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Effect of Green Manures on Organic Carbon, Nitrogen, and Aggregate Stability in Chernozems of Northern Kazakhstan

Vladimir Zabolotskich[1*], Natalya Zuyeva[2], Irina Rukavitsina[3], Sergey Zhurik[1], Irina Bulgakova[3], Lyudmila Zhloba[2]

[1]Laboratory Biological farming, "A.I. Barayev Research and Production Centre for Grain Farming" LLP, Nauchny, Kazakhstan.
[2]Laboratory of Soil and Agrochemistry Studies, "A.I. Barayev Research and Production Centre for Grain Farming" LLP, Nauchny, Kazakhstan.
[3]Laboratory Microbiology, "A.I. Barayev Research and Production Centre for Grain Farming" LLP, Nauchny, Kazakhstan.

ABSTRACT

Long-term conventional tillage in rainfed agriculture reduces soil organic matter and fertility. However, the effectiveness of different green-manure mixtures under arid conditions remains insufficiently studied. This study assessed the effects of green manures on soil organic carbon (SOC), total nitrogen (TN), aggregate-size distribution, and aggregate water stability in Chernozem soils of Northern Kazakhstan. In a two-year field experiment, mixtures of pea and oat (PA), oat and mustard (AB), and pea and mustard (PB) were compared with conventional clean fallow (CF). Soil properties were assessed before sowing and 35 days after green-manure incorporation. After incorporation, SOC was significantly higher under green manures than under CF ($P < 0.05$), reaching 1.69% in PA compared with 1.33% in CF. TN was significantly lower in PA and AB than in CF, but differences among the green-manure mixtures were not significant. SOC changes were positively correlated with incorporated biomass ($r = 0.62$). PA produced the greatest fresh biomass, reaching 27.92 t ha^{-1} in 2024, when growing-season precipitation totaled 232.2 mm. In PA, the proportion of 0.25–10 mm aggregates and aggregate water stability increased by 4.8 and 12.2 percentage points, respectively, relative to presowing values; under CF, the corresponding measures decreased by 4.2 and 2.9 percentage points. These findings support the use of green manures, particularly pea–oat mixtures, to increase SOC content and improve soil aggregation and water stability under the study conditions.

Keywords: *Aggregate stability, Chernozems, Clean fallow, Green manures, Organic carbon*

Corresponding author: Vladimir Zabolotskich
e-mail ✉ zabolotskih_vladimir@mail.ru
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INTRODUCTION

Long-term intensive agricultural practices have deteriorated arable land quality, associated with organic matter loss and a persistent decline in soil fertility (G. Wang *et al.*, 2025). Green manures are an important tool for increasing organic carbon content and regulating nitrogen nutrition in agroecosystems. Introducing green manure crops substantially increases SOC through continuous biomass inputs and enhanced microbial activity, which positively affects the productivity of subsequent crops (Salisu *et al.*, 2025). In addition, carbon distribution within soil macroaggregates improves (Gentsch *et al.*, 2024).

The effect of cover crops on SOC depends on mixture composition, biomass production, and climatic conditions. The greatest carbon accumulation is observed when grasses and legumes are combined, as such mixtures promote the formation of stable organo-mineral complexes (Wooliver & Jagadamma, 2023). Green manures have a particularly pronounced effect on increasing SOC content in the upper soil horizon, thereby reducing soil degradation and erosion (Jian *et al.*, 2020).

The effects of green manures on nitrogen nutrition are also variable. Total nitrogen content may increase when mineralization occurs at a moderate rate. However, intensive microbial immobilization of nitrogen during the decomposition of readily degradable biomass may sometimes temporarily stabilize or even reduce available nitrogen (Hu *et al.*, 2023).

In addition to changes in plant nutrient availability and microbial activity, the adverse effects of the long-term intensive use of arable land also extend to the agrophysical properties of soil, including soil structural quality and aggregate stability, water-retention capacity, and the degree of soil compaction (Chenu *et al.*, 2000; Lal, 2020). Erosion, the lack of organic matter inputs, and reduced microbial activity substantially impair soil structural quality and aggregate stability. Consequently, soil organic matter content is closely associated with soil structural stability and soil compaction (Annabi *et al.*, 2007; Mosavi *et al.*, 2012).

Green manures, as one of the most accessible means of replenishing soil organic matter, can improve soil agrophysical properties and enhance effective soil fertility under rainfed agricultural conditions. This study assessed the effects of

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different types of green manure on SOC content, total nitrogen, aggregate-size distribution, and aggregate stability under the arid conditions of Northern Kazakhstan.

MATERIALS AND METHODS

Study site and experimental design

The study was conducted at the A.I. Barayev Research and Production Centre for Grain Farming in Northern Kazakhstan (51°38′39.7″ N, 71°00′52.2″ E). The experimental site soil was a southern carbonate low-humus Chernozem with a heavy texture (USDA Soil Survey Staff., 1999). The humus content in the 0–20 cm soil layer was 3.4%; the total nitrogen and phosphorus contents were 0.22% and 0.12%, respectively; and the carbonate content was approximately 5%. The arable soil layer was alkaline (pH > 8.0). The following crop mixtures were grown as green manures: pea and oat (Pisum sativum + Avena sativa), designated PA; oat and mustard (Avena sativa + Brassica juncea), designated AB; and pea and mustard (Pisum sativum + Brassica juncea), designated PB. Before sowing, the green-manure seeds were mixed in proportions of their respective sole-crop seeding rates: 60:40 for PA, 40:60 for AB, and 50:50 for PB. The green-manure mixtures were sown during the fallow phase of a three-field fallow–wheat–wheat crop rotation. Conventionally managed clean fallow served as the control treatment (CF). The green-manure mixtures were sown between May 25 and May 30 using a seed drill fitted with cultivator sweeps and press wheels. The sowing depth was 3–4 cm. The green-manure crops were incorporated into the soil 40–45 days after sowing, when the plants had reached the budding-to-early-flowering stage. Incorporation was performed using a Catros disc tillage implement to a depth of 8–10 cm. The control treatment was managed as conventional clean fallow throughout the summer. The first tillage operation was performed during the first ten days of June using flat-cutting implements at a depth of 10–12 cm. Three subsequent tillage operations were performed at 20–25-day intervals, with the working depth gradually increasing to 16–18 cm. The final tillage operation was conducted from late August to early September at a depth of 25–27 cm using a cultivator-subsoiler without soil inversion.

Meteorological conditions

The meteorological conditions during the 2024 and 2025 growing seasons differed in precipitation amounts. They featured higher-than-normal temperatures, a pattern that has become typical of the study area over the past decade. During the three-month growing season from June to August 2024, total precipitation was 232.2 mm, compared with the climatic normal of 136.3 mm. August recorded the highest monthly precipitation, at 106.6 mm, exceeding the long-term normal by 66.8 mm. Mean temperatures in June and July were 4.3 and 1.8 °C above normal, respectively. The mean daily temperature in August equaled the long-term normal of 17.4 °C **(Figure 1)**. The combination of abundant moisture and elevated temperatures promoted intensive green-manure biomass production.

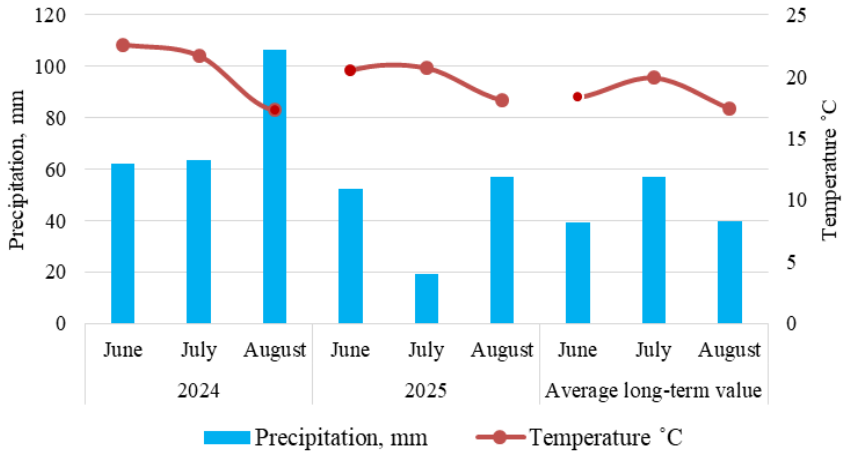


Figure 1. Meteorological conditions in 2024 and 2025 compared with the long-term average (1936–2025).

The 2025 growing season was less favorable for the growth and development of the green-manure crops. Total growing-season precipitation amounted to 128.6 mm, which was 7.7 mm below the climatic normal. The meteorological conditions in 2025 were characterized by the absence of the typical July precipitation maximum, relatively high precipitation in June and August, and elevated temperatures throughout the growing season. In June, total precipitation was 52.2 mm, compared with the climatic normal of 39.5 mm, while the mean daily temperature was 20.5 °C. July 2025 was dry, with only 19.4 mm of precipitation, 36% below normal. However, the effects of meteorological drought were not apparent because soil moisture reserves were sufficient. August again recorded the highest monthly precipitation, reaching 57.0 mm compared with the long-term normal of 39.8 mm. The mean growing-season temperature was 19.7 °C, or 1.2 °C above normal. Overall, the weather conditions during the growing seasons covered by the study were favorable and did not limit the growth or development of the green-manure crops.

Soil sampling

Soil samples from the 0–20 cm layer were collected before sowing and 35 days after incorporating green-manure biomass. Samples were collected using a soil auger, with ten subsamples from each plot combined into one composite sample. The soil samples were air-dried, ground, and passed through a 2 mm

sieve before determining their agrochemical properties. Soil organic carbon (SOC) content was determined by the Dumas combustion method using a Primacs analyzer (Skalar, the Netherlands). The method involved high-temperature combustion of the samples at 1,100 °C in an oxygen atmosphere. During combustion, carbon was converted to CO_2, and its concentration was measured using an infrared detector. Soil total nitrogen (TN) was determined by the Kjeldahl method. A weighed soil sample was digested in concentrated sulfuric acid with a catalyst, converting nitrogen to ammonium sulfate. Ammonia was subsequently distilled using a UDK 139 automatic analyzer (VELP Scientifica, Italy) and quantified by titration. The proportion of agronomically valuable aggregates measuring 0.25–10 mm was determined in the 0–10 cm soil layer using the N.I. Savvinov method. Air-dried soil was passed through a stack of sieves with aperture sizes of 10, 7, 5, 3, 2, 1, 0.5, and 0.25 mm. Aggregate stability, expressed as water stability, was determined using the I.M. Baksheev method, which involves mechanically oscillating sieves immersed in water. Immediately before green-manure incorporation, we also determined the fresh biomass produced in each experimental treatment. Plant biomass was collected from 0.25 m^2 quadrats in four replicates and converted to centners per hectare (c ha^{-1}; 1 c = 100 kg). The results were statistically analyzed using analysis of variance (ANOVA), followed by the least significant difference test at $P < 0.05$ ($LSD_{0.05}$), in the SNEDECOR statistical software package.

RESULTS AND DISCUSSION

In the two-year field experiment involving three types of green manure, SOC and TN contents, the proportion of agronomically valuable aggregates, and their water stability differed significantly compared with clean fallow without green-manure application. Under the same conditions, green-manure mixtures of different compositions produced different amounts of aboveground biomass, and biomass production differed significantly ($P < 0.05$) among treatments in both years of the study **(Figure 2)**. The PA treatment produced the greatest biomass.

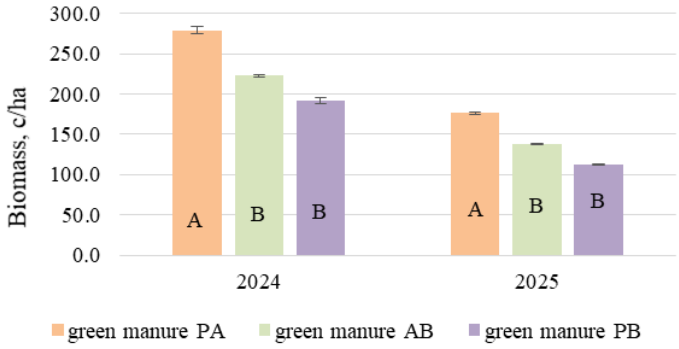
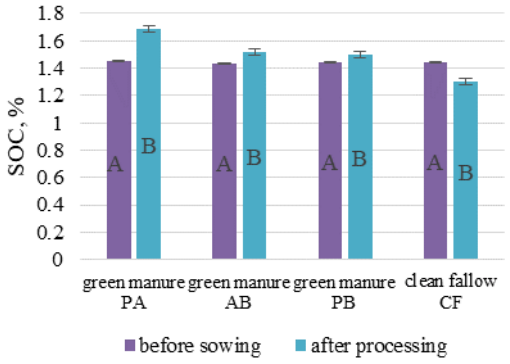
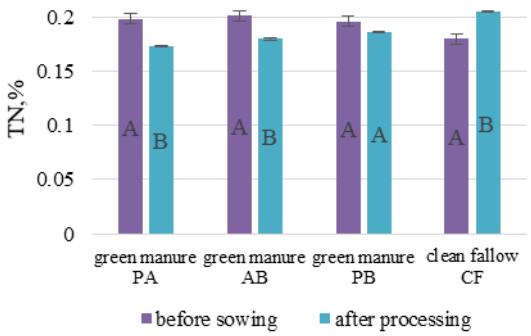


Figure 2. Biomass amount before incorporation into the soil depending on the type of green manure. *Statistical significance at $P < 0.05$.

The type of green manure and the amount of biomass produced also affected soil organic carbon (SOC) and total nitrogen (TN) contents **(Figure 3)**. Before the green-manure crops were sown, SOC content did not differ statistically among treatments. It ranged from 1.43% to 1.45%. After green-manure incorporation, the PA treatment recorded the highest SOC content (1.69%), significantly higher than both the control treatment (clean fallow, 1.33%) and the other green-manure mixtures (1.51%–1.52%). The AB and PB treatments did not differ statistically, but both had higher SOC contents than clean fallow.



a)



b)

Figure 3. Changes in SOC (a) and total nitrogen (b) contents in the 0–20 cm soil layer depending on the type of green manure. *Statistical significance at $P < 0.05$.

At emergence, mean total nitrogen (TN) content ranged from 0.180% to 0.205%. The green-manure mixture treatments had higher values than the clean-fallow treatment; however, differences among the green-manure treatments were not statistically significant.

After green-manure incorporation, TN content ranged from 0.173% to 0.205%. The highest value was recorded in the clean-fallow treatment (0.205%), which was significantly higher than those in the PA and AB green-manure treatments. No significant differences were found among the green-manure mixtures.

Before the green-manure crops were sown, the proportion of agronomically valuable 0.25–10 mm aggregates did not differ among the experimental treatments and ranged from 66.5% to 68.0%. After the green-manure crops had been grown and incorporated, soil aggregation increased by 0.3%–4.8%, whereas the proportion of aggregates in the clean-fallow treatment without green manure decreased by 4.2%. Differences in the proportion of agronomically valuable aggregates among the green-manure treatments were also significant at $P < 0.05$ **(Figure 4)**.

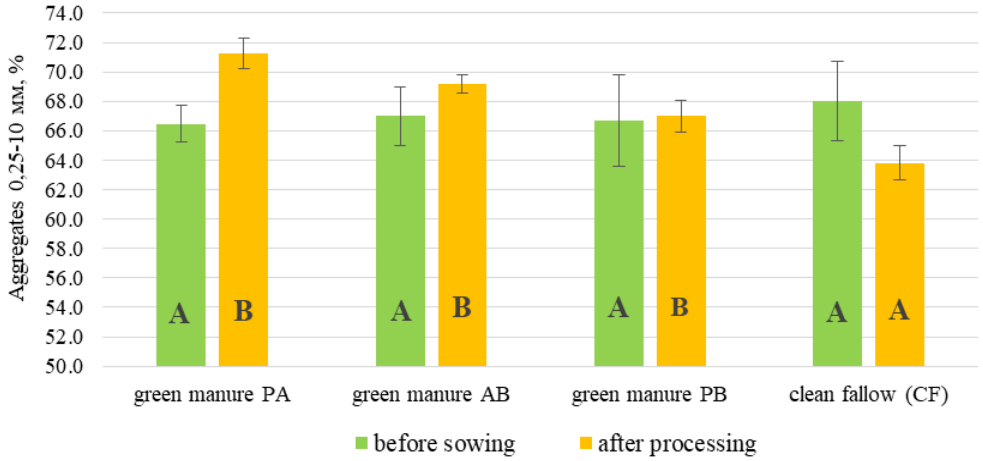


Figure 4. Effect of green manures on the proportion of agronomically valuable 0.25–10 mm aggregates compared with conventional tillage. *Statistical significance at $P < 0.05$.

As with aggregate abundance, aggregate water stability did not differ among the treatments before the green-manure crops were sown, but significant differences became apparent after their incorporation. Green-manure application increased aggregate stability by 3.3%–12.2%, whereas aggregate water stability in the control treatment (CF) decreased by 2.9% compared with the spring values **(Figure 5)**.

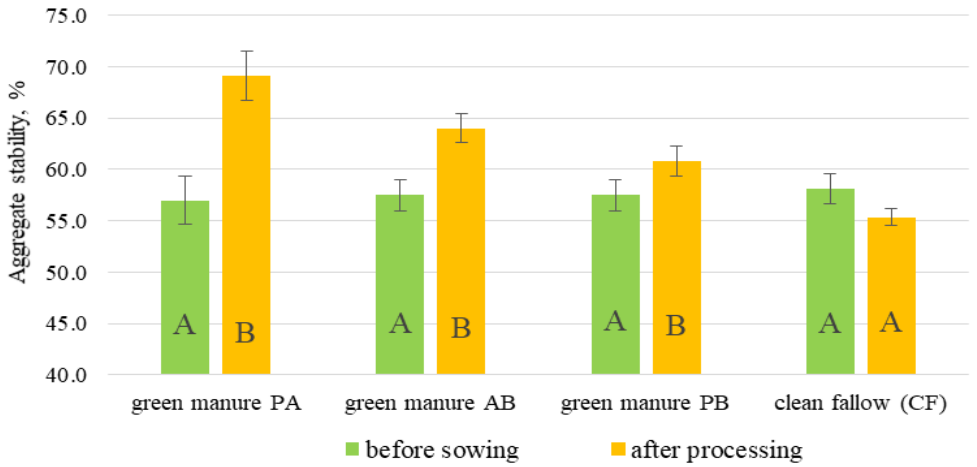


Figure 5. Effect of green manures on aggregate water stability compared with conventional tillage. *Statistical significance at $P < 0.05$.

The more green-manure biomass produced and incorporated into the soil, the higher the proportion of agronomically valuable aggregates and their water stability at the second sampling date. Regression analysis revealed a strong correlation between soil aggregation, aggregate stability, and green-manure biomass production during both years of the study **(Figure 6)**.

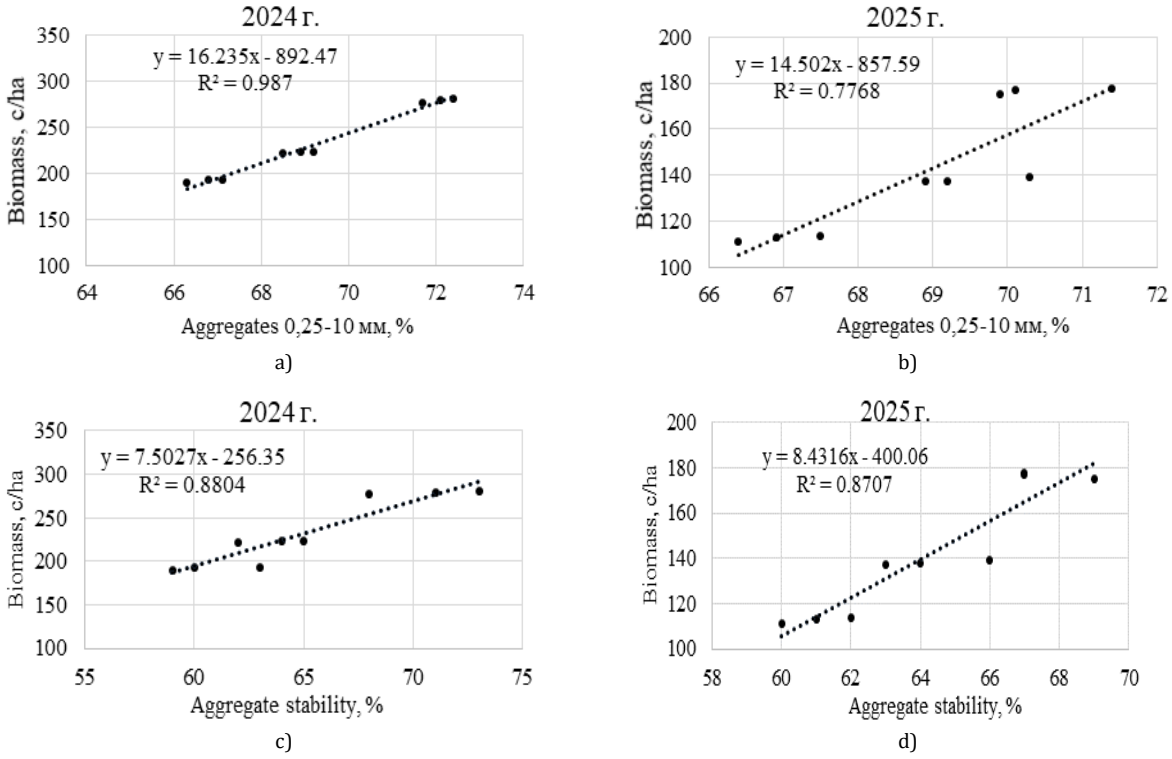


Figure 6. Relationship of the proportion of agronomically valuable aggregates and their stability with green-manure biomass after incorporation into the soil.

The strongest relationship during the study years was observed between biomass production and soil aggregation (R^2 = 0.88–0.98). The weakest relationship was recorded between aggregate stability and biomass production in 2024 (R^2 = 0.77), which may have been associated with the unusually high precipitation that year.

The results showed that green-manure application redistributed carbon and nitrogen fluxes in the soil, altering the balance between organic matter mineralization and stabilization. The observed positive relationship between biomass production and SOC dynamics (r = 0.62) is consistent with the concept of carbon balance in agroecosystems (Lal, 2004; Paustian *et al.*, 2016). Plant residue inputs are a major factor contributing to SOC accumulation and increasing the resistance of agricultural landscapes to degradation processes (Minasny *et al.*, 2017). SOC stabilization in the treatment with the greatest biomass production (AB green manure) can be explained by active aggregate formation and the physical protection of organic matter, as described in detail by Six *et al.* (2002). According to the Microbial Efficiency–Matrix Stabilization (MEMS) framework, microbial processing of plant residues determines subsequent carbon incorporation into stable organo-mineral complexes (Cotrufo *et al.*, 2013). The substantial decrease in SOC in clean fallow, accompanied by an increase in TN, indicates intensified mineralization, consistent with Fontaine *et al.* (2007), who showed that a limited supply of readily decomposable substrates may stimulate the decomposition of previously stabilized organic matter. Kallenbach *et al.* (2016) examined the mechanisms of microbial anabolic carbon stabilization in detail and emphasized the role of microbial biomass in forming stable SOC fractions. Studies under semi-arid steppe conditions in North America and the Mediterranean region have also shown that green manures increase microbial biomass and soil aggregation, thereby promoting SOC accumulation (Aguilera *et al.*, 2013; Basche & DeLonge, 2019). In arid regions, green manures often improve soil structure and water-holding capacity (Lal, 2008; Wiesmeier *et al.*, 2019). Our study in Northern Kazakhstan showed a positive effect of green-manure application in the fallow field, expressed as an increase in the proportion of agronomically valuable soil aggregates and their stability, similar to the findings of Park and Lee (2025). Under conventional fallow management without green-manure cultivation and with high precipitation, aggregate water stability may decrease, as Zeng *et al.* (2018) indicated. The improvement in soil structural composition after green-manure incorporation resulted from macroaggregate formation and a reduction in the fine fraction (< 0.25 mm), as confirmed by Y. Wang *et al.* (2025). Some studies have reported a positive effect of legumes on soil aggregation and aggregate stability (Ansari *et al.*, 2022). However, Beslemes *et al.* (2023) reported that the use of a legume crop as green manure reduced aggregate stability from spring to autumn. Increases in soil aggregation and aggregate stability were closely related to green-manure biomass production (R^2 = 0.88–0.98), consistent with the results of Kamran *et al.* (2021). Therefore, selecting green-manure crops plays an important role in producing large amounts of aboveground biomass. In the experiments of Cicek *et al.* (2014), biomass production was consistently higher when pea and oat were grown together, which fully supports our findings: the biomass of the pea–oat green-manure mixture was 20%–22% greater than that of the oat–mustard mixture and 31%–36%

greater than that of the pea–mustard mixture. In addition to green-manure crop composition, precipitation was a primary factor affecting green-manure biomass production and SOC accumulation (Gong *et al.*, 2020). Poeplau and Don (2015) showed that green manures can increase SOC stocks, although the magnitude of this effect depends substantially on climatic conditions. The high growing-season precipitation in 2024 (232 mm) promoted the maximum production of pea–oat biomass, which reached 279.2 c ha^{-1}. In contrast, only 176.5 c ha^{-1} was obtained in the same treatment in 2025, when precipitation amounted to 128.6 mm.

CONCLUSION

The data obtained indicate that, under the arid conditions of Northern Kazakhstan, green-manure application has a more pronounced effect on SOC content than on TN content. The combined sowing of pea and oat achieved the most consistent positive effect on SOC accumulation. Changes in TN content were less pronounced and statistically inconsistent. Green-manure application increased soil aggregation and aggregate water stability. The proportion of agronomically valuable aggregates, as well as their stability, increased with increasing green-manure biomass, which depended on growing-season precipitation and was consistently greater in the pea–oat treatment. Conventional clean-fallow management without green manure resulted in reduced soil aggregation and aggregate stability by the end of the growing season because of repeated mechanical disturbance.

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CONFLICT OF INTEREST: None

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ETHICS STATEMENT: None

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