Properties:
The Pearson's correlation coefficients (r) between the fundamental parameters and soil enzyme activities exhibit variability and are statistically significant at the p = 0.05 level. Total sulphur and phosphorous showed a significant negative correlation with amidase, amylase and urease. Whereas, organic carbon and total nitrogen negatively correlate with cellulose, urease, tannase, respectively (Fig. 4 & 5). Conversely, organic carbon, total nitrogen, total sulphur and total phosphorous exhibited a significant positive correlation with phosphatase, glutaminase, tannase, cellulose, amylase and amidase.

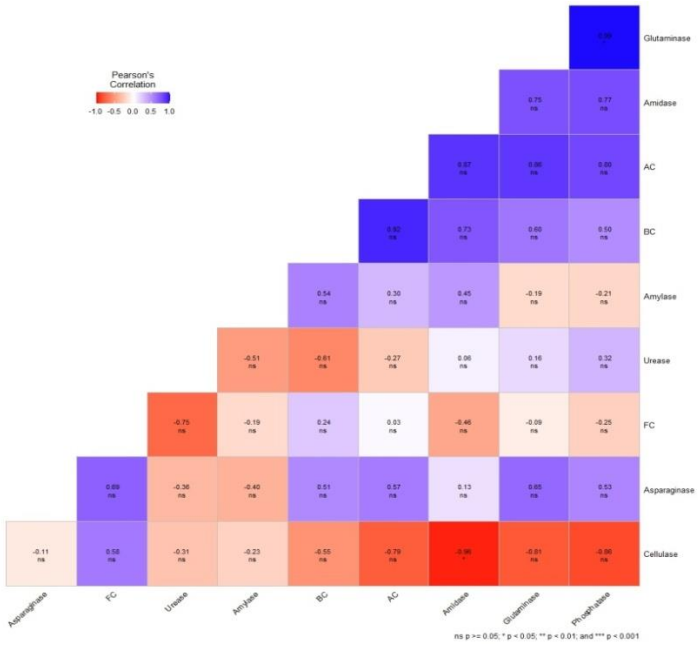

Figure 4: Relationship between soil microbial counts and enzymatic activities

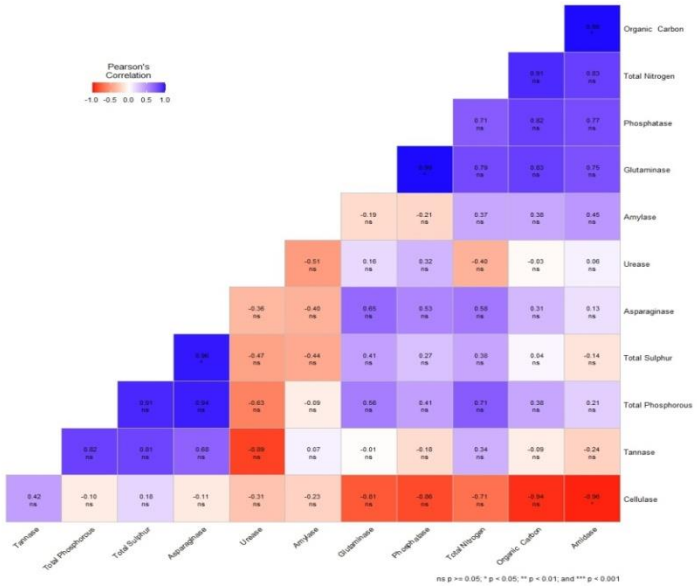

Figure 5: Relationship among physico-chemical characteristics and enzymatic activities

Furthermore, FC showed a significant negative correlation with phosphatase, glutaminase, amidase and urease. AC and BC also show negative correlation with cellulase and urease. On the other hand, a high and significant positive correlation was also observed between soil microbial counts (bacterial, fungal, and actinomycete counts) and enzyme activities (Fig. 4). The higher energy supply, enzyme substrate, and the easily accessibility of H+ ions from carbohydrates and organic acids substantiate the significant positive correlations between microbial and enzyme activities[22].

IV. CONCLUSION:

Numerous ecological studies have sought to correlate the abundance and diversity of soil organisms with habitat efficiency and ecosystem functioning. The analysis of soil physico-chemical parameters and enzyme activities provides critical insights into soil health and ecosystem dynamics. Variations in these parameters significantly affect nutrient cycling, microbial activity, and overall soil fertility. Wetlands exhibit lower pH values, which can be attributed to their undisturbed nature and high organic content, including litter, humus, and microorganisms. The pH values across all seasons indicate that the soil is slightly acidic. Elements such as carbon, nitrogen, sulfur, and phosphorus are found in higher concentrations during the post-monsoon

season, with fluctuations observed in other seasons. Soil enzyme activities including tannase, amylase, cellulase, nitrogenase, urease, glutaminase, asparaginase, and phosphatase vary seasonally, directly influencing organic matter decomposition and nutrient availability. These seasonal variations have a significant impact on element availability and cycling, microbial activity, and plant growth. Understanding these dynamics is crucial for effective wetland management, conservation efforts, and maintaining the ecological balance of these vital habitats.

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