



## Carbon sequestration and restoration of soil organic matter under giant miscanthus under different fertilisation systems in the Forest-Steppe conditions of Ukraine

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**Abstract.** The aim of the study was to determine the patterns of carbon sequestration by a perennial plantation of giant miscanthus (*Miscanthus × giganteus*), cultivar ‘Osinniy Zoretsvit’, in the soil and above-ground biomass under different fertilisation systems and foliar application of potassium humate in the Forest-Steppe conditions of Ukraine. Field experiments were conducted during 2021–2025 on strongly eroded podzolised chernozem with a light loamy texture. The two-factor experiment included three fertilisation systems (control; organic – Leonardite, 100 kg/ha; mineral –  $N_{60}P_{16}K_{80}$ ) and four foliar potassium humate application regimes. It was established that the cultivation of giant miscanthus ensured a progressive accumulation of soil organic carbon (SOC) from 0.829 to 0.876–0.934% over five years. The greatest increase in SOC (+11.9%) was recorded under the organic fertilisation system combined with double foliar application of potassium humate ( $A_2B_4$ ). Carbon stocks in the arable layer (0–30 cm) increased from 2.91 to 3.28 t C/ha. The maximum annual carbon sequestration by above-ground biomass reached 8.25 t C/ha ( $A_3B_4$ ), while the cumulative amount over five years ranged from 16.74 to 29.25 t C/ha, equivalent to 61.4–107.3 t  $CO_2$ /ha in  $CO_2$ -equivalent terms. The study confirmed the statistically significant effects of fertilisation systems ( $LSD_{05} = 0.012\%$ ;  $p < 0.01$ ) and foliar feeding ( $LSD_{05} = 0.008\%$ ). The organic fertilisation system using Leonardite in combination with double foliar application of potassium humate proved to be the most effective for restoring soil organic matter, whereas the mineral system  $N_{60}P_{16}K_{80}$  ensured the highest carbon sequestration in above-ground biomass. A functional differentiation of agrotechnological effects was established: the organic system optimises the soil carbon pool, while the mineral system enhances the bioenergy carbon pool. The practical significance of the study lies in the scientific substantiation of a differentiated choice of

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fertilisation systems for miscanthus cultivation, serving as a basis for the implementation of climate-smart agriculture and the certification of carbon credits

**Keywords:** soil organic carbon; carbon balance; potassium humate; climate-smart agriculture; bioenergy crops

## Introduction

Global climate change, caused by rising concentrations of greenhouse gases in the atmosphere, has become one of the most significant challenges facing modern civilisation. In this context, agricultural soils have been viewed as a strategic reservoir for storing atmospheric carbon and, at the same time, a significant source of its release due to inefficient agricultural practices. The development and implementation of scientifically sound carbon sequestration strategies in agroecosystems have become an integral part of measures to mitigate climate change and ensure food and energy security. This area has become particularly relevant for Ukraine, where significant areas of agricultural land have been degraded as a result of intensive exploitation, erosion and military action, accompanied by a substantial loss of soil organic matter. Perennial energy crops, primarily giant miscanthus (*Miscanthus × giganteus*), have been regarded as both an efficient producer of biomass for biofuel production and a powerful tool for long-term carbon sequestration. Through  $C_4$  photosynthesis, a robust root system and the absence of annual tillage, miscanthus has demonstrated a unique combination of high productivity with soil-protection and climate-regulating functions. However, for the conditions of the Ukrainian Forest-Steppe, the patterns of soil organic carbon (SOC) accumulation and carbon sequestration by biomass during the initial five-year period (2021-2025) of plantation cultivation under various fertilisation systems (particularly in combination with humic-like biostimulants) remain poorly understood.

Recent international studies have confirmed the high potential of perennial energy crops

as tools for climate-smart agriculture. I.A. Sid-dique *et al.* (2023) found that switching from annual crops to perennials increases SOC stocks by 16.6-23.1% in the 0-30 cm layer. Y. Wu *et al.* (2024) demonstrated that the rates of SOC sequestration under perennial energy crops depend on climate, soil type and plantation age, with the effect being most pronounced in the first 10 years after establishment. C. Bertola *et al.* (2024), using data from a European network of field experiments, showed that in the first 4 years after establishing miscanthus on marginal lands, there was a statistically significant increase in C stocks in the 0-30 cm layer, and the contribution of aboveground and belowground biomass to the formation of the soil C pool became decisive. J.L. Kane *et al.* (2023) demonstrated that miscanthus acted as an “ecosystem engineer”, increasing soil bacterial diversity and organic matter content on marginal lands through specific rhizodeposition of C. Researchers N. Namoi *et al.* (2024) found that nitrogen fertilisation of mature miscanthus plantations (60-168 kg N/ha) increased individual shoot mass by 34-77% and biomass yield by 23-106%, with the effects of pre-fertilisation persisting for a long time. N. Namoi *et al.* (2026) confirmed in the Sun Grant Trial that adequate nitrogen nutrition was a key factor in maintaining the productivity of perennial miscanthus plantations.

The role of humic biostimulants in the formation of soil carbon pools and crop productivity has been actively studied. J. Shen *et al.* (2024) demonstrated that foliar application of potassium humate significantly increased photosynthetic activity, chlorophyll content and grain yield. J. Izquierdo *et al.* (2025), in a study across

448 production plots in Uruguay, demonstrated that a single foliar application of a humic biostimulant increased the yield of major cereal crops by 7.6-15.7%. A.M.S. Elshamly & S.M.A. Nas-sar (2023), in a study of the stimulating effect of potassium humate on crops under water stress, demonstrated that this biostimulant improved nutrient use efficiency and antioxidant protection in plants. V. Pidlisnyuk *et al.* (2025) found that miscanthus produced a productive biomass of 25-35 t/ha on marginal heavy clay soils under a low-intensity management system, whilst simultaneously sequestering CO<sub>2</sub> and producing oxygen. Despite a significant number of studies, there was a lack of systematic data for the conditions of the Ukrainian Forest-Steppe regarding the dynamics of SOC and carbon sequestration by Miscanthus under different fertilisation regimes in combination with foliar application of potassium humate. Furthermore, the CO<sub>2</sub> equivalent sequestration potential under different agricultural practices had not been established, which complicated the integration of this crop into climate compensation schemes. The aim of the study was to establish the patterns of carbon sequestration by a perennial giant miscanthus plantation in soil and above-ground biomass under different fertilisation regimes and foliar application of potassium humate during the first five years (2021-2025) of vegetation in the Forest-Steppe zone of Ukraine.

## Materials and Methods

The field study was conducted between 2021 and 2025 at the Separate Subdivision of the National University of Life and Environmental Sciences of Ukraine "Velykosnitynske named after O.V. Muzychenko", Fastiv District, Kyiv Region. The soil of the experimental site was strongly eroded podzolised chernozem with a light loamy texture formed on loess-like loam. The agrochemical characteristics of the arable layer (0-30 cm) at the time of experiment establishment were as follows: humus content – 1.43%, bulk density – 1.17 g/cm<sup>3</sup>, pH<sub>KCl</sub> – 5.76, available phosphorus – 105 mg/kg,

exchangeable potassium – 78 mg/kg. Climatic conditions of the region were characterised by a mean annual temperature of +7.8°C and annual precipitation of 540-620 mm. The experiment was a two-factor design with three replicates, and the treatment arrangement was randomised in blocks. The area of the sowing plot was 50 m<sup>2</sup>, and the plot area was 35 m<sup>2</sup>. The crop was giant miscanthus (*Miscanthus × giganteus*), 'Osinniy Zoretsvit' variety. Planting was carried out in April 2021 using rhizomes weighing 25-35 g at a density of 10,000 plants/ha (1.0 × 1.0 m spacing). Factor A (fertilisation system): A<sub>1</sub> – control (no fertiliser); A<sub>2</sub> – Leonardite (0-2 mm fraction, humic acid content ≥75%), 100 kg/ha (applied once during spring tillage in the year the plantation was established); A<sub>3</sub> – mineral fertiliser N<sub>60</sub>P<sub>16</sub>K<sub>80</sub> (ammonium nitrate + ammonium phosphate + potassium chloride, localised application, annually). Factor B (foliar feeding with potassium humate 2 l/ha, solution concentration 1%): B<sub>1</sub> – no feeding (control); B<sub>2</sub> – during the tillering stage; B<sub>3</sub> – during the stem elongation stage; B<sub>4</sub> – both stages (twice). In total, the design comprised 12 treatments × 3 replicates = 36 plots. The analysis was conducted on the extreme variants for factor B (B<sub>1</sub> – no fertilisation; B<sub>4</sub> – double fertilisation), as it was precisely the contrastive comparison of these variants that allowed the nature of the effect of foliar fertilisation with potassium humate on C-sequestration indicators in each fertilisation system of factor A to be identified as clearly as possible.

Soil samples were collected annually throughout the study period at the end of the growing season (third decade of October) from three depths: 0-10 cm, 10-20 cm and 20-30 cm at five points on each plot using the envelope method in accordance with DSTU 4287:2004 (2005). A single composite sample weighing 1 kg was prepared from each plot for analysis; in total, 540 soil samples were collected and analysed over the 5-year period. Above-ground biomass yield was recorded by completely mowing the plot areas (35 m<sup>2</sup>) at the end of the growing

season (December – plants in a state of physiological dormancy). The samples were dried to air-dry condition in a drying oven at 60°C for 48 hours, and moisture content was determined in accordance with DSTU ISO 11465:2001 (2003). A biomass sample for each treatment was formed from three replicates. SOC (%) was calculated using the formula:

$$\text{SOC} = \text{Humus}/1.724, \quad (1)$$

where 1.724 – is the Van Bemmelen coefficient accounting for the average carbon content in humus (~58%).

Organic carbon stocks in the soil layer (t C/ha) were calculated according to the formula:

$$\text{SC} = \text{SOC} \times \rho \times h \times 100, \quad (2)$$

where SC – the stock of organic carbon, t C/ha; SOC – the SOC content, %;  $\rho$  – the soil bulk density, g/cm<sup>3</sup>; h – the thickness of the soil layer, m (0.30 m for the 0-30 cm layer); 100 – the conversion coefficient.

For comparative analysis, the study used the contrasting variants of factor B – without foliar feeding (B<sub>1</sub>) and double foliar feeding (B<sub>4</sub>), which enabled the clearest assessment of the effect of potassium humate within each fertilisation system. The carbon content in above-ground biomass was determined using an Elementar Vario MAX elemental analyser (Elementar Analysensysteme GmbH, Germany) (CHNS mode, combustion temperature 1,150°C, carrier gas – helium, standard – sulfanilamide). The average carbon content in dry miscanthus biomass was 46.0 ± 0.5%, corresponding to literature data for the genus *Miscanthus*. Carbon sequestration by above-ground biomass (CB, t C/ha) was calculated as:

$$\text{CB} = \text{DBY} \times \text{Cc}/100, \quad (3)$$

where DBY is the yield of absolutely dry biomass, t/ha; and Cc is the carbon content in biomass, %. Conversion of carbon into CO<sub>2</sub> equivalent was

performed using the coefficient 3.667 (molar ratio CO<sub>2</sub>/C = 44/12).

The dynamics of indicators during 2021-2025 were assessed using non-linear regression with approximation by a logistic function (for biomass carbon sequestration) and linear regression (for SOC and soil carbon stocks). The coefficient of determination R<sup>2</sup> was calculated to assess the degree of correspondence between the approximated curve and the experimental data. Statistical processing of the results was carried out using analysis of variance (two-factor ANOVA) for a split-plot design. In addition, the following were calculated: Fisher's F-test, significance level (p-value), least significant difference at the 5% significance level (LSD<sub>05</sub>), and coefficient of variation (CV, %). The normality of distribution was verified using the Shapiro-Wilk test, while homogeneity of variances was assessed using Levene's test. Differences were considered significant at p ≤ 0.05. Multiple comparisons of means were performed using Tukey's HSD test. Correlation and regression analyses were conducted with calculation of pairwise correlation coefficients (r) and coefficients of determination (R<sup>2</sup>). Calculations were performed using Statistica 13.3 (TIBCO Software, USA) and R Studio (package "agricolae"). Graphical data processing was carried out in the Python 3.11 environment (matplotlib, seaborn) with a resolution of 300 dpi. The study was conducted in accordance with the requirements of the Convention on Biological Diversity (1992).

## Results and Discussion

The assessment of SOC dynamics during the initial years of miscanthus plantation establishment was important for clarifying the directions of carbon balance formation within the agroecosystem. It was precisely at the early stage of cultivation that the differences between fertilisation systems became most clearly apparent in terms of the rate of organic compound input into the soil and their subsequent stabilisation. Analysis of the research results (Table 1)

indicated a progressive accumulation of organic carbon in the arable soil layer under the perennial plantation of giant miscanthus across all investigated treatments.

**Table 1.** SOC content under giant miscanthus, % (selected treatments, 2021-2025)

| No. | Factor A                 | Factor B                     | Years of study |       |       |       |       | Average |
|-----|--------------------------|------------------------------|----------------|-------|-------|-------|-------|---------|
|     |                          |                              | 2021           | 2022  | 2023  | 2024  | 2025  |         |
| 1   | Control                  | No foliar feeding ( $B_1$ )  | 0.829          | 0.829 | 0.835 | 0.847 | 0.858 | 0.840   |
| 2   |                          | Both growth stages ( $B_4$ ) | 0.829          | 0.835 | 0.847 | 0.858 | 0.876 | 0.849   |
| 3   | Leonardite,<br>100 kg/ha | No foliar feeding ( $B_1$ )  | 0.835          | 0.847 | 0.864 | 0.887 | 0.905 | 0.868   |
| 4   |                          | Both growth stages ( $B_4$ ) | 0.835          | 0.858 | 0.882 | 0.911 | 0.934 | 0.884   |
| 5   | $N_{60}P_{16}K_{80}$     | No foliar feeding ( $B_1$ )  | 0.829          | 0.835 | 0.841 | 0.853 | 0.864 | 0.844   |
| 6   |                          | Both growth stages ( $B_4$ ) | 0.829          | 0.841 | 0.858 | 0.870 | 0.887 | 0.857   |

**Note:** soil layer 0-30 cm.  $LSD_{05}$  (factor A) = 0.012;  $LSD_{05}$  (factor B) = 0.008;  $LSD_{05}$  ( $A \times B$ ) = 0.015;  $Cv$  = 1.9%

**Source:** compiled by the author

Three distinct trends in SOC dynamics were identified depending on the fertilisation system (factor A). On the control plots ( $A_1$ ), SOC content increased from 0.829% in 2021 to 0.858-0.876% in 2025, corresponding to an increase of 3.5% ( $B_1$ ) and 5.7% ( $B_4$ ) over 5 years. Under the mineral fertilisation system ( $A_3$ ), the increase in SOC was 4.2% ( $B_1$ ) and 7.0% ( $B_4$ ), indicating a moderate contribution of mineral fertiliser to the formation of the soil carbon pool due to the predominant flow of assimilated carbon into above-ground biomass. The highest SOC increase was achieved by the organic fertilisation system ( $A_2$ , Leonardite) – 8.4% ( $B_1$ ) and 11.9% ( $B_4$ ), with the absolute SOC value in the  $A_2B_4$  treatment in 2025 (0.934%) being the highest among all combinations studied. Analysis of variance confirmed a significant effect of factor A on SOC ( $F = 87.3$ ;  $p < 0.01$ ), factor B ( $F = 24.6$ ;  $p < 0.01$ ) and their interaction  $A \times B$  ( $F = 5.8$ ;  $p < 0.05$ ). The smallest significant difference was  $NIP_{05} = 0.012\%$  for factor A and  $NIP_{05} = 0.008\%$  for factor B. The linear regression for SOC dynamics in variant  $A_2B_4$  was described by the equation  $SOC = 0.0245 \times t + 0.810$  ( $R^2 = 0.994$ ), indicating high predictive reliability of the model and stable rates of organic carbon accumulation in the topsoil under the organic fertilisation system. For variant  $A_3B_4$ , the corresponding

equation was  $SOC = 0.0145 \times t + 0.815$  ( $R^2 = 0.989$ ). This dynamic is associated with a complex of physico-chemical processes: the direct entry of humic acids from Leonardite into the soil carbon pool, stimulation of microbial activity, intensification of the humification of fresh plant residues, and stabilisation of organo-mineral complexes. Figure 1 shows the dynamics of SOC content and the hierarchy of fertilisation systems in terms of their impact on the soil carbon pool:  $A_2B_4 > A_2B_1 > A_3B_4 > A_3B_1 > A_1B_4 > A_1B_1$ . This pattern was consistent with the findings of C. Bertola *et al.* (2024), who established that the application of organic fertilisers to miscanthus plantations significantly accelerated the formation of stable carbon pools in the topsoil. Figure 1 showed that, as early as the second year of vegetation, the curves for the Leonardite treatments diverged from the control and the mineral fertiliser system, and in 2024-2025 the gap between  $A_2B_4$  and the control reached its maximum. Importantly, two foliar applications of potassium humate enhanced the positive effect of each fertilisation system; however, the most pronounced additional increase in SOC was observed specifically with the organic system. The calculated organic carbon stocks in the 0-30 cm soil layer for the various fertilisation treatments are presented in Table 2.

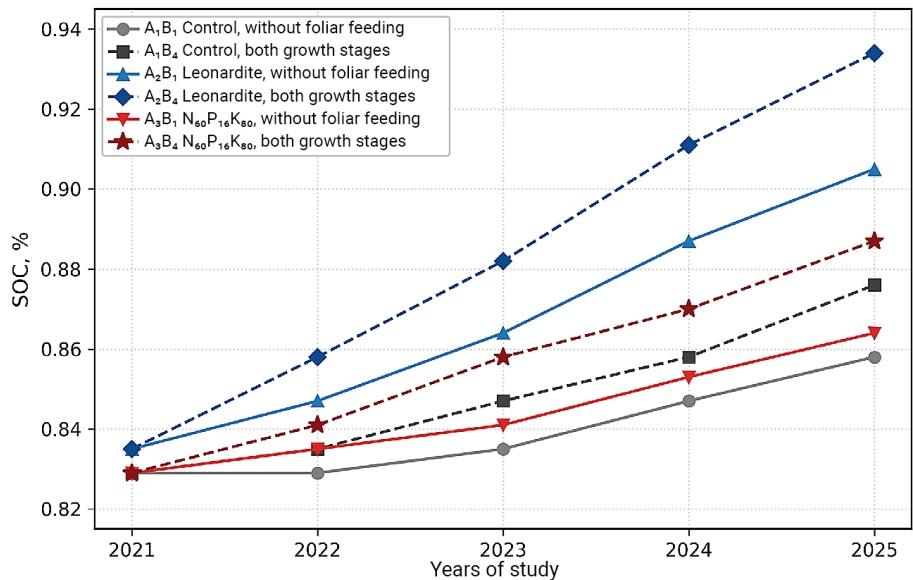


Figure 1. Dynamics of SOC content under giant miscanthus under different fertilisation systems (2021-2025)

Source: compiled by the author

Table 2. Carbon stocks in the arable layer (0-30 cm) under giant miscanthus, t C/ha (selected treatments, 2021-2025)

| No. | Factor A                                        | Factor B                             | Years of study |      |      |      |      | Average |
|-----|-------------------------------------------------|--------------------------------------|----------------|------|------|------|------|---------|
|     |                                                 |                                      | 2021           | 2022 | 2023 | 2024 | 2025 |         |
| 1   | Control                                         | No foliar feeding (B <sub>1</sub> )  | 2.91           | 2.91 | 2.93 | 2.97 | 3.01 | 2.95    |
| 2   |                                                 | Both growth stages (B <sub>4</sub> ) | 2.91           | 2.93 | 2.97 | 3.01 | 3.07 | 2.98    |
| 3   | Leonardite, 100 kg/ha                           | No foliar feeding (B <sub>1</sub> )  | 2.93           | 2.97 | 3.03 | 3.11 | 3.18 | 3.04    |
| 4   |                                                 | Both growth stages (B <sub>4</sub> ) | 2.93           | 3.01 | 3.10 | 3.20 | 3.28 | 3.10    |
| 5   | N <sub>60</sub> P <sub>16</sub> K <sub>80</sub> | No foliar feeding (B <sub>1</sub> )  | 2.91           | 2.93 | 2.95 | 2.99 | 3.03 | 2.96    |
| 6   |                                                 | Both growth stages (B <sub>4</sub> ) | 2.91           | 2.95 | 3.01 | 3.05 | 3.11 | 3.01    |

Note: initial soil bulk density  $\rho = 1.17 \text{ g/cm}^3$ ; layer depth  $h = 0.30 \text{ m}$ .  $\text{LSD}_{05} \text{ (factor A)} = 0.043 \text{ t C/ha}$ ;  $\text{LSD}_{05} \text{ (factor B)} = 0.028 \text{ t C/ha}$ ;  $\text{Cv} = 1.7\%$

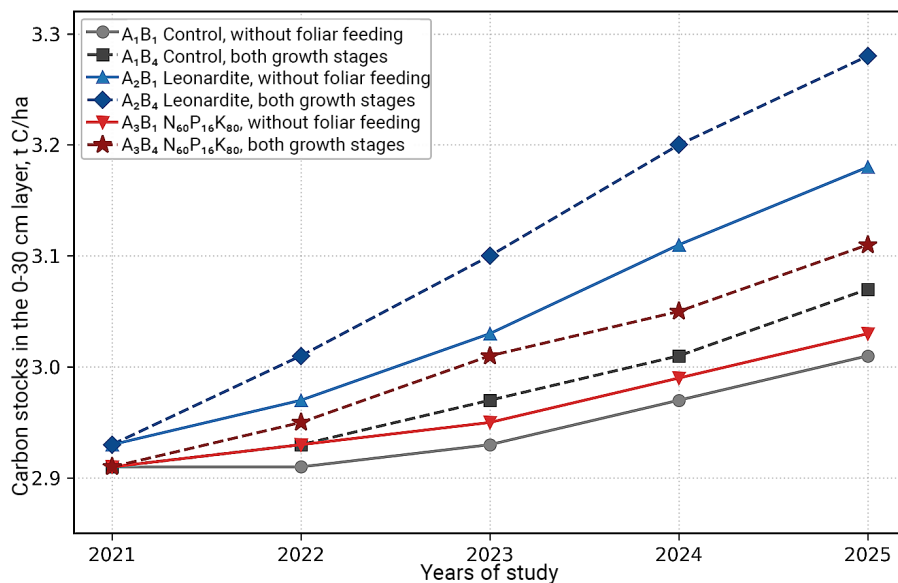
Source: compiled by the author

Organic carbon stocks in the arable layer (0-30 cm) demonstrated patterns similar to SOC dynamics. In the control plots, the absolute increase in carbon stocks amounted to only 0.10 t C/ha (A<sub>1</sub>B<sub>1</sub>) to 0.16 t C/ha (A<sub>1</sub>B<sub>4</sub>) over five years. The mineral fertilisation system ensured an increase of 0.12-0.20 t C/ha, whereas the organic system provided an increase of 0.25-0.35 t C/ha. The maximum absolute carbon stock value, 3.28 t C/ha, was recorded in treatment A<sub>2</sub>B<sub>4</sub> (Leonardite + double foliar application of potassium

humate), exceeding the control treatment A<sub>1</sub>B<sub>1</sub> by 0.27 t C/ha (+9.0%). Thus, the annual rate of carbon accumulation in the 0-30 cm soil layer ranged from 0.02 t C/ha/year (A<sub>1</sub>B<sub>1</sub>) to 0.07 t C/ha/year (A<sub>2</sub>B<sub>4</sub>), which generally corresponded to the lower limit of the range reported for long-term miscanthus plantations (0.83-1.37 t C/ha/year). The lower absolute values observed in the present study were explained by the initial stage of plantation establishment and the characteristics of the eroded chernozem soil. Analysis of Figure 2

demonstrated that the dynamics of carbon stocks closely followed the SOC trends: under the organic fertilisation system, carbon accumulation in the

soil occurred most intensively, while the effect of foliar potassium humate application in combination with Leonardite proved to be synergistic.



**Figure 2.** Changes in carbon stocks in the topsoil (0-30 cm) under giant miscanthus under different fertilisation regimes (2021-2025)

Source: compiled by the author

The graph shows that in 2023, treatment A<sub>2</sub>B<sub>4</sub> exceeded the 3.10 t C/ha threshold, whilst the control treatments remained below 3.0 t C/ha almost until the end of the experiment. In 2025, the gap between A<sub>2</sub>B<sub>4</sub> and A<sub>1</sub>B<sub>1</sub> was 0.27 t C/ha, confirming the cumulative nature of carbon accumulation specifically under the organo-mineral-stimulated

technology. Carbon sequestration by the above-ground biomass of miscanthus during 2021-2025 was characterised by steady annual growth across all fertilisation and foliar feeding treatments, with the highest values recorded in the N<sub>60</sub>P<sub>16</sub>K<sub>80</sub> treatment combined with foliar application in both phases (Table 3).

**Table 3.** Carbon sequestration by above-ground biomass of giant miscanthus, t C/ha (selected treatments, 2021-2025)

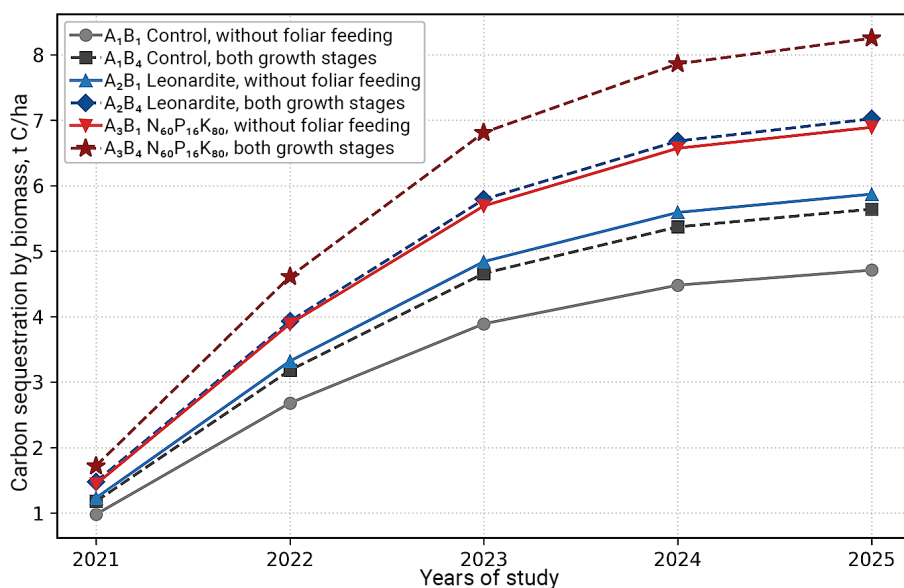
| No. | Factor A                                        | Factor B                             | Years of study |      |      |      |      | Average |
|-----|-------------------------------------------------|--------------------------------------|----------------|------|------|------|------|---------|
|     |                                                 |                                      | 2021           | 2022 | 2023 | 2024 | 2025 |         |
| 1   | Control                                         | No foliar feeding (B <sub>1</sub> )  | 0.98           | 2.68 | 3.89 | 4.48 | 4.71 | 3.35    |
| 2   |                                                 | Both growth stages (B <sub>4</sub> ) | 1.18           | 3.18 | 4.66 | 5.37 | 5.64 | 4.01    |
| 3   | Leonardite, 100 kg/ha                           | No foliar feeding (B <sub>1</sub> )  | 1.23           | 3.32 | 4.84 | 5.59 | 5.87 | 4.17    |
| 4   |                                                 | Both growth stages (B <sub>4</sub> ) | 1.48           | 3.93 | 5.79 | 6.68 | 7.02 | 4.98    |
| 5   | N <sub>60</sub> P <sub>16</sub> K <sub>80</sub> | No foliar feeding (B <sub>1</sub> )  | 1.44           | 3.89 | 5.69 | 6.57 | 6.89 | 4.90    |
| 6   |                                                 | Both growth stages (B <sub>4</sub> ) | 1.72           | 4.61 | 6.81 | 7.86 | 8.25 | 5.85    |

**Note:** carbon content in dry miscanthus biomass –  $46.0 \pm 0.5\%$  (determined using an Elementar Vario MAX elemental analyser). LSD<sub>05</sub> (factor A) = 0.18 t C/ha; LSD<sub>05</sub> (factor B) = 0.12 t C/ha; Cv = 3.4%

Source: compiled by the author

Thus, a logistic pattern characteristic of perennial C4 crops was observed: an intensive increase in carbon sequestration in 2021-2023, gradually levelling off in 2024-2025. In the first year after planting, C sequestration was minimal (0.98 t C/ha in  $A_1B_1$  – 1.72 t C/ha in  $A_3B_4$ ), due to the priority given to the formation of the root system and rhizome apparatus. By the fifth year of vegetation, C sequestration by biomass had increased to 4.71-8.25 t C/ha, with the maximum recorded under the  $N_{60}P_{16}K_{80}$  mineral fertiliser regime with two foliar applications of potassium humate ( $A_3B_4$ ). A two-factor analysis of variance confirmed the significance of the effects of both factors (A:  $F = 142.8$ ;  $p < 0.001$ ; B:  $F = 38.1$ ;  $p < 0.001$ ) and their interaction ( $F = 4.2$ ;  $p < 0.05$ ).

The logistic regression for the  $A_3B_4$  treatment was of the form  $CB(t) = 8.55 / (1 + 7.9 \cdot \exp(-0.86 \cdot t))$  with  $R^2 = 0.996$ , indicating a very high predictive ability of the model. Based on the mean values, the following hierarchy of fertilisation systems was established in terms of C sequestration by biomass:  $N_{60}P_{16}K_{80}$  (4.90-5.85 t C/ha) > Leonardite (4.17-4.98 t C/ha) > control (3.35-4.01 t C/ha). The mineral fertiliser system provided a 46.3% increase in average C sequestration compared to the control, whilst the organic system provided a 24.5% increase. Two foliar applications of potassium humate further increased C sequestration by biomass by 16.4-19.4%. Figure 3 shows the dynamics of carbon sequestration by aboveground biomass for the period 2021-2025.



**Figure 3.** Trends in carbon sequestration by the above-ground biomass of giant miscanthus under different fertilisation regimes (2021-2025)

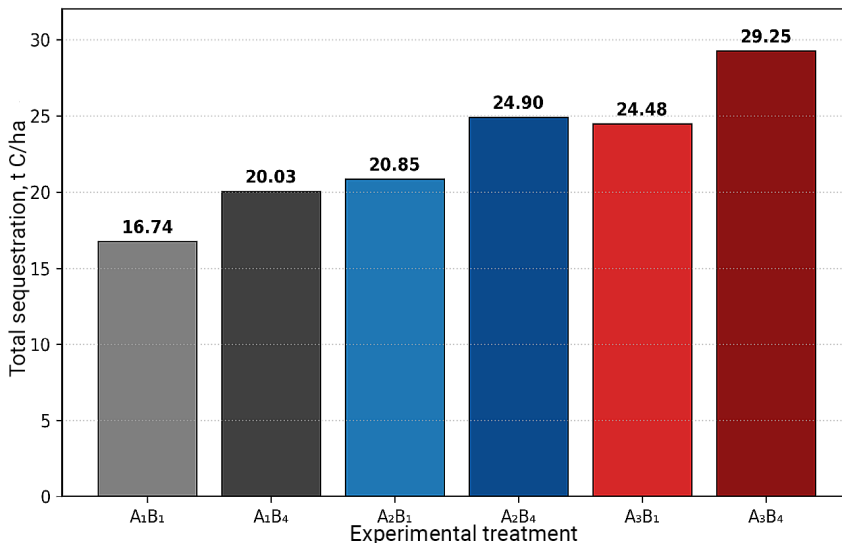
Source: compiled by the author

It was found that the mineral fertiliser regime produced the steepest rise in the curve as early as the second growing season, and the difference between  $A_3B_4$  and  $A_1B_1$  in 2025 reached 3.54 tonnes of carbon per hectare. Thus, it was the combination of full mineral nutrition with twice-yearly humic fertilisation that ensured the

highest carbon sequestration in above-ground biomass. At the same time, although the organic system (Leonardite) lagged behind the mineral system in terms of absolute biomass sequestration, it demonstrated better carbon deposition in the soil, which shaped a specific carbon balance architecture. The cumulative carbon

sequestration by above-ground biomass during 2021-2025 of miscanthus plantation vegetation

varied widely depending on the fertilisation and feeding systems (Fig. 4).



**Figure 4.** Total carbon sequestration by the above-ground biomass of giant miscanthus over five growing seasons (ranking of variants, 2021-2025)

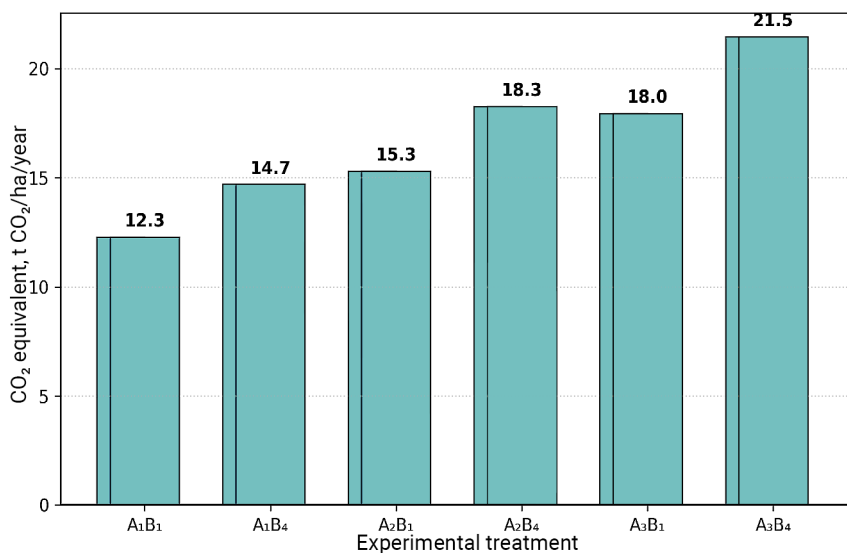
**Source:** compiled by the author

The minimum value of cumulative carbon sequestration (16.74 t C/ha) was recorded in treatment A<sub>1</sub>B<sub>1</sub> (control without foliar feeding), while the maximum value (29.25 t C/ha) was observed in treatment A<sub>3</sub>B<sub>4</sub> (mineral fertilisation system with double foliar feeding). Of particular importance is the fact that treatment A<sub>2</sub>B<sub>4</sub> (Leonardite + double foliar feeding; 24.90 t C/ha) was practically equivalent to treatment A<sub>3</sub>B<sub>1</sub> (mineral fertilisation system without foliar feeding; 24.48 t C/ha), indicating the high efficiency of combining an organic source of humic substances with a biostimulant as an alternative to intensive mineral technologies. According to O. Prysiazniuk *et al.* (2022), miscanthus under low-intensity cultivation on heavy soils of Ukraine is capable of ensuring stable dry biomass production, which generally corresponds with the obtained results regarding the high productivity of the studied crop even on soil with reduced humus content. This confirmed that technological optimisation of fertilisation was a key factor in enhancing the sequestration func-

tion of miscanthus under specific soil and climatic conditions. The conversion of the obtained carbon sequestration values into CO<sub>2</sub> equivalent was performed using the standard coefficient 3.667 (molar ratio CO<sub>2</sub>/C). The average annual CO<sub>2</sub> uptake by above-ground miscanthus biomass ranged from 12.28 t CO<sub>2</sub>/ha/year (A<sub>1</sub>B<sub>1</sub>) to 21.45 t CO<sub>2</sub>/ha/year (A<sub>3</sub>B<sub>4</sub>), exceeding the average values for the main C<sub>4</sub> crops of temperate climates – maize (8-12 t CO<sub>2</sub>/ha/year) and sorghum (10-14 t CO<sub>2</sub>/ha/year) – by 1.5-2.0 times (Fig. 5). The cumulative CO<sub>2</sub> uptake during 2021-2025 of giant miscanthus plantation vegetation ranged from 61.4 t CO<sub>2</sub>/ha (A<sub>1</sub>B<sub>1</sub>) to 107.3 t CO<sub>2</sub>/ha (A<sub>3</sub>B<sub>4</sub>). This corresponded to the upper range of values reported for the first five years of miscanthus cultivation. Double foliar application of potassium humate ensured additional CO<sub>2</sub> sequestration of 12.1-19.6% compared with treatments without foliar feeding. Figure 6 demonstrated the ranking of treatments according to their integrated climate-regulating effect: A<sub>3</sub>B<sub>4</sub> > A<sub>2</sub>B<sub>4</sub> > A<sub>3</sub>B<sub>1</sub> > A<sub>2</sub>B<sub>1</sub> > A<sub>1</sub>B<sub>4</sub> > A<sub>1</sub>B<sub>1</sub>. The difference

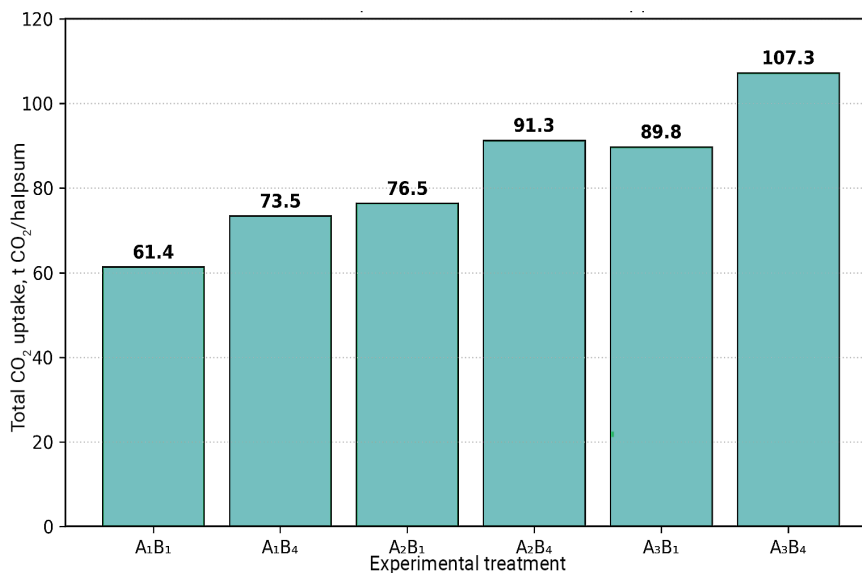
between the extreme treatments amounted to 45.9 t CO<sub>2</sub>/ha, providing a substantial argument in favour of the differentiated selection of fertilisation systems depending on the objective – maximum carbon sequestration in biomass or

in soil. The methodology for converting carbon into CO<sub>2</sub> equivalent complied with the recommendations of the IPCC (2019), which increased the suitability of the results for subsequent application in greenhouse gas accounting systems.



**Figure 5.** Average annual CO<sub>2</sub> uptake by the above-ground biomass of giant miscanthus under different fertilisation regimes (2021-2025)

Source: compiled by the author



**Figure 6.** Total CO<sub>2</sub> uptake by giant miscanthus crops over five growing seasons (2021-2025)

Source: compiled by the author

Interpretation of the obtained results required the identification of two distinct pathways of carbon balance formation depending on the type of fertilisation system. The organic system (Leonardite, 100 kg/ha) ensured the greatest increase in SOC (+11.9% in treatment A<sub>2</sub>B<sub>4</sub>) at a relatively lower level of biomass carbon sequestration. To explain this pattern, a methodological understanding of the nature of the SOC indicator was essential: D.W. Pribyl (2010) demonstrated that the traditional coefficient used for converting humus into organic carbon represented a generalisation and required careful interpretation depending on soil type. In the present experiment, the use of the constant coefficient 1.724 was justified for comparing treatments within the same soil type, while the identified trends confirmed a genuine enhancement of organic matter sequestration following Leonardite application. At the same time, the IPCC (2019) approach to assessing carbon stocks in agroecosystems emphasised the need to combine data on soil and biomass carbon pools, which fully corresponded to the logic of the present experiment. The mineral fertilisation system (N<sub>60</sub>P<sub>16</sub>K<sub>80</sub>) ensured the highest carbon sequestration by above-ground biomass (5.85 t C/ha/year) with a lower increase in SOC, indicating that assimilated carbon was predominantly directed towards biomass formation. In the work of O. Prisyazhniuk *et al.* (2022), it was shown that miscanthus under low-intensity agrotechnologies maintained high productivity stability on heavy low-productivity soils, and therefore additional mineral nutrition naturally contributed to an even fuller realisation of the crop's bioenergy potential. T. Nakajima *et al.* (2018) established that *Miscanthus × giganteus* was characterised by higher carbon accumulation and biomass productivity compared with *Miscanthus sinensis* under various growing conditions. The authors also demonstrated that perennial miscanthus plantations were capable of efficiently accumulating organic carbon in the soil, confirming the crop's potential for bioenergy and climate-oriented agriculture. Yu. Hayda *et al.* (2024) identified the high

potential of miscanthus plantations for carbon dioxide sequestration and oxygen production under the conditions of Ukraine. The researchers noted that increasing plant biomass directly influenced the intensity of CO<sub>2</sub> uptake, confirming the environmental efficiency of crop cultivation. R. Teteriuk & M. Kulyk (2024) assessed miscanthus cultivars in terms of adaptability, yield, and energy productivity under the conditions of the Left-Bank Forest-Steppe of Ukraine. According to their results, certain cultivars demonstrated high stability of yield formation and significant potential for use as an energy feedstock. I. Beznosko *et al.* (2022) investigated the quantitative composition of micromycetes in chernozem soils of the Left-Bank Forest-Steppe of Ukraine under cereal crops. The researchers established that agroecological conditions and cultivation characteristics significantly influenced the structure of the soil mycobiota and soil microbiological activity. M. Gumentyk & M. Kharytonov (2018) developed and evaluated technologies for miscanthus cultivation under the Forest-Steppe conditions of Ukraine, taking into account crop productivity and energy efficiency. Their study demonstrated that optimisation of cultivation technology elements contributed to increased biomass yield and the economic feasibility of energy crop utilisation.

The synthesis by D. Rakhmetov (2011) concerning the introduction and economic-biological characteristics of energy crops in Ukraine also confirmed that miscanthus productivity was determined by cultivar adaptability and the level of resource provision, explaining the observed superiority of the mineral system regarding the biomass carbon pool. V. Yakovenko *et al.* (2024; 2025) established that in the chernozems of the Ukrainian Steppe, the key indicators of soil condition were SOC content, humus form, and the intensity of bioturbation, and that these indicators responded distinctly to the type of land use. A. Holder *et al.* (2026) proposed the concept of a cell-wall ideotype of miscanthus aimed at maximising soil carbon sequestration, opening new perspectives for breeding. The overall potential of CO<sub>2</sub> sequestration

by miscanthus under Bioenergy with Carbon Capture and Storage systems reached 26 t CO<sub>2</sub>-equivalent/ha per year. Foliar application of potassium humate provided an additional synergistic effect through activation of photosynthetic processes, the enzymatic apparatus of carbon and nitrogen metabolism, and enhanced plant stress resistance (Terravesta, n.d.). In combination with different fertilisation systems, this resulted in the formation of two practically significant scenarios for carbon balance management: the organic system combined with potassium humate proved more effective for the restoration of soil organic matter, whereas the mineral system combined with potassium humate was more effective for maximising carbon accumulation in harvestable biomass. Consequently, the choice of agrotechnology should be determined not only by productivity but also by which particular carbon pool is prioritised by the farm or climate reporting system.

### Conclusions

As a result of the study, the patterns of carbon sequestration by a perennial plantation of giant miscanthus, cultivar 'Osinniy Zoretsvit', under the Forest-Steppe conditions of Ukraine were established. It was demonstrated that the cultivation of giant miscanthus under all investigated fertilisation treatments ensured a progressive accumulation of SOC: SOC content increased from 0.829% in 2021 to 0.858-0.934% in 2025, while carbon stocks in the arable layer (0-30 cm) rose from 2.91 to 3.28 t C/ha. The highest increase in SOC (+11.9%) and soil carbon stocks (+0.35 t C/ha over five years) was achieved under the organic fertilisation system (Leonardite, 100 kg/ha) combined with double foliar application of potassium humate (treatment A<sub>2</sub>B<sub>4</sub>), which was associated with the direct input of Leonardite humic acids into the soil carbon pool and the intensification of

humification processes. The highest carbon sequestration by above-ground biomass was ensured by the mineral fertilisation system N<sub>60</sub>P<sub>16</sub>K<sub>80</sub> combined with double potassium humate application (treatment A<sub>3</sub>B<sub>4</sub>): the maximum annual sequestration reached 8.25 t C/ha, while the cumulative amount over five years totalled 29.25 t C/ha. In CO<sub>2</sub>-equivalent terms, the average annual atmospheric CO<sub>2</sub> uptake by miscanthus crops ranged from 12.28 to 21.45 t CO<sub>2</sub>/ha/year, with the cumulative five-year uptake ranging from 61.4 to 107.3 t CO<sub>2</sub>/ha, corresponding to the highest carbon sequestration efficiency indicators among agricultural crops of temperate climates. Analysis of variance confirmed the significant effects of the fertilisation system ( $p < 0.01$ ) and foliar feeding ( $p < 0.01$ ) on all investigated parameters, while the coefficients of variation (1.7-3.4%) indicated high reproducibility of the results. The obtained findings served as a methodological basis for the implementation of climate-smart agriculture principles, the calculation of carbon credits in accordance with national and international greenhouse gas accounting standards, and the development of national decarbonisation policy for the agro-industrial sector. Prospects for further research include the long-term assessment of the stability of sequestered carbon, quantification of the total carbon footprint throughout the complete miscanthus cultivation cycle, and an integrated economic evaluation of the proposed agrotechnologies.

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### Conflict of Interest

None.

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## Секвестрація вуглецю та відтворення органічної речовини ґрунту під міскантусом гігантським за різних систем удобрення в умовах Лісостепу України

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**Анотація.** Метою дослідження було встановити закономірності секвестрації вуглецю багаторічною плантацією міскантусу гігантського (*Miscanthus × giganteus*) сорту 'Осінній зорецьвіт' у ґрунті та надземній біомасі за різних систем удобрення та позакореневого підживлення гуматом калію в умовах Лісостепу України. Польові дослідження було проведено впродовж 2021-2025 років на чорноземі опідзоленому сильнозмитому легкосуглинковому. Двофакторний дослід включав три системи удобрення (контроль; органічна – Леонардит, 100 кг/га; мінеральна –  $N_{60}P_{16}K_{80}$ ) та чотири режими позакореневого підживлення гуматом калію. Встановлено, що вирощування міскантусу гігантського забезпечило прогресивне нагромадження органічного вуглецю в ґрунті (SOC) від 0,829 до 0,876-0,934 % за п'ять років. Найбільший приріст SOC (+11,9 %) зафіксовано за органічної системи удобрення з дворазовим позакореневим підживленням гуматом калію ( $A_2B_4$ ). Запаси вуглецю в орному шарі (0-30 см) зросли від 2,91 до 3,28 т С/га. Максимальна щорічна секвестрація вуглецю надземною біомасою – 8,25 т С/га ( $A_3B_4$ ), сумарна за 5 років – 16,74-29,25 т С/га, що в перерахунку на  $CO_2$ -еквівалент становить 61,4-107,3 т  $CO_2$ /га. У роботі було підтверджено достовірність впливу систем удобрення ( $HIP_{05} = 0,012$  %;  $p < 0,01$ ) та позакореневого підживлення ( $HIP_{05} = 0,008$  %). Органічна система удобрення з Леонардитом у поєднанні з дворазовим позакореневим підживленням гуматом калію виявилася найефективнішою для відтворення органічної речовини ґрунту, тоді як мінеральна система  $N_{60}P_{16}K_{80}$  забезпечила максимальну секвестрацію вуглецю в надземній біомасі. Встановлено функціональне розмежування агротехнологічних ефектів: органічна система оптимізує ґрунтовий пул вуглецю, а мінеральна – біоенергетичний пул. Практична цінність роботи полягає у науковому обґрунтуванні диференційованого вибору системи удобрення міскантусу, які слугують основою для впровадження кліматично-розумного землеробства та сертифікації вуглецевих кредитів

**Ключові слова:** органічний вуглець ґрунту; вуглецевий баланс; гумат калію; кліматично-розумне землеробство; біоенергетичні культури