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THE EFFECT OF TILLAGE METHOD ON THE NUTRIENT REGIME OF SOIL DURING THE GROWING OF *TRIFOLIUM PRATENSE*

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The article presents the results of studying the influence of tillage methods on the number of bacteria and fungi in the arable soil layer, nutrient indicators, and, consequently, the yield of *Trifolium pratense*. The purpose of the study is to determine how tillage affects the feeding regime and yield of *Trifolium pratense*. The research was conducted at the experimental field of the Educational and Scientific Research Center of Lviv National Environmental University. For the study of microorganisms in the arocenosis of meadow clover, soil samples were taken using a cylindrical drill from arable (0–20 cm) and sub-soil (20–40 cm) layers with an average weight of an individual sample of 300 g. For microbiological analysis, average samples from five individual samples were prepared. The isolation of the main agronomically useful groups of microorganisms from the soil was carried out by the method of microbiological sowing of soil extracts on solid nutrient media: for fungi – wort-agar, for bacteria – meat-peptone agar (MPA). The dilution of the soil extract for fungi and bacteria was, respectively, 1 : 1000; 1 : 100000. Microbiological seeding was performed in triplicate. The calculation of the total number of microorganisms in the soil was carried out by Tepper´s method. The yield of crops and the results of laboratory studies were processed by the method of variance analysis. The application of a tiered tillage allowed to increase the yield of *Trifolium pratense* green mass by 4.5 t·ha^{-1} (+17.0%) compared to the control (conventional plowing). It is established that tier ploughing with the PYA-4-40 plough promotes uniform distribution of nutrients in the arable soil layer and formation of homogeneous soil fertility. In such a soil environment, much more favorable conditions are created for the development of microflora and more intensive microbiological processes compared to the options of chisel and conventional (control) tillage, and, consequently, the formation of high yields of green *Trifolium pratense*.

Keywords: green mass; soil; nitrogen; phosphorus; potassium

According to the statements of scientists, including Abbaspour Gilandeh et al. (2022), soil tillage is considered one of the main field operations in agriculture due to its influence on the physical properties of the soil and, as a result, on the production of agricultural crops. The choice of the type of soil tillage operations and their proper execution are determined by physical properties of the soil and help prevent its degradation, as well as maintain and improve physical, chemical and biological properties (Chahal et al., 2021; Abbaspour Gilandeh et al., 2022). Nouraein et al. (2020) claim that tillage also affects such soil properties as moisture and nutrient availability. Therefore, it is necessary to evaluate the methods of soil cultivation from the point of view of different farming conditions (Nouraein et al., 2020).

One of the most important indicators of soil fertility is microbiological activity, which reflects its ability to mobilize mobile nutrients and is directly related to the activity of microorganisms. Microorganisms ensure the ecological balance of agroecosystems. They are able to activate the cycle of elements that are important and poorly accessible to higher organisms (Arlauskienė et al., 2009, Demyanyuk et al., 2018). The number of microbiotas depends on the influence of various factors: soil type, climatic conditions, elements of cultivation technology, environmental reactions (pH), etc. (Krasyuk, 2011; Shuvar et al., 2021a). The most intensively developing microorganisms affect soil fertility and crop yields.

High crop yields are formed under conditions of optimal supply of the most important nutrients – nitrogen, phosphorus, potassium, and trace elements. The above nutrients (nitrogen, phosphorus, potassium) are an important component not only in plant nutrition, but

also the active life of microorganisms. Their application to the soil as part of organic and mineral fertilizers enhances microbiological activity.

The quantitative composition and ratio of individual representatives in the microbial coenosis of the soil largely depends on the method and depth of its cultivation. It significantly affects the agrophysical properties, water, nutrient regimes and forms the direction of soil-forming processes (Mishustin, 1972; Volkogon et al., 2017). One of the main tasks of the main tillage is to create optimal conditions for mineral nutrition of plants (Munkholm et al., 2013; Shuvar et al., 2021b). The use of mechanical tillage does not directly affect the accumulation of nutrients in it. However, the optimization of the ratio of solid, liquid, and gaseous phases of the soil contributes to the course of biochemical processes in a favorable direction for plants. This is due to better decomposition of root and post-harvest plant residues, as well as the use of easily accessible to plants mineral fertilizers (Slepetys, 2009; Khristenko et al., 2013; Volkogon et al., 2017).

In this process, there is an improvement of agrophysical and water properties of soil which affect the increase in the number and growth of activity of microbiological processes and comprehensively affect its effective fertility. Hence, it became necessary to carry out a scientific study on the definition and implementation of the main tillage in a scientifically based crop rotation in the specific conditions of the Western Forest Steppe of Ukraine. Therefore, the study of the dynamics of the amount of nitrogen, phosphorus and potassium in the soil depending on its cultivation in the technology of growing *Trifolium pratense*, considering the biological features of the culture and the bioclimatic potential of the region, is relevant.

The scientific novelty and innovation of the obtained results is in the effect of the main tillage method on the indicators of the nutritional regime and the yield of the green mass of *Trifolium pratense* variety Truskavchanka, adapted to the local soil and climatic conditions in five-field crop rotation, for the first time in the conditions of the Western Forest Steppe of Ukraine on dark gray podzolic light loam soil. Also, the main technological processes of *Trifolium pratense* cultivation have been improved by optimizing the main cultivation.

Material and methods

The research was conducted at the experimental field of the Educational and Scientific Research Center of Lviv National Environmental University. The study was performed during 2018–2020 in the Western Forest Steppe of Ukraine on dark gray podzolic light loam soil, which is characterized by low humus content (2.80–2.85%), slightly acidic reaction of soil solution (pH 5.7–5.8), low supply of nitrogen, which is easily hydrolyzed (106–110 mg per kg of soil), medium supply of phosphorus (188–193 mg per kg of soil), and potassium (122–126 mg per kg of soil).

The predominant fraction of dark gray podzolic soil is coarse dust (particle size 0.05–0.01 mm) and a small amount of silt, which is washed into the illuvial horizon. A large amount of dust promotes silting after a small amount of precipitation and the formation of crusts on the soil surface. The experimental study was carried out in a short rotation crop rotation (field 1 – *Trifolium pratense*, field 2 – winter wheat + post-harvest mustard for siderate, field 3 – sugar beets, field 4 – corn for silage, field 5 – spring barley with a seeding of *Trifolium pratense*). Under crop rotation, multi-depth tillage was carried out under the multi-depth system of tillage for crops.

The experiment was carried out in three repetitions with a systematic placement of plots. The total area of the plot is 220 m^2, the registered area is 160 m^2. *Trifolium pratense* was sown under spring barley, with the following variants for the main tillage: I. Conventional (control) – ploughing with a four-cabinet plough mounted with a body width of 35 cm (PLN-4-35) to a depth of 20–22 cm; II. Chisel – ploughing with a chisel plough with a working width of 4.5 m (PCh-4.5) to a depth of 20–22 cm; III. Tiered – ploughing with a tiered four-hull plough with a hull width of 40 cm (PYA-4-40) to a depth of 14–16 cm.

Immediately after harvesting the predecessor, the stubble was husked twice with heavy disc harrows BDT-7 to a depth of 6–8 cm, BDV-10 to a depth of 10–12 cm and ploughed with ploughs according to the experimental scheme. In the spring, when the soil was physically ripe, the field was harrowed in two tracks with the BZTS-1.0 harrows. The Europak combined unit was used for pre-sowing treatment. *Trifolium pratense* was sown at the same time as spring barley using a SZT-3,6 grain-grass planter.

Agrotechnical conditions for growing the *Trifolium pratense* variety Truskavchanka were generally accepted for the conditions of the Western Forest Steppe of Ukraine. The crop rotation was *Trifolium pratense*; winter wheat + mustard for sider; sweet beets; corn for silage; and spring barley with *Trifolium pratense* seeding.

Fertilizers were applied to crop rotation in the following doses: N120P80K100 (winter wheat) N20P30K30 (white mustard), 60 $t \cdot ha^{-1}$ of manure and N219P136K210 (sugar beets), 40 $t \cdot ha^{-1}$ of manure and N120P80K110 (corn for silage), and N40P40K60 kg (burnt barley). Mineral fertilizers were applied in the form of ammonium nitrate (34.5%), superphosphate (19.5%) and kalimagnesia (28%). Pesticides were not applied. The agrochemical indicators of soil were determined as following: humus – according to Tyurin in the modification of Nikitin; easily hydrolyzed nitrogen – according to Cornfield; phosphorus (P_2O_5) – by Chirikov's method; and exchangeable potassium (K_2O) – by Chirikov's method (Chirikov,1987).

For the study of microorganisms in the agrocenosis of meadow clover, soil samples were taken using a cylindrical drill from arable (0–20 cm) and sub-soil (20–40 cm) layers with an average weight of an individual sample of 300 g. For microbiological analysis, average samples from five individual samples were prepared. The isolation of the main agronomically useful groups of microorganisms from the soil was carried out by the method of microbiological sowing of soil extracts on solid nutrient media: for fungi – wort-agar, for bacteria – meat-peptone agar (MPA) (Ezhov, 1981).

The dilution of the soil extract for fungi and bacteria was 1 : 1000 and 1 : 100000, respectively. Microbiological seeding was performed in triplicate. The calculation of the total number of microorganisms in the soil was carried

out by Tepper´s method (Tepper et al., 1979). Nutrient regime indicators (mobile forms) were determined during the period of full seedlings, in the bud phase and before harvesting of *Trifolium pratense*. The yield of crops and the results of laboratory studies were processed by the method of variance analysis. Experiments were set up and performed in accordance with the requirements of the field research methodology (Dospekhov, 1985).

Results and discussion

During the research, the results established that the content of nitrate nitrogen during the growing season of *Trifolium pratense* significantly depended on the method of basic tillage and the phase of plant development (Table 1).

Over the years, the study found that in the phase of beginning of growth of *Trifolium pratense* in the soil layer 0–20 cm, there were significant reserves of nitrate nitrogen which had a positive effect on the initial growth of plants. The highest amount (0.71 mg per 100 g of absolutely dry soil) of nitrate nitrogen was found in the arable soil layer during ploughing with the PYA-4-40 plough. During this period, the process of intensive mineralization of organic substances by the maximum number of microbiota takes place.

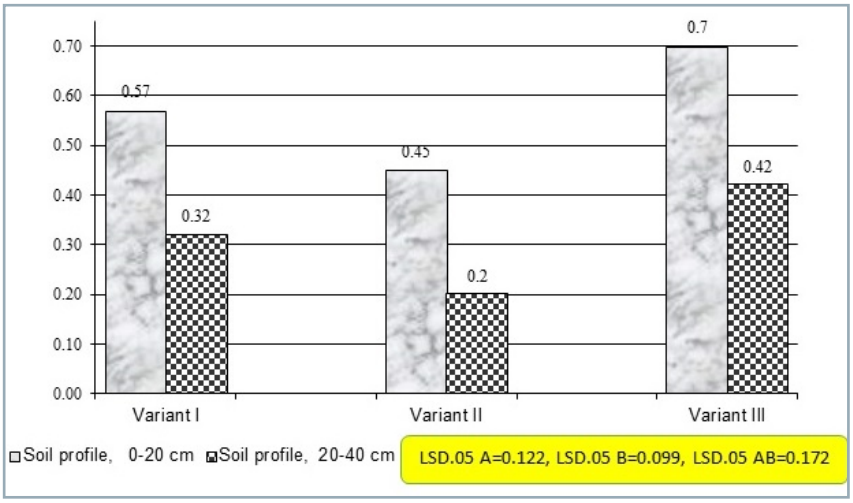


Fig. 1 Influence of the method of basic cultivation (factor A) on the content of nitrate nitrogen in the soil layers (factor B) 0–20 cm and 20–40 cm in the technology of growing of *Trifolium pratense* in mg per 100 g of abs. dry soil (average for years 2018–2020)

Over time (June), in the process of active growth of *Trifolium pratense* plants, the nitrate content decreased to 0.58 mg per 100 g of soil. At the end of the growing season of crop plants, due to a decrease in the intensity of their use of nitrate nitrogen, its amount in the soil increased to 0.82 mg per 100 g of soil.

The reduction of the amount of nitrate nitrogen in the soil layer 0–20 cm at a young leaf growth stage of *Trifolium pratense* was found in the options for conventional (0.57–0.70 mg per 100 g) and chisel tillage (0.47–0.57 mg per 100 g). This is due to a decrease in the number of microorganisms and a decrease in the intensity of the development of microbiota and apparently nitrifying microorganisms.

Research and calculations show that the methods of basic tillage affect the dynamics of nitrate content in the subsoil (20–40 cm) layer of soil in different ways. In particular, a decrease in the number of nitrate forms of nitrogen compared to the arable (0–20 cm) layer was found but the general patterns were similar. Thus, for tillage with a tiered plough, nitrate nitrogen accumulated the most (0.29–0.64 mg per 100 g of soil), and for chisel tillage – the least (0.13–0.29 mg per 100 g of soil).

Over the years, the study found that in the case of tiered ploughing during the growing season of *Trifolium pratense*, the maximum amount of nitrate nitrogen in the arable soil layer was 0.7 mg per 100 g and in the subsoil 0.42 mg per 100 g of soil (Fig. 1).

The use of conventional and chisel tillage caused a decrease in these nutrients in the arable and subsoil layers of the soil, i.e., 0.57 and 0.32 mg and 0.45 and 0.2 mg per 100 g of absolutely dry soil, respectively.

The amount of analyzed elements was not the same in the years of research. During the growing season, the amount of nitrate nitrogen in the 0–20 cm soil layer was in the range of 0.75–0.48 (2018), 0.58–0.44 (2019), and 0.78–0.52 mg per 100 g of soil

Table 1 The influence of the method of basic cultivation on the content of nitrate nitrogen in the soil in the technology of growing *Trifolium pratense* in mg per 100 g abs. dry soil (average for the period of 2018–2020)

Variant of the main tillage (factor A)	Determination period (factor B)	Soil profile (cm)	
		0–20	20–40
Conventional tillage (control)	full seedlings	0.57	0.19
	bud phase	0.45	0.28
	before harvesting	0.70	0.49
Chisel tillage	full seedlings	0.47	0.13
	bud phase	0.32	0.17
	before harvesting	0.57	0.29
Tiered tillage	full seedlings	0.71	0.29
	bud phase	0.58	0.33
	before harvesting	0.82	0.64
LSD_{05} A		0.36	0.12
LSD_{05} B		0.36	0.12
LSD_{05} AB		0.63	0.21

LSD – least significant difference

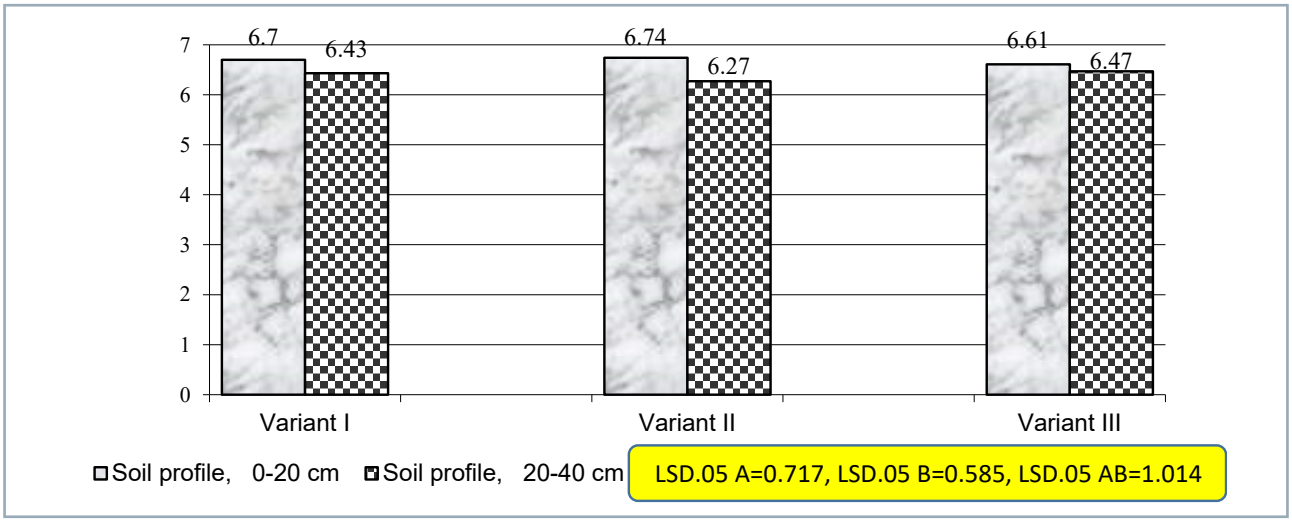


Fig. 2 Influence of the method of basic cultivation (factor A) on the content of mobile forms of phosphorus in soil layers (factor B) 0–20 and 20–40 cm in the technology of growing *Trifolium pratense* in mg per 100 g abs. dry soil (average for 2018–2020)

(2020), and in the 20–40 cm soil layer – 0.38–0.21 (2018), 0.37–0.17 (2019), and 0.51–0.22 mg per 100 g of soil (2020).

The decrease in the level of nitrate nitrogen in the soil and its distribution in soil horizons is associated with the number of microflora (Aboltins et al., 2018).

During the tiered ploughing, the number of bacteria and fungi in the arable soil layer averaged 3458.3 thousand and 43.8 thousand, respectively. In the options for chisel tillage and conventional tillage, the figures were lower and amounted to 2712.1 thousand and 37.8 thousand, and 2920.0 thousand and 41.7 thousand colony-forming units per 1 g of absolutely dry soil.

The results of the study indicate similar number and distribution of these groups of microorganisms in the subsoil. When performing tiered tillage, the accumulation and assimilation of nitrate nitrogen from the soil by *Trifolium pratense* plants is more efficient compared to other methods of cultivation in the experimental variants.

The study of the influence of tillage methods revealed different effects not only on the development of microorganisms and the content of nitrate nitrogen, but also on the content of mobile phosphorus because its lacking limits the increase in yield.

Various phosphates are concentrated in the soil: mineral forms complex compounds with mineral and organo-mineral colloids which are part of the soil organic matter. The plant easily absorbs the salts of all monovalent cations of orthophosphoric and metaphosphoric acids, worse – the monosubstituted salts of divalent cations of these acids.

The results of the study showed (Table 2) that the amount of mobile phosphorus in the soil during the cultivation of *Trifolium pratense* significantly depended on the method of basic tillage. Based on the analysis of the soil of the arable and subsoil layers during the restoration of spring vegetation, it was found that a large amount of mobile phosphorus in the arable layer of the soil was in the variant of chisel tillage. The amount of mobile phosphorus was 5.94 mg per 100 g of soil, which is a consequence of the localization of nutrients in the upper horizon for tillage without rotating the formation.

In the variant of conventional tillage (control) and tiered tillage, these amounts were lower, i.e., 5.91 and 5.92 mg per 100 g of soil, respectively.

In the subsoil layer, the soil revealed an inverse nature pattern compared to arable where phosphorus compounds were more evenly distributed, which is a sign of creating a heterogeneous layer of soil under deep chiselling of the soil. Tillage with shelf tools, especially tiered tillage, forms a homogeneous layer of soil nutrients. In this process, it was found that the amount

Table 2 The influence of the method of basic cultivation on amount of mobile phosphorus in the soil in the technology of growing *Trifolium pratense* in mg per 100 g of abs. dry soil (average for the period of 2018–2020)

Variant of the main tillage (factor A)	Determination period (factor B)	Soil profile (cm)	
		0–20	20–40
Conventional tillage (control)	full seedlings	5.91	4.88
	bud phase	7.42	7.17
	before harvesting	6.77	7.24
Chisel tillage	full seedlings	5.94	4.67
	bud phase	7.45	7.05
	before harvesting	6.82	7.08
Tiered tillage	full seedlings	5.92	4.93
	bud phase	7.10	7.20
	before harvesting	6.81	7.29
LSD_{05} A		0.47	1.6
LSD_{05} B		0.47	1.6
LSD_{05} AB		0.81	2.7

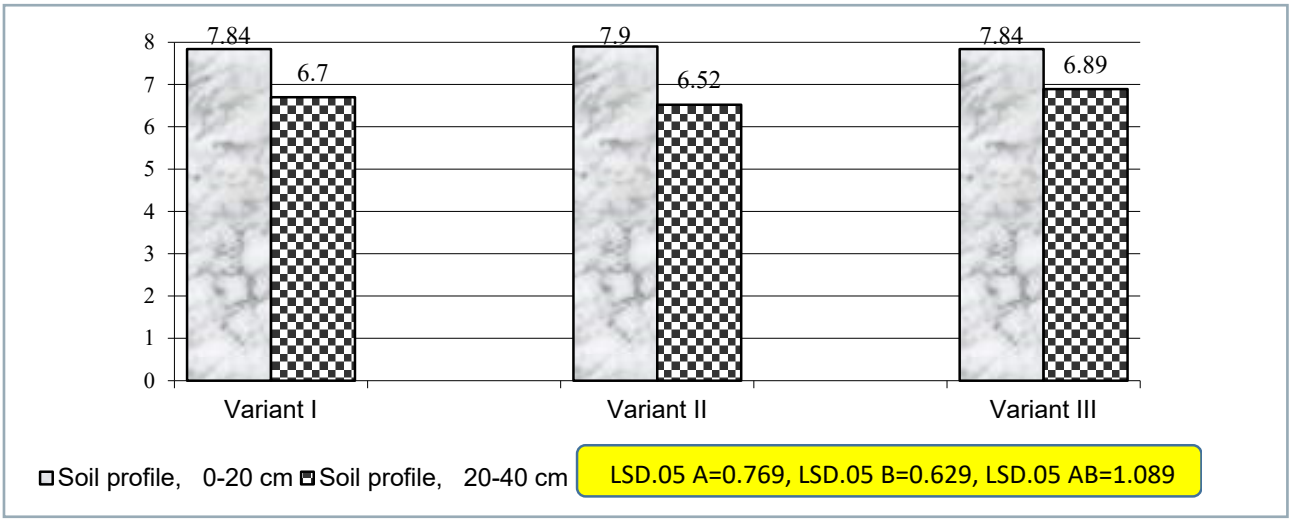


Fig. 3 The influence of the method of main cultivation (factor A) on the content of exchangeable potassium in soil layers (factor B) 0–20 and 20–40 cm in the technology of growing *Trifolium pratense* in mg per 100 g of abs. dry soil (average for 2018–2020)

of mobile phosphorus varied from spring to autumn. Before harvesting, due to the absorption of phosphorus by *Trifolium pratense* plants during the formation of the crop, the number of its mobile forms decreased on average but the previously established trends remained.

During the growing season, the amount of phosphorus in the 0–20 cm soil layer was in the range of 7.74–7.4 (2018), 5.15–5.11 (2019), and 7.31–7.29 mg per 100 g of soil (2020), and in the 20–40 cm soil layer, it was 6.66–6.51 (2018), 5.32–5.13 (2019), and 7.44–7.16 mg per 100 g of soil (2020).

The analysis of the obtained results showed that the amount of mobile phosphorus (6.74 mg) in the arable layer of the soil was in the variant for chisel tillage (Fig. 2). During conventional and tiered tillage, these values decreased to 6.7 and 6.61 mg per 100 g of absolutely dry soil, respectively. The indicators of accumulation of mobile phosphorus in the soil and the number of microorganisms did not contribute to such dependence as the production of nitrate nitrogen when the development of bacteria and fungi showed similar patterns of accumulation of nitrates in the soil.

A detailed study of potassium reserves in the soil showed that they are slightly higher than nitrate nitrogen and phosphorus. The direct source of plant nutrition is primarily water-soluble potassium. The processes of assimilation of this element by plants and replenishment of its reserves in the soil from difficult and inaccessible forms are in balance and can be at a constant level for a long time.

During the growing season of *Trifolium pratense*, the amount of exchangeable potassium in the soil varies less than mobile phosphates and nitrogen, depending on the microbiological activity which is significantly affected by mechanical tillage (Table 3).

During the chisel tillage, layer-by-layer differentiation took place according to the content of exchangeable potassium. In the upper (0–20 cm) layer of the soil, its amount is higher (full seedlings – 7.11, bud stage – 8.57, before harvesting – 8.0 mg per 100 g of soil) compared to conventional (7.01, 8.50, 8.01 mg) ploughing (control) and tiered tillage (7.05, 8.53, 7.95 mg per 100 g of soil). In the subsoil (20–40 cm) layer of the soil, the results obtained

Table 3 The influence of the method of basic cultivation on the content of exchangeable potassium in the soil in the technology of growing *Trifolium pratense* in mg per 100 g of abs. dry soil (average for the period of 2018–2020)

Variant of the main tillage (factor A)	Determination period (factor B)	Soil profile (cm)	
		0–20	20–40
Conventional tillage (control)	full seedlings bud phase before harvesting	7.01 8.50 8.01	5.15 7.45 7.51
Chisel tillage	full seedlings bud phase before harvesting	7.11 8.57 8.0	4.95 7.33 7.27
Tiered tillage	full seedlings bud phase before harvesting	7.05 8.53 7.95	5.34 7.63 7.7
LSD_{05} A		0.48	1.50
LSD_{05} B		0.48	1.50
LSD_{05} AB		0.83	2.60

Table 4 The yield of green mass of *Trifolium pratense* depending on the method of tillage (together for two slopes) (t·ha^{-1})

Variant of the main tillage	Year			The average for 2018–2020	±to control	
	2018	2019	2020		t·ha^{-1}	%
Conventional tillage (control)	59.6	47.8	54.6	54.0	–	–
Chisel tillage	55.5	44.3	50.3	50.0	-4.0	-7.4
Tiered tillage	66.5	50.7	58.3	58.5	+4.5	+17.0
LSD05 (t·ha^{-1})	2.3	2.5	2.2			

reflected the pattern inverse to the upper (0–20 cm) layer of the soil.

During the growing season, the amount of potassium in the 0–20 cm soil layer was in the range of 8.93–8.83 (2018), 6.3–6.22 (2019), and 8.44–8.36 mg per 100 g of soil (2020), and in the 20–40 cm soil layer, it was 7.08–6.69 (2018), 5.73–5.41 (2019), and 7.86–7.44 mg per 100 g of soil (2020) (Fig.3).

From the analysis of results of the study it follows that the highest rates of potassium exchange were at the beginning and in the middle of the growing season. Over the years of research, the amount of potassium before harvest decreased slightly due to its assimilation by plants in the process of crop formation.

In the variant of tillage with the PYA-4-40 plough, the average potassium content at a depth of 0–40 cm was higher compared to the control and chisel tillage.

Thus, the results of the study are a confirmation that the intensity of biological processes determine the methods of cultivation that affect the growth of soil biogenicity. Under such conditions, the amount of nutrients in the soil solution increases.

The tillage method influenced the intensity of microbiological processes. In particular, in the variant of tillage with the PYA-4-40 plough, the number of microflorae was the highest, which led to a higher intensity of nutrient accumulation.

The accumulation of nutrients with a high content of nitrogen, phosphorus, and potassium in the soil according to the experimental options led to an increase in the yield of green mass of *Trifolium pratense* (Table 4).

In the variant of chisel tillage, the yield of green mass of *Trifolium pratense* was 50.0 t·ha^{-1} (together for two slopes) which is 4.1 t·ha^{-1} less than for conventional cultivation (control). The results of the analysis of variance convincingly confirm the reliability of the study.

The application of a tiered tillage helped to increase the yield of green mass of *Trifolium pratense* by 4.4 t·ha^{-1} (+17.0%) compared to the control (conventional ploughing).

During the growing season of crop plants, both nitrogen uptake for vegetative mass growth and accumulation of its available forms due to decomposition of organic matter by soil microflora took place at the same time.

In the course of research, the influence of tillage on the nutrient regime of the soil and the yield of the green mass of meadow clover in the five-field crop rotation of the Western Forest Steppe of Ukraine on dark gray podzolized soils was discovered.

The results obtained show that the amount of mobile phosphorus in the soil during the cultivation of meadow clover significantly depended on the method of the main tillage. Based on the soil analysis of the arable and subsoil layers during spring recovery, it was established that the greater amount of mobile phosphorus in the arable soil layer was in the chisel tillage option. The amount of mobile phosphorus was 5.94 mg per 100 g of soil, which is associated with the result of the localization of nutrients in the upper horizon due to soil cultivation without rotation of the layer.

The results of this study coincide with the data obtained in the studies of Karabach (2010). The author indicates an increase in the content of mobile phosphates during flat-cut cultivation compared to conventional ploughing.

The perspective of further research is to establish the optimal variant of processing and the rate of fertilizer application in the technology of growing crops of the crop rotation link, which ensures the optimal nutritional regime and the maximum development and yield of plants.

Conclusion

Two-tier ploughing creates more favorable conditions for plant nutrition with nitrogen, hosphorus, and potassium throughout the growing season. When performing row ploughing, an increase in the average content of nitrate nitrogen during the growing season was established by 0.13–0.25 (soil layer 0–20 cm) and 0.10–0.22 mg per 100 g of soil (soil layer 20–40 cm) compared to control and chisel processing.

In the upper layer of the soil (0–20 cm), with the use of chisel cultivation, the content of mobile phosphorus and potassium increases to 6.74 and 7.89 mg per 100 g of soil, respectively, and in the bottom layer (20–40 cm), on the contrary, it decreases to 6.27 and 6.52 mg per 100 g of soil compared to conventional and row ploughing.

A significantly higher yield of meadow clover green mass (58.5 t·ha^{-1}) was obtained by two-tier ploughing than by chisel and conventional tillage.

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