SOIL SCIENCE ANNUAL
2024, 75(3), 193448

https://doi.org/10.37501/soilsa/193448

Possibilities of biological soil management in monoculture greenhouse cultivation: cover crops, organic matter replenishment and *Trichoderma* sp. application to improve soil health

Enikő Papdi[1], Flórián Kovács[1,2], István Fekete[3], Katalin Juhos[1*], Zsolt Kotroczó[1*]

[1] Department of Agro-Environmental Studies, Hungarian University of Agriculture and Life Sciences; Villanyi str. 29-43, H-1118 Budapest, Hungary
[2] Faculty of Agriculture, University of Szeged; Andrássy str. 15. H-6800 Hódmezővásárhely, Hungary
[3] Institute of Environmental Science, University of Nyíregyháza, Sóstói út 31/b. H-4400 Nyíregyháza, Hungary

* Corresponding author: PhD, Zsolt Kotroczó, kotroczo.zsolt@gmail.com, PhD, Katalin Juhos, juhos.katalin@uni-mate.hu), ORCID iD: https://orcid.org/0000-0002-5637-2802 (Z. Kotroczó)

Received: 2024-03-16
Accepted: 2024-09-16
Published online: 2024-09-16
Associated editor: Jadwiga Wyszkowska

Keywords:

Sandy soil
Soil biology
Soil Health
Cover crop
Capsicum
Trichoderma harzianum

Abstract

Today, soil degradation processes present severe challenges to farmers. This is even more typical in cultivating vegetables in growing equipment, where the intensive use of chemicals causes physical, chemical, and biological degradation of soils. Therefore, it is essential to find solutions with which we can sustainably slow down these processes in such a way as to minimize crop losses and not increase the costs of growers. One such solution could be the use of cover crops in growing equipment. Our main objective was to find a sustainable cultivation solution to increase soil biological activity, organic matter and nutrient content, thus improving soil health. The research was carried out on sandy soil with low organic matter content in a foil housing experiment. Several soil samples were taken during the experimental period. The labile carbon content (POXC), fluorescein diacetate and phosphatase enzyme activity, nitrate content and yield tests were also determined from the soil samples. Based on our results, we could not detect a significant difference between the cover crop + *Trichoderma* treatment and the cover crop treatment. However, we found that when using cover crops, the *Trichoderma* treatment had a positive effect and was significantly different from the other treatments for biological activity (FDA), phosphatase activity, and nitrogen retention. Although there was no difference in yield between the treatments, the positive characteristics mentioned above may help develop a sustainable horticultural production that will improve soil health in the long term. Overall, it can be concluded that using cover crops can be an effective method for increasing the active carbon content of the soil, which microbes can use. Furthermore, with the diversity of its root system, the well-chosen cover plant mixture provides a vast, rich surface and a habitat for soil microbes, increasing biological activity. In addition, the rhizosphere, the scene of important metabolic processes, contributes significantly to increasing the soil's organic matter stock.

1. Introduction

There is a growing demand for farm-grown vegetable crops free from fertilizers, pesticides and xenobiotics. These systems can only be maintained through an ecological approach by improving the biological properties of the soil. Improving soil biological activity is essential to increase and improve the organic matter content of soils. Organic matter preserves soil moisture, improves soil structure and attenuates climate change stresses, which are essential for good soil life (Gurmu, 2020). Growing environmental challenges show that soils cannot be seen as a simple supporting medium but as a key factor for yield, crop quality and crop safety (Juhos et al., 2019). With increasing soil degradation problems and changes in the regulation of agricultural chemicals, we need new alternatives that promote healthy crop growth and improved crop quality. New solutions include ensuring an adequate supply of organic matter, recycling available organic waste, or using alternative organic materials. The use of alternative organic materials, such as composts or organic fertilizers, makes it possible to maintain and improve soil nutrient supply (Viti et al., 2010; Rayne and Aula, 2020), allowing the use of inorganic fertilizers to be reduced, but they are becoming increasingly scarce. A particular difficulty in vegetable production is using technologies that involve growing in foil or greenhouses. There is limited scope for soil improvement, replacement or crop rotation here. This increases the chances of pathogenic organisms proliferating, depleting soil nutrients and reducing biological processes. In the case of such growing equipment, particular attention should be paid to developing cultivation technologies that consider sustainability and consumption needs.

In horticultural vegetable production, various types of enclosed growing equipment are widespread. In this practice, there is often no possibility of moving the equipment or changing the soil, and monoculture cultivation is frequently used, where the same crops are grown for several years in a row. In practice, it is common to have several crops in succession in unheated greenhouses, taking advantage of the possibility to extend the growing season for better economic use. However, when growing economically important crops, the aim is to achieve higher yields as early as possible. This often leads to soil degradation, as these technologies do not necessarily consider soil degradation and soil health (Norris and Congreves, 2018). Different mulches are essential in preventing soil degradation and restoring soil quality. A common variation is the use of different ground cover plants. Some authors say cover crops preserve soil moisture, reduce soil erosion (Alvarez et al., 2017) and improve soil quality (Koudahe et al., 2022). In addition, ground cover plants enrich the soil with organic matter through the rhizosphere effect and after their termination (or death), thus helping soil life to be active (Sharma et al., 2018). This is necessary because it provides food for soil microorganisms and has a significant nutrient supply (Dabney et al., 2010) and soil structure improvement/formation properties. Their use in closed systems is not yet widespread, but they can be a good alternative to improve soil properties. Referred because of their positive properties mentioned above; on the other hand, in the periods between main crops, plants other than the main crop can also control potential pests of the main crop in the soil by altering the rhizosphere (Berry et al., 2011; Singhal et al., 2020; Kovács et al., 2024).

Without organic matter input, microbial activity cannot be maintained (Koudahe et al., 2022). A soil with active soil life and a continuous supply of organic matter provide many natural services through soil biological activity. Therefore, replacing the necessary nutrients and macro-nutrients with living microorganisms or their metabolic products instead of fertilizers is one of the most important professional challenges of our time. The services provided by microorganisms are much broader than what we use. In their work, Shingh and Dwivedi (2022) described *Trichoderma harzianum* as having a positive effect on plant development. This can be explained by the fact that *Trichoderma harzianum* protects plants from certain pathogens and helps them survive stress (El-Katatny 2010). Furthermore, the secondary metabolites produced by the root colonization of *Trichoderma harzianum* can increase and increasenutrient the efficiency of nutrient uptake from the soil and NO_3 uptake (Singh and Dwivedi 2022). In their work, Kovács et al (2024) found that *Trichoderma harzianum* + in combination with organic matter, increased the biological activity of the soil. Their most important application is to plant nutrient element detection and ensure nutrient balance (Biró et al., 2000; Dudas et al., 2017; Kocsis et al., 2020). These practices offer a more sustainable and nature-based approach to soil management and can contribute to the conservation of topsoil and the stability of agricultural systems in the future. The rhizosphere is the area whose functioning and microbiological composition are actively regulated by the plant, species, or life stage (Reinhold-Hurek et al., 2015). It also plays a crucial role in improving plant vitality and soil fertility (Rosenberg and Zilber-Rosenberg, 2016). The plant regulates the rhizosphere pH, soil moisture and oxygen supply by releasing significant amounts of “liquid/active carbon” through other chemical attractants and antibiotics (Berg and Smalla, 2009; Shi et al., 2016). Since small changes in total organic matter are difficult to detect, the potassium permanganate oxidisability test of soil carbon content (Weil et al., 2003) is a rapidly and dynamically changing parameter and a good indicator of changes over a short time (Bongiorno et al., 2019; Pulleman et al., 2021). These measurement methods aim to estimate the “active”/”labile” – more available to plants and microbes – soil carbon content, including the carbon stored in the soil’s microbial biomass, organic matter, and carbohydrate molecules. The resulting active organic carbon (POXC) is more sensitive to soil disturbance than total organic carbon and in many cases, correlates with soil biological activity (Margenot and Hodson, 2016; Margenot et al., 2017). Other influencing factors may include soil type and condition (Marschner et al., 2004), agrochemicals, organic and inorganic fertilizers used, and tillage practices (Zobiole et al., 2011; Wu et al., 2020). However, crop production technology impacts soil biological activity through its effect on the carbon cycle. Overall, biological activity is the maintenance of soil nutrient reserves, converting nutrients into compounds available to plants.

The use of cover crops in growing equipment (such as a greenhouse or foil house) is not widespread in the literature or practice, and we aimed to test this option and compare it with various conventional methods that impact soil health and quality. Our main objective was to select soil biological parameters that correlate well with soil quality and health parameters. We evaluated the effect of different nutrient amendment methods on soil biological properties by measuring soil labile carbon, fluorescein diacetate and phosphatase enzyme, nitrate content and yield development.

2. Materials and methods

2.1. The research area, applied treatments and soil sampling

The experimental site is located at Királyhalom in Serbia (46.102046°N, 19.885812°E). The trials were carried out over two years (2022 and 2023) in an unheated foil house of 165 m^2, with 33 m^2 plots per treatment. The soil properties of the experimental area are shown in Table 1. The test plant was sweet pepper (*Capsicum annuum* L.,). Sweet peppers are important in vegetable production and are grown in large areas of the world as growing equipment. This is mainly due to its heat and light requirements. In our experiments, 110 pepper seedlings per treatment were planted in early May in both years, in 50×40 cm rows/spacing. Two treatment groups were used. For one group, we aimed to model conventional cultivation technology. In this case, the main crops (crops grown for economic purposes) follow each other during the growing season, thus taking advantage of the growing equipment's ability to extend the growing season. In our case, radish (*Raphanus sativus* L. var. *sativus*) preceded table pepper (*Capsicum annuum* 'Amy F1') in early spring. For the other treatment group, we used a cover crop that is common in field conditions but not rarely used in cultivation equipment, which, although it has an economic loss, can help in sustainable cultivation. The test plants were provided with 6.5 L/m^2 of water per day during the growing season, with sprinkler irrigation, and 10 g/m^2 of active ingredient in the form of ammonium nitrate was applied to all treatments (except dM) at the beginning of berry development. For further treatments, we used *Trichoderma* fungi, cattle manure, and fertilizer (Table 2). *Trichoderma spp*. are a group of free-living fungi that are used worldwide as biocontrol and growth-promoting agents (Pang et al., 2017). The inoculant (with a spore number of 1×10^9) was applied with irrigation water before planting the seedlings and rotated shallowly (at the depth of the root zone) into the soil. The stable manure we used consisted of cattle manure. The fertilizer treatment (F) was 34% ammonium nitrate, which is applied immediately before planting. For the soil biology studies, soil samples were always taken from a depth of 0–10 cm of soil. Samples were taken from four points per plot, which were used after thorough homogenization and from which further measurements were taken. Fresh soil samples were stored at +4°C until use for the enzyme tests, while room temperature dried soil was used for the labile carbon (POXC) and nitrate content tests.

Table 1
Soil physical and chemical properties of the experimental site

Parameters	Soil properties
Texture	sand
pH ($CaCl_2$)	7.44
$CaCO_3$ (m/m%)	3.75
Soil Organic Matter (m/m%)	0.30
P_2O_5 (mg/kg)	263
K_2O (mg/kg)	93
$NO_3 + NO_2 - N$ (mg/kg)	44

2.2. Measurement of soil enzyme activity and permanganate oxidizable carbon (POXC)

Soil phosphatase enzyme activity was measured using the method of Sinsabaugh et al (1999). The measurement of phosphatase activity is based on the determination of p-nitrophenol released during enzymatic hydrolysis of a synthetic substrate, p-nitrophenyl phosphate (pNP-PO4). The enzyme in the soil causes the substrate to form p-nitrophenol. NaOH causes the pH to become alkaline and the colourless pNP formed is converted to yellow phenolate. The intensity of the color is proportional to the enzyme activity of the soil. The determination of the enzyme fluorescein diacetate (FDA) was performed according to the description of Varma and Oelmüller (2007). During the reaction, the colorless FDA is hydrolyzed by free and membrane-bound enzymes in the samples, releasing a colored end product, which was determined spectrophotometrically at 490 nm. The measurement of activated carbon (POXC) was carried out according to the method of Weil et al (2003) using the change in $KMnO_4$ concentration to estimate the amount of oxidized carbon. The technique consists of shaking 1 g of air-dry soil in 10 ml of 0.02 M $KMnO_4$ in 0.02 M solution for 5 min and measuring the absorbance at 565 nm. Nitrate content was measured only from samples taken at the end of the growing season. Nitrate content of soil samples was determined with 1 M KCl extraction. According to the rapid determination method of NO_3^-N, the complex molecule reacts with salicylic acid in complex basic solutions formed under strongly acidic conditions and absorbs maximally at 410 nm (Cataldo et al., 1975). Enzyme activity, POXC, and nitrate measurements were performed with a Biochrom Libra S22 spectrophotometer. The soil moisture was determined from the soil sample by gravimetric method and the results of the soil biological measurements were expressed as dry soil mass. Yields were characterized by the weight of peppers harvested per unit area during the whole growing season.

2.3. Data analysis

Statistical evaluation of the results was performed using R 4.2.1. The effect of treatments on soil microbial parameters was evaluated by multi-factor analysis of variance (MANOVA). The homogeneity of the covariance matrix (homoskedasticity) was

Table 2
Recommended and applied dose of treatments expressed in kg/m^2 and l/m^2

Treatment in short	Treatment	Description and dose
F(control)	Fertilizer	0,04 kg/m^2; ammonium nitrate (34%)
M	Cattle manure	6 kg/m^2
dM	Double dose cattle manure	12,8 kg/m^2
Cc	Cover crop:	0,006 kg/m^2; composition of cover crops: *Pisum sativum subsp. arvense, Vicia sp., Avena sativa.*
CcT	Cover crop + *Trichoderma harzianum.*	0,006 kg/m^2; composition of cover crops: *Pisum sativum subsp. arvense, Vicia sp., Avena sativa.* + 0,002 L/m^2

checked by Box's test. The normality of the MANOVA model was verified for the residuals using the Shapiro-Wilk test. Levene's test was used to test for variance homogeneity as a variable, and on this basis we decided on the *post hoc* pairwise comparison method. The significance levels for the analysis of variance were determined using Bonferroni correction. The effectiveness of treatments was assessed according to Wilks' Lambda.

3. Results and discussion

3.1. Effect of treatments on soil-biological activities

In the first year, treatments had a significant effect on FDA activity ($F_{(4,22)} = 3.04$; $P<0.05$; partial $\eta^2 = 0.35$). The value of the partial η^2 indicates that the treatments affected the FDA activity by 35%. The results of Duncan's post hoc test between treatments are shown in Fig. 1. If we look at the data from the first year of the experiment, there was a significant difference in the total biological activity of the FDA enzyme between the control (F) and the double manure (dM), as well as the cover crop treatments (Cc, CcT (P<0.05). Significantly (P<0.05) lower FDA enzyme values were measured in the soil of the only artificially fertilized plots than in the dM, Cc and CcTtreatments (Fig. 1a). Overall, there was a significant difference (P<0.05) between the treatments in the first year, but no statistically significant difference was detected in the second year $F_{(4,75)} = 0.09$; $P>0.05$; partial $\eta^2 = 0.005$, however, it can be seen that for all treatments the values were higher on average compared to the previous year (Fig. 1b). FDA is a good indicator of soil biological activity because it is hydrolyzed by many different enzymes, such as proteases, lipases, and esterases (Hernández et al., 2020). The studies of Aseri and Tarafdar (2006) also confirmed that the FDA enzyme is a particularly good indicator for soils with low organic matter content and arid areas. In our case, in the first year, it was confirmed that the biological activity was lower in those treatments that did not receive organic matter or had a low organic matter content (due to the absence of the rhizosphere of the cover plants). In the treatments where we modeled conventional practice and used continuous cultivation, the sparser rhizosphere of the pre-crop (radishes) did not have a significant effect in the first year, in contrast to the cover crop treatments, which produced a particularly large rhizosphere and aboveground biomass. The exception is the dM treatment, which showed a significant difference from the F treatment, similar to the cover crop treatments. Here, the excess organic matter affected the activity. By the second year, the biological activity of the soil showed a significant increase in all treatments due to the increased content of organic matter. The significant differences between the treatments disappeared, and we showed an equalization between the treatments. Sandy soils with low organic matter content are generally characterized by lower activity (Acosta-Martínez et al., 2007; Iovieno et al., 2009). At the same time, it can be observed that the treatments rich in rhizosphere (cover crop and cover crop + *Trichoderma*) had significantly high biological activity. This is due to the rhizosphere effect (Dotaniya and Meena 2015), as the root system of the cover plants contributed to improving the soil by increasing the organic matter content, as well as providing a habitat for microbes (Nair and Ngouajio, 2012; Finney et al., 2017).

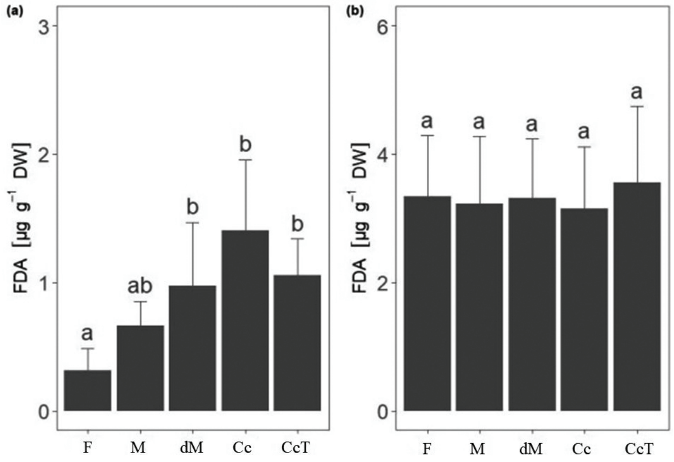

Fig. 1. Effects of treatments on FDA enzyme activity in two years a) 2022, b) 2023. The notations in the figure are F: fertilizer, M: manure, dM: double dose manure, Cc: cover crop, CcT: cover crop + *Trichoderma sp*. The results of multiple comparisons of the MANOVA model are indicated by the letters above the graph. Statistical significance was determined by post-hoc test, where *'$P<0.05$ ** $P<0.01$ ***' $P<0.001$./ Tukey *post-hoc* test

In the case of phosphatase activity, a statistically significant difference between the treatments was detected in both the first ($F_{(4,35)} = 55.13$; $P<0.001$; partial $\eta^2=0.86$), and the second ($F_{(4,114)} = 5.073$; $P<0.01$; partial $\eta^2=0.15$) year. The results of the Duncan post hoc test are shown in Fig. 2ab. The value of the partial η2 indicates that the treatments affected the phosphatase enzyme 86% in the first year, while in the second year, only 15%. The production of the phosphatase enzyme starts in the soil when the amount of phosphorus decreases or when a source of phosphorus that is difficult to absorb is present (Allison et al., 2007). However, at the same time, the high phosphorus level inhibits the enzyme's functioning (Spohn et al., 2015). In our case, in the first year (Fig. 2a), we showed a significant difference between M, dM, CcT associated to the control (F) and Cc treatment. At the same time, CcT treatment had significantly higher activity than M and dM treatments. In the second year (Fig. 2b), the phosphatase activity of the F, M and dM treatments developed very similarly compared to the previous year. This similarity was possible in the M and dM treatments due to phosphorus found in different forms in the manure (Lemanowicz et al., 2014). However, the TT treatment activity decreased and significantly differed from the dMtreatment. During their experiment, Liu et al. (2020) established that *Trichoderma harzianum* positively affected phosphatase activity. That is why, in the first year, the phenomenon could have been that although phosphorus was present in sufficient quantity in the soil, the plants needed more phosphorus in the CcT treatment. The activity of the Cc treatment developed similarly to the CcT treatment in the second year. Furthermore, it can be observed that the phosphatase activity of the F treatment was lower than the activity of the other treatments in both years. It is essential to consider during the treatments that lower

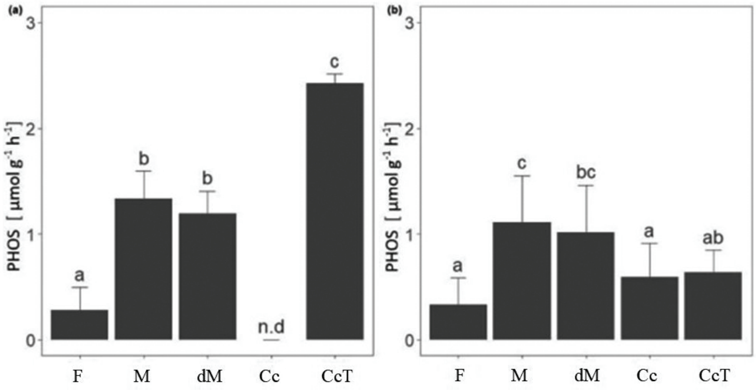

Fig. 2. Effect of treatments on phosphatase enzyme activity in two years a) 2022, b) 2023. The notations in the figure are F: fertilizer, M: manure, dM: double dose manure, Cc: cover crop, CcT: cover crop + *Trichoderma sp*. The results of multiple comparisons of the MANOVA model are indicated by the letters above the graph. Statistical significance was determined by post-hoc test, where *'$P<0.05$ ** $P<0.01$ ***' $P<0.001$./ Tukey *post-hoc* test

activity is typical on more calcareous soil, as acid phosphatase enzyme activity is negatively correlated with soil pH (Iyyemperumal and Shi, 2008).

3.2. Changes in soil permanganate oxidizable (POXC) carbon content

In the case of POXC, no statistically significant difference could be detected between the treatments in the first year ($F_{(4,25)} = 0.57$; $P>0.05$; partial $\eta^2 = 0.15$) (Fig. 3). At the same time, in the second year, there was a significant difference ($F_{(4,75)} = 3.53$; $P<0.01$; partial $\eta^2 = 0.15$) between the treatments. The results of the Duncan post hoc test between treatments are shown in Fig. 3. By the second year a significant increase in POXC values was observed for all treatments. The significant increase in POXC can be attributed to companion crops being used between the main plants in all treatments. In the case of the Cc and CcT treatments, the rhizosphere of the cover crops, while in the case of the F, M and dM treatments, the contribution of the rhizosphere of the intermediate plant widely used in conventional cultivation (in our case, radish) contributed to a significant increase in the amount of POXC in the soil. These results were supported by a similar increase in the biological activity of the soil for the second year (Fig. 1). What was even more striking in the case of labile carbon was that, for the second year, the F treatment showed significantly higher results compared to the dM, Cc, and CcT treatments. In the case of treatments with a higher root mass and organic matter, the quantity of soil microbes is more significant (Buyer et al., 2010; Nair and Ngouajio, 2012). As a result of the priming effect, these microbes can utilize the available and added organic matter more efficiently and quickly (Fontaine et al., 2003; Blagodatskaya and Kuzyakov, 2011). In contrast, this effect is not observed in the case of the F treatment, which contains a much smaller continuous root mass and low organic matter. In this case, the soil does not have the same level of microbial diversity as the other treatments. Although the overall biological activity is similar to the other treatments, presumably the amount of microbes adapted to continuous organic matter decomposition is lower than in soils with high organic matter content (Bending et al., 2002; Murphy et al., 2011).

3.3. Effect of treatments on plant nutrition and yields

Regarding the nitrate content of the soil, a significant difference could be detected between the treatments in both the first ($F_{(4,5)} = 2.05$; $P<0.05$; partial $\eta^2 = 0.62$) and the second ($F_{(4,5)} = 46.73$; $P<0.05$; partial $\eta^2 = 0.97$) year. The value of the partial η2 in the second year is 97%, while in the first year it is only 62%. In the first year, there was a significant difference in soil nitrate content between the control (F) and CcT treatments (Fig. 4). It was observed that the cover crop (Cc) treatment resulted in a higher nitrate content. In the first year, despite the differences in dosage between the M and dM treatments, there was no difference in the NO_3 content. This suggests that the extra amount of organic fertilizer that was applied as a surplus dM treatment soil did not change the leaching rate of nitrate from the soil of the other non-cover plant treatments. Our results also show that the intermediate plant (radishes) used in conventional cultivation

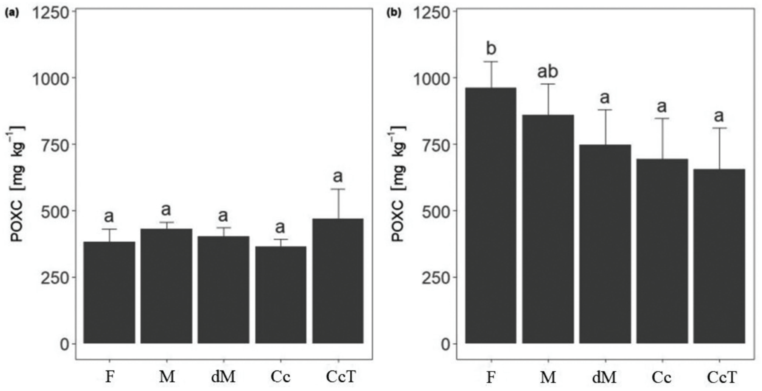

Fig. 3. Effect of treatments on the oxidizable carbon content of permanganate in two years a) 2022, b) 2023. The notations in the figure are F: fertilizer, M: manure, dM: double dose manure, Cc: cover crop, CcT: cover crop + *Trichoderma sp*. The results of multiple comparisons of the MANOVA model are indicated by the letters above the graph. Statistical significance was determined by post-hoc test, where *'$P<0.05$ ** $P<0.01$ ***' $P<0.001$./ Tukey *post-hoc* test

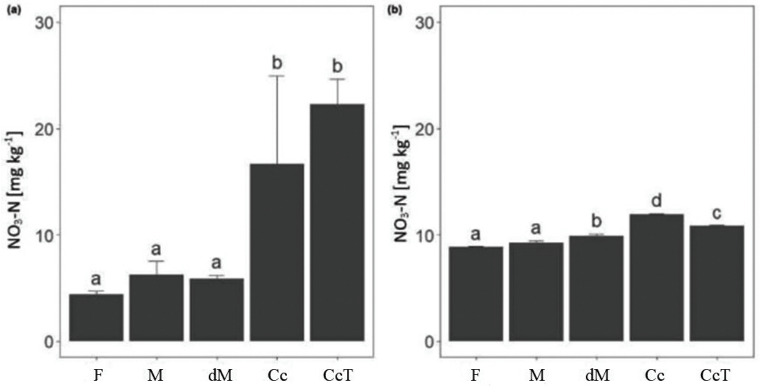

Fig. 4. Effect of treatments on NO3-N in two years a) 2022, b) 2023. The notations in the figure are F: fertilizer, M: manure, dM: double dose manure, Cc: cover crop, CcT: cover crop + *Trichoderma sp*. The results of multiple comparisons of the MANOVA model are indicated by the letters above the graph. Statistical significance was determined by post-hoc test, where *'$P<0.05$ ** $P<0.01$ ***' $P<0.001$./ Tukey *post-hoc* test

practices could not influence nitrate leaching. At the same time, the large root mass of the continuously and purposefully applied cover crops contributed to the binding of nitrate in the soil (near the rhizosphere) by the plants taking up and incorporating the nitrate into their own bodies. After terminating the cover crops, this returned to the soil and increased its nitrate content. The two cover crop treatments (Cc and CcT) significantly differed from the F, M and dM treatments in the second year. In addition, a significant difference was also detected between the Cc and CcT treatments. Gabriel et al. (2012) found in their 3.5-year experiment that the cover crop plants helped reduce NO_3 leaching. This explains why the nitrate content in the soil was higher in the cover crop treatments.

We examined the effects of the treatments on the crop yield and the vintage effect as a separate factor and their interaction. Neither the treatments ($F_{(4,30)} = 2.77$; $P>0.05$; partial $\eta^2 = 0.27$) nor the vintage as a factor had a significant effect on the yield ($F_{(1,30)} = 7.15$; $P>0.05$; partial $\eta^2 = 0.19$). The interaction effect was not significant either ($F_{(4,30)} = 1.40$; $P>0.05$; partial $\eta^2 = 0.15$) (Fig. 5). The effect of the different treatments on the soil parameters, such as soil FDA changes in organic POXC, PHOS and NO_3, can be evaluated partly through the partial η^2 values (Fig. 6). The data show that in the year 2022, the FDA value contributed significantly to the change in soil parameters with a partial η^2 value of 0.35. In the same year, the POXC and NO_3 parameters showed a moderate contribution (with values of 0.105 and 0.62, respectively),

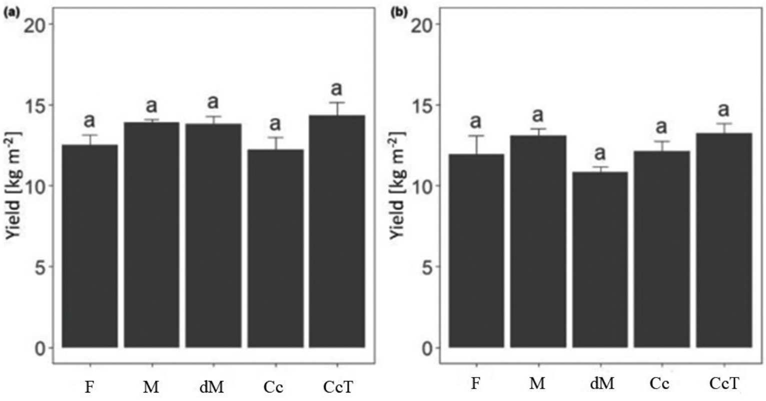

Fig. 5. Effect of treatments on yields in two years a) 2022, b) 2023. The notations in the figure are F: fertilizer, M: manure, dM: double dose manure, Cc: cover crop, CcT: cover crop + *Trichoderma sp*. The results of multiple comparisons of the MANOVA model are indicated by the letters above the graph. Statistical significance was determined by post-hoc test, where *'$P<0.05$ ** $P<0.01$ ***' $P<0.001$./ Tukey *post-hoc* test

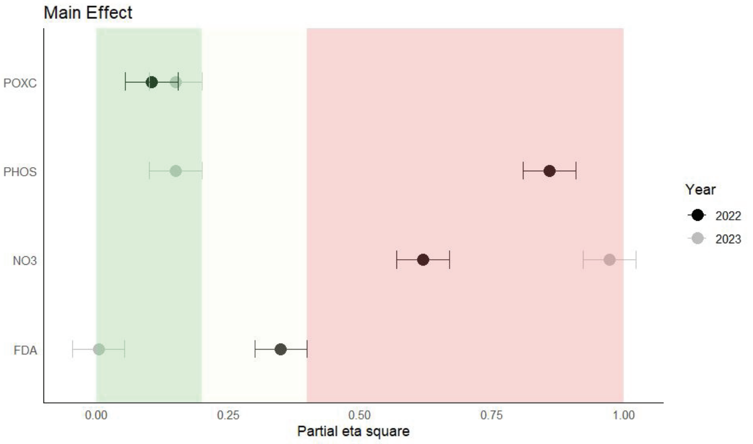

Fig. 6. Effect of treatments on partial η2 of dependent variables, with 90% confidence interval. Partial η2 values are classified and presented in green (low effect size), medium yellow (medium effect size) and red (high effect size)

while the PHOS parameter provided the largest contribution (with a value of 0.86). In the year 2023, the following trend was observed: FDA partial η^2 decreased drastically (0.004), which may mean that the role of FDA activity in the change of geological parameters is negligible in this year. On the other hand, the contribution of the POXC and PHOS parameters increased (with values of 0.15 and 0.15, respectively), although these values can still be considered moderate. The contribution of the NO_3 parameter increased to the highest, with a value of 0.973. This has a substantial effect and may indicate that the nitrogen content has become an important factor in the soil parameters studied this year. These results suggest that the importance of individual soil parameters may change over time and that changes in treatments or environmental conditions may affect soil quality characteristics in different ways. When analyzing the data, it is necessary to take into account the interactions between different parameters, as well as environmental and weather changes, which can also affect the change in geological parameters.

4. Conclusions

The use of cover crops is a sustainable practice for improving the biological and chemical parameters of the soil, thereby contributing to making water management more efficient. Numerous previous studies have confirmed this, but its use in cultivation tools is not yet widespread. Since organic manure of animal origin is available in minimal quantities, alternative solutions are needed that improve, or at least maintain, the stock of organic matter and biological activity of the soil, thus its good physical and chemical properties. This is especially true for sandy soils. From our results, we found that using cover crops can be an effective method for increasing the active carbon content of the soil, which microbes can use.

Furthermore, with the diversity of its root system, the well-chosen cover plant mixture provides a vast, rich surface and a habitat for soil microbes, increasing biological activity. In addition to creating a living space, the rhizosphere, the scene of important metabolic processes, contributes significantly to increasing the soil's organic matter stock. Special attention must be paid to retaining nitrogen nutrients and preventing leaching on sandy soils and growing equipment. With our two-year results, we have already proved the important role of cover crops in nutrient retention, thereby contributing to the practice of more sustainable fertilizer application. There were no differences between the treatments regarding yield, but their contribution as mentioned above to sustainable horticultural/agricultural practice is noteworthy. Based on our results, we could not detect a significant difference between the cover crop + *Trichoderma* treatment and the cover crop treatment. However, we found that when using cover crops, the *Trichoderma* treatment had a positive effect and significantly differed from the other treatments for most parameters. Another research direction is the reduction of pathogenic and plant pathogenic organisms, which are also a significant problem in greenhouse cultivation practices, where soil replacement cannot be solved. This is especially true for the reduced or complete absence of chemical use. In this case, an additional goal is to carry out studies in which it is established what effect the use of "transitional" plants of different types and properties between cultivated plant cultures has in reducing soil pathogens, contributing to sustainable horticultural cultivation.

Acknowledgments

This work was supported by the Collegium Talentum Programme of Hungary (E.P); The project was supported by Scientific Council of University of Nyíregyháza (I.F.). The research was supported by the MATE Research Excellence Program 2024 (Grant number: MATE-K/1011-32/2024) (Zs.K.)

References

Acosta-Martínez, V., Cruz, L., Sotomayor-Ramírez, D., Pérez-Alegría, L., 2007. Enzyme Activities as Affected by Soil Properties and Land Use in a Tropical Watershed. Applied Soil Ecology 35(1), 35–45. https://doi.org/10.1016/j.apsoil.2006.05.012

Allison, V.J., Condron, L.M., Peltzer, D.A., Richardson, S.J. Turner, B.L., 2007. Changes in Enzyme Activities and Soil Microbial Community Composition along Carbon and Nutrient Gradients at the Franz Josef Chronosequence, New Zealand. Soil Biology and Biochemistry 39(7), 1770–1781. https://doi.org/10.1016/j.soilbio.2007.02.006

Aseri, G.K., Tarafdar, J.C., 2006. Fluorescein Diacetate: A Potential Biological Indicator for Arid Soils. Arid Land Research and Management 20(2), 87–99. https://doi.org/10.1080/15324980500544473

Bending, D.G., Turner K.M., Jones, E.J., 2002. Interactions between Crop Residue and Soil Organic Matter Quality and the Functional Diversity of Soil Microbial Communities. Soil Biology and Biochemistry 34(8), 1073–1082. https://doi.org/10.1016/S0038-0717(02)00040-8

Berg, G., Smalla, K., 2009. Plant Species and Soil Type Cooperatively Shape the Structure and Function of Microbial Communities in the Rhizosphere. FEMS Microbiology Ecology 68(1): 1–13. https://doi.org/10.1111/j.1574-6941.2009.00654.x

Berry, S.D., Rhodes, R., Foster, J., Risede, J.M., van Antwerpen, R., 2011. The Effect of Cover Crops on Plant Parasitic-Nematodes of Sugarcane. International Journal of Pest Management 57(4), 363–375. https://doi.org/10.1080/09670874.2011.621984

Biró, B., Köves-Péchy, K., Vörös, I., Takács, T., Eggenberger, P., Strasser, R.J., 2000. Interrelations between Azospirillum and Rhizobium Nitrogen-Fixers and Arbuscular Mycorrhizal Fungi in the Rhizosphere of Alfalfa in Sterile, AMF-Free or Normal Soil Conditions. Applied Soil Ecology 15(2), 159–168. https://doi.org/10.1016/S0929-1393(00)00092-5

Blagodatskaya, E., Kuzyakov, Y., 2011. Priming Effects in Relation to Soil Conditions-Mechanisms. In: Gliński, J., Horabik, J., Lipiec, J. (eds) Encyclopedia of Agrophysics. Encyclopedia of Earth Sciences Series. Springer, Dordrecht. https://doi.org/10.1007/978-90-481-3585-1_128

Bongiorno, G., Bünemann K.E., Oguejiofor, C.U., Meier, J., Gort, G., Comans, R., Mäder, P., Brussaard, L., de Goede, R., 2019. Sensitivity of Labile Carbon Fractions to Tillage and Organic Matter Management and Their Potential as Comprehensive Soil Quality Indicators across Pedoclimatic Conditions in Europe. Ecological Indicators 99, 38–50. https://doi.org/10.1016/j.ecolind.2018.12.008

Buyer, J.S., Teasdale, R.J., Roberts, P.J., Zasada, A.I., Maul, E.J., 2010. Factors Affecting Soil Microbial Community Structure in Tomato Cropping Systems. Soil Biology and Biochemistry 42(5), 831–841. https://doi.org/10.1016/j.soilbio.2010.01.020

Cataldo, D.A., Maroon, M., Schrader, L.E. Youngs, V.L., 1975. Rapid Colorimetric Determination of Nitrate in Plant Tissue by Nitration of Salicylic Acid. Communications in Soil Science and Plant Analysis 6(1), 71–80. https://doi.org/10.1080/00103627509366547

Dabney, S.M., Delgado, J.A., Meisinger, J.J., Schomberg, H.H., Liebig, M.A., Kaspar, T., Mitchell, J., Reeves, W., 2010. Using Cover Crops and Cropping Systems for Nitrogen Management. Advances in Nitrogen Management for Water Quality, 231–282.

Dotaniya, M.L., Meena, V.D., 2015. Rhizosphere Effect on Nutrient Availability in Soil and Its Uptake by Plants: A Review. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 85(1), 1–12. https://doi.org/10.1007/s40011-013-0297-0

Dudas, A., Kotroczo, Zs., Videki, E., Wass-Matics, H., Kocsis, T., Szalai, M.Z., Vegvari, Gy., Biro, B., 2017. Fruit Quality of Tomato Affected by Single and Combined Bioeffectors in Organically System.' https://www.cabidigitallibrary.org/doi/full/10.5555/20183066532

El-Katatny, M.H., 2010. Enzyme Production and Nitrogen Fixation by Free, Immobilized and Coimmobilized Inoculants of Trichoderma Harzianum and Azospirillum Brasilense and Their Possible Role in Growth Promotion of Tomato.

Finney, D.M., Buyer, J.S., Kaye, J.P., 2017. Living Cover Crops Have Immediate Impacts on Soil Microbial Community Structure and Function. Journal of Soil and Water Conservation 72(4), 361–373. https://doi.org/10.2489/jswc.72.4.361

Fontaine, S., Mariotti, A., Abbadie, L., 2003. The Priming Effect of Organic Matter: A Question of Microbial Competition? Soil Biology and Biochemistry 35(6), 837–843. https://doi.org/10.1016/S0038-0717(03)00123-8

Gabriel, J.L., Muńoz-Carpena, R., Quemada, M., 2012. The Role of Cover Crops in Irrigated Systems: Water Balance, Nitrate Leaching and Soil Mineral Nitrogen Accumulation. Agriculture, Ecosystems & Environment 155, 50–61. https://doi.org/10.1016/j.agee.2012.03.021

Gurmu, G., 2020. Soil Organic Matter and Its Role in Soil Health and Crop Productivity Improvement. https://doi.org/10.14662/ARJASR2019.147

Hernández, I.Z., Natera, J.F.Z., López, P.M.G., Fuentes, E.R., Tapia M.N.T., 2020. Biological Activity in Soils Treated with Green Manures of Lupinus Spp.(Leguminosae) Using the Hydrolysis of Fluorescein Diacetate Method (FDA) in Jalisco, Mexico. Horticulture International Journal 4(5), 203–206.

Iovieno, P., Morra, L., Leone, A., Pagano, L., Alfani, A., 2009. Effect of Organic and Mineral Fertilizers on Soil Respiration and Enzyme Activities of Two Mediterranean Horticultural Soils. Biology and Fertility of Soils 45(5), 555–561. https://doi.org/10.1007/s00374-009-0365-z

Iyyemperumal, K., Shi, W., 2008. Soil Enzyme Activities in Two Forage Systems Following Application of Different Rates of Swine Lagoon Effluent or Ammonium Nitrate. Applied Soil Ecology 38(2), 128–136. https://doi.org/10.1016/j.apsoil.2007.10.001

Juhos, K., Czigány, Sz., Madarász, B. and Ladányi, M., 2019. Interpretation of Soil Quality Indicators for Land Suitability Assessment–A Multivariate Approach for Central European Arable Soils. Ecological Indicators 99, 261–272.

Kocsis, T., Kotroczó, Zs., Kardos, L., Biró, B., 2020. Optimization of Increasing Biochar Doses with Soil–Plant–Microbial Functioning and Nutrient Uptake of Maize. Environmental Technology & Innovation 20, 101191. https://doi.org/10.1016/j.eti.2020.101191

Koudahe, K., Allen, S.C., Djaman, K., 2022. Critical Review of the Impact of Cover Crops on Soil Properties. International Soil and Water Conservation Research 10(3), 343–354. https://doi.org/10.1016/j.iswcr.2022.03.003

Kovács, B., Andreolli, M., Lampis, S., Biró, B. Kotroczó, Zs., 2024. Bacterial Community Structure Responds to Soil Management in the Rhizosphere of Vine Grape Vineyards. Biology-Basel 13(4), 254. https://doi.org/10.3390/biology13040254

Kovács, F., Papdi, E., Veres, A., Mohay, P., Szegő, A., Juhos, K., 2024. More Efficient Nitrogen Utilization through Wool Pellet and Soil Inoculation. Agrosystems, Geosciences & Environment 7(2), e20534. https://doi.org/10.1002/agg2.20534

Lemanowicz, J., Siwik-Ziomek, A., Koper, J., 2014. How Fertilization with Farmyard Manure and Nitrogen Affects Available Phosphorus Content and Phosphatase Activity in Soil. Polish Journal of Environmental Studies 23(4), 1211–1217.

Liu, X., Wang, S., Deng, X., Zhang, Z., Yin, L. 2020. Comprehensive Evaluation of Physiological Traits under Nitrogen Stress and Participation of Linolenic Acid in Nitrogen-Deficiency Response in Wheat Seedlings. BMC Plant Biology 20, 1–16.

Margenot, A.J., Hodson, A.K., 2016. Relationships between Labile Soil Organic Matter and Nematode Communities in a California Oak Woodland. Nematology 18(10), 1231–1245. https://doi.org/10.1163/15685411-00003027

Margenot, A.J., Pulleman, M.M., Sommer, R., Paul, B.K., Parikh, S.J., Jackson, L.E., Fonte, S.J., 2017. Biochemical Proxies Indicate Differences in Soil C Cycling Induced by Long-Term Tillage and Residue Management in a Tropical Agroecosystem. Plant and Soil 420(1), 315–329. https://doi.org/10.1007/s11104-017-3401-z

Marschner, P., Crowley, D., Yang, C.H., 2004. Development of Specific Rhizosphere Bacterial Communities in Relation to Plant Species, Nutrition and Soil Type. Plant and Soil 261(1), 199–208. https://doi.org/10.1023/B:PLSO.0000035569.80747.c5

Murphy, D.V., Cookson, W.R., Braimbridge, M., Marschner, P., Jones, D.L., Stockdale, E.A., Abbott, L.K., 2011. Relationships between Soil Organic Matter and the Soil Microbial Biomass (Size, Functional Diversity, and Community Structure) in Crop and Pasture Systems in a Semi-Arid Environment. Soil Research 49(7), 582–594. https://doi.org/10.1071/SR11203

Nair, A., Ngouajio, M., 2012. Soil Microbial Biomass, Functional Microbial Diversity, and Nematode Community Structure as Affected by Cover Crops and Compost in an Organic Vegetable Production System. Applied Soil Ecology 58, 45–55. https://doi.org/10.1016/j.apsoil.2012.03.008

Norris, C.E., Congreves, K.A., 2018. Alternative Management Practices Improve Soil Health Indices in Intensive Vegetable Cropping Systems: A Review. Frontiers in Environmental Science 6. https://doi.org/10.3389/fenvs.2018.00050

Pang, G., Cai, F., Li, R., Zhao, Z., Li, R., Gu, X., Shen, Q., Chen, W., 2017. Trichoderma-Enriched Organic Fertilizer Can Mitigate Microbiome Degeneration of Monocropped Soil to Maintain Better Plant Growth. Plant and Soil 416, 181–192.

Pulleman, M., Wills, S., Creamer, R., Dick, R., Ferguson, R., Hooper, D., Williams, C., Margenot, A.J., 2021. Soil Mass and Grind Size Used for Sample Homogenization Strongly Affect Permanganate-Oxidizable Carbon (POXC) Values, with Implications for Its Use as a National Soil Health Indicator. Geoderma 383, 114742. https://doi.org/10.1016/j.geoderma.2020.114742

Rayne, N., Aula, L., 2020. Livestock Manure and the Impacts on Soil Health: A Review. Soil Systems 4(4), 64. https://doi.org/10.3390/soilsystems4040064

Reinhold-Hurek, B., Bünger, W., Burbano, C.S., Sabale, M., Hurek, T., 2015. Roots Shaping Their Microbiome: Global Hotspots for Microbial Activity. Annual Review of Phytopathology 53, 403–424.

Rosenberg, E., Zilber-Rosenberg, I., 2016. Microbes Drive Evolution of Animals and Plants: The Hologenome Concept. MBio 7(2), e013 95-15.

Sharma, P., Singh, A., Kahlon, C.S., Brar, A.S., Grover, K.K., Dia, M., Steiner, R.L., 2018. The Role of Cover Crops towards Sustainable Soil Health and Agriculture–A Review Paper. American Journal of Plant Sciences 9(9), 1935–1951. https://doi.org/10.4236/ajps.2018.99140

Shi, S., Nuccio, E.E., Shi, Z.J., He, Z., Zhou, J., Firestone, M.K., 2016. The Interconnected Rhizosphere: High Network Complexity Dominates Rhizosphere Assemblages. Ecology Letters 19(8), 926–936

Singh, Narayan, B., Dwivedi, P., 2022. Trichoderma-Induced Promotion of Nitrogen Use Efficiency Is Mediated by Nitric Oxide Generation Leading to Improved Growth and Yield in Pea (Pisum Sativum L.) Plants. Journal of Plant Growth Regulation 42, 6397–6412. https://doi.org/10.1007/s00344-022-10861-5

Singhal, V., Ghosh, J., Jinger, D., 2020. Cover Crop Technology – a Way towards Conservation Agriculture: A Review. The Indian Journal of Agricultural Sciences 90(12), 2275–2284. https://doi.org/10.56093/ijas.v90i12.110310

Sinsabaugh, R.L., Klug, M.J., Yeager, P.E., 1999. Characterizing Soil Microbial. Standard Soil Methods for Long-Term Ecological Research, 318.

Spohn, M., Treichel, N.S., Cormann, M., Schloter, M., Fischer, D., 2015. Distribution of Phosphatase Activity and Various Bacterial Phyla in the Rhizosphere of Hordeum Vulgare L. Depending on P Availability. Soil Biology and Biochemistry 89, 44–51. https://doi.org/10.1016/j.soilbio.2015.06.018

Varma, A., Oelmüller, R., 2007. Advanced Techniques in Soil Microbiology. Vol. 11. Springer Science & Business Media.

Viti, C., Tatti, E., Decorosi, F., Lista, E., Rea, E., Tullio, M., Sparvoli, E., Giovannetti, L., 2010. Compost Effect on Plant Growth-Promoting Rhizobacteria and Mycorrhizal Fungi Population in Maize Cultivations. Compost Science & Utilization 18(4), 273–281. https://doi.org/10.1080/1065657X.2010.10736966

Vivas, A., Barea, J.M., Biró, B., Azcón, R., 2006. Effectiveness of Autochthonous Bacterium and Mycorrhizal Fungus on Trifolium Growth, Symbiotic Development and Soil Enzymatic Activities in Zn Contaminated Soil. Journal of Applied Microbiology 100(3), 587–598.

Weil, R.R., Islam, K.R., Stine, M.A., Gruver, J.B., Samson-Liebig, S.E., 2003. Estimating Active Carbon for Soil Quality Assessment: A Simplified Method for Laboratory and Field Use. American Journal of Alternative Agriculture 18(1), 3–17. https://doi.org/10.1079/AJAA200228

Wu, L., Jiang, Y., Zhao, F., He, X., Liu, H. and Yu, K., 2020. Increased Organic Fertilizer Application and Reduced Chemical Fertilizer Application Affect the Soil Properties and Bacterial Communities of Grape Rhizosphere Soil. Scientific Reports 10(1), 1–10.

Zobiole, L.H.S., Kremer, R.J., Oliveira Jr, R.S., Constantin, J., 2011. Glyphosate Affects Micro-Organisms in Rhizospheres of Glyphosate-Resistant Soybeans. Journal of Applied Microbiology 110(1), 118–127.