

The Influence of No-till Farming on Durum Wheat Mycorrhization in a Semi-Arid Region: A Long-Term Field Experiment

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Abstract

Maintaining a reliable and sustainable agricultural production system has become one of the major concerns of producers in arid and semi-arid regions. Simplifying farming techniques and practicing Direct Seedling (DS) could contribute to insure the sustainability of agriculture, preserving the natural resources and the environment. Direct Seedling based on limiting soil plowing has a potential number of benefits, including reduced production costs and soil erosion. Associated with the organic mulch, this technique improves the soil fertility and favors the establishment of root symbioses. Given the importance of the no-till farming techniques, the present research work aims to compare the effects of DS and those of Conventional Seedling (CS) on the evolution of arbuscular mycorrhizal symbiosis in durum wheat (*Triticum durum* Desf) roots, cultivated in the field for five years. Soil and root samples were collected during three different cropping stages at two different treatments. The results of Arbuscular Mycorrhizal Fungi (AMF) root colonization kinetics have shown an increase in the percentage of arbuscules and a decrease in vesicles for plants sampled from a DS field compared to those from CS. Effects of the DS on the mycorrhizal parameters appears clearly in the fourth year of the experiment and continues in the fifth year, with an arbuscule percentage reaching 80% in a DS field and not exceeding 21% in a CS field. Soil phospholipid fatty acids (PLFA) C16:1 ω 5 (biomarker of AMF) and C18:2 ω 6, 9 (biomarker of saprophytic/ectomycorrhizal fungi) demonstrate that no-till practice improves AMF biomass and saprotrophic/ectomycorrhizal fungal biomasses by 52 and 159%, respectively, in comparison with those found in a CS field. In both treatments, no-till farming and CS plots, the AMF biomass is higher than saprotrophic/ectomycorrhizal biomasses. The natural biodiversity of AMF is also enhanced in a no-till field. In addition, an increase in the relative abundance of six families of Glomeromycota (Gigasporaceae, Diversisporaceae, Scutellosporaceae, Entrophosporaceae, Acaulosporaceae, Dentiscutataceae) was observed. To summarize, the present study highlights the importance of no-till practice as an approach to restore the microbiome in soils disturbed by tillage in semi-arid regions.

Keywords: arbuscular mycorrhizal fungi, no-till farming, conventionnel seedling, lipid biomarkers, root endophytes, semi-arid region

1. Introduction

Maintaining a reliable and sustainable agricultural production system has become one of the major concerns of producers in arid and semi-arid regions (Lyons et al., 1996; Bruelle et al., 2017). Simplifying farming techniques and using no-till techniques contribute to ensure the sustainability of agriculture, preserving the natural resources and the environment. To cope with the various constraints, including drought, many countries around the world (The United States, Argentina, Chile, Brazil, and Australia), have adopted the simplification of cultural techniques, known as SCT (Freitas et al., 2013; Bellotti et al., 2014). In 2007, the extension of this system was about 95 million hectares (Lal et al., 2007), and presently it exceeds 125 million hectares (Bruelle et al., 2017).

In Algeria, cereals are rainfed crops that occupy an annual area of 3.5 million hectares (Rouabhi et al., 2019). They are mainly located in the east of the country, in the semi-arid regions of the High Plateaus, where yearly rainfall levels are less than 400mm. Soils of these regions are predominantly calcareous with low levels of soil organic matter (Batouche & Labiod, 1991). The combined effects of all these factors affect cereal production, and yields remain low even though the cultivated varieties are improved (Lahmar & Ruellan, 2007). Cultivation of cereals is conducted according to the soil conventional tillage system, based mainly on soil plowing to control weeds, preparing seedlings, incorporating residue crops and fertilizers, and increasing water infiltration. Conventional farming system increases grain production costs and threat of soil erosion (about 2000 to 4000 tons/km² of soil are lost) (Kaci et al., 2017; Arabi & Roose, 1989), especially when combined with overgrazing.

Compared to other farming systems, conventional soil tillage (CT) influences the soil physical characteristics. It increases the soil pulverization at 0-10cm depth leading to lower bulk density and higher macroporosity, which facilitates water erosion. In layers below 10 cm depth, CT increases bulk density and reduces the macro-porosity to critical levels for crop growth (Tuzzin de Moraes et al., 2016), which increases the risk of soil compaction, penetration resistance and affects their biological activity (Babujia et al., 2010; Aslam, 1999).

Conservation agriculture (CA) is a more recent innovation that combines soil cover-organic-mulch, reduced plowing or no-till practice and crop rotation. It is currently promoted as a means to reduce soil degradation in the Mediterranean semi-arid areas (Lahmar & Bouzerzour, 2011). A number of experiments and research results from Spain, Morocco and Tunisia, underlines the prominent role of the organic mulch in the improvements that occur in the soil, and suggest that no-till practice is a plausible alternative to conventional tillage (Mrabet, 2001; Lahmar & Arrúe, 2007; Lahmar, 2010; Vega et al., 2015). However, the adoption of no-till farming should be done in an integrated approach, allowing good management of rotations, judicious control of weeds, diseases and the attack of herbivores while minimizing the use of chemical inputs (Karuma et al., 2014). This will promote biological activity and microbial biomass in soils (Labreuche et al., 2007). Celik et al. (2011) have specified that no-tillage practices under a semi-arid climate have considerably increased the number of arbuscular mycorrhizal fungi (AMF) spores in the soil. Indeed, for cultivated cereal species, direct seedling promotes interactions between the root system and the symbiotic fungi such as Dark Septated Endophytes (DSE) or AMF (Jansa et al., 2006; Schalamuk et al., 2006; Duponnois et al., 2012).

AMF symbiotic interactions allow the host plant to tolerate abiotic stress through a structural change of the root system and especially by providing them better water and mineral nutritions (Rodriguez et al., 2004; Soka & Ritchie, 2014; Wu, 2017). Root Dark Septate Endophytes (DSE) are fungi that locate and grow between root tissues (Vega et al., 2008). They are characterized by having dark pigmentation, microsclerotia and melanized septated hyphae that colonizes the epidermis as well as the inter and intracellular root cortex of the host plant (Peterson et al., 2008). Many of these fungi are able to colonize the root cells of plants, promoting growth without causing pathologies (Clay, 2004; Yuan et al., 2010; Vergara et al., 2018). DSE are found worldwide and coexist often with different mycorrhizal fungi (Mandyam & Jumpponen, 2005, 2008).

In Algeria, only 5,559 ha of cereal areas are converted to no-till farming as a result of sensitization campaigns carried out by agricultural research and development institutions. They encourage farmers to adopt the no-till farming system in order to reduce the environmental and economic costs of agricultural production (Zeghouane et al., 2015, 2016).

Consequently, whilst aiming to compare the effect of no-till farming and conventional seedling systems on two important types of fungal root symbiosis-arbuscular mycorrhiza and dark septated endophytic fungi of durum wheat in a field experiment, the present study lasted five years.

2. Material and Methods

2.1 Experimental Site

This study was carried out from 2008 to 2013, at the agronomic experimental station of the Technical Institute of Field Crops (ITGC) of Sétif (latitude, 36°08' N; longitude, 5°20' E and altitude 962 m. The climate is Mediterranean and semi-arid, characterized by cold wet winters and hot dry summers. During the experiment, the average annual rainfall did not exceed 400 mm and presented some irregularity in distribution, with large inter-month and inter-annual variations. The dry season was usually noted from May to September (Figure 1).

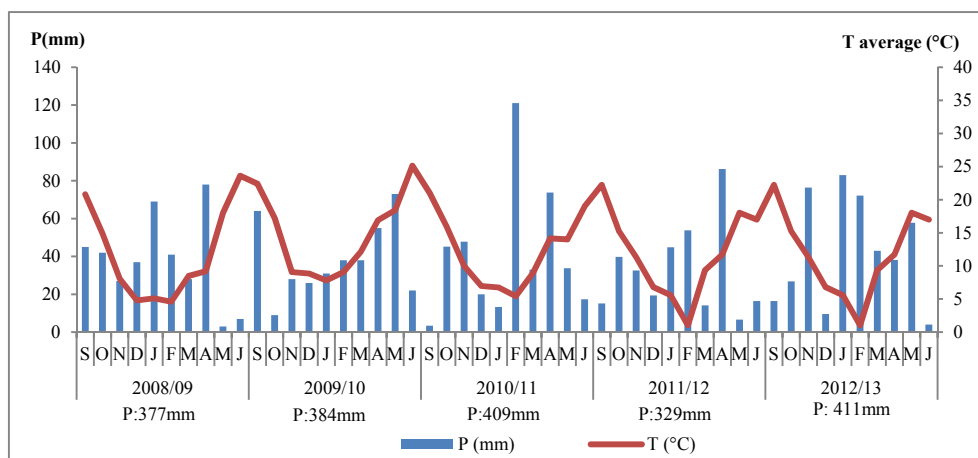


Figure 1. Evolution of precipitation and temperature during the five years of field experimental trial conducted at the agronomic experimental station of the Technical Institute of Field Crops (ITGC) of Setif (ONM National Office of the meteorology).

Soil characterization analyses of the experimental plot were made by the Technical Institute of Field Crops (ITGC) at the beginning of the project. They indicated that on the horizons which delimited the first thirty centimeters of topsoil, the soil of the experimental plot had a clay-sandy-loamy texture, with an average organic matter content of 2.6%. Basic pH (8.26) justified the strong presence of total and active limestone (51.58%; 21.25%), which weakened the availability of absorbable phosphorus (7.33 ppm) (Table 1).

Table 1. Physicals and chemicals characteristics of the soil in the experimental field

Physical characteristics %			Chemical characteristics					Organic characteristics	
Silt	Clay	Sand	CE (mmho/cm)	pH	Total CaCO ₃ (%)	Actif CaCO ₃ (%)	Available P ₂ O ₅ (ppm)	OM (%)	C org (mg g ⁻¹)
34.66	34.66	30.66	0.175	8.26	51.58	21.25	7.33	2.6	1.51

Note. Available phosphorus (Olsen Phosphorus) was determined by the Olsen et al. (1954) method.

The soil organic carbon was determined by means of the Anne method.

The total and active carbon dosages were made by the methods of Bernard's calcimeter and that of Drouineau, respectively. Soil Granulometry by the Robinson Method.

2.2 Plant Material and Field Conditions

The studied variety of durum wheat used in the present study is named "Boussalem". It was obtained from the Sétif experimental station (Heider/Marli/Heider Cro ICD4141BLCTR4AP) on an ICARDA-CIMMYT introduced material. The experimental design was based on the application of two soil management practices: 1) No-till with seedling (SD) carried out directly in the residue of the previous crop, using a combined seeder (SEMEATO) which opened only a seed line with 2 cm of width; 2) Conventional seedling (CS), where the soil was prepared with a moldboard plough followed by a cover crop and a disc harrow. Seedling was carried out by means of a conventional seed drill (Figure 2).

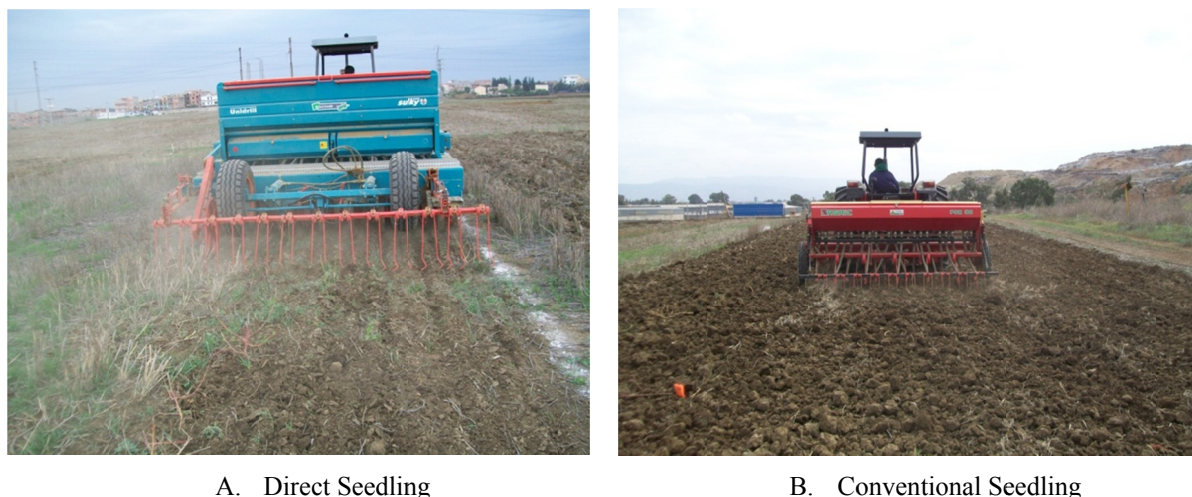


Figure 2. Photos of the both seeders used in the experiment

Note. The previous crop is a durum wheat, two treatments are conducted as follows: Direct Seeding (A): No Tillage; Conventional Seeding (B): Mouldboard plough + Cover Crop + disc harrow.

Seedling rate was of 130 kg/ha. Farming techniques applied on the fields during the five years of the experiment culture are described in Table 2.

Table 2. Culture conditions applied to the plots of the field trial

Years of study	Soil tillage		Herbicides Treatment		Fertilizers		Sowing dates	
	DS	CS	DS	CS	DS	CS	DS	CS
A1 (2008/09)			Topic 0.75 l/ha				10/11/2008	
-----			Grand star 15 g/ha				-----	
A2 (2009/10)			Glyphosate 2 l/ha		*TSP (46%) 100 kg/ha		03/11/2009	
			Glyphosate 2 l/ha		Before sowing		-----	
A3 (2010/11)	No tillage	Mouldboard plough + Cover Crop + disc harrow	Rymbi 0.75 l/ha				28/11/2010	
			Grand star 15 g/ha		Urée (46%) 100 kg/ha		-----	
A4 (2011/12)			Glyphosate 3 l/ha		Tillage Stage		12/01/2012	
-----			Pallas OD				-----	
A5 (2012/13)			(Pyroxilam 45 g/l) 0.5 l/ha				09/01/2013	

Note. DS: no-till farming system; CS: Conventional Seeding; TSP: Tri Sulfate of Phosphate.

*The high presence of weeds in no-till plots necessitated a total weeding before sowing with glyphosate and at post-emergence with Grand star (anti dicotyledonous) mixed with Brumby (anti monocotyledons) or with Pallas OD (anti dicotyledonous and monocotyledons).

* The conventional seeding trial was only subjected to post-emergence weeding.

* The change of products for the last two campaigns is justified by the appearance of Brome plants (*Bromus rubens*) which is controlled by the Pallas OD herbicide and not by the other previous products.

For the two treatments, the plots had 180m² of area with three replicates per treatment, and they were distributed following a completely randomized bloc design (Figure 3).

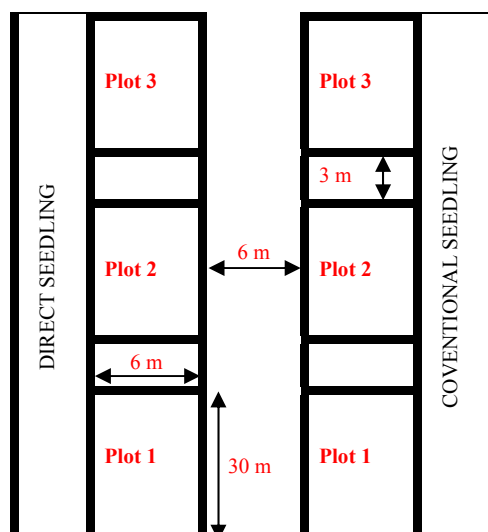


Figure 3. The experimental design applied during the five years of experimentation

Note. Test with six plots with 180 m² (6 m × 30 m) arranged as a complete random block (CRB).

2.3 Plant and Soil Sampling

The experimental trial was field-installed in 2008, but our observations started in the third year of testing (2010-2011) and proceeded as follows:

2.3.1 Soil Fatty Acid Analysis

Two soil samples were taken from each plot at a depth of 10 cm with an auger, following the harvest of the 2010/2011 trial (third year of application of the two different operating systems). After removing plant debris from soil samples, four replicates of 3 g of freeze-dried soil (from each soil sample) were analysed to measure the phospholipid fatty acid (PLFA) contents. Amounts of PLFA and neutral fatty acid (NLFA) specific to AMF (C16:1 ω 5) and to saprotrophic/ectomycorrhizal fungi (C18:2 ω 6.9) were measured.

Lipid extraction was carried out following the method suggested by Frostegård et al. (1991). The extracted lipids were fractionated into neutral lipids, glycolipids and polar lipids on a silica acid column by successively eluting with chloroform, acetone and methanol (1:2:1, V:V:V). Chloroform fraction (containing the neutral lipids) and the methanol fraction (containing the phospholipids) were subjected to a transesterification using a base solution (KOH 0.2 M) prepared in methanol to transform the PLFA and the NLFA into free fatty acid methyl esters. Final extracts were analysed by using a PerkinElmer Auto system gas chromatograph (GC) equipped with a flame-ionization detector (Norwalk, CT) and an EC-1000 (Alltech) capillary column (30 m × 0.53 mm i.d.) with hydrogen as a carrier gas (3.6 ml min⁻¹). The temperature program included a fast rising from 50 °C to 150 °C at 15 °C min⁻¹ and then a rise from 150 to 220 °C at 5 °C min⁻¹. Fatty acid quantification was made by using nonadecanoic acid methyl ester (C19:0) as an internal standard. Their identification relied on the retention times of a wide range of standards (fatty acid methyl ester mixtures C4-C24:1, Sigma Aldrich) and mass analysis. The system was operated in the constant linear velocity (70 cm s⁻¹) using helium as a carrier gas. Fatty acids were identified by comparing their mass spectra with the standard mass spectra in the NIST MS library.

2.3.2 Determination of Arbuscular Mycorrhizal Fungal Colonization

To estimate the root colonization by AMF, during three stages of the vegetative cycle of wheat (tillering, grain filling and grain maturity), 6 plants/plot/stage for each treatment were sampled in 2011, 2012 and 2013 (last three years of the experiment). The measurement was performed on fresh roots. Roots were soaked during 5 min in an H₂O₂ (30%) bath to remove pigments, cleared in KOH (10%) and stained with Trypan blue (0.05%) (Phillips & Hayman, 1970). Root mycorrhizal rates were calculated using a magnified grid line intersect method (Mc Gonigle et al., 1990). For each repetition three microscopic slides with ten randomly root fragments (1 cm) were observed under an optical microscope (× 100). Intersections were counted in the following categories: negative (no fungal material in root), arbuscules, vesicles and hyphae. This made it possible to quantify the following rates:

$$H\% = (G - p)/G \times 100; Arb\% = (q + s)/G \times 100; Ves\% = (r + s)/G \times 100 \quad (1)$$

where, G: total number of intersections; p: no mycorrhizal structure; q: presence of arbuscules; r: presence of vesicles; s: presence of arbuscules and vesicles.

2.3.3 Determination of Dark Septated Endophytic Fungal Root Colonization Rate

The estimation of root colonization was completed in 2013 by the observation of structures specific to dark septated endophytic fungi (DSE). Microscopic observations were performed on the same slides used for AMF but focused on the structures specific to DSE (dark septated hyphae, microsclerotia) in the root cortex and the central cylinder. The rate of DSE (DSE%) was calculated according to the following formula:

$$DSE\% = E/G \times 100 \quad (2)$$

where, E: presence of DSE structures; G: total number of intersections, using the method suggested by McGonigle et al. (1990).

2.3.4 Biodiversity of AMF Species

Soil samples were collected during the grain filling stage in the last year of the study. Two soil samples were taken from the rhizosphere zone per plot for each treatment. A mixed homogenized sample was made for each plot to form three samples per treatment. AMF spores were extracted from 100 g of soil using the wet-sieving method (Gerdemann & Nicolson, 1963) followed by flotation-centrifugation in 50% sucrose (Dalpé, 1993). The spores were isolated from supernatant on a filter paper and were counted using a stereomicroscope at $\times 40$ magnification to determine the total density of AMF (density of spores per 100 g of soil collected in each studied plot). Spores were thus classified by spore morphotype (size, color, hyphae) (Gerdemann & Trappe, 1974) and then mounted in polyvinyl alcohol-lacto-glycerol (PVLG) with or without Melzer's reagent on microscopic slides. Observations were carried out under an optical microscope ($\times 100$ magnification). Identification, based on morphological characters, was performed using the INVAM website: <http://invam.wvu.edu>. Following identification, AMF species richness was evaluated based on the number of species present in each treatment.

The relative abundance of each species in each treatment was calculated following the method provided by Johnson et al. (1991):

$$RA\% = (n/N) \times 100 \quad (3)$$

Where, n = number of identified spores, N = total number of spores. This made it possible to determine the relative abundance of each spore family.

2.3.5 Carbon and Nitrogen Stocks

An analysis of organic matter and mineral nitrogen was performed on the soil samples after harvesting during the last year of the experiment. Two soil samples per plot and replicate were taken from a 10-cm depth for each treatment. The soil organic carbon was determined by means of the Anne method (Aubert, 1978). The quantification of total nitrogen was determined according to the KJELDAHL method (Bremner et al., 1982).

2.3.6 Grain Yield Evaluation

The grain yield was determined after harvesting for each plot area, during the five years of testing (Grignac, 1986).

2.4 Statistical Analysis

An analysis of variance with a Newman and Keuls test for the comparison of means was applied to the data ($p < 0.05$) with Statbox 2012 software. The Percentage data was transformed by a square root procedure.

3. Results

3.1 Effect of Farming System on Wheat Mycorrhizal Colonization

The results of the three years of observation showed that the total mycorrhizal rate was about 90% at the tillering stage and during grain filling. At the grain maturity stage, this rate decreased until reaching 26% in the last year. However, during this latter stage, no-till treatment showed a higher mycorrhizal root colonization compared to conventional seedling. This increase was about 22% during the second year of observation, and it reached 45% during the last year (Figure 4). Arbuscules were higher in plant roots sampled from the no-till farming plots than those from the conventional seeding plots during all the time of the trial. At the second year of observation, the arbuscules reached in no-till plots 65% and 45% at tillering and filling stages of the grain, respectively. However, in the conventional seedling system, it did not exceed 25%. While this increase continued during the last year of the experiment until reaching 80% at tillering stage, it remained stable in conventional seedling (Figure 4).

Contrary to this evolution, the vesicles were found to have increased in the conventional seedling plots, especially in the grain filling stage (Figure 4).

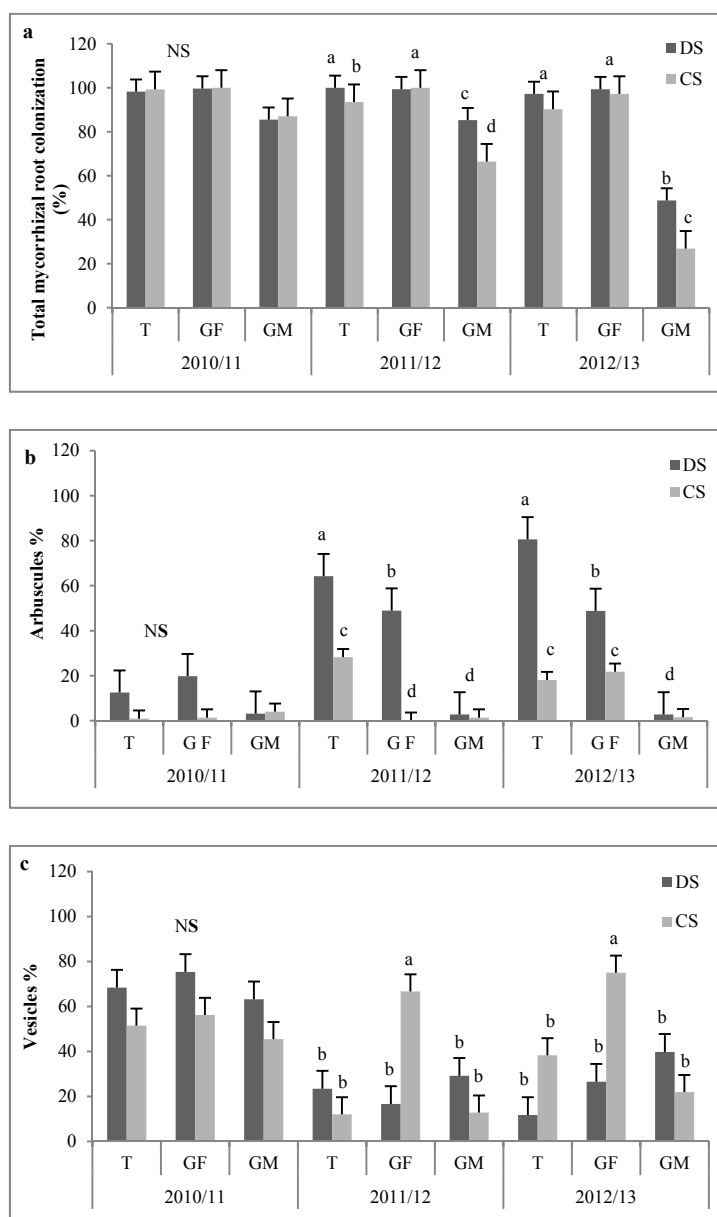


Figure 4. Effect of conventional seedling (CS) and no-till farming system (DS) on total mycorrhizal root colonization (a), arbuscules (b) and vesicles(c) percentage during the last three-years of the experiment for the three plant growth stages

Note. T: tillering, GF: grain filling, GM: Grain maturity. Means were obtained from 3 replicates. Significant differences between CS and DS are reported according to the Newman-Keuls test ($P < 0.05$).

3.2 Effect of Cultivation System on AMF Diversity

Spores density varied from 1280 spores to 2114 spores isolated from the soil of conventional and in direct seedling plots respectively. A total of 21 AMF species belonging to 7 families were identified. The AMF richness values of direct and conventional seedling soils were estimated respectively to 85.71 and 66.67% of the total estimated richness.

Seven species were found exclusively in direct seedling soil. Among them five species of Acaulosporaceae (*Acaulospora scrobiculata*; *Acaulospora koskei*; *Acaulospora thomii*; *Acaulospora capsicula*; *Acaulospora denticulata*) and two species of Gigasporaceae (*Gigaspora albida* and *Gigaspora margarita*) with respective relative abundance (38 and 33%) for two families. Three species of Glomeraceae (*G. mossae*, *G. aggregates* and *G. ambisporum*) were occurred only in the conventional seedling soil.

Eleven species were present in both treatments but more abundant in plots conducted in the no-till system (Figure 5). The respective relative abundance in DS/CS was as follows: Gigasporaceae (13.4%/7.4%) with one species (*Gigaspora decipiens*); Diversisporaceae (12.8%/5.5%) with the species *Diversispora tortuosa*; Scutellosporaceae (24%/10.5%) with the species *Scutellospora scutata* and *Scutellospora calospora* and Entrophosporaceae (15.6%/3.7%) with *Entrophospora infrequens*. Dentiscutataceae were rarely present in the conventional seedling plots and are poorly present in the no-till plots (7.8%/0.2%) with *Dentiscutata erythropha* and *Dentiscutata nigra*. Glomeraceae had a dominant presence in the soil of both treatments, but their relative abundance is higher in conventional seedling (58.7%/76.6%) with (*G. intraradices*; *G. fasciculatus*; *G. gerdemanni* and *G. multicaule*) (Table 3 and Figure 5).

Table 3. AMF spore species isolated from the soil of the experimental field plots, for each treatment (DS: no-till farming system; CS: conventional seedling) in the last year of the trial

Family/Species	DS	CS
Glomeraceae		
<i>G. intraradices</i>	x	x
<i>G. fasciculatus</i>	x	x
<i>G. gerdemanni</i>	x	x
<i>G. multicaule</i>	x	x
<i>G. mossae</i>		x
<i>G. aggregates</i>		x
<i>G. ambisporum</i>		x

Acaulosporaceae		
<i>A. koskei</i>	x	
<i>A. thomii</i>	x	
<i>A. capsicula</i>	x	
<i>A. denticulata</i>	x	
<i>A. scrobiculata</i>	x	

Gigasporaceae		
<i>Gg. Albida</i>	x	
<i>Gg. margarita</i>	x	
<i>Gg. decipiens</i>	x	x

Dentiscutataceae		
<i>D. erythropha</i>	x	x
<i>D. nigra</i>	x	x

Diversisporaceae		
<i>D. tortuosa</i>	x	x

Scutellosporaceae		
<i>S. scutata</i>	x	x
<i>S. calospora</i>	x	x

Entrophosporaceae		
<i>E. infrequens</i>	x	x

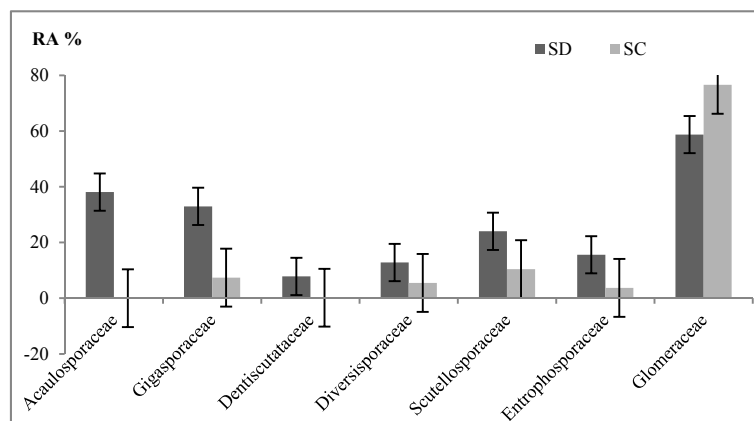


Figure 5. Influence of conventional seedling (CS) and no-till farming system (DS) on the relative abundance (RA%) of Glomales families. Means were obtained from 3 replicates

3.3 Arbuscular Mycorrhizal Fungi and Dark Septate Endophytic Root Colonization

The comparative measurement of wheat root colonization by symbiotic structures specific to AMF and those of dark septate endophytic fungi (DSE) showed that mycorrhizal colonization remained more important in both treatments in comparison with DSE root colonization. The latter reached 40% in conventional seedling and 57% in the no-till farming plots, while AMF root colonization exceeded 90% in both treatments (Figure 6).

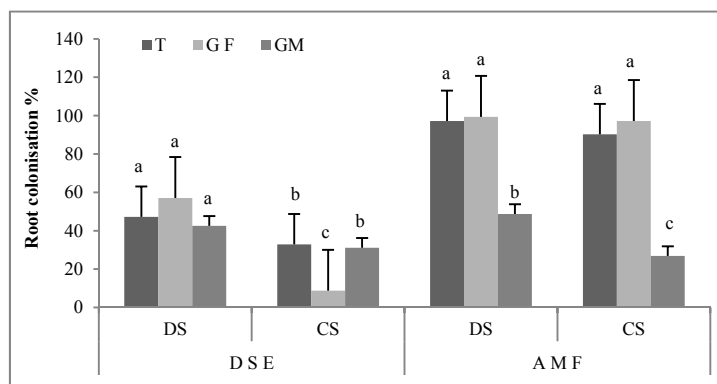


Figure 6. Influence of conventional seedling (CS) and no-till farming system (DS) on mycorrhizal and dark septated endophytic fungi root colonization, after five years of experimentation for the principal stages of plant growth

Note. T: tillering, GF: grain filling, GM: Grain maturity. Means were obtained from 3 replicates. Different letters indicate significant differences between DS and CS according to the Newman-Keuls test ($P < 0.05$).

3.4 Effect of the Farming System on Soil Fungal Biomass

Quantification of microbial biomass by means of specific lipid biomarkers showed that the amounts of PLFA C16:1 ω 5, a specific fatty acid of AMF, increased by 52% in the no-till plots (DS) compared to the conventional seedling ones with respective values of (DS 1.07/CS 0.70) nanomoles g^{-1} of soil. Similarly, the amount of PLFA C18:2 ω 6.9 used to quantify saprotrophic and ectomycorrhizal fungal biomasses increased by 159% with respective values of (DS 0.96/CS 0.37) nanomoles g^{-1} of soil. C16:1 ω 5 PLFA amounts were higher than C18:2 ω 6.9 PLFA both in the no-till and conventional seedling plots (Figure 7). In contrast, the content of C16:1 ω 5 NLFA representing mainly the storage structures of the AMF (vesicles and spores) was almost the same for both treatments: 1.6 nanomoles/g of soil.

The NLFA/PLFA ratio of C16:1 ω 5 fatty acid was superior to 1 indicating that the C16:1 ω 5 fatty acid was originating from AMF and not bacteria.

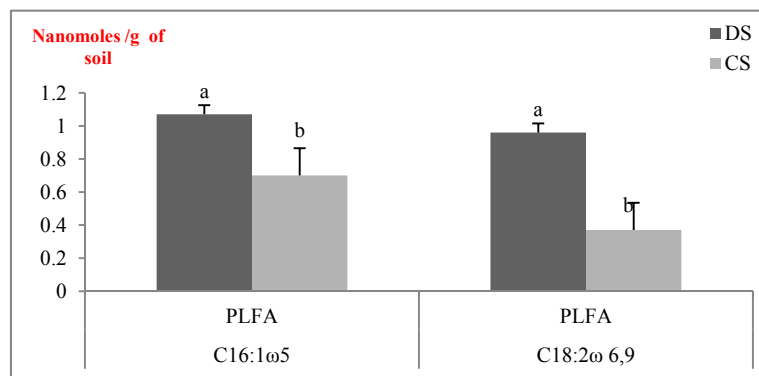


Figure 7. Amounts of phospholipid fatty acid (PLFA) (C16:1ω5) specific to AMF and (C18:2 w 6,9) specific to saprotrophic/ectomycorrhizal fungi in root-free soil in no-till farming system (DS) and conventional seedling (CS), after three years of experiment. Means were obtained from 6 replicates. Different letters indicate significant differences between DS and CS according to the Newman-Keuls test ($P < 0.05$)

3.5 Organic Matter and Nitrogen Amounts in the Soil

The results showed that after five years of no-till farming, the soil organic matter and organic carbon contents increased by 29% compared to conventional seedling. However, this increase was statistically insignificant, and the soil organic matter (SOM) remained low for respectively the no-till and conventional seedling systems (3.71 and 2.62%) (Figure 8). The mineral nitrogen content remained similar for the two cultural systems (0.17%) (Figure 8). The C/N ratio was improved by 27%.

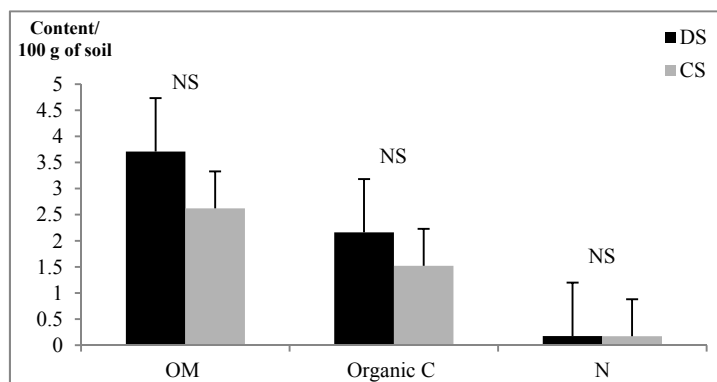


Figure 8. The effect of conventional seedling (CS) and no-till farming (DS), after five years of experimentation, on soil organic matter organic carbon and nitrogen amounts. Means were obtained from 3 replicates. (NS) No significant differences between DS and CS according Newman-Keuls test ($P < 0.05$) were observed

3.6 Grain Yields

In the first three years of the experiment, the wheat grain yield was higher in plots conducted in the direct seedling system compared to the conventional seedling system (Figure 9). The recorded grains are 1.6; 3.6 and 3 t/ha respectively for the three years of trial. However, in the last two years, cereal yields were similar for both systems.

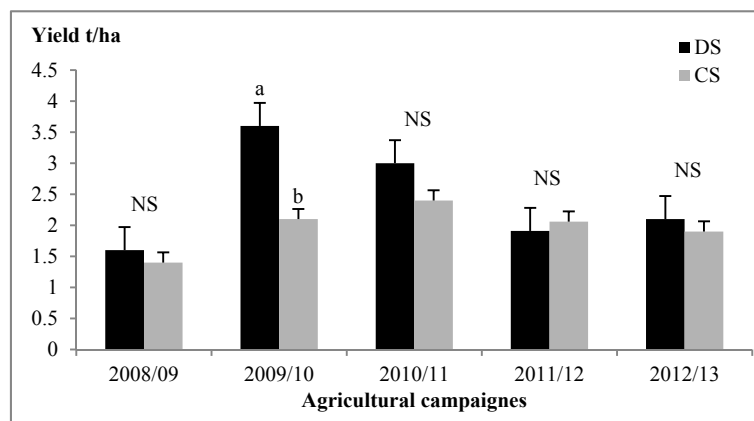


Figure 9. Wheat grain yield during the five years trials in no-till farming system and in conventional one. Means were obtained from 3 replicates. Different letters indicate significant differences between DS and CS according to the Newman-Keuls test ($P < 0.05$)

4. Discussion

This long-term study of durum wheat rainfed crop in the east of Algeria, in no-till farming and in the conventional seedling system, has allowed us to determine the effect of these two farming systems on the establishment of a mycorrhizal symbiosis and the diversity of AMF in the field.

After three years of no-till practice, the amounts of PLFA biomarkers (C16:1 ω 5 for AMF) showed an increase in AM fungal biomass in the soil compared to those found in the conventional plots (Figure 7). This reflects a better growth and establishment of extra-radical AMF mycelium (Olsson et al., 1998). Alguacil et al. (2008) reported that the persistence of AM fungi in the soil depends on the survival of their active propagules (e.g. spores, hyphens, colonized roots). Our findings are in agreement with those of Roldan et al. (2007) who observed the highest levels of mycorrhizal propagules in the soil of maize and bean crops without tillage compared to tillage soils.

Under the same conditions, an improvement of saprotrophic and ectomycorrhizal fungi biomass was observed through the measurements of PLFA biomarkers: C18:2 ω 6.9. Consequently, one may say that the no-till system promotes the presence of a mulch of crop residues on the soil surface over a thickness varying from 5 to 30 cm, which may cause the increase in the biomass of the microbial community (Aslam et al., 1999; Balota et al., 2004; Babujia et al., 2010; Kabiri et al., 2016). On the other hand, tillage in conventional seedling causes a dilution of the organic matter and disturbs the fungal mycelium biomass. Our results are consistent with those reached by Bourgeois et al. (2015) and Lienhard et al. (2014), who claim that stopping tillage avoids physical disruption of mycelial hyphae and preserves microbial habitat.

Mycorrhizal and saprophytic fungi constitute the major component of the microbial community of unplowed soils or those with reduced soil tillage (Fontaine et al., 2003). The most intensively managed soils have the highest relative abundance of bacteria and actinobacteria (García-Orenes et al., 2013).

Our results showed that AMF biomass was higher than saprotrophic and ectomycorrhizal biomasses in both no-till farming and conventional seedling plots. Similar findings have been reported by Olsson et al. (1999) in cultivated soils. Van Groenigen et al. (2010) have shown, using both ergosterol and PLFA analyses, that while reduced tillage increased biomass of saprotrophic fungi in the 0-5 cm soil layer, it increased AMF biomass across the whole plough layer. The aridity of the study area (a rainfall close to 400 mm) may explain the dominance of the AMF biomass (Rodriguez et al., 2004; Duponnois et al., 2012; Nadjj et al., 2017). Similarly, some characteristics of the study area soil such as the low total phosphorus level (Nadjj et al., 2017) and the alkaline soil pH in relation with limestone mother rock (Djebaili et al., 1984; Halitim et al., 1988) also contributes to the AMF installation. Coutinho et al. (2015) reported that low levels of phosphorus allow production by AMF of a large number of propagules in an environment with few nutrients. This highlights the adaptation of endogenous mycorrhizal fungi strains to the soil physicochemical characteristics (Meglouli et al., 2018). Moreover, the monoculture facilitates the AMF installation because the presence or absence of host plant is important in the occurrence of colonization and sporulation of AMF (Cavalcante et al., 2009; Coutinho et al., 2015).

The root colonization also demonstrated a predominance of the number of intra-radicular structures specific to AM compared to the dark septated endophytic fungi (Figure 6). This is in accordance with the work of Jansa et al. (2006), who have observed a competitive effect of mycorrhizal fungi on non-mycorrhizal fungi and have claimed that this effect is promoted by reduced tillage. Olsson (1998) maintains that there is a negative impact of AMF on the growth of saprotrophic fungi in calcareous dune sand. However, Keenan et al. (2007) and Liu et al. (2011) showed that the presence of saprotrophic fungi in Gramineae crops reduced the density of AMF spores in the soil and mycorrhizal root colonization could be decrease by 50%.

The emergence of arbuscules in the root cortex of the no-till farming plants at the fourth year of this experiment is likely to reflect a good AMF activity, as put forward by Jansa et al. (2006) and Curaqueo et al. (2010). Their significant positive evolution from the early stage to the last year of the vegetative cycle demonstrates the efficiency of no-till farming in comparison with the conventional seedling system (Figure 4). In fact, arbuscules are the main symbiotic structures ensuring the exchanges between both symbiotic partners (Brundrett et al., 1996, Balestrini et al., 2015).

In our experiment, we observed a large production of vesicles in the cortical compartments of the plant roots grown under the conventional seedling system. The vesicles are carbon storage structures in particular lipids, obtained from the host plant (Brundrett et al., 1996; Olsson, 1999, Trépanier et al., 2005). Vesicle formation is abundant at the end of the growth cycle of the host plant (Al-Karaki et al., 2004). It is a mean of conservation and propagation of fungi (Staddon & Fitter, 2001). At this stage, AMF will be disconnected from the root of their host plant and deprived of their usual source of energy, thereby catabolising the lipids stored in the vesicles (Olsson, 1998). Under the conditions of the conventional sowing system, the vesicles formation occurred at the grain filling stage reflected a negative partnership between the host plant (durum wheat) and the AMF.

AMF are obligate biotrophs and constitute a strong sink for plant carbon (Gavito et al., 2019) they take advantage of 3% to 20% of the carbon substances produced by the host plant (Strullu et al., 1991; Smith & Read, 2008; Garbaye, 2013). These reserves stored in the vesicles will allow the AMF to develop extended extraradical hyphal networks for efficient soil exploration (Keymer et al., 2017). The growth of the mycelial networks requires a large amount of energy and carbon building blocks, which are transported mostly as lipid droplets and glycogen to the growing hyphal tips (Bago et al., 2002, 2003). The destruction of the hyphal network by tillage explains the high presence of vesicles in the roots of plants growing under the conditions of conventional seeding.

AMF biodiversity studied in the last year of the trial showed the positive effect of no-till farming on the values of spore density and total species richness. Several studies reported the effect of soil tillage on the diversity and structure of AMF in temperate (Jansa et al., 2002, 2003), tropical (Boddington & Dodd, 2000) subtropical (Alguacil et al., 2008) and Mediterranean soils (Brito et al., 2012). Our findings are in agreement with these studies indicating that intensification of agriculture practices affects negatively AMF abundance and diversity.

The twenty-one species belonging to the seven families of Glomales recorded in the soil of the experimental plot reflect a high AMF diversity. Nadji et al. (2017) reported the importance of diversity and abundance of mycorrhizal propagules in the eastern wheat fields of Algeria. However, a specific richness is observed in the no-tillering treatment soil, linked mainly to the presence of five species of Acaulosporaceae (*Acaulospora scrobiculata*; *Acaulospora koskei*; *Acaulospora thomii*; *Acaulospora capsicula*; *Acaulospora denticulata*) and two species of Gigasporaceae (*Gigaspora albida* and *Gigaspora margarita*) which completely disappeared in conventional seedling. Similarly Jansa et al. (2006) have reported that, after thirteen years of non-tillage testing, there is the presence of some species of AMF, especially those not belonging to the genus *Glomus* such as *Acaulospora paulinae*. Soka and Ritchie (2014) have explained that the hyphae of *Acaulospora laevis* completely lose their infectivity when the soil is disturbed.

In the Grime's classification of functional groups (Chagnon et al., 2013), the Acaulosporaceae family corresponds to the stress-tolerant AMF which produce less extraradical hyphae and intraradical root structures as compared to Glomeraceae and Gigasporaceae (Maherali & Klironomos, 2007; Chagnon et al., 2013) which may reduce the exposure to abiotic factors (i.e. drought, salinity, heavy metals, acidity) (Chagnon et al., 2013). These AMF are capable to complete their life cycle producing low biomass, which disappears under plowing in the conventional seedling soil.

Seven species were present in both treatments but more abundant in plots conducted in the no-till system. This is the case of (*Gigaspora decipiens*; *Diversispora tortuosa*; *Scutellospora scutata*; *Scutellospora calospora*; *Entrophospora infrequens*; *Dentiscutata erythropha* and *Dentiscutata nigra*). This finding is in line with the study of Jansa et al. (2006) which reported that *Scutellospora pellucida* and *Entrophospora infrequens* were most

abundant in no tilled soils. The work of Alguacil et al. (2008) showed an interaction between the tillage system and the host plant showing very significant differences between AM fungal populations for different managed tillage systems. They concluded that no-tillage system had in fact a higher number of fungal types colonizing bean and sorghum crops than the other management.

Spore diversity may also be related to root density which increases favorably under no-till conditions (Cardoso et al., 2003). Similarly, the more abundant weeds in direct seedling (Karkour, 2012) can be an intermediate host between AMF and the main crop (Kabir, 2005).

The Glomeraceae are predominant in both treatments, but their relative abundance was higher in conventional seedling. Three species of Glomeraceae (*G. mossae*, *G. aggregates* and *G. ambisporum*) were occurred only in the conventional seedling soil. The predominance of spores belonging to the family of Glomeraceae has also been recorded in some countries of Africa, particularly in the semi-arid zones of Senegal (Diallo et al., 1999) and Burkina Faso (Guissou, 2001). Schalamuk et al. (2006) have noted that Glomeraceae were more abundant in the conventional system, compared to the no-till farming system. The ubiquity of Glomeraceae family in disturbed environments is related to their ability to grow fast (de Souza et al., 2005), to sporulate rapidly and to restore a network of hyphae by quickly forming anastomoses between the different mycelial branches of the same or neighboring genotype (Giovannetti et al., 1999; Oehl et al., 2003; de la Providencia et al., 2005; Voets et al., 2006). They are therefore considered ruderal AMF (Van der Heijden & Scheublin, 2007).

Most Glomeraceae have been classified on the basis of their life-history strategies (LHS) as r-strategist because they invest mainly in the production of many offspring, favoring their development in unstable environments (Ijdo et al., 2010). In this sense (Johnson et al., 1992; Wang et al., 2008) reported in the tillage soil system, the plowed horizon is mainly occupied by AMF species less useful for crops and investing a large part of their resources in the production of spores.

In addition, Glomeraceae colonize via fragments of mycelium or mycorrhizal root pieces, and therefore have the ability to high root colonization and to develop larger extraradical hyphae (Declerck et al., 2001). In contrast, Gigasporaceae invest more energy in the production of extraradical biomass than in intraradical fungal structures (Hart et al., 2002; Maherali & Klironomos, 2007), they produce few and large spores (Johnson et al., 1992, de Souza et al., 2005; Wang et al., 2008) and propagate via sporal dispersal or infection from an intact mycelium (Biermann & Linderman, 1983, Daniell et al., 2001). Such differences clearly explain the dominance of Glomeraceae over Gigasporaceae in environments characterized by repeated severe physical disturbances, such as tillage agricultural practices and clearly explain the loss of species richness of Gigasporaceae by the deep burial of their propagules following the plowing (Oehl et al., 2004) (Jasper et al., 1991; Hart & Reader, 2004).

The presence of plant residues (mulch) contributes in the increase of the organic soil matter. This hypothesis is in line with Van Groenigen et al. (2010) study which has reported that, after twenty years of no-till operations, the soil organic carbon increases by 16% in humid temperate regions and by 10% in dry temperate regions, compared to conventional seedling. In our study, the soil organic matter and organic carbon contents showed a tendency increase of 29% in no-till plots (Figure 8). Contents of soil organic matter (SOM) and total nitrogen remained low for respectively the no-till and conventional seedling systems respectively (Figure 6), which reflects soil poor fertility.

This situation could be explained by the low amounts of cereal crop residues to return to the soil. In North Africa, often cereal residues are used as a pasture source for sheep farming (Lahmar & Ruellan, 2007). The monoculture (wheat/wheat), applied in our experiment, implies a slow-down of organic matter mineralization in comparison to crop rotation (cereal/legume).

An increase in yields observed in our experiment during the first three years of the no-tillage farming system (Figure 9), is consistent with the results reached by Angar et al. (2010), who have reported that durum wheat yields, during ten years of no tillage in a sub-humid zone of Tunisia, increased by 0.8 t/ha, compared to conventional seedling. In a semi-arid zone, this difference was lower (0.35 t/ha), and it reached 0.7 t/ha in the upper semi-arid areas.

Consequently, one may say that late sowing (12/01/2012 and 09/01/2013) (Table 3) during the last two years of experimentation does not allow a good early planting of the crop and limits the H₂O intake of the rainfall during the autumn season. These effects are to be added to the low temperatures of January and February that occur just after sowing, which prevents an adequate growth of plants by affecting their physiological development of meristems (Chassot et al., 2002). Chapman et al. (2001) have pointed the negative effect of low temperatures on the rate of mineralization of nitrogen in the soil. These factors together seem to be at the origin of the suppression of the positive effect of the no-till farming system during these two agricultural campaigns.

5. Conclusion

The results of our study demonstrated that the no-till farming system has a positive impact on the AMF symbiosis, as demonstrated by the increase of AMF biomass in the soil and the arbuscular root colonization percentages. Besides, in conventional seedling, root colonization was characterized by a strong presence of vesicles. The biodiversity of AMF was improved by the relative abundance of six families of Glomales and the emergence of new AMF species. These results are in favor of the adoption of the no-till system as an approach to restore the microbiome in agro-systems disturbed by tillage.

The aridity of our study area, the monoculture and the presence of a high level of soil limestone facilitated the predominance of the AMF root colonization over the other endophytic fungi.

For the sake of productivity and sustainability, it is desirable that a similar study be carried out using co-culture of wheat/legume rotation to improve nitrogen and soil organic matter levels. An economic study is also needed to assess the impact of no tillage technique on the cost of production and profitability of the crop.

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