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Effect of soil regenerative techniques on representative soil health indicators in organic farming

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PhD THESIS BOOKLET

Eszter TÓTH

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Name of Doctoral School: Horticultural Sciences Doctoral School

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Head of Doctor's School	Dr. Éva Németh Zámboriné, Professor, DSc
	Head of Department, University Professor
	Hungarian University of Agriculture and Life Sciences
	Department of Medicinal and Aromatic Plants
Supervisor:	Dr. Zita Szalai, CSc.
	Associate professor retired
	Hungarian University of Agriculture and Life Sciences
	Department of Agroecology and Organic farming
Co-Supervisor:	Dr. Borbála Biró, DSc.
	Professor emerita
	Hungarian University of Agriculture and Life Sciences
	Department Agro-Environmental Studies
Signature	Signature
Head of Doctor's School	ool of supervisors

1. INTRODUCTION AND OBJECTIVES

Today, the greatest challenge for humans is to *produce foods* for a growing population, while reducing the environmental impact of agricultural practices. Beside this to sustain resources, and to adapt to the new, emerging conditions of *global warming*. The extensive use of fertilisers, synthetic pesticides and over-fertilisation of soils has led to soil degradation worldwide. Soil, where our food and life come from, is our most precious resource. It is not only a production medium, a substrate, but also a habitat for living organisms, that participate in the geochemical cycles of key environmental elements. Soil conservation is important to us not only for our food production, but also for its role in the ecosystem. Today, this recognition is gaining interest for preserve and conserve of soil quality and soil health.

Organic farming principles and practices are the most effective way to address this aspect. The principles of organic methods include the maintenance and preservation of soil fertility and health as one of their primary objectives. Numerous studies reports that soils in organically managed areas have higher humus content, greater soil life and better structure. However, organic farms do not necessarily live up to expectations in terms of their environmental impact. Certified farms comply with standards, for example by excluding synthetic inputs, but do not always focus on improving soil quality and properties as much as possible. Recent trends are not only concerned with fertility, but also with maintaining and improving soil health. Conservation agriculture (CA) is one such trend and regenerative agriculture (RA) is also spreading. The main pillars of both methods are minimum tillage, continuous mulching and increasing crop diversity. Even under organic farming conditions, there is a strong need to place more emphasis on soil health, but reducing tillage and continuous crop cover in organic farming is more challenging than in conventional farming. The main means of weed control in organic farming is mechanical cultivation of soils and the termination of cover crops can only be achieved in this way, because herbicide-application is not possible. The technological shift from conventional farming to low-input farming can lead to yield losses in the short term, which is a strong constraint. Little information is available to farmers about the conservation tillage methods and the successful application of cover crops. It can be also difficult how to disseminate good practices for soil conservation, preservation and regeneration in organic farming. In case of introducing those practices, the early changes of soil-health could be necessary, to detect the beneficial changes in soil-plant systems by the farmers an land-users.

The future of agricultural support system is required "results-based" funding, where it is also crucial to detect beneficial changes in the soil, as early as it is possible with appropriate and trustable soil-quality indicators. Traditional soil tests focus on mainly the soil-nutrient availability - based on instrumental chemical data - in the soil-tillage advisory systems, developed to farmers. This approach is useful for increasing the yields and potential income of farmers, however beside the physical-chemical soil data, no any information is known about the soil-biological parameters. Concept of soil health is that soil must be considered as a living environmental element, a living entity, in which it is the soil-life, the biology can be responsible all its functionality and environmental services. The assessment of soil-biological composition and functionality must be a crucial objective to better understand soil health. Soil biological parameters are considered to be more sensitive to see changes by different land users and can therefore be good indicators both of the early and later changes in soil environment.

As described above several questions and tasks were addressed in this PhD work:

Scientific questions, hypotheses:

- i. Among soil regenerative practices and conservation tillage methods, the use of cover crops, and increasing crop diversity how able to result in positive changes and measurable parameters of soil quality and soil health. The changes can be seen in the short term, about in 2-5 years of periods among the organic farming practices?
- ii. How the effects of soil regenerative methods developed, and is there any monitoring indicators to be detected beside the basic physico-chemical properties at different soil-characteristics (texture, pH, organic matter)?
- iii. Are there any indicators among the assessment methods used, which can be easily measured even by the farmers of their own; to detect the beneficial changes in the soil in a short-term periods of soil regenerative tillage?
- iv. Based on farmers' experiences, what are the advantages or disadvantages of using soil regenerative methods?
- v. Which types and species of cover crops could result the most positive effects on soil-plant properties, based on some measured parameters?

Based on those scientific questions, the following objectives and backgrounds were set:

- Comparing the different cultivated practices of the organic farming the potential effect of variable farming histories was studied at the Soroksár Experimental Farm (MATE). Investigation was performed to identify the main research directions and to select possible indicators to be applied throughout in the dissertation.
- A short-term field experiment was designed and used to test the impact of regenerative soil methods (conservation tillage and cover crops) and to compare the results among the conventional tillage practices and the organic farming.
- A pot experiment was used to study the potential effects of the most common cover crops in a controlled greenhouse conditions and to verify their impact and significance in the plant-microbe relationships.
- Among on-farm study conditions the practical results of soil regeneration methods were compared in four Hungarian organic farmers' sites. The comparison with conventional farming results can be very helpful for farmers to change of their attitudes and transition in a trustable way.

2. MATERIALS AND METHODS

The preliminary research was carried out at the Hungarian Agricultural and Life Sciences University (MATE) in Soroksár, in the Ecological Farming Unit. Six different plots with different cultivation history were compared. The fields were variable on the basis of the time-periods of last soil disturbance and the crops grown on them. Of the 6 plots, the 'Arable field land disturbed' plot had been cultivated every year, the 'Pasture' had been uncultivated for 2 years, the 'Perennial legumes' for 2 years, the 'Recultivated plot with pasture' for 4 years, the 'Meadow orchard' for 8 years and the 'Hedgerow' for 20 years. An average, composite sample was taken from the different sites by homogenising 40 sampling points, originating from a depth of 0-20 cm.

The short-term field experiment was conducted between 2018 and 2020 at the MATE Soroksár Experimental Farm and Agricultural Research Station within the Ecological Farming Unit. The

experimental site encompassed an area of 2820 m², where 3 types of soil cultivation were implemented: 1) traditional *ploughing-based cultivation*, which was the mainly applied technique in the experimental farm (T), 2) *conservation tillage*, which in this case involved the abandonment of ploughing (CT), and 3) no-till cultivation combined with *cover crops* (NT). The experimental plots were arranged in a striped, random layout. Each treatment was implemented in two strips, which were further divided into three sections for soil sampling, with each section treated as a separate replication. One replication covered an area of 156 m², meaning that each treatment was associated with six replications.

The pot experiment was established in the greenhouse of the MATE Department of Vegetable and Mushroom Production, located within the Budai Arboretum. The experiment was set up in October 2020. Five plant species were sown into 5000 g plastic pots filled with humus-rich sandy soil sourced from the university's Soroksár Experimental Farm and Agricultural Research Station: Ethiopian mustard (*Brassica carinata*), phacelia (*Phacelia tanacetifolia*), sandy oats (*Avena strigosa*), purple vetch (*Vicia benghalensis*), and broad beans (*Vicia faba*). Each pot contained ten plants of each species, cultivated over a period of eight weeks with four replications. In addition, a mixture of the five plants was also sown, including a variant inoculated with arbuscular mycorrhiza (AM) (Danuba Ltd.), and this mixed treatment was subjected to greenhouse conditions (19 °C during the day, 10 °C at night, 52% humidity). After eight weeks, the experiment was terminated for soil and plant analyses.

To explore the applicability of conservation tillage and other soil regenerative techniques in organic farming, we examined various fields in four organic farms. For three of the farms, samples were also taken from adjacent conventional farms, which served as controls. The research was conducted between 2020 and 2021 and included two sampling periods (autumn 2020 and spring 2021). The 4 farms involved in the study was:

- Kömlőd (Komárom-Esztergom County),
- Szár (Fejér County),
- Bugac (Bács-Kiskun County), and
- Füzesgyarmat (Békés County).

The primary characteristics of the soils from the studied fields are presented in *Table 1*.

Soil sampling was conducted at two time points, in autumn 2020 and spring 2021. The examined fields were divided into 4 sections, and 8 samples were collected from each section, which were then homogenized to create average, composite samples within the 4 sections. Each field yielded 4 replications.

Soil samples for all field research were taken from a depth of 0-20 cm, while samples for microscopic examinations were collected from a depth of 10 cm. A portion of the soil samples was stored in a refrigerator at 5 °C until enzyme activity, nematode density, and microscopic bacterial counts could be assessed. Biological analyses were conducted within one week of sampling.

Another portion of the soil samples was air-dried for *physical-chemical analyses*, which were performed in the laboratory of the MATE Institute of Environmental Sciences, Department of Agroecology. The measured parameters from the research are described in *Table 2*., where it is indicated which analyses were performed in each study.

Table 1. Brief description of the fields in different settlements.

	Org-conserv1	Conv-ploughed	Conv-conserv	Org-conserv2		
Soil texture	silt	silt	sand	sand		
Org/Conv	3 years	conventional	conventional	2 years		
Soil tillage	5 years of	conventional	5 years of shallow,	5 years of shallow,		
system	shallow, non-	tillage with	non-inversion	no-till farming		
	inversion	ploughing	farming			
	farming					
Cover crops	legume	-	green manure	legume		
	intercropping			intercropping		
	Org-conserv	Conv-ploughed	Org-ploughed	Extensive		
Soil texture	sand	sand	silt	sandy silt		
Org/Conv	5 years	conventional	5 years	5 years		
Soil tillage	5 years of	conventional	5 years of shallow,	5 years of shallow,		
system	shallow, non-	tillage with	non-inversion	no-till farming		
	inversion	ploughing	farming			
	farming					
Cover crops	cover crops	-	-	legumes in the cro		
				rotation		
	Org-conserv1	Conv-ploughed	Org-conserv2	Conv-conserv		
Soil texture	clay loam	clay loam	clay loam	clay loam		
Org/Conv	20 years	conventional	20 years	conventional		
Soil tillage	8 years of non-	conventional	8 years of non-	non-inversion		
system	inversion	tillage with	inversion farming	tillage		
	farming	ploughing				
Cover crops	-	-	-	-		
	Org-graze	Orgconserv1	Org-conserv2	Org-cover crop		
Soil texture	sandy loam	sandy loam	sand	sand		
Org/Conv	18 years	18 years	18 years	18 years		
Soil tillage	minimum	minimum tillage	minimum tillage (6	minimum tillage (6		
-	tillage (6 cm)	(6 cm)	cm)	cm)		
system	image (o cm)	()				

In addition to analysing soil samples taken from the fields of the studied farms, we conducted semi-quantitative interviews with the owners of the farms, recording any additional information that emerged during the structured questionnaire.

For data processing, we utilized IBM SPSS Statistics version 26. In the studies, we employed both one-way and multivariate analysis of variance (ANOVA and MANOVA) for the statistical comparison of different treatments and comparable areas. The homogeneity of variance was assessed using Levene's test, while the normality of residuals was evaluated through the

Shapiro-Wilk or Kolmogorov-Smirnov tests. For pairwise comparisons involving more than two treatments, we conducted Tukey or Games-Howell tests. We examined the relationships between various parameters using Pearson's correlation coefficient, also with a significance level.

The effects of soil properties and land use on aggregate stability, as the response variable, were analysed using a "random forest" classification approach, considering results with an accuracy of at least 80% on the testing dataset.

Table 2. Brief description of the soil tests used, indicating in which research they were applied. The different numbers indicate: 1 = Preliminary study, 2 = Field experiment, 3 = Pot experiment, 4 = Onfarm experiment.

Soil analysis	Soil analysis short description and source					
Soil organic matter (SOM)	Hargitai, 1963	1,2,3,4				
Humus quality (Hargitai)	In the two solvent test methods, an extract of 0.5% NaOH and 1% NaF is prepared from the soil (Hargitai 1963)	4				
Humus quality (E4/E6)	The study investigates the light absorption of humus extracts at wavelengths of 465 and 665 nm (Kononova, 1961).	4				
Natrium NO₃, Ammonium NH₄	Salicylate extraction method (Kempers & Zweers, 1986). NH4-N and NO3-N are quantified by a spectrophotometer at 655 nm and 410 nm wavelength (absorbance mode) respectively.					
Phosphorus	ammonium lactate extraction at 438 nm (Egnér et al., 1960),	1				
Potassium	ammonium lactate extraction at 438 nm (Egnér et al., 1960),	1				
Active, labile carbon (POXC)	KMnO4 oxidation method (Weil et al., 2003)	2,4				
Soil moisture	Füleky, 2011	1,2,3,4				
Aggregate Stability Index (ASI)	SIAKES smartphone app (Fajardo and McBratney, 2016)	4				
Dehydrogenase-enzyme activity (DHA)	A 2,3,5 triphenyl tetrazolium chloride solution (TTC) method (Veres et al., 2013). DHA is measured by spectrophotometer at a wavelength of 546 nm	1,2,3,4				
Fluorescein-diacetate (FDA) hydrolisis	We used the FDA (fluorescein diacetate) assay as defined by Schnürer and Roswall (1982) and validated by Villányi et al 2006. Spectrophotometry was performed at a wavelength of 490 nm.	1,2,3				
Glomalin content (GRSP)	Easily extracted glomalin related soil proteins (EE-GRSP) were measured by the Bicinchoninic acid method (BCA) proposed by Stoscheck (1990). Glomalin concentration was assessed spectrophotometrically at 562 nm wavelength.	2,3,4				
Free living nematode density	Nematodes were extracted from the soil using a developed Baermann funnel run method modified by Szakálas et al. (2015).	4				
Bacteria abundance	Counting bacterial cells by light microscope	1,2,4				
Microscopic fungi index	Index formation based on counting fungal filaments by light microscopy	1				

3. RESULTS AND DISCUSSION

Short-term field experiment

The soil chemical analyses (H%, POXC, pH, NO3, NH4) did not reveal any significant differences between the treatments after two years. The humus content, which has proven to be a reliable indicator for assessing soil management methods and soil health, demonstrated the positive effects of various conservation tillage practices, as supported by numerous studies (e.g., Juhos et al., 2024). However, such studies generally indicate changes in organic matter content over the long term, as soil organic matter changes relatively slowly. A period of at least 3 to 5 years is typically required to observe significant changes. This may explain why the humus content did not show detectable changes within two years. The pH is also a soil health indicator that changes slowly in response to alterations in soil management practices, and in our case, no significant differences were found. In contrast, the labile fraction of soil organic matter can respond rapidly to environmental impacts, making it suitable for demonstrating the effects of conservation tillage even within a few years (Weil et al., 2003). However, we did not observe significant differences in labile carbon measurements among the three soil treatments.

Among the biological indicators, significant differences were found in soil enzyme activities (Table 3). Dehydrogenase (DHA) enzyme activity proved sensitive to differences among the various soil treatments (ploughed-P, conservation-C, and conservation with cover crops-CC) in the CC plots. The presence of living roots from cover crops enhances soil biological activity, as evidenced by both DHA and the Fluorescein diacetate (FDA) enzyme. A meta-analysis conducted in 2023 (Wen et al.) indicated that conservation tillage has a significantly positive effect on soil enzyme activity. Dehydrogenase is the sixth most commonly measured enzyme in such studies. The FDA enzyme has been used less frequently because it is non-specific and reflects the overall degradative capacity (catabolic activity) of the soil.

In a 2-year-experiment, Szostek et al. (2022) also demonstrated significant differences in DHA enzyme activity, which we subsequently employed in our further investigations. Measurements of glomalin did not show significant variations in response to different soil treatments at any sampling time, contrary to the findings of Koberski et al. (2020). Glomalin is primarily associated with the quantity of mycorrhizal fungi and root colonization, which are further influenced by soil structure, clay mineral content, and organo-mineral complexes. In sandy soils, glomalin content is more resistant to change. Increasing the use of mycotrophic plants in cover crops may be one method to enhance glomalin levels and, thus, indirectly improve soil structure.

Table 3. The development of various soil parameters over the two sampling years. P - conventional tillage, C - reduced tillage with cover crops. The values marked in red indicate significant differences with different lowercase letters (p<0.05)

So	il	Physical-chemical properties						Soil biological properties						
properties		Soil mois	sture	-NO₃ mg/kg		-NH ₄ mg/kg	DHA TPFμg/g			FDA µg/g		GRSP mg/g		
		%												
		mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	
2019	Р	8,67	1,11	7,43	2,41	7,01	0,45	3,12 a	0,50	12,14	4,18	1,12	0,04	
	С	7,72	0,87	6,40	0,88	6,22	0,32	2,59 a	0,90	2,59	0,90	1,11	0,03	
	СС	7,47	0,98	5,69	0,66	6,74	0,74	4,60 b	0,44	11,68	5,17	1,04	0,09	
2020	Р	7,47	1,87	4,08	0,12	6,31	0,41	2,07	0,92	12,14	4,18	0,90	0,04	
	С	6,57	0,91	5,32	0,54	5,32	0,54	1,56	1,10	12,43	3,23	0,89	0,04	
	CC	6,99	1,41	5,98	2,67	5,96	0,57	1,92	1,49	11,68	5,17	0,90	0,04	

Results of the pot experiment

Different plant species had varying effects on *soil enzyme activity*, with the presence of roots of several plant species increased soil enzyme activity; however, none of these differences were statistically significant. Notably, sandy oats, broad beans, and the mixture exhibited significantly higher values. The presence of living roots increases enzyme activity by promoting microbial activity through root exudates. According to the literature, leguminous plants tend to have a more positive impact on enzyme activity compared to other plant species, attributed to their nitrogen-fixing capability and the superior C/N ratio of their plant residues. The positive effect of broad beans on enzyme activity has also been demonstrated in field conditions, where higher activities of dehydrogenase, protease, and urease were measured in broad beans compared to spring wheat (Siczek et al., 2018). The enzyme activity (phosphatase) was significantly enhanced by the diverse cover crop mixture, which included species such as phacelia, oats, vetch, radish, and buckwheat (Reynolds et al., 2017).

The highest *glomalin levels* were recorded for purple vetch. Glomalin, is a protein secreted by mycorrhizal fungi, correlates with the degree of mycorrhizal presence. The choice of plant species in cover cropping plays a crucial role in the mycorrhizal colonization of subsequent main crops. Different cover crop species offer various advantages; for example, oats and rye produce substantial biomass and are economical, while clovers and vetches fix nitrogen, and radishes help loosen the soil and suppress weeds. Literature suggests that such mixtures are generally more beneficial for soil parameters and main crop yields (Chu et al., 2017). However, a recent meta-analysis (Florence & McGuire, 2020) indicated that only 2% of the 21 studies examined showed that mixtures containing multiple species were more advantageous. These findings can be significantly influenced by the conditions under which cover crops are sown, as well as whether the chosen species are appropriately selected.

Broad beans produced the greatest biomass and root mass, utilizing the most nutrients based on nitrate content, thus demonstrating the highest potential for nutrient retention in the short term and effectively reducing nitrogen leaching in our experiment. The DHA results indicated a positive effect on soil enzyme activity, corroborated by Siczek et al. (2018). In contrast, purple vetch, with low root mass and biomass and moderate nitrate uptake, positively affected glomalin and FDA enzyme activity. Sandy oats, which produced small root mass and biomass, had a moderate nitrate uptake and the lowest ammonium uptake, positively influenced DHA but yielded low glomalin content. Ethiopian mustard formed extremely low root mass and biomass, resulting in the least nitrate uptake relative to its growth. Based on measurements of both enzyme activities, it exhibited the lowest values, even compared to the control. The mixtures (both AM fungus-inoculated and non-inoculated) demonstrated moderate root mass and biomass, with high nitrate uptake but had no effect on glomalin content; however, they achieved positive results in enzyme activity measurements compared to the control.

The result of the on-farm experiment

There is a scarcity of on-farm research investigating soil regenerative methods, yet such research enables the examination of soil under real agricultural conditions. While established plot experiments reduce the number of confounding variables, they often overly simplify the complexity of the system. However, operating farms do not adhere to strict management categories; instead, they modify soil management practices annually based on crop and rotation types, soil and weather conditions, residue amounts, and weed pressure.

The farms across the four sites exhibited diverse soil textures (Table 10). Notable heterogeneity was observed within farms based on the texture categories according to the Arany yarn number and nutrient availability. The data measured on various plots, along with the analysed results and correlation coefficients, help elucidate the properties of indicators concerning different soil management practices and soil textures.

Examining all sites reveals a trend where lower organic matter content was consistently measured in soils with lower Arany yarn number values. This suggests that the quantity of organic matter was more influenced by the physical type of soil than by the soil management practices employed. However, the chosen soil management approach could have long-term effects, as demonstrated in the soils of the Füzesgyarmat plots. All plots belonged to the same soil type, yet only the plots employing deep tillage showed significantly lower soil organic matter (SOM). Accordingly, the impact of conservation practices was already evident in the organic matter content over a longer term (8 years).

Several parameters (moisture content, POXC, GRSP) positively correlated with SOM, and it was similarly observed that the basic properties of the soil influenced their development, although to a lesser extent than with organic matter content, as significant differences were still evident between plots. This assertion also held true for dehydrogenase activity (DHA), but with greater sensitivity in highlighting differences between soil management practices (figure 1.). In Kömlőd, a significant difference was observed between Org-conserv1 and Conv-ploughed, as well as between Conv-conserv and Org-conserv2 plots, where the organic matter content was also comparable. In Szár, differences were found between Org-conserv and Conv-ploughed based on the spring sampling, as well as between Org-ploughed and Extensive plots, where the Extensive plot recorded significantly higher values based on the autumn sampling, despite having lower organic matter content and compaction, plasticity (AK). Thus, in this case, the recent soil management practices played a more decisive role in shaping enzyme activity.

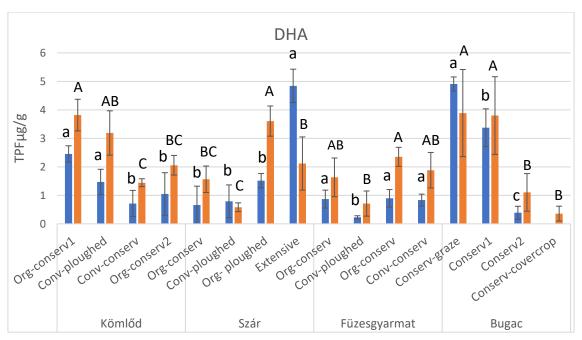


Figure 1. The variation in dehydrogenase enzyme activity (DHA) across fields in different sites during the two sampling periods (2020-2021). Different lowercase letters indicate significant differences within sites at the first sampling period (p<0.05), while different uppercase letters denote significant differences at the second sampling period (p<0.05).

Aggregate stability exhibited a different trend compared to the other parameters (Figure 2). We found a significant difference between the conservation-tillage and conventional-tillage areas, with Kömlőd showing notably lower values in the conventional tillage plots. In Szár, the two conventional tillage areas (Conv-ploughed, Org-ploughed) demonstrated the lowest values, which were significantly different from those of the Org-conserv plots.

Similarly, at Füzesgyarmat, the plot based on traditional tillage also recorded the lowest values. These findings suggest that the physical type of soil had a lesser influence on the results of aggregate stability than on the development of other parameters. This is consistent with the research conducted by Williams et al. (2020). Correlation analysis reflected these differences in relation to the other parameters, as this indicator displayed the weakest correlation with the other parameters assessed.

For aggregate stability, we also conducted a random forest statistical analysis. Our inquiry focused on the contribution of agrotechnical elements, soil properties derived from soil type, and soil biological factors resulting from management practices to the formation of soil structure. Based on the so-called variable importance, it was evident that aggregate stability was primarily determined by the intensity of tillage and the transition to ecological farming, while the significance of soil properties appeared to be considerably less impactful (Figure 3).

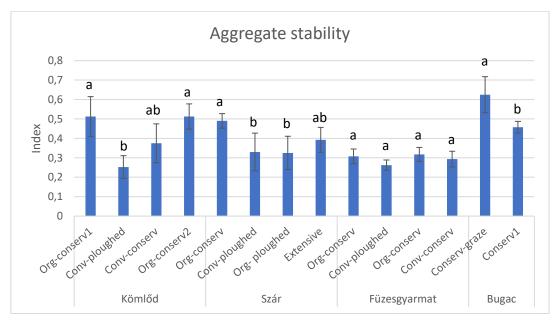


Figure 2. Evolution of the aggregate stability index (ASI) in the tables of the different municipalities. The different lowercase letters show significant differences within sites (p<0.05) (On-farm experiment, 2021)

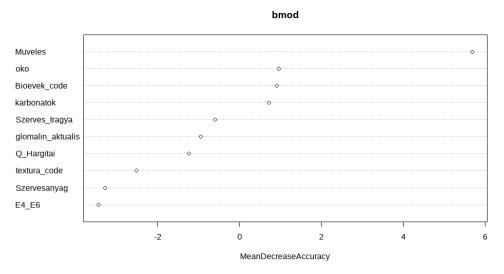


Figure 3. Random forest model on factors influencing the development of aggregate stability. (Muveles-tillage, oko-eco, Bioevek-years, karbonatok-carbonates, szerves-tragya-manure, glomalin, Q, texture, szervesanyag-SOM)

In the correlation analyses, if the parameters measured at two sampling times correlated with themselves, we can infer that they can provide consistent data. Based on this, it can be stated that soil moisture content, dehydrogenase activity (DHA), glomalin content, and nematode density yielded similar results across both sampling times. Notably, moisture content exhibited correlations with the majority of parameters, followed by DHA. The only significant correlation with aggregate stability was found with nematode density.

Within the framework of the on-farm research, we specifically analysed three pairs of adjacent plots, where one plot was managed conventionally using traditional tillage practices, while the other employed ecological farming methods focused on soil regeneration. This setup allowed us to compare the two different soil management systems. For clarity, I will refer to the conventional management system as *CONV* (conventional farming, traditional tillage) and the ecological system as *ORG* (ecological farming using soil regenerative methods) in the subsequent discussion.

The summary table (*Table 4*) indicates that, across all three locations, we recorded significantly higher values in the ORG plots for four different indicators. These indicators varied by location. However, it can be stated that Hargitai's humus quality, pH, glomalin content, and nematode density showed significant differences at one site, while aggregate stability, moisture content, and labile carbon demonstrated significant differences at two sites, and DHA exhibited significant variation at three sites.

It is also noteworthy that most parameters, while not necessarily significant, tended to have higher values in the ORG plots, as indicated in *Table 4*.

Table 4. The significant difference of soil parameters on the conventional (CONV) and organic (ORG) fields of the three sites (Kömlőd, Szár, Füzesgyarmat)

	Kömlőd				Szár				Füzesgyarmat			
Soil analysis	CONV OR		.G CO		NV OF		RG CO		NV	ORG		
Soil analysis	mean	±SD	mean	±SD	mean	±SD	mean	±SD	mean	±SD	mean	±SD
SOM (m/m%)	3.85	0.22	4.41	0.56	1.34	0.61	1.44	0.32	2,14	0.47	2.41	0.87
Q	6,07	0.97	6.35	0.52	1.93	1,02	3,14	1.79	1.00	0.23	2,01	0.77
E4/E6	7,11	0.26	7,29	0.27	6,14	0.49	6.33	0.48	4.79	0.29	4.81	0.30
рН	7.325	0.15	7.225	0.05	6.78	0.93	7,03	0.69	5.95	0.13	6.63	0.378
ASI	0.25	0.06	0.51	0.10	0.33	0.10	0.49	0.04	0.26	0.03	0.31	0.04
Soil moist1 (m/m%)	20.22	0.19	20.33	0.18	12.69	1.56	13.74	0.88	22.39	1.63	22.78	0.25
Soil moist2 (m/m%)	14.60	0.49	14.41	0.48	7.85	0.68	10,21	0.83	21.57	0.45	23.27	0.73
POXC1 (mg kg ⁻¹)	615.38	34.71	574.49	27.61	314.09	129.50	263.43	98.78	485.62	59.52	478.51	40.48
POXC 2 (mg kg ⁻¹)	650.04	172.93	1030.42	161.57	314.09	95.98	238.55	37.40	228.77	29.37	410.96	121.03
GRSP ősz (mg g ⁻¹)	0.96	0.06	1,01	0.09	0.81	0.13	0.83	0.06	1,22	0.05	1,13	0.08
GRSP (mg g ⁻¹)	0.97	0.08	1,12	0.08	0.65	0.18	0.66	0.09	0.98	0.03	0.80	0.13
DHA1(TPFµg g ⁻¹)	1.47	0.44	2.45	0.29	0.79	0.58	0.63	0.69	0.23	0.06	0.87	0.31
DHA2 (TPFμg g ⁻¹)	3,19	0.78	3.82	0.55	0.51	0.15	1.57	0.46	0.92	0.44	1.63	0.68
Nem1 (db/25g talaj)	552.33	86.75	687.75	114.15	540.67	49.66	611.00	122.56	323.50	67.34	453.00	101.47
Nem2 (db/25g talaj)	220.25	81.35	427.75	172.66	262.00	67.74	481.67	52.54				

Five years of shallow tillage, transitioning to ecological farming, and applying double cropping have already shown changes in sensitive indicators over the short term. It is clear that the more sensitive indicators, as noted in the literature, also indicated significant differences in our study (e.g., Weil et al., 2003). Many studies have demonstrated the positive effects of soil regenerative techniques, but mostly in long-term experiments (Kobierski et al., 2020; Littrell et al., 2021; Cooper et al., 2016). Only a few short-term studies have reported differences in certain parameters, such as enzyme activity, microbial biomass, and aggregate stability within just a few years (Pittarello et al., 2021).

Numerous studies have indicated that soil organic matter (SOM) changes relatively slowly, often taking 3 to 10 years before significant differences can be detected (e.g., Cardoso et al., 2013). Therefore, in the cases of Kömlőd and Szár, it is likely that there hasn't been enough time for significant differences to emerge. This finding aligns with observations in other studies (Puerta et al., 2018). In the case of Füzesgyarmat, the ORG plot was converted to ecological farming 20 years ago and also showed no significant differences compared to the CONV plot. This may be because the CONV plot regularly received manure, which can increase SOM (Mäder et al., 2002), potentially reducing the difference between the ORG and CONV plots.

The E4/E6 ratio is considered an important measure for characterizing humic acids and is also sensitive to the effects of farming practices. A lower E4/E6 ratio indicates greater stability in

humus fractions as a result of soil regenerative methods, as seen in long-term studies by Juhos et al. (2024). In our experiment, the three- and five-year conservation tillage and organic farming practices at Kömlőd and Szár did not result in changes to the E4/E6 ratio. However, according to the Hargitai humus quality index, we found significantly higher values in the ORG field at Füzesgyarmat, which had transitioned to ecological farming 20 years ago. Since there was no significant difference in SOM between the two treatments at this location, we can hypothesize that soil pH influenced the Q value. In this study, the Füzesgyarmat ORG plot, which has been under ecological farming for 20 years, resulted in a significant difference in pH, likely because acidic fertilizers were not used during nutrient management.

The labile carbon content of the soil is a portion of organic matter that responds sooner to farming practices than SOM (Weil et al., 2003). The labile carbon content indicated significant differences due to regenerative methods in the ORG plots based on spring sampling at two locations. The higher labile carbon values align with the literature, where organic farming, tillage, and conservation agricultural practices have also increased the active carbon fraction of SOM (Juhos et al., 2023). Our results suggest that the sampling time can influence labile carbon. During the fall sampling, the CONV plot had already undergone stubble cultivation and primary tillage (ploughing), while the ORG plot had not. The aeration of the soil and incorporation of organic matter may have temporarily increased the values of the sensitive parameters in the CONV plot, leading to no significant differences in labile carbon across different farming practices, even though this parameter is known to change easily due to agronomic interventions. The longer period of inactivity before the spring sampling may have reduced the impact of interventions, allowing for significant differences to emerge favouring the ORG approach. In the fall sampling, we observed lower labile carbon values at Kömlőd and Füzesgyarmat in the CONV plots. The deeper tillage likely resulted in greater aeration and faster decomposition of microbial biomass, which is reflected in the lower values.

Aggregate stability was significantly higher in the ORG plots at Kömlőd and Szár. Our results align with the literature indicating that organic farming practices, conservation tillage, and diverse crop rotations enhance aggregate stability (Williams et al., 2020; Littrell et al., 2021), a trend that was also significantly confirmed using the SLAKES test. These soil regenerative practices positively affect soil biological activity, which contributes favorably to aggregate formation (Lehmann et al., 2017). At Füzesgyarmat, aggregate stability was also higher in the ORG plot, but the difference was not statistically significant. This plot had been under organic farming for the longest time; however, the CONV plot received manure every year, which may also have positively impacted its aggregate stability.

We measured significantly higher *dehydrogenase (DHA) enzyme activity* in the ORG plots during the autumn sampling at Kömlőd and Füzesgyarmat, and during the spring sampling at Szár. Numerous studies support the notion that conservation tillage practices increase microbial activity, which can serve as a good indicator of soil improvement in response to farming practices - often changing much sooner than other soil properties (Cardoso et al., 2013). DHA can be easily influenced by the most recent tillage or the quantity and quality of plant residues, making it advisable to conduct measurements multiple times a year. Biological activity is closely linked to labile carbon stocks, as these serve as the most readily available energy source for microbes. Additionally, dead microbial biomass (necromass) can later contribute to increasing labile carbon stocks. Our findings support this approach, as we observed

significantly higher labile carbon content in the spring where we previously found higher enzyme activity in the autumn (Kömlőd, Füzesgyarmat).

The glomalin content showed significant differences only in the Kömlőd plots during the spring sampling, where the ORG plot had significantly higher glomalin levels compared to the CONV plot. Literature reports that conservation tillage, organic farming, and cover crops increase glomalin content in soil. The trend of regenerative tillage positively influencing glomalin content was also observed in Kömlőd, consistent with findings by Kobierski et al. (2020). In Füzesgyarmat, the application of large amounts of organic manure may have resulted in higher glomalin content in the KONV plot.

Our results indicated that *nematode density* was higher in the ORG plots than in the CONV plots, but these differences were significant only in Kömlőd and Füzesgyarmat during the spring sampling. Nematode population analysis can serve as an indicator of soil health, as it responds quickly to changes in soil management systems. The composition of nematode populations is an important parameter in studies examining the effects of different soil management practices on soil health, as some nematodes are root-feeding species. Moreover, nematodes at various trophic levels are crucial for maintaining the balance of the soil food web. However, some studies have also reported increased nematode density as a result of soil regenerative methods (Henneron et al., 2016), a trend reflected in our study.

To determine whether the sustainable management systems we examined had an effect on soil health compared to conventional management, we standardized and statistically analysed the data from the plots. Based on our studies and analyses, we can conclude that there are statistically significant differences between the two management systems and methods (p=0.011). Among the measured parameters, three showed significant differences between the two management systems: aggregate stability (p=0.000), DHA enzyme activity in autumn (p=0.025) and spring sampling (p=0.048), and nematode density (p=0.032).

4. CONCLUSIONS AND RECOMMENDATIONS

Based on the short-term field trial, it can be concluded, that the soil regenerative methods integrated into organic farming cannot not show prompt and detectable changes in short (2-years) period, that were supported by literary data. It was only the enzyme activity, that could reveal significant differences in the conservation tillage plots, with cropping, compared to conventional tillage. This activity can indicate the potential improvements in other soil parameters in the future.

Among the studies, the on-farm experiment yielded the most useful results regarding soil regenerative practices. The three pairs of plots, comparing conventional traditional tillage with ecological regenerative farming, clearly demonstrated the relevance and legitimacy of more sustainable farming methods. Significant differences in sensitive indicators were already observable in favour of regenerative practices in most parameters, likely due to the combined application of various soil regenerative methods (conservation tillage and increased plant diversity) during the transition to organic farming. This assumption holds true when compared with existing literature.

After evaluating and comparing the results with literature, we can also conclude that regenerative methods can complement each other, and the negative effects of one method can be mitigated by the application of another. Minimizing tillage presents a significant challenge in organic farming; however, this issue can be alleviated through the use of cover crops and double cropping. Skilful techniques, such as inter-seeding or under-sowing cover crops with the main crop, can help avoid the challenges associated with cover cropping. A common reason for the unsuccessful application of cover crops is that their sowing period coincides with the driest and hottest time of year, following the summer crop harvest. In double cropping, cover crops are sown at a more favourable time, either simultaneously with or after the main crop, providing immediate cover after the main crop is harvested.

The farmer in Kömlőd successfully implemented double cropping methods on two of the plots we studied, allowing the land to remain covered with plants for one and a half years without skipping a year in main crop cultivation. This likely contributed to the significant differences we detected in soil health indicators when comparing the neighbouring conventional plot with traditional tillage.

The results also indicated that if a farmer employs one soil regenerative method but continues other practices that degrade the soil, the positive effects of the regenerative method may be completely negated. In Füzesgyarmat, the farmer using conventional tillage applied a large amount of organic manure annually; however, the anticipated positive effects were not reflected in the results. Instead, we measured better outcomes in the adjacent ecological farm. This discrepancy could be attributed to intensive and deep tillage, which may have obscured the benefits of the manure due to its detrimental structural impact.

The scientific evaluation of on-farm research is complicated by numerous variables; however, real agricultural conditions, particularly involving farmers whose livelihoods depend on their practices, can yield more grounded and practical scientific investigations. This is especially true when farmers have the opportunity to share their experiences. Analysis of samples from different municipalities suggests that the impact of regenerative methods varies across soil textures. Specifically, when assessing soil health, the physical type of soil is more crucial than the agricultural practices applied to it. Most parameters we examined showed differing correlations across soils of varying physical types.

Interviews with farmers in our study revealed that they too have observed positive effects of regenerative methods on their soils. For instance, areas that previously experienced waterlogging no longer exhibit this issue due to altered soil management practices. Additionally, the use of cover crops has resulted in greater moisture retention in the soil and reduced deflation and erosion damage.

A clear advantage of organic farming conditions—characterized by minimized external inputs and increased reliance on natural resources—is that they render farmers less vulnerable. Reduced input use and minimal tillage lower expenses. Although farmers noted a decrease in crop yields after transitioning to organic practices, this was offset by the ability to sell their products at higher prices. Diversifying crop rotations mitigates economic loss during extreme weather years, as a greater variety of plants increases the likelihood of having crops that are

less affected by conditions like drought. Moreover, minimizing inputs means that even in poor yield years, the economic impact on the farm budget is less severe.

From the interviews, we can also conclude that developing a suitable machinery equipments are essential for implementing regenerative methods, as traditional tools may limit their application. Farmers with larger fields find it easier to adopt these methods, as they typically have access to better machinery that facilitates soil-friendly practices. For instance, the conventional farm in Füzesgyarmat, which have several hundred hectares, may show better or similar results in some parameters compared to the organic farm that has been operational for 20 years, due to access to modern machinery capable of performing multiple operations efficiently, including easy incorporation of crop residues into the soil.

The conversations also indicated that farmers need to acquire significant information to implement regenerative methods, which requires a higher level of self-improvement and consumes time. Patience and time are necessary for farmers to successfully adapt the techniques they have learned to their own fields.

Considering the indicators, we can conclude that more stable parameters, such as Soil Organic Matter (SOM), humus quality, and pH, are not sensitive enough to soil management practices in the short term (within 3-5 years) periods. In our on-farm experiments, regenerative soil management showed significantly higher values in several parameters, yet no significant differences in organic matter levels were observed across most soils. The only exception was found in a field that had been under ecological management for 20 years (Öko-sekély1), where slightly higher average values were noted even in areas that transitioned to regenerative practices within 3-5 years. Our findings corroborate many studies suggesting that SOM is a good long-term indicator of changes in soil management.

Humus quality did not demonstrate a strong correlation with humus content. In the case of Füzesgyarmat, we obtained a more complex picture of soil conditions, where comparable humus content yielded much lower humus quality measurements. Therefore, humus quality can provide valuable supplementary data. As an indicator of soil health, humus quality may effectively reflect the impacts of soil management over the long term, although the physical type of soil significantly influences measurement outcomes. Regarding pH, we observed striking results in preliminary studies; however, in both the experiment and on-farm research, this parameter did not show differences among various soil management practices in the short term. In the Füzesgyarmat samples, which have been under a different management system for 20 years, significant changes in pH were evident. Thus, pH may serve as a suitable long-term indicator for changes occurring in the soil.

Soil moisture is a straightforward measurement closely linked to soil structure and organic content. It is essential not only as a research parameter but also as a key goal of soil conservation practices. Maintaining soil moisture is crucial for cultivation, especially in mitigating the effects of climate change. In our research, it proved to be a reliable parameter, showing strong correlations with most measured parameters in preliminary studies and also in on-farm research in Kömlőd and Szár. Measurements taken at two different sampling times consistently showed positive correlations across all municipalities. However, extreme soil textures demonstrated fewer clear correlations with other parameters. Notably, we recorded significantly higher results

for regenerative soil practices in Szár and Füzesgyarmat, indicating its potential as an early indicator.

One limitation is that moisture can only serve as an indicator when a control area is available nearby; it is not applicable for monitoring without this context. Aggregate stability is considered a good indicator for assessing the effects of differing soil management practices, and our results supported this. We evaluated aggregate stability from two perspectives: as a soil parameter and as a measurement method. The SLAKES smartphone application, which was recently introduced in 2023, was applied only in the on-farm trial. The measurement is straightforward and requires minimal equipment. This investigation provided the most significant results in our on-farm research, demonstrating the strongest significance level in differentiating between fields that transitioned to ecological practices within 3-5 years and those under conventional management.

Correlation studies suggest no necessarily direct connection between traditionally recognized indicators and those functioning effectively in our research, particularly among soils with higher clay fractions (humus quantity and quality, enzyme activity). This feature is advantageous, as aggregate stability may detect changes in the soil before other parameters do. Correlations also indicated that it positively correlates with nematode density, bacterial quantity, and dehydrogenase activity. This suggests that it is more closely associated with sensitive, rapidly changing parameters, a finding supported by literature. Our results, particularly from the Random Forest analysis, indicated that aggregate stability is sensitive to management effects, often showing the lowest index values in traditionally tilled soils.

The labile carbon, a form of organic matter that changes rapidly in the soil, may serve as an early indicator of the impact of changing management practices, as our research demonstrated. In the on-farm trial, we observed significant variations due to different soil management practices, with differences evident even within 3-5 years. However, results from autumn and spring sampling times showed considerable variability, and correlation studies revealed no correlation between the two sampling periods for labile of carbon. This may result from the influence of short-term practices, such as crop residue management, which can show differences even within a single growing season. When comparing results or monitoring soil development, careful selection of sampling times or repeated measurements is crucial.

Dehydrogenase enzyme activity (DHA) was measured in all four studies. Based on the findings, we found this indicator to be the most suitable for detecting changes arising from soil use. In the short-term trial, DHA was the only measurement that showed significant differences in response to regenerative practices compared to conventional methods. In the pot trial, it demonstrated varying effects based on plant species' enzyme activity. The on-farm trial also revealed differences among treated fields. Correlation studies showed positive correlations with most parameters, maintaining high correlation across sampling times. The results consistently indicated correlations based on DHA measurements, although differences between fields were significant only in one sampling, as various soil management interventions may temporarily mitigate differences in enzyme activity.

Therefore, careful selection of *sampling times is essential* for DHA; ideally, it should occur after a period free from recent soil disturbances. Regarding glomalin content, it provided

consistent data, and correlation results indicated positive relationships with several other parameters. Among sensitive soil parameters, glomalin showed the least significant differences in the on-farm study. Our research did not support the established correlations in the literature suggesting that glomalin presence is related to aggregate stability, as we recorded higher values on a field in Füzesgyarmat compared to its neighbour, even when most other correlated parameters showed lower values. In light of this, I regard glomalin as a conditional indicator, potentially revealing significant differences between differing soil management practices only over periods exceeding five years and with repeated measurements.

Based on our findings, *nematode density* proved to be a better indicator than expected according to literature. Most studies examine nematode composition, often not considering quantitative parameters as crucial indicators. Unfortunately, we could only apply this in the on-farm trial, limiting comparisons with other studies. Nonetheless, in our research, it served as a significant indicator, consistently providing correlation data. Its results were less associated with persistent indicators but consistently correlated with bacterial quantity in all measured instances and also with aggregate stability in several cases. Thus, it appears to be more strongly correlated with rapidly changing parameters, especially other biological indicators. In analysing the *statistical data* from the three pairs of fields, where conventional tillage was compared with ecological and regenerative systems, *nematode density* was one of three parameters showing significant differences, with trends evident in both sampling periods. This suggests it is an undervalued indicator with greater potential than currently recognized in the literature.

Nonetheless, in line with existing research, it may be advisable to supplement *nematode density* data with information about its population composition. The Baermann funnel method requires only a simple stereo microscope and a few easily accessible tools, making it feasible for farmers to conduct their measurements without any laboratory background

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5. NEW SCIENTIFIC RESULTS

- 1. **On-farm Research Findings.** During the on-farm research conducted at various locations across the country, I found that the transition to ecological farming, complemented by regenerative soil management practices such as conservation tillage, increased plant diversity, and the use of cover crops demonstrated significant differences in most of the studied parameters compared to conventional tillage practices, even in the short term.
- 2. **Indicator Sensitivity**: The dehydrogenase enzyme activity (DHA), labile carbon content (POXC), abundance of nematodes, aggregate stability, and soil moisture content are sensitive indicators; capable of reflecting the positive effects of regenerative soil management practices, even in the studied short-term period.
- 3. **DHA as an Early Indicator:** Based on the results of four different experiments, the measurement of dehydrogenase enzyme activity (DHA) is the most useful early indicator, that is supported by Hungarian background data. The DHA indicated the positive impacts of transitioning to regenerative methods as early as two-years-periods, providing a solid basis for comparing accurately of the various farming practices.
- 4. **Aggregate Stability:** I found that aggregate stability reflects differences between various agricultural practices independently from basic physical characteristics of the soil. The values of aggregate stability were most influenced by soil management practices, which distinguishes this finding from other studies. The employed measurement method (SLAKES test) proved to be straightforward and simple, it did not require laboratory conditions. This method can be recommended for the direct use by farmers.
- 5. **Sampling Timing:** I established that the suggested indicators, including DHA enzyme activity and labile carbon, are particularly sensitive to soil interventions, highlighting the importance of carefully selecting sampling times. I demonstrated that a well-developed timing is necessary for the proper comparative studies. It is suggested at late spring, as the most suitable sampling time, as soils are typically in an undisturbed state for a longer period at that time.
- 6. **Effect of Cover Crop Species:** Based on the pot experiment, I found that among the chosen cover crop species, the leguminous, N₂-fixing faba bean and its mixture in the used other five plant species had the most positive effects on soil health in terms of enzyme activity (DHA, FDA), glomalin content (GRSP), and nutrient retention (-NO₃, -NH₄, EC). Therefore, I recommend their use as cover crops in ecological farming practices.

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7. KEY PUBLICATIONS AND SCIENTIFIC ACTIVITIES RELATED TO THE THESIS

Journal articles (IF)

Kabalan, S., Juhos, K., **Tóth, E**., & Biró, B. (2022). Impact of symbiotic mycorrhiza interrelation in some soil biological parameters and growth of five cover crops. *Agrokémia és Talajtan*. 71(1): 135-147. - Q4, IF=0,151

Kabalan, S., Kovács, F., Papdi, E., **Tóth, E.**, Juhos, K., Biró, B. (2024): Residues of symbiont cover crops improving corn growth and soil-dependent health parameters. *Agriculture*, 14, 1601. - Q1 IF=3,3

Tóth, E., Csambalik, L., Biró, B., Gere, A., Koren, D., Kotroczó, Zs., Szalai, Z. (2024): Are the Nutritional Properties of Organic Tomatoes Altered by Single and Combined Microbial Soil Inoculants? A Multiperspective Approach. *J. Plant Growth Regulation* (on line available) – Q1 IF:3,35

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Journal articles (lectored, refereed, without IF)

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