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The effect of biological fertilisation on the intensity of nitrogen -carbon cycling in crop rotations in the forest-steppe

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ABSTRACT

The aim of the study was to determine the parameters of a novel integrated stoichiometric model of the interrelated nitrogen and carbon cycles under conditions involving the implementation of different types of crop rotations, alternative types of organic fertilisation and varying methods of mechanical soil cultivation in the agrocenoses of the forest-steppe of Ukraine. The research methodology was based on a combination of long-term field observations and an integrated stoichiometric framework for evaluating nitrogen, as well as carbon balances under different fertilisation systems and soil tillage practices. It was established that the type of organic fertiliser directly correlates with the level of CO_2 emissions resulting from mineralisation processes: when manure is applied, the range of emissions is usually 25–85 tonnes, whereas the use of by-products increases this level to 80–160 tonnes. In the first scenario, a more intensive nitrogen removal relative to its input was observed, whereas in the second, a progressive increase in total stocks was recorded. An increase in CO_2 emissions in such cases is accompanied by an improvement in the soil carbon balance and an increase in the overall yield of the system. An assessment of the impact of black soil tillage methods showed that with non-inversion tillage technology - regardless of the type of crop rotation or fertiliser – a positive trend in soil carbon balance is observed, whereas with ploughing, the nature of the increase in organic matter deficit has a non-linear (parabolic) form. Ploughing is characterised by a wider range of CO_2 emissions, indicating more active mineralisation. The results of the study confirmed the high efficiency of using shredded crop by-products in combination with appropriate nitrogen compensation via mineral fertilisers. The practical significance of the results lies in substantiating the effectiveness of using crop by-products and non-inversion tillage to stabilise nitrogen-carbon cycling, restore the fertility of chernozems and increase the productivity of agrocenoses in the forest-steppe of Ukraine.

Keywords: carbon dioxide, nitrogen balance, manure, crop residues, ploughing, non-inversion tillage.

INTRODUCTION

Optimisation of the soil nitrogen regime and mineral nutrition of plants constitutes a key component of modern agriculture and sustainable agroecosystem management. Nitrogen forms part of amino acids, proteins, nucleic acids, chlorophyll, and enzymes, and accounts for an average of 1.5–5.0% of plant dry matter. Owing to the narrow range of plant uptake, this element is regarded as the most labile among nutrients, while both its deficiency and excess lead to reductions in crop productivity and product quality, as emphasised by Prysiazhniuk et al. (2022) and Yanitskyi (2024).

Climate destabilisation substantially transforms the biogeochemical relationship between the accumulation and mineralisation of organic matter in agricultural landscapes. Under intensified decomposition processes, the rate of carbon dioxide release may exceed the potential for its biological fixation, thereby turning agricultural systems into net sources of greenhouse gas emissions, as demonstrated in the studies of Sturman and Quénol (2023) as well as Tang et al. (2025). At the same time, according to Kalbande et al. (2023), contemporary models of biogeochemical processes that account for the coupling of carbon and nitrogen cycles indicate the possibility of increasing the terrestrial carbon pool under rising temperatures. The principal driver of this process is the intensification of organic compound decomposition, which promotes the accumulation of mineral nitrogen required for enhanced photosynthetic fixation of CO_2 by plant communities.

In the context of global biogeochemical transformations, as noted by Tkalenko et al. (2021), nitrogen and carbon cycles are regarded as fundamental coupled mechanisms determining the functional stability of terrestrial ecosystems and agricultural landscapes. A substantial proportion of carbon assimilated by phytomass (up to 55%) is directly involved in nitrogen metabolism processes, whereas organic carbon compounds and CO_2 emissions act as key determinants of soil microbiota activity. The scientific community emphasises the critical necessity of integrating the nitrogen cycle into climate models, since nutrient limitation (deficiency of mineral nitrogen in soil) represents the principal barrier to effective sequestration of atmospheric carbon by plant communities, as highlighted in the works of Jones (2021) and Parray (2023).

As noted by Dalias and Christou (2022), the central role in the mechanism of interaction and synchronisation of carbon and nitrogen cycles belongs to the soil microbial biomass, which functions as an active "biological hub" for the transformation of organic matter. The microbial pool serves not only as a temporary reservoir of nutrients, but also as the principal mediator determining the direction of processes between accumulation and mineralisation. The biogeochemical thresholds identified by Quan et al. (2021) as well as Sturman and Quénol (2023) constitute critical determinants of the functioning of the destructive component of agroecosystems. These numerical values are based on the physiological requirements of microbial communities, which, in order to maintain their own homeostasis, require a specific ratio between the energy substrate (C) and the structural material (N). Net mineralisation (C:N < 20): when organic matter with a narrow elemental ratio is incorporated into the soil (for example, legume residues or green manure crops), microorganisms receive an excess of nitrogen relative to the available carbon. This results in the release of ammoniacal nitrogen forms into the soil solution, thereby increasing nutrient availability for plants. Biological immobilisation (C:N > 30): when substrates with a high cellulose and lignin content (such as cereal straw) are incorporated into the soil, a nitrogen deficiency arises for the synthesis of microbial proteins. Under such conditions, the soil microflora becomes a strong competitor to plants by absorbing mineral nitrogen forms from the soil solution, leading to a temporary reduction in soil fertility – the phenomenon known as "nitrogen starvation". The rate of C-CO_2 release (heterotrophic respiration) is considered an appropriate proxy indicator of gross nitrogen fluxes, since both processes are limited by the rate of decomposition of the carbon skeletons of organic molecules. However, as noted by Kucher et al. (2022), this relationship remains linear only under the conditions of substrate metabolic stability.

Globally, the optimisation of carbon (C) and nitrogen (N) cycling in agroecosystems is recognised as a fundamental priority for ensuring long-term agricultural sustainability and maintaining stable soil fertility (Lal, 2020). Recent international studies highlighted that traditional intensive farming practices often lead to the rapid depletion of soil organic matter, necessitating a shift towards conservation agriculture. Consequently, the integration of reduced tillage systems - such as non-inversion or minimum tillage - with the strategic management of crop residues has been widely advocated. These practices not only enhance soil structural stability but also significantly improve carbon sequestration and nitrogen use efficiency across various temperate agroecosystems (Blanco-Canqui and Ruis, 2020; Mondal et al., 2020).

Despite the substantial theoretical foundation, the issue of quantitative synchronisation of nitrogen and carbon fluxes under the specific conditions of Forest-Steppe agrocenoses of Ukraine remains debatable. Previous models predominantly evaluated nitrogen and carbon fluxes independently or relied on static emission factors. The novelty of the study lies in integrating nitrogen and carbon balance calculations into a unified stoichiometric framework that simultaneously evaluates nutrient inputs, outputs, soil carbon balance, and CO_2 emissions under contrasting fertilisation and tillage systems. Unlike previous studies that assessed nitrogen and carbon cycling separately, the proposed framework enables their integrated evaluation within a single balance approach, providing a more comprehensive assessment of agroecosystem functioning under long-term field conditions. Thus, the aim of the study was to determine the standardised indicators of this new coupled nitrogen-carbon cycling model under variable crop rotation types and alternative organic fertilisation sources, influenced by different soil tillage technologies.

The objective was to reveal the principal trends in nitrogen-carbon cycling, utilising a novel measurement approach to quantify the regeneration of natural pedogenic processes to maintain long-term soil fertility and organic matter stability.

MATERIALS AND METHODS

The study was conducted during 2014–2024 in the central part of the Left-Bank Forest-Steppe of Ukraine under the conditions of a long-term stationary experiment at the Drabiv Experimental Field of the Cherkasy State Agricultural Research Station of the National Scientific Research Centre "Institute of Agriculture of the National Academy of Agrarian Sciences of Ukraine" (Figure 1). The region is characterised by a temperate continental climate. The average annual air temperature ranges from 7.5 to 8.5 °C. The average annual precipitation is approximately 500–550 mm, with the majority (about 60–70%) falling during the active vegetative period from April to September. However, the region frequently experiences prolonged dry spells and high summer temperatures, leading to periodic drought conditions that significantly influence soil moisture dynamics and the rate of organic matter mineralisation.

Experimental design and reproducibility

The field research utilised a randomized complete block design (RCBD) structured systematically by year to capture both spatial and temporal variability over the 10-year study period. The experiments were established on plots with a total

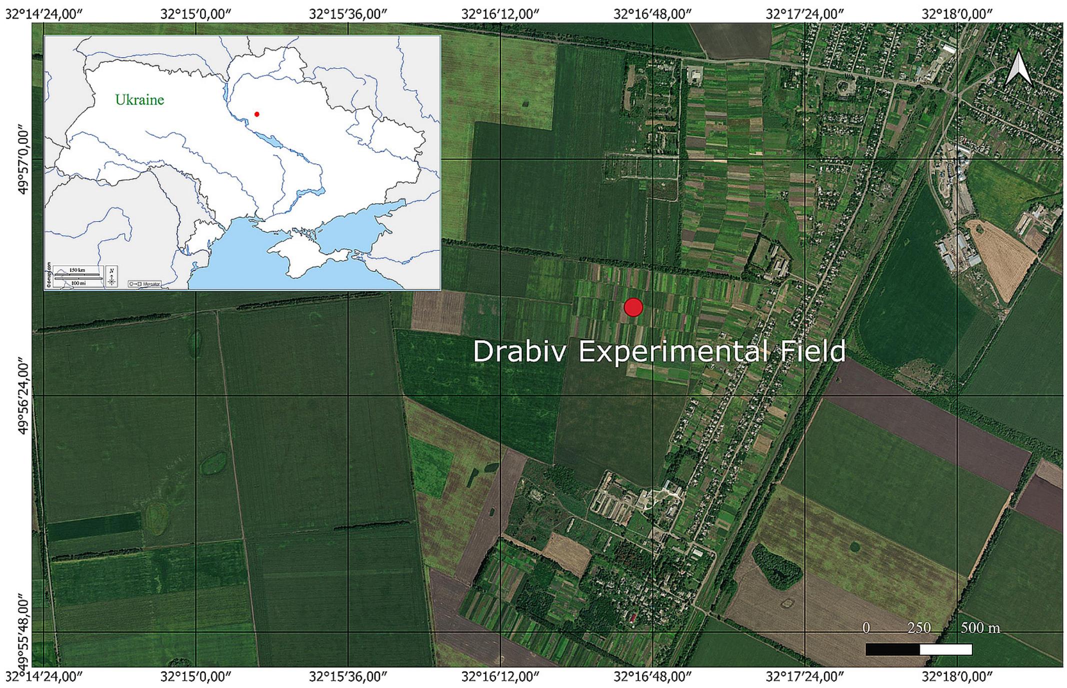

Figure 1. Location of the Drabiv experimental field study area. Coordinate system: WGS 84 (EPSG:4326). Map created in QGIS based on high-resolution satellite imagery

sowing area of 160 m² and an accounting area of 100 m². Each treatment was evaluated in three replications. Baseline data were collected in 2014 before the establishment of the experimental treatments. Thereafter, from 2015 to 2024, 15 composite soil samples were collected annually after harvest for agrochemical analysis, resulting in a total of 150 samples. Composite soil samples were collected from the 0–20 cm soil layer. The experimental factors included crop rotation systems, fertilisation treatments, and tillage methods. Experimental plots remained at the same location throughout the study period. Baseline measurements conducted in 2014 indicated an initial winter wheat yield of 4.5 t ha^{-1}. To standardise the assessment of agricultural productivity across different crop types within the rotations, the absolute crop yields were converted into 'feed units'. One feed unit is defined as the nutritional equivalent of the energy content of one kilogram of oats. The total feed unit yield (t ha^{-1}) for each experimental plot was calculated by multiplying the physical yield of the harvested crop (grain or biomass) by its respective standard conversion coefficient according to established regional agrotechnical evaluation guidelines. Determination of the agrochemical indicators of soil quality was carried out in accordance with current national standards: pH according to DSTU ISO 10390:2007 (2007); total humus and carbon content according to the method of Tyurin as modified by Simakov (DSTU 4289:2004, 2004). Baseline indicators established a total humus content of 4.2%. The initial soil organic carbon () was 2.44%, and initial total nitrogen (N_{tot}) was 2200 mg kg^{-1}. The soil was well supplied with available phosphorus compounds (140 mg kg^{-1} soil) and had an elevated level of available potassium compounds (100 mg kg^{-1} soil) according to the method of Chyrykov (DSTU 4115-2002, 2002). The balance of soil organic matter and humus was calculated according to Balyuk et al. (2011). The soil exhibited a neutral active acidity reaction with a pH of 6.8–7.0. This range is considered optimal for most agricultural crops, as it ensures maximum nutrient availability and favourable conditions for soil microbiota activity.

The objects of analysis were two types of specialised crop rotations. The first model (A) was based on the alternation of peas, winter wheat, sugar beet, and grain maize (two phases), where the proportions of cereals, industrial crops, and legumes constituted 60%, 20%, and 20%, respectively. The second model (B) included perennial grasses, winter wheat, sugar beet, maize, and barley with grasses, maintaining a similar proportion of cereal and industrial crop groups with a 20% share of the grass component. The field experiment was structured to evaluate the combined effects of agricultural practices on agroecosystem functioning. The design included crop rotation models, organic fertilisation variants, and soil tillage methods. Specifically, regarding fertilisation, two organic sources were evaluated at a rate of 6 t ha^{-1}- manure versus crop by-products - both integrated with a background mineral fertiliser application of $N_{62}P_{66}K_{82}$ per 1 ha of crop rotation area. The tillage methods included conventional ploughing, non-inversion tillage, and minimum (surface) tillage. All organic fertilisation variants and soil tillage methods were systematically combined across both crop rotation models to assess their interactive and main effects.

Stoichiometric modelling and balance calculations

The calculation of integrated indicators of nutrient cycling was performed using the balance method. The calculations were based on the methodology proposed by Skrypchuk et al. (2020), which integrates humus formation, mineralisation, crop productivity, and nutrient removal by phytomass. Nitrogen input from manure (N_{manure}) was calculated based on the application rate, and nitrogen input from crop residues ($N_{residues}$) was estimated using crop productivity and residue-to-yield ratios according to the adopted balance methodology. Nitrogen removal ($N_{removal}$) was determined from the harvested yield using crop-specific nutrient removal coefficients. Carbon dioxide emissions (ECO_2) and crop biomass production were estimated according to the published methodology. The nitrogen balance (B_N) within the agrocenosis was calculated using the following deterministic formula (Equation 1):

$$B_N = (N_{manure} + N_{residues} + N_{min}) - \\ - N_{removal} \quad (1)$$

where: inputs include nitrogen from manure (N_{manure}), crop by-products ($N_{residues}$), and mineral fertilsers (N_{min}), and $N_{removal}$ is the biologically extracted nitrogen via harvested crop yield.

Assessment of carbon dioxide (CO_2) emissions and carbon sequestration was performed taking into account the actual productivity of agricultural crops in multi-rotation crop rotations (Anishin et al., 2014). Regression equations differentiated according to productivity levels were used to determine the volumes of crop by-products, post-harvest residues, and below-ground biomass (Rechcigl, 2018). The regression coefficients were selected according to crop species and productivity level in accordance with the published methodology. The net carbon balance *(Bc*) was evaluated as shown in Equation 2:

$$B_C = (M_{res} \times K_c) - E_{CO_2} \quad (2)$$

where: M_{res} is the quantified mass of crop residues and below-ground biomass, K_c is the stoichiometric coefficient of 3.7 utilised for converting carbon pools into carbon dioxide, and E_{co2} represents total quantified carbon dioxide emissions.

The quantity of formed humus (C_g) as a stabilised carbon pool was calculated systematically depending on the volumes of incoming organic substrate as described in the methodology of the Intergovernmental Panel on Climate Change (IPCC) (2008). To evaluate the overall sustainability of the agrocenoses, the balance intensity index (*Ib*) was computed using Equation 3:

$$I_b = \left(\frac{\sum \text{Nutrient Inputs}}{\sum \text{Nutrient Removal}}\right) \times 100 \quad (3)$$

All primary laboratory data were statistically processed using Microsoft Excel and Statistica 10. Data were subjected to analysis of variance (ANOVA) to evaluate the main effects and interactions of crop rotation, fertilisation, and tillage methods. Treatment means were compared using Tukey's Honestly Significant Difference (HSD) post-hoc test, with statistical significance established at $p < 0.05$. To accurately reflect data dispersion and effect sizes, standard errors are integrated directly alongside the average values (presented as Mean ± SE). The processed data were subsequently used for calculating nitrogen and carbon balances and for evaluating the direction and intensity of biogeochemical cycling in the studied agroecosystems.

RESULTS AND DISCUSSION

The results of the feed unit analysis indicate that the use of by-products is preferable to manure in both types of crop rotation (Figure 2). The highest average feed unit value (33.0 t) was recorded in the crop rotation with peas, provided that 6 t ha^{-1} of by-products were applied.

Nitrogen removal with yield correlated with productivity: the highest values are observed in treatments with catch crops, reaching 718 kg in the crop rotation with peas and 564 kg in the crop rotation with grasses (Figure 3).

Total N removal was highest in the pea-based crop rotation with manure application (935 kg), indicating intensive utilisation of nitrogen resources within the agrocenosis; however, the level of nitrogen input from crop by-products was the lowest among all treatments, reflecting substantial removal of the element with the harvested yield. In contrast, the use of crop by-products (6 t ha^{-1}) as the principal fertiliser contributed to optimisation of the nitrogen regime. N removal with yield increased to 717 ± 37 kg, while

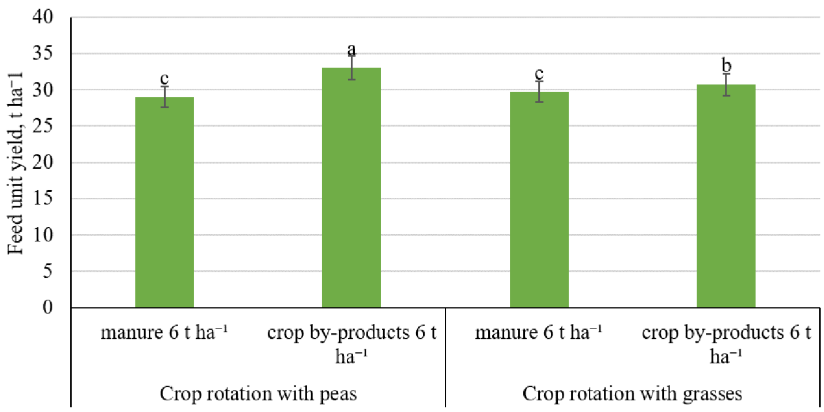

Figure 2. Feed unit yield in agrocenoses depending on the type of crop rotation and fertilisation system, t ha^{-1}. Different lowercase letters indicate statistically significant differences according to Tukey's HSD test ($p < 0.05$)

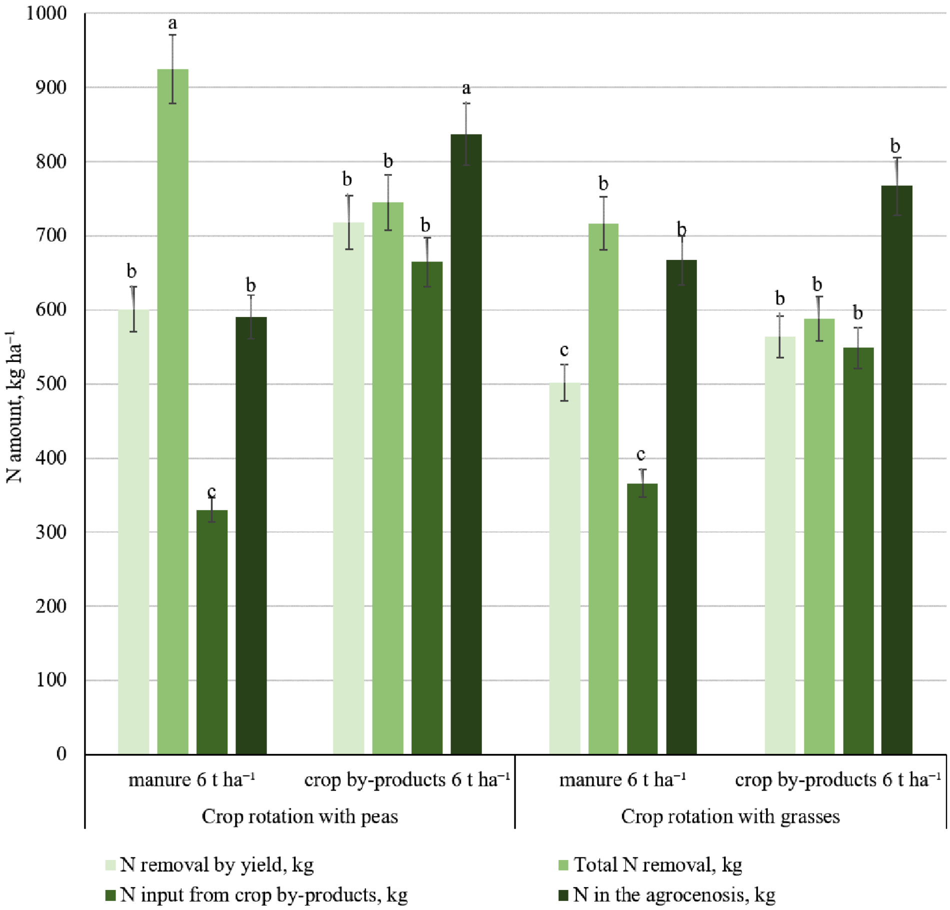

Figure 3. Nitrogen cycle indicators in agrocenoses depending on the fertilisation system and crop rotation type. Different lowercase letters indicate statistically significant differences according to Tukey's HSD test ($p < 0.05$)

nitrogen input into the soil from plant residues doubled compared with the manure treatment, reaching 664 ± 34 kg. This resulted in the highest total nitrogen content within the agrocenosis – 837 ± 42 kg – indicating the high nitrogen-fixing capacity of peas under conditions of biomass return to the soil.

A similar trend was observed in the grass-based crop rotation, although with slightly lower absolute values of nitrogen fluxes. The manure treatment was characterised by the lowest N removal with yield (503 ± 25 kg) and total N removal of 716 ± 38 kg. Application of crop by-products in this crop rotation ensured a balanced distribution of nitrogen: N removal with yield (564 ± 28 kg) was nearly equivalent to its return to the system (549 ± 28 kg). The total nitrogen content within the agrocenosis amounted to 767 ± 39 kg, which was 15% higher than in the manure treatment within the same crop rotation. Thus, comparison of the two crop rotation types demonstrates that the inclusion of peas provides more intensive nitrogen cycling than grasses. The key factor in nitrogen accumulation within the system proved to be the use of crop by-products (6 t ha^{-1}).

The statistically significant advantage of crop by-products over manure (at a rate of 6 t ha^{-1}) with respect to nitrogen accumulation in the agrocenosis is explained by the direct return of organic matter that has undergone a cycle of biological transformation *in situ*. The obtained data confirm the hypothesis that, under the conditions of limited application of traditional organic fertilisers, the use of plant residues in combination with leguminous crops represents the most effective strategy for maintaining soil nitrogen homeostasis. The results of the nitrogen balance assessment (Figure 4) demonstrate a fundamental difference in the direction of nitrogen cycling depending on the form of organic matter returned to the soil.

In the treatments involving manure application in both crop rotation types, a negative nitrogen balance was recorded, indicating that nitrogen removal exceeded its input. In the pea-based crop rotation, the nitrogen deficit was critical, amounting to -345 kg. Such a substantial negative value is explained by the high level of nitrogen removal with the harvested yield of the leguminous crop, which was not compensated for by the low manure application rate. In the grass-based crop rotation, the deficit was considerably lower at -61.4 kg, owing to less intensive nitrogen removal compared with the legume-based rotation.

The transition to the use of crop by-products (6 t ha^{-1}) as the principal fertiliser source ensured a positive nitrogen balance in both investigated systems. In the pea-based crop rotation, nitrogen accumulation amounted to 97.8 kg. The return of plant residues combined with the biological nitrogen fixation capacity of the crop made it possible not only to offset nitrogen removal but also to generate a positive balance. The maximum nitrogen surplus was recorded in the grass-based crop rotation, where the indicator reached 189 kg. This demonstrates the highest efficiency of crop by-products precisely within this crop rotation component for increasing the nitrogen potential of the soil. Analysis of the obtained data suggests that manure application at a rate of 6 t ha^{-1} is insufficient to maintain a balanced nitrogen budget in intensive crop rotations. By contrast, the use of an equivalent amount of crop by-products (6 t ha^{-1}) radically alters the direction of the processes – from degradational (depletion of reserves) to accumulative.

Assessment of the dynamics of organic carbon (Corg.) made it possible to establish patterns in the transformation of organic matter depending on crop rotation structure and sources of carbon input (Figure 5). All investigated treatments were characterised by a strongly negative overall balance of organic carbon within the agrocenosis. In the pea-based crop rotation, the carbon deficit ranged from -26 t (manure treatment) to -38.5 t (crop by-products treatment). In the grass-based crop rotation, these values were even lower, reaching a minimum of -39 t under the application of crop by-products. These findings indicate substantial carbon losses through respiration (CO_2 emissions) and removal with yield, exceeding the total organic matter input.

The indicators of the organic carbon (Corg.) balance directly within the soil demonstrated a different trend, which is critically important for assessing humus status. In the treatments with manure application in both crop rotations, a deficit soil carbon balance was recorded (from -1 to -3.1 t). This indicates that a manure application rate of 6 t ha^{-1} is insufficient to compensate for humus mineralisation processes. The use of plant residues ensured a transition to a positive soil organic carbon (Corg.) balance. In the pea-based crop rotation, the surplus amounted to approximately +2.7 t, while in the grass-based crop rotation it reached around +2.0 t. Despite the overall carbon deficit within the agrocenosis system (caused by biological activity and biomass removal), the return of crop by-products to the soil acts as an effective mechanism for carbon sequestration. The superiority of crop by-products over manure (at a rate of 6 t ha^{-1}) with respect to the restoration of soil carbon reserves was statistically confirmed. This is explained by the more uniform distribution of plant residues within the plough layer and their gradual humification, which ensures a stable input of organic carbon (Corg.) into the soil matrix. By contrast, low manure application rates are unable to halt the degradation of carbon reserves, resulting in a negative balance even in crop rotations, including perennial grasses.

Organic carbon (Corg.) reserves constitute a fundamental indicator of the potential fertility and environmental sustainability of agricultural landscapes. The research findings (Figure 6) revealed substantial differentiation in the Corg. content

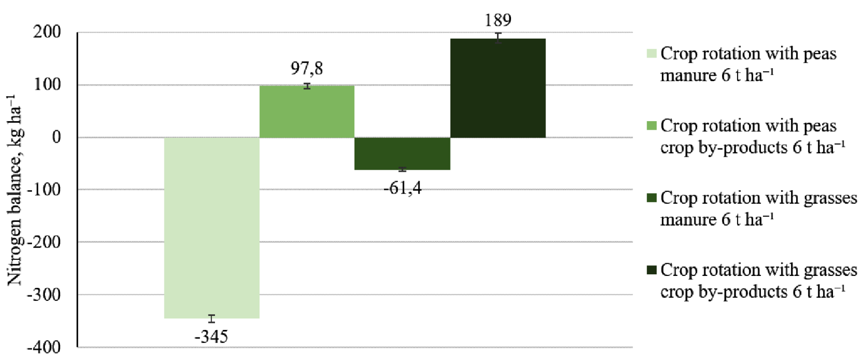

Figure 4. Nitrogen balance in agrocenoses depending on the fertilisation system and crop rotation type, kg ha^{-1}

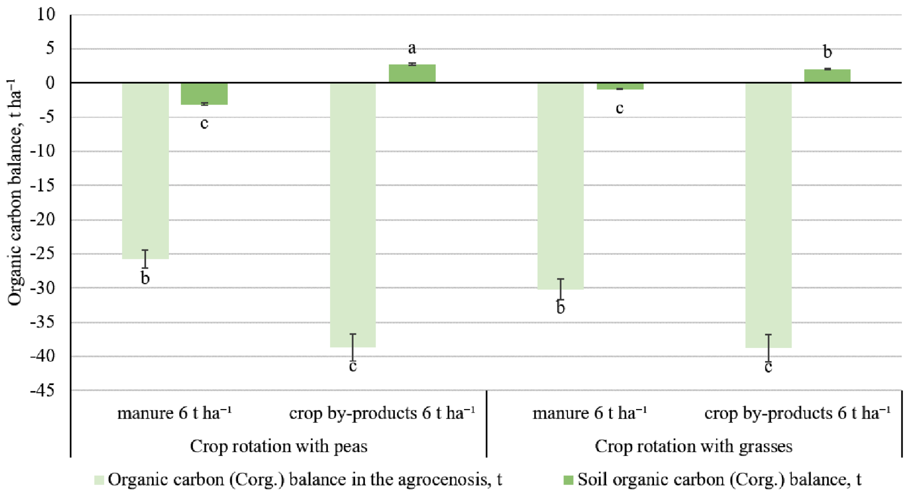

Figure 5. Carbon balance as a function of fertilisation systems and crop rotation. Different lowercase letters indicate statistically significant differences according to Tukey's HSD test ($p < 0.05$)

depending on the type of organic fertiliser and the structure of the crop rotation. The obtained data confirm the hypothesis regarding the high efficiency of crop by-products (plant residues) as a principal factor of carbon sequestration in modern agricultural systems. The considerable difference in the Corg. content between the manure and crop by-product treatments (despite the identical physical application rate of 6 t ha^{-1}) indicates the qualitative superiority of biomass transformed directly within the soil environment. Such dynamics indicate the transition of agrocenoses to a higher level of organic matter stabilisation through the use of plant residues, which is strategically important for mitigating the effects of climate change and preventing soil degradation.

The intensity balance index (Ib) makes it possible to assess the level of replenishment of nutrients and organic matter within the system. According to the research findings (Figure 7), a critical dependence of Ib on the source of organic fertilisation was established. Under manure application in both crop rotations, a deficit level was recorded (Ib = 66–97%). The transition to the use of crop by-products (6 t ha^{-1}) ensured a surplus balance: 108 ± 5% in the pea-based crop rotation and the highest value of 130 ± 6% in the grass-based crop rotation.

The use of crop by-products ensured enhanced reproduction of soil Corg. (Ib > 100%), whereas manure application resulted in a carbon deficit, with the level of compensation for mineralisation losses not exceeding 60–102%, depending on the crop rotation. In all treatments, the intensity of the overall carbon balance within the agrocenosis system remained low, which is explained by the substantial expenditure of energy and biomass required to maintain agroecosystem functioning. Thus, the application of crop by-products at a rate of 6 t ha^{-1} represents a considerably more effective approach for ensuring a sustainable nitrogen balance and carbon sequestration in soil compared with an equivalent rate of manure. The statistical significance of the results ($p < 0.05$) confirms the advantage of the biogenic transformation of plant residues *in situ*. The calculations demonstrate that, alongside the influence of the type of organic fertiliser and crop rotation system, tillage also affected N cycling (Table 1).

Data are presented as Mean ± SE. Different lowercase letters in the same row indicate statistically significant differences between tillage methods according to Tukey's HSD test ($p < 0.05$).

The application of different tillage technologies demonstrated a direct relationship between the intensity of soil loosening and nitrogen removal. The highest feed unit yield (31.7 t) and corresponding nitrogen removal with yield (645 kg) were recorded under conventional ploughing. Transition to non-inversion tillage resulted in only a slight decrease in productivity (to 31.0 t), whereas minimum tillage caused a more substantial reduction in productivity, to 28.1 t. Total nitrogen removal from the agrocenosis was greatest under ploughing (813 kg), exceeding the values for non-inversion tillage by 17 kg and for minimum tillage by 252 kg. This indicates a greater mineralisation capacity of nitrogen compounds under the conditions of intensive soil aeration associated with ploughing.

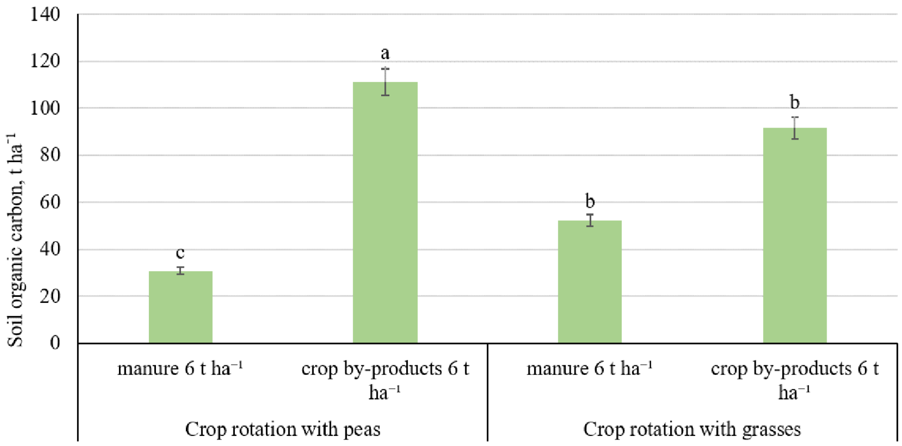

Figure 6. Soil organic carbon content as a function of fertilisation and crop rotation, t ha^{-1}. Different lowercase letters indicate statistically significant differences according to Tukey's HSD test ($p < 0.05$)

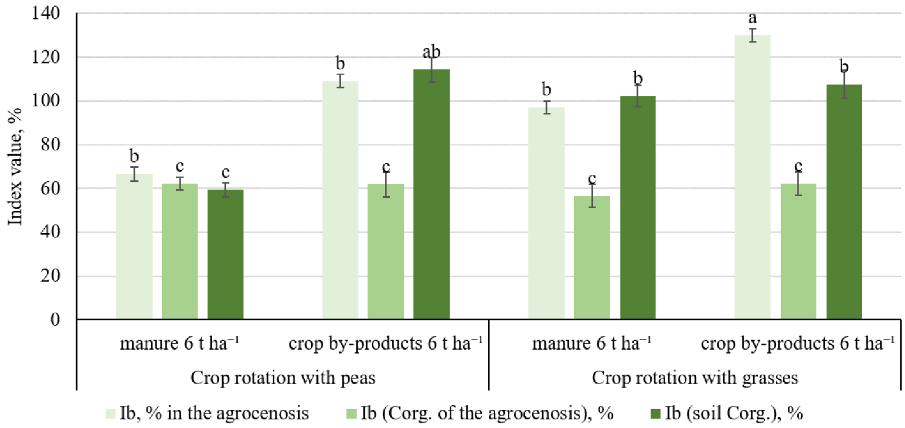

Figure 7. Nitrogen and carbon balance index as a function of fertilisation system and crop rotation type. Different lowercase letters indicate statistically significant differences according to Tukey's HSD test ($p < 0.05$)

Table 1. Influence of tillage methods on the standardised parameters of nitrogen-carbon cycling in agrocenoses of the Forest-Steppe of Ukraine

Cycling parameters	Soil tillage method		
	Ploughing	Non-inversion tillage	Minimum tillage
	($\bar{X} \pm S$)		
Feed unit yield, t ha^{-1}	31.7 ± 1.7	31.0 ± 1.9	28.1 ± 2.0
N removal with yield, kg	645 ± 21.5	628 ± 52.3	447.5 ± 36.8
Total N removal, kg	813 ± 103.0	796 ± 82.3	561 ± 49.0
N input from crop by-products, kg	488 ± 74.8	488 ± 79.3	450 ± 38.5
N content in the agrocenosis, kg	742 ± 126.3	717 ± 113.8	637 ± 147.5
N balance, kg (±)	-68.0 ± 82.5	-80.4 ± 98.5	103 ± 106.8
Ib, % in the agrocenosis	94.6 ± 9.9	94.1 ± 12.8	122 ± 4.8
Corg. balance in the agrocenosis, t	-39.8 ± 3.8	-29.5 ± 3.0	-27.3 ± 2.8
Soil Corg. balance, t	-0.75 ± 1.2	1.4 ± 1.1	0.71 ± 0.9
Ib (agrocenosis Corg.), %	62.7 ± 16.5	55.3 ± 16.0	57.8 ± 15.5
Ib (soil Corg.), %	93.9 ± 9.8	97.3 ± 14.5	93.6 ± 14.3
Corg. (CO_2), t	77.5 ± 15.4	67.9 ± 8.7	60.2 ± 13.3

Note: $\bar{X}$ – mean value of the indicator; S – standard deviation; Ib – balance intensity.

The study revealed a fundamental difference in the formation of the nitrogen balance depending on the tillage method. A deficit nitrogen balance was observed under deep tillage systems: ploughing (-68.0 kg) and non-inversion tillage (-80.4 kg), where balance intensity amounted to only 94.1–94.6%. A positive nitrogen balance (+103 kg) was achieved exclusively under minimum tillage. Despite the lower productivity, the balance intensity in this treatment reached 122%, indicating enhanced reproduction of the nitrogen potential of the agrocenosis. The dynamics of organic carbon (Corg.) demonstrated the environmental advantages of reduced tillage intensity. The soil Corg. balance under ploughing was deficit-based (-0.75 t), whereas non-inversion (+1.4 t) and minimum (+0.71 t) tillage promoted carbon accumulation (sequestration). CO_2 emissions, as an indicator of the intensity of organic matter mineralisation, showed a clear decreasing trend: from 77.5 t under ploughing to 60.2 t under minimum tillage. This confirms that reducing tillage depth slows carbon losses to the atmosphere. The balance intensity indicator for soil carbon under all tillage methods was close to optimal (93.6–97.3%), indicating relative stability of humus status under the application of crop by-products. Thus, minimisation of soil tillage in combination with the use of crop by-products makes it possible to simulate the natural carbon cycle. Surface localisation of organic residues under non-inversion and minimum tillage contributes to the restoration of organic carbon (Corg.) reserves and to a substantial reduction (by 12.6–22.3%) in greenhouse gas (CO_2) emissions. This brings agrocenoses closer to their natural functional status and correlates with the findings concerning reductions in total emissions along the sequence "arable land – fallow – virgin land", as confirmed by the research of Demydenko (2025).

The interaction between carbon and nitrogen cycles in agrocenoses significantly affects the nitrogen and carbon content in agricultural crops, detritus, and soil organic matter, while the intensity of nitrogen and carbon cycling is determined by the C:N ratio both in the soil and within agrocenoses as a whole, as emphasised by Daly and Hernandez-Ramirez (2020), Fenice (2021), and Jesmin *et al*. (2021). The use of different types of organic fertilisers, as noted by Luxhoi *et al.* (2006), influences the removal of both nitrogen and carbon. From the perspective of nutrient balance and environmental sustainability, non-inversion tillage is considered optimal, as according to Canadell *et al.* (2007) and McNeill and Unkovich (2007), it combines high productivity with a positive soil carbon balance and moderate CO_2 emissions. Based on the data presented in Figures 8–10, an analysis was conducted of the organic carbon-to-nitrogen ratio indicators, which represent key indicators of mineralisation intensity, humus formation, and agrocenosis stability in the Forest-Steppe zone. In the presented calculations, the parameter is expressed in standardised units: kg Corg per 1 kg N.

The type of fertiliser proved to be the most significant factor determining differentiation in the carbon-to-nitrogen ratio. Crop by-products were characterised by a considerably wider ratio

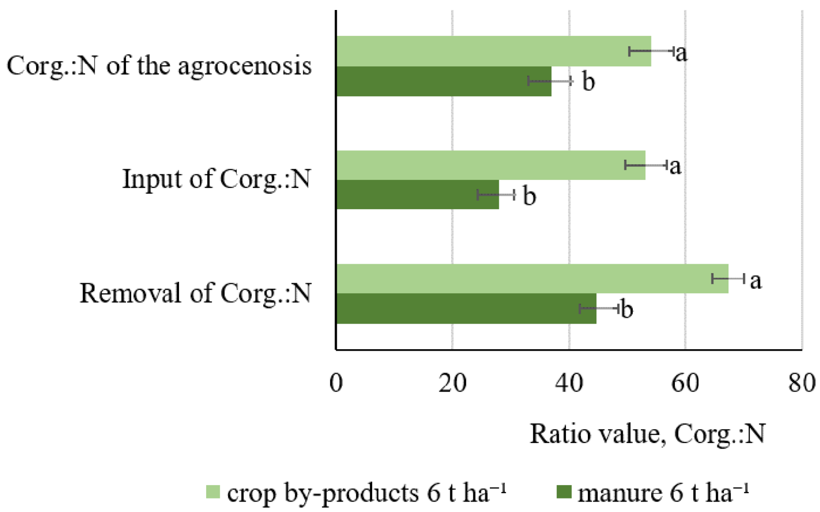

Figure 8. Carbon-to-nitrogen (C:N) ratio in agrocenoses under the application of different organic fertilisers. Different lowercase letters indicate statistically significant differences according to Tukey's HSD test ($p < 0.05$)

across all components of the cycling process. The C:N input ratio reached 53.2, which was almost twice as high as the value recorded for manure (28.0). This resulted in a higher level of carbon stabilisation within the agrocenosis (C:N = 54.2) and slower mineralisation. Manure provided a narrower ratio (C:N = 36.9 within the agrocenosis), indicating a higher nitrogen concentration and more rapid mobilisation of nutrients, but a lower potential for long-term carbon sequestration. According to Kucher *et al.* (2019), the type of agricultural crop determines the chemical composition of biomass and differences in nutrient removal. The grass-based crop rotation demonstrated higher C:N ratio values both in yield removal (67.8) and across the agrocenosis as a whole (50.5). This is explained by the high cellulose and lignin content of grass residues. Owing to the nitrogen-fixing capacity of legumes and the higher protein content of their biomass, the C:N ratio was considerably lower – 44.3 (removal) and 40.6 (within the agrocenosis) – thereby promoting more intensive nitrogen cycling.

Mechanical impact on the soil exerted a less pronounced, though statistically significant, effect on nutrient transformation. Ploughing promoted the formation of a slightly wider ratio within the agrocenosis (47.7) compared with non-inversion tillage (44.3). This may result from more intensive mixing of the soil profile and activation of aerobic processes. The minimum tillage treatment occupied an intermediate position, demonstrating a C:N ratio of 46.0 within the agrocenosis and 56.5 regarding yield removal, which indicates a moderate impact on the transformation of organic matter. Non-inversion tillage, by minimising soil disturbance, reduced the range of values (32.5–53.5 compared with 35.5–54.0 under ploughing), indicating greater stability of nitrogen–carbon cycling under this technology. Thus, for the sustainable management of Forest-Steppe agrocenoses, it is advisable to combine minimum tillage with the use of crop by-products, making it possible to maintain an optimal C:N ratio for preserving chernozem fertility.

There are numerous publications presenting a sceptical perspective on the role of intensive chemical amendments in the long-term preservation of soil fertility. According to Schulze (2000) and Breza and Grandy (2025), although mineral fertilisers accelerate biomass accumulation, they frequently lead to disruption of soil system homeostasis and intensification of humus mineralisation. However, under the conditions of the contemporary Ukrainian agricultural market, which requires resource conservation, increasing attention is being directed towards alternatives to costly mineral fertilisers (Tsentylo *et al.*, 2019; Tsentylo and Tsyuk, 2022). Particular interest is focused on the methods of managing natural nitrogen and carbon cycles through the use of crop by-products and optimisation of tillage practices.

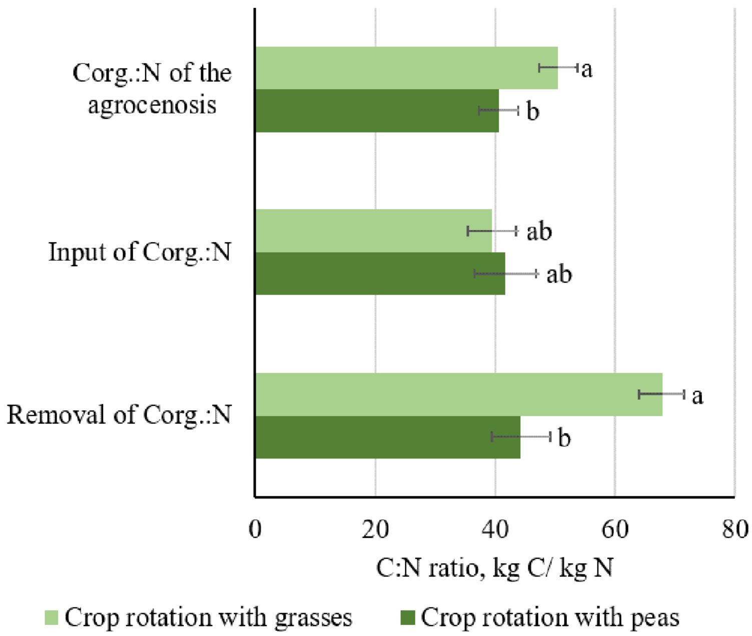

Figure 9. Carbon-to-nitrogen ratio in agrocenoses of different types.
Different lowercase letters indicate statistically significant differences according to Tukey's HSD test ($p < 0.05$)

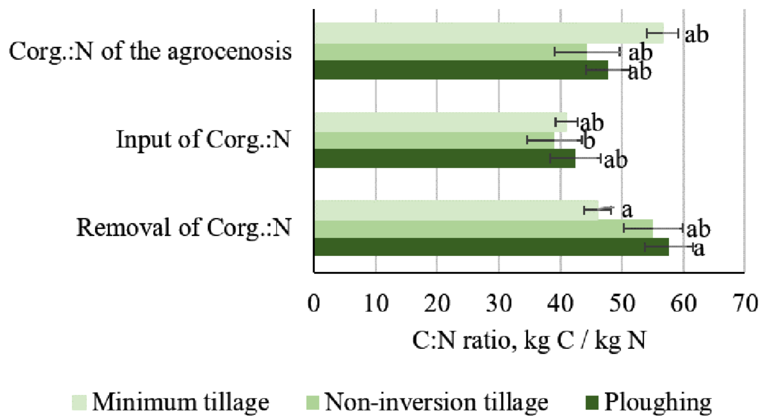

Figure 10. Carbon-to-nitrogen (C:N) ratio in agrocenoses under different soil tillage methods (kg C / kg N). Different lowercase letters indicate statistically significant differences according to Tukey's HSD test ($p < 0.05$)

The present study confirmed that the use of crop by-products (6–7 t ha^{-1}) is a more effective tool for managing the nitrogen-carbon cycle compared with manure. Under manure application, a deficit nitrogen balance was observed (-345 kg in the pea-based crop rotation), which is explained by intensive nutrient removal not compensated by nutrient input. By contrast, the incorporation of crop by-products ensured a linearly increasing input of total nitrogen, generating a positive balance (+97.8 to +189 kg). This is consistent with recent findings by P. Shao *et al.* (2020), demonstrating that organic residues are capable of "tightening" the nitrogen cycle through stimulation of microbial immobilisation.

Effective management of chernozem fertility in the Forest-Steppe zone requires consideration of the stress imposed by climate warming. It was established that under intensified tillage (ploughing), the rate of soil respiration increases, resulting in a deficit carbon balance (−0.75 t). The transition to non-inversion tillage makes it possible to simulate the natural carbon cycle, ensuring sequestration of organic carbon (Corg.) in the soil (+1.4 t) and reducing CO_2 emissions by 12.6–22.3%. This confirms the role of tillage as a subordinate regulatory subsystem within the overall cycling of nutrients (Brodowska *et al*., 2022).

Particular importance is attached to the C:N ratio as an indicator of agrocenosis stability. In the current research, the use of crop by-products ensured an expansion of the C:N ratio to values almost twice as high as those observed for manure. Such a wide ratio under the use of crop by-products and grasses creates favourable conditions for humus accumulation, since the excess carbon limits rapid mineralisation. Similar patterns were also reported by Friedlingstein *et al.* (2006) and D. Meng *et al.* (2022), who indicated that nitrogen availability is a key driver of carbon sequestration in plant communities. Thus, comparison of the present findings with those of other authors confirms the high efficiency of adaptive soil restoration measures. The combination of minimum tillage with the use of shredded crop by-products makes it possible not only to stabilise crop productivity, but also to restore the natural status of nitrogen-carbon cycling, which is critically important for the food and environmental security of Ukraine. Further research should focus on the long-term (over 2–3 crop rotation cycles) effects of crop by-products on the fractional composition of humus and soil microbiological activity. Particular attention should also be devoted to investigating the synergistic effects of combining different types of plant residues with microbiological preparations to accelerate cellulose decomposition and optimise nutrient release during the critical stages of crop development.

While this long-term study was conducted in the Forest-Steppe zone of Ukraine, the observed patterns of nitrogen and carbon cycling have broader implications for sustainable agriculture in other temperate agroecosystems. Regions with similar climatic and soil conditions, such as Central and Eastern Europe or parts of the North American Great Plains, face comparable challenges regarding soil organic matter depletion and climate change adaptation. The demonstrated effectiveness of combining non-inversion tillage with the application of crop by-products can serve as a highly adaptable strategy. Implementing these practices can help optimise the C:N balance, enhance carbon sequestration, and maintain long-term soil fertility across various temperate agricultural zones globally.

CONCLUSIONS

The study established that manure application at a rate of 6 t ha^{-1} does not compensate for nitrogen removal with yield and leads to a substantial nitrogen deficit (from - 61.4 to -345 kg). By contrast, the use of an equivalent rate of crop by-products (6 t ha^{-1}) fundamentally alters the direction of the processes, ensuring a positive nitrogen balance (+97.8 to +189 kg) and a balance intensity of 108–130%. The highest level of nitrogen accumulation in the agrocenosis (837 kg) was recorded in the pea-based crop rotation under the return of plant residues to the soil. Crop by-products proved to be a considerably more effective resource for carbon sequestration compared with manure applied at low rates. Despite the overall negative carbon balance within the agrocenosis system (caused by biological CO_2 emissions), the use of plant residues ensured enhanced reproduction of organic carbon (Corg.) directly in the soil, with an intensity exceeding 100%. This made it possible to achieve maximum carbon reserves of 111 ± 5.6 t in the pea-based crop rotation, which was three times higher than the values recorded in the manure treatments.

It was established that crop rotation structure plays a decisive role in synergy with organic fertilisation. The pea component demonstrated greater intensity of nitrogen and carbon cycling, which is explained by the combination of biological nitrogen fixation and the high quality of residual biomass. In the crop rotation with perennial grasses, greater stability of the nitrogen balance was observed; however, in terms of carbon accumulation potential, it was inferior to the legume-based rotation. The obtained results confirm that, under the conditions of limited availability of traditional organic fertilisers, the complete return of crop by-products combined with leguminous crops represents the most rational strategy for maintaining soil homeostasis. This approach makes it possible not only to prevent degradation of humus status but also to actively increase fertility potential in intensive agrocenoses.

The advantage of non-inversion tillage over ploughing in the management of nitrogen-carbon cycling in chernozem soils lies in its ability to ensure a progressively positive carbon balance, whereas under ploughing this process is characterised by a parabolic relationship with an increasing deficit. It was established that inversion tillage intensifies mineralisation processes, as confirmed by the wider range of CO_2 emissions compared with reduced tillage methods. Future research will focus on long-term modelling of soil organic matter dynamics under varying agro-climatic extremes, as well as evaluating the economic and energy efficiency of the studied crop rotation models in the Forest-Steppe region.

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