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Aging increases arbuscular mycorrhizal fungal diversity in iron ore mine sites

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ABSTRACT

Arbuscular mycorrhizal (AM) fungal colonization, spore density, and AM fungal richness were assessed with mine age. Iron ore mines with ages ranging from 10 to 50 years located in the state of Goa constituted the study sites. Most of the selected plant species assessed from recently mined areas also had higher colonization levels. However, AM fungal spore density increased with the increase in the age of mines. Spores of a total of 39 AM fungal species were isolated and characterized from various mine sites. Among the AM fungal species *Acaulospora spinosa* was most dominant (30-90%) followed by *Glomus macrocarpum* (20-80%), *Acaulospora scrobiculata* (60%), *Racocetra gregaria* (20-60%), *Cetraspora pellucida* (30-50%), and *Gigaspora margarita* (10-40%). Shannon's diversity index was highest at the oldest mine site and least at the recently mined site. In contrast, Simpson's dominance index was highest in the recently mined area and least at the oldest site. Diversity indices were more significant in the well-established mine than in the recently degraded mine. The study indicates that the severity of disturbance, the harshness of the site, low inoculum levels, edaphic characteristics, and time are known to influence the rate of AM fungi. Thus, revegetation of any disturbed site can occur over time with high species richness and diversity of AM fungi. They are essential in establishing a healthy plant community and facilitating plant succession.

Keywords: Arbuscular mycorrhizal fungi, Disturbance, Diversity, Mine spoils

INTRODUCTION

Ecosystem destruction by mining for minerals to meet the demand of industries is an inevitable part of civilization (Sonter *et al.,* 2018). Mining renders the land unproductive, and the situation is particularly alarming in the tropical areas where the mining areas are on or near a forest. The mineral extraction drastically alters the physical and biological nature of the mining area (Žibret *et al.,* 2018). The establishment of vegetation on mined soils is hindered by physical factors such as high temperature, low availability of soil moisture, uncertain structure, unstable slopes due to hilly terrain and compaction, low organic matter, and low levels of plant-available nutrients, particularly phosphorus (P), nitrogen (N), and potassium (K) (Singh *et al.,* 2002). The most common response to the resurgence of ecological disturbance has been abandonment or dependence on natural succession to replenish lost soil fertility, species richness, and biomass productivity (Dybzinski *et al.,* 2008; Furey and Tilman, 2021). However, since the topsoil is removed, soil seed banks and rootstocks are eliminated, and the soil profile is disturbed, the process of natural succession on surface-mined soils is very slow (Ahirwal and Maiti, 2016; Chaturvedi and Singh, 2017; Feng *et al.,* 2019).

The mutualistic relationship of AM fungi supports the successional establishment of vegetation in widely diversified environments such as abandoned agricultural fields (Hedlund, 2002), mine sites (Van der Heijden *et al.,* 2008; Yang *et al.,* 2017, Kumar *et al.,* 2010), and, desert soils (Wu *et al.,* 2007; Apple, 2010). Thus, they play a vital role in the sustainable conservation of tropical gene pools and diversity. Furthermore, through years of succession, evolution, selection, and co-existence, AM fungi have helped refine the soil quality, texture, structure, fertility, and compatibility to suit the indigenous plant species. The microbial component, especially AM fungi of the plant rhizosphere, endows major tasks in determining plant diversity and helps in stabilizing the highly complex diversity regime of the tropical forests.

The Indian state of Goa has been a prime exporter of iron ore since the 1950s. Around 18% of Goa's land area (66,300 ha of 370,000 ha) has been leased out for open cast mining of iron ores in the northern parts of Goa. This indiscriminate mining, mainly in the forest areas, has destroyed around 50,000 ha of forests (D'Souza and Nayak, 1994). Several studies have investigated the AM fungal occurrence and distribution in iron ore mining sites (Teixeira *et al.,* 2017; Prabhu and Rodrigues, 2019; Rodríguez *et al.,* 2021). Sastri and Johri (1999) reported the diversity of AM fungi from iron ore sites in the Bastar region of Madhya Pradesh, India. These authors found natural AM colonization levels between 25% and 90% from various soils and recovered 89 AM fungal species.

Suppose reclamation programmes allow for the rapid establishment of mycorrhizae in these highly disturbed habitats, then information is needed on the AM fungal species diversity that occurs at such sites. There are several reports on the role of AM fungi in the revegetation of mining (Hazarika *et al.,* 2010, 2014; Agus *et al.,* 2019). It has been reported that soil disturbances associated with mining activity reduce the colonization of AM fungi in vegetation to different extents, depending upon the mining operation and environment (Agus *et al.,* 2019). Reports on the successional establishment concerning AM fungal association from mine wastelands are scarce (Kikvidze *et al.,* 2010; Logaprabha and Tamilselvi, 2014). Thus, the present investigation attempts to study the colonization, species richness, and spore density of AM fungi associated with some dominant and commonly occurring plant species from iron ore mines of varying ages.

MATERIALS AND METHODS

The investigation was carried out at four iron ore mine sites viz., Dadivaril Sodo "Xelpi" mine situated in Satari (15°29'45" N and 74°7 '49" E) in northern Goa., where mining started ten years ago in about 100 ha with an area of 45 ha under active mining. Sonshi, where mining started 35 years ago, is situated in Satari (15°32'15" N and 74°3'46" E) in northern Goa, spread over 62 hectares, with the entire area

under mining. Codli is situated in Sanguem (15°20'53" N and 74°8'33" E) in southern Goa, where mining started 30 years ago in about 300 ha with an actual area of 290 ha under mining. Sanquelim, a 50-year-old mine situated at Sanquelim in Bicholim, (15°34'2" N and 74°1'12" E) in northern Goa. This mine is spread over an area of 203.5 hectares with an actual size of 102 hectares under mining.

Sample collection

Plant roots and soil samples of 10 species were collected during the early monsoon from July to August from all the sites. The selected species were common to all four sites. Identification of plant species was carried out using relevant floras (Rao, 1985, 1986; Matthew, 1991; Mohanan and Henry, 1994; Naithani *et al.,* 1997).

Care was taken while collecting individual plants so that roots could be identified as belonging to the particular plant. For this, herbs were usually dug out, and samples of trees or shrubs usually were made from saplings if available, or the roots were traced back to the stem. The collected roots were transported to the laboratory for processing. Rhizosphere soil shaken from roots and adjacent to roots was collected. Soil samples collected from individual plants for a species were packed individually in polyethylene bags and stored at 4^0C until processing. The soil samples were used for assessing soil chemistry, enumeration, and isolation of AM fungal spores.

Determination of soil characters

Soil pH was determined at 1:1, soil: water soon after the soil samples were brought to the laboratory. Electrical conductivity (EC) was determined in 1:1 water: waste extracts (Bower and Wilcox, 1965). The total N and available P were determined according to Jackson (1971), and exchangeable K was determined after extraction with ammonium acetate (Jackson 1971). Organic matter was determined by Walkley and Black's rapid titration method (Jackson, 1971).

Assessment of AM colonization and spore densities

Roots were observed under a dissection microscope for AM fungal spores attached to roots. After examination, the roots were cut into 1 cm bits, cleared in 10% KOH, acidified with 5N HCl, and stained with Trypan blue in lactophenol (Phillips and Hayman, 1970). The roots were kept overnight immersed in the staining solution. Roots that remained dark after clearing were bleached in alkaline H_2O_2 before acidification. The stained roots were examined under a compound microscope (200-400) for the presence of AM fungal structures. The percentage of root length colonization was estimated according to the magnified intersection method (McGonigle *et al.,* 1990).

Twenty-five grams of rhizosphere soil of each plant was assayed for spore count using wet sieving and decanting technique (Gerdemann and Nicolson, 1963). Estimation of spore density was carried out according to Gaur and Adholeya (1994). Sporocarps and spore clusters were considered as one unit. Intact AM fungal spores were transferred using a needle on a wet filter paper for establishing trap cultures or to polyvinyl alcohol-lactoglycerol (PVLG) (Koske and Tessier, 1983) with or without Melzer's reagent on a glass slide for identification.

Identification of AM fungal species

Spore colour was examined under Leica stereomicroscope using intact spores immersed in water. Intact and crushed spores in PVLG with or without Melzer's reagent were examined under a Leica compound microscope and identified based on spore morphology and sub-cellular characters (Almeida and Schenck, 1990; Schüßler and Walker, 2010; Rodrigues and Muthukumar, 2009; Redecker *et al.,* 2013), and compared with original descriptions available at http://www.amf-phylogeny.com/. Voucher specimens of AM fungi have been retained in the Botany Department, Goa University, Goa. AM fungal nomenclature follows http://www.speciesfungorum.org.

Establishment of Trap cultures

Trap cultures were established from fresh field rhizosphere soils collected from all four sites. The soil samples were mixed with autoclaved sand in a ratio of 1:2 (v/v) and filled in 15-cm diameter pots. Pots were planted with one of the four hosts (i) *Eleusine coracana* (L.) Gaertn, (ii) *Solanum lycopersicum* L. (iii) *Allium cepa* L., and (iv) *Coleus* sp. Pots were irrigated from the top when the top one cm of the surface became dry. After three months, the plants were cut back to the soil surface and reseeded with the same host. The plants were allowed to grow for an additional three months, at which time they were sampled to determine AM fungal species. The spores isolated from the trap cultures were later used to confirm the identified spores recovered during the study period.

Determination of AM fungal diversity

The diversity of AM fungi at four sites was assayed based on diversity indices (Simpson, 1949, Shannon and Wiener, 1949).

Species richness is the number of species present in a particular site.

Simpson's index is an index of dominance since the maximum value is obtained when there are only one or few species.

Simpson's index: $D' = \Sigma (pi)^2$

Shannon Wiener index (H') is an index of diversity in which the higher the value, the greater the diversity, less the site is dominated by one or few species $H' = \Sigma (pi \ln pi)$

Where, pi is the proportion of individuals that species i contribute to the total.

Species evenness (J') indicates the distribution of the individuals within species designations

$$J' = \frac{H'}{H'_{max}}$$

Where H' max is the maximum value of diversity for the number of species present.

The frequency (%) of each species was calculated as (si/S)

100; where si = number of soil samples containing spores of the ith species and S = total number of soil samples examined.

Relative abundance (%) of each species in each soil sample was calculated as (ni/N) 100; where ni = the number of spores from the ith species and N = the total number of spores examined from the sample.

Statistical analysis

The data on mycorrhizal colonization were arcsine square-root transformed, and spore numbers were log-transformed prior to statistical analysis. Data on soil factors, AM colonization, and spore numbers were subjected to Analysis of Variance (ANOVA) to investigate the influence of mine age. The means were separated using Duncan's Multiple Range Test (DMRT).

RESULTS

Soil characteristics: Soil pH, EC, N, P, K, and organic matter varied significantly (P< 0.01) between the sites. Soil N and organic matter increased with mine age except at the Sonshi mine (**Table 1**).

Table 1: Soil characteristics at iron ore mines of varying ages

			Nutrients (mg 100g^{-1})			
	pH	**EC**	**N**	**P**	**K**	**OM (%)**
Xelpi (10*)	6.48 b (0.04)	0.08 c (0.002)	145.0 d (2.45)	145.2 d (3.96)	32.6 a (1.82)	0.03 a (0.003)
Codli (30*)	6.06 a (0.09)	0.122 d (0.004)	47.2 b (2.05)	121.2 b (2.77)	55.6 c (2.51)	0.386 c 0.024)
Sonshi (35*)	6.04 b (0.13)	0.070 c (0.001)	36.49 a (4.16)	138.6 c (3.05)	56.40 c (2.61)	0.189 b 0.021)
Sanquelim (50*)	7.74 c (0.13)	0.056 a (0.002)	68.20 c (4.32)	107.40 a (7.70)	43.20 b (3.42)	0.506 d (0.027)
F Statistics	**34.94****	**83.05****	**77.75****	**62.66***	**91.13****	**505.4***

Significance at: ** - P< 0.01
In a column, means followed by the same letter are not significantly different according to DMRT (P< 0.05).
Values in parenthesis indicate Standard deviation
*- indicates the age of mine in years
EC - Electrical conductivity, N - Nitrogen, P - Phosphorus, K - Potassium, OM- Organic matter

AM fungal colonization: AM colonization levels significantly varied between plant species at all the four sites (F9,40 = 83.54; P<0.01). Mining activity had a significant effect on AM colonization levels at all the sites (F3, 40 = 224.82 P<0.01), and the interaction plants sites were also significant (F27, 40= 49.33; P<0.01). AM colonization levels in all the plant species were significantly lower in highly disturbed sites. In contrast, no significant impact of disturbance on AM colonization levels was observed in plants at Sanquelim sites. Further, the colonization levels in Sanquelim, the oldest mine site, were significantly higher than in the other three mine sites. The colonization levels of selected plant species at Xelpi ranged from 10% to 45%, Codli 09% to 50%, and Sonshi between 07% and 73%. Likewise, AM colonization levels ranged between 20% and 72% in plant species at the Sanquelim mine site (**Table 2**).

AM fungal spore numbers: AM fungal spore numbers varied significantly between the selected plant species at all four sites (F9, 40 = 52.54; P<0.01). Spore numbers were significantly affected by mining activity at all four sites (F3, 40 = 355.28; P<0.01), and the interaction plants sites were also significant (F27, 40= 12.95; P<0.01). Disturbance reduced spore numbers by one to several folds in all the sites. Spore numbers in soils of selected species ranged from 5 to 14 spores 25g^{-1} soil in Xelpi, 9 to 34 spores 25g^{-1} soil in Codli, 12 to 55 spores 25g^{-1} soil in Sonshi, and 12 to 122 spores 25g^{-1} in Sanquelim (**Table 3**).

AM fungal species composition: In the present study, 39 AM fungal species belonging to 13 *Glomeromycota* genera *viz., Acaulospora* (7), *Archaeospora* (1), *Cetraspora* (1), *Claroideoglomus* (2), *Dentiscutata* (2), *Funneliformis* (3), *Gigaspora* (3), *Glomus* (12), *Racocetra* (3), *Rhizoglomus* (1), *Rhizophagus* (2), *Septoglomus* (1), and *Scutellospora* (1) were recorded from the four mine sites with species number given in parenthesis (**Table 4**). Of these 39 species recovered, six were specific to Sanquelim, three were specific to Sonshi mine sites, and nine were common to all the sites. Though trap cultures provided fresh, healthy spores for identification, they did not contain any additional AM fungal species other than those in the field samples.

Pooled data indicated that *A. spinosa* was most dominant (30-90%), followed by *G. macrocarpum* (20-80%), *A. scrobiculata* (60%), *R. gregaria* (20-60%), *C. pellucida* (30-50%), and *Gi. margarita* (10-40%). Of the several AM fungal species recovered from the four study sites, *A. spinosa, A. scrobiculata, Rh. fasciculatus, G. macrocarpum, F. geosporus, C. pellucida, Ra. gregaria, Ra. weresubiae* and *Gi. margarita* was common in all the study sites (**Table 4**).

AM fungal diversity: AM fungal species richness, Shannon's diversity index, and species evenness was higher at the 50-year-old Sanquelim mine, whereas the same diversity indices were least at the 10-year-old Xelpi mine. However, Simpson's dominance index was greater in a 10-year-old mine than in a 50-year-old mine site (**Fig.1**). In all the four mine sites, maximum species diversity was observed in the genus *Glomus*, followed by *Acaulospora*.

DISCUSSION

The present study indicates that spore diversity and AM colonization are influenced by the age of the mine, host species, and soil type. There were significant differences in soil properties between different mine sites. Soil N and organic matter increased with mine age. The difference in AM fungal population at the study sites may be attributed to differences in the chemical properties of the soil (Ong *et al.,* 2012; Dhumal and Shinde, 2020). According to Kardol and Wardle (2010), revegetation is presumed to enhance soil fungal diversity of target mine wastelands by increasing the diversity of microhabitats and food resources.

Table 2: Arbuscular mycorrhizal colonization in plant species at the study sites.

Plant species	Family	Root colonization (%)			
		Xelpi (10)*	Codli (30)*	Sonshi (35)*	Sanquelim (50)*
Acacia auriculiformis A. Cunn. ex Benth	Mimosaceae	28.00 bc	9.00 d	7.00 f	35.00 e
Acacia mangium Willd	Mimosaceae	17.00 d	13.00 d	26.00 d	60.00 b
Anacardium occidentale L.	Anacardiaceae	45.00 a	50.00 a	17.00 c	70.00 a
Calotropis gigantea (L.) R. Br	Asclepiadaceae	33.00 b	23.00 c	41.00 c	50.00 cd
Calycopteris floribunda (Roxb.) Lam.	Combretaceae	17.00 d	33.00 b	24.00 d	57.00 bc
Chromolaena odoratum (L.) King & Robinson	Asteraceae	43.00 a	23.00 c	60.00 b	72.00 a
Ischaemum semisagittatum Roxb.	Poaceae	40.00 a	33.00 b	40.00 c	51.00 cd
Mimosa pudica L.	Mimosaceae	40.00 a	38.00 b	73.00 a	49.00 d
Terminalia crenulata Roth.	Combretaceae	10.00 a	25.00 c	60.00 b	20.00 f
Trema orientalis (L.) Blume	Ulmaceae	23.00 c	20.00 c	70.00 a	33.00 e

F statistics	d.f.	F Stats
Plant Species (S)	9	83.54 **
Mine sites (M)	3	224.82**
S X M	27	49.33**

Significant at : ** - P< 0.01.
In a column, means followed by a common letter are not significantly different according to DMRT (P<0.05).
*- indicate age of the mine in years
d. f. - Degrees of freedom.

Table 3: Arbuscular mycorrhizal spore density in plant species at the study sites

Plant species	Spores 25 g $^{-1}$ soil			
	Xelpi (10)*	Codli (30)*	Sonshi (35)*	Sanquelim (50)*
Acacia auriculiformis	7.00 c	9.00 e	12.00 f	12.00 f
Acacia mangium	12.00 ab	15.00 bcd	16.00 ef	20.00 e
Anacardium occidentale	11.00 ab	20.00 b	26.00 cd	122.00 a
Calotropis gigantea	9.00 bc	11.00 de	33.00 bc	47.00 bc
Calycopteris floribunda	11.00 ab	15.00 bc	20.00 de	28.00 d
Chromolaena odoratum	14.00 a	30.00 a	55.00 a	101.00 a
Ischaemum semisagittatum	5.00d a	34.00 a	38.00 b	44.00 bc
Mimosa pudica	9.00 bc	30.00 a	35.00 bc	51.00 b
Terminalia crenulata	12.00 ab	13.00 cd	30.00 bc	42.00 bc
Trema orientalis	10.00 bc	16.00 bc	37.00 b	36.00 cd

F statistics	d.f.	F Stats
Plant Species (S)	9	52.54 **
Mine sites (M)	3	355.28 **
S X M	27	12.95**

Significant at: ** - P< 0.01.
In a column, means followed by a common letter are not significantly different according to DMRT (P<0.05).
*- indicate age of the mine in years
d. f. - Degrees of freedom.

Table 4: Frequency and relative abundance of arbuscular mycorrhizal fungi at the study sites

AM fungal species	Frequency (%)				Relative abundance (%)			
	Xelpi	Codli	Sonshi	Sanquelim	Xelpi	Codli	Sonshi	Sanquelim
Acaulospora								
A. foveata Trappe & Janos	10	-	-	20	2.0	-	-	1.12
A. laevis Gerd. & Trappe	-	10	-	10	-	0.52	-	0.40
A. mellea Spain & Schenck	-	-	10	-	-	-	0.33	-
A. morrowiae Spain & Schenck			-	10	-	-	-	0.40
A. rehmii Sieverding & Toro	-	-	10	-	-	-	0.66	-
A. scrobiculata Trappe	60	60	60	60	34.4	17.10	15.40	13.52
A. spinosa Walker & Trappe	70	90	80	30	23.0	15.54	20.36	9.69
Archaeospora								
Archaeospora undulata (Sieverd.) Sieverd., G.A. Silva, B.T. Goto & Oehl	-	-	10	10	-	-	0.66	0.20
Cetraspora								
C. pellucida (Nicol. & Schenck) Oehl, Souza & Sieverd	30	40	50	50	8.0	6.74	8.44	7.75
Claroideoglomus								
C. claroideum (N.C. Schenck & G.S. Sm.) C. Walker & A. Schüßler	-	30	10	20	-	2.07	0.33	1.74
C. etunicatum (W.N. Becker & Gerd.) C. Walker & A. Schüßl er	-	30	10	20	-	3.63	0.83	0.65
Dentiscutata								
D. nigra (Redhead) Sieverd, Souza & Oehl	-	-	10	-	-	-	0.33	-
D. reticulata (Koske, Miller & Walker) Sieverd., Souza & Oehl	-	10	20	20	-	0.52	2.98	0.80
Funneliformis								
*F. monosporum (*Gerd. & Trappe) Oehl, Silva & Sieverd	-	10	20	10	-	1.19	1.16	0.50
F. mosseae (Nicol. & Gerd.) Walker & Schuessler	-	-	10	-	-	-	0.33	-
F. geosporus (Nicol. & Gerd.) Walker & Schuessler	10	50	40	40	2.0	9.84	2.65	4.67
Gigaspora								
G. albida Schenck &Smith	-	10	10	10	-	1.04	0.66	0.20
G.decipiens Hall & Abbott	-	-	10	-	-	-	0.33	-
G. margarita Becker & Hall	30	10	40	40	4.0	0.78	4.97	4.98
Glomus								
G. clavispora (Trappe) Almeida & Schenck	-	-	-	10	-	-	-	0.99
G. coremioides (Berk. & Bromme) Morton & Redecker	-	-	10	10	-	-	0.33	0.20
G. formosanum Wu & Chen	-	-	-	10	-	-	-	0.60
G. globiferum Koske & Walker	-	-	10	20	-	-	0.66	1.39
G. glomerulatum Sieverding	-	10	10	10	-	0.52	0.33	0.40
G. hoi Berch & Trappe	-	-	-	20	-	-	-	0.60
G. macrocarpum Tul. & Tul.	20	80	70	60	9.0	21.37	15.65	9.05
G. microcarpum Tul. & Tul.	-	10	-	10	-	0.52	-	0.20
G. multicaule Gerd. & Bakshi	-	-	-	10	-	-	-	0.40
G. rubiformis (Gerd. & Trappe) Almeida & Schenck	-	10	-	20	-	0.52	-	2.58
G. sinuosum (Gerd. & Bakshi) Almeida & Schenck	-	20	10	50	-	1.04	0.66	4.17
G. taiwanensis (Wu & Chen) Almeida & Schenck	10	10	-	40	2.0	1.55	-	4.57
Racocetra								
R. gregaria (Schenck & Nicol.) Oehl, Souza & Sieverd	20	40	20	60	3.0	5.96	2.65	6.85
*R. persica (*Koske & Walker) Oehl, Souza & Sieverd	-	-	-	40				2.38
R. weresubiae (Koske & Walker) Oehl, Souza & Sieverd	10	30	50	20	2.0	2.59	6.62	1.39

Contd...

Contd...

Rhizoglomus								
Rhizoglomus microaggregatum (Koske, Gemma & P.D. Olexia) Sieverd., G.A. Silva & Oehl	10	-	-	-	1.0	-	-	-
Rhizophagus								
R. aggregatus (N.C. Schenck & G.S. Sm.) C. Walker	-	10	-	10	-	1.04	-	0.40
R. fasciculatus (Thaxter) Walker & Schuessler	30	40	40	50	10.0	3.63	12.25	17.30
Scutellospora								
S. calospora (Nicol. & Gerd.) Walker & Sanders	-	-	-	10	-	-	-	0.40
Septoglomus								
Septoglomus constrictum (Trappe) Sieverd., G.A. Silva & Oehl	-	10	10	10	-	1.55	0.66	0.40

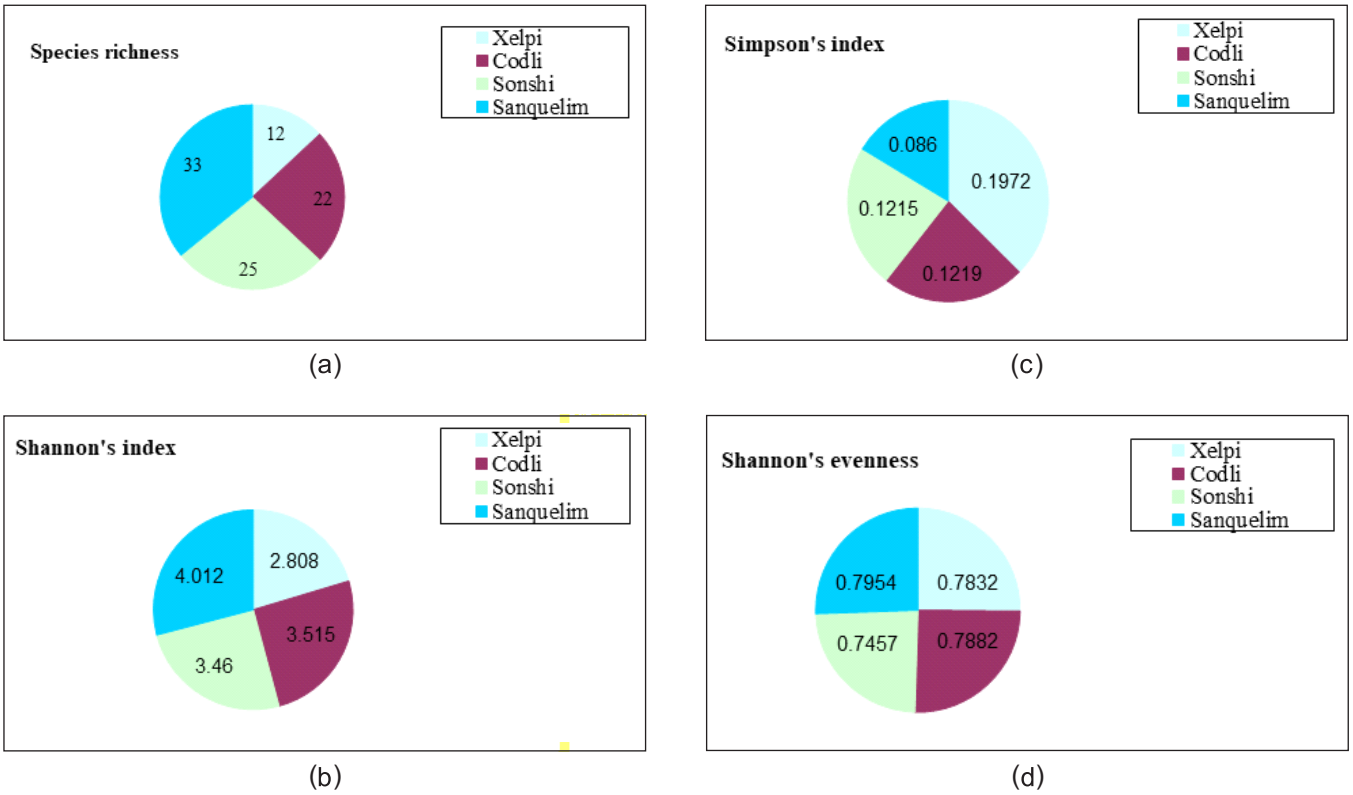

Fig. 1: Species richness (a); Diversity indices viz., (b) Shannon's, (c) Simpson's, and (d) Shannon's evenness, at the study sites.

In the present study, the extent of AM colonization and spore density varied between the plant species at each site, confirming the earlier findings of Lee *et al.* (2009). The recently degraded mine site harboured the least number of AM fungal species. In contrast, the old disturbed sites had maximum AM fungal species, suggesting that mycorrhizal formation depends not only on the plant species but also on on-site conditions and nutrient availability (Lekberg *et al.,* 2007; Fitzsimons *et al.,* 2008). Based on the overall level of AM fungal population distribution in terms of spore density, species diversity, and species richness, it is evident that AM fungal population generally increases during plant succession with mine age (Brundrett *et al.,* 1996).

The reduction in AM fungal diversity would result because disturbance eliminates many intolerant fungi. In contrast, others with more resistance may not be affected because they have more capacity to adapt to major changes in the environment. The increased diversity at the established mine could be due to increased organic matter content, thus encouraging AM development (Kardol and Wardle, 2010). The continuous and constant disturbance on the recently degraded mine and Sonshi mine, which were under active

mining operations, might have severely affected the seedling establishment and colonization of AM fungi. It may be pointed out that the below-ground diversity of AM fungi is one of the major factors contributing to the maintenance of plant biodiversity and adding to the ecosystem's functioning. In the present study, it has been observed that well-established mines had high AM fungal diversity with decreased dominance. Hence, it can be argued that the spores are more in number in a stable ecosystem with equal dominance and greater diversity. In the recently degraded mine, the conditions are not favourable regarding vegetation cover and nutrient status. So only a few species that can tolerate the adverse conditions become more dominant, whereas the rest gets eliminated. As a result, the probability of the dominant species increases, and the diversity decreases. The diversity indices decrease with an increase in the dominance of species. The increased AM fungal activity in established mines may be attributed to the moderate degree of disturbance, supporting Connell's (1978) view of the intermediate disturbance hypothesis. This hypothesis supports the present findings, which report increased diversity and reduced dominance with the increase in age of the mine site. Thus, mycorrhizae may influence succession by regulating competition between plants of different successional stages, which has often been cited as a succession mechanism (Krüger *et al.,* 2017; Dickie, *et al.,* 2013). The mycorrhizal association can probably modify the structure and functioning of a plant community in a complex and unpredictable way (Kardol and Wardle, 2010). Albert (2015) emphasized the positive effect of vegetation on soil formation and organic matter accumulation.

In the recently degraded mine, the possible reasons for low propagule density and diversity could be low soil fertility, organic matter, soil texture, soil moisture, and severe soil compaction. According to Agus *et al.* (2019), soil disturbance depends upon mining operations and the environment, thus lowering the AM fungal colonization level. Any shift in the AM fungal population could affect the composition of plant communities, causing changes in the biology of natural ecosystems. Therefore, knowledge of the different factors influencing the population biology of AM fungi is essential in any attempt to use them in environmental conservation (Melo *et al.,* 2019), biotechnology (Begum *et al.,* 2019), or sustainable agriculture (Khanam *et al.,* 2006).

In conclusion, our data indicate that the severity of disturbance, the harshness of the site, low inoculum levels, edaphic characteristics, and time certainly influence the rate of AM fungi. Thus, revegetation of any disturbed site can occur over time with high species richness and diversity of AM fungi. They are essential in establishing a healthy plant community and facilitating plant succession. This type of inventory is a much-needed area of research that is better to understand AM fungal role in mine restoration function and provide data for their establishment.

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