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Influence of *Piliostigma reticulatum* on the diversity of indigenous vesicular-arbuscular mycorrhizal in Senegal

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Abstract

Piliostima reticulatum shrubs constitute "islands of fertility" in the soil beneath the canopy with better availability of water and more intense biological activity. Assessment of diversity and understanding factors underlying species distribution are fundamental themes in ecology. However, the diversity of native Vesicular Arbuscular Mycorrhizal (VAM) species in sudano-sahelian agro-ecosystems are weakly known. This research was carried out to understand the contribution of VAM on "islands of fertility" by (i) assessing the morphological diversity of indigenous VAM species in soils within and outside the influence of the shrub *Piliostigma reticulatum* in Senegal and (ii) examining the effects of soil chemical properties on this diversity. Soils were collected from a depth of 0 - 30 cm in the beneath of canopy (SC) and outside canopy (HC) at ISRA experimental station located at Nioro du Rip in Kaolack, Senegal. Morphological diversity of VAM spores was done after extraction by wet sieving. The effect of the presence or absence of *P. reticulatum* were analyzed with a one-factor ANOVA. Principal Components Analysis (PCA) was performed to determine relationships between morpho-species diversity parameters and soil chemical properties. Results showed that the genus *Glomus, Scutellospora* and *Gigaspora* were found in both soils (SC and HC) and *Sclerocystis* spores were only found outside canopy (HC). VAM spores were more abundant and more diverse in soil outside the influence of *P. reticulatum* (HC) than the soils beneath canopy (SC). Ultimately, this study tends to confirm that soil chemical properties have effect on VAM diversity.

Key words: *Piliostigma reticulatum*; *Glomus; Scutellospora; Gigaspora, Sclerocystis*; Senegal

1. Introduction

The Sudano-Sahelian region of West Africa, like many other arid and semi-arid environments, is characterized by perennial woody shrubs (Diakhate *et al*., 2016). Among these shrubs, *Piliostigma reticulatum* (DC) Hochst and *Guiera senegalensis* JF Gmel, are the most common native shrub commonly found in farmers' fields where they are traditionally cut and burned (Lufafa *et al.*, 2008). *P. reticulatum* shrub, commonly found in many parts of Sub-Saharan Africa, significantly promotes crop growth and improves soil quality (Bright *et al.,* 2017). They are known as “islands of fertility “also termed “resource islands” under and near the vegetation tufts (Wezel *et al*., 2000). These “resource islands” under the influence of *Piliostigma reticulatum* shrub is characterized by high water availability and soil nutrients such as nitrogen, phosphorus, and potassium. it also creates a favourable environment to increase microbial population and diversity hence resulting to high decomposition of organic materials (Diedhiou *et al.,* 2009; Dossa *et al.,* 2010). According to Kizito *et al*., (2006) and Diakhate *et al.,* (2013) *P. reticulatum* and *G. senegalensis* surrounding soils showed a high level of water availability and nematode population and diversity compared to soil outside the cover. *P. reticulatum* shrub promotes also carbon sequestration in the intercropping system (Bright *et al.,* 2017).

P. reticulatum shrub is known to influence the diversity of Vesicular Arbuscular Mycorhizza (VAM) in the soil (Maurer-Troxler et al., 2006 ; Bender, Wagg & van der Heijden, 2016). VAM play a key role in plant hydro-mineral nutrition and health. Indeed, several studies have shown the beneficial effects of the VAM on the agro-ecosystems such as improving crop growth, improving soil structure, and increasing plant resistance against biotic and abiotic stresses (Gonzalez-Chavez *et al*., 2009). However, to date, there has not been any systematic study done in this sector of central Senegal to ascertain the influence of *P. reticulatum* shrub cover on the diversity of VAM. The objective of this study was to compare the diversity of VAM of soils within and outside the influence of the shrub *P. reticulatum*, and to characterize the diversity of spore of VAM within and outside the influence *P. reticulatum* and to determine the effect of soil chemical properties on the abundance of VAM spores. The main hypothesis being tested is that the presence of *P.* reticulatum shrub influences the diversity of VAM in the soil.

2. Material and Methods

2.1. Study area

The study was conducted in pearl millet (*Pennisetum glaucum*) field at the experimental station of ISRA (Senegalese agricultural research institute) located at Nioro du Rip situated in the Southern region of the Senegalese Peanut Basin (13° 45 N, 15° 47 W, 18 m above sea level). The annual rainfall average is 750 mm per year distributed from July to September, and a mean annual temperature ranging from 20˚C in December-January to 35.7˚C in April-June characteristic of semi-arid climate. The soil is a fine-sandy, mixed Haplic Ferric Lixisol type (Iuss, 2006) , locally called Deck-Dior (Badiane *et al*., 2000). The site is dominated by the shrub *P. reticulatum* (DC.) Hochst (*Caesalpinioidea*) with a density of about 185 shrubs per hectare.

2.2 Soil sampling

Random soil sampling was done after the wintering period in January on ten shrubs of *P. reticulatum* found in the field to obtain a composite soil samples. Soil samples were collected beneath canopies (0 - 30 cm depth) of *P. reticulatum* shrubs (SC). The surrounding soil samples were collected fifteen meters away from the shrub (HC) using a soil auger. Soil samples are then mixed and homogenized to obtain a composite sample representative of the field. The distance selected for the soil sampling of the surrounding soils were chosen to minimize the influence the shrub on soil nutrients (Diedhiou *et al*., 2013). The soil samples were sieved through a 2 mm mesh sieve and packed into sample bags and kept for soil analysis. The spores were characterized in the LCM lab (Laboratoire Commun de Microbiolgie/ IRD/ISRA/UCAD) in the research center of ISRA in Bel Air (Dakar / Senegal).

2.3 Determination of VAM

The spores of VAM were isolated by wet sieving combined with a density gradient technique Gerdemann & Nicolson, (1963) and determined under an optical microscope with 400 times magnification (Błaszkowski, 2012). Systematic was used in comparison with specimens from INVAM (2018) (West Virginia University, Morgantown, USA) and with reference to descriptions provided by Morton & Benny, (1990). Density of the spores was determined for each species in number of spores per 100 g of air-dried soil

2.4 Statistical analysis

In order to characterize the diversity, the diversity index was calculated according to Shannon-Weaver for each soil (SC and HC), using the formula:

$$H = -\sum\left(\frac{ni}{N}\right)\ln\left(\frac{ni}{N}\right).$$

Where *ni* represents the density of the spores of the species *i* and N the total density of the spores of all the species in a sample. A low H value generally suggests a site with few species and a few dominant species, while a high H value suggests considerably more species. The effect of the presence / absence of *P. reticulatum* were analyzed with a one-factor ANOVA. Fisher's test (LSD) was used to compare the means ($P < 0.05$). Linear regression clarified the influence of soil chemical parameters on communities of VAM fungi. Statistical analyzes were performed using XLSTAT software (Version 2013.1). Principal Components Analysis (PCA) was performed using Minitab 17 software to determine relationship between soil chemical properties (C, N and P) and spores abundance. Scores of samples in Axis 1 and Axis 2 were further analyzed using permutational multivariate analysis of variance (PerMANOVA) (Anderson, 2001) to determine the statistical significance of any treatment.

3 Results

3.1 Soil properties

Soil chemical properties are shown in Table 1. Total C, P and N contents were significantly ($P < 0.05$) higher beneath than outside the influence of the shrub. The shrub had no significantly effect on soil pH.

Table 1: Chemical properties of the soils beneath and outside the influence of shrubs in senegal (± standard error).

Soil parameters	**Soil location**	
	Outside canopy (HC)	**Beneath canopy (SC)**
Total C (mg C g^{-1})	2.50 ± 0.77 a*	4.00 ± 0.40 b
Total N (mg N g^{-1})	0.22 ± 0.01 a	0.36 ± 0.01 b
Total P (µg P g^{-1})	42.3 ± 3.23 a	59.5 ± 5.77 b
pH (H2O)	5.5 ± 0.1 a	5.5 ± 0.1 a

*: means followed by different letter within a row indicate significant difference between treatments (HC/SC) at $P < 0.05$.

3.2 Effect of presence or absence of *P. reticulatum* on the diversity and abundance of VAM's spores

The isolated fungal species belong to the family of *Endogonaceae* (Morton & Benny, 1990). A total of 4 morphotypes of VAM fungi were identified, including 3 in the soil (SC) and 4 in the soil (HC) (table 2). *Sclerocystis rubiformis* was found exclusively in HC soil samples. A comparison of the soil types showed that diversity index is higher in the soil (HC) (H = 1.00) than in the soil (SC) (H = 0.71; table 2).

The comparison of the means also showed that the number of spores of VAM is significantly higher in HC (300 ± 84 per 100 g of dry soil) than in SC (201 ± 42 per 100 g of dry soil) $p < 0.01$.

The abundance of the spores of VAM was significantly higher in soil outside the shrub's influence (Table 2; $P<0.05$). Spores are more abundant and more diverse in HC soil samples compared to soil samples SC (Table 2).

Three morpho-species were found in beneath of *P. reticulatm* in this study. These species belonged to three genera. One species belonged to the *Glomeracea* family: *Glomus aggregatum* and *Sclerocystis rubiformis*. There were two species from the *Gigasporaceae* family: *Gigaspora margarita*, *Scutellospora gregaria* (Fig1).

Among the 4 morpho-species found, 2 species had a relatively high density of spores (*Glomus aggregatum* and *Gigaspora margarita*) and 2 genera a rather low density of spores (*Sclerocystis rubiformis* and

Scutellospora gregaria). *Glomus aggregatum, Gigaspora margarita* and *Scutellospora gregaria* were found in all soils. Only *Sclerocystis rubiformis* was found exclusively in the HC soils.

Table 2: Number of spores per 100 g of soil per fungal type and soil type (± standard error)

Treatments	*Gigaspora margarita*	*Glomus aggregatum*	*Sclerocystis rubiformis*	*Scutellospora gregaria*	Mean	Species number	Shannon Index (H)
SC	65 ± 30 a	131 ± 76 a	0 ± 0 a	7 ± 2 a	201 ± 87 a	3	0.71 a
HC	73 ± 24 a	171 ± 55 a	54 ± 41b	3 ± 2 a	300 ± 84 b	4	1.00 b

*: Different letters within a row indicate significant difference between treatments according to the Turkey test at P < 0.05 different.

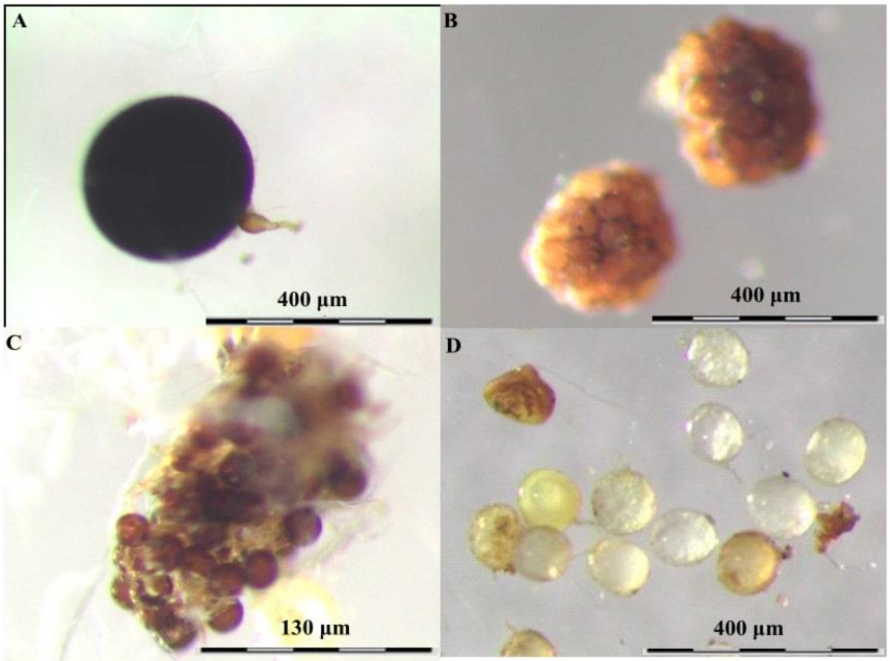

Figure 1: Spore diversity in soil within and outside the influence of *P. reticulatum* shrub. (Photo: Abdoulaye Fofana Fall)

A: Spore of *Scutellospora gregaria* is found in all soils. Its spores are large and visible to naked eye, black and have a suspending bulb. **B**: *Sclerocystis rubiformis* is the species characteristic of soil outside the influence of *P. reticulatum*. The spores are inseparable cluster and brown. **C**: Spore of *Glomus aggreagatum*, is present in all soils. It forms spores in brown clusters D: Spore of *Gigaspora margarita* is found in all soils. The spores are medium-sized, whitish and yellowish with a suspending bulb.

3.3 Effect of soil chemical properties on the abundance of VAM

At the outset, it is noted that total Carbon, Nitrogen and Phosphorus contents were significantly ($P < 0.05$) higher beneath than outside the influence of the shrub (table1). The pH was not influenced by the presence of *P. reticulatum* in the soil. The correlation of soil chemical properties (C, N and P) and the abundance of spores showed that the abundance of VAM's spores was negatively correlated with the high rate of Carbon ($r = -0.28$, $p < 0.001$), Nitrogen ($r = -0.47$, $p < 0.001$), and Phosphorus ($r = -0.67$, $p < 0.01$). There is a negative influence of soil chemical elements (C, P and N) on the abundance of the spore of VAM in another word, it indicated that when C, P and N increase, the number of spores decreases.

Multivariate analysis of all data showed a strong clustering by soil location ($p < 0.01$). Principal component analysis of the abundance of the spore of VAM showed a distinct separation of clusters along the first axis (54.5% of the total variance) based on the soil chemical properties. The second axis accounted for only 17.1% of the variability. Samples were clustered separately between data from beneath and outside the canopy influence. Total N as well as P and C were negatively correlated with the abundance of spores of *Glomus*, *Sclerocystis* and *Gigaspora* ($p < 0.01$).

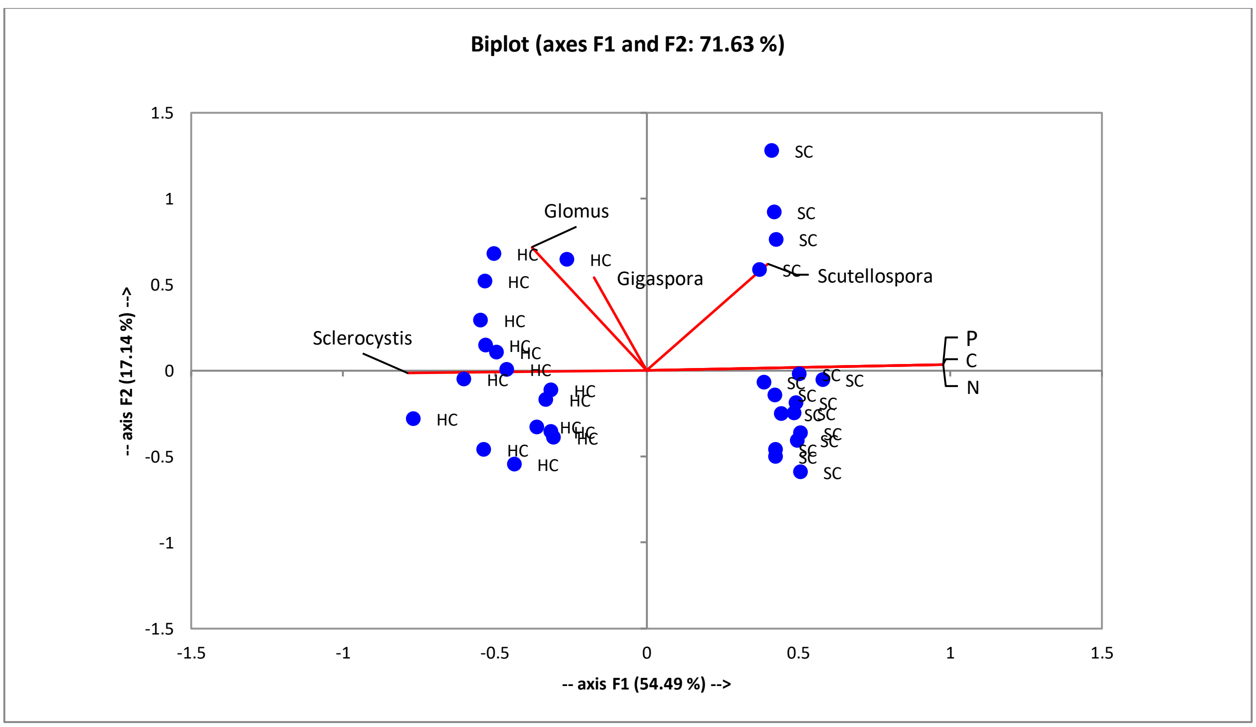

Figure 2: Principle component analysis of the abundance of VAM spore from soil beneath and ouside the *P. reticulatum* camopy influence. HC: ouside beneath of canopy, SC : beneath canopy C: carbon; P: phosphorus and N: nitogen

4. Discussion

4.1 Effect of presence or absence of *P. reticulatum* on the diversity and abundance of the spores of VAM

The density of soil VAM spores in beneath of *P. reticulatum* was relatively low (on average 201 spores per 100 g of dry soil). The density of spores was significantly lower than that found in plantations of *Acacia holosericea* and *Acacia mangium* in the southern and northern regions of Sudan in Burkina Faso (Bâ, Dalpé & Guissou, 1996) and that of *Acacia albida* in different regions from Senegal (Diop *et al.,* 1994). The density of *Glomeromycota* spores was generally higher in HC *P. reticulatum* soils than in SC *P. reticulatum*. According to Tchabi the density of spores in forest soils was consistently higher than that of mixed crops (Tchabi *et al*., 2008). Morphotypes belonging to the *Glomeraceae* family dominated in both soils (HC and SC). The predominance of *Glomeracea* has also been reported in some research based on VAM morphotypes in various tropical soils (Bâ, Dalpé & Guissou, 1996; Diallo, Samb & Ducousso, 1999; Houngnandan et al., 2009) and in certain agricultural soils of temperate zones (Mathimaran *et al.,* 2005).

In this study, spores of the genus *Glomus* are more abundant in both SC and HC soils. Indeed, in the semi-arid zones in Senegal *Glomus* genus is numerically more important than others genus (Diallo, Samb & Ducousso, 1999 ; Diop, 1995). We found four (4) out of nine (9) species found by Diallo et al., (1999) in the semi-arid regions of Senegal. The absence of *P. reticulatum* is significant ($p = 0.04 < 0.05$) on the abundance of spores of VAM. In another word, spores are more abundant when *P. reticulatum* is absence rather than when it is present. The characteristic species that could be identified for HC soil is *Sclerocystis rubiformis* because it is only found there.

4.2 Effect of soil chemical properties on the diversity of VAM

VAM fungi have an ecological specificity (Diop, 1995). Soil properties are important factors that influence VAM fungal community (Bever *et al*., 2001). However, according to Koske, (1987) the relationships between the density of the spores of VAM and the chemical properties of the soil are not stable, but they would vary according to the composition of the community of *Glomeromycota* (Johnson et al., 2013). Season, soil physical properties, host dependency, age of the host plant, VAM sporulation capacity and patterns of the spores of VAM distribution in soils (Bever *et al.,* 1996) and certain chemical properties of the soil were previously reported as being among the factors (pH, available phosphorus, and organic matter) (Mohammad, Hamad & Malkawi, 2003 ; Isobe *et al*., 2007). The influence of the presence or absence of *P. reticulatum* shrub on the VAM fungus community is thus manifested indirectly through the chemical properties of the soils. In this study, results on richness and abundance in fungal populations seem to be negatively influenced by the richness of phosphorus, carbon and nitrogen. When phosphorus, carbon, and nitrogen levels in the soil decrease, fungal richness increases, this is observed in soil (HC). On the other hand, when the level of phosphorus, carbon and nitrogen in the soil increases, the fungal populations decrease the case recorded outside the beneath (SC). The results of the PCA (fig. 2) confirm those of the Pearson correlation when it comes to explain the abundance of spores in relation to the soil chemical properties. The abundance of *Glomus aggregatum*; *Gigaspora margarita* and *Sclerocystis rubiformis* seem to be linked to the chemical properties of the soil while *Scutellospora gregaria* behaves unlike these three species and the properties mentioned above. These results confirm those of Cuenca & Meneses, (1996) and Isobe *et al*., (2007) who also indicated that the abundance and the diversity of VAM communities associated with cocoa were negatively correlated with the P available in the soils. According to Johnson *et al.*, (2013), certain morpho-species are more sensitive to available P and become less frequent and undetectable in soils with high level of available P. The low species richness in both soils (HC and SC) could be due to limited or non-existent sporulation in the soils of certain species since many VAM species can end their life cycle with sporulation only towards the end of the wet season, to survive during the dry season. Another reason is pearl millet's monoculture on the site and it is known that this plant is not mycotroph. Therefore, the use of soil DNA and root DNA extraction techniques on one hand combined with pot trap culture on the other hand is highly recommended to successfully trap VAM in tropical ecosystems.

Declarations

Ethic approval and consent

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The authors want to declare that they can submit the data at any time based on publisher's request. The datasets used and/or analyzed during the current study will be available from the authors on reasonable request.

Competing interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Authors' contributions

AFF contributed on inception of the paper, field methods, analysis and writing. HF contributed on inception, field methods and reviews of the paper. SD contributed on inception, field methods and reviews. SD contributed on inception and reviewed the work. IN contributed on data management and analysis and on write up.

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Figures

A: Spore of *Scutellospora gregaria* is found in all soils. Its spores are large and visible to naked eye, black and have a suspending bulb. **B**: *Sclerocystis rubiformis* is the species characteristic of soil outside the influence of *P. reticulatum*. The spores are inseparable cluster and brown. **C**: Spore of *Glomus aggreagatum*, is present in all soils. It forms spores in brown clusters D: Spore of *Gigaspora margarita* is found in all soils. The spores are medium-sized, whitish and yellowish with a suspending bulb.

Figure 1

Spore diversity in soil within and outside the influence of P. reticulatum shrub. (Photo: Abdoulaye Fofana Fall)

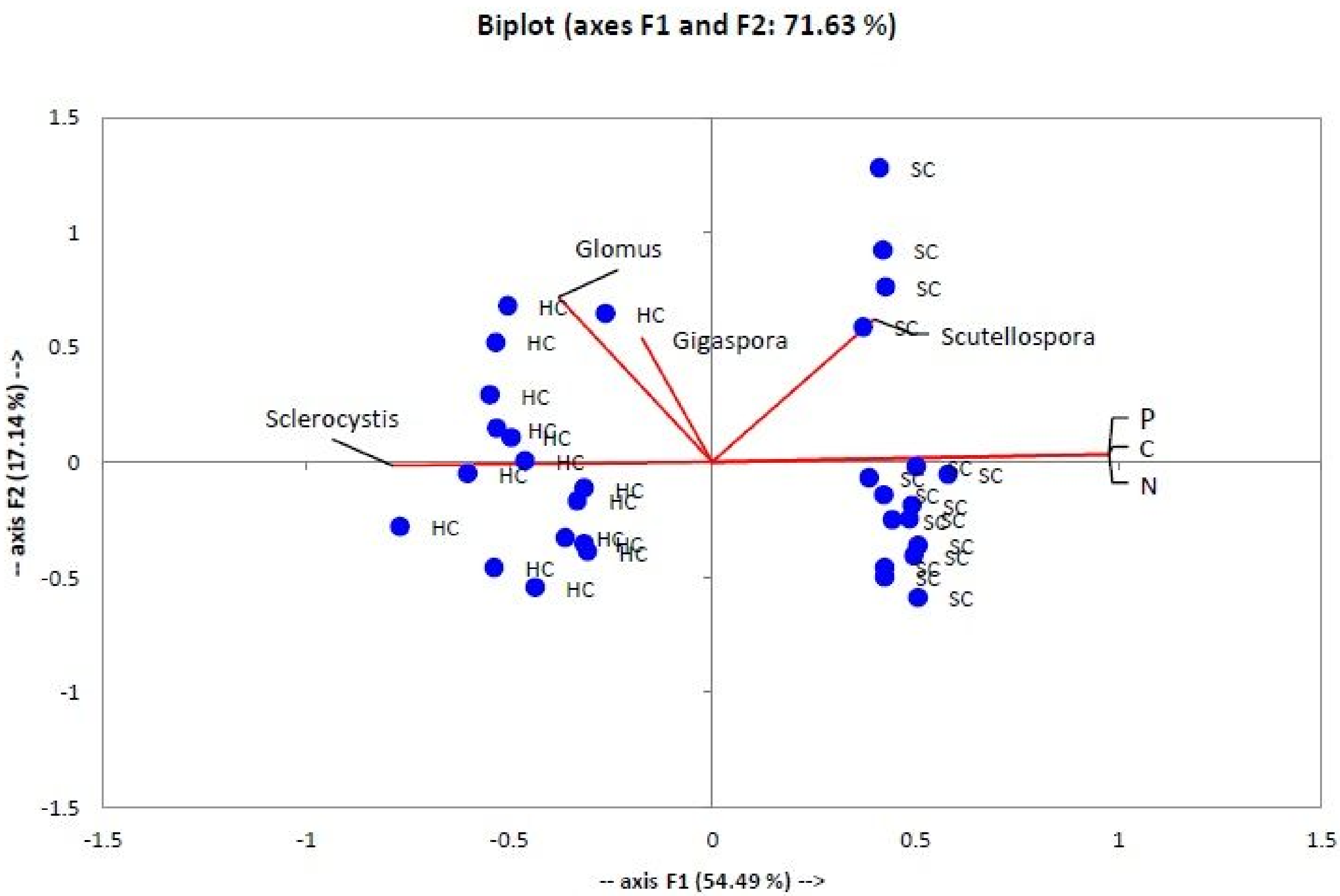

Figure 2

Principle component analysis of the abundance of VAM spore from soil beneath and ouside the P. reticulatum camopy influence. HC: ouside beneath of canopy, SC : beneath canopy C: carbon; P: phosphorus and N: nitogen