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Effects of Two Cover Crops [Arachis Repens (L.) Handro And Desmodium Adscendens (SW.) DC.] on The Density of Arbuscular Mycorrhizal Fungi in Soils Under Industrial Banana Plantations in Côte d'Ivoire

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Abstract

This study aims to evaluate the effects of two leguminous plants *Arachis repens* and *Desmodium adscendens*, used as cover crops, on the proliferation of arbuscular mycorrhizal (AM) fungal spores in soils under industrial banana plantations. Soil samples were collected at two depths (0-10 cm and 10-20 cm) before, 6 and 12 months after the cover crops installation in a three-treatment Fisher block design. After laboratory analysis of the collected soil samples, the results showed that *A. repens* strongly contributed to the increase of AM fungal spores. Indeed, before planting this legume, the average number of spores which was 882.50 at 0-10 cm of the soil, increased to 1502.50 and then to 2390.00 in 100 g of soil respectively after 6 and 12 months. At the depth of 10-20 cm, the number of spores was 790.00, 1177.50 and 1270 spores/100 g soil, respectively. *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora* were the main genus obtained among the identified spores. Among them, *Glomus* and *Acaulospora* were the most abundant. *A. repens* could be used as a cover crops for the sustainable management of biological soil fertility.

Keywords: Dessert banana, Cover crops, Arachis repens, Desmodium adscendens, mycorrhizae.

Introduction

Côte d'Ivoire is the leading African producer-exporter of dessert banana with a production of 397,305 tons in 2018 (FAO, 2018). The resources generated by this sector contributed 8 % to agricultural GDP and 3 % to national GDP (Cheickna *et al.*, 2018).

However, one of the major problems faced by dessert banana producers in Côte d'Ivoire is plot weediness. Tano *et al.* (2016) noted that weeds cause losses estimated at 9.7 % of global agricultural production and about 10-56 % in Africa. In fact, weeds promote the presence of pests and parasites such as nematodes, weevils, fungi, bacteria and viruses. This leads to diseases such as cercosporiose, cladosporiose, bacterial wilt and viral diseases (Dave, 1993).

In addition, the root system of banana plants is superficial like that of weeds, resulting in competition for nutrients and water, causing a delay in the vegetative cycle of banana plants and a decrease in the average weight of the bunches (Traoré *et al.*, 2009).

To limit crop losses due to weeds, the use of synthetic herbicides is the most common control method in industrial banana plantations with intensive production systems. However, these products represent a potential health hazard through their carcinogenic effects on humans (Rautiainen and Reynolds, 2002) They are a source of environmental pollution and threaten

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the health of the applicator and the consumer (Sténuit *et al.*, 2004). Indeed, their use causes the modification of the carbon cycle with impacts on the climate (Houghton and Hackler, 2001), the alteration of water resources (Green *et al.*, 2004) and soil degradation (Wood *et al.*, 2000). In addition, during application and depending on the stage of development of the plant, 10 to 70 % of the product is lost to the soil and leads to a strong decrease in the development of soil microorganisms (Jensen and Spliid, 2003 ; Pimm, and Raven, 2000).

Thus, protective measures must be taken regarding the harmful effects of these chemicals on the soil microfauna, which is essential for maintaining soil fertility. To this end, the use of innovative cropping systems, through the use of cover crops to firstly control weeds and secondly improve the biological quality of the soil, is a promising avenue. For several decades, numerous studies have presented the importance of cover crops, particularly legumes, in soil fertilization by improving the growth of microorganisms, particularly arbuscular mycorrhizal fungi (Lamy, 2019 ; Savana *et al.*, 2021). In banana plantations, several works undertaken have highlighted the impact of arbuscular mycorrhizal fungi on the growth and development of this crop of interest (Jaizme-Vega *et al.*, 2002 ; Lebisabo *et al.*, 2019). In fact, according to the work of Stenström *et al.* (1997), under natural conditions, the vast majority of plants live in symbiotic association with mycorrhizal fungi that not only supply their hosts with water and mineral elements and also provide root protection against pathogenic fungi. It is in this context that this study was conducted. The main objective of this study was to evaluate the effects of two leguminous plants, *Arachis repens* and *Desmodium adscendens*, as cover crops on the development of microorganisms such as arbuscular mycorrhizal fungi (AMF) under industrial banana plantations.

Materials And Methods

Study Site

The experiment was conducted in the locality of Aboisso in Côte d'Ivoire, located 116 km east of the city of Abidjan. The geographical coordinates establish it at 5°28′04″ North latitude, 3°12′25″ West longitude, and 12 m altitude above sea level. The soil is ferralitic and heavily leached at the base under heavy rainfall (Koua, 2007). The department of Aboisso has an Atrean type climate with four seasons. A long dry season from December to February ; a long rainy season from March to July ; a short dry season from August to September and a short rainy season from October to November (Koua, 2007). The trials were conducted in an industrial banana plantation in Akressi, a village located 18.6 km from the town of Aboisso, at latitude 5°37′100″ North and longitude 3°12′25″ West.

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Plant material

Banana plants

The plant material used for the establishment of the banana plantation consisted of *in vitro* plants of the variety Grande naine of the Cavendish AAA group. These in vitro plants were planted after a stay of 11 weeks in the nursery.

Cover crops used

Two perennial cover crops (legumes), *A. repens* and *D. adscendens*, have been introduced into dessert banana plantations. *A. repens* is an exotic species. However, *D. adscendens* was collected in Côte d'Ivoire in the locality of Aboisso.

Preparation of the soil and plantation of the banana plants

Cultivation beds 10 m wide and 110 m long were made using the bulging method. These were then subdivided into three elementary plots, each with dimensions of 36 m × 10 m, to house the trials. The micro-sprinkler irrigation system was installed before planting. The banana plants were planted in double rows, in any row, at a density of 1820 plants per hectare with a spacing of 2.2 m between rows and 1.7 m between rows. They were watered by sprinkling at a rate of 2 h every two days. Three months after the establishment of the vitroplants, the cover crop cuttings were introduced into the plot after two months in the greenhouse. For this purpose, a staking operation with the simultaneous realization of 10 cm deep holes was used to materialize the locations of the cuttings. The cuttings were transplanted on the whole surface of the elementary plots in the spaces between the banana trees at a density of 111111 plants/ha, i.e. a spacing of 30 cm × 30 cm.

Experimental design

The experimental design chosen was a Fischer block with three treatments repeated three times. The trial consisted of elementary plots. These plots contained 64 planted banana trees. The treatments were as follows :

- T0 : Control or conventional practice with herbicide applications ;
- T1 : *A. repens* associated with banana plants ;
- T2 : *D. adscendens* combined with banana plants.

Soil sample collection

Prior to the installation of the cover crops under the dessert bananas, soil samples were taken. Six and twelve months after the installation of these plants, two other samples were taken respectively. For this purpose, 30 cm from each banana plant, blocks of monoliths were taken using a metal frame 25×25×20 cm3 deep. The monoliths were then cut into two layers according

to depth : 0-10 cm and 10-20 cm. Each layer was mixed gently to obtain a homogeneous sample.
Quantities of 100 and 500 g of soil per treatment (elementary plot) were taken for physicochemical analysis and for extraction of mycorrhizal spores, respectively.

Extraction of spores from AMF

Spores were extracted from soil samples following the wet sieving method described by Gerdemann and Nicolson (1963) followed by centrifugation in a biphasic water/sucrose medium (Daniels and Skipper, 1982). Thus, 100 grams of soil were suspended in water. Most of the soil particles sedimented faster than the mycorrhizal spores, which were then recovered by sieving the supernatant on a series of four sieves with decreasing mesh sizes (710 µm, 500 µm, 90 µm and 45 µm). The rejects from the last three sieves (500 µm, 90 µm, 45 µm) were collected in buckets and centrifuged at 3000 rounds for 5 min. After that, the supernatants were removed and replaced with 70 % sucrose solution. Centrifugation was again performed under the same conditions as the previous one. Then, they were filtered through the 45 µm sieve to recover the spores, which were rinsed with tap water to remove the sucrose.

Determination of the average number of spores of AMF

The extracted spores were distributed in squared Petri dishes containing cellulose wadding paper. They were observed with a binocular magnifying glass and counted according to their size (500, 90 and 45 µm).

Identification of AMF genera

Spores isolated from soil samples were sorted manually under a binocular microscope according to morphological characters such as color, shape and size. In each homogeneous batch, a few spores were mounted between slide and coverslip in a mixture of glycerine and Melzer's reagent in equal proportion and observed under the light microscope. Genus were determined with reference to the identification keys defined by Artib *et al.* (2016), Abbas (2014) and Moreira *et al.* (2015). The relative abundance of each identified genus was also determined by the following formula :

$$\text{Relative abundance (\%)} = \frac{\text{Number of spores of an identified genus}}{\text{Total number of spores of identified genus}} \times 100$$

Statistical analysis of the data

The data obtained were processed using STATISTICA software version 7.1. An analysis of variance one-way was used to study the effects of cover crops according to soil depth on the number of arbuscular mycorrhizal (AM) fungal spores. In case of significant differences between treatments, the

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Student and Newman Keuls multiple comparison test at the 5 % threshold was used to classify the means into homogeneous groups. For the comparison between the two depths, the Mann-Whitney test was used instead.

Results

AM fungal spore densities associated with cover crops

The effects of cover crops on AM fungal spore density were assessed using ANOVA. Thus, prior to cover crop placement, ANOVA revealed no significant difference between plots (cover crop and control) at soil depths 0-10 and 10-20 cm ($p = 0.26$ and $p = 0.33$ respectively). The number of AM fungal spores ranged from 687.50 to 882.50/100 g soil at the first depth (0-10 cm). At the second soil depth (10-20 cm), the number of spores fluctuated from 607.50 to 790 individuals in all plots (Figure 1).

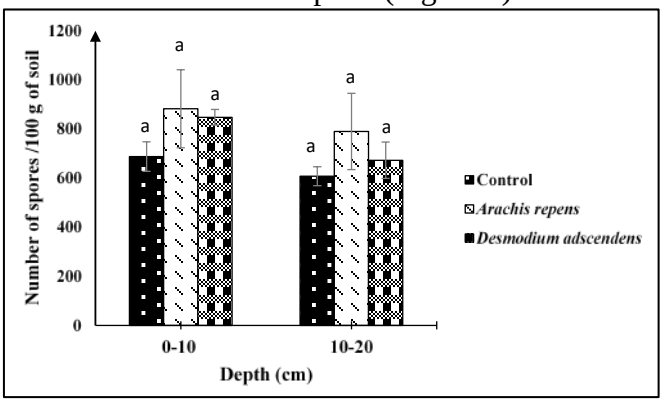

Figure 1. Mean numbers of arbuscular mycorrhizal fungals spores present in soil samples at depths 0-10 and 10-20 cm as a function of cover crops before trial establishment

Bars bearing same letter on the histograms are not significantly different at the 5 % threshold (Student's t test and Newman Keuls).

Six months after cover crop establishment, a significant difference was observed between treatments at each depth ($p=0.02$ and $p=0.01$ 0-10 cm and 10-20 cm, respectively). Indeed, at the 0-10 cm soil depth, a high number of AM fungus spores was obtained in the *A. repens* plots (1502.50±130.81 spores/100 g soil). However, in bare soil (control) and *D. adscendes* soil a low spore density was obtained at the same depth (1045.00±98.99 and 1030.00±35.35 spores/100 g soil).

Similarly, at 20 cm soil depth, *A. repens* plots showed the highest spore count, with a relatively downward trend (1177.50±201.52 spores/100 g soil). However, the lowest average number, 705.00±63.64 spores/100 g soil, was observed at this depth (20 cm soil) of the bare control plots (Figure 2).

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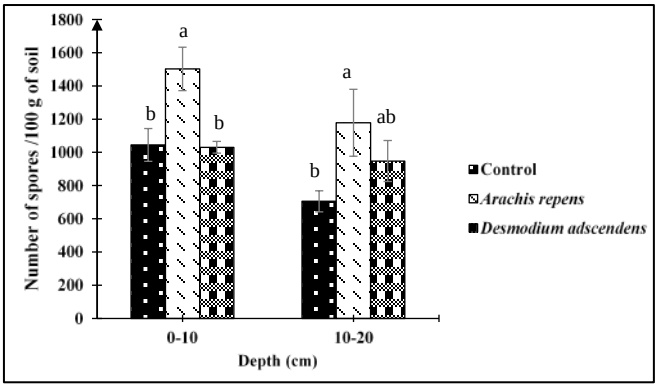

Figure 2. Average numbers of arbuscular mycorrhizal fungals spores present in soil samples at levels 0-10 and 10-20 cm according to cover crops 6 months after trial establishment

Bars bearing same letter on the histograms are not significantly different at the 5 % threshold (Student's t test and Newman Keuls).

Twelve months after the establishment of the cover crops (Figure 3), a significant difference was observed between the soils of the plots (p=0.017) with respect to the average number of mycorrhizal spores counted. Indeed, at a depth of 10 cm, a greater number of spores were observed in the soil samples taken from the *A. repens* plots (2390.00±70.71). In contrast, at the same depth, control and *D. adscendens* covered soils had the lowest average spore counts (1217.50±74.24 and 1400±318.19 respectively).

At 20 cm soil depth, a significant difference was also observed between treatments (p=0.001). Soils covered with *A. repens* obtained the most spores with a mean number of 1270±42.42. In contrast, bare soil and *D. adscendes* soil had the lowest spore numbers (867.50±24.74 ; 730.00±24.74).

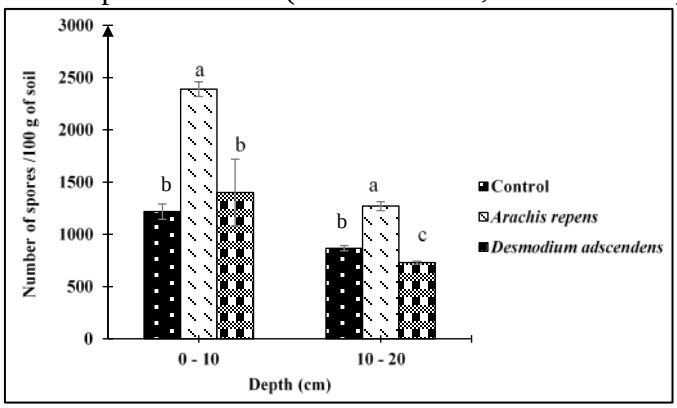

Figure 3. Average numbers of arbuscular mycorrhizal fungals spores present in soil samples at levels 0-10 and 10-20 cm depending on cover crops 12 months after trial establishment

Bars bearing same letter on the histograms are not significantly different at the 5 % threshold (Student's t test and Newman Keuls).

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AM fungal spore densities as a function of soil depth

An analysis of variance was performed on the average number of AM fungus spores as a function of soil depth before, 6 and 12 months after cover crop placement (Figure 4). This analysis revealed that during the first two collection periods, i.e. before and 6 months after cover crop placement, soil depth did not influence AM fungal spore density (p= 0.11 and p= 0.10 respectively). However, 12 months after cover crop placement, a significant difference was observed in soil depth (p= 0.02). Indeed, a large number or 1669.17±583.75 of AM fungus spores were observed at the first soil depth (0-10 cm). On the other hand, the lowest number was obtained at the 10-20 cm soil depth (955.83±252.02).

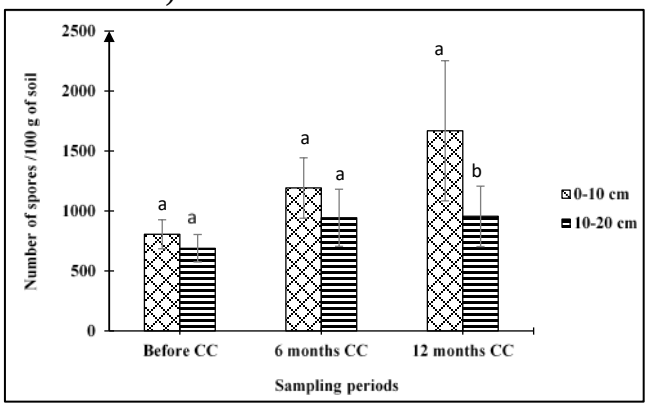

Figure 4. Mean numbers of arbuscular mycorrhizal fungals spores present in soil samples at levels 0-10 and 10-20 cm before, 12 months and twelve months after cover crops placement

Bars bearing same letter on the histograms are not significantly different at the 5 % threshold (Student's t test and Newman Keuls).

Measurements of AM fungal spores associated with cover crops

In Table 1 are presented the average numbers of spores per 100 g of soil according to their sizes determined by the diameter of the sieve meshes (500 µm, 90 µm and 45 µm). The difference between spore sizes was highly significant at the 5 % threshold. Indeed, the results showed that the soils of the different plots contained spores of varying sizes. The majority of the spores extracted were 45 and 90 µm in size regardless of the plots and the dates of observation.

Genus of AM fungi associated with cover crops

Four main genera were identified among the observed spores. These were *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora* (Figure 5). In all plots, the genera *Glomus* and *Acaulospora* were the most abundant at all sampling times and soil depths with values of more than 80 % (Table 2, 3 and 4). The last two genera, *Gigaspora* and *Scutellospora*, had relative abundances of less than 20 %.

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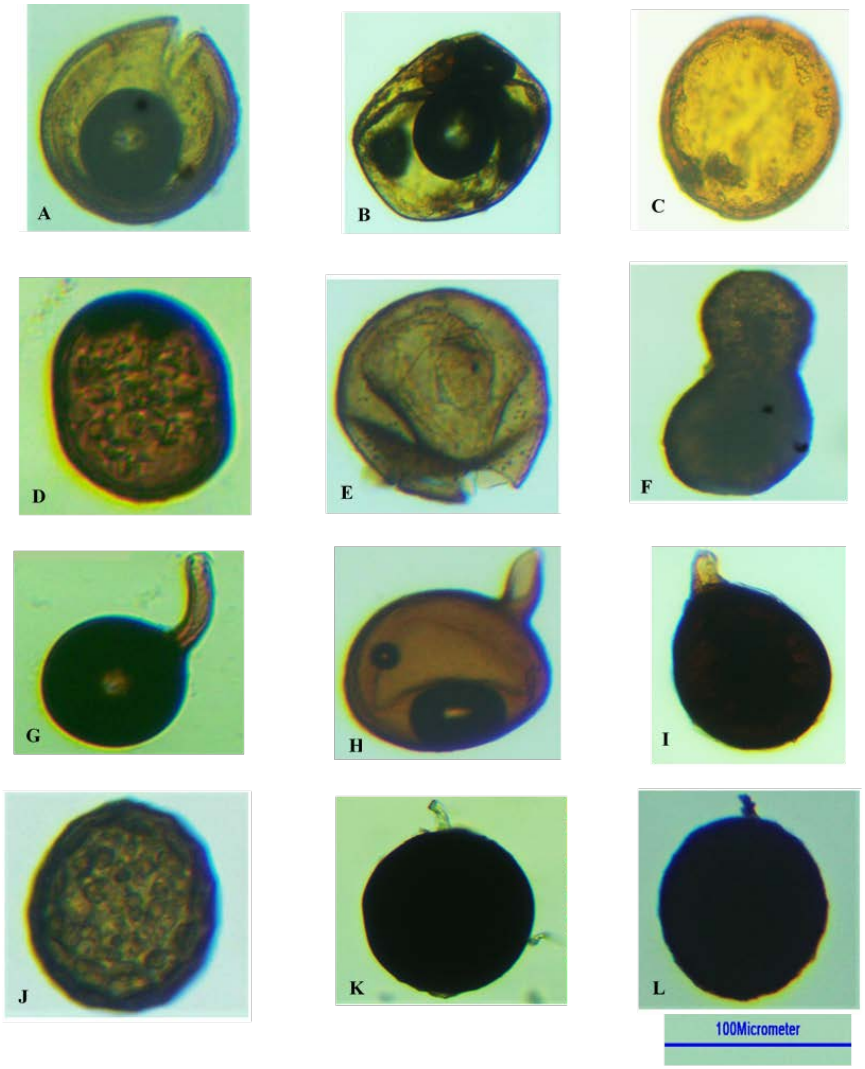

Figure 5. Genus of arbuscular mycorrhizal fungi identified

Table 1. Number of arbuscular mycorrhizal fungal spores according to their size in the soils of cover crops plant fields in banana plantations

		Before CC			6 months after CC			12 months after CC		
		Size of spores (µm)			Size of spores (µm)			Size of spores (µm)		
Treatments	**Depth (cm)**	**500**	**90**	**45**	**500**	**90**	**45**	**500**	**90**	**45**
Control	**0-10**	5.00 ± 2.89 c	206.67 ± 30.60 b	476.67 ± 7.26 a	13.33 ± 4.41 b	446.67 ± 9.28 b	586.67 ± 30.60 a	5.00±0.00 c	742.50±88.38 a	470.00±14.14 b
	10-20	0.00 ± 0.00 c	176.67 ± 12.58 b	428.3 ± 12.02 a	1.67 ± 1.67 c	266.67 ± 25.22 b	435.0 ± 5.77 a	02.50±03.53 b	400.00±07.07 a	465.00±28.28 a
Arachis repens	**0-10**	8.33 ± 1.67 c	385.0 ± 57.95 b	486.67 ± 9.28 a	25 ± 7.64 b	575 ± 60.62 a	911.67 ± 13.64 a	12.50±03.53 c	950.00±35.53 b	1427.50±31.82 a
	10-20	3.33 ± 1.67 c	361.67 ± 25.22 b	421.67 ± 39.19 a	18.33 ± 4.41 c	455 ± 10.41 b	720.0 ± 78.10 a	05.00±0.00 c	545.00±56.56 b	720.00±14.14 a
Desmodium adscendens	**0-10**	5.00 ± 2.89 c	356. 67 ± 25.22 b	476.67 ± 38.98 a	15.0 ± 2.89 b	458 ± 33.71 a	560 ± 18.03 a	07.50±03.53 b	742.50±53.03 a	650.00±268.70 a
	10-20	5.00 ± 2.89 c	315.0 ± 26.46 b	355.00 ± 7.64 a	11.67 ± 4.41 c	395 ± 12.58 b	545 ± 60.83 a	05.00±0.00 c	405.00±07.07 a	320.00±07.07 b

Before CC: before cover crops installation; 6 months after CC: 6 months after cover crops installation; 12 months after CC: 12 months after cover crops installation;

In each column, means followed by the same letter are not significantly different at the 5 % level (Student's t test and Newman Keuls). NS: Not significant, S: Significant

Table 2. Relative abundances of arbuscular mycorrhizal fungal genus identified in soils collected before cover crops installation in banana plantations

Treatments	Depth (cm)	Genus proportions (%)			
		Glomus	*Acaulospora*	*Gigaspora*	*Scutellospora*
Control	0-10	43.99	50.42	0.00	5.59
	10-20	30.20	56.84	12.96	0.00
Arachis repens	0-10	55.40	33.41	0.00	11.19
	10-20	49.02	36.21	5.29	9.48
Desmodium adscendens	0-10	56.63	29.94	6.51	6.92
	10-20	56.01	28.51	5.16	10.32
Total proportions of genus (%)		48.54	39.22	04.99	07,.25

Table 3. Relative abundances of arbuscular mycorrhizal fungal genus identified in soils collected 6 months after cover crops installation in banana plantations

Treatments	Depth (cm)	Genus proportions (%)			
		Glomus	*Acaulospora*	*Gigaspora*	*Scutellospora*
Control	0-10	46.83	46.46	6.71	0.00
	10-20	39.12	46.64	4.34	9.90
Arachis repens	0-10	55.77	30.25	3.05	10.92
	10-20	56.07	31.04	9.70	3.19
Desmodium adscendens	0-10	55.41	36.15	5.41	3.03
	10-20	64.34	35.66	0.00	0.00
Total proportions of genus (%)		52.92	37.70	04.87	04.51

Table 4. Relative abundances of arbuscular mycorrhizal fungal genus identified in soils collected 12 months after cover crops installation in banana plantations

Treatments	Depth (cm)	Genus proportions (%)			
		Glomus	*Acaulospora*	*Gigaspora*	*Scutellospora*
Control	0-10	56.00	38.40	0.00	5.59
	10-20	35.63	61.34	0.00	3.03
Arachis repens	0-10	45.85	37.73	9.50	6.91
	10-20	52.84	34.37	2.98	9.81
Desmodium adscendens	0-10	26.18	40.13	17.84	15.85
	10-20	44.17	42.50	3.75	9.58
Total proportions of genus (%)		43.45	42.41	05.68	08.46

Discussion

The results of this work revealed a very high number of AM fungal spores in plots covered by *A. repens* and *D. adscendens* compared to bare soil (control). This suggests that the plant cover could strongly influence the increase of AM fungal spores. The plants of *A. repens* and *D. adscendens* during photosynthesis would have made sugar available to the AM fungi for

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their nutrition. This would have favored the increase of these spores over time in soils covered with these two legumes as indicated by the recent work of Lamy (2019).

Furthermore, AM fungi are obligate symbionts, meaning they cannot thrive in the absence of a host plant. To establish mycorrhization, these fungi colonize a plant's root system with their mycelium and they produce spores, which allow them to survive in the absence of the host plant and reproduce (Hopkins, 2003; Smith and Read, 2008). Thus, the disappearance of the herbaceous stratum, would necessarily lead to the biological degradation of the soil (Requena et al., 2001; Azcon-Aguilar et al., 2003). This degradation is manifested by a reduction in diversity or telluric microbial activity (Kennedy and Smith, 1955). The work of El Mrabet et al. (2017) also showed that the total number of AM fungal spores isolated from rhizospheric soils of two accompanying species of argan tree namely *Chamaecytisus albidus* and *Ononis natrix*, was significantly higher than that of bare soil. Moreover, in the current study showed that the soils covered with *A. repens* had the highest number of mycorrhizal spores compared to *D. adscendens*. This finding could be due to the root system of *A. repens* that allows for the multiplication and entrapment of mycorrhizal fungi. These results confirm that some plant species have the ability to facilitate the development of fungal propagules in their rhizosphere (Eom et al., 2000 ; Azcon-Aguilar et al., 2003 ; Lovelock et al., 2003).

Twelve months after cover crop establishment, the distribution of arbuscular mycorrhizal fungal in soil was not homogeneous. The 0-10 cm layer was more densely populated than the 10-20 cm layer. These observations could be explained by the heterogeneity of the soil matrix in terms of physicochemical properties and distribution of carbon resources. Indeed, the depth of the soil (different soil horizons) induces gradients in nutrient availability, which would contribute to modify the density of microorganisms (Fierer et al., 2003).

Small and medium-sized spores were most abundant in all plots studied. These results are consistent with those of Dione (2007) who reported that AMF spore numbers are inversely proportional to their size.

Spores of four genera of AMF were identified in the soils from the different plots. These are the genera *Glomus, Gigaspora, Scutellospora* and *Acaulospora*. These four genera of AM fungi found during this study are identical to those observed in Côte d'Ivoire by Anguiby et al. (2019). These have been generally isolated, in the rhizosphere of other crops, by different microbiologists in the central West African (Ngonkeu et al., 2013) and Mediterranean (Bouamri et al., 2006) regions, in the humid forest zone in Cameroon and in the Sudanic zone in Burkina-Faso. Several works have reported the presence of endomycorrhizae of the genus *Glomus*, in general, in

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the rhizosphere of plants in other warm regions of the globe (Bousselmame et al., 2002 ; Zhao et al., 2003 ; Laminou, 2010). In a study conducted by Jamil et al. (2002) in the arid and semi-arid regions of Jordan, seven species of the genus *Glomus* were found. The predominance of the genus *Glomus* in most ecosystems suggests a better adaptation of this genus either to the most hostile conditions such as drought, salinity and other environmental stresses (Pande and Tarafdar, 2004), or to a wide range of ecological niches (Houngnandan et al., 2009). Indeed, the genera *Glomus* would preferentially spread by spores which are forms of resistance of MACs to harsh conditions; while *Gigaspora* and *Scutellospora* would spread more with other types of propagules such as hyphae and extra-root mycelial fragments (Brito et al., 2012).

Conclusion

At the end of this study, it was clear that the two leguminous plants, *A. repens* and *D. adscendens*, used as cover crops had an effect on the development of soil microorganisms. *A. repens* strongly contributed to the multiplication of AM fungal spores. In contrast, reduced spore number was observed in the bare soil. It would, therefore, be advantageous to use cover crops, particularly *A. repens*, for the maintenance of biological soil fertility, especially in industrial dessert banana plantations where synthetic chemical fertilizers and herbicides are widely used.

Conflict Of Interests

The authors have not declared any conflict of interests

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