Effects of sustainable agricultural practices on functional diversity of soil microbial community in the context of global climate change

SUMMARY OF Ph.D. THESIS

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INTRODUCTION

1. The structure of the PhD thesis

The PhD thesis comprises a total of 129 pages and has been structured in two parts, Current State of Knowledge and Personal Contribution. The current state of knowledge spans 41 pages and has been divided into two chapters presenting updated knowledge on the effects of sustainable agricultural practices on the functional diversity of the soil microbial community in the context of global climate change. The second part of the thesis totals 88 pages, being divided into five distinct chapters that present the purpose and objectives of the work, the materials and methods used, the results of the doctoral thesis and discussions based on them, the conclusions and recommendations, and last but not least, the originality and its innovative contributions.

The thesis includes a total of 20 figures, 42 tables and 140 bibliographic titles.

2. Aim and objectives of the research

2.1. The aim of the research

The phD thesis entitled "Effects of sustainable agricultural practices on the functional diversity of the soil microbial community in the context of global climate change" aims to investigate the effects that tillage systems, as well as specific habitat conditions, can have on diversity functions of the soil microbial community and the processes mediated by the microbial community.

At the same time, the possibility of determining soil microbial biomass and soil bacteria : fungi ratio by a new and less expensive method will be investigated. This new approach will allow the evaluation of many samples in a short period.

2.2. Research objectives

To achieve the proposed goal, in the framework of the doctoral thesis we proposed a series of specific objectives:

1. **Characterization of the quality of the soils studied based on physical, chemical, and biological characteristics and the description of the soil work systems at the established points.**
2. **Evaluation of the influence of the sampling depth on the biological and physico-chemical characteristics of the soil in the conventional work system and the minimum work system.**
3. **Determining the effects of the conventional tillage system (CT) and the soil's minimum tillage system (MT) on the metabolic activity and the**

functional diversity of the microbial community in arable lands in Transylvania, in the context of global climate change.

4. **Analysis of the possibility of improving and using the MicroResp method for determining the microbial biomass and the ratio of bacteria: fungi in the soil.**

3. Material and method

3.1. Experimental design

To achieve the proposed objectives, 8 work points were established, located in Cluj, Mureș, Sălaj, and Satu Mare counties. The sampling points were selected in fields cultivated with winter wheat. Two sampling points were established at each location, differentiated by tillage type. In the conventional tillage system, the seedbed was prepared by plowing to 25 cm, followed by harrowing or tilling with an agricultural cultivator, before sowing. In the minimum work system, soil mobilization was done only superficially, using the chisel plow or rotary harrow. In the case of the MT system, the furrow was not turned, and the organic residues from the previous crop remained on the soil surface.

Since at one of the locations (Meseșeni, Sălaj county), it was not possible to identify a sampling point characteristic of the MT tillage system, the samples were taken only from the CT system. In this way, 15 sampling points were established in the central-northern area of Transylvania, with a temperate-continental climate that ensures an average annual precipitation of around 700 mm and an average annual temperature of 9°C.

At each sampling point thus established, soil samples were collected to determine the physical-chemical and biological parameters evaluated.

Sampling was carried out in the spring season, in April and May, of 2018, under suitable climatic conditions.

3.2. Soil sampling methodology

To assess the functional diversity of the soil microbial community, samples were collected at three depths (0 - 10 cm, 10 - 20 cm, and 20 - 30 cm) using a soil sampling probe. For sampling, a square with a side of 5 m was described in the selected wheat lots, in the corners of which the soil sample was collected from the established depths.

3.3. Analysis of the physico-chemical properties of the soil samples

Soil texture

The quantitative determination of the proportion of different granulometric fractions in the soil requires the completion of two main stages: the treatment of soil samples and the separation of granulometric fractions, analysis carried out in the Pedology Laboratory of the Faculty of Agriculture.

Apparent density (volumetric mass) of the soil

The determination of the volumetric weight of the dry soil, in a natural setting, which follows the volume occupied by the solid particles, but also the porous space between them, was made from samples collected with metal cylinders of known volume.

Soil acidity (pH)

The acidity of the soil, determined by the totality of H^+ ions that circulate freely in the soil solution, was measured using a pH meter in a soil suspension prepared in a ratio of 1 : 2, 5 mass/volume, using ultrapure water as an extractant.

Organic carbon and total nitrogen in the soil

The determination of organic carbon and total nitrogen in the soil was carried out by the dry combustion method, measuring the amount of carbon and nitrogen released during the combustion of a sample.

The analyses were carried out in the laboratories of the Johann Heinrich von Thünen Institute in Braunschweig, Germany.

Phosphorus and potassium in the soil

The determination of phosphorus and potassium in the soil was carried out by the Egner-Riehm method at the Soil Analysis Laboratory in Gotingen, Germany.

3.4. Analysis of soil microbial biomass and functional diversity

MicroResp – the soil microbial community analysis method

The evaluation of the functional diversity of the microbial community in the studied sites was carried out by the Microresp method (CAMPBELL et al., 2003). The method was developed as an alternative that combines the advantages of the Biolog technique, using the microplate system, and the advantage of the SIR method to measure CO2 production during short incubation periods (SASSI et al., 2012).

MicroResp is a colorimetric method based on the principle of respiration of the microbial community as a result of the metabolism of different carbon sources in a 96-well microplate system. 15 substrates are recommended to be used, but their number may vary depending on the objectives of the study. This method provides an immediate response to these substrates and reflects microbial activity rather than numerical growth as a result of measuring responses within the first 4 - 6 hours. The reading of the samples is carried out using a multipoint spectrophotometer at the wavelength of 570 nm before and after the 6 hours of incubation of the analyzed soil.

Determination of microbial biomass and soil bacteria : fungi ratio by adapting the SIR method to the MicroResp system

The potential of the MicroResp method for microbial community profiling in soil ecology studies is well known and accepted, as well as its considerable contribution in

providing relevant data for soil microbiological activity. However, this potential is not sufficiently exploited, which is why testing the possibility of using this method in order to determine the microbial biomass of the soil and the bacterial biomass:fungal biomass ratio in agricultural soils, was the objective of this thesis.

To achieve this, the following activities were carried out:

1. Testing glucose as a nutrient substrate, so that it can be used in the determination of soil microbial biomass using the MicroResp kit.
2. Use of antibiotic and antifungal substances to estimate soil bacterial and fungal biomass and bacteria:fungi ratio in arable agricultural soils, following the methodology used in the SIR method.

4. Results and discussions

4.1. Results and discussions regarding the physico-chemical properties of the studied soils

The soils studied in the doctoral thesis presented important differences in morphological, physical and chemical characteristics, as well as in terms of their fertility and productive potential. The dominant soils in the studied area are cernisols and luvisols, in the network of farms studied, three types of soils have been identified: faeozom, preluvosol and luvosol. Phaeozoic soil was characteristic for six farms, luvosol for two farms, and preluvosol was described for only one location.

The physico-chemical characterization of the soils from the studied farms revealed, for most locations, a clay-clay or clay-clay texture, with high values of the clay content in most cases. Since the texture significantly affects the aero-hydric regime of the soil and that the presence of clay in the proportion of 40 – 50 % is associated with quality soils, we can consider the studied soils as soils in which the limiting effect of the texture on the biological processes in the soil is reduced.

The apparent density determined at two depths (0 - 10 cm and 10 - 20 cm) had subunit values that varied between 0, 78 g / cm^3 and 0, 93 g / cm^3 at the surface, and at the depth of 10 - 20 cm, the values determined were between 0, 78 g / cm^3 and 0, 96 g / cm^3. Therefore, no significant differences were reported between the conventional tillage system and the minimum tillage system.

Regarding the chemical properties of the studied soils, it is highlighted that the values of organic carbon (Corg) in the soil were high in the vast majority of locations, ranging between 0, 94 % (Lipău) and 4, 18 % (Viișoara). High values of Corg in soil signal a high microbial biomass with intense metabolic activity, which can lead to a rapid process of mineralization of soil organic matter. No depth differences in organic carbon values were reported, even if in the conventional system a tendency of slightly

higher values can be observed at the depths of 10 - 20 cm and 20 - 30 cm compared to the depth of 0 - 10 cm.

Similar to organic carbon values, total nitrogen (Nt) values follow the same trends. In most locations, nitrogen supply is good, with values between 0, 08 % and 0, 35 %. The C:N ratio in the soil is an indicator of the quality of the soil organic matter and can be indicative of the microbial processes in the soil.

An extremely important parameter for the microbial activity in the soil, but also for the productivity of agricultural systems, is the soil reaction expressed in pH units. The results of the pH determinations of the soils taken in the study revealed a pH range between the minimum value of 4, 97 (Lipău - CT) and the maximum of 8, 20 (Turda - MT). Therefore, in the study carried out, they ranged from soils with a strongly acidic reaction to moderately alkaline soils, but most of the measured values were around the neutrality point. Regarding the depth, the pH values show a slight increase from the surface to the depth (20 - 30 cm). This trend is more visible in the minimum tillage system compared to the conventional tillage system.

4.2. Results and discussion regarding the effects of tillage systems (MT and CT) on the metabolic activity and functional diversity of the soil microbial community

Metabolic activity in the analyzed soils

The results obtained in this study highlighted the fact that the metabolic activity, expressed by the metabolic rates of the different substrates, was significantly influenced by all the factors considered: the type of management, the location, and the depth of sampling.

The pattern of use of the main groups of substrates was the same in the case of all analyzed variants, carboxylic acids having the highest metabolism rate, followed by carbohydrates, amino acids, and amino sugars. Within the same substrate group, utilization rates of different carbon sources varied by location, management type, and sampling depth.

Regarding the soil management variants, the pattern of use of the main groups of substrates was the same in both variants, with a more intense use recorded for carboxylic acids, followed by carbohydrates, amino acids, and amino sugars, but there were differences in the use of carbon sources from within the mentioned groups. Therefore, comparing the metabolism rates of different carbon substrates in the two tillage systems, it was found that the carbon sources with the highest metabolism rate were α-ketoglutaric acid, citric acid, malic acid, galactose, cysteine, acetylglucosamine.

Functional diversity of the soil microbial community

Using the metabolic rate of each substrate by the soil microbial community, expressed in CO_2 production, was used to calculate the catabolic diversity of the

microbial community using the Shannon - Weaver and Simpson indices. The ratio of the metabolic rates of each substrate to the total metabolic rate calculated as the sum of the amount of CO_2 produced by each substrate was used to calculate these indices. The calculated indices were used to compare the functional diversity of the microbial community in CT and MT soil management systems but also between different depths. The results obtained did not indicate significant differences between the considered variables, but there is a trend that confirms the results presented above.

4.3. Results and discussions regarding the possibility of improving and using the MicroResp method for the determination of microbial biomass and the bacteria : fungi ratio in soils

The MicroResp method can be used to evaluate the total microbial biomass of the soil, but also to estimate the bacteria:fungi ratio in the soil.

The results obtained during the incubation period highlighted the inhibitory activity of the substances used for all considered soils. The types of inhibitors chosen (gentamicin and cycloheximide) exerted inhibition of the target groups. Even though this inhibitory activity was not as expected, we believe that modification of the work protocol can improve this shortcoming. We can consider the concentrations of 4000 ppm in soil for gentamicin and 8000 ppm in soil for cycloheximide as values that can be used in further studies since no stronger inhibitory activity was determined when the concentration of the inhibitor was doubled.

The total microbial biomass recorded the highest values in the organo-mineral fertilized soil type, while the lowest values were recorded in the mineral soil. The ratio of bacteria : fungi in the soil, estimated with the help of the MicroResp method, reflects the dominance of fungi in the studied soil samples.

5. Conclusions and recommendations

The studies carried out within this doctoral thesis aimed at evaluating the effects of tillage systems on the metabolic activity and functional diversity of the microbial community in arable soils of the Transylvania region. The microbiological indicators used in this study were the microbial community physiological profile (CLPP), basal respiration, and microbial biomass.

The obtained results allow us to formulate the following **general conclusions**:

1. The soils selected for this study presented physical - chemical differences and were culturally important, which is why the results obtained show high variability. Among the physical-chemical parameters determined, we consider as relevant for microbiological activity: the clay content of the soil, the pH, and the quantity, respectively the quality of the organic matter in the soil. These aspects justify dividing

the studied soils into two categories, namely soils with high microbiological potential (Sănduleşti, Bădeni, Viişoara, Cheţani, Cojocna) and soils with lower microbiological potential (Lipău, Turda, Meseşeni).

2. Considering the fact that the conventional system is often replaced by that of minimum tillage to counteract the effects of global climate change, this has major influences on the soil microbial community. In our study, no farms were identified that practice the minimum tillage system continuously, but only for limited periods of time (2 - 3 years), after which plowing intervenes. This practice affects the results regarding the microbiological characteristics, because every 2 - 3 years the studied soils are returned to an initial state characteristic of the conventional tillage system.

3. The sampling depth did not reveal important changes in physical or chemical parameters of the soil at different depths, although soil pH, organic carbon, as well as total nitrogen show trends of change with depth.

4. The location of the soil sample significantly influences the rate of consumption of different organic substrates, without considering the type of management applied to the soil. Distinctive soil characteristics, such as pH, soil organic carbon, or nitrogen supply, can provide a physiological profile of the microbial community specific to each sampling point.

5. The tillage system did not significantly influence the total rate of metabolism of the carbon substrates used in the CLPP analysis. An intense microbiological activity was determined in both the conventional and the minimal tillage systems. A tendency to increase the metabolic activity is evident for the soils cultivated in the conventional system, which can be associated with the loosening and increase of the porosity of the soil in this type of system, as well as with the reduction of the structuring state of the soil aggregates in this case. As previously mentioned, these characteristics can also be present in the MT tillage system, in which plowing occurs at certain time intervals. Probably, for this reason, the differences between the CT and MT system are not statistically significant.

6. In the case of both management variants considered in this study, the model of the main groups of organic substrates used in the CLPP analysis was the same, with a high metabolism rate for carboxylic acids, followed by carbohydrates, amino acids, and amino sugars. The use of carboxylic acids by the microbial community is associated with the presence of species with r-type strategy, species that occur predominantly in soils without functional stability. The management of agricultural soils ensures a continuous disturbance of this type of habitat, which is why these species are associated with intensive metabolism of carboxylic acids in the CLPP analysis.

7. It could be seen that there are specific substrates that were used predominantly in the conventional tillage system, while other substrates were used more intensively in the minimum tillage system. The obtained data allow us to point

out the fact that, in the conventional tillage system, α-ketoglutaric acid, citric acid, N-acetylglucosamine, and galactose were more intensively metabolized, while malic acid and cysteine were predominantly consumed in the of minimal soil tillage.

8. Sampling depth significantly influenced the rate of metabolism of organic substrates, with the most intense metabolic activity being reported for the depth of 10 - 20 cm. The more pronounced presence of the plant root system as well as the distribution of soil organic matter at this level can explain this result.

9. The pattern of use of organic substrates at different depths was the same as and in the case of the type of management, carboxylic acids being the most intensively metabolized, followed by carbohydrates, amino acids, and amino sugars.

10. The MicroResp method has the potential to be used in biomass assessment of total soil microbes. The great advantage is that a considerable number of samples can be analyzed in a short time and at low cost. However, it is necessary to continue the process of adapting and improving the work protocol, which is why studies in this direction must be continued.

11. Estimation of the ratio of bacteria: fungi with MicroResp methods did not give the expected results. Although the inhibitory activity of gentamicin and cycloheximide was determined in the samples analyzed, the differences in the induced inhibitory activity were unclear and difficult to explain. We consider this result to be due to the small amount of soil used in this analysis.

Recommendation

The results obtained in this study, but also the challenges related to the biological diversity of soils justify the formulation of some recommendations for the research methodology in the field, but also for agronomic practice.

Therefore, **for microbiological research**, we propose the following:

1. The need to investigate microbiological parameters in agricultural soils to complete and improve the data on the structure and functions of the soil microbial community.

2. Creation of indices for evaluating biotic processes in soils, indices that allow monitoring the processes of decomposition and mineralization of organic matter in soils. The use of certain types of substrates for the evaluation of a certain biological process is of great interest for applied research.

3. Intensification of studies regarding the effects of some agricultural practices on soil microbial community and dissemination of relevant scientific results among farmers and other stakeholders.

4. Use of the MicroResp method for quantification of microbial biomass a soil to monitor soil health.

Based on the data obtained, **for agronomic practice**, we can recommend as follows:

1. Assisting farmers in making soil management decisions, to ensure the long-term preservation of the health of the soils. However, the advantages of soil biodiversity conservation must be correlated with economic or productivity benefits to show interest from farmers. Cost reduction in the minimum work system, correlated with the advantages of the correct management of organic residues to improve fertility can be a good example of this.

2. Adaptation of the soil works system to the local conditions specific to each area. In this sense, for the specific conditions of the studied area, the tillage systems must consider the metabolic potential of the microbial community to decompose and mineralize the organic matter in the soil or on the surface so that the release of nutrients can be done at the right time and with limited effects on soil carbon losses.

3. The implementation at the farm level of new agricultural practices, based on more advanced scientific knowledge in the field of technologies, aiming at the sustainable and sustainable conservation of soil fertility, in the context in which farmers must be aware that their economic interests in obtaining profitable productions must be harmonized with the requirements regarding the protection and preservation of the environment.

6. Originality and innovative contributions of the thesis

Tillage is the agricultural practice with the most significant impact on soil quality and soil fertility. This impact can be positive through beneficial effects on soil parameters, but also negative if we consider soil organic matter, soil structure, or soil nutrient loss. It is the reason why new tillage systems are adopted by farmers and evaluated from the perspective of agricultural productivity.

The presented PhD thesis approaches a different perspective and evaluates the effects of conventional (CT) and minimum tillage (MT) systems on the functional diversity of the soil microbial community. The importance of the metabolic activity of this community for ensuring soil functions and fertility in the case of agricultural soils is discussed.

The way of organizing the research is original, which focused on soils from farms and not from experimental lots, as well as a wide distribution of sampling points that ensured a high heterogeneity of the studied soils. Another aspect of originality concerns the study of the microbial community at different depths in the soil. The tillage system creates different habitat conditions at depth and thus influences the structure and metabolic activity of the microbial community. This aspect is reflected in soil quality parameters and can affect its fertility.

The microbiological indicators used in this study were the physiological profile of the microbial community, basal respiration, and soil microbial biomass. To study these parameters, only one method was used, the MicroResp method, whose working principle allows the evaluation of several microbiological indicators in a single analysis. The adaptation of the work protocol and the interpretation of the results in this sense is an innovative contribution to soil microbiology studies which, in our opinion, can open multiple research perspectives in this field.

Soil microbial biomass and the bacteria:fungi ratio in soil are two of the most commonly used indicators in soil quality monitoring. Determining these indicators, however, is difficult to achieve and often takes a long time. The method used in this work allows the estimation of microbial biomass for a large number of samples without too much financial effort or human resources. The adaptation of this method could ensure the monitoring of microbial biomass in agricultural soils and the use of this indicator in soil health assessment. In addition, the prospect of new research directions was opened that could allow the evaluation of the bacteria: fungi ratio in the studied soils. Even if the results obtained did not reach the level of our expectations, the use of other inhibitors of the evaluated groups or the increase in the amount of soil used in the analysis could allow the correct estimation of this ratio.

Sooner or later, soil microbial activity will be used in evaluating the potential fertility of soils, and future research in this direction will certainly bring important results in this regard. These results will be able to be used in the future, in particular, to reduce or stop the negative effects of anthropogenic impact on soils, a major objective of environmental policies around the world.

SELECTIVE REFERENCES

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