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THE ROLE OF SYMBIOTIC INTERRELATIONS IN THE AVAILABILITY OF NUTRIENTS FOR FIVE COVER CROPS

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***Abstract**: Biomass production of cover crops and relation with electrical conductivity (EC), soil glomalin content and the mycorrhiza frequency (F%). Cover crops (CC) serve as essential source of nutrients in the soil and improving of the soil properties. The production of cover crops is considered as a benefit of the soil quality; by protecting of the soil from erosion, reduce the weeds and the so-called soil-borne plant pathogens and increase soil biological activity. There are different varieties of cover crops that can be grown: such as legumes, non -legumes, brassica and grass-type of plants. Each of these cover crops have their own benefits to the soil system, the presence of cover crops helps in maintaining the nitrogen cycle as well as bolster the growth of nitrogen and phosphorus producing and/or solubilizing bacteria. Our examinations were carried out in pot experiments at MATE Buda Campus, in a heated greenhouse. The pot experiment was designed with five types of cover crops species (Phacelia tanacetifolia, P.t.; Brassica carinata, B.c.; Vicia faba, V.f.; Avena strigosa, A.s.; Vicia benghalensis, V.b.) and a mixture of the five species, placed in sandy soil (arenosol) in plastic pots in 4 repetitions. We measured soil biological activity, which can be the first step to achieve healthy soils. We assessed the saline content of the soils, i.e., the quantity of salts in the soil-plant environment. This parameter is considered as an indicator of soil health and fertility. Considering of 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 formation of cover crops might support also the growth of the symbionts, such as the mycorrhiza fungi. Different plants can have variable interactions with the AM Fungi. We have measured the Frequency of mycorrhizal infection (F%). The percentage of mycorrhiza colonization was good in all CC except the mustard, B.c. (0%), which is known as a non-symbiotic plant. Wetch is responding positively to AM fungal inoculation. It is well known that the AMF has the ability to improve the uptake of the phosphorus (P) of the hosting plants as well as the performance of its growth (Finlay 2008). The hyphae of arbuscular mycorrhizal fungi produce a protein called glomalin. The glomalin concentration along with the cover crop can be very important in maintaining the soil quality and the aggregation-stability. It was found in the pot experiment, that the vetch (V.b.) has the highest capacity to retain glomalin concentration followed by mustard (B.c.) and the mixture of cover crops. Further aim is to test the benefits of mycorrhiza symbiosis and interrelation with their "helper" bacterial community in the secondary structure of soil.*

Keywords: cover crops, mycorrhiza symbiosis, soil nutrients, sustainable agriculture

INTRODUCTION

Cover crops which also known at some cases as catch crop plants are mainly cultivated after harvesting of 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 in retaining the water capacity of the soil, improve of the activity of natural flora and fauna in the soil as well as contribution in maintaining the natural ecosystem processes. The presence of cover crops helps in maintaining the nitrogen cycle as well as bolster the growth of nitrogen and phosphorus producing and/or solubilizing bacteria (Dabney et al. 2010; Tonitto, David, and Drinkwater 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. Cover crop rotation can increase microbial diversity (Schonbeck and Morse 2006), enhance the biodiversity of the agro-ecosystem, ensure organic matter, increase soil biological activity and can prevent soil from nutrient leaching (Kaye and Quemada 2017). It is, also known, that microbial community of the soil improve by the cover crops. It is also intensively studied, how the cover crops might provide mycorrhizal fungal abundance and in parallel, the microbial biomass of affected soils. The phosphorus availability of soils and activity of phosphatase enzymes was also reported (W. Zhang et al. 2016; Dudás et al. 2017). 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 agriculture (CA) practices. The approach has a wider advantage when looking at the resilience of the agro-systems and the livelihoods of those farmers who adopt it. The soil will be more resilient to disasters such as droughts and heavy rainfall from an ecosystem perspective, and at the same time, the soil will 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 (FAO 2012).

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 symbionts at about 80% of higher plants. It is well known that the AMF has the ability to improve the uptake of the phosphorus (P) of the hosting plants as well as the performance of its growth (Finlay 2008). These benefits of the arbuscular mycorrhizal fungi can easily be enhanced through studied agricultural management (Gosling et al. 2006; Ryan and Graham 2018).

The objective of the present study was to compare the effect of some most frequently applied cover crops on the soil microbiological activity, especially mycorrhiza (AM) fungi, and how these effects depend on the physical and chemical properties of the soil, which is the first step towards the soil-health improvement. Two different soil-enzymes were selected for the comparison study.

MATERIALS AND METHODS

Test plants and growth characteristics: As a test plant we used five cover crops (V.f.: Vicia faba also known as Broad bean, P.t.: Phacelia tanacetifolia, A.s.: Avena strigose also called as Black oat, B.c.: Brassica carinata, known as Ethiopian mustard and V.b.: Vicia benghalensis, known as Purple vetch) and a mixture of the five species. The plants were grown in sandy soil (Arenosol, Soroksar) in plastic pots. 10 plants were put in each pot in heated greenhouse at SZIE Buda Campus. The pot experiment was conducted from 12/10/2020 to 24/11/1010. The average temperature was 19℃ and the humidity was 52%. After one and a half month of growth, the plants were harvested.

Biomass-production: Fresh and dry shoot and root biomass above ground shoot weight were measured, the weight refers to the “dry weight” value after the plant tissue has been dried to a constant weight at 60°C, and the soil moisture was measured, tow enzymatic activities in the soil samples were determined at the end of the experiment. Four replicates per assay were used.

Enzyme activities: Dehydrogenase activity (DHA) was determined using the assay by Casida method (Casida, Klein, and Santoro 1964) on the basis of the reduction of triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF). One gram of pots-moist soils was mixed with 1 ml 1.5% TTC thoroughly into test tubes. The samples were mixed on a vortex and the suspension was incubated for 24 at 30°C, the control contains only 1 ml tris buffer (without TTC). Then 4 ml methanol was added for each tube for extraction of TPF reflecting in red coloration. Samples incubated again for 2 h at room temperature.

Finally, the suspension was filtrated, and the density of the suspension was measured with the spectrophotometer at a wavelength of 485 nm using methanol as a blank. DHA was expressed as µg TPF per 1g dry soil matter. Average of 4 replicates is shown of DHA activities. Total microbial activity in the soils was measured by hydrolysis of fluorescein diacetate (FDA). FDA was determined using the assay by

(Green, Stott, and Diack 2006). During the process 7.5 ml was added of 60 mM phosphate buffer to each tube which containing 1 g of soil, to suspend the solution it is need to vortex, then 100 µl of stock solution FDA was added to each tube, shake it by hand and incubated for 3 h in the shaker incubator at 30℃ (200 rpm) after incubation 7.5 ml as added of acetone to each tube, shake again, then the solution was filtered into a clean falcon tube. The intensity of the fluorescent was measured by the coloration of samples, including the control on a spectrophotometer (wavelength 490 nm).

Electrical conductivity of soils: Electrical conductivity (EC) was measured in the soils of 5 cover crops and its mixture in a pot experiment, compared to a non-planted control soil. Four replications for each crop were measured by using electrical conductivity meter (EC meter) which measured the electrical conductivity in a soil solution.

Frequency of mycorrhiza (F%): general Mycorrhiza was measured which is root colonized by AMF + VMF+ hyphae, for the roots of 5 cover crops and its mixture, 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 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 24h at room temperature. The stained roots were stored in lactoglycerol until they were used for slide preparation. Four parameters of mycorrhizal colonization were evaluated microscopically using thirty 1-cm root fragments per sample and calculated as percentages: frequency of mycorrhization of root fragments (F%).

Glomalin content of soils: Glomalin content was mesured in the soil of 5 cover crops and its mixture in a pot experiment in four repetation, compared to a non-planted, non-AMF-inoculated control plant. 50 ml of a 2 mol / l KCl solution were added to 10 g of dry soil, (2 M KCl: 1 liter of distilled water for 149 g of KCl salt), the suspension was shacked for1 hour, then centrifuge it. The intensity was measured by spectrophotometer (wavelength 595 nm).

Statistical analysis: IBM SPSS 23 program was used. Normality assumption was proven by Kolmogorov-Smirnov test ($p>0.05$) and the homogeneity of variance was checked by Levene's test ($p>0.05$). For evaluation of the results multivariate analysis of variance (MANOVA) test was applied.

RESULTS AND DISCUSSION

Biomass production of cover crops

Legume cover crops have better biomass production, than the other studied, non-legume plants. It is especially the bean, which was producing the greatest biomass (Figure 1), due to its complex root system, which might absorb water and minerals from the soil.

The hydraulic system can transport the nutrients to the whole plant; also the growth rate of it plays an important role with increasing the biomass. Biomass production differed substantially among the used mixes of the cover crops. Generally, the legumes of having simple and double symbiotic systems, might produce more cover crop biomass, than the mustard, which plant cannot be supported neither the N2-fixing or the P-mobilizing symbiotic activities.

B. carinata usually do not produce as much biomass as other brassica leguminous plants. Biomass production of faba bean by shoot height, number of branches, root length, absolute growth rate, and number of leaves can be significantly higher than most legumes and non-legumes, the growth and biomass production by mustards in the winter is not usually as reliable as that of other cover crops such as legume or the mixture of the five crops.

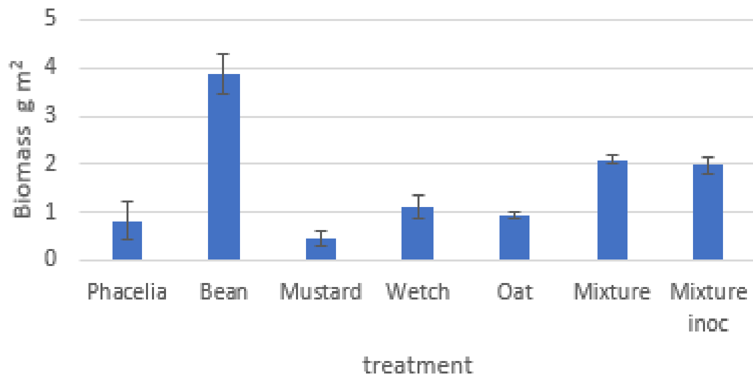

Figure 2. Plant biomass production, as dry weight (g m^2) of 5 studied cover crops species and their mixture, studied among green-house condition in pot experiment.
Cover crops: Phacelia-P.t., Bean-V.f., Mustard-B.c., Wetch-V.b., Oat-A.s.

Soil Enzyme Activities of DHA and FDA enzymes

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; *Figure 2*) were detected after termination of the experiment. DHA values showed a significant ($p < 0.05$) difference between oat and other plants that's maybe because of hull acts as a physical barrier duringoat germination and prevents the loss of moisture, lipids, protein, carbohydrates and their seed derivatives.This fact may have positive effects on the activity of enzymes.

Furthermore, the mixture of five cover crops showed the biggest effect on the enzyme activities compared to the other samples. Among the 5 cover crops the mustard (B.c.) has the lowest enzyme activitycomparing of the other crop species. The mustard is the only cover crop plant, which cannot show any symbiotic interrelation with soil-microorganisms, influencing perhaps of the smaller growth.

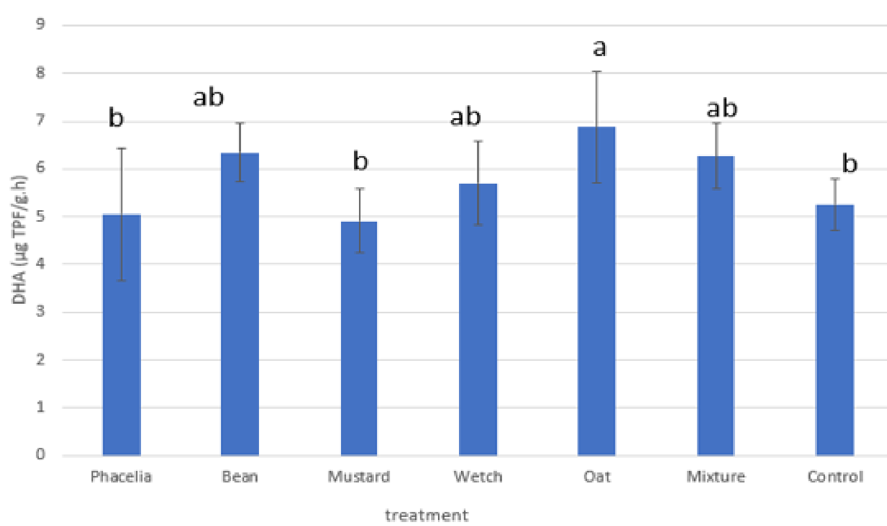

Figure 3. Dehydrogenase activity in the soil samples before the establishment and after the end of the experiment. Error bars indicate standard deviation, n = 3 (ANOVA; $p < 0.05$).
Cover crops: Phacelia-P.t., Bean-V.f., Mustard-B.c., Wetch-V.b., Oat-A.s.

The measured hydrolysis of fluorescein diacetate (FDA) was determined to assess the total microbial activity (Figure 3). FDA value were rather variable character (minimum=2 µg FDA/g.3h; the maximum=3.12) The wetch and mixture has the highest value in comparison to other variants. It is assumed, that the wetch has a strong root system that might contain N2-fixing nodules at an early stage, and those nodules might provide for the plants sufficient nitrogen and it is also can accumulate significant amounts for the following crops. (De Ron 2015). The mustard on the other hand might has 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 (Nikaeen et al. 2015).

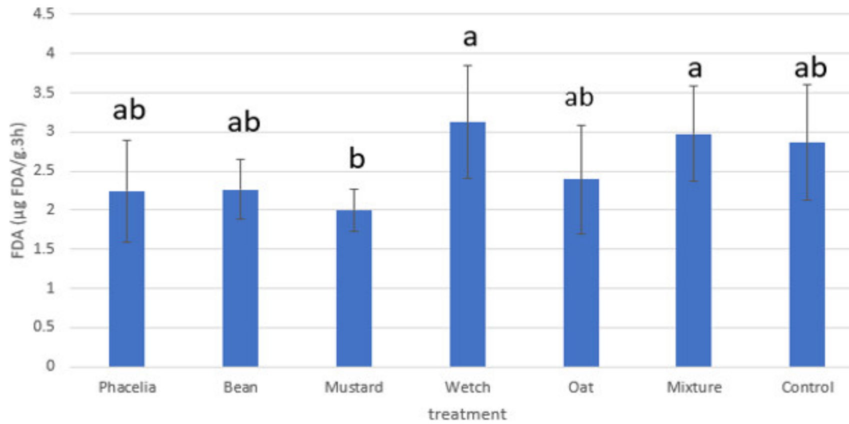

Figure 4. Hydrolysis of fluorescein diacetate (FDA) in the soil samples at finishing of the experiment.
Error bars indicate standard deviation, n = 3 (ANOVA; $p < 0.05$).
Cover crops: Phacelia-P.t., Bean-V.f., Mustard-B.c., Wetch-V.b., Oat-A.s.

As a summary for the two enzyme activity methods the mixture of the five crops showed higher enzyme activities compared to the other samples, cropping that's mean incorporation 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 comparing to other cover crops because mustard reduced total porosity of the soil to a higher extent. The porosity of soils is improving in case of a mycorrhiza symbiosis and also the mucigel production of N2-fixing symbiosis. The mustard cover crop cannot show such symbiotic microbes. The legume type of cover crops can better improve of the soil nutrient capacity, affecting as biomass-production and also as providing biological origin of nitrogen and other elements.

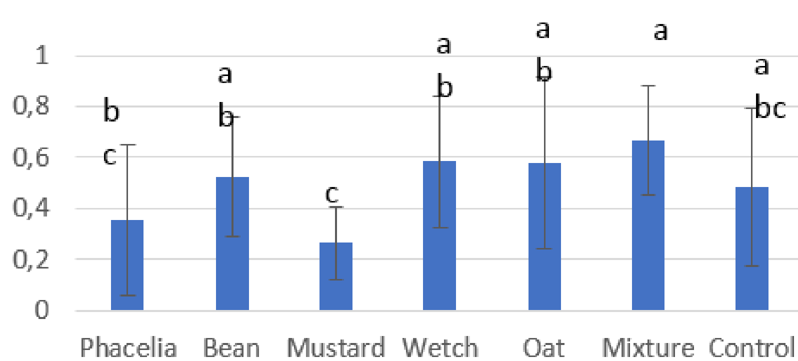

Figure 4. Summarized average result of the soil enzymatic activities, the dehydrogenase (DHA) and the fluorescein diacetate (FDA) of 5 cover crops and its mixture in a pot experiment, compared to non-planted control soils

Electrical conductivity of soils

Electrical conductivity level of soils gives as a potential of measuring the saline content of the soils. It measures the quantity of salts in the soil-plant environment, and it is considered as an indicator of soil

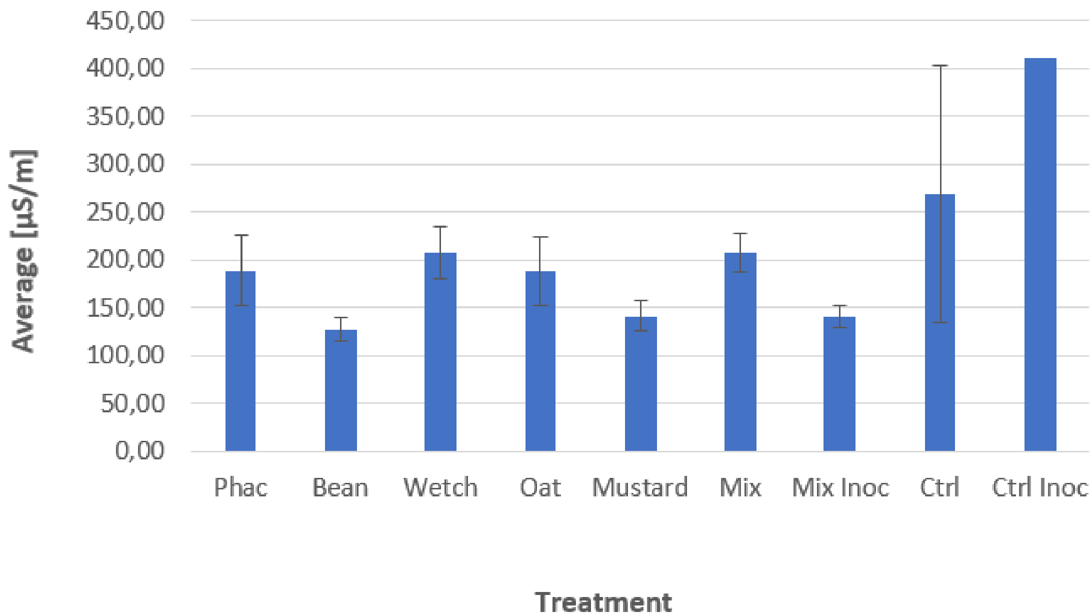

Figure 5. Electrical conductivity (EC) in the soils of 5 cover crops and its mixture in a pot experiment, compared to a non-planted control soil.

Frequency of mycorrhiza (F%):

Mycorrhiza enhance the production of cover crops. Type of Cover crops is the key-important factor in AM fungal colonization. Bean, as double symbionts have the highest mycorrhizal activity, and the mustard, as non-symbionts has 0% of mycorrhiza was good in all CC except the Mustard (0%). The Mustard is a non-symbiotic plant. Wetch is responding positively to AM fungal inoculation.

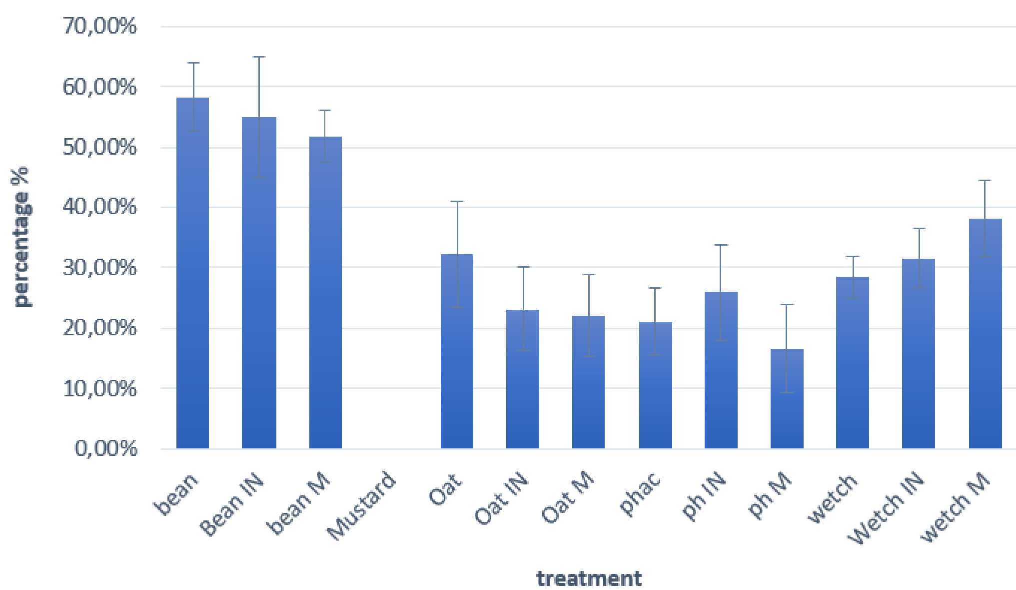

Figure 6. Frequency of mycorrhiza (F%) for the roots of 5 cover crops and its mixture

Glomalin concenteration:

Glomalin is a protein-like substance produced by the AM fungi in the soil-plant ecosystems. The glomalin concentration along with the cover crop can be very important in maintaining the soil quality and the aggregation-stability. It was found in the pot experiment, that the vetch (Vb) has the highest capacity to retain glomalin concentration followed by mustard (Bc) and mixture of the cover crops.

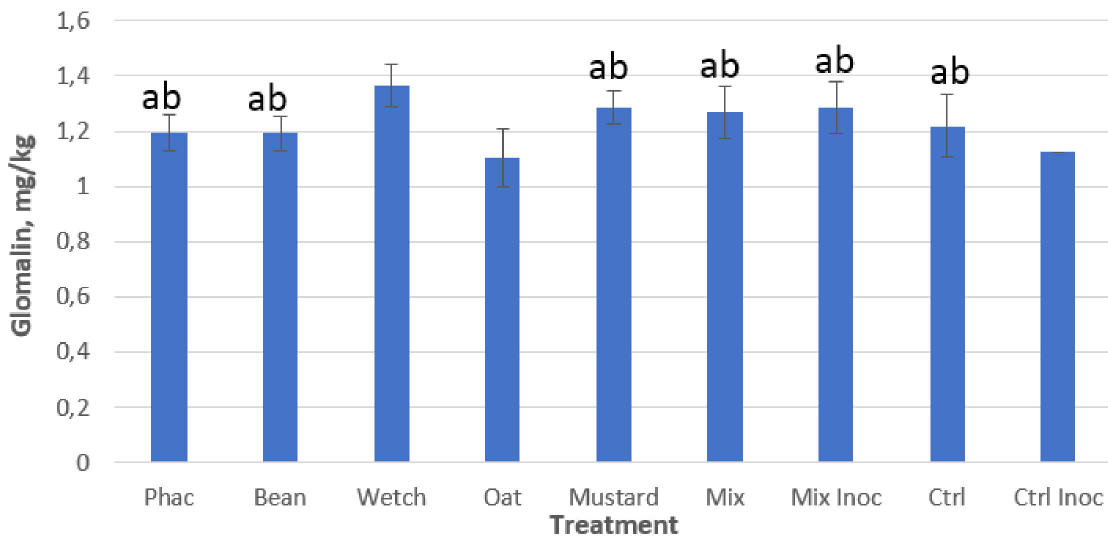

Figure 7. Glomalin content in the soil of 5 cover crops and its mixture in a pot experiment, compared to a non-planted, non-AMF-inoculated control plant.

CONCLUSIONS AND RECOMMENDATIONS

Based on our results cover crops can increase the soil nutrient status by their biomass production. Regarding the soil-biological functioning, the tested enzymes showed also improved activities and this result was also significant at certain cover crops. Legume and mixed cover crops tended to have more biomass production, comparing with the other studied cover crops. Mustard has the lowest enzyme activity, due to the missing symbiotic interrelation.

Total microbial catabolic activity in the soils was measured by monitoring the hydrolysis of fluorescein diacetate (FDA). This enzyme can show a great relation with the degradative capacity of microbial community, the degradation of soil organic matter is one of the main soil quality indicators. So, both the biomass production of cover crops and also the soil enzymatic activities can help to develop a sustainable growth, which is an important aspect, more particularly among organic agricultural productions. Mixed cover crop application can show the best performance of considering those aspects.

Mycorrhiza enhance the production of CC. Type of CC is the key-important factor in AM fungal colonization. Bean, as double symbionts has the highest mycorrhizal activity, and the mustard, as non-symbionts has 0%. Vetch (Vb) has the highest capacity to retain glomalincontent of soil. The new practice in the future needs to focus more efficiently on reducing of Fertilizersand find perfect replacement from the natural sources.

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