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Impact of multi-cropping on some soil physical properties, nutrient proportion and enzymatic activity

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ABSTRACT
Crop growing with multifunctional companions can maintain soil fertility and agrotechnological inputs, but the combinations of multi-crops are still not sufficiently investigated. For this reason, in Lithuania (54°53′7.5″ N, 23°50′18.11″ E), in humid continental climate conditions (Dfb), maize, technical hemp and faba bean were grown as single, binary and ternary crops. The aim was to determine their effect on the variation of soil structural proportion, penetration resistance, macronutrient contents and enzymatic activity continuously over three years. In most multi-crops soil aggregates stability decreases by 16–23%, NPK levels by 1–20% and urease activity by 70–87%, compared to the initial conditions. In the ternary crop the amounts of the main macronutrients in the soil decreased the most (about 2.7 times), because its biomass was 2–2.5 times higher, compared to other diversified crops. The maize-faba bean binary crop had the lowest negative influence or sometimes positive change in the soil parameters studied. Results suggest the cultivation of multi-crops during a single vegetative period, by including them in existing crop rotations or growing them continuously with more frequent fertilisation.

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KEYWORDS
Chemical-physical soil parameters; faba bean; maize; soil enzymatic activity; technical hemp

Introduction

Despite of biodiversity losses and climate change, multi-cropping can ensure higher yields, thus generating higher incomes for the marketing of products and the provision of food for humanity (Lithourgidis et al. 2011; Ehrmann and Ritz 2014; Heuermann et al. 2019; Aly and Borik 2023; Ahakwa 2024).

In mixed cultivations, maize ensures high biomass productivity for different needs, especially, biofuel production (Yasin et al. 2021). Similarly, hemp is increasingly grown not only for its fibre, oil and seeds, but also for its high biomass yield, which can be utilised for energy production (Malayil et al. 2024). After seed harvest, faba bean biomass can be also used for energy production (Gomez et al. 2017). Beyond these uses, faba beans are able efficiently fix atmospheric nitrogen, thereby improving soil fertility and preventing degradation such as erosion and compaction (Karkanis et al. 2018). However, the separate harvesting of maize, hemp and faba bean biomass or seeds – along with the removal of crop residues from the field – can lead to a decline in soil fertility (Luo et al. 2024). Multi-cropping enhances soil quality by stabilising and restoring soil fertility, because reduce nutrient leaching into deeper soil layers, protect the soil from erosion caused by wind and water, and improve overall soil parameters (Mu-chun et al. 2012; Panda et al. 2020; Raza et al. 2021). The soil aggregate size distribution is one of the main soil health indicators and depends on the dynamic interactions between plants, microorganisms and soil components (e.g. organic matter). Here, plant roots are the key factor in all size classes of soil aggregates, as well as changing the pore size distribution and pore geometry of the soil, thereby altering the physical-mechanical soil parameters. The type and extent of structural effects of roots vary depending on the type of rooting, timing (alive or decomposed), environmental conditions (e.g. granulometric composition, moisture, temperature)

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and plant family (Bodner et al. 2021). Multi-crops produce higher root biomass, compared to single crops due to cross-species synergy among plants (Kumar et al. 2006). The roots of multi-crops are located at different soil depths and take up water and nutrients from different layers of the soil (Salama and Abdel-moneim 2021). The faba bean roots generate symbiosis with specific *Rhizobium* bacteria, thus resulting in biological nitrogen fixation and, therefore, reduced fertiliser requirements in arable soils and increased biological activity (Fageria et al. 2005; Brooker et al. 2015). The nitrogen content that can be fixed by faba bean mainly depends on the variety, local farming practices (e.g. nitrogen and phosphorus fertilisation), soil parameters and the symbiont content (Adak and Kibritci 2016; Argaw and Mnalku 2017).

Diversity of multi-crops affects the whole soil biota by increasing the abundance, diversity and activity of soil microorganisms (Song et al. 2007; Zhou et al. 2011). Technical hemp can absorb heavy metals (Linger et al. 2002; Hernandez-Allica et al. 2008; Golaszewska and Golaszewski 2020), as well as absorb more CO_2 from the environment, due to its larger surface area (Shen et al. 2022). This may have important practical implications for sustainable and healthy soil use through the inclusion of hemp in the composition of multi-crops.

In Lithuania, faba bean (*Vicia faba* L.), hemp (*Cannabis sativa* L.) and maize (*Zea mays* L.) show the highest biomass productivity, a high food and energy potential, as well as a good theoretical potential to grow together. This is very beneficial to growing a lot of biomass for biofuel production and to becoming increasingly independent on fossil fuels imported from the Russian Federation. More often, biomass production have been studied in single and binary agroecosystem experiments, while in this research, in addition to single and binary crops, a ternary maize-hemp-faba bean crop was also assessed. In presented study the effects of single and multi-crops on the physical, chemical soil parameters and enzymatic activity during the three-years of continuous cultivation of crops were tested. The research hypothesis states that continuous multi-cropping, especially including faba bean, will use nutrients more efficiently and their amounts in the soil will stabilise, as well as the physical-mechanical and biological soil parameters will be enhanced due to the higher biodiversity.

Materials and methods

Site description

The field experiment was started in 2020 at the Vytautas Magnus University, Agriculture Academy, Experimental Station (54°53′7.5″ N, 23°50′18.11″ E, altitude a.s.l. of 110 m), and it was carried out until 2022. The experimental field soil was Planosol (*Endohypogleyic-Eutric Planosol-Ple-gln-w*) (World Reference Base for Soil Resources 2022). The soil pH_{KCl} (0–25 cm) varied from 7.3 to 7.8, the total nitrogen content was 0.08–0.13%, the organic matter content was from 2.6 to 2.9%, the available potassium was from 97 to 118 mg·kg^{-1}, the available phosphorus was from 189 to 280 mg·kg^{-1}, the available sulphur was from 1.2 to 2.6 mg·kg^{-1} and the available magnesium varied from 436 to 790 mg·kg^{-1}.

Even if Lithuania is a country with high surplus precipitation rates, during the last 156 years, the precipitation rates have become uneven, with many short or long drought periods. For example, in 2020, the lack of moisture after sowing stopped seeds sprouting and negatively affected crop growth and development (Table 1). In 2021, the meteorological conditions were suitable for crop germination, thus allowing them to form strong roots in the initial stages of crop growth. However, the distribution of moisture was

Table 1. Weather conditions: Kaunas meteorological station.

Month / Year	2020	2021	2022	Long-term average
Air temperature (°C)				
April	6.9	6.2	6.2	6.9
May	10.5	11.4	11.0	13.2
June	19.0	19.5	17.7	16.1
July	17.4	22.6	17.9	18.7
August	18.7	16.5	20.9	17.3
Precipitation rate (mm)				
April	4.0	33.7	38.4	41.3
May	94.4	121.6	84.0	61.7
June	99.3	40.3	77.6	76.9
July	60.5	48.4	100.5	96.6
August	92.8	122.2	38.7	88.9

uneven and the lack of moisture and the high temperatures in July delayed crop biomass development. Even if in 2022, as in 2020, there was an initial lack of moisture until crop germination, sufficient precipitation rates and suitable air temperatures in later stages helped to compensate for this lack.

To summarise, the precipitation rates during May–July were closer to the long-term average. The riskiest periods of the season were April and August, when plants suffered a lack or surplus of precipitation and the uneven distribution of precipitation had a negative impact on both the crop productivity indices and the physical-mechanical soil parameters.

Experimental design and crop operations

Maize (*Zea mays* L.) ('Pioneer' hybrid P7034), technical hemp (*Cannabis sativa* L.) (cultivar 'Austa SK') and faba bean (*Vicia faba* L.) (cultivar 'Vertigo') were grown as single, binary and ternary crops for biomass production aimed at generating solid biofuel (Table 2). The experiment consisted of 7 treatments, 21 experimental plots in total, arranged with three replications in a randomised complete block design (RCBD). The size of the plot was 8 m^2. In spring, the soil was tilled by means of a germinator-type cultivator and the inter-rows were tilled twice by hand. Seeding was done by hands too (Table 3). Multi-crops were sown according to prepared sowing schemes (Balandaitė et al. 2022). The experiment was fertilised with complex fertilizer NPK 15:15:15 (200 kg ha^{-1}) in the first year, NPK 5:15:30 + 5S (200 kg ha^{-1}) in the second and third years of the experiment.

Synthetic pesticides were not included in the agro-technics of the experiment. In Table 4, the biomass productivity is shown. The biomass productivity indices are presented in more detail in an earlier publication (Balandaitė et al. 2022).

Methods and analysis

Every year, after the harvest of cultivated plants, soil samples were taken to determine chemical parameters: from at least 10–15 spots, soil samples were taken at 0–25 cm layers in each experimental plot by means of a Nekrasov auger. An average sample was made, in which the levels of the main macronutrients (N, P, K, Mg) were determined via laboratory analyses. Soil pH was determined using the potentiometric method (in KCl

Table 2. Experimental treatments and their abbreviations.

Biodiversity level	Crops	Abbreviation
Single crop	Maize, hemp, faba bean (separate crops)	M (1), H (2), FB (3)
Binary crop	Maize + hemp	M + H (4)
	Maize + faba bean	M + FB (5)
	Hemp + faba bean	H + FB (6)
Ternary crop	Maize + hemp + faba bean	M + H + FB (7)

Table 3. Crop operations.

Crop operations	Execution time		
	2020	2021	2022
Seedbed preparation	22/04/2020	22/04/2021	18/04/2022
Seeding	30/04/2020	28/04/2021	22/04/2022
Interrow tillage	29/05/2020	08/06/2021	18/05/2022
	15/06/2020	25/07/2021	08/06/2022
Biomass harvest	03/09/2020	24/08/2021	29/08/2022

Table 4. Impact of multi-cropping on 2020–2022 average data of fresh and dried biomass productivity (g m^{-2}).

Treatments	M	H	FB	M + H	M + FB	H + FB	M + H + FB
Fresh biomass	4414.8a	1863.0d	800.0e	2469.4c	3421.5b	1874.4d	2557.2c
Dried biomass	239.9d	402.0d	487.3cd	956.2b	747.8bc	833.5b	1868.0a

Note: M – maize single crop, H – hemp single crop, FB – faba bean single crop, M + H – binary maize and hemp crop, M + FB – binary maize and faba bean crop, H + FB – binary hemp and faba bean crop, M + H + FB – ternary maize, hemp and faba bean crop. Different lowercase letters indicate significant differences between treatments at $P < 0.05$.

extract with a glass electrode) (ISO 10390:2005). Total nitrogen (%) determined by the Kjeldahl method, available phosphorus and available potassium (mg kg^{-1}) were determined by the Egner-Rim-Domingo (A–L) method. Mobile magnesium was determined by LVP D-13:2016 method. Soil tests were carried out at the Lithuanian Agricultural and Forestry Research Center, Kaunas.

Samples (300 g of soil) for the determination of soil aggregate-size distribution and stability in each experimental plot were taken by means of a spade after the cultivated plants were sown and at the end of the vegetative season. Sampling was carried out from 0 to 25 cm layers in at least four spots, so that average samples were formed. The soil samples were dried in laboratory conditions and then they were sieved for 2 min by means of a sieving amplitude of 60%. To determine the soil aggregate-size distribution, a Retsch sieving machine (Retsch Lab Equipment, VERDER Group, The Netherlands) and a set of sieves were used. The sieve set was assembled by hole diameter in descending order: 10, 7.1, 5.6, 4, 2-, 1-, 0.5- and 0.25-mm. Soil structure is classified according to the size of the aggregates: mega-aggregates are larger than 10 mm, macro-aggregates are 0.25–10.0 mm, and micro-aggregates are smaller than 0.25 mm.

The stability of the soil aggregates was determined by means of wet sieving with the Retsch wet sieving apparatus only on a previously dry-sieved soil fraction of 1 mm.

Soil cone penetrometer resistance was determined at the beginning of each vegetative period, after intercrops sowing. At least four measurements per experimental plot were performed by means of an Eijkelkamp electronic penetrometer (penetrologger) (Royal Eijkelkamp, Giesbeek, The Netherlands). The upper soil layer is 0–20 cm, and the deep soil layer is called the depth >20 cm.

Soil samples for the soil enzyme activity tests were taken at the same time, together with samples for the chemical soil parameters. Natural moisture content samples were dried in open boxes at laboratory temperature (20–22 °C). After drying the samples in laboratory conditions, the activity of urease was assessed according to the Hofmann and Schmidt methods, while saccharase activity was assessed according to the Hofmann and Seegerer methods, modified by Chunderova (1973). The studies were carried out in the Laboratory of Food Raw Materials, Agronomic and Zootechnical Research of VMU Agriculture Academy.

The fresh and dried biomass of the crops were determined at harvest: plants were cut from five spots in each plot in a 0.5 m row, so that an average sample was formed. The samples were weighed to determine their fresh biomass, while the samples were dried at 105 °C temperature to determine the absolutely (totally) dry biomass.

For the statistical evaluation of the data, a one-factor analysis of variance was used. The limits of significant differences for the probability levels R_{05} and R_{01} were determined using the computer program ANOVA according to the F criterion. Correlation and regression analyses were performed using STAT and SIGMA PLOT, and calculations were carried out at Vytautas Magnus University Agriculture Academy. The changes in soil aggregates, chemical elements and enzyme activity were calculated by subtracting the results obtained in the corresponding year of the experiment (2020–2021; 2021–2022; 2020–2022). To assess the impact of multi-cropping on agroecosystems, an integrated assessment model according to G. Lohmann and K.U. Heyland was used. An example of the CEI (Complex Evaluation Index) calculations is shown in Supplementary materials, Table S1 (Kimbirauskienė et al. 2024).

Principal Component Analysis (PCA) was used to establish the correlation between different indicators in single, binary and ternary crops. This analysis creates new artificial variables (principal components) based on the analysed variables. Its main premise was the ability to visualise the relationships between individual variables in a two-dimensional diagram showing the coordinate system of the first two principal components. Based on the position of the vectors in space, it is possible to determine which variables are correlated with each other. Lower angle between the vectors shows the stronger positive correlation. When the vectors are aligned on the same line but in opposite directions, there is a strong negative correlation between the variables. However, when the vectors are aligned at an angle close to 90 degrees, there is no correlation (Jolliffe and Cadima 2016).

The results of investigations were also grouped using cluster analysis. The clustering of all tested single, binary and ternary crops considering the estimated physical-mechanical soil parameters, macronutrients content and enzymatic activity was carried out according to the Ward criterion using Euclidean distance matrices. Statistical analysis was performed using the Statistica software package Statistica 10 (TIBCO Software Inc., Palo Alto, CA, USA).

Results and discussion

Changes in physical-mechanical soil parameters

Soil aggregate-size distribution and water stability

Over three years of the continues cropping (Figure 1), the amounts of soil mega-size aggregates in almost all plots of multi-crops decreased by 1.5-fold on average, while the amounts of macro- and micro-size aggregates, on the contrary, increased by 1.1 and 1.6 times, respectively; the only exception was M + H binary crop with converse results. According to comprehensive evaluation, a macrostructure was the main indices in M + H multi-crop (Figure 2). The volume of macroaggregates was partly related to the amount of available magnesium in the soil ($r = 0.50$, $P \leq 0.05$). George et al. (2012) also claimed that multi-crops improve soil structure by increasing the amounts of macroaggregates and reducing the amounts of micro- and mega-size aggregates in the soil. Mottin et al. (2018) clarified that *Poaceae* family crops promoted higher levels of macro-size aggregates, compared to the *Fabaceae* family.

In our experiment, soil water stability decreased over the 3-year experimentation (Figure 1), probably due to the drastically uneven distribution of precipitation during the vegetative period (Table 1). Sarah (2005) also confirmed that amount of rainfall has a significant impact on the stability of soil aggregates. In general, the average stability of soil aggregates in our experiment was 43.5%, so that the soil could be regarded as insufficiently structured. Aggregate stability was partially dependent on the mega-size aggregates content in the soil ($r = 0.510$, $P \leq 0.05$). The highest decreases in soil stability after the three years of the experiment were found in the faba bean single crop (19.7%) and the binary M + H crop (13.8%). However, according to the comprehensive evaluation, a n aggregate stability was the most important indices in single hemp H crop (Figure 2). According to other researchers, soil aggregate stability is usually lower in faba bean single crop compared to multi-crops (Sánchez-Navarro et al. 2019), because interspecific competition among crops promotes plant rooting (Hauggaard-Nielsen and Jensen 2005).

Soil cone penetrometer resistance

The highest soil cone penetrometer resistance resulted in lower than 2.5 MPa, i.e. in the deepest layer (21–25 cm), 2.28 MPa for maize in spring 2020, 2.26 MPa for hemp in spring 2021 and 2.04 MPa for double cultivation maize + hemp in spring 2022. Therefore, the soil was not compacted in all plots (Figure 3), because cone penetrometer resistance exceeding on average 2.5 MPa (range from 1.5 to 4.2 MPa) linked to detrimental effects on plant growth (Van Orsouw et al. 2022). Moreover, the lowest soil cone penetrometer resistance resulted in the topsoil layer (0–5 cm) for M + H + FB, i.e. 0.50 MPa in 2020, 0.36 MPa in 2021 and 0.24 MPa in 2022. Therefore, ternary cultivation was the best solution for avoiding topsoil compaction. However, in the deepest layer (21–25 cm), the lowest soil cone penetrometer resistance resulted 1.44 MPa for M + H + FB in 2020, 1.18 MPa for M + FB in 2021 and 1.52 MPa for H in 2022. Furthermore, in 2020–2021, the soil cone penetrometer resistance increased only for single hemp cultivation. In 2021–2022, in multi-crops the soil cone penetrometer resistance decreased only in the top-soil layer (0–5 cm), while it increased in all the other deeper layers (totally 6–25 cm), above all for double cultivations.

During three years of continued cropping, in all cultivations, the soil cone penetrometer resistance decreased, except in H + FB and ternary cultivations, in which some deeper soil layers remained compacted (Figure 3).

Comprehensive assessment of multi-cropping showed that the binary H + FB and M + FB crops, which had the highest CEI values, had the strongest effect on the studied indicators shown in Figure 2. Although the scores of the ternary crop did not reach the assessment threshold, they were very close to it in all cases.

Changes of chemical soil parameters

Total nitrogen

Crops in individual cases had a significant influence on the amount of total nitrogen, but these results were not consistent throughout the years of the experiment. The change in total nitrogen in multi-crops was negative in most cases (Figure 4). The binary M + H and ternary cropping were characterised by higher productivity, which resulted in higher nitrogen depletion. However, comprehensive evaluation showed that in

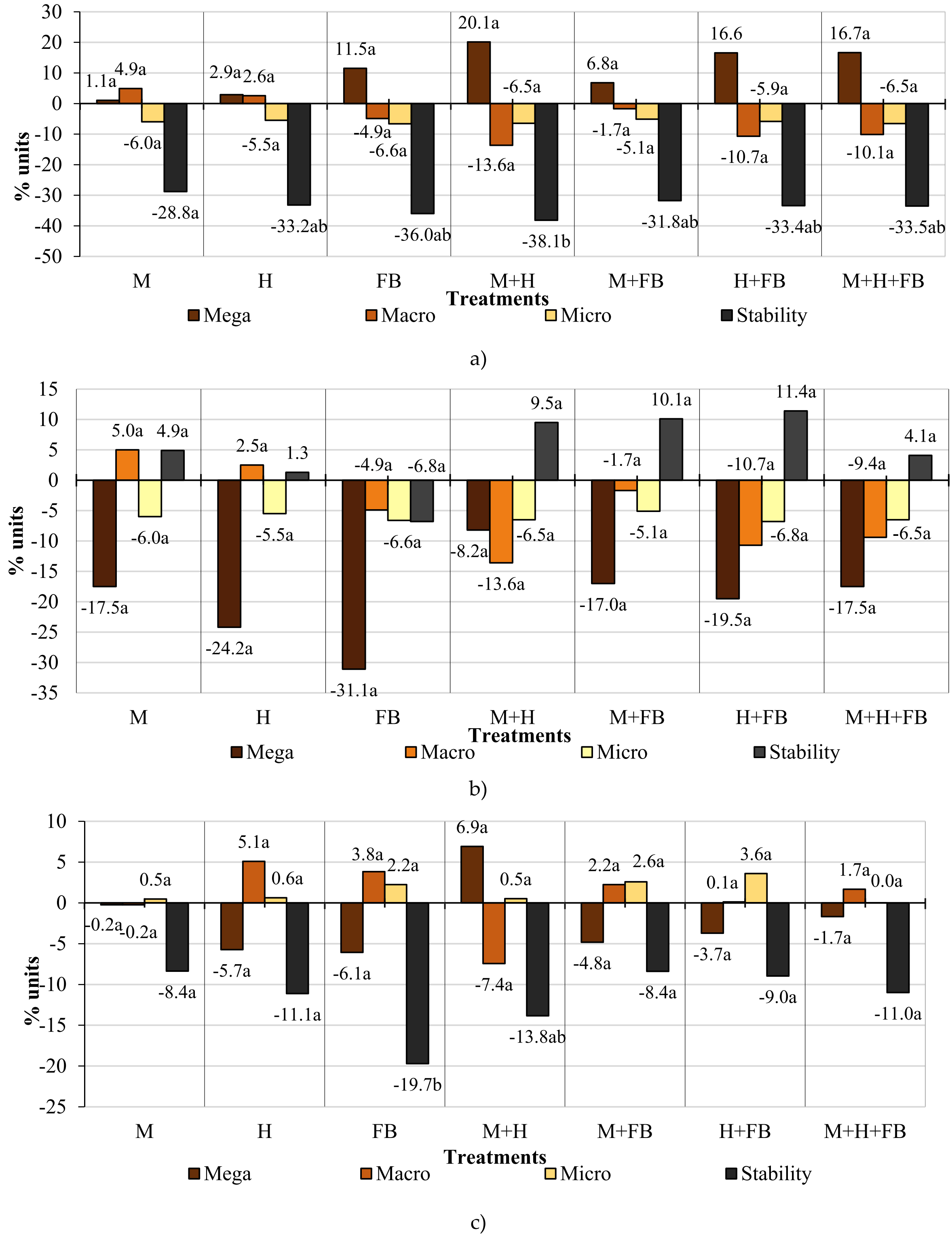

Figure 1. Impact of multi-cropping on the soil aggregate-size distribution and its stability changes in 2020–2021 (a), 2021–2022 (b) and 2020–2022 (c).

Note: M – maize single crop, H – hemp single crop, FB – faba bean single crop, M + H – binary maize and hemp crop, M + FB – binary maize and faba bean crop, H + FB – binary hemp and faba bean crop, M + H + FB – ternary maize, hemp and faba bean crop. Different lowercase letters indicate significant differences between treatments at $P < 0.05$.

single hemp H crop changes of nitrogen were the most important (Figure 5). A moderately significantly positive correlation was found between total nitrogen and mobile phosphorus contents ($r = 0.47$, $P < 0.05$). Liu et al. (2024) obtained the opposite results and indicated that after a three-year field experiment where maize

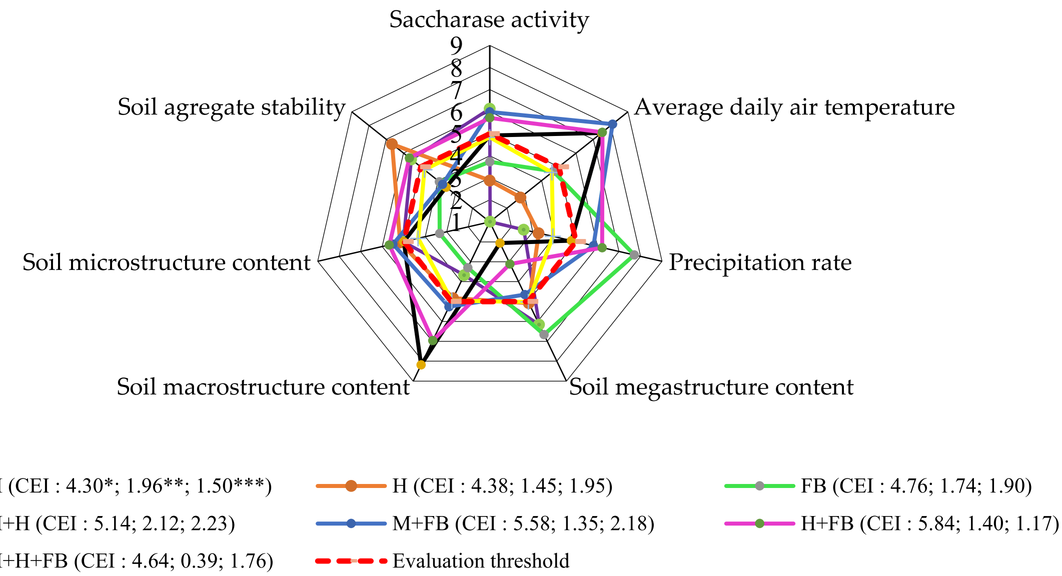

Figure 2. Comprehensive assessment of soil properties and main meteorological indices impact on different cultivations in 2020–2022.

Note: M – maize single crop, H – hemp single crop, FB – faba bean single crop, M + H – binary maize and hemp crop, M + FB – binary maize and faba bean crop, H + FB – binary hemp and faba bean crop, M + H + FB – ternary maize, hemp and faba bean crop. CEI–complex evaluation index, * –average of evaluation points (EPs), ** –standard deviation of EPs, *** –standard deviation of the average of the evaluation points below the evaluation threshold. In the figure: soil aggregate stability.

was intercropped with common vetch and pea, the total nitrogen content of the binary crop increased significantly (up to 14.3%), compared to the maize single crop. Rudinskienė et al. (2022) also found nitrogen increase in binary and ternary crops by alternating them.

Available phosphorus

In 2020–2021, the presence of most multi-crops tended to reduce the amount of this macronutrient. In 2021–2022, the highest significant increase in phosphorus content was found in the H + FB binary crop (29.5 mg kg^{-1}), compared to the hemp single crop. Comprehensive evaluation showed that variation in phosphorus, potassium and magnesium contents was the most important indices in single maize M cultivation (Figure 5). According to Li et al. (2011; 2014), faba bean plants grown in mixtures can secrete a higher amount of phosphorus-mobilising root exudates, which support the phosphorus nutrition of other plant species in multiple crops. Similarly in other experiments, in maize crops accompanied by pea and faba bean, higher levels of available phosphorus were found in the soil (Latati et al. 2014; Liao et al. 2020).

Over the three years, in the maize single crop, the amount of phosphorus in the soil increased, although insignificantly. Instead, in the multi-crops it decreased, except for the years 2021–2022, when, due to drought, part of the phosphorus was not absorbed by the plants (Figure 6).

Available potassium

After three years of continued cropping, the highest potassium content decrease was found in ternary cultivation, as it produced the highest plant biomass (Balandaitė et al. 2022). H + FB and M + FB binary crops had stable non-significant positive effect on potassium content (Figure 7). Li et al. (2023) found similar results in faba bean and maize multi-crops. In Wang et al. (2015) studies with multi-crops, multi-crops remove 200–300 kg ha^{-1} of available potassium from the soil. Nasar et al. (2022) suggest balancing the amount of available potassium in the soil to grow multi-crops including cereal and bean, which significantly increases the amount of available potassium in the soil compared to single crops.

Available magnesium

The diversification of crops in some cases had a significant impact on the amount of available magnesium. It is important to note that the amount of this nutrient increased significantly in all crops for all treatments

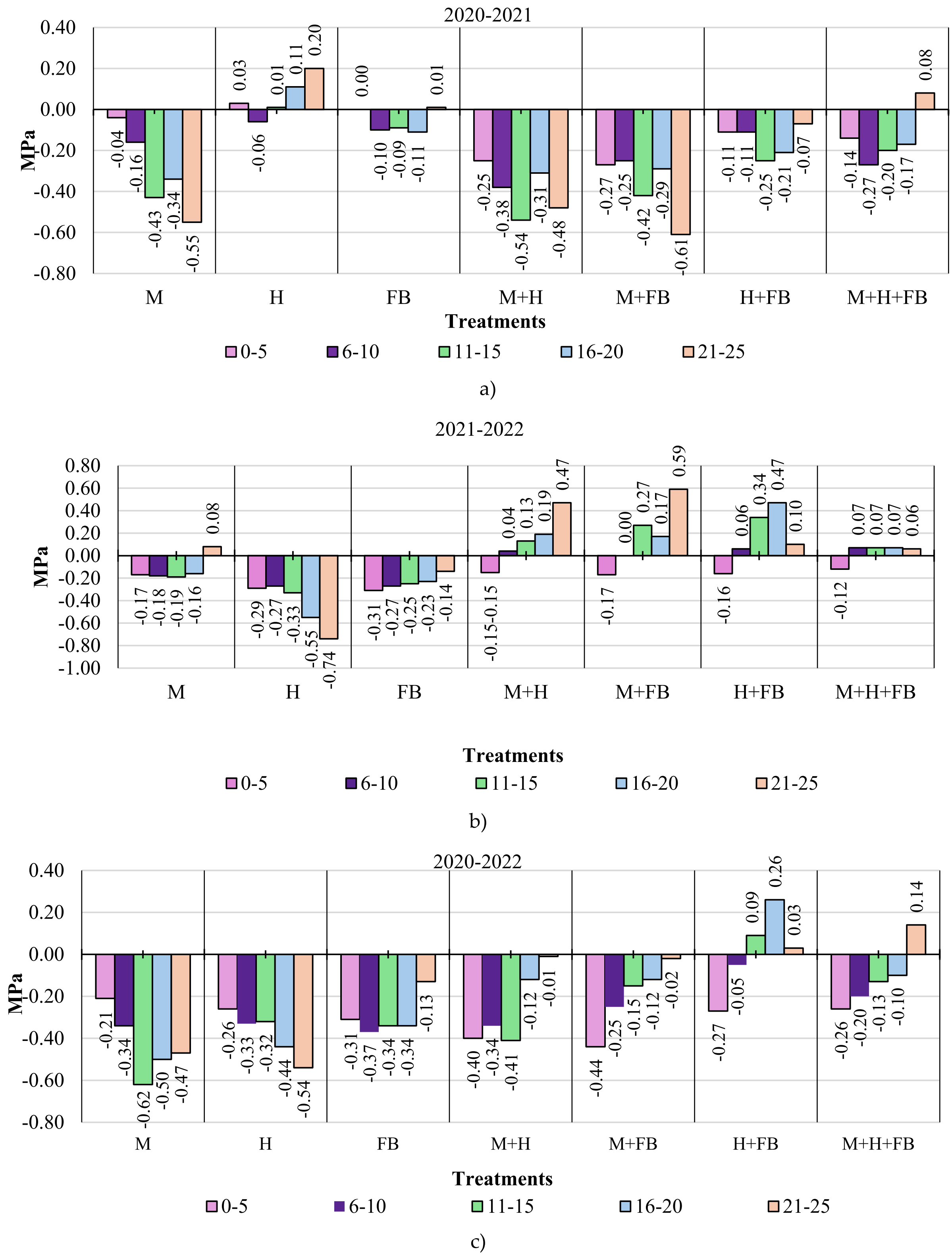

Figure 3. Impact of multi-cropping on the soil cone penetrometer resistance in different soil layers in 2020–2021 (a), 2021–2022 (b) and 2020–2022 (c).

Note: M – maize single crop, H – hemp single crop, FB – faba bean single crop, M + H – binary maize and hemp crop, M + FB – binary maize and faba bean crop, H + FB – binary hemp and faba bean crop, M + H + FB – ternary maize, hemp and faba bean crop.

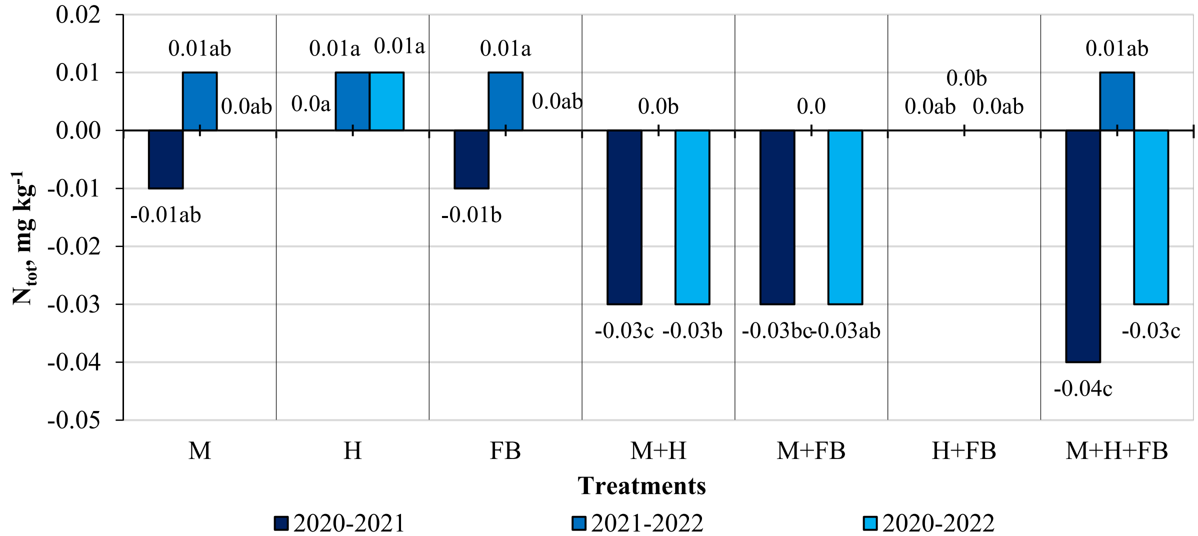

Figure 4. Impact of multi-cropping on total nitrogen changes.

Note: M – maize single crop, H – hemp single crop, FB – faba bean single crop, M + H – binary maize and hemp crop, M + FB – binary maize and faba bean crop, H + FB – binary hemp and faba bean crop, M + H + FB – ternary maize, hemp and faba bean crop. Different lowercase letters indicate significant differences between treatments at $P < 0.05$.

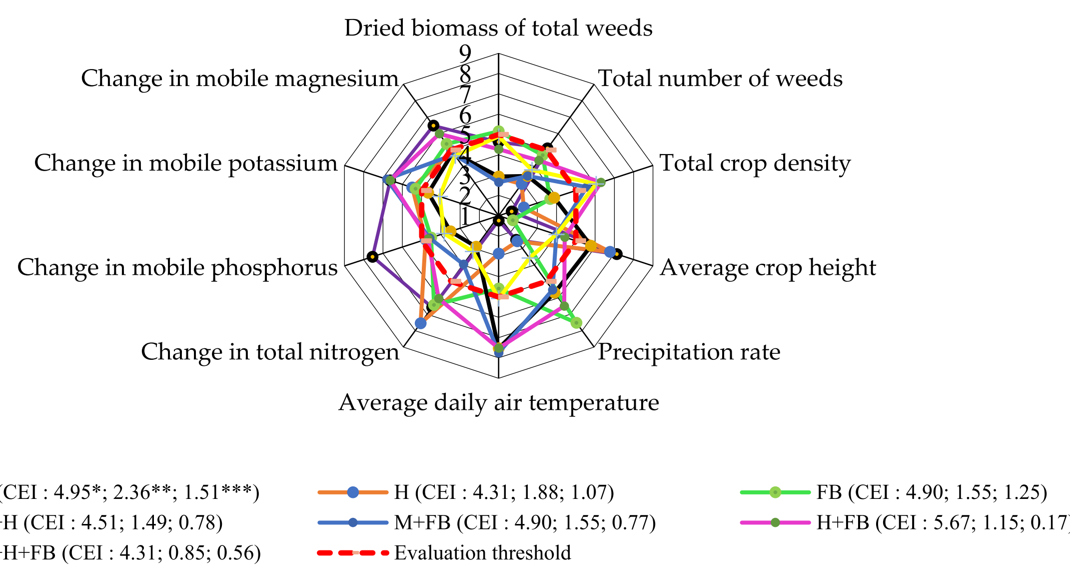

Figure 5. Comprehensive assessment of soil chemical, meteorological and crop development conditions impact on different cultivations in 2020–2022.

Note: M – maize single crop, H – hemp single crop, FB – faba bean single crop, M + H – binary maize and hemp crop, M + FB – binary maize and faba bean crop, H + FB – binary hemp and faba bean crop, M + H + FB – ternary maize, hemp and faba bean crop. CEI–complex evaluation index, * –average of evaluation points (EPs), ** –standard deviation of EPs, *** –standard deviation of the average of the evaluation points below the evaluation threshold.

(Figure 8). After three years of experimentation, the highest positive change was found in the maize single and H + FB binary crop. The lowest increase was established under ternary cultivation, probably due to the highest amount of biomass being removed, as this crop was also the most productive one. Positive correlations were found between the amount of available magnesium and the amounts of available phosphorus ($r = 0.49$, $P \leq 0.05$) and available potassium ($r = 0.45$, $P \leq 0.05$).

When comparing the effects of multi-crops on the content of total nitrogen, mobile potassium and magnesium in the soil, the evaluation scores of the binary H + FB crop exceeded the evaluation threshold, except for the mobile phosphorus scores that did not reach the evaluation threshold (Figure 2). When evaluating the effects of the binary M + H and M + FB on the same soil macronutrients, it was found that their scores were

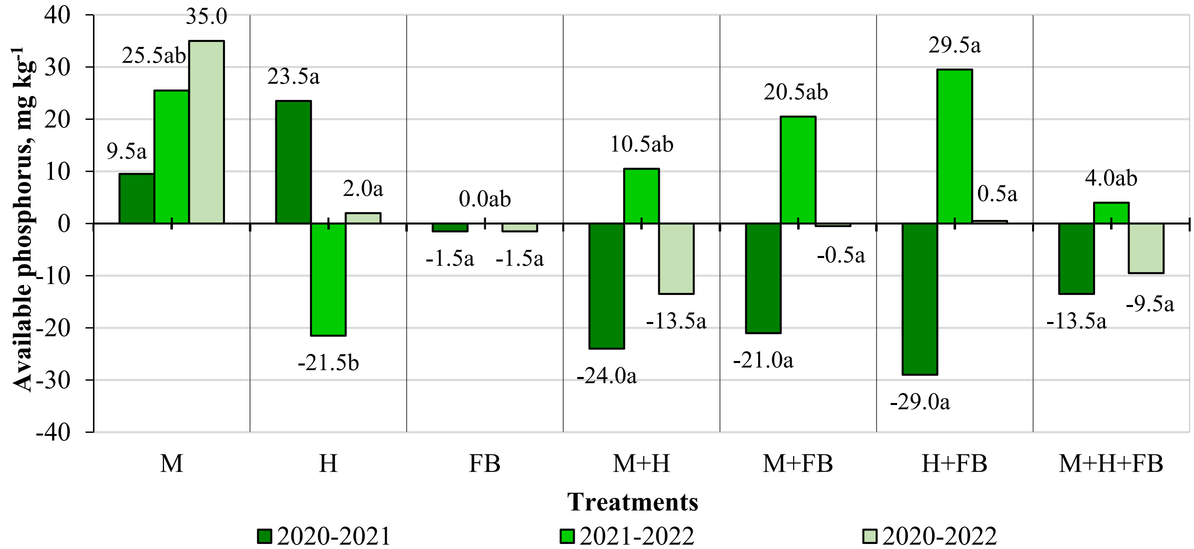

Figure 6. Impact of multi-cropping on available phosphorus changes.

Note: M – maize single crop, H – hemp single crop, FB – faba bean single crop, M + H – binary maize and hemp crop, M + FB – binary maize and faba bean crop, H + FB – binary hemp and faba bean crop, M + H + FB – ternary maize, hemp and faba bean crop. Different lowercase letters indicate significant differences between treatments at $P < 0.05$.

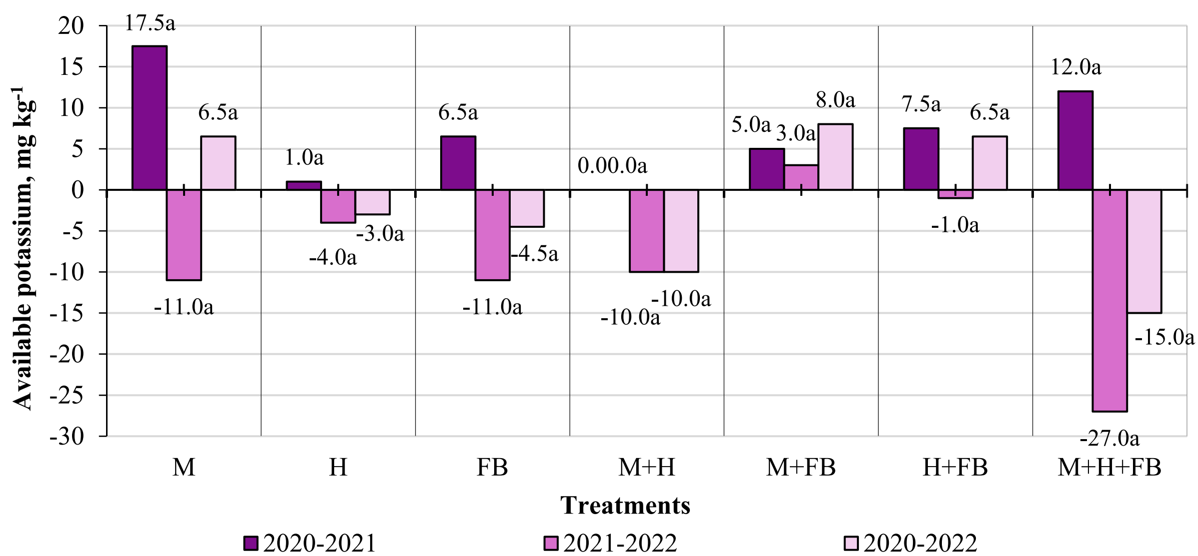

Figure 7. Impact of multi-cropping on available potassium changes.

Note: M – maize single crop, H – hemp single crop, FB – faba bean single crop, M + H – binary maize and hemp crop, M + FB – binary maize and faba bean crop, H + FB – binary hemp and faba bean crop, M + H + FB – ternary maize, hemp and faba bean crop. The lowercase letter (a) indicates insignificant differences between treatments at $P > 0.05$.

lower than the evaluation threshold, except for the binary M + FB crop, which had a positive effect on the content of mobile potassium in the soil. The evaluation scores of the ternary crop in all cases did not reach the evaluation threshold, so that its CEI value, like that of the single H crop, was the lowest one.

Changes of soil enzymatic activity

Saccharase activity

In the first year of experimentation, the activity of the enzyme saccharase in the soil decreased in the plots for all treatments (Figure 9). The enzymatic activity in the soil increased in the second year. This means that multi-crops can stimulate the growth and development of plant roots, thus improving the biological and

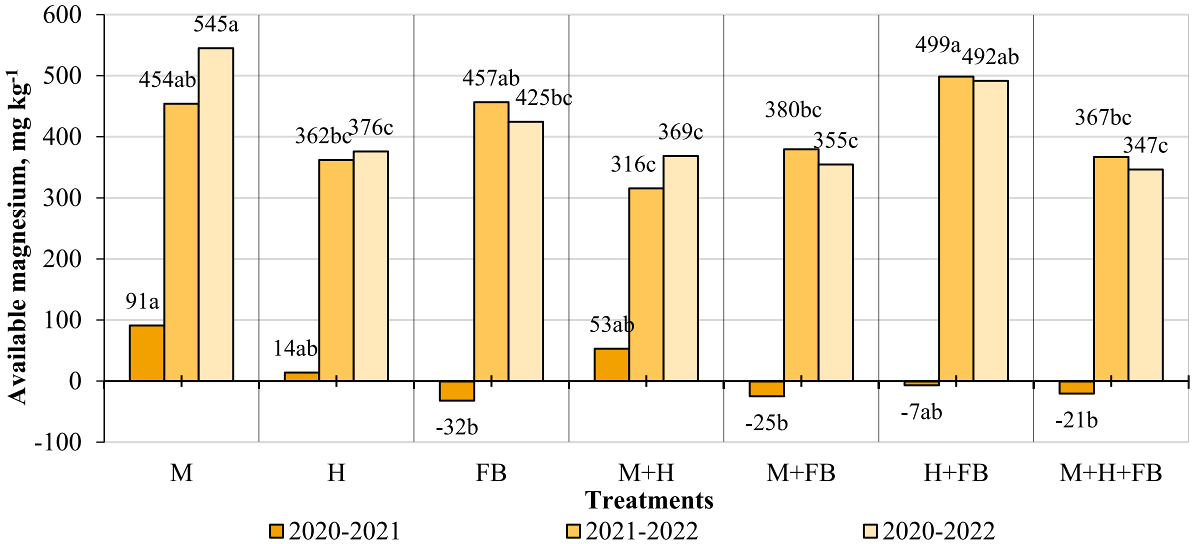

Figure 8. Impact of multi-cropping on available magnesium changes.

Note: M – maize single crop, H – hemp single crop, FB – faba bean single crop, M + H – binary maize and hemp crop, M + FB – binary maize and faba bean crop, H + FB – binary hemp and faba bean crop, M + H + FB – ternary maize, hemp and faba bean crop. Different lowercase letters indicate significant differences between crop diversification levels at $P < 0.05$.

chemical soil parameters (Rankoth et al. 2019). After three years of experimentation, most treatments maintained a positive saccharase balance, except for the single hemp and binary H + FB crops. According to the indicators' comprehensive evaluation, mixture of M + FB showed the highest impact of saccharase activity compared to other treatments (Figure 2). Li et al. (2022) also pointed out that multi-crops can also improve soil nutrient metabolism by improving the activity of the soil enzyme saccharase. The increased activity of saccharase indicates the acceleration of the decomposition of hydrolytic carbohydrates and the intensification of the mineralisation of organic substances in the soil (Saha et al. 2008).

Urease activity

In the first years of research, the amount of enzyme urease increased, while in subsequent years, it decreased in all treatments, as the amount of nitrogen in the soil decreased (Figure 10). Chen et al. (2018), Breza et al.

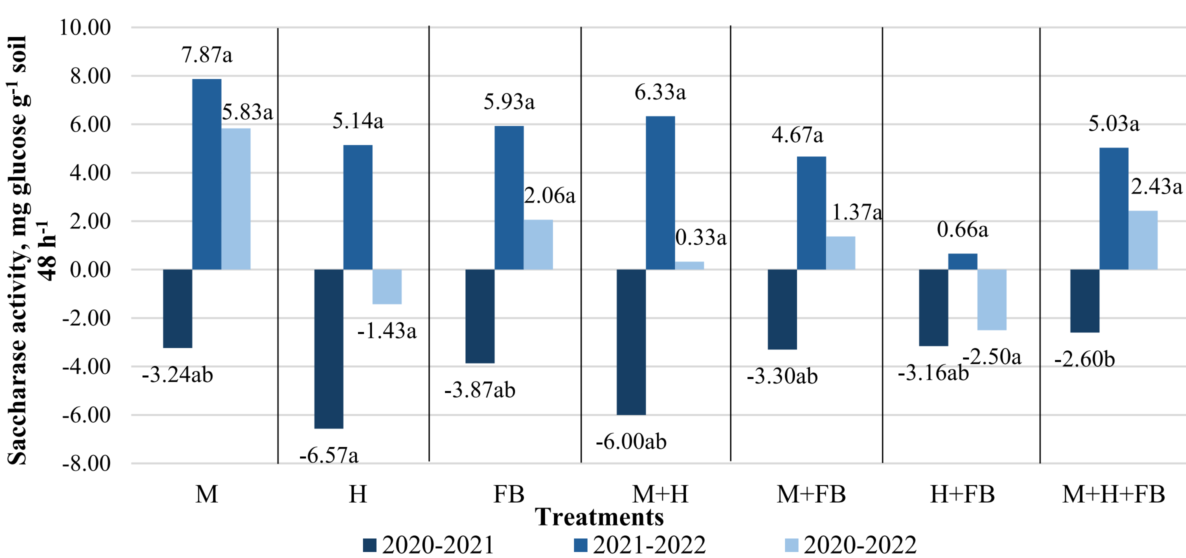

Figure 9. Impact of multi-cropping on saccharase activity changes.

Note: M – maize single crop, H – hemp single crop, FB – faba bean single crop, M + H – binary maize and hemp crop, M + FB – binary maize and faba bean crop, H + FB – binary hemp and faba bean crop, M + H + FB – ternary maize, hemp and faba bean crop. Different lowercase letters indicate significant differences between treatments at $P < 0.05$.

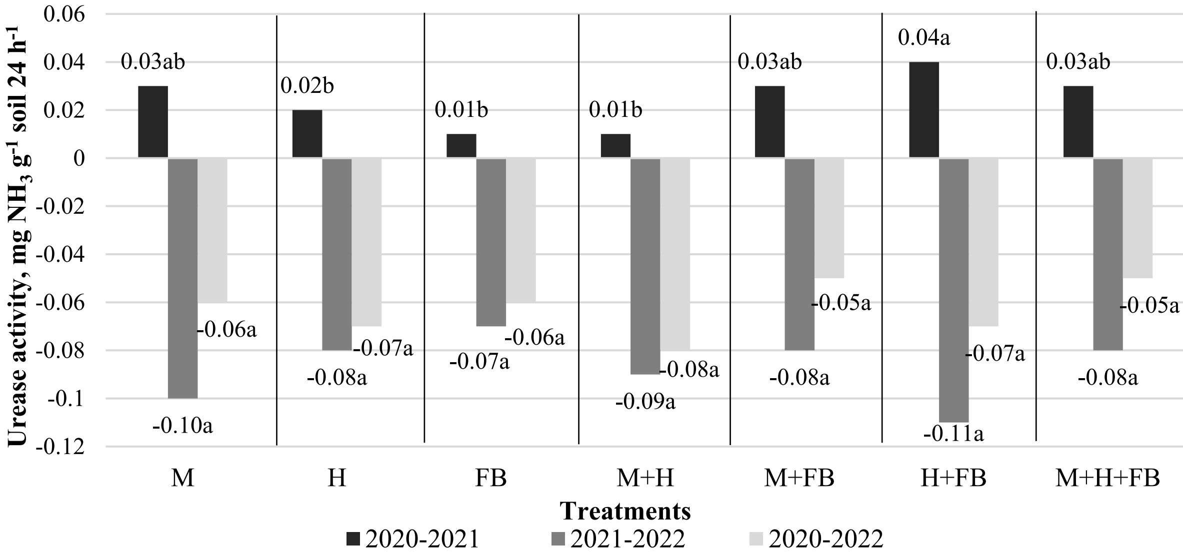

Figure 10. Impact of multi-cropping on urease activity changes.

Note: M – maize single crop, H – hemp single crop, FB – faba bean single crop, M + H – binary maize and hemp crop, M + FB – binary maize and faba bean crop, H + FB – binary hemp and faba bean crop, M + H + FB – ternary maize, hemp and faba bean crop. Different lowercase letters indicate significant differences between crop diversification levels at $P < 0.05$.

(2023) and Bian et al. (2023) also found correlations between urease activity and the amount of nitrogen in the soil. Ben-chuan et al. (2022) found that the soil protease, urease and nitrate reductase activities were significantly higher in a binary crop of maize and soybean than when grown in single crops. In addition, Wang et al. (2023) reported that intercropping maize with potato increases the activity of C-degrading enzymes such as β-glucosidases.

PCA and cluster analysis

PCA analysis showed that in 2020, the activities of the soil enzymes saccharase and urease were closely correlated with the content of total nitrogen, mobile phosphorus, soil stable aggregates and microaggregates (Figure 11). The content of soil mobile potassium, mobile magnesium and mega aggregates was negatively correlated with soil penetration resistance.

In 2021, the activity of saccharase was strongly positively influenced by the amount of mobile potassium and mobile magnesium in the soil. The activity of the urease was influenced by the amounts of total nitrogen and mega aggregates. The amount of mobile potassium in the soil was negatively correlated with soil

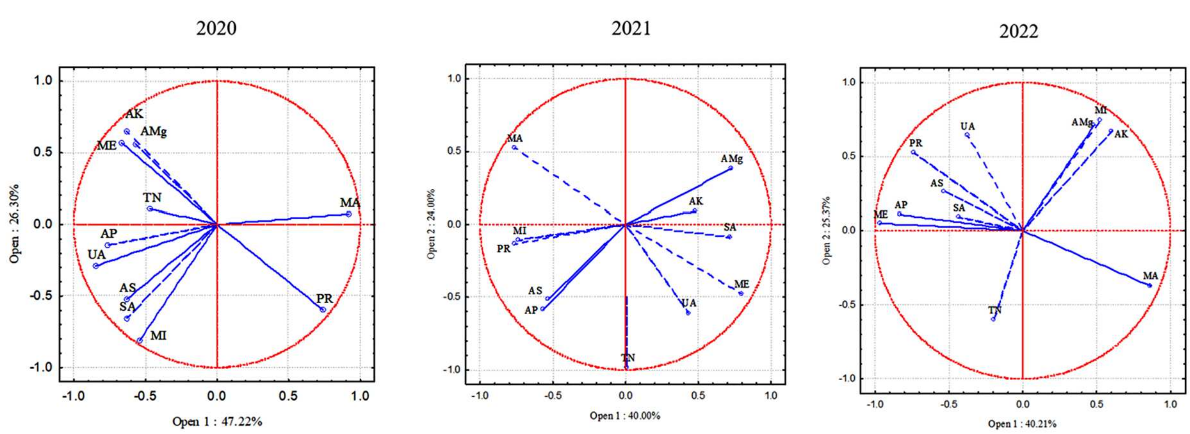

Figure 11. PCA of single, binary and ternary crops in 2020–2022.

Note: TN – total nitrogen; AP – available phosphorus; AK – available potassium; SA – saccharase activity; UA – urease activity.

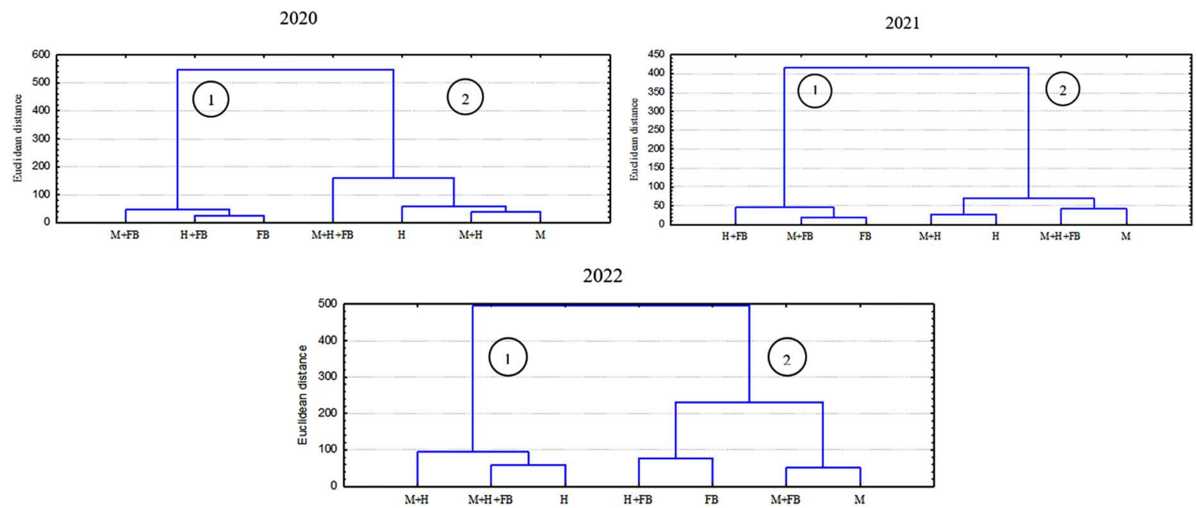

Figure 12. Hierarchical cluster analysis (Ward method, Euclidean distance matrices) of single, binary and ternary crops, based on soil parameters.

Note: 1, 2 – groups of multi-crops indicate similar soil parameters.

penetration resistance and the amount of microaggregates. In 2022, the activity of saccharase and urease depended on the amounts of mobile phosphorus, soil stable aggregates and mega aggregates, and the penetration resistance. The second group of correlated parameters consisted of mobile phosphorus, mobile potassium and the amount of microaggregates.

Cluster analysis showed that in 2020 and 2021, single, binary and ternary crops were divided into two groups, based on their impact on soil parameters (Figure 12).

The first group consisted of a single faba bean crop, binary maize and faba bean and hemp and faba bean crops. The second group consisted of a single maize and hemp crop, a binary maize and hemp and a ternary crop. The first group of crops was distinguished by a higher impact on the amount of soil mobile magnesium and the activity of saccharase. In 2022, crops were also divided into two groups according to their impact on soil parameters (Figure 12). The first group consisted of single maize, a binary maize and hemp and a ternary crop. The second group consisted of a single faba bean and hemp, a binary hemp and faba bean, and maize and faba bean crops. The first group of crops was distinguished by a higher impact on the amount of soil mobile phosphorus. The second crop group was distinguished by a higher effect on the amount of mobile magnesium and mobile potassium, and on the activity of the enzyme saccharase.

Conclusions

Crop diversification did not significantly affect changes in the soil aggregate-size distribution. After three years of experimentation, a significant 1.5 times decrease in soil stability was found in the faba bean single crop and the binary maize–hemp (M + H) crop. This led to the increase of the soil cone penetrometer resistance.

During three years of continue cropping, the highest reductions in total nitrogen, available phosphorus and potassium were obtained in the soil of the binary maize–hemp crop and the ternary crop, but only nitrogen significantly decreased (19%). These crops were characterised by higher productivity, which is why the amount of material removed was higher. It was found that among the diversified crops, intercropping with faba beans had a more stable effect on the amounts of mobile potassium and magnesium, and saccharase activity in the soil. The activity of urease decreased by 87% in the binary maize–hemp crop, compared to the other diversified crops. In general, when assessing the changes in the physical-mechanical, chemical and biological soil parameters, among the diversified crops in this study, the best indicators were obtained not in ternary crops, as expected, but when single maize and binary maize-faba bean crops were grown. Finally, the changes in soil parameters were influenced more by weather conditions than by crop diversification level.

Author Contributions

Conceptualisation, K.R., A.K. and J.B.; methodology, K.R., J.B. and A.M.; software, J.B. and U.G.; validation, J.B., R.K., A.S., A.K. and K.R.; formal analysis, J.B., A.M., U.G. and R.K.; investigation, J.B., A.Š., U.G., R.K., A.S., A.M. and K.R.; resources, A.Š, R.K., A.C. and A.S.; data curation, J.B. and U.G.; writing – original draft preparation, J.B., R.K., A.S., A.Š, A.C. and K.R.; writing – review and editing, K.R., J.B., R.K. and A.S; visualisation, J.B., R.K., K.R. and A.S; supervision, K.R. All authors have read and agreed to the published version of the manuscript.

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Availability of data and materials

Data and materials are available from the corresponding author upon request.

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