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The impact of green manure and intercropping in organic production on soil nitrogen content and carbon sequestration

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Summary: The adverse effects of climate change are impacting agricultural production, necessitating adaptation and mitigation measures. Sequestering atmospheric carbon into the soil represents potential strategy to reduce CO_2 concentrations in the atmosphere. The aim of this research was to investigate the effects of green manure and intercropping of winter wheat and winter forage pea on soil organic carbon and nitrogen content, with the goal of achieving carbon neutrality and sustainable agricultural production. The study was conducted over three growing seasons (2021/22, 2022/23, and 2023/24) on experimental plots at the Institute of Field and Vegetable Crops in Novi Sad, Serbia. Different combinations of treatments with winter wheat, winter forage pea with biomass incorporation were applied. Sowing was done in autumn, and in early May the crops were mowed, chopped and incorporated into the soil. Soil sampling was conducted at a depth of 0-30 cm. Data were statistically analyzed using a two-way ANOVA. The results indicate that green manure significantly contributes to increased nitrogen and organic carbon content in the soil, particularly when combined with other sustainable agricultural practices like intercropping. The highest organic carbon (14.89 g/kg) and nitrogen (0.195%) content in the soil were observed in these treatments. Intercropping of forage pea and wheat + biomass incorporation, compared to the control, increased soil carbon content by an average of 1.4 g/kg, meaning that the SOC stock increased by 0.5 t/ha/yr. The implementation of climate-smart agricultural practices enhances the resilience of agricultural production to climate change while preserving soil as an exceptionally important, non-renewable natural resource.

Key words: carbon sequestration, forage pea, green manure, intercropping, soil organic carbon, winter wheat

Introduction

Climate change and the decreasing use of organic fertilizers in agricultural production are leading to a decline in soil quality, which negatively affects plant productivity. Soil plays a significant role in mitigating climate change due to its high potential for carbon storage through organic matter. The carbon sequestration strategy is based on a positive organic carbon balance, where carbon input into the soil exceeds its losses (Jakšić, 2021a). The total global organic carbon stock in the upper soil layer (up to 100 cm depth) is approximately 1500 Pg, which is about twice the amount in the atmosphere. The estimated value of organic carbon stocks up to a soil depth of 2 m is 2060 ± 215 Pg (Batjes, 2015).

Maintaining an adequate level of organic matter in the soil (minimum 3% of humus) is a prerequisite for sustainable agricultural production, which involves the application of organic fertilizers and soil amendments. However, in recent years, there has been a noticeable decline in organic matter content, particularly humus, in the soil of Serbia. The results of the project "Status, trends and possibilities to increase the fertility of agricultural land in the Vojvodina Province" indicated the loss of soil organic matter. Humus depletion is still the norm on many agricultural soils and countermeasures have not yet arrived in practice. For example, arable soils in northern Serbia have lost an average of 0.5% humus over the last 30 years (Vasin et al, 2021). This is a consequence of the reduced use of organic fertilizers in intensive agriculture due to the lack or inability to apply manure and other organic fertilizers of animal origin, as well as the demands of production itself and insufficient education of farmers regarding the importance of organic fertilizers and the potential use of plant-based organic fertilizers. Intercropping and green manure offer potential solutions to this problem, particularly in organic production where fertilization options are limited. These practices can easily be integrated into existing crop rotations, and their positive effects on soil properties become evident within a few years (Jakšić, 2021b).

Intercropping involves growing two or more different species simultaneously on the same land, a practice that has been used for decades in Serbia (Dolijanović et al., 2015). This method gained wider adoption in Western countries at the beginning of the 20th century (Lithourgidis et al., 2011), while in Serbia it has traditionally been used for crops like maize grown with beans or pumpkins, and beans grown with pumpkins (Dolijanović, 2008). According to Kovačević (2003), the main advantages of intercropping include more efficient use of soil resources, nutrients, and water; better utilization of light, heat, oxygen, and carbon dioxide; improved tolerance to adverse climatic conditions; increased resistance to diseases, pests, and weeds; and favorable effects on soil structure and fertility, especially when legumes are included in the mix. When the forage pea is included in intercropping, with minimal nitrogen fertilizer application, the annual emission of nitrous oxide is 0.65 kg/ha. In forage production, various species of vetch, clover, and other legumes are often intercropped with alfalfa or different types of clover, yellow star grass, and sainfoin (Krga, 2022). Due to the mutual benefits of combined plant species, intercropping is particularly significant in organic production.

Annual species from the *Poaceae* family (cereals: oats, barley, rye, wheat, and triticale) are often intercropped with legumes such as field pea, forage pea, faba bean, lupin, and soybean. Sometimes, annual legumes are combined with other legumes or perennial grasses, though this is less common compared to standard mixtures. One of the most commonly used annual legumes for intercropping with cereals is forage pea (*Pisum sativum ssp. arvense* L. Poir) (Chapagain & Riseman, 2014), which is typically grown in mixtures with oats, barley, wheat, or triticale.

Green manure (sideration) involves the incorporation of fresh plant biomass into the soil, where plants are specifically grown for this purpose to enrich the soil with organic matter, thereby improving its physical, biological, and chemical properties. Green crops are mowed immediately after flowering and left to dry in the field for two to three days, then tilled and incorporated into the soil at a depth of 10–15 cm. The incorporation of green biomass is best done one month before sowing the next crop and can be combined with pre-sowing preparation. Plants used for green manure should have the following characteristics: they should produce a large amount of above- and below-ground biomass, to be nitrogen-fixers (capable of utilizing atmospheric nitrogen and transferring it to the soil through symbiosis with microorganisms), to have low requirements for nutrients and water, to possess a strong root system for protecting soil from erosion, and have high seed germination rates even under reduced moisture conditions. Leguminous plants such as faba bean, field pea, forage pea, and clover are considered the best for green manure and can also be used in intercropping systems (Ninkov, 2023).

The aim of this research was to investigate the effects of green manure and intercropping of winter wheat and winter forage pea on soil organic carbon and nitrogen content, with the goal of achieving carbon neutrality and sustainable agricultural production.

Material and Methods

Soil Type

Field experiments were conducted on experimental plots at the Institute of Field and Vegetable Crops at Rimski Šančevi site, near Novi Sad, Serbia, where the soil is classified as calcareous chernozem. This soil is characterized by exceptionally favorable chemical and physical properties (Škorić, 1986; Miljković, 1996; Ćirić et al., 2012), and the application of intensive cultivation practices, water management, and rational fertilization enables high yields.

Agrometeorological Conditions

Weather conditions in Serbia from October 2021 to September 2022 showed some deviations from the average characteristics for the region (Table 1 and 2). The production year was warmer in comparison with the long-term average (1981-2010), with typical precipitation levels, though rainfall was not evenly distributed spatially or temporally. Additionally, several climatic extremes were recorded during the year (late spring frosts, drought in spring months, and heatwaves in summer, which occurred during critical vegetative and generative phases of agricultural crops. All weather data have been taken from the Republic Hydrometeorology Service of Serbia (https://www.hidmet.gov.rs).

From October 2022 to September 2023, weather conditions in Serbia also deviated from the average characteristics for the region. Agrometeorological conditions during this period were not optimal for all agricultural crops in all phenological phases and across all regions. The production year was warmer, with typical precipitation levels, compared to the long-term average (1981-2010). However, rainfall distribution was uneven, and moisture conditions during part of the 2022/23 production year were unfavorable for many crops, resulting in lower yields and quality than expected. Several climatic extremes were recorded (cold and wet spring with daily rainfall, strong storm winds, hail, and heatwaves in summer), which occurred during critical phases of crop development.

Table 1. Average monthly temperature °C

Average monthly temp. °C	10.	11.	12.	1.	2.	3.	4.	5.
2021/22	10.6	7.2	3	1.5	5.7	5.5	10.9	19.1
2022/23	13.7	7.9	5.5	4.9	3.6	9.0	10.4	17.2
2023/24	16.6	8.4	5.3	2.7	9.7	11.5	15.3	18.9

Table 2. Average monthly precipitation (mm)

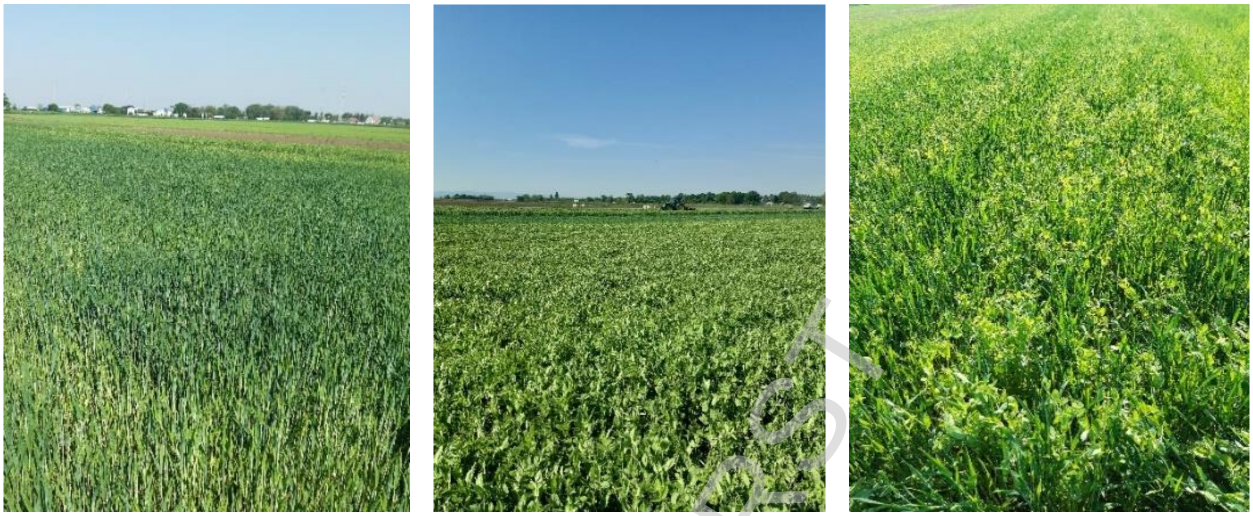
Average monthly precipitation (mm)	10.	11.	12.	1.	2.	3.	4.	5.
2021/22	88	114.6	88.9	11.6	23.7	1.1	54.5	17.9
2022/23	31	58.7	44.7	66.4	57.2	25.3	63.9	124.8
2023/24	11.4	83.8	33.9	36.7	9.1	15.0	21.5	78.0

From October 2023 to September 2024, weather conditions in Serbia showed significant deviations from the average characteristics for the region. Agrometeorological conditions during this period were unfavorable for many crops in all phenological phases and across all regions. The production year was warmer, with typical precipitation levels across most of Serbia compared to the long-term average (1981-2010). However, rainfall distribution was extremely uneven, and given the very high temperatures, the amounts of rainfall did not meet the water requirements for plants, making the year drought-prone. This was also the warmest growing season in the last fifty years. From April to September, average precipitation in Serbia was around 350 mm, which is about 10% less than the average for the growing season.

Applied Cultivation Technology

The research was conducted over three years (2021/22, 2022/23, and 2023/24) on experimental plots at the Institute of Field and Vegetable Crops, National Institute of the Republic of Serbia, located at Rimski Šančevi site, near Novi Sad, Serbia. The experiment was conducted following the principles of organic production. According to the experimental design, a completely randomized block system with three replications was applied. Each experimental plot within a block represented one of the following treatments (Picture 1): 1. Control treatment – bare soil; 2. Treatment I – winter wheat + biomass incorporation (WW+BI); 3. Treatment II – winter forage pea + biomass incorporation (WFP+BI); 4. Treatment III – intercropping of winter wheat and winter forage pea + biomass incorporation (IC+BI).

In late summer, deep tillage was performed at a depth of 25 cm. Before deep tillage, soil samples were taken to determine baseline soil fertility (pH, $CaCo_3$, humus, P_2O_5, K_2O). After soil preparation, sowing was done in October using a seed drill at a depth of 5 cm for forage pea and 3 cm for wheat. The amount of certified organic seed used was 100 kg ha^{-1} for wheat and 130 kg ha^{-1} for forage pea. In early spring, soil samples were taken for Nmin analysis to determine the content of mineral nitrogen in the soil. In early May, after flowering phase, the crops were mowed, chopped and incorporated into the soil. Soil was sampled and analyzed. No other fertilizers or soil amendments were applied in the experiment, except for the incorporated biomass. The following varieties, developed at the Institute of Field and Vegetable Crops in Novi Sad, were used in the experimental field trial: winter wheat NS 40S and winter forage pea NS Pionir.



a) b) c)

Picture 1. a) Treatment I – Winter wheat; b) Treatment II - Forage pea; c) Treatment III - Intercropping of forage pea and wheat

Methods of Soil Analysis

Soil analyses were conducted in the Laboratory for Soil and Agroecology of the Institute of Field and Vegetable Crops in Novi Sad, accredited according to the SRPS ISO/IEC 17025 standard. The following soil property analyses were performed: Determination of free calcium carbonate ($CaCO_3$) – volumetric method using Scheibler's calcimeter; Determination of humus content – Tyurin's method by oxidation of organic matter; Determination of total nitrogen and organic carbon content (CNS elemental analysis by total sample combustion) using an automatic method - CHNS analyzer; Determination of ammonium lactate P_2O_5 – determination of readily available phosphorus by spectrophotometry; Determination of ammonium lactate K_2O – determination of readily available potassium by flame photometry; Determination of active acidity - pH in water - in a soil-water suspension (10g:25cm^3), potentiometrically using a pH meter; Determination of potential acidity - pH in 1 M KCl - in a soil-potassium chloride suspension (10g:25cm^3), potentiometrically using a pH meter.

Statistical Analyses

The research results were analyzed using a two-way analysis of variance (ANOVA). Factors were year and treatment. The significance of differences between mean values was tested using the LSD test at a significance level of $p < 0.05$.

Results and Discussion

Basic Chemical Properties of the Soil

The soil was classified as a calcareous chernozem, and its basic chemical properties at a depth of 0-30 cm are presented in Table 3. According to the pH value determined in a 1M KCl suspension, the soil of this chernozem belongs to the slightly alkaline soil class. The $CaCO_3$ content varied significantly, ranging from slightly calcareous to calcareous classes. Humus content ranged from low to high. The values of available P_2O_5 and K_2O indicated high variability in terms of supply, ranging from optimal to harmful levels.

Soil Organic Carbon Content

The average SOC content across the entire experiment was 13.97 g/kg (Table 4). According to research by Jakšić S. (2015) at the same location, the SOC content was 12.70 g/kg. Dozet (2010), in a study of chernozem soils in the Srem region, recorded humus content ranging from 7.88 g/kg to 30.05 g/kg.

Table 3. Basic chemical properties of the soil at a depth of 0-30 cm

pH-KCl	pH-H_2O	$CaCO_3$ (%)	Humus (%)	P_2O_5 (mg/100g)	K_2O (mg/100g)
7.41±0.28	8.23±0.16	3.10±0.23	2.40±0.33	27.04±3.04	25.50±2.76

Table 4. Total SOC content in soil at a depth of 0-30 cm (g/kg)

	Control	Treatment I WW+BI	Treatment II WFP+BI	Treatment III IC+BI	Average
2021/22	14.18^{c}	14.08^{cd}	14.44^{bc}	15.37^{a}	14.52^{A}
2022/23	14.04^{cd}	13.38^{de}	15.05^{ab}	15.11^{ab}	14.37^{A}
2023/24	11.85^{f}	11.99^{f}	12.93^{e}	13.96^{cd}	12.68^{B}
Average	13.49$^{\mathbf{A}}$	13.26$^{\mathbf{C}}$	14.25$^{\mathbf{B}}$	14.89$^{\mathbf{A}}$	13.97

WW+BI=winter wheat + biomass incorporation; WFP+BI= winter forage pea + biomass incorporation; IC+BI= intercropping of winter wheat and winter forage pea + biomass incorporation. Treatments marked with the same letters do not have statistically significant differences (Fisher's LSD test at a significance level of 0.05). **A**-capital, bold letters- differences between treatment; *A*-capital, italic letters- differences between years; a- small letters- interactions.

Analysis of variance showed a statistically significant difference between the factors examined. The average organic carbon content in the soil of Treatment III (IC+BI), intercropping of forage pea and wheat + biomass incorporation, was significantly higher (14.89%) compared to the other treatments. The statistically significantly lower SOC content was observed in the soil under forage pea crop + biomass incorporation (14.25%). The SOC content under winter wheat + biomass incorporation was not significantly higher compared to the control and was lower than in Treatments II and III. Treatment III (IC+BI) had the highest SOC content across all years (13.96 g/kg in 2021/22; 15.11 g/kg in 2022/23; and 15.37 g/kg in 2023/24). Results from other studies indicate similar results. According to Bedoussac et al. (2015), intercropping of wheat and pea increases soil organic carbon content through enhanced biomass production and better resource utilization. Cong et al. (2015) also concluded that intercropping increases soil organic carbon content. The authors state that intercropping can increase SOC by 10-20% compared to monocultures. According to Lithourgidis et al. (2011), intercropping of legumes and cereals can significantly improve soil fertility, including an increase in SOC. A meta-analysis shows that intercropping increases SOC by 10-20% compared to monocultures (Li et al., 2020).

Treatment II (WFP+BI) showed intermediate SOC values: 14.44 g/kg in 2021/22, 15.05 g/kg in 2022/23, and 12.93 g/kg in 2023/24, which were higher than the control and Treatment I in the second and third years of the study but lower than Treatment III in the first and third years. Legumes, such as peas, contribute to increased SOC content through nitrogen fixation and enhanced biomass production (Sainju et al., 2017). However, the results show that intercropping has a greater impact than pea monoculture, which aligns with the findings of these authors. According to Herridge et al. (2008), legumes can significantly increase SOC, but the effects are often greater when legumes are combined with other crops, confirming our results.

Treatments with biomass incorporation (WFP+BI, IC+BI) positively influenced SOC content, with results higher than the control. The greatest effect was observed in Treatment III. Cherr et al. (2006) conclude that green manures, especially legumes, increase soil organic carbon content. However, the effects are often greater when green manures are combined with other practices, such as intercropping (Crews and Peoples, 2004). There was no difference compared to Treatment II.

Annual variations in SOC content were observed, with lower values in 2023/24 (12.68 g/kg) compared to the previous two years (14.52 g/kg in 2021/22 and 14.37 g/kg in 2022/23). Climatic conditions, such as precipitation and temperature, can significantly influence the accumulation and mineralization of organic carbon in the soil (Vanlauwe et al., 2019). the majority of the incorporated biomass ads only to the labile C soil pool and its quickly released to the atmosphere. The 2023/24 growing season had significantly worse climatic characteristics compared to the previous two years. It was the warmest growing season in the last fifty years, with a 10% precipitation deficit compared to the average precipitation for the growing season.

Intercropping of forage pea and wheat, compared to the control and wheat monoculture, contributed to an increase in soil carbon content by an average of 1.4 g/kg and 1.63 g/kg, meaning that SOC stock increased by 0.50 t/ha/yr and 0.58 t/ha/yr. Compared to variant with forage pea, carbon sequestration increased by 0.64 g/kg, and SOC stock was higher by 0.23 t/ha/yr. Intercropping of forage pea and wheat, compared to the control, contributed to an increase in soil carbon content by an average of 1.4 g/kg, meaning that SOC stock increased by 0.5 t/ha/yr. This is consistent with previous research. Data from a literature review (Blanco-Canqui et al., 2015) reported that this practice increases soil organic C stocks by 0.1-1 t/ha/yr. A higher potential was found in woody crops in warm temperate dry climates (about 1.00 t C/ha/yr), and lower values in arable crops, mostly in temperate and tropical climates (0.32-0.56 t C/ha/yr). C storage potential in arable systems was 0.71, 0.51, and 0.46 t C/ha/yr in tropical, temperate, and arid climates, respectively (Jian et al., 2020). Soil C sequestration rates are highest during the first years after practice implementation and progressively decrease as C stocks approach equilibrium.

Soil Nitrogen Content

The average total N content in the soil was 0.181%, indicating that the tested soil was moderately supplied with this nutrient (Table 5). In studies by Džamić and Stevanović (2000), the total nitrogen content in the surface layer (0-30 cm) of chernozem was 0.22%. Jakšić (2015) reported an average nitrogen content of 0.19% in the surface layer (0-30 cm) of chernozem at the same location. These values are slightly higher than the control but lower than Treatment III.

Analysis of variance showed a statistically significant difference between the factors examined. Statistically significant differences in total N content were observed among the treatments. The highest average nitrogen content, 0.195%, was found in Treatment III. Significantly lower N content was observed in Treatment II (0.187%). There was no statistically significant difference in average N content between the control and Treatment I, and these values were significantly lower compared to Treatments II and III. Many studies show that intercropping legumes (such as peas) with cereals (such as wheat) can increase soil nitrogen content due to the ability of legumes to fix atmospheric nitrogen. This aligns with our results, where Treatment III had the highest nitrogen content.

Table 5. Total nitrogen content in soil at a depth of 0-30 cm (%)

	Control	Treatment I WW+BI	Treatment II WFP+BI	Treatment III IC+BI	Average
2021/22	0.177^{bc}	0.178^{bc}	0.184^{b}	0.196^{a}	0.184^{A}
2022/23	0.170^{cd}	0.171^{cd}	0.200^{a}	0.193^{a}	0.184^{A}
2023/24	0.167^{de}	0.161^{e}	0.174^{cd}	0.196^{a}	0.175^{B}
Average	0.172$^{\mathbf{C}}$	0.171$^{\mathbf{C}}$	0.187$^{\mathbf{B}}$	0.195$^{\mathbf{A}}$	0.181

WW+BI=winter wheat + biomass incorporation; WFP+BI= winter forage pea + biomass incorporation; IC+BI= intercropping of winter wheat and winter forage pea + biomass incorporation. Treatments marked with the same letters do not have statistically significant differences (Fisher's LSD test at a significance level of 0.05). **A**-capital, bold letters- differences between treatment; *A*-capital, italic letters- differences between years; a- small letters- interactions.

According to Bedoussac et al. (2015), intercropping of wheat and peas increases nitrogen utilization and improves soil fertility. Additionally, in the study by Cong et al. (2015), intercropping increased the content of nitrogen and organic matter in the soil. Treatment II (OSG+ZB) shows a lower nitrogen content (0.184% in 2021/22, 0.200% in 2022/23, and 0.174% in 2023/24) compared to Treatment III but higher than the control and Treatment I. According to Jensen et al. (2012) and Herridge et al. (2008), legumes such as peas increase soil nitrogen content through nitrogen fixation, but the effects can vary depending on growing conditions and crop management. However, our results show that intercropping has a higher impact than pea monoculture, which may be due to the synergistic effect between peas and wheat. The intercrops increased the contribution of N_2 fixation to total N accumulation of pea crop in the mixture compared to pea sole crop (Pelzer et al, 2012). This is also related to higher DM yield incorporated with mixture of wheat and pea (8.03 t/ha) compared to monoculture of forage pea (4.37 t/ha).

Research by other authors indicates that incorporating green biomass increases soil nitrogen content. This is consistent with the results of the C/N ratio, where it was the highest on the control variety (7.84), followed by wheat (7.7), and followed by peas and intercropping (7.6). According to Cherr et al. (2006) and Crews and Peoples (2004), green manures, especially legumes, increase soil nitrogen content and improve soil fertility. This supports the results obtained in our study, as treatments with green manure had higher nitrogen content compared to the control, except for Treatment I, where pure wheat was grown. Green manure increases N content in N-fixing species, reduces N leaching, and prevents nutrient erosion through runoff (Kaspar & Singer, 2011). The results show that in the 2023/24 growing season, the nitrogen content was slightly lower compared to the previous two years, which may be related to the specific climatic conditions of that year.

Conclusion

Forage pea contributes to increasing soil organic carbon and nitrogen content, but intercropping has the greatest positive impact on these soil fertility parameters. Green manure significantly contributes to increasing soil nitrogen and organic carbon content, especially when used in combination with other sustainable agricultural practices, such as intercropping. Intercropping of fodder pea and wheat, compared to the control, wheat monoculture, and pea monoculture, contributed to an increase in SOC stock by 0.5 t C/ha/yr, 0.58 t C/ha/yr, and 0.23 t C/ha/yr, respectively. Annual variations in organic carbon and nitrogen content are, among other factors, a result of different climatic conditions, which influence biomass production and the mineralization of organic matter. Intercropping and green manure have the potential to contribute to overall carbon sequestration as a form of mitigation and adaptation to climate change.

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Uticaj zelenišnog đubrenja i združene setve u organskoj proizvodnji na sadržaj azota u zemljištu i sekvestraciju ugljenika

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Sažetak: Negativni efekti klimatskih promena utiču na poljoprivrednu proizvodnju, što zahteva mere prilagođavanja i ublažavanja. Sekvestracija atmosferskog ugljenika u zemljište predstavlja potencijalnu strategiju za smanjenje koncentracije CO_2 u atmosferi. Cilj ovog istraživanja bio je da se ispitaju efekti zelenišnog đubrenja i združenog useva ozime pšenice i ozimog stočnog graška na sadržaj organskog ugljenika i azota u zemljištu, sa ciljem postizanja karbonske neutralnosti i održive poljoprivredne proizvodnje. Istraživanje je sprovedeno tokom tri godine (2021/22, 2022/23 i 2023/24) na eksperimentalnim parcelama Instituta za ratarstvo i povrtarstvo, Novi Sad, Srbija. Primenjene su različite kombinacije tretmana sa ozimom pšenicom, ozimim stočnim graškom sa inkorporacijom biomase. Setva je obavljena u jesen, a početkom maja usevi su pokošeni, tarupirani i inkorporirani u zemljište. Uzorkovanje zemljišta izvršeno je na dubini od 0-30 cm. Za statističku obradu podataka korišćena je ANOVA. Rezultati pokazuju da zelenišno đubrenje značajno doprinosi povećanju sadržaja azota i organskog ugljenika u zemljištu, posebno kada se kombinuje sa drugim održivim poljoprivrednim praksama poput združene setve. Najveći sadržaj organskog ugljenika (14,89 g/kg) i azota (0,195%) u zemljištu zapažen je u ovim tretmanima. Združena setva stočnog graška i pšenice + inkorporacija biomase, u poređenju sa kontrolom, povećao je sadržaj ugljenika u zemljištu u proseku za 1,4 g/kg, što znači da se zaliha organskog ugljenika u zemljištu povećala za 0,5 t/ha/god. Primena klimatski pametnih poljoprivrednih praksi povećava otpornost poljoprivredne proizvodnje na klimatske promene, istovremeno čuvajući zemljište kao izuzetno važan, neobnovljiv prirodni resurs.

Ključne reči: organski ugljenik, pšenica, sekvestracija ugljenika, stočni grašak, zelenišno đubrenje, združeni usevi