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# Effect of mineral and organic fertilizers on some soil chemical and biological properties in a 90-year-old long-term experiment

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## Abstract

The Westsik's long-term crop rotation experiment was set up in 1929 at the Nyíregyháza Experimental Station (NE Hungary) on a slightly acidic Arenosol. Besides fallow crop rotation (CR), effects of different organic amendments (lupine as green manure, lupine as main crop, straw manure, and farmyard manure (FYM) were studied with or without N or NPK-fertilizers. The crop rotation consisted of rye, potato, lupine, and oat with common vetch. The soil of potato plots was analysed in 2019 at the 90th anniversary of Westsik's crop rotation experiment.

The following chemical and microbiological soil parameters were determined: soil pH, available nutrient contents, organic carbon (OC) and nitrogen (ON) contents, microbial biomass carbon (MBC) and nitrogen (MBN), soil respiration, net nitrification, and activity of some soil enzymes.

In the CRs, the soil $pH_{H2O}$ varied from acidic to weakly alkaline and it largely differed from $pH_{KCl}$. The results showed a significant increase in the content of nitrate, available phosphorus and potassium in most of the fertilized plots. Applying straw, green manure, or FYM significantly increased the OC and ON contents. The total count of cultivable bacteria increased upon the application of the organic manures. Combined application of straw manure and N-fertilization heavily improved the abundance of the microscopic fungi.

While all the applied organic manures significantly enhanced the MBC, the MBN increased only by the green manure amendment. Our results revealed higher soil respiration rate in the plots receiving straw or FYM than in the control. Both green manure and FYM elevated the net nitrification rate. Phosphatase, saccharase, urease, and dehydrogenase enzymes showed a hesitating response to the manure application in the different CRs.

The soil respiration and dehydrogenase activity correlated to most of the measured chemical parameters. Among microbiological properties, the MBC and MBN, as well as dehydrogenase and other enzyme activities displayed a positive correlation. Results proved the need for the exogenous application of organic matter in the form of organic manures to enhance the nutritional status and health of the soil.

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## Introduction

One of the greatest challenges nowadays in crop production is to maintain soil fertility to ensure the production of an appropriate amount of feed and food raw materials of good quality. Natural and ecological conditions (climate, climate change, soil formation, parent material) are less controllable factors for farming, but agro-technical processes (nutrient supply, use of chemicals) can be professionally selected for more successful productivity (SÁNDOR, 2006). Experiments older than 20 years can be termed long-term experiments (RASMUSSEN et al., 1998) studying crop production, nutrient cycling, and the environmental effects of agriculture. Long-term experiments are suitable for monitoring the effects of regular treatments on soil's physical, chemical, and biological properties (GEISSELER & SCOW, 2014; HUNGRIA et al., 2009). The balanced supply of minerals and organic matter (e.g., plant residues, manure, green manure, compost, etc.) added into the soil improves the nutrient cycling, dynamics, abundance, and activity of soil microorganisms. They contribute directly and indirectly to the growth and development of plants (HICKS et al., 2020; KÁTAI, 1999, 2006; KÁTAI et al., 2014, 2020; SZILI-KOVÁCS et al., 2009, 2011; ZHAO et al., 2013), where the degraded organic matter acts as a nutrient source for the soil heterotrophic organisms. Another positive effect of soil organic matter supply and its transformation is the part of the organic matter which is converted to humus, which can, consequently, improve the soil-water-air relationship (MEURER et al., 2020).

The intensity of the organic matter conversion in soils depends on the humus content and the activity of soil organisms, which are decisively influenced by the soil properties, the environmental factors, and the applied agro-technologies (FÜLEKY & RAJKAINÉ, 1999). ANDERSON (2003) attributed the sustainability of soil fertility to the living soil organisms taking part in the organic matter decomposition.

In long-term experiments, the dual application of mineral fertilizers and different organic materials was the most effective for soil biochemical processes (BÖHME et al., 2005; INUBUSHI et al., 2020; SIMON & CZAKO, 2014). The beneficial effect could be due to the long-term stimulative effect of the combined nutrient replenishment.

Sandy soils generally have low nutrient content and usually belong to arid and semi-arid regions. In these soils, successful crop production, with higher crop yields, requires proper management practices with the complex utilization of mineral nutrients and organic matter, as well as a balanced water supply (MAYER et al., 2015).

The Westsik's Crop Rotation Experiment on sandy soil (Arenosol) in Nyíregyháza, Hungary, uniquely models the impact of fallow, straw, farmyard, and green manure application, as well as chemical fertilization on soil fertility. The obtained results provided important information for the sustainable farming (LAZÁNYI, 2001; ISZÁLYNÉ, 2007; ROMHÁNYI et al., 2012). Besides reporting the variations in chemical properties of soil, yield information was also reported by HENZSEL et al. (2011).

The present study aimed to conduct a complex chemical and microbiological examination of the soil in the 90-year-old experiments. Therefore, chemical, biochemical, and microbiological traits of soil were measured. Correlations between the measured soil properties were statistically calculated.

## Materials and methods

*Westsik's long-term crop rotation experiment and soil sampling*

The Westsik's long-term fertilisation experiment was established in 1929 with 12 crop rotations at the Nyíregyháza Experimental Farm (Nyíregyháza, Hungary, the predecessor of the University of Debrecen, IAREF, Research Institute of Nyíregyháza). Nowadays, altogether 15 crop rotations (CR) have still been maintained (LAZÁNYI, 2001, HENZSEL, 2012). *Table 1* shows the description of the 15 CRs.

Briefly, the 15 CRs were: CR I was used as periodical fallow in the experiment and as a control in data evaluation. In CR II lupine was the main crop and it was cultivated for the green manure. In CR III lupine was cultivated for the grain yield. In CRs IV, V, VI and VII straw manure was added to the soil, CR VIII lupine was cultivated for the grain yield and as a second crop for green manure. In CR IX lupine was cultivated as a fodder, CRs X and XI received farmyard manure, CRs XII, XIII, XIV, and XV were based on secondary green manure cultivation.

The CRs I, VII, X, and XV did not receive any chemical fertilizers at any growth stage. The fertilized CRs received a total of 94 kg $ha^{-1}$ of $P_2O_5$ and 84 kg $ha^{-1}$ of $K_2O$-fertilizer in every three and four years, respectively. We applied lower dose of N for the CRs II, III, XI, and XII at a rate of 43 kg $ha^{-1}$ (spread over 3 years). We added higher dose of N (86 kg $ha^{-1}$, spread over 3 or 4 years) to the CRs VIII, IX, XIII and XIV. We applied the largest amount of N to the CRs IV, V, and VI amended with straw at a rate of 108 kg $ha^{-1}$, spread over 3 years.

The soil type of the experiment is Lamellic Arenosol, Dystric (WRB 2015) with low humus content. The texture of the soil is sand, with 89.13% sand, 2.21% loam, and 8.66% clay (HENZSEL, 2012). Potato was cultivated in every crop rotation. Soil samples were taken from the experimental plots from the 2–20 cm layer, on 27 June 2019 at the flowering stage of potato (BBCH 65). In every crop rotation, a composite soil sample of 15 subsamples collected from about 20 $m^2$ in front of the hill, split into three subsamples.

*Determination of chemical and microbiological soil characteristics*

Collected soil samples were transported immediately to the laboratory and sieved by <2 mm sieve. Moisture content was measured by drying the soil at 105 $^{o}$C for 24h (BUZÁS, 1988). Soil pH was measured in 1:2.5 ratio of soil:water and soil:1M potassium chloride (KCl) suspensions, respectively (MSZ 08-0206/2:1978). The $NO_3^-$-N, ammonium lactate (AL) soluble phosphorous and potassium contents were determined according to the Hungarian Standard MSZ 20135:1999. Soil organic carbon (SOC) was estimated according to the MSZ-08-0210:1977, while organic nitrogen (ON) content was measured based on the MSZ-08-0458:1980.

*Table 1*
*Treatments of the Westsik's long-term experiment in Nyíregyháza*

| Crop Rotations ID | Organic fertilization of the crop rotation | Applied mineral fertilizers to the model plant, potato* |
|---|---|---|
| I | fallow | no fertilizers |
| II | lupine green manure as a main crop | N-fertilizer |
| III | lupine for grain | N-fertilizer |
| IV | straw manure | N, P, K-fertilizers |
| V | fermented straw manure with N | N, P, K-fertilizers |
| VI | fermented straw manure with water | N, P, K-fertilizers |
| VII | fermented straw manure with water | no fertilizers |
| VIII | lupine green and root manure | P, K-fertilizers |
| IX | lupine for green forage | N-fertilizer |
| X | farmyard manure | no fertilizers |
| XI | farmyard manure | N-fertilizer |
| XII | lupine green manure as a second crop | N-fertilizer |
| XIII | lupine green manure as a second crop | P, K-fertilizers |
| XIV | lupine green manure as a second crop | P, K-fertilizers |
| XV | lupine green manure as a second crop | no fertilizers |

**Ratios of chemical fertilizers are described within the text.*

The microbial biomass carbon (MBC) and nitrogen (MBN) were measured by chloroform fumigation-extraction method (VANCE et al., 1987). The $CO_2$-production was determined after a 10-day incubation period according to ÖHLINGER (1996). The net nitrification rate was measured after 14-day incubation with a reagent of Na-salicylate, same way as by the analysis of $NO_3^-$-N content of soil (Hungarian Standard MSZ 20135:1999). The total number (Colony Forming Unit, CFU) of bacteria and quantity of microscopic fungi were determined by plate dilution method according to SZEGI (1979) on Buillon plate and pepton-glucose agar, respectively. The CFU was counted with a Leica-type manual counter. The saccharase activity was measured by the method of FRANKENBERGER & JOHANSON (1983), while urease enzyme activity was quantitatively determined according to SZEGI (1979). Along with the determination of phosphatase activity, the hydrolysed phosphoric acid was measured colorimetrically by KRÁMER-ERDEINÉ (SZEGI 1979) at the original soil pH, after hydrolysis of disodium phenyl phosphate. Dehydrogenase activity was determined based on the reduction of the used artificial hydrogen acceptor (MSZ-08-1721-3:1986).

*Statistical evaluation*

The means, standard deviation and variance analyses (followed by Post Hoc Multiple Comparisons and Duncan test; $p \leq 0.05$) were statistically calculated with SPSS 27.0 program. *Pearson's correlation* analyses $(n = 45)$ were used to highlight the relationships between soil properties at 0.01 level.

## Results

Results of soil moisture, pH and mineral nutrient content are shown in *Table 2*. Soil moisture content was extremely low at the sampling time and ranged between 3.88–8.73%. Most of the soil samples had a moisture content between 4–6%.

Based on the results of soil $pH_{H2O}$, seven soil samples were acidic (CRs I, II, III, IV, VI, VIII, XIV), four were slightly acidic (CRs V, VII, IX, X), one was neutral (CR XV), and three were slightly alkaline (CRs XI, XII, XIII). The soil $pH_{KCl}$ values were lower by 0.92–1.75 pH units than $pH_{H2O}$.

Concentrations of the available nutrients in the non-fertilized CRs (CRs I, VII, and XV) were significantly lower than in the other CRs (*Table 2*).

The soil $NO_3^-$-N content increased significantly in the CRs II, III, IV, VI, and XI. Among them, extremely high values were measured in the CRs II, VI and XI compared to the control (CR I). Soil $NO_3^-$-N content was significantly lower in the CRs IX, X, and XV. Low soil $NO_3^-$-N content was determined in the secondary green manure crop rotations (e.g., CRs XII, XIII, and XV).

The lowest available P content corresponded to the CR XV. The 11 CRs fertilized with P showed 2–4.5 times higher P content than the CR I. The highest P content linked to the CR XI, where N and+FYM were applied. This nutrient supply resulted in a 6.5-time higher soil P content than the CR I.

The lowest K content corresponded to non-fertilized CRs I, VII, and XV. The soil K content significantly increased in ten CRs compared to the control, from which three CRs (i.e., CRs III, X and XI) showed 3–5 times higher K content.

*Table 2*
*Moisture content and some chemical characteristics of soils from Westsik's crop rotation experiment*

| CRs | Moisture content % | pH $H_2O$ | pH KCl | $NO_3$ mg kg$^{-1}$ | AL $P_2O_5$ mg kg$^{-1}$ | AL $K_2O$ mg kg$^{-1}$ |
|---|---|---|---|---|---|---|
| I | 7.71 f | 5.15 b | 4.39 b | 23.73 de | 64.36 b | 31.35 ab |
| II | 4.32 b | 4.79 a | 3.93 a | 117.61 j | 160.93 h | 47.92 d |
| III | 8.22 g | 5.10 b | 3.85 a | 33.10 g | 136.41 f | 102.12 g |
| IV | 4.40 b | 5.07 b | 3.92 a | 40.32 h | 221.27 j | 43.94 d |
| V | 8.73 h | 5.85 c | 4.51 bc | 23.44 de | 133.28 e | 33.81 bc |
| VI | 4.82 c | 5.79 c | 4.87 d | 137.13 k | 144.84 g | 45.69 d |
| VII | 5.67 d | 6.26 e | 4.51 bc | 27.93 fg | 66.64 b | 34.92 bc |
| VIII | 6.65 e | 5.09 b | 3.81 a | 19.21 cd | 116.68 d | 55.21 e |
| IX | 4.39 b | 6.08 d | 4.61 c | 23.77 de | 112.98 c | 43.04 d |
| X | 4.53 bc | 6.35 e | 4.93 d | 16.72 bc | 139.97 fg | 161.19 h |
| XI | 3.88 a | 7.45 i | 6.51 h | 48.73 i | 419.97 l | 157.28 h |
| XII | 5.70 d | 7.27 h | 7.20 g | 11.04 ab | 273.34 k | 37.59 c |
| XIII | 4.63 bc | 7.86 j | 6.31 g | 14.55 abc | 204.68 i | 44.34 d |
| XIV | 5.34 d | 6.69 f | 5.15 e | 18.20 cd | 200.39 i | 67.00 f |
| XV | 6.52 e | 6.93 g | 5.56 f | 8.81 a | 33.48 a | 26.92 a |

*Means followed by different letters in the same columns are statistically significant at $p < 0.05$.*

*Soil organic matter and its degradation*

Low content of SOC, measured in the CRs III, VII, and IX ranged between 4.32–4.53 g $kg^{-1}$ soil (*Table 3*). The CRs (IV, V, and VI) which received straw manure showed high SOC contents ranging between 7.54–7.78 g $kg^{-1}$ soil. The FYM and secondary lupine treatments (the CRs X-XIV) in combination with chemical fertilizer resulted in the highest SOC content (7.77–9.76 g $kg^{-1}$ soil).

The ON content of soils ranged between 0.56–1.02 g $kg^{-1}$ soil. The lowest ON was measured in the CR I, while the highest ON content was determined in the FYM + fertilizers treated CR XI. Soils can be divided into two groups based on their ON content: one group (CRs I–V and XV) where ON content ranged between 0.56–0.63 g $kg^{-1}$ soil and the other group included the CRs VI-XIV, where the ON content ranged between 0.68–1.02 g $kg^{-1}$ soil. Similar to the SOC, the straw, FYM and different lupine treatments (CRs VI–XIV) combined with fertilizers provided higher ON content than the other treatments.

Based on the soil MBC, the CRs can be divided into three groups. First group included the CRs I, III, IV, V, VI, and VIII with low MBC values ranging between 71.00–87.67 μg C $g^{-1}$ soil. Second group included the CRs II, XI, XIII, and XIV with MBC of 103.33–149.33 μg C $g^{-1}$ soil. Third group included the CRs II, XI, XIII, and XIV with an MBC ranging from 170.33-213.33 μg C $g^{-1}$. In the CRs, where lupine was used as green manure, the soil MBC was usually higher than in the other CRs.

Based on the MBN results, the CRs could be grouped in three categories. The CRs I, V, VI, VII, X, and XV showed the lowest MBN values (<2.02 μg N $g^{-1}$ soil). The CRs IV, IX, XI, and XII had MBN contents ranging between 2.28–2.60 μg N $g^{-1}$ soil. The CRs II, III, VIII, XIII, and XIV displayed the highest content of MBN that ranged between 3.8–6.20 μg N $g^{-1}$ soil. Based on both the MBC and MBN results, the CRs I, II, V, VI, IX, XII, XIII, and XIV belonged to the same category.

The $CO_2$ production of soils indicated that the various forms of applied organic manures resulted in different rates of $CO_2$ emissions. This might be caused by the degradation of OM in various ways and rates. Similar $CO_2$ emission of CR I was found in the CRs VIII, IX, X, and XV. The other CRs showed significantly higher $CO_2$ emissions ranging from 127.1–139.7 mg $CO_2$ $kg^{-1}$ soil. Outstanding $CO_2$ production was measured in the fermented straw and FYM treated CRs (VI and XI). High $CO_2$ production was measured in the straw-treated CRs (IV–VII) and green manure-treated CRs (XII–XIV).

The nutrient stock of soil was low in the experiment, so the net nitrification was also low ranging between 2.53–19.34 mg $kg^{-1}$ NO3$^{-}$, as expected. Slightly higher or lower net nitrification values corresponded to the CRs IV, V, VII, and XIV compared to the CR I. The net nitrification was significantly higher in the CRs applying green manure (CRs II, III, XII) and fermented straw (CR VI) with values between 13.60–19.34 mg $kg^{-1}$ nitrate.

*Table 3*
*Some parameters of soil C and N cycles from Westsik's experiments*

| CRs | Hu % | OC (g C $kg^{-1}$) | ON (g N $kg^{-1}$) | MBC (μg C $g^{-1}$) | MBN (μg N $g^{-1}$) | $CO_2$ (mg $kg^{-1}$ 14 $days^{-1}$) | Net nitrification (mg $NO_3$ $kg^{-1}$) |
|---|---|---|---|---|---|---|---|
| I | 0.91 b | 5.18 c | 0.56 a | 72.33 a | 2.02 ab | 115.9 ab | 3.88 ab |
| II | 0.91 b | 5.23 c | 0.62 b | 173.00 e | 5.42 d | 135.2 e | 17.34 h |
| III | 0.77 a | 4.50 b | 0.58 a | 87.67 a | 4.82 d | 122.7 bc | 16.07 h |
| IV | 1.30 e | 7.54 f | 0.62 b | 77.00 a | 2.28 ab | 127.1 cd | 4.13 ab |
| V | 1.38 f | 7.69 f | 0.63 b | 83.33 a | 1.48 a | 132.8 de | 5.03 bc |
| VI | 1.38 f | 7.78 fg | 0.71 cd | 71.00 a | 1.64 a | 155.3 g | 19.34 i |
| VII | 0.77 a | 4.32 a | 0.70 cd | 128.33 c | 2.01 ab | 138.2 f | 5.38 bc |
| VIII | 1.25 d | 6.96 e | 0.78 g | 72.00 a | 3.80 c | 114.9 a | 11.71 f |
| IX | 0.79 a | 4.53 b | 0.74 f | 149.33 d | 2.60 b | 119.0 ab | 8.15 de |
| X | 1.36 f | 7.77 g | 0.71 ef | 105.33 b | 1.65 a | 118.3 ab | 6.64 d |
| XI | 1.62 h | 9.33 i | 1.02 h | 170.33 e | 2.39 ab | 151.8 g | 6.57 cd |
| XII | 1.37 f | 7.89 g | 0.81 g | 146.67 d | 2.45 ab | 139.7 f | 13.60 g |
| XIII | 1.71 i | 9.76 j | 0.72 ef | 191.33 f | 6.20 dc | 134.5 e | 6.17 cd |
| XIV | 1.48 g | 8.37 h | 0.68 c | 213.33 g | 5.06 d | 130.2 de | 2.53 a |
| XV | 1.08 c | 6.31 d | 0.63 b | 103.33 b | 1.63 a | 118.3 ab | 9.45 e |

*Means followed by different letters in the same columns are statistically significant at $p < 0.05$*

*Microbial counts and enzyme activities in the soil*

The total number of bacteria varied between 2.64–13.36 x $10^6$ CFU $g^{-1}$ soil (*Table 4*). Similar to the CR I, a low number of bacteria was determined in the CRs II, IV, VIII, and XIII. Significantly higher total bacterial numbers were counted in the other CRs ranging from 5.27–13.36 x $10^6$ CFU $g^{-1}$ soil. The highest bacterial count (13.36 x $10^6$ CFU $g^{-1}$ soil) can be linked to the CR XII, where N, P, and K- fertilizers and secondary sown lupine were applied.

In ten CRs of the experiment, the population of microscopic fungi varied between 11.67–19.00 x $10^3$ CFU $g^{-1}$ soil; however, the control (CR I) displayed a 16.00 x $10^3$ CFU $g^{-1}$ soil. The CRs II, IV, VI, and VIII exhibited significantly higher population of microscopic fungi ranging between 30.33–78.33 x $10^3$ CFU $g^{-1}$ soil.

Phosphatase activity ranged between 12.65-27.79 mg $P_2O_5$ $g^{-1}$ soil $2h^{-1}$ (*Table 4*). The enzyme activities in the CRs VII and XII were similar to CR I as they ranged between 17.35–18.80 mg $P_2O_5$ $g^{-1}$ soil. The activity decreased significantly in the CRs II, IV, V, and VI; nevertheless, increased activities corresponded to the CRs III, VIII, IX, X, XI, XIV, and XV.

The activity of the saccharase enzyme fluctuated between 22.1–61.3 mg glucose $kg^{-1}$ soil $24h^{-1}$. A significant decrease was noticed in the CRs II, III, and VIII compared to the CR I. Saccharase activity increased significantly in the CRs V, X, XIV, and XV, while the other seven CRs did not significantly differ.

The urease activity varied over a wide range (676.2–1323.4 mg $NH_4^+$ $kg^{-1}$ soil). There was a small but significant decrease compared to the control (CR I) in the CRs III, IV, VII, VIII, and IX, in the group amended with straw and green manure. The urease activity significantly induced in the other CRs, especially where root, green, and FYM manures were applied (CRs II and X–XV).

Activity of soil dehydrogenase enzyme significantly increased in all the fertilized treatments. Approximately, 1.5–2-fold increase in dehydrogenase activity was determined in eight CRs (i.e., CRs V, VII and from X to XV) compared to the CR I.

*Table 4*
*Population dynamics and some enzymes activity from Westsik's experiments*

| CRs | Number of bacteria ($\times 10^6$ CFU $g^{-1}$ soil) | Microscopic fungi ($\times 10^3$ CFU $g^{-1}$ soil) | Phosphatase (mg $P_2O_5$ $g^{-1}$ $2h^{-1}$) | Saccharase (mg glucose $kg^{-1}$ soil $24h^{-1}$) | Urease (mg $NH_4^+$$kg^{-1}$ soil $2h^{-1}$) | Dehydrogenase (µg INTF $g^{-1}$soil $2h^{-1}$) |
|---|---|---|---|---|---|---|
| I | 2.64 ab | 16.00 a | 17.35 d | 41.7 de | 877.5 d | 34.97 a |
| II | 2.77 ab | 30.67 b | 12.65 a | 25.4 ab | 1072.3 i | 47.30 c |
| III | 11.05 f | 15.00 a | 20.83 e | 22.1 a | 755.1 b | 48.75 c |
| IV | 3.45 abc | 78.33 d | 16.09 c | 35.1 cd | 751.9 b | 40.44 b |
| V | 7.59 d | 19.00 a | 14.48 b | 56.4 f | 937.0 e | 59.80 d |
| VI | 5.27 c | 68.67 c | 16.78 c | 44.7 e | 930.6 e | 41.89 b |
| VII | 9.50 de | 13.00 a | 18.80 de | 47.6 ef | 785.7 c | 73.99 f |
| VIII | 1.77 a | 30.33 b | 21.91 ef | 31.9 bc | 753.5 b | 59.36 d |
| IX | 9.95 e | 18.67 a | 22.63 ef | 41.7 de | 676.2 a | 47.40 c |
| X | 8.05 de | 18.67 a | 26.54 f | 61.3 g | 1323.4 l | 78.34 g |
| XI | 8.00 de | 14.00 a | 21.38 ef | 46.6 ef | 1202.7 k | 72.30 f |
| XII | 13.36 g | 10.33 a | 14.54 b | 36.7 cd | 1003.0 g | 60.50 de |
| XIII | 3.82 bc | 11.67 a | 17.54 d | 45.6 ef | 999.8 f | 74.97 f |
| XIV | 8.32 de | 16.00 a | 27.79 g | 51.5 f | 1035.2 h | 67.70 e |
| XV | 9.64 de | 12.67 a | 20.16 e | 53.9 f | 1156.0 j | 62.50 de |

*Means followed by different letters in the same columns are statistically significant at $p < 0.05$*

According to the results presented in *Tables 2–4*, only the CR XIV showed positive changes recording higher values for all the measured parameters. In this CR, the secondary sown lupine and NPK were jointly applied. The second most successful nutrient supply technique was the combined FYM and N-fertilization (CR XI). Only five soil parameters changed positively in the CR V, which seems to be the least successful nutrient supply method.

*Correlations between soil chemical and microbiological properties*

Positive and negative statistical correlations were found among soil chemical and microbiological properties (*Table 5*). The $CO_2$ emission and the dehydrogenase activity correlated with most of the chemical parameters. All correlations were positive, except between dehydrogenase activity and $NO_3^-$-N content. In contrast, net nitrification correlated only with $NO_3^-$-N content. The CFU of soil bacteria positively correlated with pH, while negatively with $NO_3^-$-N. The CFU of microscopic fungi negatively correlated with pH and positively with $NO_3^-$-N. Phosphatase activity positively correlated with K content and negatively with $NO_3^-$-N. Saccharase and urease activities positively correlated with soil pH and OC; moreover, urease activity positively correlated with AL-$P_2O_5$.

*Table 5*
*Correlations between soil chemical and microbial properties.*
*Correlation significant at 1%-level; n = 45*

| Soil properties | pH $H_2O$ | pH KCl | $NO_3^-$-N | AL phosphorus | AL potassium | Organic carbon | Organic nitrogen |
|---|---|---|---|---|---|---|---|
| $CO_2$-emission | | 0.439 | 0.562 | 0.575 | | 0.418 | 0.469 |
| Net nitrification | | | 0.608 | | | | |
| CFU of bacteria | 0.433 | 0.423 | –0.386 | | | | |
| CFU of fungi | –0.515 | –0.407 | 0.573 | | | | |
| Phosphatase | | | –0.412 | | 0.547 | | |
| Saccharase | 0.560 | 0.468 | | | | 0.442 | |
| Urease | 0.424 | 0.484 | | | 0.525 | 0.617 | |
| Dehydrogenase | 0.545 | 0.491 | –0.448 | | 0.435 | 0.574 | 0.495 |

The MBC positively correlated with the MBN, while negatively correlated with net nitrification (*Table 6*). Saccharase activity negatively correlated with net nitrification and MBN, while positively with urease and dehydrogenase activity. Dehydrogenase activity negatively correlated with CFU of fungi and positively with phosphatase, saccharase, and urease activity. Negative correlation was reported between CFU of bacteria and microscopic fungi.

*Table 6*
*Correlations among the soil microbiological properties.*
*Correlation significant at 1%-level; n = 45*

| Soil properties | Net nitrification | MBC | MBN | CFU of bacteria | CFU of fungi | Phosphatase | Saccharase | Urease | Dehydroge-nase |
|---|---|---|---|---|---|---|---|---|---|
| Net nitrification | ------- | –0.409 | | | | | | | |
| MBC | –0.409 | ------- | | | | | | | |
| MBN | | 0.505 | -------- | | | | | | |
| CFU of bacteria | | | | -------- | | | | | |
| CFU of fungi | | | | –0.401 | ---- | | | | |
| Phosphatase | | | | | | ------ | | | |
| Saccharase | –0.541 | | –0.468 | | | | ----- | | |
| Urease | | | | | | | 0.509 | ------ | |
| Dehydrogenase | | | | | –0.533 | 0.429 | 0.532 | 0.541 | ------ |

## Discussion

In long-term experiments, the same treatments have been applied for decades. The real effects of different treatments considering slow changes in some soil properties, and the cumulative effects are expected after long time. Therefore, long-term experiments help us to understand the consequences of agronomical practices on soil properties and functions (STUMPF et al., 2021).

*Changes in soil pH and available nutrient supply in CRs*

Soil texture of the Westsik's CRs experiment is draft sand, where the clay and silt content was <10% (HENZSEL, 2012). The low soil moisture (3–8%) observed in these CRs could be partially attributed to the close correlation between soil texture and moisture; however, soil moisture was mainly affected by the precipitation. Very low soil moisture content was noticed in the CRs X and XI, where the FYM was added. Overall, the OM addition could elevate the soil moisture content (MINASNY& MCBRATNEY, 2018) resulting in higher potato yield (HENZSEL, 2011) due to increasing water uptake by plants. Furthermore, cultivated plants could modify the soil moisture content (YANG et al., 2016).

The application of chemical fertilizers usually decreases the soil pH (GE et al., 2018). However, the mean pH of unfertilized CRs I, VII, X, and XV was 4.85, while the mean pH value of fertilized CRs with N, PK or NPK-fertilizer was 4.97. This result confirms that the long-term utilisation of different forms of OM can mitigate the negative effect of chemical fertilizers on soil pH (KÁTAI et al., 1999). Generally, higher $NO_3^-$-N concentrations were measured in the N-fertilized treatments. Nitrate ion is a labile form of soil N pool, its current value is primarily determined by OM decomposition, soil aeration, plant demand, and leaching (GILES et al., 2012; SEBILO et al., 2013). Moreover, lupine as a previous crop, could fix $N_2$ up to 300 kg $ha^{-1}$

(SULAS et al., 2016), which also could contribute to the $NO_3^-$-N content of soil. Considering the earlier results of KÁTAI et al. (1999) and HENZSEL et al. (2012), the FYM+N-fertilizer (CR XI) had substantial impact on this parameter, particularly in a long-term observation.

The dual application of OM and chemical N-fertilizer showed a significant potential on the available P and K contents in comparison to the control (I). The relatively high K content of FYM (RAYNE & AULA, 2020) and the high adsorption capacity of OM (WANG & HUANG, 2001) could increase the available P and K content. Long-term application of FYM, green and straw manure proved that the FYM could be used more effectively for maintaining high concentration of available P and K content, particularly in acidic sandy soils. Moreover, WANG et al. (2019) found that mineral and organic matter content of soils were both increased when organic and mineral fertilizations were jointly applied.

*Organic C and N content and their transformation in CRs*

Our results revealed that the effect of organic manures to increase soil organic matter can be improved with chemical fertilizer addition. According to MUHAMMED et al. (2018) the OC stock continuously decreased in the arable lands, while it increased in grasslands in the UK from 1800. This result also draws attention to the decline in OM content of arable lands, which is one of the main threats of soils (HUBER et al., 2008). Sandy soils with low humus content are extremely sensitive to the decline in OM content. Therefore, our results are of importance to prove that all the applied manure types (FYM, green, straw) had a positive effect on soil OM content, especially when chemical fertilizers were added to the organic manure.

The MBC and MBN refer to the quantity of bacteria and fungi in the soil. Organic manures usually increase the microbial population of soil resulting in better transformation of nutrients (RAYNE & AULA, 2020). The growing rate of microbial community depends on the added OM (KÁTAI et al., 2018) and the microbial necromass carbon is the primary constituent of stable carbon stored in the soil (BUCKERIDGE et al., 2020). MCLAREN & BUCKARIDGE (2019) found that the N fertilization had no effect on the MBC but it reduced the MBN. Our results are in contradiction with this finding, where the MBC and MBN increased in the CRs receiving chemical fertilizers.

Increased microbial population could raise the quantity of mineralizable N and strong relationship was found between the OM, microbial activity and N mineralization (N'DAYEGAMIYE & CÔTÉ, 1989). Negative correlation between net nitrification, MBC, and saccharase were reported, while no significant correlations were found between the other microbiological parameters.

An important parameter of the carbon cycle in the soil-plant-atmosphere system is the $CO_2$ production of soil, originating from the decomposition of OM and plant respiration. According to RAVN et al. (2017) the fertilization contributed to the acceleration of the carbon cycle. Through the measurement of $CO_2$ changes caused by microbes, their amount and activity can be estimated. In an earlier investigation of this experiment (KÁTAI et al., 1999), the application of straw and FYM stimulated the $CO_2$ emission. HENZSEL et al. (2012) measured higher $CO_2$ emission in the CRs

X–XIV, while MAKÁDI et al. (2012) reported lower $CO_2$ emission in the CRs VI, and XIII–XV. The main abiotic factors controlling soil respiration are soil temperature, soil moisture content and substrate concentrations (TRUMBORE, 2006). Therefore, in the Westsik's experiment, the applied different organic manures determined mainly the substrate concentrations and influenced the soil moisture content (*Table 2*).

*Quantitative dynamic of microorganisms and changes in some enzyme activities*

Healthy soils require stable and active microbial community to sustain nutrient cycling, improve soil structure, and contribute suppressiveness against plant pathogens. Soil organic matter functions as a biotope, shelter and substrate for soil microbes (PAUL, 2007). Therefore, the addition of any type of the OM to the soil could affect the size and activity of microbial community. In the Westsik's long-term experiment, the regular application of different organic matters had similar effects within different sampling times. In 1999 (KÁTAI et al., 1999) and in 2019, the CFU of bacteria was higher after the OM addition compared to the control (CR I). Fermented straw and FYM increased the number of bacteria (CRs V, VI, VII, X, XI, *Table 4*), while the effect of green manure was not so unambiguous. Whereas green manure increased the microbial diversity and changed the microbial richness and community composition in sandy silt soil (LONGA et al., 2017), our results revealed that the time of sowing and application or lack of chemical fertilizers in CRs could cause different results than green manure application on bacterial and fungal numbers. The number of microscopic fungi increased upon the dual application of straw and N-fertilizer. However, changes in the bacterial and fungal communities (abundance and diversity) were different in other fertilization studies (HICKS et al., 2020; WANG et al., 2019). These results indicated that the effect of the applied treatment could be modified by other factors like soil type, cultivation methods, and plant species. Our results showed lower bacterial counts coupled with higher fungal counts.

A common method for measuring the intensity of soil microbiological processes is the determination of enzyme activities that play a key role in the processes of degradation and energy flow taking place in soils. Dehydrogenase activity is a good indicator of the increasing substrate supply of microbes, where the quality of OM is a strong factor (CHU et al., 2016). Our results showed that the activity of dehydrogenase according to the applied manures was as follows: FYM > green manure > straw manure ≥ lupine main crop > control. Although earlier results revealed the importance of the added N-fertilizer to organic manure in dehydrogenase activity (CHU et al., 2016), this is not proved by the correlation analysis of our results. Besides the source of OM as a substrate, the environmental conditions have also strong effects on dehydrogenase activity (KUMAR et al., 2013). Regarding phosphatase activity, a significant increase was observed in some green manure-amended CRs (III, VIII, IX, XIV and XV). There was no correlation between phosphatase activity and available P content, similar to those reported by BALOTA et al. (2004). The higher level of available P could decrease the phosphatase activity (SPIERS & MCGILL, 1978) as in the case of CRs XI and XIV.

According to an earlier comparative study (ANTAL & ANTON, 1986), the main factor affecting saccharase activity was the cellulose content of soil, which was altered by the NPK treatment. Our results and the earlier findings of KÁTAI et al. (2018) have also confirmed these conclusions, proving the important role of the OM addition to the soil. According to the correlation analysis, the SOM content significantly and positively correlated with the measured soil enzymes, except for the phosphatase enzyme. Similar correlation was reported for dehydrogenase activity and the other measured soil enzymes. Soil tillage and fertilization heavily affect soil microbial properties (HABING & SWANEPOEL, 2015; MEHRA et al., 2018). The SOM could have high impact on soil microbial properties, especially enzyme activities (KWIATKOWSKI et al., 2020). Our results proved that the long-term application of different organic manures could result in differences between microbial properties of soils amended with FYM, straw manure, or green manure.

## Conclusion

In the Westsik's crop rotation (CR) experiment, the same nutrient and OM supply methods (straw, green and farmyard manure with or without different chemical fertilizers) have been applied for 90 years in 15 CRs.

On potato plots of the Westsik's CRs, 19 soil chemical and soil biological properties were examined in 2019 and the relationships between these variables were explored.

The control (fallow, CR I), fermented straw (CR VII), organic manure (CR X) and lupine main + second crop (CR XV) treatments did not receive mineral fertilizers. Even so, it showed a significant increase in seven parameters in the CR VII, 13 parameters in the CR X, and 11 parameters in the CR XV out of the 19 parameters examined.

In most treatments, there was a significant increase in pH, P and K, OC, and ON, MBC, $CO_2$, net nitrification, number of bacteria, activity of dehydrogenase and urease enzymes.

The most effective CRs were the CR VI, CR X, CR XI, CR XII, CR XIII, and CR XIV, where 12–14 out of the 17 parameters examined were significantly increased. The combined application of OM and chemical fertilizers could improve soil chemical and microbiological properties in long-term experiments. The results suggested that the application of green manure and FYM could effectively increase the soil fertility and microbiological activity compared to the straw manure.

Our results proved that the Westsik's long-term experiment was able to reveal more soil chemical and microbiological processes, concerning the effects of OM application to the acidic sandy soil.

**Keywords:** long-term experiment, crop rotation, nutrient supply, microbial activity, Westsik's crop rotation

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