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Long-Term Impact of Biodynamic, Organic, and Conventional Farming Systems on Soil Fertility: Implications for Sustainable Agriculture

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Due to the environmental and human health concerns of the conventional agricultural practices, organic and biodynamic represent eco-friendly alternative farming systems that increase crop productivity while sustaining soil health. Present study is dedicated to examine the soil fertility as affected by long term application of three different farming systems, bio-dynamic, organic and conventional (DOK). To do that, the rhizosphere soil of wheat plants (W-Rh) grown under DOK farming systems were collected and the total population numbers of bacteria, fungi, diazotrophs, phosphate and potassium solubilizing bacteria were assessed, as well as the chemical and physical properties of W-Rh-soils. To evaluate the bacterial functional capabilities, we obtained a number of bacterial isolates, representing the three farming systems, and characterized their direct and indirect plant growth promoting (PGP) activities. A bioassay test was performed to determine the capacity of DOK soils to support the germination and growth performance of wheat plant. The results revealed that D-W-Rh samples harbored significantly higher bacterial populations in comparison to O-W-Rh and K-W-Rh samples, whereas the opposite pattern was observed for fungal populations. Bacterial isolates from the three systems exhibited diverse capabilities, without a clear correlation between a specific function with a particular system. Bioassay test revealed that wheat plants grown in soil from biodynamic system displayed higher germination rate, root and shoot fresh and dry weights followed by those from organic then conventional systems. Thus, different systems had distinct impacts on soil fertility with the biodynamic having a superior influence on the measured plant growth parameters.

Keywords: DOK, plant growth, microbial population, PCA, bioassay.

1. Introduction

The need to feed the world growing populations has led to the intensification of agricultural practices which, in turn, exhibited severe environmental consequences, such as climate change and biodiversity loss (**Arkhipova et al., 2007, Greiner et al., 2017, Steffen et al., 2015**). In recent years, more emphasis has been given to assess the impacts of the agricultural practices as a key component driving soil health and quality (**Mäder et al., 2002**). Although conventional farming systems can significantly increase the productivity, they cannot be considered as a strategy to sustained soil quality in the future for the next generations (**Singh et al., 2011**). Sustainable agricultural systems, that aim to ensure food security while preserving soil fertility and quality, are desperately needed to be implemented (**Reganold & Wachter, 2016, Lorenz & Lal, 2016**). Changes in soil quality develop slowly, in a way that would not be detectable in short-term studies. Therefore, DOK trials, (D; bio-Dynamic, O; Organic and K; Conventional (K for German: "konventionell")), are very valuable in providing long-term, well-designed, and controlled systems that allow to follow and document soil quality changes (**Alexander et al., 1996**).

Agricultural practices have a direct effect on soil microbial community. For example, organic farms had higher soil fauna and microbes compared to conventional systems (**Tuck et al., 2014**). In addition, increases in microbial activity, soil aggregate stability, microbial biomass, and root colonization by mycorrhiza were reported for the organic farming systems when compared to conventional (**Mäder et al., 2002**). In DOK trials, biodynamic farms reported significantly higher levels of organic matter content and higher microbial activity (**Fließbach et al., 2007**). Several studies (**Zelles et al., 1994, Marschner et al., 2004**) showed that farming systems with regular organic manure application developed higher concentrations of soil microbial biomass and different community structures than in systems with mineral fertilization.

Soil microbial community is a key component responsible for soil activity and fertility. Although culture-dependent techniques are restricted to the cultivable proportion of the soil microbial communities and does not provide a precise assessment of the uncultivable proportion **(Preston-Mafham et al., 2006)**, it has been successfully applied in comparative soil analyses and has shown to be a powerful and a sensitive low-cost analytical tool for exploring differences or even changes in soil microbial characteristics **(Widmer et al., 2001)**.

Therefore, the present study is dedicated to assessing the influence of DOK farming systems on the soil fertility from different perspectives. To achieve that, rhizosphere soil of wheat plants grown under DOK farming systems were collected and analyzed regarding the population numbers of different microbial groups including the total population numbers of bacteria, fungi, diazotrophs, phosphate and potassium solubilizing bacteria. The chemical and physical properties of the collected rhizosphere soils were assessed as well. To evaluate the functional capabilities, we obtained a number of bacterial isolates, representing the DOK farming systems, and characterized their direct and indirect plant growth promoting (PGP) activities. Additionally, a bioassay test was performed to determine the capacity of DOK soils to support the wheat plant germination and growth as a sole nutrient source thorough a pot experiment.

2. Material and Methods
2.1 Microbial Culture Media
Different media were used for different purposes. Plate count agar (PCA, Merck; Germany) medium was used for bacterial enumeration and isolation. Rose Bengal agar (RBA, HI- MeDIA, Pvt. ltd., Mumbai, India) medium was used for fungal enumeration **(King et al., 1979)**. For enumerating and characterizing the phosphate solubilizing, potassium solubilizing, and nitrogen fixing bacteria, National Botanical Research Institute Phosphate (NBRIP, Sigma), Aleksandrov and N-deficient Combined Carbon sources (CCM) media were used, respectively **(Nautiyal, 1999, Sun et al., 2020, Hegazi et al., 1998).** Bacterial isolates were characterized for their antagonistic activities against tested phytopathogens using Potato Dextrose Agar (PDA) medium (Merck KGaA, Darmstadt, Germany) **(Ali et al., 2020)**.

2.2 Site history and description
The DOK long-term field experiment began in May 2016 at the SEKEM farm in Bilbes-Sharqia. The goal of this field experiment is to compare three farming systems: "Bio-Dynamic" (D), "Organic" (O), and "Conventional" (K for German: "Konventionell") in a practice-oriented trial design under semiarid climatic circumstances at the same site. The design and crop rotation for this long-term experiment were created by Dr Sabine Zikeli, coordinator for the organic farming and consumer protection section at the faculty of agriculture science, University of Hohenheim, in collaboration with the SEKEM agriculture sector, to compare with the results obtained from the DOK trail of FiBl, the world's most significant and important long-term field trial comparing organic and conventional cropping systems in Switzerland.

The Experimental area is divided into 27 plots of 234 m^2 each, for a total area of 6,318 m^2. Within this area, the three agricultural systems (biodynamic, organic, and conventional) were implemented, each involving three different crops including wheat plant. The selected tested plant was cultivated in 9 plots, 3 for each agricultural system. Fertilization and plant protection practices are varied depending on the farming system. In the organic and biodynamic farming systems, compost was applied (24 tons/ha) during soil preparation, and Herobiofert, a locally produced fertilizer by SEKEM, was used at the recommended dose (714.29 L/ha), divided four times. The biodynamic system followed the same practices as the organic system, with additional treatments including the application of horn manure (357.14 g/ha) via sprayer immediately after planting and adding silica in the amount of 2 g/fed three times. In the conventional system, mineral fertilization, including ammonium nitrate (34N-0P-0K), superphosphate 15.5 P, calcium nitrate 15 Ca, and potassium 48 K, were applied **(Sabahy et al., 2024)**.

2.3 Samples collection and preparation
Two-months old wheat plants grown in the experimental site under the DOK farming systems were harvested. A total of 27 samples were collected representing 3 replicates/plot, 9 samples/farming system. The collected plants were placed in plastic bags and transferred to the lab. Prior to the analysis, a composite sample was prepared by mixing three replicates obtained from the same plot. Rhizosphere soil was obtained from each composite sample as follows: a total of 5 g of the entire root system with the tightly adhering soil was placed into a flask containing 15 ml of 0.85% NaCl and vigorously vortexed at the highest speed. The rhizosphere cell suspension was collected, and the previous step was repeated twice each with 15 ml 0.85% NaCl. In total, 45 ml of cell suspension was collected in 50 ml sterilized Falcon tube and used for the analysis **(Shintani et al., 2020)**.

2.4 Enumeration of cultivable bacterial, fungi, diazotrophs, potassium and phosphate solubilizing bacterial counts
Serial dilutions were prepared for each rhizosphere sample, obtained as described above, using 0.85% NaCl (three replicates for each farming system). Appropriate dilutions were plated on different selective media. For enumerating the total bacterial and fungal CFU counts, PCA and RBA media were used, respectively, while CCM, NBRIP and

Aleksandrov were used to enumerate the CFU counts of N_2 fixing (diazotrophs), phosphate solubilizing and potassium solubilizing bacteria, respectively. Inoculated plates were incubated at 30°C. Developed colonies were counted after 3 and 5 days for bacterial, diazotrophs and fungal plates, respectively. The NBRIP and Aleksandrov inoculated plates were incubated at 30°C then after 5 to seven 7 days, the colonies displayed clear zone was counted.

2.5 Bacterial isolation, purification and characterization

Bacterial colonies displayed distinct colony morphology, developed from rhizosphere samples representing the DOK farming systems, were picked. These bacterial isolates were then purified and preserved in NB medium with 20% glycerine at -20°C for further analysis. Obtained bacterial isolates were characterized for their direct and indirect plant growth promotion-related activities (PGPAs). The isolates were screened for their ability to fix nitrogen, solubilize phosphate and/or potassium as direct PGPAs. Nitrogen fixers were determined through their ability to grow on N-deficient CCM medium for three successive sub-cultures **(Hegazi et al., 1998)**. Screening the bacterial isolates for phosphate and/or potassium solubilization capacity was carried out through spot inoculation of 5 μL of fresh culture of each bacterial isolates onto NBRIP and Aleksandrov agar media with triplicates, respectively **(Nautiyal, 1999, Sun et al., 2020)**. Inoculated plates were incubated at 37°C for 5 to 7 days **(Batool & Iqbal, 2019)**. Bacterial isolates exhibited a clear zone around their colonies were recorded as positives.

An in vitro assessment of the antifungal activity of obtained bacterial isolates was conducted, through a dual culture assay on PDA medium, against two common phytopathogen *Aspergillus* sp. and *Fusarium* sp. as an indirect PGPA. Mycelial agar discs (0.5 cm diameter) of the tested phytopathogens, taken from 7-day-old fungus plates, were placed on one side of the PDA plates, about 3 cm from the edge. A loopful of each bacterial isolate was streaked 3 cm away from the mycelia disc from fresh cultures. Plates with only fungal mycelial discs for each of the tested phytopathogens were inoculated as controls. The antagonistic activity was recorded for the isolates that showed growth inhibition zone after 5–7 days of incubation at 25°C **(Guerrieri et al., 2020)**.

2.6 Chemical Analysis

Composite samples were prepared from the soil under the influence of wheat roots which grown under the DOK farming systems. A composite sample from each farming system was applied for physicochemical analysis including pH, electrical conductivity (EC), available nitrogen (N), phosphorus (P) and potassium (K) according to **(Vikram et al., 2023)**.

2.7 Bioassay test-based assessment for the soil fertility

Wheat seeds were brought from El-Mizan Plant Raising Company (Belbes, El Sharkeya, Egypt). The seeds were planted on pots (10 seeds/pot) containing soil obtained from the plots representing the three DOK farming systems from the experimental site, where no external nutrients were applied. A total of 15 pots, representing 5 pots replicates per treatment, were kept at room temperature under day/night light. The pots were watered every couple of days or when needed. The number of germinated seeds was recorded every two days. Two months after planting, wheat plants were harvested, and the shoot and root fresh weights were recorded for the plants from each pot. The dry weights of the corresponding samples were determined after drying at 70°C for 24 h until the weight remained constant.

2.8 Data Analysis

Obtained data were subjected to one-way analysis of variance (ANOVA) using STATISTICA software version 10.0 and Prism 0.4. The differences among various treatment means were compared using Fisher's LSD at 5% ($p \leq 0.05$). The correlation between the effects of the three DOK farming systems on the measured parameters were subjected to principal component analysis (PCA) using Past 4.03 **(Srivastava et al., 2020)**. Clustering of the obtained bacterial isolates based on their functions was constructed via a neighbor-joining method using Past 4.03 **(Yan et al., 2022)**, and the tree was saved in Newick format and visualized with an interactive tree of life (iTOLv4) (https://itol.embl.de) for formatting **(Letunic & Bork, 2019)**.

3. Results

3.1. Impact of DOK farming systems on cultivable microbial populations

Total bacterial CFU counts were estimated for the rhizosphere soil of wheat plants grown under DOK farming systems. Rhizosphere soil from wheat plants grown under biodynamic (D-W-Rh) system recorded significantly higher bacterial CFU counts (7.66 log cfu/g) compared to the other two systems. Lower CFU counts were recorded for organic (O-W-Rh) and conventional (K-W-Rh) samples with no significant differences among them as they showed almost identical average numbers (7.39 and 7.38 log cfu/g, respectively) as shown in **figure** 1.A. The samples (O-W-Rh) and (K-W-Rh) exhibited significantly higher fungal population counts (5.14 and 5.12 log cfu/g, respectively) with no significant difference among them in comparison to (D-W-Rh) samples (4.85 log cfu/g) **(Figure** 1.B).

Estimation of different functional groups showed that the highest numbers of N_2 fixing populations were recorded for the (O-W-Rh) samples (5.63 log cfu/g) followed by (D-W-Rh) then (K-W-Rh) samples (5.50 and 5.41 log cfu/g, respectively), although that, the

differences were not significant (**Figure** 1.C). Regarding phosphate solubilizing bacteria, there were no significant differences between (K-W-Rh) and (D-W-Rh) samples (3.47 and 3.22 log cfu/g, respectively) while (O-W-Rh) samples exhibited

significantly lower CFU counts (2.65 log cfu/g) (**Figure** 1.D). However, the potassium solubilizing bacteria were below detection threshold in all tested samples, (D-W-Rh), (O-W-Rh) and (K-W-Rh).
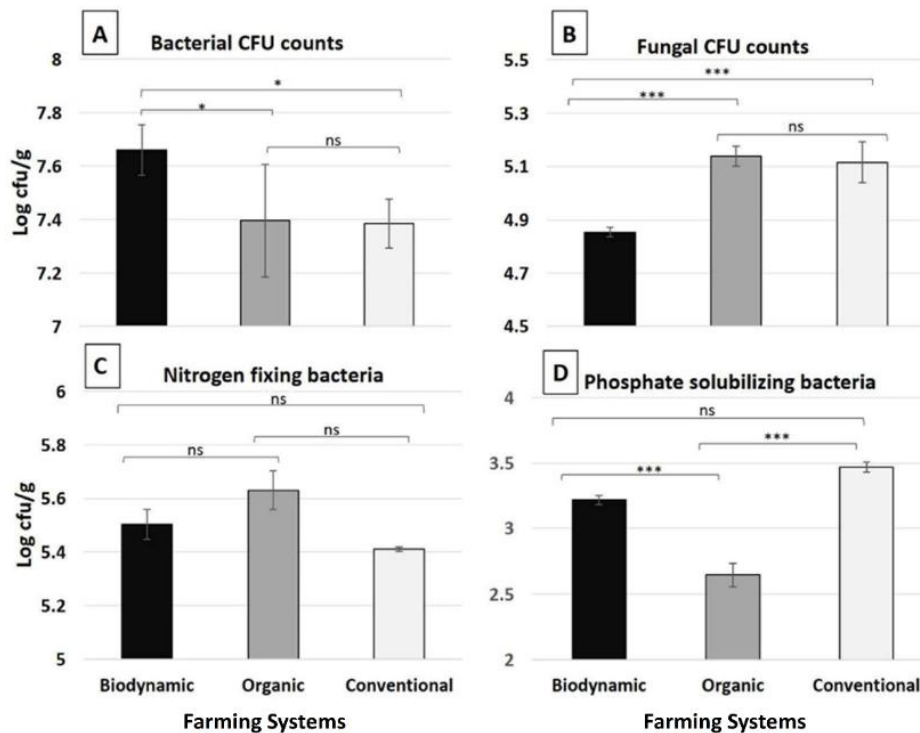

Fig. 1. Total cfu counts of bacteria; A, fungi; B, nitrogen fixing bacteria; C and phosphate solubilizing bacteria; D estimated for the rhizosphere soil of two-month-old wheat plants grown under DOK farming systems (black; Biodynamic, dark gray; Organic, and gray; Conventional). (*); significant difference and ns; no significant difference.

3.2. Isolation and characterization of bacterial isolates associated to the rhizosphere of wheat plants from DOK farming systems

A total of 32 (DWRh1 to 32), 24 (OWRh 1 to 24) and 20 (KWRh 1 to 20) bacterial isolates were selected from the (D-W-Rh), (O-W-Rh) and (K-W-Rh) samples, respectively, based on the difference of colony morphology. Then, in vitro characterization was conducted to evaluate the capacity of tested isolates to perform functions related to direct and/or indirect plant growth promotion. As direct PGPAs, bacterial isolates were screened for their ability to fix N_2, solubilize phosphate and/or solubilize potassium. Among these 19, 13 and 16 bacterial isolates out of 32, 24 and 20, respectively were able to grow on N deficient medium representing 59.38, 54.17 and 80%,

respectively. Regarding phosphate solubilization, 9, 10 and 5 bacterial isolates out of 32, 24 and 20, respectively exhibited phosphate-solubilizing abilities representing 28.13, 41.67 and 25%, respectively. None of the bacterial isolates displayed potassium solubilization abilities (**Figure** 2).

Furthermore, the antagonistic potential of these bacterial isolates was tested against two common phytopathogen, *Aspergillus* sp. and *Fusarium* sp. serving as an indirect PGPA. Out of the 32, 24 and 20 bacterial isolates, 1, 6 and 2 displayed antagonistic effect against *Aspergillus* sp., representing 3.13, 25 and 10%, respectively. For *Fusarium* sp., a total of 21, 14 and 7 bacterial isolates displayed antagonistic effect, representing 65.63, 58.33 and 35%, respectively (**Figure** 2).
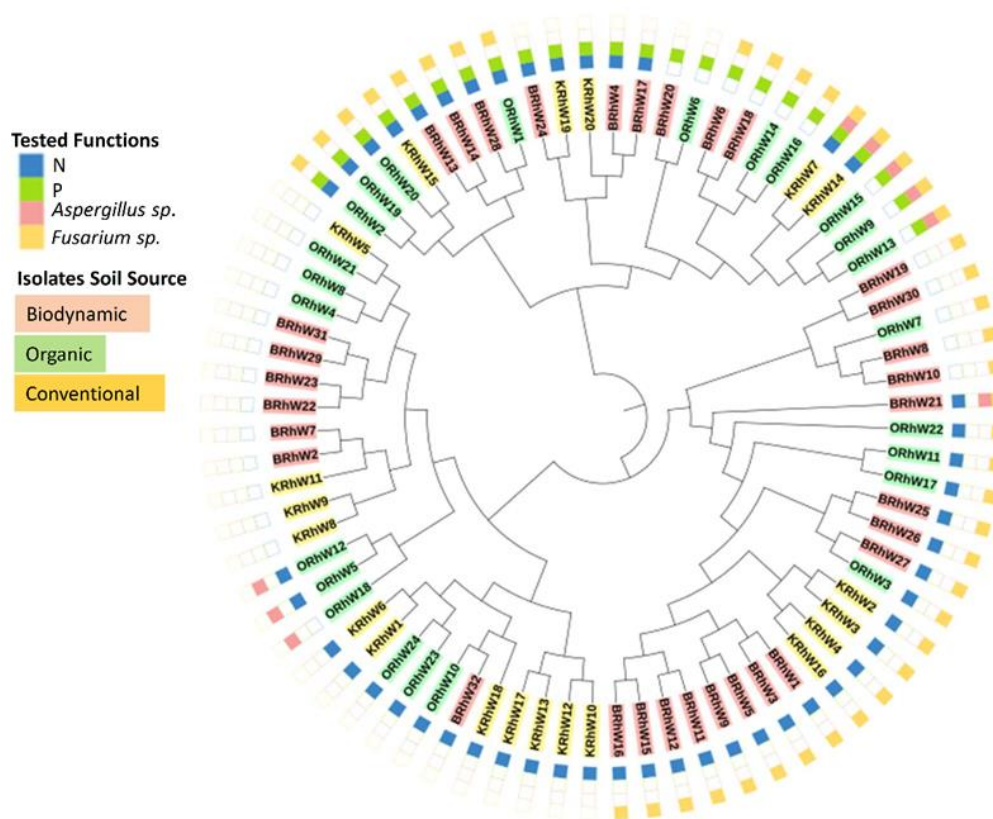
Fig. 2. Phylogenetic tree of a total of 72 bacterial isolates constructed based on their direct (blue; N_2 fixation and green; P solubilization) and indirect (antagonistic effect against: red; *Aspergillus* sp. and orange; *Fusarium* sp.) PGPA(s) using iTOL. A number of 32, 20 and 20 bacterial isolates were obtained from (red; Biodynamic, green; Organic, and gold; Conventional). Farming systems are shown as highlights and PGPAs are displayed as square shapes with different colors.

3.3. Physiochemical analysis of soils from DOK farming systems

After harvesting, the soil under the influence of wheat root that grown under DOK farming systems were sampled and subjected to physiochemical analysis. Total available nitrogen (NH4 + NO3), phosphorus and potassium as well as the soil pH and salinity were determined. The pH values showed slightly less alkaline conditions in the biodynamic system (7.96) compared to organic and conventional systems (8.46 and 8.32, respectively). Salinity levels (EC) were lower in the organic system (0.323 dS/m) compared to the biodynamic and conventional systems (0.436 and 0.412 dS/m, respectively). The availability of nitrogen, phosphorus, and potassium was slightly differed among the farming systems, with conventional and organic systems showing closer amounts to each other. The results of the chemical analysis are shown in table 1. This analyzed soil was further used in the bioassay experiment conducted in the present study.

Table 1. Chemical analyses of the soil under the influence of wheat roots which were grown under the DOK farming systems.

Three farming systems (DOK)	Soil reaction	Salinity	Available macro-nutrients (mg/kg)		
	pH (1:2.5) (H_2O)	EC dS/m (1:5)	N	P	K
Conventional	8.32	0.412	44	36	577
Organic	8.46	0.323	44	35	595
Biodynamic	7.96	0.436	50	43	542

N; total available nitrogen (NH_4+NO_3). P; available phosphorus. K; available potassium
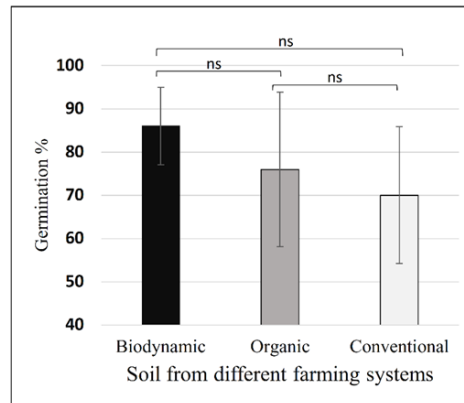
Fig. 3. Germination percentage (%) of the wheat plant seeds (10 seeds per pot), planted in soils from different farming systems (DOK: black; Biodynamic, dark gray; Organic, and gray; Conventional). ns; no significant difference.

3.4. Bioassay test-based soil fertility estimation

We assessed the capacity of the soils from the three farming systems (DOK) to support the wheat seed germination and growth performance, as a sole nutrient source without adding any external supplementation. While the soil from the biodynamic system exhibited the highest germination percentage (86%) followed by organic (76%) then conventional (70%) systems, these differences were not significant (**Figure** 3).

Two months after planting, the wheat plants grown in soil from different systems were collected and both fresh and dry weights were determined for the roots and shoots. Wheat plants grown in soil from biodynamic system exhibited the highest root fresh and dry weights (1.51 and 0.23 g) followed by organic (1.21 and 0.20 g) and conventional (0.9 and 0.18 g) systems, respectively, (**Figure** 4 A and B). Despite that, the differences were significant between all treatments only in root fresh weights while the observed differences among root dry weights were not significant (**Figure** 4 A and B).

Comparable patterns were also observed for the shoot fresh and dry weights. The highest weights were recorded for the wheat plants grown in soil from biodynamic system (1.60 and 0.26 g) followed by organic (1.38 and 0.25 g) and then conventional (1.14 and 0.19 g) systems (**Figure** 4 A and B), respectively. For fresh weights, the significant differences were observed only between biodynamic and conventional while regarding the dry weights both biodynamic and organic where significantly higher compared to conventional systems (**Figure** 4 A and B).
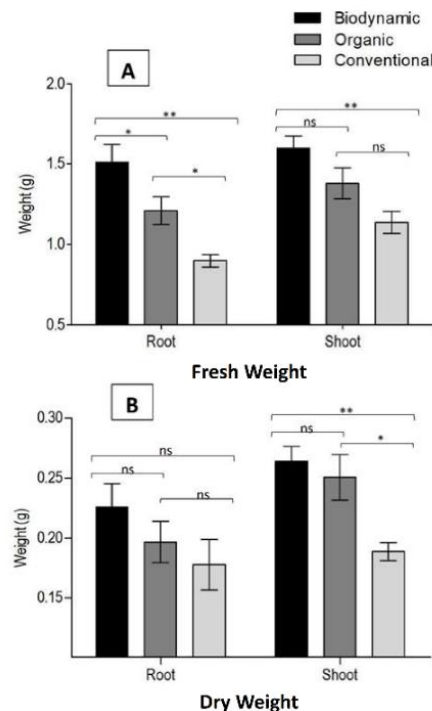

Fig. 4. Fresh (A) and dry (B) weights recorded for the wheat plants root and shoot when planted in soils obtained from different DOK systems (black; Biodynamic, dark gray; Organic, and gray; Conventional). (*); significant difference and (ns); no significant difference.

3.5. Correlations between all tested parameters for DOK farming systems using principal component analysis

In the present study, biplot principal component analysis (PCA) was also employed to explore the overall correlations between the tested farming systems (DOK) and all tested parameters including both plant growth (R-FW, Sh-FW, R-DW and Sh-DW) and microbial (bacteria, fungi, diazo. and phos.) parameters. As shown in **figure** 5, the two principal components (PCA 1 and PCA 2) explained 80.32% of total variation in tested parameters as they explained 55.61% and 24.71% of the variability, respectively. They showed a clear partitioning between the conventional (at the left), biodynamic (at the right) and organic (at the middle) samples along the PCA 1 in the PCA biplot (**Figure** 5). Accordingly, R-FW, Sh-FW, R-DW, Sh-DW, bacteria and diazo. variables were strongly correlated with the biodynamic systems while phos. and fungi were correlated with conventional system (**Figure** 5).
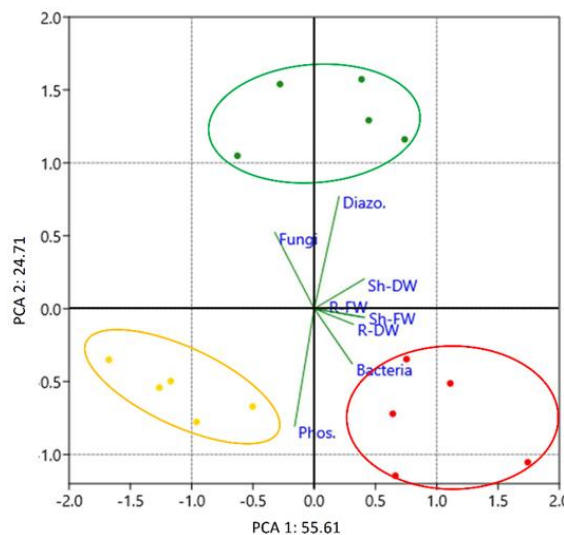

Fig. 5. Biplot principal component analysis (PCA) for PC1 and PC2 showing the correlation between the tested farming systems (red; Biodynamic, green; Organic, and gold; Conventional) and the measured parameters (R-FW; root fresh weight, Sh-FW; shoot fresh weight, R-DW; root dry weight, Sh-DW, shoot dry weight, bacteria; bacteria, fungi; fungi, diazo.; diazotrophs and phos.; phosphate solubilizing bacteria). Green arrows represent the tested variables, and circles in color around the dots represent the farming systems samples.

4. Discussion

While the impact of agricultural practices on the soil microbial community has been frequently assessed employing culture independent methods, the impact on the abundances and activity of cultivable proportion represents a valuable indicator for the soil vitality. Therefore, in the present study, we assessed the influence of the long-term DOK farming systems on the abundances and activities of various soil microbial groups in the rhizosphere of wheat plants grown under the DOK systems. Moreover, the reflected influence on the plant growth performance was also investigated. The results showed that the different farming systems significantly influenced the abundances of the tested microbial groups in a different manner. Biodynamic farming displayed significantly higher bacterial counts compared to both organic and conventional systems. Conversely, organic and conventional systems had higher fungal populations compared to the biodynamic system. These results suggest that the domination of a certain population negatively impact of the numbers of the other population. Similar patterns were recently reported by **(Zhang et al., 2022)** as they showed that

when fungal community was more prevalent or exhibits a particular pattern, the bacterial populations tend to show the opposite trend. The high bacterial numbers reported by biodynamic system suggesting that its practices may provide conditions promote bacterial abundances **(Chen et al., 2019)**. However, the prevalence of bacterial populations was reported to have a positive impact on nutrient cycling that contributes to soil health improvement **(Chen et al., 2019, Jangid et al., 2008, García-Orenes et al., 2016, Zhang et al., 2022)**. The lack of significant differences between conventional and organic systems in some of the tested microbial groups highlights the complexity of microbial responses to farming practices as well as suggests that methods other than cultivation methods are needed to detect the differences **(Zhang et al., 2022)**.

The impact of the three farming systems was also reflected on the soil chemical characteristics with biodynamic being closer to organic farming in nitrogen, phosphate available content and the pH compared to conventional system **(Cuartero et al., 2021)**. The elevated total available nitrogen in conventional system may be attributed to the chemical fertilizers application **(Gu et al., 2017)**.

To get insights into the influence of the different practices on the soil microbial activities, bacterial isolates were obtained from the rhizosphere of wheat plants grown under DOK farming systems and characterized. Bacterial isolates from different farming systems exhibited diverse nutrient cycling capabilities with no discernible correlation of a specific function with a particular system **(Arkhipova et al., 2007)**. However, the correlation between specific bacterial functions and particular farming systems can be complex and variable as it can be influenced by various factors **(Zhang et al., 2022)**. The bacterial isolates from biodynamic and organic systems displayed the highest percentage of isolates with antagonistic activity against *Fusarium* sp. (65.63%) and *Aspergillus* sp. (25%) phytopathogen, respectively, when compared to conventional system. The increased antagonists percentage indicates the soil health **(Sammauria et al., 2020, Suman et al., 2022)**.

Bioassay test was employed to evaluate the fertility of the soils under the DOK practices. Notably, wheat plants grown in soil from biodynamic system displayed higher germination percentage, root and shoot fresh and dry weights followed by organic then conventional systems, which was significant in case of the shoot fresh and dry weights in addition to the root fresh weight. Although significant differences exist among the three DOK farming systems, the results suggest that the specific practices employed in the biodynamic farming system, such as the use of specific preparations, composts, or other biodynamic techniques, likely contributed to enhancing soil fertility that was reflected on the tested plant growth parameters **(Turinek et al., 2012)**. However, this enhancement might be attributed to the increased bacterial numbers in the biodynamic system (**Figure 1A**) that might improve the nutrient availability and/or enriched beneficial microbial activity **(Turinek et al., 2012)**. The distinct impacts among the three DOK farming systems on the tested parameters were further confirmed via the PCA biplot. Among the distinct impacts, the biodynamic system displayed the strongest impact on the most tested parameters. However, further analysis is needed to explore the specific factors contributed to the soil improvement as a response to the application of the biodynamic practices.

5. Conclusion

Present study showed that the three DOK farming systems exhibited significantly distinctive impacts on the soil microbial abundances, activities as well as the chemical characteristics. Among them, Biodynamic practices may provide conditions that promote the abundances of bacterial populations, which play a vital role in enhancing the soil nutrient availability and contributes to soil health improvement. The impact of the biodynamic practices was further reflected on the tested plant growth parameters as shown through the bioassay test. It seems that the long-term application of biodynamic displayed the most sustainable and conducive system for soil fertility that was followed by the organic farming practices. However, the soil from conventional farming system exhibited the least favourable impact on the soil fertility.

Conflicts of interest: Authors have declared that no competing interests exist.

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