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Ph.D. Theses

Aggregate stability, humus and microbial biomass tests in a long-term fertilization experiment

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1. Introduction and objective

A key element of sustainable agricultural practice is to conserve, maintain and improve the quality of soil resources. Therefore, a large amount of research is being carried out worldwide to explore and identify soil properties, their interactions, and the various processes we can characterize soil functions. The ultimate goal of this research is to preserve, restore and maintain the fertility of our soils. It is well known that soil fertility is related to its biological state and activity, organic matter content, nutrient content, and structure, among many other factors. In this research, I have investigated some of these parameters and their relationships.

The effects of fertilization and different tillage and cultivation practices can be studied in many ways. However, the most valuable results for agricultural practice are those obtained from long-term fertilization and cultivation experiments, which make it easier to assess the causal relationships between the results.

As the most common tool in current agricultural practice, mineral fertilization has a reliable effect on increasing soil nutrients, thus increasing yields and, at the same time, having a positive effect on soil fertility. Barn manure have long been used in agriculture, but because their effects are less predictable and variable over time than mineral fertilizers, their use has been marginalized. The addition of barn manure can be an effective fertilization strategy to improve and maintain soil fertility, structure, and biodiversity.

The biological properties of soils are based on the physico-chemical state of the soil, and therefore, to improve soil quality, it is necessary to develop an optimal soil structure. Soil structure consists of organic and inorganic elementary particles and the structural elements formed by their interconnection, known as aggregates. Aggregates are essential for maintaining soil porosity and aeration, which are necessary for plant growth, microbial activity, and microbial respiration.

Soil organic matter is the engine of soil life and the various humic substances, which are modified and transformed by microorganisms from plant and animal residues that are deposited in the soil (BIRÓ, 2018). The quality of humus is mainly influenced by how fertilization is applied, the quantity and quality of organic matter in the soil, the type of cultivation, and the soil type.

Microorganisms play an essential role in soils' biological and chemical processes, and their activity causes measurable changes in soil structure and organic matter content. The microbial biomass is sensitive to cultivation and tillage practices and exhibits high seasonal dynamics and spatial variability. Enzyme activities measured in soils reflect microbial activity and can be used as an index of microbial functional diversity, including a wide range of metabolic processes.

Many studies investigate the above parameters but fewer examples of their collaborative study in a tank experiment. In addition to the correlations already established, there are many new questions, furthermore the locations of the studied areas have decisive influence on the data. Hopefully, the more data we have, the more detailed our understanding of soil processes, interactions, and geographical variability will become. With a better understanding of long-term impacts and relationships, sustainable agricultural practices can be developed.

In my research, I investigate whether organic amendments (manure; straw incorporation + green manure) and N rates used in addition to fertilization affect the physical/chemical and biological properties. I am also looking for correlations between the parameters studied.

Accordingly, I aimed to answer the following main questions:

- Do organic amendments and N rates affect aggregate stability, humus quality and quantity, and microbial biomass quantity and activity?
- Is there a correlation between aggregate stability and microbial biomass mass?
- Is there a correlation between the amount and activity of soil microbes?
- Does organic matter quality and quantity influence microbial biomass and aggregate stability?
- Is there a correlation between the estimated amount of organic carbon input and the measured parameters?
- Are the yields affected by the treatments applied, and can a relationship be detected with any of the parameters studied?

2. Materials and methods

My studies were carried out in the IOSDV (Internationale Organische Stickstoff-dauerdüngungsversuch, International Organic- and Nitrogen Fertilisation Experiment) experimental field, set up in 1983. The soil type of the site is a Ramann brown forest soil on sandy loess, classified as Eutric Cambisol according to the WRB classification; the physical composition is sandy loam. The soils of the experimental area are poor in humus and phosphorus and medium in potassium. At the start of the experiment, it was characterized by low organic carbon content (SOC ~ 1 %), medium ammonium lactate soluble K_2O content 140-160 mg kg^{-1}, and low P_2O_5 content 60-80 mg kg^{-1}, pHKCl: 6,8-7,0.

The crop sequence of the two-factor strip-tillage cereal rotation experiment, designated IOSDV, was maize - winter wheat - winter barley; the number of replicates: 3. Gross plot size: 48 m^2. The factors of the experiment included increasing N fertilizer rates and different organic fertilizers applied as amendments. In terms of fertilization, all experimental plots (including the N control) receive uniform basal fertilization of 100 kg ha^{-1} of P_2O_5 and K_2O active ingredients, while N is applied in 5 equally increasing rates (N0, N1, N2, N3, N4) depending on the crops in the rotation. The N rates are 0-70-140-210-280 kg ha^{-1}, for maize, 0-50-100-150-200 kg ha^{-1}, for wheat and 0-40-80-120-160 kg ha^{-1} for winter barley. In addition to the application of fertilizer (NPK) alone (control without organic fertilizer supplementation), fertiliser+plant fertilizer (NPK+IST) and fertiliser+stem residue+green manure (NPK+SZ+ZT) are included. In the manure treatments, manure is applied at 35 t ha^{-1} dose once per rotation (every three years) before maize. In the case of stem residue application, 10 kg N active ingredients are also added per hectare for every 1 t of stem residue. In the stem residue replenishment variants, once the second-seeded oil radish green manure crop (*Raphanus sativus* var. *Oleiformis*) sown in winter, barley stubble was plowed during the rotation.

Soil samples were collected from plots of this experiment between 2014 and 2016 and used to determine aggregate stability, humus quality (E4/E6), microbial biomass (MBC), and fluorescein diacetate (FDA) degrading enzyme activity. I also used total soil organic carbon (SOC) content and crop yields to evaluate the results and estimated the total organic carbon released to the soil.

Aggregate stability was carried out according to the methodology of *Kemper and Koch* (1966), using an Eijkelkamp "Wet Sieving Apparatus" from a soil fraction of 1 to 2 mm obtained during dry sieving. The soil organic carbon content was determined according to the Hungarian standard MSZ 08-0452:1980. The humus quality was measured using the E4/E6 method (KONONOVA, 1966, SCHNITZER, KAHN, 1989). The microbial biomass was measured by chloroform fumigation extraction as described by *Vance* et *al.* (1987), and fluorescein diacetate degradation activity measurements were performed according to the method of *Alef and Nannipieri* (1998).

Measurement results were evaluated with SPSS Student Version 15.0 statistical software and Microsoft Office Excel using the following statistical methods:

- analysis of variance (two-, three- and four-factor),
- coefficient of variation (CV),
- Duncan test
- correlation analysis

3. Results and conclusions

Organic additions had a significant effect on **aggregate stability**. The highest aggregate stability was found where the straw incorporation - green manure were used. (spring 2015, summer, spring 2016). Only in spring 2015, the manure shows a significantly higher stability value than the fertilizer-only version, so the structure improving effect was not observed except for this measurement, which is probably due to the high dispersing power of the manure.

The difference in aggregate stability results was also influenced by the year and sampling date. Regarding the seasons, the highest values were measured in spring, lower values in summer and autumn. The differences are significant. The trend in my studies is that where the monthly mean temperature was close to or higher than the long-term average and precipitation were lower than average, I measured higher stability values and obtained significant differences (spring 2015, summer, spring 2016).

The N dose did not significantly affect aggregate stability.

There were several significant positive correlations between microbial biomass mass and aggregate stability, but examining samples from one year only in case of 2016 spring. The correlation between MBC and aggregate stability would support aggregate models that emphasize the critical importance of microorganisms, but further studies are needed to clarify this.

At the time of the tank experiment setup in 1983, the soil **humus content** was 1.6-1.7% (organic C, **SOC** content 0,93-0,99 %). Organic fertilization increased the organic matter content. Soil organic carbon content was significantly the highest in the manure treatment, with no significant difference between the two treatments. I also found that even in the fertilizer-only treatments with no organic amendment, the soil organic matter content increased compared to the baseline of the experiment under conventional tillage. Thus, even under the experimental conditions, the exclusive use of fertilizers maintained the soil's organic carbon content. However, it is essential to note that organic matter remained in the soil even in this case since stubble and root residues were rotated, which was strongly influenced by the fertilizer.

Organic manure amendments improved the **humus quality (E4/E6)**. According to the 2014 results, the manure also resulted significantly better humus matter, but there was a notable effect of plant residues, with no significant difference between them. Overall, the quality of the humus material is poor in the whole experimental area, as the E4/E6 method is considered to have a good quality of humus material for the ratio between 3 and 5.

The N dose did not influence the values for humus quantity and quality.

Microbial biomass was correlated with soil organic matter content in several cases. In our experiments, soil organic matter content varied narrowly between 1.13 and 1.29 %, with a significant positive effect on manure treatment. The positive correlation may be due to the similarity of other factors influencing microbial biomass (climate, vegetation, cultivation, soil type).

Contrary to expectation, soil organic carbon (SOC) was not correlated with the other parameters studied, although soil organic matter plays a dominant role in developing almost all soil properties. The present study shows that the relationship between soil carbon content and other soil properties is not linear.

Besides the quantity of organic matter, I also examined its quality in relation to microbial biomass mass and activity, where I found a significant correlation. The negative correlation between the two variables means that lower E4/E6 values (presence of complex, high molecular weight humic acids) are associated with higher microbial biomass and FDA, so an increase in the proportion of more easily degradable, less condensed, lower molecular weight humic matter in the soils reduced the amount of microbial biomass and activity. If we consider humic matter only as a carbon source, this is contrary to our expectations. If we consider that higher molecular weight, more condensed humic matter improves soil structure to a greater extent, this result is more meaningful. However, the correlation analysis does not show a significant relationship between humus quality and aggregate stability, so there may be another indirect explanation for the effect of humus quality.

The statistical evaluation showed that the effect of vintage had an influential role in the variation of **microbial biomass (MBC)**. There were significant differences between the mean values of years and sampling dates, but no trend was observed between the available meteorological data and the change in MBC. There was no difference between the seasons (spring, autumn) averaged over the three years, but when examined separately, two years (2014, 2015) had higher MBC values in autumn measurements compared to spring in 2016, so no clear trend for a seasonal maximum was detected.

My research results confirm that organic amendments used in addition to fertilizer increase microbial biomass. In 2014 and 2015, manure was found to be the most effective in terms of MBC. Although there was no significant difference between the fertilization methods in the spring 2014 measurements, there is a trend that the manure supplementation gave the highest average value. At the time of the spring 2014 measurement, only the initial effect of the manure appeares, as the application was made in November 2013, and therefore presumably did not cause a relevant difference. The trend changed in 2016, and the application of straw incorporation and green manure resulted the highest MBC when I measured an outlier value in this treatment. This high value could be because crop residues were applied to the soil every year, while manure was only applied every third year (in this case, autumn 2013 deep plowing). Also, in the case of the SZ+ZT supplementation, oil radish applied as green manure were rotated into the winter barley stubble, i.e., before maize. However, oil radish is considered a readily degradable organic matter, so its effect was expected to be visible in 2014. N rates did not significantly affect MBC.

Based on the evaluation of three years, the treatment that stabilized the microbial community the most was the organic supplementation of straw incorporation and green manure, since the coefficient of variation (CV) was the lowest here compared to the other two treatments (NPK; NPK+IST), i.e., NPK+SZ+ZT had the most homogenizing effect on microbial biomass values.

The **FDA hydrolysis activity** assay results showed a statistically verifiable positive effect of the organic amendments applied in the experiment on the microbial activity. In 2015, the highest activity was found in plots receiving manure. In spring 2016, the values were much higher, but there was no significant difference between organic amendments. In autumn 2016, the highest activity value was measured in the SZ+ZT amendment, 35% higher than the IST amendment and more than three times higher than the activity of the fertilizer-only (NPK) plots. N dosages did not significantly affect the evolution of FDA activity.

The results suggest that organic inputs to the soil increase the decomposition potential of microorganisms and the microbial community's amount and activity, while the quantity and quality of soil organic matter and carbon dynamics are positively affected.

FDA hydrolysis activity and MBC together reflect the ratio of active to dormant microbes. In two cases, the activity of microorganisms correlated with the mass of microorganisms (using the same sample). This shows that the proportion of metabolically active and passive (dormant, enduring) microorganisms did not change significantly during the treatments in autumn 2015 and 2016. However, specific FDA activity also showed a treatment effect for these two measurements. The results, therefore, suggest that as the number of active microbes increased, the number of passive microbes also increased.

Although the N rates did not directly affect any of the soil parameters I studied significantly, they did have an indirect effect on the amount of plant biomass and thus the number of plant parts remaining in the soil and rotated into the soil. This is important to note even in the case of fertilizer-only and manure-only treatments, where root and stubble residues are also a factor. Consequently, the microbial community is also affected, as root and rhizodeposition carbon is an important food source for micro-organisms, and the roots are completely permeated, so their even distribution makes their uptake much more even. In addition, N fertilizer is also a nutrient for micro-organisms.

Unlike the other parameters studied, the **yield** is affected by the N dose, but significant differences are mainly due to the total or partial absence of nitrogen. In the case of organic supplementation, the only relevant difference was in 2015, with winter wheat as a marker crop, where the manure had a significant positive effect on yield. In this case, we are talking about a 2nd-year effect of the manure.

The (estimated) effect of **organic carbon inputs** in a given year was examined on the following year, but most of the results show a relationship with not the following year rather than the year after. The amount of C incorporated in 2013 strongly correlates with the 2015 MBC and 2015 FDA values. The 2014 C amount strongly correlates with the 2016 spring MBC and 2016 spring and fall FDA values. For the 2015 C amount, the following year's effect is observed, with the 2016 spring MBC and 2016 spring and fall FDA values. I hypothesize a prolonged organic matter build-up behind the longer-term effect. (The effect of manure applied to the soil in autumn 2013 is still significant in year 2, but less so in year 3. Due to the higher amount of maize stalk residues and higher lignin content of the maize straw plowed in 2014, the effect on MBC is still noticeable in 2016, and stalk residues are incorporated in the soil every year with the SZ+ZT treatment, and the drought in previous years could also have led to prolonged uptake). However, the amount of carbon incorporated in 2015 correlates with 2016 (stable manure in year 2, wheat straw plowed in 2015).

Based on my results, I recommend using any of the organic amendments I have studied under field conditions. However, to different extents, both have had a positive effect on several parameters, and root and rhizodeposition biomass remaining in the soil by mineral fertilization alone may maintain some soil parameters at a level.

This research could be helpful to understand better the relationship between fertilization strategies and soil, biochemical properties, and overall health, which could contribute to the development of a more effective sustainable nutrient management system. However, I also consider investigating the environmental and economic impacts of the fertilization methods used under different environmental conditions.

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4. New scientific results

1. The results showed that the straw incorporation and the use of green manure plants stabilized the microbial biomass amount the most, as the CV value of NPK + SZ + ZT treatment was significantly lower compared to NPK and NPK + IST treatments.
2. Compared to the control, among the organic fertilization methods, straw incorporation and green manure supplementation increased the aggregate stability and the volume of the microbial biomass the most. In the case of aggregate stability, although both organic supplements (manure; straw incorporation + green manure) had a measurable effect, a significant effect was attributed to the NPK + SZ + ZT treatment, in the case of a significant difference it had the highest stability value in all cases. In the case of microbial biomass, NPK + IST showed significantly higher values several times in the first two years compared to the other treatments. However, NPK + SZ + ZT treatment gave remarkably high MBC results in the last year compared to NPK and NPK + IST treatments.
3. Under the experimental conditions (IOSDV), the N dose, unlike the method of organic matter replacement, did not significantly affect the weight and activity of soil microbial biomass, the stability of the aggregate, or the quality and quantity of humus. In this experiment, the effect of N dose was only indirect, as it had a significant effect on plant biomass, thus influencing the quantity of roots left in the soil and the amount of incorporated straw and green manure.
4. In the case of the results from the exact sampling, twice in three cases (autumn 2015 and 2016), the proportion of metabolically active and passive (dormans) microbes does not show any change due to using organic supplements. These two strong correlations show that the number of passive microbes also increased with the growth of active microbes.
5. Humus quality is strongly correlated with FDA activity showing microbial activity twice out of 3 cases. This can be explained by the microbial metabolism theory that, even if nutrient sources are available, mineralization is only enhanced when a more complex substrate enters the soil (ALLISON, VITOUSEK, 2004).

   Humus quality was correlated with MBC in only one of 6 measurements, so the present study could not detect a clear relationship between the two parameters.

5. Own publications on the topic of the dissertation

Kökény M., Tóth Z., Csitári G. (2015): Mikrobiális biomassza és a humuszminőség alakulása trágyázási tartamkísérletben, 57th Georgikon Scientific Conference 2015, 235-241

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Poszterek: „Talajok szervesanyag tartalom változásának korai előrejelzése trágyázási tartamkísérlet alapján” (Növényvédelmi Fórum, 2015)

„Mikrobiális biomassza tömeg, talajszerkezet és humusz vizsgálatok szerves- és nitrogéntrágyázási tartamkísérletben” (Növényvédelmi Fórum, 2016)

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