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DEAD WOOD INHABITING FUNGAL DIVERSITY IN FOREST ECOSYSTEM ASSOCIATED WITH WOOD DECAY AND FOREST HEALTH

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ABSTRACT

Forest ecosystems harbor large amount of dead woods form of branches, snags and stumps. Deadwoods offer habitats for a variety of organisms including bacteria, fungi and vertebrates. The diversity of organism in the habitat are correlated with the health of natural forest. In the fungal diversity study of Achanakmar Bioresearve, Chhattisgarh state of India total of 53 fungal species belonging to 42 genera was recorded during the year 2018-2021. Seasonal variation and dominance analysis suggests that the diversity is greatly influenced by the seasonal change. However significant numbers of fungal species remain consistent in the habitat in all season.

Keywords: Biodiversity, fungal diversity, forest ecosystem, wood decay, enzyme assay

INTRODUCTION

Microbial diversity play an important role in natural forest ecosystem as it provide mediating nutrient and water uptake, protecting roots from pathogens and environmental extremes, and maintaining soil structure and forest food webs **[1]**. Number in fungal species increases with increasing dead tree size **[2]** and are able to maintain more stable microclimatic conditions required by some specialized species **[3]**. However, also branches with a small diameter are important to support fungal species diversity, likely because they have a high surface area per volume, thereby supporting more sporocarps compared to large logs **[4]**. Also variation in wood traits within trees can influence fungal community composition and decay rates **[5]**. For instance, different fungal communities were found in heartwood and sapwood within the same tree species after one year of wood decay **[6]**. Besides size and variation in wood traits within dead wood, also the position of the wood is affecting fungal diversity. Fungal diversity usually is overlooked during consideration of management of forest ecosystems. Foresters, ecologists, and managers are recognizing now that forest productivity, recovery and stability depend on organisms and processes belowground **[1]**. In India, forests are largely managed and it was estimated that the volume of dead wood is reduced tremendously as compared to natural forests. Such a reduction in available habitats has a negative effect on the diversity of species depending on dead wood **[7, 8]**. It has been suggested that the presence of large dead logs and the continuous supply of new dead wood is important to protect fungal biodiversity in managed forests **[9]**. Existence of fungal population in forest soil and decaying wood is correlated with the nutrient supply to the tree and are indicators of good forest health **[10-12]**. Nowadays, nature conservation organizations in many countries are creating, maintaining and managing dead wood habitats to improve the condition of the forest ecosystem **[13]**.

MATERIALS AND METHODS

Study area

The study was carried out in Achanakmar Wildlife Sanctuary forest, located in Mungeli District of Chhattisgarh India. It is situated on the east boundary of Satpura Hills and north of the Mahanadi River. The sanctuary covers an area of ≈ 551.55 square kilometres, and lies between the latitudes of 22°-24’-22°-35’N and the longitudes of 81°34’3”-81°85’ E. The vegetation of sanctuary is primarily tropical deciduous forest. Samplings were done between 2018 and 2021.All three seasons of year i.e. winter, summer and rainy was chosen for the sampling. The average minimum temperature in month of December was between 12-15°C and in May were 40. The maximum rain falls noted during the study period were 225.35 mm and 236.55 mm in 2019 and 2020 respectively. The average wind speed was between 6.0-7.4 kmph in December and 9.8-10.5kmph in May. The humidity remained between 44-48% in December and 19-20% during the month of May.

Sample collection

Wood samples were taken using a sterilised Knife and hand driven drill and were collected in properly labeled zip locked polybags. The sample covered by bryophytes, were removed before sampling and thus only woody material was included in sample. Five sub-samples were obtained per log, and sub-samples were pooled before analyses. Bigger wood pieces were broken to short pieces and in cases where it was not possible to obtain five sub-samples. Collected the minimum three numbers of sub-samples obtained per log and Kept in refrigerator until analysis.

Isolation of Fungi

Solidified Potato Dextrose Agar plate was used for isolation of fungi. The serially diluted dried and milled samples were placed on solidified Potato Dextrose Agar (PDA) plates (90mm diameter) aseptically. Inoculated plates were incubated at 28±30c for 7 days. Morphologically distinct cultures were subcultures and maintained on PDA slants.

Identification of the Fungal Isolates

One to four weeks pure cultures of the fungal isolates were identified using cultural and morphological features such as colony growth pattern, conidial morphology and pigmentation, by slide culture techniques **[14]**. The Morphological characteristics and appearance of the fungal isolates were confirmed and authenticated with the help of Color atlas of mycology by Love and Ribs **[15]**.

Molecular identification of fungal isolates

Fungal isolates were identified to its nearest species based on partial ITS sequence. Genomic DNA was isolated from the sample provided by the scientist. The ~0.6-0.8kb, ITS fragment was amplified using high–fidelity PCR polymerase. The PCR product was sequenced single direction. The sequence data was analyzed to identify the sample and its closest neighbors.

Enzyme assays

All enzyme activities were assayed in the extracts prepared as follows. MilliQ water (4 ml) was added to 1 g milled, fresh wood sample. The tubes were shaken for 1 h at room temperature. Out of this the enzyme extracts were made by pressing the moistened, milled wood over an stainless filter (pore size 2 mm dia). Filtrates were transferred to Eppendorf tubes and after centrifuging for 10 min at 10,000 rev min−1. The supernatants were frozen (−20°C) until analysis of enzyme activity. Laccase and manganese peroxidase activities were measured as indicators for lignin degradation **[16]**. Laccase activity was measured via oxidation of ABTS (2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid)) according to Bourbonnais and Paice **[17]**. Manganese peroxidase activity was measured via the oxidative coupling of DMAB (3-dimethylaminobenzoic acid) and MBTH (3-methyl-2-benzothiazolinone hydrazine hydrochloride) in the presence of Mn2+ and H_2O_2, as described by Daniel *et al*. **[18]**. One ml of the final assay mixture contained 50 mM sodium malonate (pH 4.5), 1 mMMnSO4, the culture filtrate, and DMP. Cellulose was estimated using carboxymethyl cellulose linked with Remazol brilliant blue R. The reaction mixture contained 200 µl of 2% Azo-CM-Cellulose in MQ and 200 µl enzyme extract. Samples were incubated at 40°C for 30 min and the reaction was stopped by adding 1 ml of precipitation solution (20%sodium acetate trihydrate and 3% zinc acetate in100 ml MQ). The formation of blue dye was measured spectrophotometrically at 590 nm. The conversion to activity was based on a calibration curve of Remazol brilliant blue (RBB) and expressed as mmol RBB/24 hours/gram dry weight. The activity of endo-1,4-ß-xylanase (xylanase) as indicator of hemicellulase was estimated by a similar procedure using birchwood xylan linked with Remazol brilliantblue as a substrate. The reaction mixture contained 200 µl of 2% Azo-CM-Cellulose in MQ and 200 µl enzyme extract. Samples were incubated at 40°C for 30 min and the reaction was stopped by adding 1 ml of precipitation solution (20% sodium acetate trihydrate and 3% zinc acetate in 100 ml (MQ). The formation of blue dye was measured spectrophotometrically at 590 nm. The conversion to activity was based on a calibration curve of Remazol brilliant blue (RBB) and expressed as mmol RBB/24 hours/gram dry weight.

RESULTS AND DISCUSSION

In order to study in fungal diversity in wood logs and stumps wood samples from study sited sites of Achanakmar biosphere were collected. An average of 3-4 samples were collected from each sampling sites for three years from 2018-2021 in all seasons (winter, summer and rainy). Overall 53 species belonging to 47 fungal genera were recorded in the study area **(Figure 1)**. During winter season of the first year 2018-19 it was observed that there was no clear-cut dominance of any one genus/species in the habitat. The maximum of 5.56% and minimum of 1.11% population share in the diversity was seen. *Ascocoryne sp., Dacrymyces sp*., and *Xylaria sp.,* shared 5.56% population percentage in diversity.

Following these, *Calocera sp., Hypochnicium sp.,* and *Phlebiopsis sp.,* was the second highest populated genus each with 4.44% share in diversity. Eight genera namely *Hypholoma sp., Panellus sp., Peniophorella sp., Pestolotiopsis sp., Phaeomollisia sp.,* and *Scytalidium sp.,* along with two unidentified species 3.3% of individual share in the diversity. Rest 25 out of 41 genera shared between 1.112-2.22% each in the population diversity. The case was different in the next year 2019-20 Hypholoma sp was found to be most populated genus with 7.14% frequency followed by *Dacrymyces* sp. comprising with 5.71 % share in the population. *Calocera sp*. and *Hypochnicium sp.,* both were the third most populated genus each with 4.29% population in the habitat. During this season no significant dominance was seen and the population in the diversity ranged between 1.43-7.14 % only. Next year in 2020-21 *Chloridium sp.* and *Fusarium incarnatum* were found to be most populated genus in the habitat with their individual share of 8.0% population. The second most populated genus was *Calocera* (*C. cornea, C. viscose*) with 6.67 % share. *Cinereomyces lindbladii* remained the third most populated fungal species with 5.33% existence. It appears that there is no seasonal dominance of a particular genus. It is also clear that the genus found in relatively higher population in one year is not consistent in the next year.

In summer of the first year *Hypochnicium* was found to be relatively higher population in the diversity with only 6.00 % shared in the diversity. This was followed by the fungal species belonging to eight different genera namely *Ascocoryne sp., Calocera sp., Dacrymyces sp., Infundichalara sp. Rhodonia sp., Scytalidium sp. Stereum sp.*, and 1 unidentified genus. Each of them shared 4.00 % population in diversity. Rest all belonging to 31 genera shared 2.0% population in diversity. During this season no significant dominance by single/few genus was noted. Second year in 2019-20 *Mycema galapus* was the highest (8.0%) populated fungal species recorded during this season, which was followed by *Hypochnicium sp.* along with one unidentified genus. Each of them had 6.0% population share in the diversity. Among remaining 31 genera 9 member had their individual share of 4.0% and the other 22 had individual share 2.0 % in the diversity. *Chloridium sp.* and *Fusarium incarnatum*, each with 6.25% individual share was the top most populated species/genus in the habitat in the third year and followed by *Ascocoryne* (*A. cylichnium, A. sarcoides*) and *Calocera*

(C. cornea and *C. viscose*) 5.0% being second most populated genus of the habitat. Rest all (35 out of 38) present in the habitat shared 84.75% population with individual share percentage from 3.75 to 1.25 %. This indicates that there was no clear cut dominating species / genus present in the habitat during the study season.

In rainy season of the first year frequency in the population ranged from 1.43 - 7.14% population. There was no significantly big figure to show the dominancy of particular species or genus during the season. However, *Hypochnicium* (7.14%) were found to be relatively higher in population among others. This was followed by *Ascocoryne* (5.71%) with two species *A. sarcoides* and *A. cylichnium* and *Dacrymyces sp.* and *Scytalidium sp*. were the third most populated genus sharing 4.29 % each. Almost 79% of the population of in the diversity was shared by 38 genus and 41 species with their individual share between 1.43 – 2.86 % only. Next year the percentage population of these species ranged between 1.43% to 7.14%. Genus *Calocera* (*C. cornea and C. viscose*) was found to be highest populated (7.14%) in the habitat. This was followed by *Scytalidium sp*. (5.71%) and *Panellus sp*., along with an unidentified genus with their individual share of 4.29%. Rest all 35 genera were populated in the range between 1.43 to 2.86% with total sharing of 78.57 % population stating that there was no significant dominance of any single species/genus in the habitat. Whereas in the third year *Hyphodontia sp*. (*H. radula, H. capnoide* and, *H. fasciculare*) was relatively highest populating genus contributing 7.78% in the habitat. The second most populating was *Fusarium incarnatum*, 5.56%, whereas, *Infundichalara sp*., was a third most populating (4.44%) genus in the population. Rest 82.22% population was contributed by species of 37 different fungal genera. This indicates that there is no clear-cut dominancy of single species/genus and the diversity was almost equally contributed during the study period.

Based on the observation and critical data analysis it was noted that a significant numbers of fungal species belonging to various genera were consistently present throughout the year during the study period and the seasonal changes mealy influenced their existence. Among those the highest populated fungi was subjected to molecular characterization and identified as *Fusarium incarnatum* **(Figure 2)**. The following is the ITS sequence (492bp) of the test organism.

TTGGACATACCTATACGTTGCCTCGGC
GGATCAGCCCGCGCCCCGTAAAACGG

GACGGCCCGCCCGAGGACCCCTAAAC
TCTGTTTTTAGTGGAACTTCTGAGTAA
AACAAACAAATAAATCAAAACTTTCA
ACAACGGATCTCTTGGTTCTGGCATCG
ATGAAGAACGCAGCAAAATGCGATAA
GTAATGTGAATTGCAGAATTCAGTGA
ATCATCGAATCTTTGAACGCACATTGC
GCCCGCCAGTATTCTGGCGGGCATGC
CTGTTCGAGCGTCATTTCAACCCTCAA
GCTCAGCTTGGTGTTGGGACTCGCGGT
AACCCGCGTTCCCCAAATCGATTGGC
GGTCACGTCGAGCTTCCATAGCGTAG
TAATCATACACCTCGTTACTGGTAATC
GTCGCGGCCACGCCGTTAAACCCCAA
CTTCTGAATGTTGACCTCGGATCAGGT
AGGAATACCCGCTGAACTTAAGCATA
TCTAAAGGGCGGAGGA

Out of 53 species belonging to 42 different fungal genera 51% were found laccase positive **(Figure 3)**. Two species each of genera *Ascocoryne), Calocera, Dacrymycesi* and *Stereum species* and 4 out of 5 of *Hypochnicium* along with *Heterobasidion annosum, Mollisia sp., Fusarium incarnatum, Peniophorella praetermissa, Phaeomollisia piceae, Postia tephroleuca, Rhodonia placenta, Sistotrema brinkmannii* and *Skeletocutis amorpha* were found laccase positive. Manganese peroxydase, another enzyme involved in wood decay was found in 47% of the diversity in the population. Among those all isolates of genera *Ascocoryne, Scytalidium, Calocera* and one unidentified genera were Mn peroxydase positive. Two out of 5 species of genera *Hyphodontia* along with *Lenzites, Stereum, Chloridium, Fibulomyce, Heterobasidion, Lenzites, Moristroma, Pezicula, Phlebia, Postia , Skeletocutis , Fibulomyce , Xylaria* were Mn peroxydase active. A 57% species belonging to 29 genera were found cellulase positive. Among those one each of genus *Ascocoryne, Calocera, Dacrymyces, Lenzites, Scytalidium,* and *Stereum* was cellulase positive. Two out of five species of *Hypholoma* and nineteen more species belonging to *Athelia, Candida, Cinereomyces, Daedalea, Gymnopilus, Infundichalara, Mollisia, Nodulisporium, Panellus, Pestolotiopsis, Phaeomollisia, Phlebiopsis, Resinicium, Rhodonia, Serpula, Sistotrema, Sordariomycetes, Phlebiopsis, Xylaria* were cellulase positive. Similarly, 57% species belonging to 27 genera exhibited hemicelluloses activity. Four species of *genus Hyphodontia,* one each from genus *Ascocoryne, Dacrymyces, Stereum* and twenty two other species belonging to genera *Athelia, Candida, Chloridium, Cinereomyces, Dacrymyces, Fibulomyces, Heterobasidion, Infundichalara, Megacollybia, Fusarium,* *Panellus, Peniophorella, Pestolotiopsis, Pezicula, Phlebiopsis, Resinicium, Rhodonia, Serpula, Skeletocutis, Stereum, Fibulomyces, Xylaria were* found hemicellulase positive. It was noted that 4% of the total species were producing all four enzymes in test, 24.5% species were positive for three enzymes, 50% of the fungal species produced two enzyme and only 21% of species in the diversity population were active for single enzyme tested.

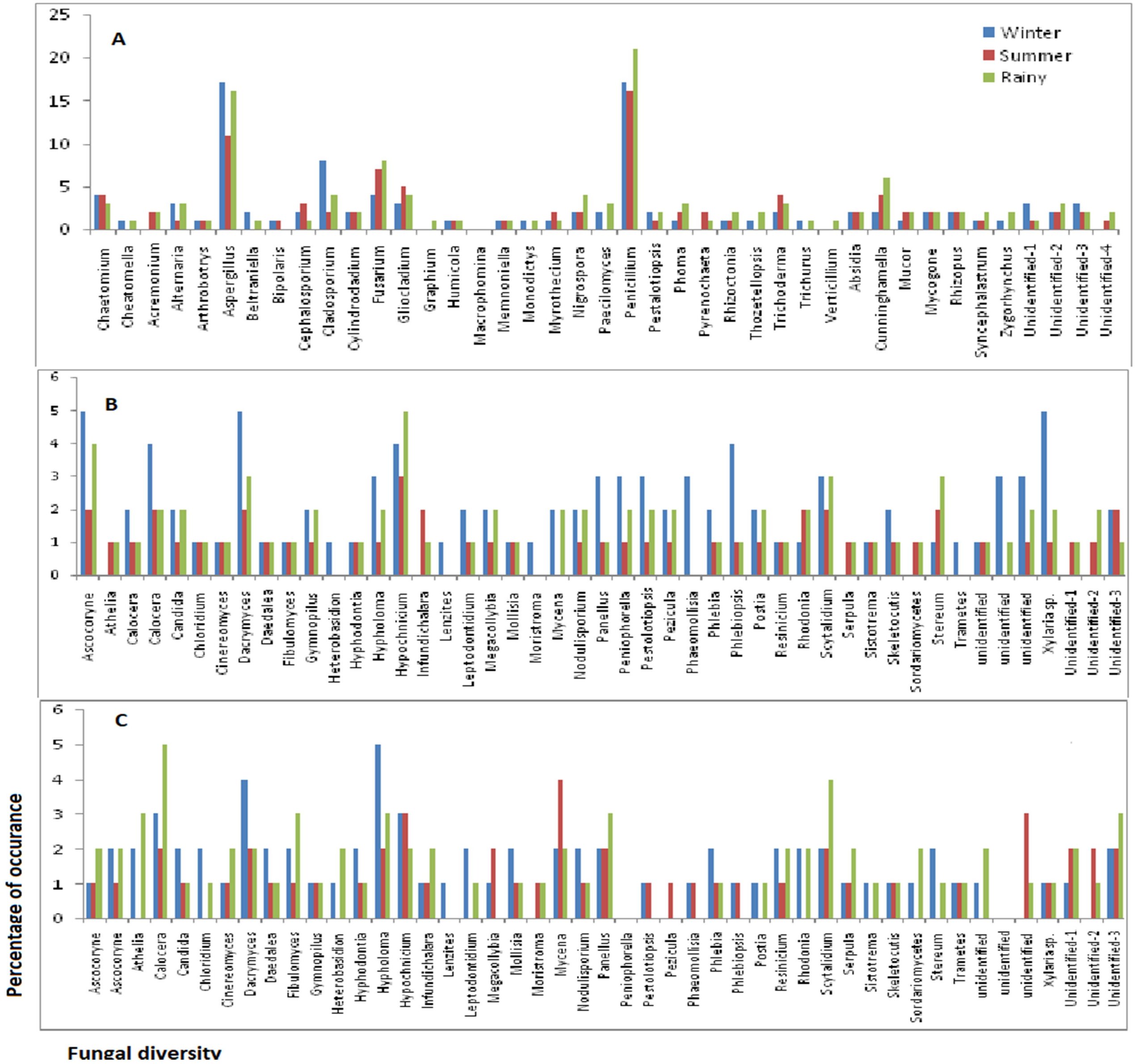

Figure 1: Fungal diversity percentage in the population (A) year 2018-2019 (B) year 2019-2020 (C) year 2020-2021

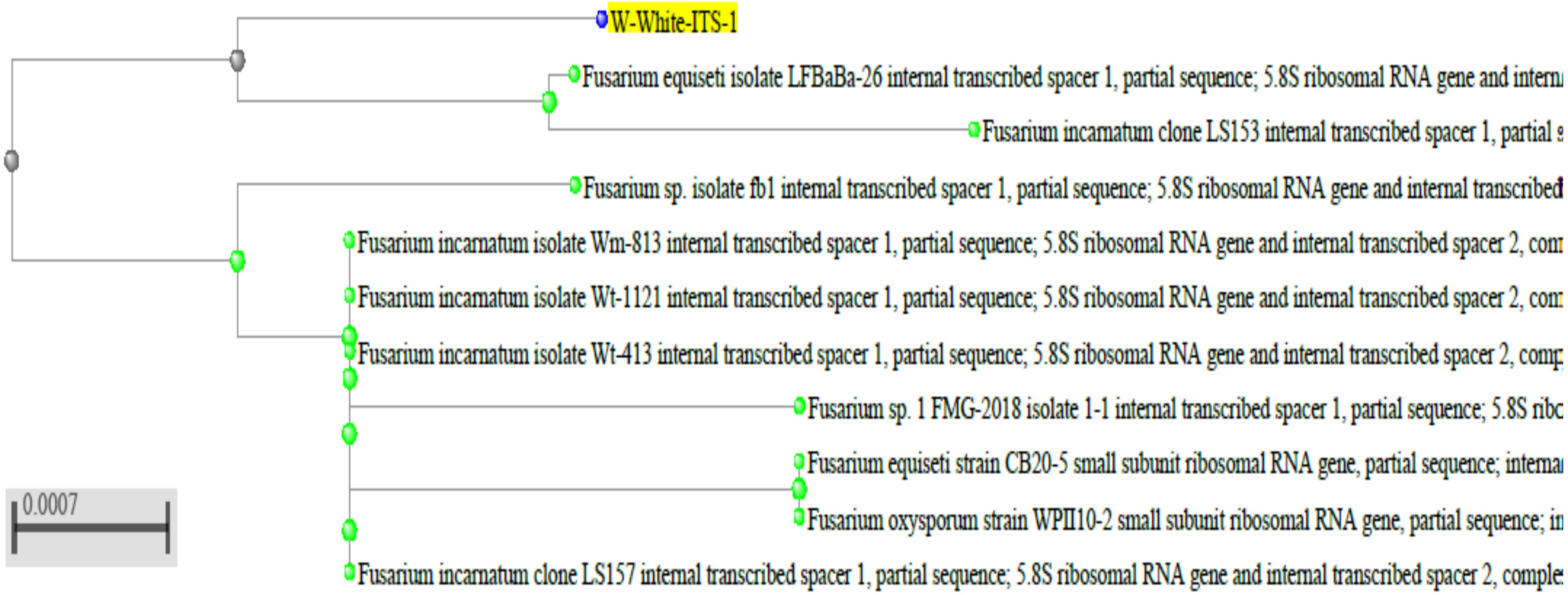

Figure 2: Phylogenetic tree of white fungus (*Fusarium incarnatum*)

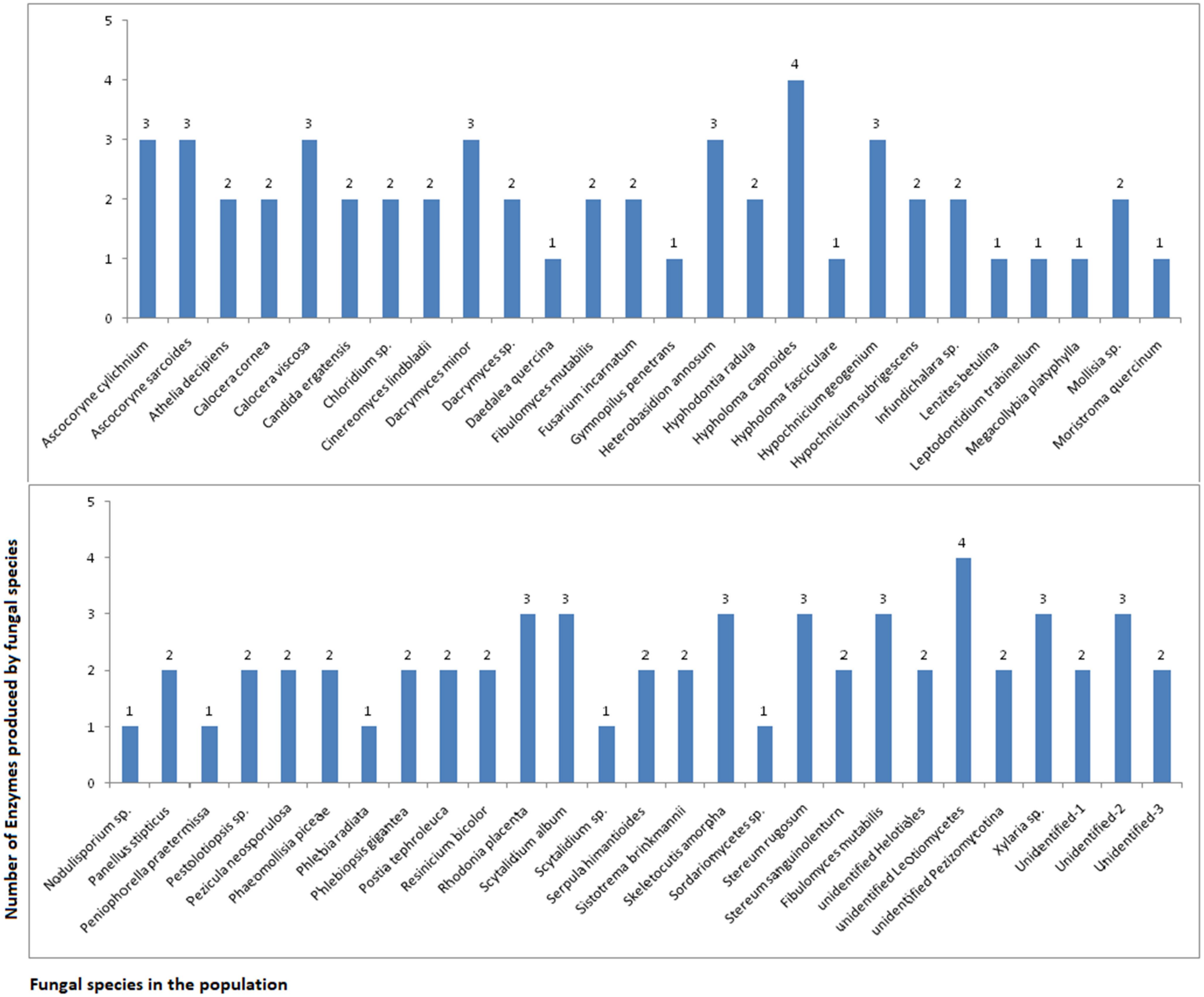

Figure 3: Wood decaying production pattern among the fungal population

CONCLUSION

Fungal diversity in the study site revealed 53 fungal species belonging to 46 genera suggest that the forest ecosystem is rich in fungal diversity. The effect of seasonal variation on the diversity is partially influenced. Dominance of species/genera in the population is not so clear with the data. However, there are few species that remained persistent throughout the study period. Correlation of diversity richness and forest health is still to be studied for study site.

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REFERENCES

[1] Amaranthus, Michael P. 1998. The importance and conservation of ectomycorrizal fungal diversity in forest ecosystems: lessons from Europe and the Pacific Northwest. Gen. Tech. Rep. PNW-GTR-431. Portland, OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station. 15p.

[2] Heilmann-Clausen, J. and Christensen, M. (2004) Does size matter?: On the importance of various dead wood fractions for fungal diversity in Danish beech forests. *Forest Ecology and Management* 201(1):105-117.

[3] Nordén, M. Ryberg, F. Götmark, B. Olausson (2004) Relative importance of coarse and fine woody debris for the diversity of wood-inhabiting fungi in temperate broadleaf forests Biol. Conserv., 117, 1-10.

[4] Stokland J., H. Kauserud (2004) Phellinus nigrolimitatus—a wood-decomposing fungus highly influenced by forestry For. Ecol. Manage., 187, 333-343.

[5] Van der Wal A, E. Ottosson, W. de Boer (2015) Neglected role of fungal community composition in explaining variation in wood decay rates Ecology, 124-133.

[6] Van der Wal A., P.J. Klein Gunnewiek, J.H.C. Cornelissen, T.W. Crowther, W. Boer (2016) Patterns of natural fungal community assembly during initial decay of coniferous and broadleaf tree logs Ecosphere, 7 (2016) 1393.

[7] Penttilä, R. Siitonen, J. Kuusinen. M. (2004) Polypore diversity in managed and old-growth boreal Picea abies forests in southern Finland Biological conservation, 117, 271-283.

[8] Ódor P., J. Heilmann-Clausen, M. Christensen, E. Aude, K. Van Dort, A. Piltaver, I. Siller, M. Veerkamp, R. Walleyn, T. Standovár (2006), Diversity of dead wood inhabiting fungi and bryophytes in semi-natural beech forests in Europe Biol. Cons., 131, 58-71.

[9] Heilmann-Clausen, J. and Christensen, M. (2003) Fungal diversity on decaying beech logs–implications for sustainable forestry. Biodivers. Conserv. 953-973.

[10] Rajala Tiina, Peltoniemi Mikko, Pennanen Taina, and Ma kipaa Raisa (2010) Relationship between wood-inhabiting fungi determined by molecular analysis (denaturing gradient gel electrophoresis) and quality ofdecaying logs Can. J. For. Res. 40: 2384–2397.

[11] Bebber DP, Watkinson SC, Boddy L, Darrah PR. (2011). Simulated nitrogen deposition affects wood decomposition by cord-forming fungi. Oecol 167: 1177–1184.

[12] Philpott TJ, Prescott CE, Chapman WK, Grayston S.J. (2014). Nitrogen translocation and accumulation by a cord forming fungus (*Hypholoma fasciculare*) into simulated woody debris. For Ecol Manag 315: 121–128.

[13] François Kremer (2015) What does it mean to have a forest in a Natura 2000 area? Natura 2000 and Forests, Part III-Case study Luxembourg: Office for Official Publications of the European Communities.

[14] Oyeleke, A. and Manga, S.B. (2008).*Essential of Laboratory Practice*, 3rd edition, Tobest Publishers, Minna, Niger State, Nigeria, Pp.12-29.

[15] Love, Gordon L. and Ribes Julie A. (2018) Color Atlas of Mycology Pub College of American Pathologists.

[16] Leonowicz, A., Matuszewska, A., Luterek, J., Ziegenhagen, D., Wojtas-Wasilewska, M., Cho, N. S., et al. (1999). Biodegradation of lignin by white rot fungi. *Fungal Genet. Biol.* 27, 175–185.

[17] Bourbonnais, R. and Paice, M.G. (1990) Oxidation of Non-Phenolic Substrates—An Expanded Role for Laccase in Lignin Biodegradation. FEBS Letters, 267, 99-102.

[18] Daniel G, Volc J, Kubatova E (1994) Pyranose oxidase, a major source of H2O2 during wood degradation by *Phanerochaete chrysosporium, Trametes versicolor* and *Oudemansiella mucida Appl. Environ. Microbiol.* 60: 2524–2532.