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Impact of symbiotic mycorrhiza interrelation in some soil biological parameters and growth of five cover crops

*Sundoss KABALAN, Katalin JUHOS, Eszter TÓTH, *Borbála BIRÓ

Doctoral School of Horticulture, Hungarian University of Agriculture and Life Sciences (MATE), Budapest, Hungary

Abstract

Cover crops serve as an essential source of nutrients in the soil and generally improve the soil's properties. Cover crops' production is considered a benefit of the soil quality; by protecting the soil from erosion, reducing the weeds and the so-called soil-borne plant pathogens. Different varieties of cover crops can be cultivated such as legumes, non-legumes, brassica, and grass-type of plants with a variability of the symbiosis. A pot experiment was carried out with five cover crops, as non-symbiont (*Brassica carinata* B.c.), single-symbiont with arbuscular mycorrhiza fungi (AMF) (*Phacelia tanacetifolia* P.t., *Avena strigosa* A.s.) and double symbiont with AMF and nitrogen-fixing bacteria (*Vicia benghalensis* V.b., *Vicia faba* V.f.) crops; and a mixture of the five species, placed in sandy soil (arenosol) in plastic pots (5000 g soil) in 4 repetitions. One of the pots with mixed cover crops was inoculated by AM fungi industrial product. We measured soil biological activity of dehydrogenase (DHA) and fluorescein-diacetate (FDA) enzymes, the frequency of AM fungi (F%), the all protein, glomalin content and electrical conductivity (EC) of the soils. Mixture of all the cover crops resulted maximum EC and significantly enhanced the enzymatic, DHA, FDA activities in comparison with single plants. Mycorrhiza colonization frequency was high in all cover crops except the mustard (B.c.), as non-symbiont. Vetch (V.b.), as double symbiont was responding very positively to AMF inoculation, and enhanced the performance of its growth. It was found in the pot experiment, that vetch, has the highest capacity to retain soil-protein, glomalin concentration, as well. The mixture of five cover crops could be suggested to use, due to the synergistic positive performance of the individual crops, and the better functioning of beneficial fungal / bacterial symbiosis.

Keywords: cover crops, mycorrhiza, soil-protein, soil enzymes, sustainable agriculture

Introduction

Soil health is defined as the ability of soil to function as a living ecosystem that maintains biological health and biological productivity and promotes plant, animal, and human health by preserving air or water. This finite, nonrenewable, and dynamic living resource affects the humans and animals through the quality of the crop

**Corresponding authors:* SUNDOSS KABALAN, BORBÁLA BIRÓ, Hungarian University of Agriculture and Life Sciences, Villányi út 29–43, H-1118 Budapest, Hungary.
E-mails: kabasuzana@gmail.com; pacsutane.biro.borbala@uni-mate.hu

(WARKENTIN, 1995). The biological activities in soil can improve the soil health and soil quality. For example, the use of cover crops (CC) can boost soil quality by protecting soil from decomposition, reduce weeds, and promote the development of nitrogen- and phosphorus-forming and dissolving bacteria. Cover crops which are used in some cases as catch crop plants, are mainly cultivated after harvesting a primary plant (ABDALLA et al., 2019). Cover crops consisting of mixtures of different species have recently become popular, as mixing species with different properties produces a single crop that can perform various functions (STORKEY et al., 2015). There are different benefits of cover crops such as retaining the water capacity of the soil, improving the activity of natural flora and fauna in the soil, and contributing to maintaining the natural ecosystem processes. The presence of cover crops helps maintain the nitrogen cycle and bolster the growth of nitrogen and phosphorus producing and/or solubilizing bacteria (TONITTO et al., 2006; BRENNAN and BOYD, 2012). Different crop alternatives are available to be used as vegetative cover such as legumes, and they have a great benefit to the soil. Crop rotation of the cover increases the biological activity and biodiversity of the agricultural ecosystem by increasing microbial diversity and ensuring organic matter, and prevents leaching of soil nutrients (KAYE and QUEMADA, 2017). It is, also known, that the microbial community of the soil improve by the cover crops. Intensive research is also being conducted on how cover crops can promote mycorrhizal fungal density, and in parallel, microbial biomass in the affected soils. Improving the phosphorus availability of soils and the activity of phosphatase enzymes was also reported by several authors (ZHANG et al., 2016; DUDÁS et al., 2017). Enzymes play an important role in the cycling of nutrients in nature. Because soil enzyme activity is sensitive to agricultural practices, it can be used as an index of soil microbial activity and fertility (HAMIDO and KPOMBLEKOU, 2009). Thus, they are involved in soil mineralization processes (TATE et al., 1988) and have been related to other soil biological properties (FRANKENBERGER Jr and DICK, 1983). Understanding the dynamics of enzyme activity on cover crop residue could give insight into the timing of cover crop residue C influx into the soil matrix (TABATABAI et al., 2010), which has potential to lead to N immobilization and decreased plant available N supply for the cash crop. Furthermore, the assessment of cover crop residue enzyme activities in conjunction with measurements of cover crop biomass accumulation and soil ammonium concentrations over time could elucidate the timing of cover crop biomass N release and how it relates to the N demand of corn during growth and development. It is important to keep in mind that farm management decisions have large effects on the biological activity in the soil. In general, more activity is better for plant productivity and the use of cover crops is also suggested as part of the conservation agricultural practices. The approach has a wider advantage when looking at the resilience of the agro-ecosystems and the livelihoods of those farmers who adopt it (GAZDAG et al., 2019). The soil would be more resilient to disasters such as droughts and heavy rainfall from an ecosystem perspective, and at the same time, the soil would improve its quality. This improvement will benefit farmers, as they will be able to increase crop yields and be less vulnerable in terms of food security, many types of plants can be used as cover crops, so it is important to know that there are four main categories:

legumes (e.g., broad bean and vetches), non-legumes (e.g., spinach and mustards), brassicas (e.g., radishes and turnips) and grasses (e.g. oat and wheat) (FAO, 2012). The experiment plants used in this study as CCs, are *Vicia faba*, *Phacelia tanacetifolia*, *Avena strigosa*, *Brassica carinata* and *Vicia benghalaensis* which selected based on their symbiotic status, so as to assess the hypothesis, that symbiosis ratio might improve soil-quality and some of the soil health parameters.

Fungi coexist with the roots at most of the crops, monocots and dicotyledons, herbaceous plants, and some fruit trees such as citrus fruits and apples... etc. The most common form of mycorrhiza is the arbuscular mycorrhiza fungi (AMF), which is known as beneficial symbionts at about 80% of the higher plants (TAKÁCS et al., 2018). It is well known that the AMF can improve the uptake of the phosphorus (P) of the hosting plants (SCHMIDT et al., 2010), as well as the performance of its growth. Their potential as indicators of soil health stems from the fact that they are ubiquitous in soil-plant systems. They play an important role in the uptake of nutrients at both macro and micro levels and thus have significant impacts on the nutritional value of plants. In addition, AMFs improve plant water balance through several mechanisms, possibly contributing to increased drought resistance in plants (AUGÉ, 2001; FÜZY et al., 2007). It is well known that AMFs are important organisms that perform a variety of ecosystem functions. They simultaneously inhabit two habitats: the root-systems of host plant and also the soils around of the plants. The structures formed in the soil include a network of thicker hyphae that serve as conduits for the delivery of nutrients taken up by thin, highly branched hyphae called absorption hyphae (BRUNDRETT and ASHWATH, 2013), these benefits of the arbuscular mycorrhizal fungi can easily be enhanced through studied agricultural management (GOSLING et al., 2006). The potential application of managing the abundance and diversity of AMF to improve soil quality and health to enhance the productivity of agricultural ecosystems has steadily evolved into a next-generation mycorrhizal technology that holds great promise (BAGYARAJ and REVANNA, 2017). It is also promising soil microorganisms that improve soil health by absorbing nutrients, controlling disease, and phytoremediation (VIVAS et al., 2006). In addition to continually monitoring and evaluating physicochemical and biological processes to improve soil health, it is important to remember that soil microorganisms and their interactions are important players in nutrient cycling and have complex interactions with plants. A soil glycoprotein has been discovered as another important agent for stabilizing soil aggregates (SINGH et al., 2013). It is called glomalin and it is thought to be produced mainly by AM fungi. Glomalin is a soil fraction defined by its extractability and immunoreactive properties rather than by a specific gene product or chemically homogeneous molecular species (ROSIER et al., 2008). Glomalin and glomalin-related soil proteins (GRSPs), as well as the all soil proteins have recently received a boost in the literature, but their development and main functions remain largely unknown. Nevertheless, they are an important determinant of soil quality and a very stable carbon sink with a half-life of several years to decades. Electrical conductivity (EC) level of soils gives as a potential of measuring the saline and the soluble nutrient content of the soils. It measures the quantity of salts (primarily negatively charged ions, e.g., $-NO_3$, $-SO_4$) in the soil-plant environment, and it is considered as an indicator somehow of the soil fertility.

This review focuses on an integrative view of soil health indicators that can be used as tools for predicting sustainability in production systems by comparing the effect of some most frequently applied cover crops on the soil microbiological activity, especially mycorrhiza (AM) fungi, and how these effects depend on some physical and chemical properties of the soil. The objective of the present study was to compare the biomass production of 5 cover crops and assess their effects on some soil / plant quality indicators focusing for the symbiosis status of those crops with the AM fungi, and the performance of used cover crops, either symbionts or not.

Materials and Methods

Test plant and growth condition

The experiment plant including *Vicia faba,* V.f. (known as broad bean), *Phacelia tanacetifolia,* P.t., *Avena strigosa,* A.s. (also known as black oat), *Brassica carinata,* B.c. (known as Ethiopian mustard) and *Vicia benghalensis*, V.b. (known as purple vetch) were grown separately in plastic pots (V = 5000 g) with sandy soil, slightly humous arenosol (pH = 7.4). Also there was a mixed pot including all of five mentioned plants. 10 plants were put in each pots. In the mixed pots there were 15 plants altogether (5×3 plants from each cover crops). The plants were grown in heated greenhouse. The average temperature was 19℃ during the day and 10℃ at night; the humidity was 52%. After 8-weeks of growth, the plants were harvested.

Biomass and enzymatic activity

Biomass production of shoots and roots (fresh and dry) were measured. Two enzymatic activities in the soil samples were determined at the end of the experiment: dehydrogenase activity (DHA) was determined by the method of (VERES et al., 2015). Total microbial activity in the soils was measured by hydrolysis of fluorescein diacetate (FDA), determined using the assay by validated to specific condition by (VILLÁNYI et al., 2006). Four replicates per assay were used.

Electrical conductivity

Electrical conductivity (EC) of 1:5 suspension of soil/distilled water was measured in the soils of 5 cover crops and its mixture in a pot experiment, compared to non-planted control soil.

Mycorrhiza colonization assay

Mycorrhiza was measured which is root colonized by AMF of 5 cover crops and its mixture, either inoculated or not with 2% industrial AMF inoculums (Danuba, Hungary). For the estimation of mycorrhizal development, roots were prepared according to the modified method of (PHILLIPS and HAYMAN, 1970). After careful washing with tap water, the roots were softened in 7% KOH solution for 24 h, washed in water, acidified in 5% lactic acid in water for 1–24 h, and stained with 0.01% aniline blue in 5% lactic acid for 24 h at room temperature. The stained roots were stored in lactoglycerol until they were used for slide preparation. Parameters of mycorrhizal colonization were evaluated microscopically using thirty 1 cm root fragments per sample and calculated as percentages: frequency and arbusculum content of mycorrhization of root fragments (F%).

Glomalin measurement

Total glomalin-related all soil protein content was measured in the soil of 5 cover crops based on (HADDAD and SARKAR, 2003). The soil dried for 4 days and sieved, 2 g of soil were used, 8 ml of 50 mM Citrate Buffer (pH = 8), then the samples were autoclaved and centrifuged. The liquid was transferred to 15 ml sterile falcon tubes and then standard solution was prepared (Protein Standard, P0834-10*1 ml; sigma; 2 mg ml^{-1}; Lot SLBS3852) with 6 dilutions (0, 0.2, 0.4, 0.6, 0.8, 1 mg ml^{-1}) in 6 replicates of using Bradford reagent (Sigma, B6916-500ml). Evaluation was assessed with spectrophotometer at 595nm wavelength.

Statistical analysis

IBM SPSS 23 program was used. Normality assumption was proven by the Kolmogorov-Smirnov test ($p > 0.05$) and the homogeneity of variance was checked by Levene's test ($p > 0.05$). Univariate analysis of variance (ANOVA) test was applied to evaluate the results.

Results and Discussion

Biomass production of used cover crops

Legume cover crops have greater biomass production, than the other studied, non-legume plants. This fact was supported previously by (FINNEY et al., 2016). It is especially the bean, which was producing the greatest biomass (*Table 1*), due to its complex root system, which might absorb water and minerals from the soil. Generally, the legumes of having mainly the double symbiotic systems might produce more cover crop biomass, in comparison with the mustard, which plant cannot be supported either the N_2-fixing bacteria or the P-mobilizing symbiotic AM fungal activities in soils. Symbiosis on the other hand can support multiple benefits in plant nutrition. This declare the effect of CC on increasing amount of P and fungal population which it makes nutrients more available for CC, therefore, the biomasses will increase. This correlation between AM and biomass production is obvious as well, especially in leguminous crops. Cover cropping enhances soil microbial community abundance, activity, and in particular, benefits from increases in AM abundance in soils (NELSON and JANKE, 2007).

Table 1
Plant biomass production (shoot and roots), as dry weight of 5 studied cover crops species and their mixture (Mix), without and with inoculation (Inoc) by arbuscular mycorrhiza fungi, grown in a pot experiment. Average and standard deviation of using 4 replicates

Treatments	**Mean shoot biomass weight** ($g \cdot m^{-2}$)	**Mean root biomass weight** ($g \cdot m^{-2}$)
Phacelia, P.t.	0.83±0.413	0.15±0.049
Bean, V.f.	**3.88±0.403***	**14.28±1.151***
Vetch, V.b.	0.45±0.142	2.01±0.551
Oat, A.s.	1.12±0.234	1.26±0.186
Mustard, B.c.	0.93±0.079	0.15±0.035
Mix	2.09±0.091	**3.54±0.738***
Mix Inoc.	1.97±0.174	**3.21±1.115***

Soil enzyme activity

The soil enzymes have been used as sensitive indicators of the fertility of the soil, soil productivity, and quality. In addition, their operations can indicate microbial activity, decay rates, and substrates for microbial or plant uptake availability (ZHANG et al., 2015). The highest values of dehydrogenase activity (DHA; *Table 2*) were detected after the termination of the experiment. DHA values showed a significant ($p < 0.05$) difference between oat and the other plants. This fact may have positive effects on the activity of enzymes. Furthermore, the mixture of 5 cover crops showed the biggest effect on the enzyme activities compared to the other samples. Among the used cover crops, the mustard (B.c.) has the lowest enzyme activity compared to the other crop species. The mustard is the only cover crop plant, which cannot show symbiotic interrelation with the studied beneficial soil-microorganisms, influencing also of the smaller growth of plants.

Table 2
Total enzyme activity, the hydrolysis of fluorescein diacetate (FDA) and dehydrogenase activity (DHA) in the soil samples at the end of the experiment of 5 studied cover crops species and their mixture, as inoculated (inoc) with arbuscular mycorrhiza fungi (n = 4)

Treatments	**Mean DHA and STDV** (TPF $\mu g \cdot g^{-1} h^{-1}$)	**Mean FDA and STDV** ($\mu g \cdot g^{-1} h^{-1}$)
Phacelia, P.t.	5.05±1.366	2.25±0.420
Bean, V.f.	**6.33±0.514***	2.26±0.303
Mustard, B.c.	4.91±0.563	2.01±0.218
Vetch, V.b.	5.69±0.778	**3.12±0.648***
Oat, A.s.	**6.87±1.195***	2.39±0.513
Mixture	6.27±0.669	2.98±0.614
Mixture, inoculated	**5.68±2.005***	**3.29±0.773***

The measured hydrolysis of fluorescein diacetate (FDA) was determined to assess the total microbial activity (*Table 2*). FDA values were rather a variable character (the minimum = 2 and the maximum = 3.12 μg FDA $g^{-1} h^{-1}$). The vetch and mixture of CC have the highest value in comparison to other variants. It is assumed, that the vetch has a strong root system that might contain N_2-fixing nodules at an early stage, and those nodules might provide for the plant's sufficient nitrogen and it is also can accumulate significant amounts for the following crops (DE RON, 2015). The mustard on the other hand might have the lowest effect on the soil microbial activity. Hydrolysis of fluorescein diacetate (FDA) is performed by a variety of enzymes including esterase, proteases, and lipases. Moreover, FDA hydrolysis may be used as an indicator of microbial catabolic activity in soils.

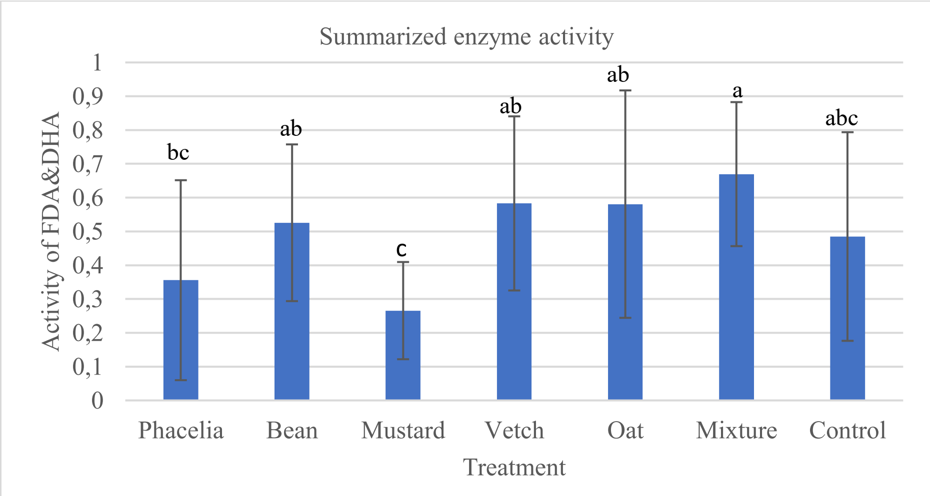

Figure 1
Summarised mean activity of fluorescein diacetate (FDA) and dehydrogenase (DHA) enzymes in the soil samples of 5 cover crops species and their mixture in comparison with the control, without any plants.
Error bars indicate standard deviation, n = 4 (ANOVA; p < 0.05).

As a summary for the two enzyme activity methods the mixture of the five crops *(Figure 1)* showed higher enzyme activities compared to the other samples, cropping which means the incorporation of the cover crops into the field may increase enzyme activities and microbial community in soil and therefore improve soil quality and health. Thus, there may be qualities unique to each cover crop that increases the activity of specific enzymes and play an important role in ecosystem function and cycling of soil nutrition. However, mustard has low enzyme activity compared to other cover crops as mustard reduced the total porosity of the soil to a higher extent. The porosity of soils is improving in the case of a mycorrhiza symbiosis and also the mucigel production of N_2-fixing symbiosis. Enhanced enzymatic activity suggests that biomass is also improved through cover plants. This increase in soil biological activity is considered a dynamic indicator reflecting the enhancement of ecosystem services and soil properties. Thus, increasing in biomass makes the enzymes more active. While, plants with the highest potency to remove ions from soil, showed the lower EC activity as compared to the others. According to present study leguminous plants tend to show low EC levels. It is potentially about the greater biomass production of leguminous plants.

Electrical conductivity of soils

Electrical conductivity (EC) level of soils gives as a potential of measuring the saline and the soluble nutrient content of the soils. It measures the quantity of salts (primarily negatively charged ions, e.g., $-NO_3^{2-}$, $-SO_4^{2-}$) in the soil-plant environment, and it is considered as an indicator of soil fertility.

Table 3
Electrical conductivity (EC) in the soils of 5 cover crops and its mixture in a pot experiment compared to a non-planted control soil (n = 4)

Treatments	**Mean EC and STDEV** (μ m^{-1})
Phacelia, P.t.	188.43±36.88
Bean, V.f.	127.13±12.59
Vetch, V.b.	207.13±26.91
Oat, A.s.	188.88±36.05
Mustard, B.c.	141.43±16.01
Mix	207.33±20.32
Mix Inoc	141.03±11.69
Ctrl	**268.90±134.01***

Considering the pot experiment the mixture of all the cover crops tended to show the maximum electrical conductivity quite significantly compared to the other cover crops. The beans showed the lowest EC activity as compared to the other cover crops. Thus, leguminous plants tend to show low EC levels, therefore those plants might offer the highest potency to remove ions from the soils. It is potentially about the greater biomass production of leguminous plants. *Table 3* is showing the measured EC of soil samples and the control sample tends to show the highest EC compared to the other samples with cover crops. Cover crop treatments improved saturated hydraulic conductivity, attributable to improved structural stability and porosity, which in turn could improve soil water storage. Also there are some similar reports (AHUJA et al., 1984; DEMIR and IŞIK, 2019).

Mycorrhiza colonization (F%)

Mycorrhiza enhances the production of cover crops (CHEN et al., 2018). Type of Cover crops is the key-important factor in AM fungal colonization. Bean, as a double symbiont has the highest mycorrhizal activity in agreement with the result of (FARZANEH et al., 2009). The mustard, as non-symbiont plant has 0%. percentage of mycorrhiza, shown also the same by (ALBERTI, 2017). Vetch is responding rather positively to AM fungal inoculation, as well (*Figure 2*). It is well established that intercropping systems favour the AM fungal communities' abundance and diversity compared to conventionally managed systems. The positive effects of cover crops on the mycorrhization parameters are similar to several studies' findings performed in other climatic zones (MUKUMBAREZA et al., 2015; BATTIE-LACLAU et al., 2020; MORTIER et al., 2020)

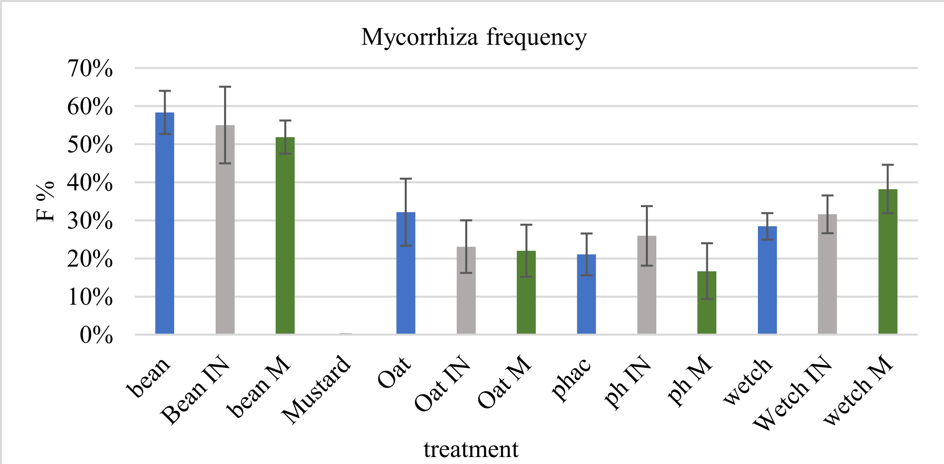

Figure 2

Frequency of mycorrhiza (F%) (mean and standard deviation) for the roots of 5 studied cover crops and their mixture grown in a greenhouse. Cover crops were either inoculated (IN) or not (M) with arbuscular mycorrhiza fungi (n = 4). Cover crops: Bean-V.f., Mustard-B.c., Oat-A.s. Phacelia-P.t., Vetch-V.b.

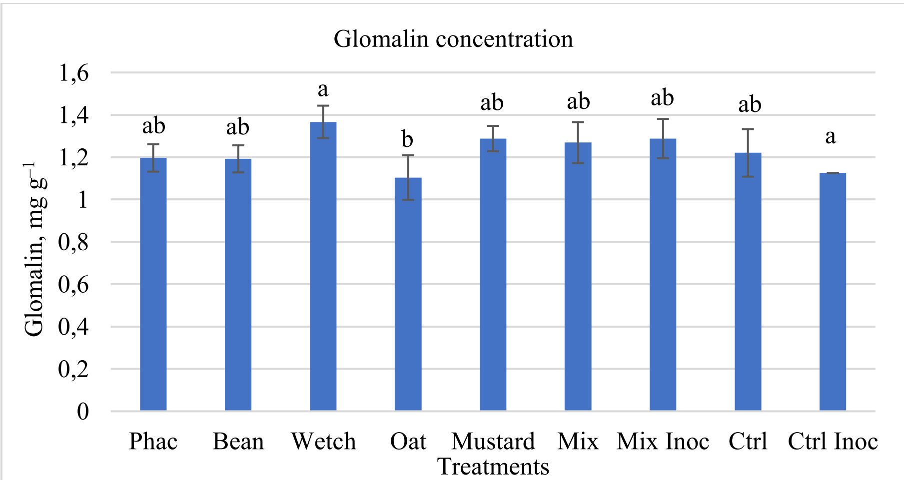

Figure 3

Glomalin, all protein content (mean and standard deviation) in the soil of 5 cover crops and its mixture in a pot experiment (mg g^{-1}) (n = 4). Cover crops: Phacelia-P.t., Bean-V.f., Mustard-B.c., Vetch-V.b., Oat-A.s. and the mixture of all. Control was either inoculated (Inoc) or not with AM fungi. Different letters represent significant differences within each group ($p < 0.05$)

Glomalin concentration

Glomalin is a protein-like substance produced by AM fungi in the soil-plant ecosystems (WANG et al., 2017). The glomalin (soil protein) concentration along with the cover crops can be very important in maintaining the soil quality and aggregation stability. It was found in the pot experiment, that the vetch (V.b.) has the highest capacity to retain glomalin (soil protein) concentration followed by the mustard (B.c.) and a mixture of the cover crops *(Figure 3)*. This result could be explained by favourable factors for AM with this pot experiment which was free of any fungicides, over-fertilization, and the other unfavourable factors. By comparing the results of soil protein (glomalin), concentration and biomass, can understand the direct correlation between them. Also, there are some similar reports to approve this (YANG et al., 2017; LI et al., 2022).

Conclusions and recommendations

Based on our results cover crops can increase the measured soil characteristics by their biomass production and physiological characteristics. Regarding the soil-biological functioning, the tested enzymes showed improved activities and this result was significant at certain cover crops. Legume and mixed cover crops with leguminous plants tended to have more biomass production, compared with the other single studied crops. Mustard has the lowest enzyme activity. It is assumed that a missing symbiotic interrelation might limiting of those parameters. On the basis of literary survey, the FDA enzyme activity can show a great relationship with degradative capacity of microbial community and the decomposition of soil organic matter. It is suggested, therefore, as a soil quality indicator. Both the biomass production of cover crops and also the soil enzymatic activities can be key-important in the sustainable growth. Mycorrhiza fungal symbiosis enhanced the production and physiology of used cover crops. Bean (V.f.), as a double symbiont crop had the highest mycorrhizal activity, and the vetch (V.b.) has the best power to retain the all protein (glomalin) content of the soil. The new practice in the future needs to focus more efficiently on reducing fertilizers and finding a perfect replacement from natural sources. According to our results, a mixture of the five cover crops could be recommended, because it has the optimal performance with the synergistic interactions of the applied individual plants. By improving these suggested finding on farm experiment, we can achieve to a desired sustainable in production system.

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