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Diversity and Phosphate Solubilization Potential of Rhizospheric Fungi from different Land-use of Mokokchung district, Nagaland, India

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***Abstract*:** This study investigates the rhizospheric fungal communities in soil samples from Mokokchung, Nagaland, India, collected from five land-use types: natural forest (NF), tree plantation (TP), bamboo plantation (BP), jhum fallow (JF), and shifting cultivation (SC). Various soil parameters, including temperature, pH, organic carbon, moisture, available nitrogen, phosphorus, and potassium, were analyzed. Soil fungal species were isolated in rose Bengal agar (RBA) and potato dextrose agar (PDA), and the diversity, richness, evenness, and abundance were estimated using diversity indices such as Shannon, Simpson, Berger-Parker, and Pielou's evenness. Results depict significant variations in soil properties influencing fungal populations across the different land-use types. NF exhibited the highest fungal diversity, taxa, and richness, while SC had the lowest, as reflected by the diversity indices. *Aspergillus* and *Penicillium* were the predominant genera. Additionally, eight fungal isolates demonstrated the ability to solubilize phosphate, with *Penicillium* emerging as a particularly promising candidate for further evaluation due to its high solubilization potential. This study highlights the impact of land-use on fungal diversity and the potential of phosphate-solubilizing fungi for sustainable agriculture.

***Keywords*:** Land-use, Fungal diversity, Phosphate solubilizing fungi, Diversity indices, North East India

The plant root system influences the soil rhizosphere and micro-organisms present in the soil are subsequently influenced by the plant-root metabolites (Sharma and Shrivastava 2017). Therefore, depending on the land-use, different fungal flora is present. The fungal population regulates the ecosystem, stabilizes habitat, and controls various soil processes (Frąc et al 2015). However, reports on the loss of biodiversity globally, which ultimately lowers ecosystem functionality, extinctions, and even ecosystem collapse in extreme situations are reported (Dunne and Williams 2009). The primary driver of this rapid loss of fungal biodiversity is converting forest land to agricultural systems and land-uses characterized by regular anthropogenic disturbances (Temjen et al 2021). Therefore, inventory is a valuable resource and ensures natural resource efficiency and sustainable utilization to prevent further biodiversity loss. The main instrument for ensuring ecological monitoring and addressing the biodiversity crisis is the use of diversity indices, which enable measurement of the two essential aspects of an ecosystem, i.e., richness and evenness (Stirling and Wilsey 2001, Morris et al 2014).

Phosphorus constitutes about 0.2% of the plant's dry weight and is essential for plant growth and metabolism (Widawati and Suliasih 2006). However, plants can only utilize a trace amount of the chemical P and a substantial amount is immobilized and left inaccessible to plants (El Attar et al 2022). The economically and environmentally beneficial solution would be a microbial inoculant capable of dissolving sparingly soluble inorganic soil P (Alori et al 2017). Phosphate solubilizing fungi (PSF) can solubilize insoluble phosphate in the soil. Fungi, in particular, have a more remarkable ability to solubilize insoluble phosphate than bacteria (Zhang et al 2018). Mokokchung district is a hilly region in Nagaland state, North-East India. The ever-increasing population in the state has amplified the pressure on the land. There is a trend of rapid conversion of natural forests into different land-use systems, which negatively affects the fungal community (Miah et al 2010, Temjen et al 2021). Therefore, the present work aims to study the diverse rhizospheric fungal populations from various land-use sites in Mokokchung district, Nagaland, India and estimate those fungi with the capacity to solubilize P for sustainable agriculture.

MATERIAL AND METHODS

Site selection: Five land-use sites under Mokokchung district, Nagaland, India, were selected (Table 1). The major type of soil in the region have alluvial soil, non-laterite red soil, and forest soil, with an average temperature of 27°C and 2500 mm of rainfall annually (Temjen et al 2022**).**

Soil sample: Composite soil layers at 0-30 cm were collected from the rhizospheric region of each site during spring 2023. Soil temperature and pH were recorded on-site, soil organic carbon (SOC) was estimated using air-dried sieved soil samples (Walkley and Black 1934), and soil moisture was measured via the gravimetric method. The available nitrogen (N) was estimated as per the Kjeldahl method (1883), the available phosphorus (P) as per Bray's no. 1 extract method (Bray and Kurtz 1945), and exchangeable potassium (K) as per Trivedy and Goel (1986). All tests were performed in triplicates. Soil fungal species were isolated in rose bengal agar (RBA) and potato dextrose agar (PDA) plates supplemented with streptomycin sulphate (0.03g/L) and prepared following the serial dilution method (Selman and Waksman 1921). PDA and RBA plates were incubated at 25±1°C for 5-7 days. The fungi were identified with the help of standard literature (Gillman 1957, Nagmani et al 2006, Webster and Weber 2007), and the colony-forming unit was expressed as CFU/g^{-1}(Johnson and Case 2006).

Phosphate solubilizing fungi (PSF): Pikovskaya (PVK) agar (Himedia) was utilized to screen their ability for phosphate solubilization. PVK agar was autoclaved at 121°C, and the subsequently sterilized PVK agar was transferred to fixed Petri plates. Fungal isolates from the pure culture were transferred to the PVK agar in three replicates. The mean values of the clearing zone diameter/halo formed around the PSF colony were recorded on the 5th, 10th and 15th days of incubation. The phosphate solubilization index (SI) was recorded by measuring the area of the clearing zone/halo area (Premono et al 1996).

Statistical analysis: Data were analysed using SPSS version 26.0 to perform the Duncan's multiple range test (DMRT). PAST 4.03 was utilized to calculate the number of taxa (S), individuals (N), dominance (D), Simpson (1-D), Shannon (H), Pielou's evenness (J), and Berger-Parker indices to determine the diversity, richness, evenness, and abundance of the fungal population.

RESULTS AND DISCUSSION

Soil properties: Soil temperature varied significantly among the sites, with the highest at JF, followed by SC (Table 2). This increased temperature at JF and SC may be attributed to the loss of vegetation cover at both sites and the resultant exposure of the soil layer to the sun. The highest pH was observed at SC, while the lowest was reported at NF and TP, respectively. The increased pH level at SC is attributed to the burning of the soil, which has been reported to raise pH (Temjen et al 2021). In contrast, the decreased pH levels at NF and TP may be attributed to the increased organic matter input from aboveground biomass at the sites. There was an also significant variation in soil moisture. Site NF possessed the highest moisture content. This is attributed to the increased vegetation and water-holding capacity of the site. The decreased soil moisture at SC may be due to the heating of the soil by slash and burning activities. SOC was found to be highest in BP and NF, while the lowest was reported under

Table 1. Description of study sites

Site	Vegetation	Period	Disturbances
Natural Forest (NF) 26.31203°N, 94.49328°E	*Ageratum conyzoides, Amaranthus* sp, *Angiopteris* sp, *Artemisia vulgaris, Azadirachta indica, Eupatorium* sp, *Sonchus wightianus, Macaranga denticulate, Mikania cordata, Pueraria* sp, *Terminalia myriocarpa* and *Thysanolaena maxima*	12th year of fallow	No anthropogenic disturbance
Tree plantation (TP) 26.3123°N, 94.4942° E	*Duabanga grandiflora*	5th year of plantation	Little or no anthropogenic disturbance
Bamboo plantation (BP) 26.3130°N, 94.4949°E	*Dendrocalamus* sp	5th year of plantation	Little or no anthropogenic disturbance
Jhum fallow (JF) 26.3130°N, 94.4959° E	*Ageratum conyzoides, Eupatorium* sp, *Amaranthus* sp	1st year of fallow	Little or no anthropogenic disturbance
Shifting cultivation site (SC) 26.31065°N, 94.49405°E	*Manihot esculenta*	4th cycle of cultivation	High anthropogenic disturbance

Table 2. Soil parameters across the during study sites

Site	Temperature (^{0}C)	pH	Moisture (%)	SOC (%)	N (Kg ha^{-1})	P (Kg ha^{-1})	K (Kg ha^{-1})
NF	22^{a}	5.4^{ab}	24^{d}	3.42^{c}	478^{d}	190^{c}	31^{c}
TP	23^{b}	5.1^{a}	19^{c}	3.01^{b}	316^{c}	170^{b}	23^{c}
BP	21^{a}	5.5^{ab}	17^{b}	3.37^{d}	303^{c}	169^{b}	24^{bc}
JF	24^{a}	5.6^{b}	18^{b}	2.64^{a}	240^{b}	116^{ab}	21^{b}
SC	24^{b}	5.7^{b}	16^{a}	2.47^{a}	114^{a}	98^{a}	19^{a}

*Values in the same column with different superscripts are significantly different at the 5% level by DMRT

SC. This increased SOC at BP and NF may be attributed to the increased build-up of organic matter from both root and aboveground biomass at the respective sites. Bamboos, in particular, have high carbon sequestration capacity owing to their large biomass density and fast growth rate (Devi and Singh 2021). Meanwhile, lower SOC under SC and JF may be due to high rates of continuous cultivation for an extended period without a sufficient fallow phase, reducing the amount of organic material available in the soil (Yimer et al 2007). Similar trend was reported for the macronutrients in the study, i.e., N, P, and K. We report higher values under NF, TP, and BP as compared to JF and SC. This may be attributed to the increased mineralization process under the soils of the undisturbed sites as compared to the disturbed sites, i.e., SC and JF (Temjen et al 2021).

Fungal diversity and diversity indices: Thirty-one fungal species were isolated from the various land-use sites (Table 3), and the CFU is depicted in Table 4. *Aspergillus* and *Penicillium* were the most dominant genera, while *Absidia* sp. and *Fusarium* sp. were the least occurring genera. The

Table 3. Fungal diversity of the different land-use sites

Fungal species	Land-use				
	NF	TP	BP	JF	SC
1. *Absidia* sp.*				+	
2. Acremonium murorum ***	+	+	+		
3. A. strictum *			+		
4. A candidus *	+				
5. A. flavus ****	+		+	+	+
6. A. fumigatus ***	+	+	+		
7. A. versicolor *	+				
8. *A,* ***	+	+			+
9. *Aspergillu*s sp. 1 **	+	+			
10. *Aspergillus* sp. 2	+		+		+
11.Chladosporium chaldosporiodes *****	+	+	+	+	+
12. Eupenicillium javanicum ****	+	+	+		+
13. *Fusarium* sp. *				+	+
*14. Geotrichum candidum****	+	+	+		
15. *Humicola* sp. **			+	+	
16. *Mortierella* sp. *****	+	+	+	+	+
17. *Mucor circinelloides*	+			+	+
18. M.hiemalis ****	+	+	+		
*19. Paecilomyces carneus****		+	+	+	
20. P. farinosus ****	+	+	+		+
21. Penicillium brevicompactum ***	+		+	+	
22. P.citrinum ***		+	+	+	
23. P. digitatum *	+	+			
24. *Penicillium* sp. 1 ***	+			+	+
25. *Penicillium* sp. 2 ***	+	+	+		
26. *Penicillium* sp. 3 ****	+	+	+		
27. *Penicillium* sp. 4 ***	+	+		+	
28. *Rhizopus* sp. ***	+		+	+	
29. *Scopulariopsis* sp. *	+				
30.Trichoderma viridie ***	+	+	+		
31.Trychophyton sp. ***		+	+	+	

Species distribution is categorized based on their presence across the land-use sites: ***** = very broad (present in all five sites); **** = broad (present in four sites); *** = moderate (present in three sites); ** = narrow (present in two sites); * = very narrow (present in one site) distribution of the fungi in the present study may be attributed to a combination of their host-specificity and suitable environmental conditions that enable their establishment. Further, there are observations that fungal colonies with better sporulation features dominate and colonize the culture plates better, resulting in more spores (Jena et al 2015). NF had the highest fungal taxa, followed by BP, TP, JF, and SC (Table 5). The dominance value, indicating the evenness of taxa distribution, was highest at SC and lowest at NF. The Simpson index of diversity was highest at NF, suggesting greater species dominance, while SC had the lowest. The Shannon index, reflecting species richness and evenness, was also highest in NF. Pielou's evenness index showed the highest evenness at JF and the lowest at SC. The Berger-Parker dominance index indicated that the most abundant species were more dominant at SC compared to other sites. The most fungi live in symbiotic association with their respective host, a change in land-use is expected to change the fungal community. Overall, NF exhibited the highest fungal diversity, likely due to its undisturbed nature and diverse vegetation. In contrast, SC had the lowest diversity, potentially due to anthropogenic activities like slash-and-burn practices, which lead to lower soil moisture and reduced fungal evenness. Miah et al (2010) also reported lower fungal populations in disturbed sites than in forests. The dominance of certain species at SC highlights the impact of land-use changes on fungal communities and underscores the importance of understanding the entire fungal population for ecosystem sustainability (Dai et al 2013)

Phosphate solubilizing fungi (PSF): Eight isolates were identified from different land-use sites with the capacity for phosphate solubilization. The SI varied significantly among the isolates (Table 6). The largest halo zone was observed under *Penicillium* sp. 3, while the smallest was reported under *Aspergillus* sp. 1. *Aspergillus* and *Penicillium* genera were particularly notable for their phosphate solubilizing capabilities. Turan (2006) and Alori et al (2017) also reported that these genera significantly reduced pH during phosphate solubilization. The process is understood to occur due to organic acids lowering pH or by complexing cations bound to phosphates (Johan et al 2021). The NF site also had the highest concentration of PSF, while JF and SC had the lowest concentrations. The greater abundance of PSF in NF may be attributed to diverse vegetation and increased organic matter, while the reduced PSF population in SC is likely due to high anthropogenic disturbances and burning practices, making the environment less suitable for these fungi (Temjen et al 2021).

CONCLUSIONS

The present findings identified eight fungal species capable of solubilizing phosphate, with higher PSF recorded in the natural forest and lower in shifting cultivation and *jhum*

Table 4. Fungal colony forming unit of the different land-use sites

Site	Fungal CFU
NF	76 $x10^4/g^a$
TP	40.5 $x10^4/g^b$
BP	45 $x10^4/g^b$
JF	26 $x10^4/g^c$
SC	24.5 $x10^4/g^c$

*Values with different superscripts are significantly different at the 5% level by DMRT

Table 6. Solubilization index of PSF

PSF	Phosphate solubilisation index (cm)		
	5th day	10th day	15th day
Acremonium murorum	2.56±0.12	2.78±0.02	2.84±0.23
Aspergillus sp. 1	1.02±0.23	1.42±0.13	1.53±0.22
Aspergillus sp. 2	1.05±0.53	1.65±0.02	1.71±0.31
Mucor hiemalis	1.56±0.11	1.72±0.13	1.81±0.09
Penicillium sp. 1	1.14±0.14	2.58±0.25	2.94±0.18
Penicillium sp. 2	1.81±0.13	2.22±0.36	2.52±0.17
Penicillium sp. 3	3.20±0.25	3.50±0.24	3.54±0.22
Rhizopus sp.	2.12±0.05	2.64±0.22	2.87±0.09

Table 5. Diversity indices of different land-use sites

Indices	NF	TP	BP	JF	SC
Taxa (S)	21	17	20	15	12
Individuals (N)	152	81	90	52	49
Dominance (D)	0.077	0.096	0.085	0.088	0.132
Simpson (1-D)	0.922	0.903	0.914	0.912	0.868
Shannon (H)	2.815	2.564	2.681	2.566	2.221
Pielou's evenness (J)	0.924	0.905	0.894	0.947	0.893
Berger-Parker	0.190	0.185	0.155	0.173	0.224

fallow sites. Among these, species from *Penicillium* sp. 3 demonstrated superior solubilization potential, making it a promising candidate for further evaluation. Given the rising costs and adverse effects of chemical fertilizers, PSF offers a sustainable alternative for agriculture. Developing high-quality inoculants and consistently monitoring biological resources is essential. Future research should focus on field trials and large-scale experiments to assess the viability of PSF.

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