

Communication

# Enumerating Indigenous Arbuscular Mycorrhizal Fungi (AMF) Associated with Three Permanent Preservation Plots of Tropical Forests in Bangalore, Karnataka, India

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**Abstract:** The establishment of Permanent Preservation Plots (PPPs) in natural forests has a significant role in assessing the impact of climate change on forests. To pursue long-term studies on climate change, PPPs were established during the year 2016 in two major forest areas in Bangalore to conduct ecological studies to monitor the vegetation changes. One of the objectives of the study was to understand the drivers of diversity, such as soils, in terms of nutrients and physical and biological properties. The native tropical forest of Bangalore, which houses Bannerghatta National Park (BNP) on the outskirts, is relatively underexplored in terms of its microflora, particularly arbuscular mycorrhizal fungi (AMF). Hence, the present study was aimed at the quantitative estimation of arbuscular mycorrhizal fungi (AMF) in the three 1-ha PPPs which were established in Bannerghatta National Park (BNP) and Doresanipalya Reserve Forest (DRF) as per the Centre for Tropical Forest Sciences (CTFS) protocol. In BNP, two plots were established, one in the Thalewood house area (mixed, moist, deciduous type) and the other in the Bugurikallu area (dry, deciduous type). In DRF, one plot was established in dry, deciduous vegetation. Each one-hectare plot (100 m × 100 m) was subdivided into twenty-five sub-plots (20 m × 20 m). Composite soil samples were collected during two seasons (dry and wet) and analyzed for AMF spore and available phosphorus (P) content. The results revealed the presence of AMF in all the three plots. Doresanipalya plot had the highest spore number, followed by the Bugurikallu plot and Thalewood house plot. The available phosphorus and AMF spore numbers showed correlations in all the three plots. Among the AMF spores, the *Glomus* species was found to dominate in all the three plots. The study shows that the dry, deciduous forests accommodated more AMF spores than the mixed, moist forests.

**Keywords:** arbuscular mycorrhizal fungi; spore number; permanent preservation plots; available phosphorus; Bannerghatta National Park; Doresanipalya Reserve Forest



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## 1. Introduction

Arbuscular mycorrhizal fungi (AMF) are ubiquitous soil microbes and have an important role in terrestrial ecosystems by forming obligate symbiotic associations with most plant root systems, responsible for enhancing growth and improving soil and plant health [1,2]. AM fungi are one of the important components in soil microbial biomass as they influence some of the essential processes in the soil and plant interface [3]. AM fungi play a crucial role in the mineral nutrition of forest trees; they represent an important nutrient-acquiring mechanism [4]. AMF benefit from carbon substrates from plants, and in turn, plants are provided with nutrients, especially phosphorus compounds from soil solutions through the hyphal network of the fungi, in addition to the increased absorptive surface area of roots [5–8]. AMF were also reported to mobilize other nutrients, viz., K, Ca, Mg, S, Zn, Cu and Fe from soil. In addition, AM fungi are involved in soil aggregation and biotic and abiotic stress management in plants [9–11]

A significant increase in anthropogenic disturbances and forces driving human-mediated climate change makes it much more imperative for the scientific community to pay attention to understanding the diversity and structure of tropical forests. The establishment of Permanent Preservation Plots (PPPs) in natural forests has a significant role in assessing the impact of climate change on forests. Ecological studies would help us to observe and record the changes in species diversity, composition and growth patterns due to climate change over a period of time. EMPRI, as a state nodal agency for climate change, is strengthened by a project sanctioned by the Department of Science and Technology (DST) to establish a Karnataka strategic knowledge center for climate change (KSKCCC) with advanced research capabilities to take up research studies on climate change issues [12].

In the pursuit of long-term studies on climate change, it was decided to establish Permanent Preservation Plots (PPPs) in two major forest areas in Bangalore. Discussions were held by the study team from EMPRI, scientists from Indian Institute of Science (IISc), the Deputy Conservator of Forests, BNP and staff of the forest department regarding criteria for establishment of PPPs, and the methodology for laying sample plots and the assessment and selection of plots based on Forest Vegetation types was established [12]. One of the important objectives of the proposed research is to understand drivers of diversity such as soils in terms of nutrients and physical and biological properties.

The biological and functional diversity of AMF is vitally important to forest ecosystems [13]. Most of the studies related to AM fungal diversity have been conducted in grasslands [14,15], farmlands [16,17] and pot culture experiments [18,19], and less information is available for tropical forests, especially native tropical forests [20]. Previous studies on AMF diversity in forests have been conducted mainly in Brazil [21,22], Mexico [23], the USA [24], India [25,26], Bangladesh [27], Ethiopia [28] and China [29]. Indications of the arbuscular mycorrhizal fungal (AMF) population in soil have often been based on spore enumerations, which have been taken as an estimate of the number of AMF propagules [30–34]. Given the scarcity of AMF data from tropical native ecosystems, two different vegetations were selected to investigate the quantitative estimation of arbuscular mycorrhizal fungi in three Permanent Preservation Plots established in the native tropical forests of Karnataka, India.

The native tropical forest of Bangalore houses Bannerghatta National Park (BNP) on the outskirts and is relatively underexplored in terms of its microflora, particularly arbuscular mycorrhizal fungi (AMF). There are reports available regarding the distribution of AM fungi in disturbed soils, whereas reports on AM fungal composition in undisturbed soils in tropical forests are very meagre.

This article details our efforts to provide quantitative information on the microflora (particularly AM fungi) of BNP. For comparison studies, a permanent plot was established in the Doresanipalya Reserve Forest (DRF) which has human interference due to its close vicinity to the city of Bangalore. The results will provide novel insight into the AMF community's composition in natural tropical forests.

## 2. Results

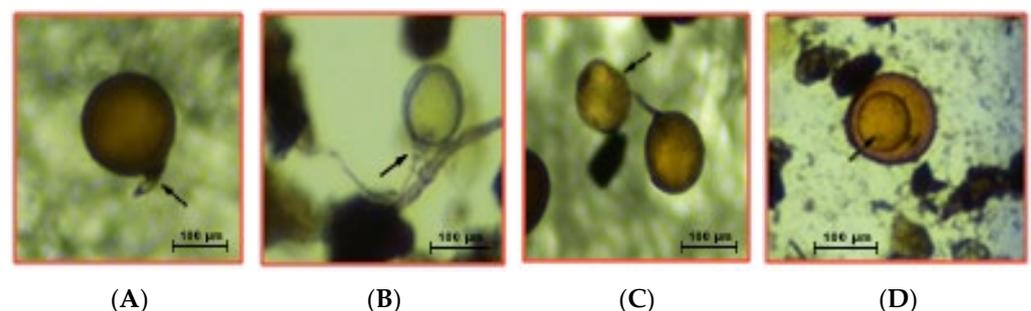
### 2.1. Quantification of AM Fungal Spores

The estimation of AM fungal spore numbers in the native tropical forests of BNP and DRF revealed that all three plots harbored AMF spores with variations observed in two different seasons (Table 1). AMF spores belonging to five different genera, viz., *Glomus*, *Gigaspora*, *Acaulospora*, *Scutellospora* and *Sclerocystis* were commonly recorded from the soils of three plots of BNP and DRF forests (Figure 1). However, the genera of *Glomus* was observed to be dominant in all the plots when compared to other genera.

**Table 1.** Estimation of AMF spores in Permanent Preservation Plots (PPPs) during dry and wet seasons.

PPP	AMF Spore Number Per Gram of Soil					
	ThalewoodHouse Plot (TWH)		Bugarikallu Plot (BGKL)		Doresanipalya Plot (DSP)	
	DS	WS	DS	WS	DS	WS
Sub-Plot 1	14.1	18.86	21.15	10.20	12.96	21.12
Sub-Plot 2	5.92	11.08	28.60	25.22	7.52	21.00
Sub-Plot 3	15.40	<b>31.00</b>	24.40	5.47	6.72	14.08
Sub-Plot 4	7.20	10.14	28.40	6.48	9.72	<b>8.70</b>
Sub-Plot 5	12.22	16.94	18.00	15.40	9.90	16.32
Sub-Plot 6	11.88	4.80	15.40	1.44	8.48	27.44
Sub-Plot 7	5.64	9.84	<b>35.56</b>	14.52	<b>4.00</b>	24.12
Sub-Plot 8	<b>4.44</b>	5.46	26.88	7.52	6.08	12.22
Sub-Plot 9	12.04	20.02	11.52	10.36	5.76	15.04
Sub-Plot 10	12.32	14.70	9.12	20.88	18.54	11.84
Sub-Plot 11	4.48	-	21.44	20.46	15.84	11.70
Sub-Plot 12	7.68	5.20	8.26	20.88	19.62	11.84
Sub-Plot 13	8.32	6.24	13.58	8.46	16.74	12.96
Sub-Plot 14	12.76	<b>1.56</b>	11.62	15.54	23.20	27.36
Sub-Plot 15	11.96	4.40	13.44	24.48	24.30	22.80
Sub-Plot 16	6.76	14.7	13.92	<b>1.25</b>	28.16	27.00
Sub-Plot 17	6.72	9.90	15.40	19.96	24.32	16.56
Sub-Plot 18	<b>19.44</b>	6.82	8.12	21.11	18.72	25.92
Sub-Plot 19	5.04	5.46	16.40	1.29	33.12	<b>34.00</b>
Sub-Plot 20	8.64	5.88	15.82	21.84	20.70	22.50
Sub-Plot 21	18.72	6.33	11.64	10.62	23.20	25.50
Sub-Plot 22	13.60	14.72	<b>6.08</b>	14.80	22.32	23.36
Sub-Plot 23	12.60	6.16	8.90	15.60	31.20	30.72
Sub-Plot 24	13.12	12.74	9.00	25.22	31.02	25.68
Sub-Plot 25	16.56	6.24	8.96	<b>26.60</b>	<b>34.56</b>	30.48
SD	<b>4.38</b>	<b>6.57</b>	<b>7.73</b>	<b>7.97</b>	<b>9.33</b>	<b>7.15</b>
<b><i>t</i>-test Values for the Three Plots during Dry and Wet Seasons</b>						
	TWH		BGKL		DSP	
<i>t</i> -test value	0.3188		0.6483		−1.0804	
<i>p</i> = 0.05	0.7512		0.5199		0.2854	

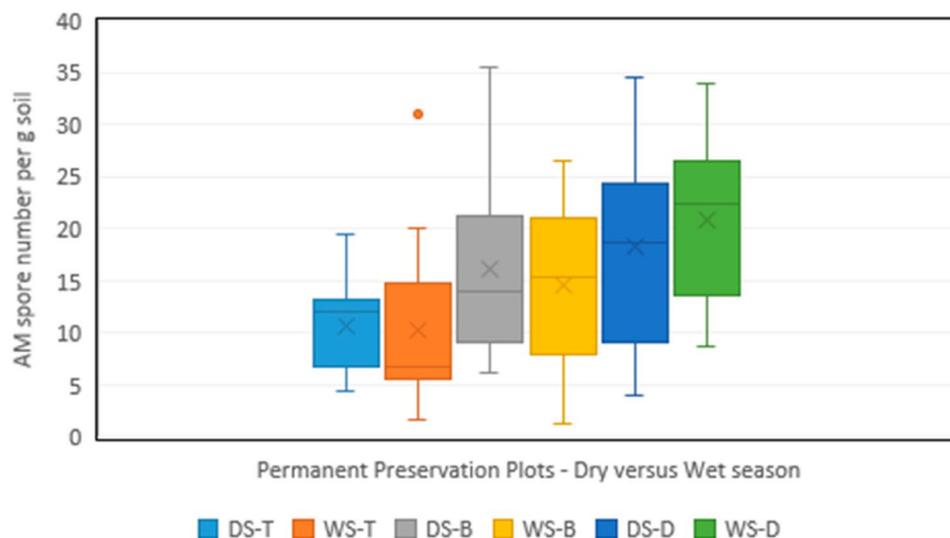
Note: PPP—Permanent Preservation Plot; DS—dry season; WS—wet season; SD—standard deviation. Bold values: AMF spores in TWH plot during dry season ranged between 4.0 to 19.0 per g soil whereas during wet season the range was 1.5 to 31.0 per g soil. Similarly, AMF spores in BGKL plot ranged from 6.0 to 35.0 and 1.0 to 26.0 during dry and wet seasons respectively. Also, in DSP plot had AMF spore number in the range 4.0 to 34.0 and 8.0 to 34.0 during dry and wet seasons respectively.



**Figure 1.** AM fungi spores observed under a compound microscope belonging to Permanent Preservation Plots in Bannerughatta National Park and Doresanipalya Reserve Forest. (A) *Glomus* spore with hyphal attachment (arrow), (B) *Gigasporoid* spore with bulbous base (arrow), (C) *Acaulosporoid* spore with its saccule (arrow) and (D) *Scutellosporoid* spore with vacuole content (arrow).

The soils in the DSP plot had higher numbers of AMF spores in both the seasons, followed by BGKL and TWH plot soils (Figure 2). The DSP plot, which is a dry, deciduous forest type, had AMF that ranged from 4.0 to 34.5 and 8.7 to 34.0 spore  $g^{-1}$  soil in dry

and wet seasons, respectively (Table 1). Meanwhile, in BGKL soil, the AMF spore number ranged from 6.08 to 35.56 spores  $g^{-1}$  soil in the dry season and 1.25 to 26.6 spores  $g^{-1}$  soil in the wet season. However, the lowest number of spores was recorded in the TWH plot, which is a moist, deciduous type, with a range of 4.4–19.44 spores  $g^{-1}$  soil during the dry season and 1.56–31.0 spores  $g^{-1}$  soil in the wet season (Table 1).



**Figure 2.** AMF spore number in permanent preservation plots during dry and wet seasons. The box and whisker graph represents average values of the AMF spore number enumerated in 25 sub-plots of 1 ha plot. DS—dry season; WS—wet season; T—Thalewood house PPP; B—Bugarikallu PPP; D—Doresanipalya PPP.

The average values of the AMF spores in the 25 sub-plot samples revealed that the highest spore numbers were recorded in the DSP soil, with 18.27 and 20.81 per gram soil in the dry and wet seasons, respectively (Table 2), followed by BGKL soil (16.06 spores  $g^{-1}$  soil in the dry season and 14.62 spores  $g^{-1}$  soil in the wet season). The lowest number of AMF spores was observed in the TWH plot during both the seasons (Table 2).

**Table 2.** Estimation of AMF spore number and available phosphorus (P) in Permanent Preservation Plots (PPPs) during dry and wet seasons.

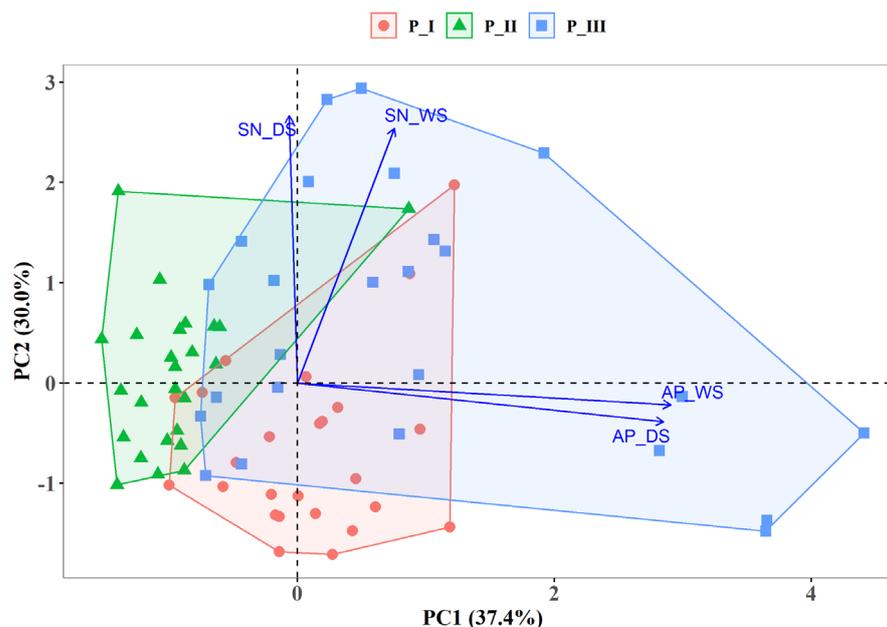
PPP	Dry Season		Wet Season	
	AMF Spore Number Per Gram of Soil	Available P(mg/kg)	AMF Spore Number Per Gram of Soil	Available P (mg/kg)
TWH	10.70	5.82	10.20	6.04
BGKL	16.06	4.97	14.62	3.28
DSP	18.27	6.92	20.81	7.20
SD	3.89	0.97	5.33	2.01

Note: PPP—Permanent Preservation Plot; SD—standard deviation. The values are the means of the 25 sample plots of 1 ha plot.

## 2.2. Estimation of Available Phosphorus in Soil

The results on the content of available phosphorus (AP) in the PPPs revealed that the highest concentration of AP was recorded in the DSP soil during the dry and wet seasons (6.92 mg/kg and 7.20 mg/kg, respectively), followed by the TWH and BGKL soils (Table 2). The concentration of AP was lower in the BGKL plot in the dry and wet seasons with 4.97 mg/kg and 3.28 mg/kg, respectively (Table 2). The principal component analysis (PCA) conducted on the variations recorded for spore number (SN) and available phosphorus (AP) during the wet season (WS) and dry season (DS) across the three plots (TWH (PI), BGKL (PII) and DSP (PIII)) showed total variations of 67.4%. As illustrated in Figure 3, the first

dimension (PC1) represented the highest variation of 37.4%, followed by the second (PC2) dimension representing 30.0% variations, respectively. With a significance threshold of 0.05, the correlation coefficient calculated using PC1 for AP\_WS and AP\_DS were found to be 0.848 and 0.842, respectively, followed by SN\_DS and SN\_WS, which were 0.788 and 0.750, calculated using PC2. The variations across plots were better represented by PC1, wherein the highest variation in AP was found to be in the sequence P III > P II > P I. A similar variation was also observed for SN.



**Figure 3.** Representation of parameters of spore number and available phosphorus during wet season and dry season using principal component analysis (PCA) biplot. Note: SN—spore number; AP—available phosphorus; DS—dry season; WS—wet season; P\_I—Thalewood house plot; P\_II—Bugarikallu plot; P\_III—Doresanipalya plot.

### 3. Materials and Methods

#### 3.1. Establishment of Permanent Preservation Plots (PPPs)

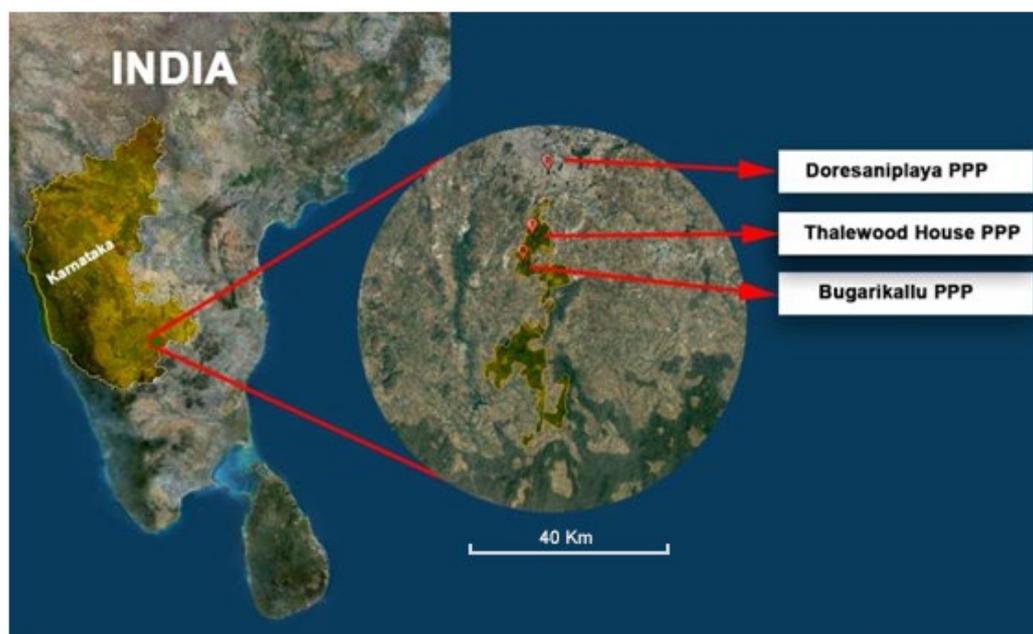
Permanent Preservation Plots of 100 m × 100 m (1 ha) in size were established in the forests of Bannerughatta National Park (BNP) and Doresaniplaya Reserve Forest (DRF) following the Centre for Tropical Forest Sciences (CTFS) protocol, which is standardized in accordance with the international monitoring of forest resources. As per the CTFS protocol, the plot was gridded into blocks of 20 m × 20 m with the help of theodolite, and each corner was marked with a semi-permanent pole. Hence, in a one-hectare plot, there were twenty-five sub-plots.

Three one-hectare plots were established, viz., two plots each in the Thalewood house (TWH) and Bugarikallu areas of BNP and one in the Doresaniplaya area of DRF. The Thalewood house (TWH) plot represented a relatively moist forest, and the Bugarikallu (BGKL) forest plot represented a drier forest with stunted trees, whereas the Doresaniplaya (DSP) plot largely contained plantations and a dry scrub type.

#### 3.2. Location of the Plots

##### 3.2.1. TWH PPP

The plot is a tropical, mixed, moist deciduous forest within Bannerughatta National Park near Bangalore, Karnataka, situated at 12°45′52.236″ N latitude and 77°33′33.023″ E longitude (Figure 4). The climate is seasonal with an average annual temperature of 26.85 °C and rainfall of 356.33 mm. The soil is silty clay loam with a pH ranging from 5.2 to 6.5. The most dominant tree species recorded were *Olea dioica*, *Cipadessa baccifera* and *Ziziphus oenoplia*.



**Figure 4.** Map showing the location of Permanent Preservation Plots in Bannerughatta National Park and Doresaniplaya Reserve Forest.

### 3.2.2. BGKL PPP

The plot is a tropical, dry, deciduous forest within Bannerughatta National Park near Bangalore, Karnataka, situated at  $12^{\circ}42'47.689''$  N latitude and  $77^{\circ}32'25.422''$  E longitude (Figure 1). The climate is seasonal with an average annual temperature of  $26.85^{\circ}\text{C}$  and rainfall of 356.33 mm. The soil is sandy clay with a pH ranging from 5.5 to 7.4. The most dominant tree species recorded were *Ixora nigricans*, *Anogeissus latifolia* and *Erythroxylum monogynum*.

### 3.2.3. DSP PPP

The plot is a tropical, dry, deciduous forest within Doresaniplaya Reserve Forest in Bangalore, Karnataka, situated at  $12^{\circ}53'32.525''$  N latitude and  $77^{\circ}35'26.520''$  E longitude (Figure 1). The climate is seasonal with an average annual temperature of  $23.51^{\circ}\text{C}$  and rainfall of 1116.17 mm. The soil is sandy loam with a pH ranging from 5.0 to 6.4. The most dominant tree species recorded were *Santalum album*, *Shorea roxburghii* and *Ziziphus oenoplia*.

### 3.3. Sample Collection

The one-hectare (ha) plot of  $100\text{ m} \times 100\text{ m}$  was subdivided into  $20\text{ m} \times 20\text{ m}$  sub-plots, and the soil samples collected from the twenty-five sub-plots stood as the replicates of the one ha plot. Soil samples were collected from each subplot at a depth of 15–20 cm during April (dry season) and December (wet season). The composite sample was made from soils collected from four corners and the center, mixed well and used for analysis. Samples were air dried, sieved and stored at  $4^{\circ}\text{C}$  until further processing.

### 3.4. Quantification of AM Fungal Spores

The soils containing AM fungal spores were isolated by using a wet sieving and decanting technique [35]. In this method, 100 g of an air-dried soil sample was dispersed in 500 mL (approximately) of water in a beaker, and the suspension was kept undisturbed for five minutes. After the settlement of heavier particles, the suspension was carefully poured on a stack of sieves (710, 250, 75 and  $45\ \mu\text{m}$  mesh size) arranged in descending order. The soil was re-suspended in water, and the process was repeated 2–3 times. The AM fungal spores on the bottom sieves, viz., 75 and  $45\ \mu\text{m}$ , were transferred into a petri plate and examined under a microscope (Olympus). The quantification of AM fungal spores from the

rhizosphere sample was expressed as the spore number per gram of soil (SN g<sup>-1</sup> soil). The identification of AM spores was based on the descriptions given by Schenk and Perez [36] and referring to the invam website ([www.invam.caf.wvu.edu](http://www.invam.caf.wvu.edu), accessed on 30 December 2021). AM fungal spores of different genera were recorded based on the spore morphology.

### 3.5. Estimation of Available Phosphorus in Soil

The available phosphorus in the soil samples of PPPs was determined using Bray's method No.1 [37]. A total of 5 g of air-dried soil was mixed with 50 mL of Bray extractant No. 1 solution. After shaking for five minutes, the contents were filtered through Whatman No. 42 filter paper. To the 5 mL of the soil extract, 5 mL of ammonium molybdate reagent was added, and the volume was made up to 20 mL with distilled water. To this, one mL of dil. Stannous Chloride (SnCl<sub>2</sub>) was added, and the final volume was made up with distilled water. The intensity of the blue color was measured after 10 min using a spectrophotometer at a wavelength of 660 nm against a blank (prepared similarly to the method described but without the soil extract). The absorbance obtained was compared with the standard curve prepared using the phosphorus standard, and the concentration of the available phosphorus was calculated by using the formula given below. The twenty-five samples collected from the subplots were considered as the replicates of the one ha plot.

$$\left\{ \frac{\text{Available P}_2\text{O}_5 (\text{kg ha}^{-1}) = \text{Graph value} \times \text{Vol. of extract} \times \text{Vol. made up} \times 2.24 \times 10^6 \times 2.29}{10^6 \times \text{Weight of soil} \times \text{aliquot of the extract taken}} \right\}$$

### 3.6. Statistical Analysis

All data were analyzed using the analysis of variance (ANOVA) and Student's *t*-test when appropriate. Data analysis was performed using descriptive statistics and principal component analysis for the AMF population and the available phosphorus in Excel ([www.socscistatistics.com](http://www.socscistatistics.com), accessed on 31 December 2021). Values of *p* < 0.05 were considered statistically significant. Pearson's correlation test was performed to assess the relationship between spore population and available P in two different seasons. Data of three plots were compared by analysis of variance for the AMF spore population and the different seasons.

## 4. Discussion

AM fungi are most abundant and ubiquitous in soil, and they are reported to establish mutualistic associations with the roots of 90% of terrestrial plants. AMF form symbiotic associations with most of the plant kingdom and provide many beneficial effects, viz., nutrient mobilization from soil [38]; protecting plants from soil-borne pathogens and nematodes [39,40]; providing resistance to plants against various stresses, i.e., drought, salinity and toxic metals, etc. The amalgamation of all the above beneficial effects of AMF increases the plant growth [9,10].

The AMF in all three plots indicated the presence of *Glomus* as the dominant group followed by *Gigaspora*, *Sclerocystis* and *Acaulospora*. The genus *Glomus* has been reported to be the dominant AM fungi in a number of forest communities [41–50]. AM fungi belonging to six genera, namely *Acaulospora*, *Enterophospora*, *Gigaspora*, *Glomus*, *Sclerocystis* and *Scutellospora*, were recorded in the natural forests of Arunachal Pradesh, India [26]. There are reports indicating that the genus *Glomus* was dominating in most of the regions as it is highly adaptable to ecological variations and can form mycorrhizal associations with plants under a wide range of soil properties [41,44,51–53].

Among the three permanent preservation plots in Bangalore, the samples collected from the DSP plot recorded significantly higher AMF spore numbers than the other two plots, and this variation may be due to the difference in edaphic factors, the rhizosphere effect, host preference, the availability of P in soil and climate factors [54,55]. The comparative assessment of AMF spore numbers in three PPPs showed that tropical dry forests harbor more AMF spores than tropical moist, deciduous forests.

The average values of the AMF spore number revealed that the DSP soil recorded the highest number of spores, with 18.7 and 20.81 number  $g^{-1}$  soil in the dry and wet seasons, respectively. The evaluation of the percent population of AMF spores in the rhizosphere soils of the eight tree species revealed values of SD in the range of 4.38–76.38 per g soil, which are much higher than those reported in the Hazarikhil forest in Bangladesh (0.35–4.32 spore number  $g^{-1}$  soil) [27] and Amazonian terra firme forest in Brazil (1.5–9.4 spore number  $g^{-1}$  soil) [56], comparable with those found in the tropical rainforest of Xishuangbana in China (0.6–19.1 spore number  $g^{-1}$  soil) [57] and the subtropical forest of Huangshan in China (0.45–32.50 spore number  $g^{-1}$  soil) [58].

A few native soils have higher spore populations [30,59]. It was suggested that because of year-round adequate soil moisture and temperature, actively growing roots are always present, and the AM fungi present need not sporulate [60]. This is typical of the undisturbed plots (TWH and BGKL). However, in the disturbed plot of DSP, several factors may have contributed to the higher spore count as compared to that of the other plots. A change in plant composition following disturbance, a change in pH and root death may induce sporulation [61]. In our study, it was found that the available P content was correlated with the AM spore number. A previous study showed that soil P was the most significant factor affecting the AMF communities [62]. The AMF spore was negatively correlated with soil P in the planted forest of eastern China [63]. Similar work [64] showed that lower concentrations of phosphorus increase the AM spore population. The mobilization of immobile elements such as Zn and P from the root depletion zone by AMF was reported by several researchers [65–68].

## 5. Conclusions

AM fungi are ubiquitous and occupy a wide range of ecological niches. They occur in different environments from arctic to tropic regions. The present study on Permanent Preservation Plots of tropical forests of Bangalore, Karnataka, viz., Bannerughatta National Park (BNP) and Doresanipalya Reserve Forest (DSP), indicated that the genus *Glomus* was observed to be the dominant in the three plots. Additionally, other genera of AMF *Gigaspora*, *Acaulospora*, *Sclerocystis* and *Scutellospora*, were commonly recorded from the soils of the Permanent Preservation Plots. The highest AMF spore number was found in the dry deciduous forest in the DSP plot (during the wet season) when compared to the mixed moist, deciduous forest in the TWH plot. The available P content was found to be correlated significantly with the AMF spore number in the BGKL plot during the dry season ( $r_s = 0.55$ ,  $p < 0.05$ ). Hence, the present findings suggest that tropical forests harbor more AMF spore numbers during the wet season when compared to the dry season.

For the first time in Karnataka, India, the soils of Permanent Preservation Plots were analyzed for the presence of AMF spores in different seasons. The present study findings may be used to provide insight on the importance of AM fungi for the sustainable management of forests. The development of a baseline database of the microflora, particularly the AMF population, in Permanent Preservation Plots established in tropical forests of Bangalore for long-term studies on climate change would create new knowledge on climate-related aspects of diverse forest ecosystems.

Insight into the population dynamics of AMF spores has been elucidated. However, obtaining a detailed overview of AMF diversity using nuclei acid probes should be considered for future studies.

**Author Contributions:** This work was carried out in collaboration with all authors. S.B. conceptualized the work, estimated the VAM spore number, compiled the data, performed the statistics, and wrote the original draft; P.P. carried out sampling and isolation of spores; N.M. and B.S. analyzed the samples for available phosphorus and provided the methodology for the phosphorus content and helped in the statistics to plot the graphs; R.O. supervised the project and reviewed the paper. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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