Understanding Arbuscular Mycorrhizal Colonization in Walnut Plantations: The Contribution of Cover Crops and Soil Microbial Communities

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Abstract: Soil microorganisms play a central role in biological soil functioning. One of the beneficial microbiota that has a symbiotic association with most of the plants is arbuscular mycorrhizal fungi (AMF). Nevertheless, little is known about the impact of cover crops—widely used in conservation agriculture or organic farming—on native mycorrhizal fungi. This study was conducted in Southern France, in 20-year-old walnut orchards, where faba bean (*Vicia faba* Roth) was intercropped. To find whether the native AM fungal community associated with walnut trees was influenced by cover crops and soil microbial communities, analyses of soil physicochemical and microbiological indicators were carried out with roots and soil samples collected from four modalities (walnut in conventional farming with and without cover crops, and walnut in organic farming with and without cover crops). Our results showed that the presence of cover crops mainly influenced the soil microbial abundance and activities in conventional plots. In contrast, cover crops stimulated AM fungal colonization of walnut roots in organic plots, reaching 35% and 54% for arbuscule abundance and mycorrhizal intensity, respectively. In conventional plots, ergosterol and mineral nitrogen contents were mainly correlated with mycorrhizal colonization, while only acid phosphatase activity in soil was positively correlated with mycorrhizal colonization in organic plots. The use of the faba bean showed the great role played by cover crops in the enhancement of walnut trees' mycorrhizal colonization. Identification of the functional traits of AM fungi sensitive to walnut trees is required to inform decisions in specific agricultural practices.

Keywords: *Juglans regia*; arbuscular mycorrhizal fungi; cover crops; soil; organic farming; conventional

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1. Introduction

Walnut tree (*Juglans regia* L., Fam.: Juglandaceae) is the most widespread nut tree in the world and the predominant tree species in current European agroforestry systems [1]. The world production of walnut is 3.458 million tons, with China having the highest production (45%) followed by the U.S.A., Iran, Turkey and Mexico [2]. According to the Food and Agriculture Organization of the United Nations, and International Nut and Dried Fruit Council data, walnut is the second largest orchard in France in terms of surface area, with 21,000 hectares under cultivation. Conventional cultivation is very widespread in the departments selected for this study (Dordogne, Correze and Lot), yet organic farming is becoming more and more important, with 30% of the walnut orchards in these regions using it [3]. Given the constant increase in the price of inputs, maintaining sufficient chemical fertilization for good production has a considerable impact on soil life. That is why good organic matter mineralization by soil microorganisms as well as the optimal use of mineral and water resources by the plant is a real challenge for walnut orchards. Furthermore,

the use of beneficial soil microorganisms is a promising strategy for optimizing plant growth and agricultural sustainability. The composition and activity of soil microbial communities largely determine biogeochemical cycles, organic matter renewal processes, and soil fertility and quality [4]. Although microorganisms play a key role in the success of cropping systems through plant nutrition and health, their community composition continues to change with plant developmental stages [5]—especially with soil type and cropping practices [6–8]. Currently, the evaluation of biological indicators of soil quality is necessary to link the abiotic properties of the soil to changes in its functions in terms of biochemical and biophysical transformations [9,10]. One of the most important groups of microorganisms, due to the relevant ecological services they provide in ecosystems, is the arbuscular mycorrhizal fungi (AMF). Nearly 270 species are described worldwide [11], and their occurrence depends mainly on soil type and farming practices. AM fungi live in symbiosis with most plant species and provide many benefits to host plants, such as improved nutrient uptake—especially of poorly mobile nutrients, such as phosphorus (P)—and increased plant tolerance to biotic and abiotic stressors [12]. Therefore, the productivity and sustainability of agroecosystems can be improved by boosting native AMF while reducing reliance on fertilizers [9]. In agroforestry systems, most of the plant species use a form of arbuscular mycorrhizae. It should be noted that studies on AMF colonization in an agroforestry system, and not just with a single tree species, are rare [13,14]. However, these studies already show us that this association allows an increase in AMF spore abundance as well as colonization of plant roots. The management of agricultural practices should promote a mixture of diverse AM fungi species to increase the multifunctionality of the symbiosis. However, the community assembly of AMF species is often limited in intensively managed agricultural systems [15].

Moreover, the integration of intercropping cover crops in walnut plantations is an innovative practice yet still underdeveloped. Despite their beneficial impacts in various agroecosystem services [16], cover cropping is not widely adopted by farmers. Previous studies showed a positive impact of this practice on mycorrhization [17–21]. The nutritional exchanges between the plant and the symbiotic fungus depend not only on the colonized plant, but also on neighboring plants [22]. The presence of intercropping cover crops, such as legumes, can act as a relay for mycorrhization if the introduced species are favorable [23]. Other authors showed an increase in the mycorrhization rate and hyphae production at the interface between tree rows and crop pathways, indicating a positive effect of the presence of a perennial system and plant diversity [24].

Few studies were carried out on the impact of mycorrhization on walnut trees, and apparently none in interaction with associated crops. It is, therefore, important to identify the main factors that motivate the maintenance of the AM fungal community in walnut plantations. Although cover crops are widely used in conservation agriculture or organic farming [20], there is little knowledge on the impact of cover crops on native mycorrhizal fungi.

The objective of this study is to evaluate the effect of cover crops and soil microbial communities on AM fungal colonization in walnut plantations under organic and conventional farming systems.

2. Materials and Methods

2.1. Study Site

The study was conducted in walnut tree plantations located in Southwestern France. The selected plots are located in three departments: two plots in Dordogne (Nouvelle-Aquitaine region), six plots in Lot (Occitanie region) and one plot in Correze (Nouvelle-Aquitaine region). The climate in these areas is described as an altered oceanic climate, a transition zone between the oceanic climate and the mountain climates, with mean annual rainfalls ranging from 800 to 900 mm. They are spread over an area of approximately 500 km^2. The walnut plots selected in this study have common characteristics: a predominantly clay-limestone soil, a grassy inter-row, and a variety of Franquette walnut trees on a Regia rootstock. Four modalities of agricultural production systems were studied:

conventional without cover crops, conventional with cover crops, organic without cover crops and organic with cover crops, and each modality has three different sample plots (Table S1). Soil sampling was carried out in June 2018 for each modality of walnut orchards.

2.2. Sampling and Analyses

2.2.1. Soil Physicochemical Analyses

Physical and chemical characteristics of all soil samples are summarized in Table 1. In the walnut plots, the presence of the trees and the contrast between row and inter-row led us to adopt an identical operating mode per plot. Five soil samples (0–15 cm depth) for each plot per modality were sampled. Each soil sample consisted of 16 auger samples, which were mixed in a plastic bag. We stood on two rows of trees and took 5 soil samples from each row and 6 samples from the inter-row (pool of 16 samples). Composite soil samples were air dried and sieved to 2 mm particle size in the laboratory. Nutrient concentrations were determined according to French and international standards (NF T90–023, NF EN ISO 11732, and NF EN ISO 13395). Soil pH was determined according to the ISO standard NF ISO 10390, and soil total nitrogen was determined with the Kjeldahl method [25]. Total soil organic carbon (SOC) was measured using a TOC analyzer Shimadzu SSM-5000A/TOC-VCSH Carbone (Shimadzu, Japan). Permanganate oxidizable carbon (POXC) in soil was estimated by reaction with a dilute permanganate solution [26]. Mineral nitrogen corresponds to the sum of the different N forms (nitrates NO_3^- and ammonium ions NH_4^+). The amount of mineral nitrogen was determined from 25 g of fresh soil sieved to 2 mm, to which 100 mL of potassium chloride (KCl) at 2 mol/L was added to extract ammonium and nitrates.

Table 1. Mean values of physicochemical parameters of soils.

Elements	Farming System			
	Conventional		Organic	
	Cover	without Cover	Cover	without Cover
pH (H_2O)	7.65 ± 0.26 a	7.17 ± 0.16 a	7.34 ± 0.28 a	6.78 ± 0.01 a
SOM (%)	4.08 ± 0.63 a	2.84 ± 1.70 b	6.40 ± 1.98 a	4.74 ± 0.84 b
SOC (%)	2.37 ± 0.37 a	1.65 ± 0.99 b	3.72 ± 1.15 a	2.76 ± 0.49 b
POXC ($g \cdot kg^{-1}$)	1.01 ± 0.10 a	0.80 ± 0.15 a	1.22 ± 0.14 a	1.06 ± 0.13 a
Total Nitrogen (%)	0.31 ± 0.01 a	0.20 ± 0.07 a	0.32 ± 0.06 a	0.32 ± 0.03 a
C/N ratio	7.47 ± 1.19 a	7.29 ± 1.76 a	11.04 ± 1.70 a	8.43 ± 1.03 b
Mineral nitrogen ($mg \cdot kg^{-1}$)	8.62 ± 0.47 a	3.82 ± 1.44 b	31.90 ± 3.06 a	8.15 ± 1.63 b

Values are expressed as mean ± standard deviation (n = 5); For each farming system, mean values in rows followed by the same letter do not differ significantly ($p > 0.05$).

2.2.2. Microbiological Analyses

In the walnut plots, each repetition is defined by a tree, and three repetitions were performed per plot. The samples were taken from 2/3 of the tree crown, which is the position in which a sufficient and simultaneous quantity of walnut roots and cover crops was found. Rhizospheric soil samples with an intact root system were collected with nine replicates per modality, with and without cover crops (a total of 36 samples). Each sample was placed in a plastic pot (diameter 15 cm, height 12 cm) using 2.5 kg of soil and stored at 4 °C for further biological analyses. Samples were frozen at −80 °C after going through liquid nitrogen for enzymatic tests, DNA extractions, glomalin and ergosterol analyses. Soil enzymatic activities of β-D-glucosidase (GLU; EC: 3.2.1.21), *N*-acetyl-β-glucosaminidase (NAG; EC: 3.2.1.30), alkaline phosphatase (PAK; EC: 3.1.4.1) and acid phosphatase (PAC; EC: 3.1.4.1) were performed in 96-well microplates in triplicates using the procedure detailed in the ISO 20130:2018. The total soil DNA was extracted from 0.5 g of soil using the FastDNA™ Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) according to

manufacturer's instructions. The soil DNA concentrations were assayed by fluorimetry using the Fluorescent DNA quantitation Kit Hoechst 33258 (Biorad, Hercules, CA, USA), following the manufacturer's instructions. They were stored at 20 °C until use. Bacterial 16S rRNA and fungal 18S rRNA gene copy numbers per gram of dry soil were estimated for each sample using the real-time PCR conditions [27]. The glomalin content was estimated by the measurement of the easily extractable glomalin, which is considered the most recent deposition fraction [28]. We applied a microwave-assisted extraction (MAE) and high-performance liquid chromatographic (HPLC) procedure to measure ergosterol in the soil sample. The total amount of ergosterol was measured after saponification according to the procedure described by Montgomery et al. [29]. To measure the mycorrhizal root colonization, root samples from walnut trees were gently washed under tap water, bleached (KOH, 10%) at 80 °C for 30 min, and stained in 0.05% blue ink at 80 °C for 30 min following the method of Phillips and Hayman [30]. The percentage of the root length colonized by AMF was assessed at ×40 magnification using 30 fragments of lateral roots for each sample (approximately 1 cm length) on microscopic slides. Mycorrhizal root colonization was evaluated by using the method of Trouvelot et al. [31]

2.3. Statistical Analysis

ANOVA and Kruskal–Wallis analyses, as well as respective post hoc Tukey and Mann–Whitney tests, were used on the biological data, according to the normality of the data, to examine the significant differences between the agricultural practices. Multivariate analysis based on principal component analysis (PCA) was used to describe the correlation between biological and soil physicochemical parameters in each farming system. All statistical analyses were computed using the R package stats [32], and statistical significance was set at $p < 0.05$.

3. Results

3.1. Bacterial and Fungal Abundance in Walnut Rhizosphere Soil

Results on microbial biomass (total soil DNA), bacterial abundance (16S rRNA), fungal abundance (18S rRNA) and ergosterol content for each modality are presented in Figure 1. Farming systems—conventional and organic—had no significant effect ($p > 0.05$) on rhizosphere microbial biomass, whatever the parameters measured (Figure 1D). Significant differences were observed on bacterial abundance ($p < 0.001$) (Figure 1A), fungal abundance ($p < 0.01$) (Figure 1B) and microbial biomass ($p < 0.05$) (Figure 1D) related to vegetal cover in conventional plots. Conventional plots with cover had the highest microbial biomass (37.49 $\mu g \cdot g^{-1}$), bacterial (3.25×10^9 copies g^{-1}) and fungal (6.15×10^7 copies g^{-1}) abundance compared with plots without cover. In contrast, in the organic plots, the results showed no significant differences ($p > 0.05$) between the modalities with and without cover, whatever the parameters evaluated. Ergosterol content (Figure 1C) and fungal abundance (Figure 1B) did not show the same pattern of variation. No significant difference ($p > 0.05$) was found for ergosterol content between cover and without cover, regardless of the farming system.

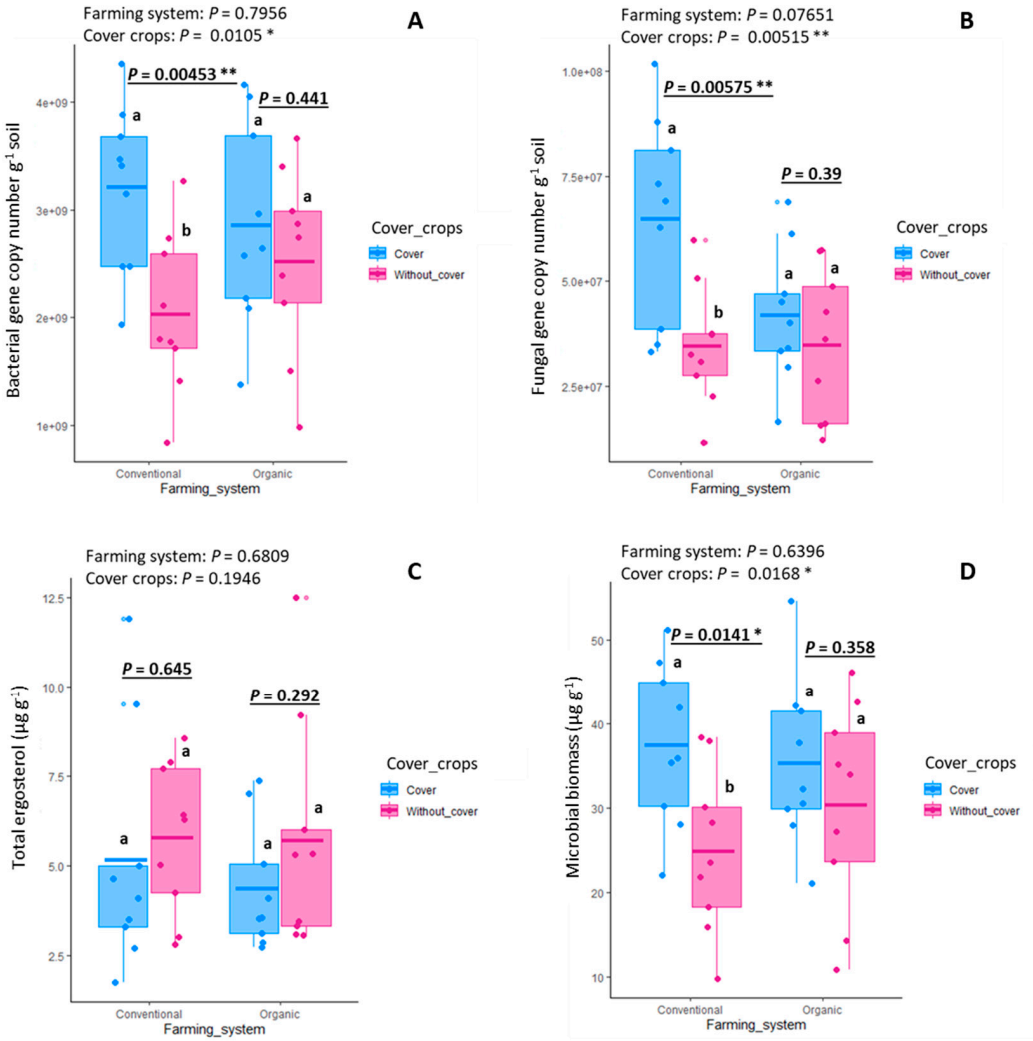

Figure 1. Effect of cover crops on bacterial abundance (16S rRNA) (**A**), fungal abundance (18S rRNA) (**B**), ergosterol content (**C**) and microbial biomass (**D**) in soil from the two farming systems (conventional and organic). The boxplot with p-values represents means of each modality using ANOVA ($n = 36$). Means followed by the same letter do not differ significantly under the Tukey test at the 0.05 level; significant levels: * $p < 0.05$; ** $p < 0.01$.

3.2. AM Fungal Community Colonization in Walnut Plantations

Arbuscular mycorrhizal fungal structures in a walnut root are presented in Figure 2. Conventional and organic farming systems had a significant effect on arbuscule (Figure 2A) abundance ($p < 0.001$) (Figure 3A), mycorrhizal intensity ($p < 0.01$) (Figure 3B) and soil glomalin content ($p < 0.05$) (Figure 3C). Root-colonizing mycorrhizal parameters were higher in organic plots with cover, reaching 35% and 54% for arbuscule abundance (Figure 3A) and mycorrhizal intensity (Figure 3B), respectively. Beneficial effects of cover crops on mycorrhizal intensity and arbuscule abundance were observed in both walnut farming systems. In organic plots, mycorrhizal intensity and arbuscule abundance varied significantly ($p < 0.001$) between with and without cover, while the glomalin content was not significantly different ($p > 0.05$) (Figure 3C). In the conventional plots, both mycorrhizal intensity and glomalin content were higher in plots with cover crops, while arbuscule abundance did not show any difference ($p > 0.05$).

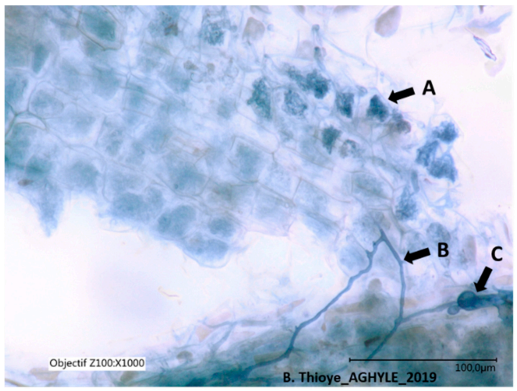

Figure 2. Walnut tree root (*Juglans regia* L.) colonized by arbuscular mycorrhizal fungi: (**A**) Arbuscules for nutrient exchange; (**B**) Hyphae colonizing the roots; (**C**) Vesicles for nutrient storage. Roots were stained with blue ink.

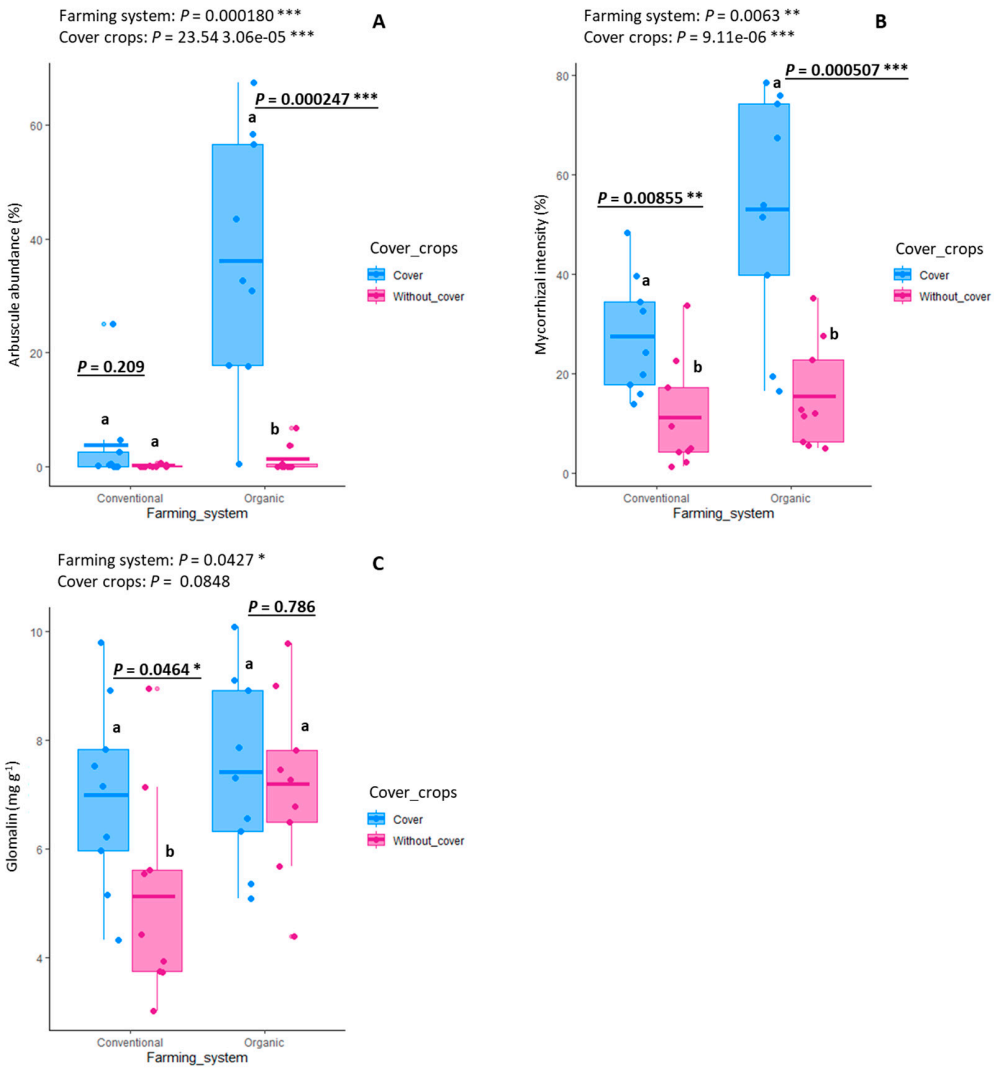

Figure 3. Effect of cover crops on arbuscule abundance (**A**) and mycorrhizal intensity (**B**) of walnut roots and glomalin content (**C**) in soil from the two farming systems (conventional and organic). The boxplot with p-values represents means of each modality using ANOVA (n = 36). Means followed by the same letter do not differ significantly under the Tukey test at the 0.05 level; significant levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3.3. Soil Enzyme Activity

Conventional and organic farming systems had no significant effect ($p > 0.05$) on soil enzymatic activities (Figure 4) related to the carbon cycle (β-glucosidase—β-GLU), nitrogen cycle (*N*-acetyl glucosaminidase—NAG) or phosphorus cycle (Acid phosphatase—PAC and Alkaline phosphatase—PAK). In conventional farming, all these enzymes showed similar profiles and were higher in plots with cover (Figure 4). The averages of the enzymatic activities were 155 nmol PNP·min·g^{-1} for β-GLU (Figure 4A), 17 nmol PNP·min·g^{-1} for NAG (Figure 4B), 105 nmol PNP·min·g^{-1} for PAC (Figure 4C) and 270 nmol PNP·min·g^{-1} for PAK (Figure 4D). In organic plots, only the acid phosphatase activity varied significantly ($p < 0.01$) in the presence of cover crops, reaching 120 nmol PNP·min·g^{-1} (Figure 4C).

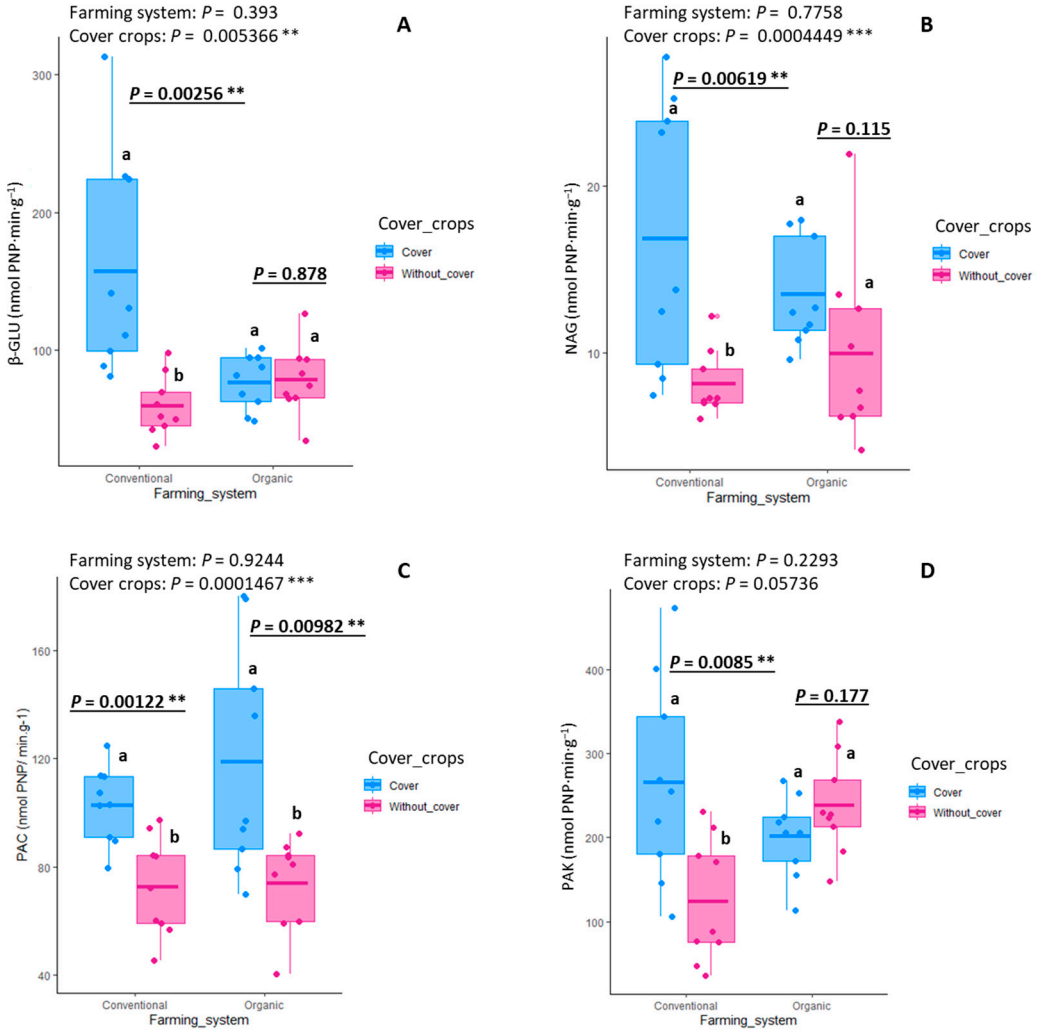

Figure 4. Effect of cover crops on activity of β-glucosidase (β-GLU) (**A**), N-acetylglucosaminidase (NAG) (**B**), acid phosphatase (PAC) (**C**) and alkaline phosphatase (PAK) (**D**) in soil from the two farming systems (conventional and organic). The boxplots with p-values represents means of each modality using the Kruskal–Wallis test ($n = 36$); means followed by the same letter do not differ significantly under the Wilcoxon test at the 0.05 level; significant levels: ** $p < 0.01$; *** $p < 0.001$.

3.4. Principal Component Analysis of Cover Crops, Soil Microbial Communities and Physicochemical Soil Properties

For each farming system, PCA biplot was done in between physicochemical (POXC, SOM, SOC, total nitrogen, mineral nitrogen, pH) and biological variables such as mycorrhizal intensity, arbuscule abundance, microbial biomass, total ergosterol, glomalin concentration, soil enzymatic activities, bacterial and fungal abundance (Figure 5). In

the conventional plots, the total variance of 71.1% could be explained by the principal component 1 (PC1) representing 57.1% and the principal component 2 (PC2) representing a variance of 14.6% (Figure 5A). The variables such as mycorrhizal intensity, arbuscule abundance, mineral nitrogen, PAC, β-GLU and POXC showed a positive response only toward plots with cover, whereas total ergosterol, glomalin, microbial biomass, bacterial and fungal abundance, NAG, PAK, SOM, SOC, total nitrogen and pH were positively correlated both with cover and without cover. Interestingly, only mineral nitrogen and ergosterol were positively correlated with the mycorrhizal parameters (arbuscule abundance and mycorrhizal intensity) in plots with cover (Figure 5A). In the organic plots, the total variance of 50.4% could be explained by the PCA with PC1 representing 31.8% and PC2 representing a variance of 18.6% (Figure 5B). Soil pH showed a positive response only towards plots without cover. Interestingly, only acid phosphatase activity was positively correlated with mycorrhizal intensity and arbuscule abundance in plots with cover (Figure 5B). All the other variables were positively correlated both with cover and without cover.

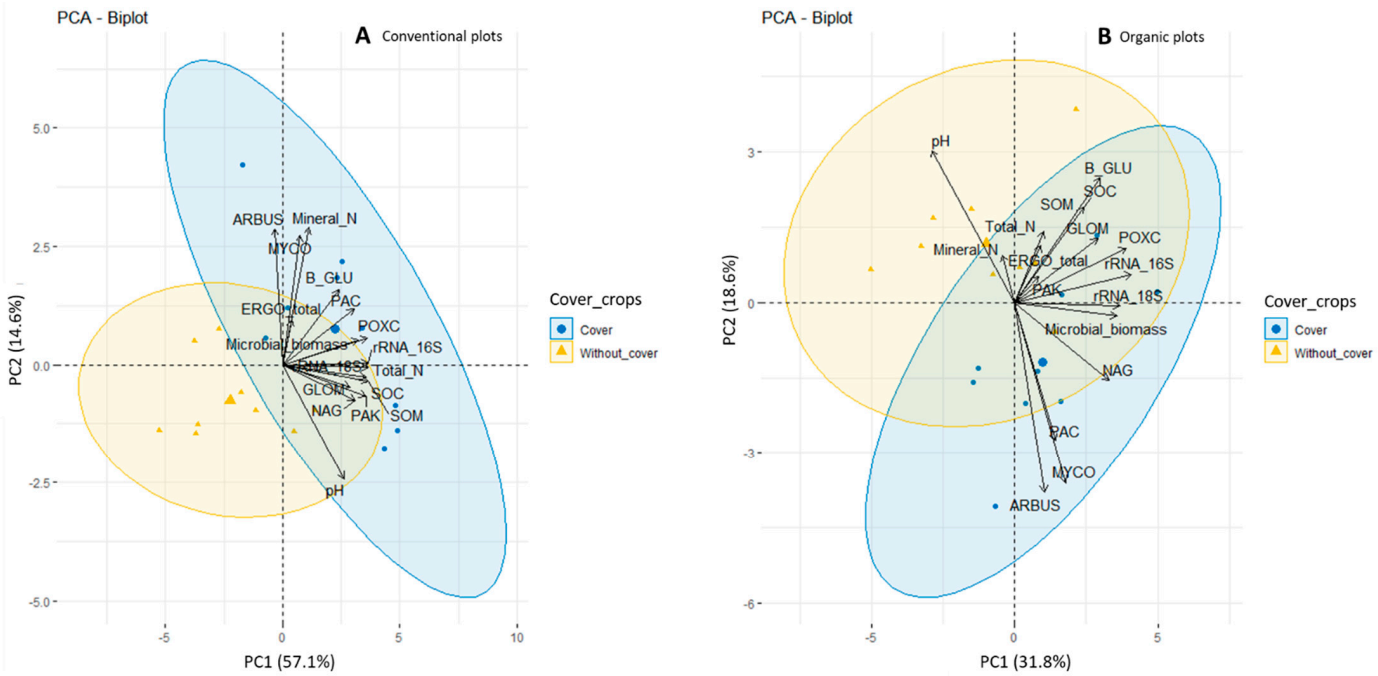

Figure 5. PCA biplot of biological and physicochemical data from conventional (**A**) and organic (**B**) plots with or without cover. Only the first two axis are shown on the biplot. MYCO—Mycorrhizal intensity; ARBUS—arbuscule abundance; ERGO total—total ergosterol; GLOM—glomalin; rRNA_16S—bacterial abundance; rRNA_18S—fungal abundance; β-GLU—β-glucosidase; NAG—N-acetylglucosaminidase; PAC—acid phosphatase; PAK—alkaline phosphatase; POXC—permanganate oxidizable carbon; SOM—soil organic matter; SOC—soil organic carbon; Total_N—total nitrogen; Mineral_N—mineral nitrogen. Values in X and Y axes indicate percentage contribution by PC1 and PC2 by using the Vegan package of R software version 3.6.2.

4. Discussion

This research establishes a clear link between cover crops, arbuscular mycorrhizal fungi and soil microbial communities (activity and abundance) under organic and conventional farming systems. The state of mycorrhization in walnut orchards, using cover crops in intercropping, without any inoculation is also addressed in this work. Furthermore, the linkage built between walnut mycorrhization with cropping practices, by identifying the main levers that promote this symbiosis, enables us to study the AM symbiosis and the services it renders in agricultural practices [33].

The results showed that in conventional plots, the presence of cover crops led to a significant increase in soil bacterial and fungal populations. Bacterial and fungal abundance as well as the total biomass follow the organic status of the soil, where SOM, SOC and mineral nitrogen contents are higher in the plots with cover crops. Microbial activity is the main link between soil organic matter and plant nutrient availability [22]. Although the organic status of soil in organic farming is higher in plots with cover, no effect of cover crops on bacterial and fungal abundance was observed.

The agricultural practices used in conventional and organic orchards positively affected the natural mycorrhization of walnut trees. In fact, walnut trees in organic farming showed a higher intensity of mycorrhization and arbuscule abundance than that in the conventional one, respectively 35% and 54%, under the influence of cover crops. It is well established that intercropping systems favor the AM fungal communities' abundance and diversity compared to conventionally managed systems [24,34,35]. The positive effects of cover crops on the mycorrhization parameters are similar to several studies' findings performed in other climatic zones [36,37]. Nevertheless, it is difficult to establish a direct link with specific practices—however, herbicides and fungicides used conventionally can have a negative impact on AM fungal communities. Moreover, walnut trees act as reservoirs of AM fungi for crops, as was shown in other agroforestry systems [38,39]. It is well known that the nutritional exchanges between the plant and the symbiotic fungus depend not only on the colonized plant, but also on neighboring plants [22]. Cultivated plants play a beneficial role to the association of AM fungi with the walnut trees' roots by enhancing their absorption of nutrients from the soil [40]. Our results indicate the involvement of AM fungi in the redistribution of nutrients between roots and crops. It was also shown that the fungal mycelium by forming an underground network interconnecting plant roots, facilitate the direct exchange between them [41]. Consequently, the integrity of the fungal mycelium is vital for walnut trees' nutrition and health.

Whether they are conventionally or organically managed orchards, the natural mycorrhization of walnut trees is more important when a plant cover is sown. Most studies agree that leguminous plants are much better placed than other plants for this type of symbiosis as they are able to play a relay role favoring these networks in the rotation [18,19,42]. The largest gains were associated with the faba bean in conventional plots and with faba bean–oat intercrops in organic plots. The plant cover, which is commonly used by nuciculturists, in this study is generally built up around the faba bean, which is known for its positive influence on mycorrhization. Thus, the presence of cover crops, such as legumes, in cross-crops can act as a relay for mycorrhization [23,43]. The use of faba bean-based cover crops would significantly reduce nitrogen fertilization inputs and stabilize the nitrogen content in the soil, in contrast with conventional mineral fertilization where nitrogen rapidly declines. Unlike walnut plots with natural grass cover, roots that have had controlled plant cover would have more specific species of mycorrhizal fungi. Intercropping plant cover is essential to provide a relay for photosynthesis and to maintain active mycorrhizal networks, even during a period of vegetative rest, as here in the walnut plots. Maintaining the presence of continuously active plants could enable the functional maintenance of the AMF hyphae and the permanent stimulation of spores.

Although cover crops have stimulated the intensity of mycorrhization of walnut roots and the concentration of glomalin in conventional plots, symbiotic exchanges have decreased with a relatively low arbuscule abundance. This result could be explained by the characteristics of the conventional plots and the sensitivity of mycorrhizae to unfavorable factors for mycelium development, such as fungicides [44], tillage [45], nitrogen and phosphorus over-fertilization, and herbicides [46]. In these plots, soil enzymatic activities were higher in the ones with cover. Similar results were obtained on β-glucosaminidase and other enzymatic activities, showing an increase in soil biomass through plant cover [47,48]. This increase in soil biological activity is considered a dynamic indicator reflecting the enhancement of ecosystem services and soil properties [36]. In organic plots, our results did not show a direct effect of plant cover on enzymatic activities except for acid phosphatase ac-

tivity. This result is confirmed by the positive correlations between walnut mycorrhization and acid phosphatase activity. Walnut trees in organic farming can use organic phosphate from the soil by secreting acid phosphatase through the extra-matrix hyphae associated with the roots [49]. Furthermore, positive correlations between mycorrhization parameters, mineral nitrogen and ergosterol contents were obtained in conventional plots. In view of the properties of AMF species on plant nutrition, it can be expected that the presence of a fungal partner stimulates the uptake of both forms of mineral nitrogen NO_3^- and NH_4^+ by the walnut root system. Several studies have shown that ergosterol is a specific biomarker of fungi in soil [29,50] and an indicator for comparing the fungal abundance of various AM fungi in soil and roots [51]. However, very few studies have shown correlations between AM fungal abundance and ergosterol content of soil. The relationship between AM fungi and ergosterol remains a controversial and complex subject [52] as the estimation of fungal abundance varies according to the fungal species and growing conditions [53]. We also noticed that the presence of plant cover did not affect the ergosterol content regardless of the farming system. Our findings do not corroborate to those of Dinesh et al. [54], who observed a higher fungal abundance from ergosterol content in coconut plantations grown with leguminous cover plants compared with a control group.

5. Conclusions

In this work, we demonstrated that the natural mycorrhization of walnut trees may be explained by soil microbial communities and the presence of cover crops. Mycorrhizal colonization was explained by mineral nitrogen and ergosterol contents in conventional plots with cover, while only acid phosphatase activity in soil could explain it in organic plots with cover. Our findings provide a good understanding of the role of soil microbial status in agricultural practices, and particularly for mycorrhizal symbiosis. This work showed that cover crops have different influence in walnut plantations according to the farming systems. Although the qualities of the faba bean were highlighted in this work, it is not the only solution or alternative to plant cover. This plant needs to be replaced by other species—such as alfalfa—that will not only provide biodiversity, but also other services in such agroecosystems.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture12010001/s1, Table S1: Geographical locations and characteristics of walnut trees plantations.

Author Contributions: Conceptualization, B.T., M.L. and I.T.-G.; methodology, B.T. and L.C.; formal analysis, B.T.; investigation, F.H. and N.C.; resources, M.L. and B.T.; data curation, L.C.; writing—original draft preparation, B.T., M.L. and I.T.-G.; writing—review and editing, B.T. and I.T.-G. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

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

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