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Soil Organic Carbon Sequestration and Distribution, Soil Biological Characteristics, and Winter Wheat Yields Under Different Tillage Practices in Long-Term Field Experiment

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Abstract: The organic carbon content of soil (SOC) is considered a key factor for soil health and plays an important role in climate change. Conservation tillage systems promote carbon sequestration and reduce greenhouse gas emissions. A long-term field experiment with different soil tillage practices (conventional tillage—CT; reduced tillage—RT; and no tillage—NT) has been conducted in Prague-Ruzyně (Czech Republic) since 1995. The soil's organic carbon content, microbial biomass (C_{mic}), and enzymatic characteristics were evaluated in four-year crop rotation periods from 2005 to 2024. The crop rotation was as follows: winter oil seed rape, winter wheat, pea, and winter wheat. The following soil layers were studied: 0–10, 10–20, and 20–30 cm. Crop residues remained in the field and were incorporated into the soil according to the used tillage—completely under CT, partly under RT, and the remaining mulch under NT. Under RT and NT, the SOC, C_{mic}, and enzymatic activity were concentrated in the top soil layer and decreased in deeper layers, whereas all these characteristics were evenly distributed across the soil layers under the CT practice. The SOC content increased gradually in the whole soil profile (0–30 cm) from 51.0 t ha^{-1} on average in 2005–2008 to 56.0 t ha^{-1} in 2021–2024 under CT. An SOC increase from 57.4 to 63.1 t ha^{-1} under RT and from 61.1 to 65.7 t ha^{-1} under NT was noted in 2017–2020, after which the stagnation in SOC content was observed in the years of 2021–2024. Similarly, a lower C_{mic} and enzymatic activity were found in the same period. The highest C sequestration was found under NT; an increase of 571 kg C ha^{-1} year^{-1} was recorded from the establishment of the experiment in 1995 to 2024. This was followed by the RT and CT practices (462 and 221 kg C ha^{-1} year^{-1}, respectively). The average winter wheat yields and nitrogen content in grain were higher under CT (8.67 t ha^{-1}, 2.16% N) and RT (8.97 t ha^{-1}, 2.13% N) than under NT (8.23 t ha^{-1}, 2.03% N). The weather conditions during the year (abundance of precipitation) influenced crop yields significantly more than the tillage practices. Conservation tillage practices increase the organic carbon and microbial activity in soils, but climate change associated with higher average temperatures can affect these processes.

Keywords: soil; conventional tillage; reduced tillage; no tillage; soil organic carbon; microbial biomass; enzymatic activity; winter wheat yields; N content in grain

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1. Introduction

Soil organic carbon (SOC) content is considered a key factor for soil health [1] and is influenced by soil tillage, which is an important factor affecting the functionality of soil [2]. Conventional tillage (CT) is often replaced by conservation tillage (reduced and no tillage) due to its positive effects on the sequestration capacity of soil organic carbon (SOC). Conventional plow tillage turns over the surface soil, which causes strong soil disturbance and accelerates the mineralization of SOC [3]. Less intensive practices, mainly reduced-tillage (RT) and, to a lesser extent, no-tillage (NT) practices without soil inversion, are already being widely used worldwide as they mitigate the negative effects of conventional tillage [2,4]. Reducing tillage intensity increases SOC, nitrogen stocks, and dissolved organic C content [5].

Although many results have been published on the agricultural use of CT, RT, and NT practices, this topic is still highly relevant due to ongoing climate change. Sanginés de Cárcer et al. [6] reported that climate change is widely discussed in agriculture because it causes crop yield variability and yield losses [7]. Central Europe, in the past 50 years, has experienced shifts toward a warmer and drier agroclimate [8]. Since the year of 2000, six major droughts within Central Europe have been reported, among which those during the years of 2015–2019 can be considered the worst in 500 years [9–11]. An increase in temperatures can negatively affect the carbon cycle in farms using agro-technologies due to the accelerated decomposition of soil organic matter (SOM) with enhanced microbial respiration and enhanced carbon output to the atmosphere [12–14]. For this reason, conservation tillage practices are used to reduce carbon losses and sequester carbon in soils.

In fact, the mean annual precipitation, mean annual temperature, soil type, pH, soil total nitrogen content, N application rate, and experiment duration were identified as the key factors affecting SOC content [15]. The stabilization of SOC in agricultural soils thus merits critical attention [16]. Furthermore, an important role is played by carbon in climate regulation [17]. However, it is not necessarily simple, or even possible, to increase SOC stocks in all locations. Clearly, in some places, it can be more difficult to increase C inputs due to limited access to necessary resources such as fertilizer or water. The need to accept new agrotechnical practices is based on the need for C sequestration, which, in turn, also depends on the actual climate; the need for new practices is much higher, for instance, in water-limited and hot areas than in cool moist climates [18].

The decline in SOC in soils as a result of the conventional tillage of crop lands, mostly associated with moldboard plowing, and the enhanced losses in soil organic carbon due to mineralization processes and the resulting CO_2 emissions from soils have been widely reported [4,5,19–21]. As a consequence, conventional tillage leads to the disruption of soil aggregates and contributes to soil erosion [22]. Inputs of easily decomposable crop residues in soils can affect (depending on the amount of added substrate) soil microbial activity and induce an increase in extra CO_2 fluxes from soils [23]. The inversion of soils by plowing enhances these processes by adding oxygen. The reduction in soil operations and the use of RT or NT practices have resulted in an increase in SOC over a period of decades [4,20,23,24]. The practice of NT has shown a higher capacity to retain soil carbon than CT. Furthermore, the soil under the wheat residue left on the soil surface is able to store soil water and decrease the high soil temperatures responsible for carbon losses from soils [24,25]. Thus, the long-term use of NT, along with mulch application, enhances carbon sequestration and soil health in terms of the soil's physical, chemical, and biological properties [26,27]. On the other hand, conventional tillage does improve other important soil properties, such as soil aeration, the ease of seed emergence, effective weed control, and the incorporation of crop residue into the soil [4]. In addition, the oxidation and mineralization of soil organic matter lead to a higher release of soil nutrients beneficial for plant growth [2].

Higher carbon stocks in the soil surface layer and their utilization by soil microorganisms can cause nitrogen immobilization [28,29] and result in an unavailability of N to growing plants such that final crop yields are ultimately affected. The conditions of a given site together with a chosen tillage practice can play an important role in crop yields.

Lundy et al. [30] found decreases in yields under NT in comparison with CT, which are smaller in temperate regions (−3.7%) than in subtropical areas (−10.7%). An analysis of many European studies has shown the potential negative effect of the number of years undertaken of conservation tillage (RT, NT) on relative crop yield, an effect that can be reduced using a good crop rotation that includes crops other than cereals [31].

In the context of climate change, rising temperatures, and increasingly frequent droughts, new soil-friendly measures are needed in agriculture to store carbon in the soil, reduce water loss, and thus stabilize soil fertility. One of the appropriate techniques is to limit the depth and intensity of soil cultivation while leaving crop residues on its surface. Although many studies have been published on different tillage practices, including conservation ones, there is not enough experience with the long-term use of different tillage practices under continental climate conditions in Europe. The field experiment with various soil tillage practices established in 1995 in Prague-Ruzyně (Czech Republic) is unique in this area and allows the evaluation of the influence of different long-term tillage and agrotechnical practices on the soil characteristics of organic carbon content, selected microbial characteristics, and the yield and quality of cultivated crops under the conditions of climate change. These measurements need to be continued under the current conditions of a changing climate which will bring higher temperatures, warmer winters without frost and snow, and a higher incidence of dry periods. Mineralization, nutrient and water availability, and yields of both main and side products are significantly affected.

The aim of this work was to evaluate the effect of different tillage practices during the twenty-year period of the long-term experiment on soil carbon sequestration and changes in SOC and microbiological characteristics in different layers of the soil profile up to 30 cm, winter wheat grain yields, and nitrogen content in grain. The results will be used in further research and innovation of tillage practices in conventional and conservation agriculture.

2. Materials and Methods

2.1. Site Description

A long-term field trial was established on an arable field in 1995 in the Crop Research Institute (CRI) of Prague (Czech Republic: 50°05′ N; 14°17′ E). The site and soil characteristics [texture, nutrient contents, cation exchange capacity (CEC)] were described by Mühlbachová et al. [32]. The soil is loess mixed with highly weathered chalk classified as illimerized luvisol [33,34]. The SOC content was 1.3%, corresponding to 50 t C ha^{-1}, in the 0–30 cm layer at the beginning of the field experiment. The climatic region is warm and moderately dry.

2.2. Field Trial

The experimental design was described by Mühlbachová et al. [21,32]. Two main blocks (A and B) of 95 m × 95 m in size were established, with each divided into three parts: CT = moldboard plowing down to 20–22 cm; RT = chisel plowing of the surface soil layer to a depth of 8–10 cm; and NT = without tillage. The crop rotation was as follows: winter oilseed rape–winter wheat–pea–winter wheat. The postharvest crop residues were incorporated into the soil according to the used tillage practice [21,32]. The data were collected every year in the second half of May from the A field. Data of the complete crop rotation (2005–2008, 2009–2012, 2013–2016, 2017–2020, 2021–2024) were evaluated together to obtain more robust information. N fertilizers (calcium ammonium nitrate and urea ammonium nitrate) were applied in doses of 120–160 kg N ha^{-1} during winter wheat and rapeseed growth. Phosphorus, potassium, and magnesium were applied annually in autumn as diammonium phosphate (23 kg P ha^{-1}), potassium chloride (46–66 kg K ha^{-1}), and magnesium sulfate (15 kg Mg ha^{-1}) [32]. Pesticides during the winter wheat growth were applied as needed in individual years uniformly across the entire field [21].

Soil to a depth of 30 cm was studied in 10 cm layers. The fresh soil samples were sieved with a 2 mm sieve. The soil moisture was determined before the determination of microbial biomass and enzymatic activities. The SOC analyses were carried out on air-dried soils. The winter wheat harvest was performed using a SAMPO harvester, SR2010 Plot Combine, designed for experimental fields and seed breeding (SAMPO Rosenlew, Pori, Finland) at the time of grain maturity, and yields were calculated in tons per ha at a grain dry matter content of 86%.

2.3. Analytical Procedures

2.3.1. Soil Organic Carbon (SOC)

SOC was determined by a modified oxidation–reduction method using sulfuric acid and potassium dichromate [35]. Briefly, 1 g of soil sample was digested in 10 mL of 2 M $K_2Cr_2O_7$ and 10 mL of concentrated H_2SO_4. The solution was heated for 1 h at 125 °C, diluted by distilled water up to 100 mL, and subsequently measured by colorimetry at a wavelength of 580 nm. When converting the carbon weight per hectare, the annually determined soil bulk density (BD) was considered:

$$\text{SOC (t ha}^{-1}) = \text{SOC (\%)} \times \text{BD (g cm}^{-3}) \times 1000 \times 10{,}000/100/1000$$

The bulk density was determined by soil sampling in 100 cm^3 metallic cylinders from the middle of each 10 cm layer. The bulk density was determined as soil weight on an oven-dry basis (at 105 °C) and was used for the calculations [36].

2.3.2. Microbial Biomass C (C_{mic})

Measurements of the soil C_{mic} were performed by use of the fumigation–extraction method [37]. Briefly, 25 g of the moist soil was extracted with 80 mL of 0.5 M K_2SO_4. An equal portion of the moist soil was fumigated in an exsiccator with ethanol-free chloroform, boiled for 2 min, and left for 24 h. Thereafter, the chloroform was removed, and soils were extracted equally as non-fumigated soils. C_{mic} was calculated from the relationship B_c= 2.64 E_c, where E_c is the difference between the organic C extracted from the fumigated and non-fumigated treatments.

2.3.3. Dehydrogenase Activity

The dehydrogenase activity of the soil samples was determined by the reduction of TTC (triphenyl tetrazolium chloride) to TPF (triphenyl formazan) [38]. Briefly, 6 g of the moist soil was mixed with 0.1 g of $CaCO_3$, 1 mL of 3% TTC, and 2.5 mL of distilled water. Soils were left in tightly closed tubes for 24 h at 37 °C. Thereafter, the extracts were removed from the tubes and further extracted and diluted with ethanol up to 100 mL. The red color intensity of the samples was measured by colorimetry at a wavelength of 485 nm.

2.3.4. Urease Activity

Urease activity was determined using urea hydrolysis on CO_2 and NH_3 [39]. Briefly, 2.5 mL of 80.0 mM urea was added to 5 g of soil and incubated for 2 h at 37 °C. A 5 g sample of soil of the same treatment with 2.5 mL of distilled water served as a control. After incubation, 2.5 mL of distilled water was added to the experimental sample and 2.5 mL of 79.9 mM urea was added to the control samples, and all samples were immediately extracted with 50 mL of 2 M KCl containing 10 mL of 1 M HCl 1^{-1} solution. After a reaction of sodium salicylate with NH_3 in the presence of sodium dichloroisocyanurate–

sodium nitroprusside as the catalyst, the extracts were measured by colorimetry at a wavelength of 660 nm.

2.3.5. Nitrogen Content in Grain

The nitrogen content in grain was determined using the Kjeldahl method consisting of digestion, distillation, and titration. A sample of 1 g was heated up to 400 °C with 12.5 mL of concentrated sulfuric acid and catalyst (Kjeltabs ST (3.5 g K_2SO_4 + 3.5 mg Se)). This process converted any nitrogen in the sample to ammonium sulfate. The digestate was neutralized by the addition of NaOH, which converted the ammonium sulfate to ammonia, which was distilled off and collected in a receiving flask with an excess of 0.05 M sulfuric acid. The residual sulfuric acid was then titrated with 0.1 M NaOH standard. The N content in the grain sample was calculated based on the NaOH consumption for titration.

2.4. Meteorological Data

Temperature and rainfall data were collected by the CRI Prague-Ruzyně Meteorological Station. The average daily air temperatures and the rainfall sum totals were calculated as averages from the partial 15 min datasets of 7, 14, 28, 42, and 61 days before sampling. The year averages of temperatures and precipitation were also collected.

2.5. Statistical Analyses

Statistical analyses were carried out by the use of Statistica 14.0 software (TIBCO, Santa Clara, CA, USA), with the results expressed as mean values from 5 replicates for a single year and mean values for the 4-year period. The same lower-case letters at the bottom of the histograms in the figures represent statistically identical values of examined tillage practices according to factorial ANOVA Tukey's test ($p < 0.05$) considering crop rotation cycles, tillage, and depth. Error bars represent the standard deviation. The correlation coefficients (r) based on Spearman's equations between soil characteristics, precipitations, and temperatures were calculated. The confidence level was as follows: * $p \leq 0.05$, the differences are significant at the 95% confidence level; ** $p \leq 0.01$, the differences are significant at the 99% confidence level; *** $p \leq 0.001$, the differences are significant at the 99.99% confidence level; and $p > 0.05$, there are no significant differences (ns—differences are significant at less than the 95% confidence level).

3. Results

3.1. Soil Organic Carbon (SOC)

The SOC under CT was more evenly distributed across all soil layers in the soil profile of 0–30 cm than under RT and particularly NT (Figure 1). The SOC content under CT in the surface layer of 0–10 cm increased from 1.25 to 1.38%, similar to the layers of 10–20 cm (1.29–1.34%) and 20–30 cm (1.24–1.32%). A significant increase in SOC content in the 0–10 cm layer was found under RT (1.55 to 1.70%) and especially NT (1.61 to 1.80%). A clear stratification and decrease in SOC in the deeper layers of RT (10–20 cm: 1.29–1.43% SOC; 20–30 cm: 1.15–1.28% SOC) and NT (10–20 cm: 1.21–1.29% SOC; 20–30 cm: 1.10–1.19% SOC) were observed.

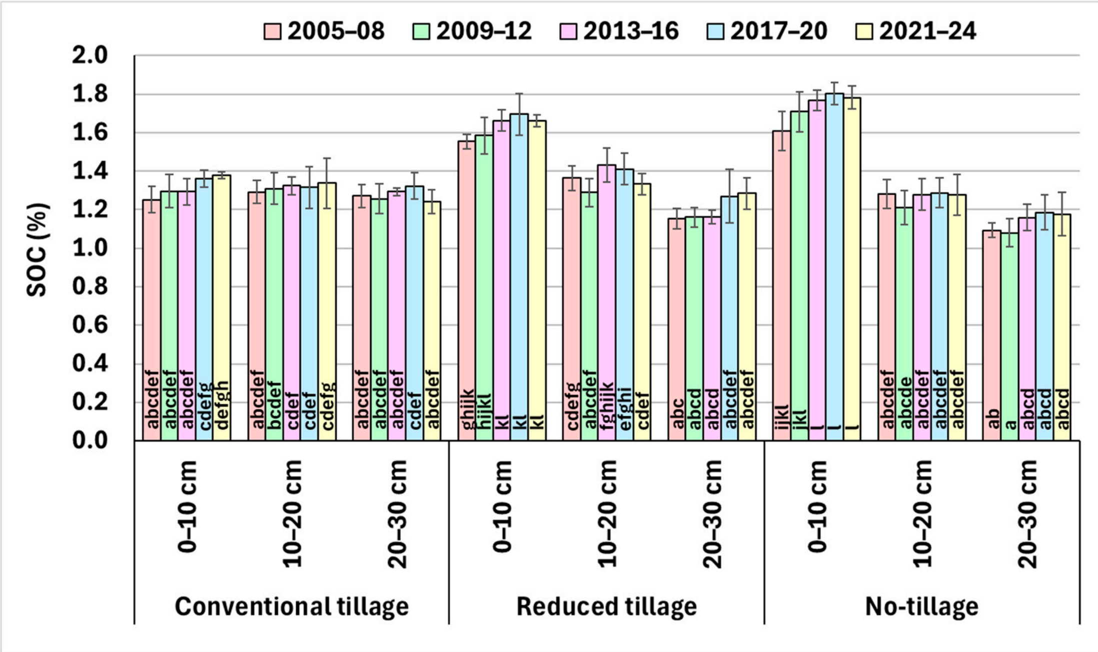

Figure 1. Dynamics of soil organic carbon (SOC) under different soil tillage practices in the years of 2005–2024. The same letters at the bottom of the histograms represent statistically identical values of examined tillage practices according to factorial ANOVA Tukey's test ($p < 0.05$). Error bars represent the standard deviation.

An increasing trend in SOC contents was noted with a certain variability in the layer of 0–10 cm in the first four rotations under all tillage practices, being higher under RT and especially NT. After that, a stagnation in SOC contents was observed in the last crop rotation. While SOC under RT and NT in the layer of 10–20 cm did not show a clear trend, an increase was noted in the 20–30 cm layer from the third and fourth rotation under RT and NT, respectively. The factorial ANOVA test showed significant differences among tillage practices, mainly in the layer of 0–10 cm, whereas, despite the clear trend, it did not indicate significant changes within the same evaluated layer among the crop rotations.

The bulk density was used to calculate the overall amount of carbon in the soil profile and land area in differently tilled soils, which changed according to the tillage practice used and also with depth of the soil profile. The upper soil layer of 0–10 cm was cultivated under CT (1.22–1.28 g cm^{-3}) and RT (1.22–1.33 g cm^{-3}), resulting in a lower bulk density compared to NT (1.43–1.49 g cm^{-3}), where only minimal local disruption of the soil surface occurred as a result of sowing. The bulk density of the soil under CT increased continuously with increasing soil depth, and increased below a depth of 10 cm under RT (1.50 to 1.56 g cm^{-3}), similarly to NT (from 1.57 to 1.60 g cm^{-3}), due to the lack of soil operations.

The total carbon weight per hectare, also considering soil bulk density, was shown to increase in the soil layer of 0–30 cm from 51.0 t ha^{-1} in the first studied crop rotation to 56.0 t ha^{-1} in the last crop rotation under CT (Figure 2). Despite the stratification shown in SOC content in the soil layers under RT and NT (Figure 1), the carbon weight per hectare increased under RT from 57.4 up to 63.1 t ha^{-1} and under NT from 61.1 to 65.7 t ha^{-1} from the first (2005–2009) to fourth crop rotations (2017–2020). The carbon weight per hectare in the soil in the first rotations after the establishment of the experiment increased faster than in the final years. While between 1995 and 2024, the increase in the carbon weight per hectare in the soil reached 0.22, 0.46, and 0.57 t C ha^{-1} year^{-1} under CT, RT, and NT, respectively, in the period of 2010–2024, the annual SOC increments decreased to values of

0.20, 0.32, and 0.44 t C ha^{-1} $year^{-1}$, respectively. These data showed a stagnation or slight decline in SOC contents in the last crop rotation.

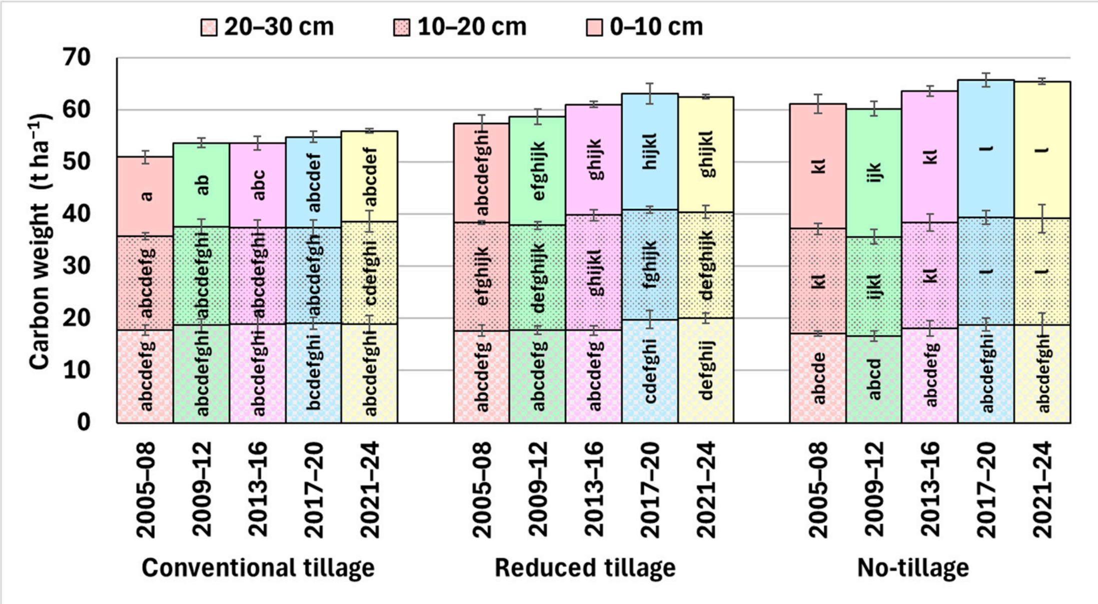

Figure 2. Soil organic carbon (SOC) in the soil profile of 0–30 cm in a given land area under different soil tillage practices in the years of 2005–2024. The same letters at the bottom of the histograms represent statistically identical values of examined tillage practices according to factorial ANOVA Tukey's test ($p < 0.05$). Error bars represent the standard deviation.

3.2. Soil Microbial Biomass (C_{mic})

Similarly to that of SOC, the content of soil microbial biomass (C_{mic}) was evenly distributed within soil layers under the CT practice, whereas an increase in C_{mic} in the top layer was observed under RT and NT (Figure 3). A clear trend of increasing C_{mic} contents was observed in the top layer (0–10 cm) of soils under RT and mainly under NT until the fourth crop rotation despite the fact that an increase was mostly not significant, due to a high variance of data. The trend of increasing C_{mic} contents up to the fourth crop rotation with a subsequent decrease in the fifth one in the deeper soil layer of 10–20 cm, as well as in the case of RT in the layer of 20–30 cm, was similar to that in 0–10 cm. A stratification in C_{mic} content in the studied soil layers was observed under RT and NT. The C_{mic} content in the deeper layers was approximately one-third (10–20 cm) to a half (20–30 cm) lower than in the surface layer of 0–10 cm under RT and NT. The total C_{mic} content in the 0–30 cm profile was higher under NT than RT. The C_{mic} in the layer of 20–30 cm slightly increased under NT, and its dynamics will be carefully observed in future periods of study. No significant correlations were found between SOC and C_{mic} in CT. In the case of RT and NT, the increase in C_{mic} correlated with SOC and confirmed a linear relationship between these soil characteristics (Table 1).

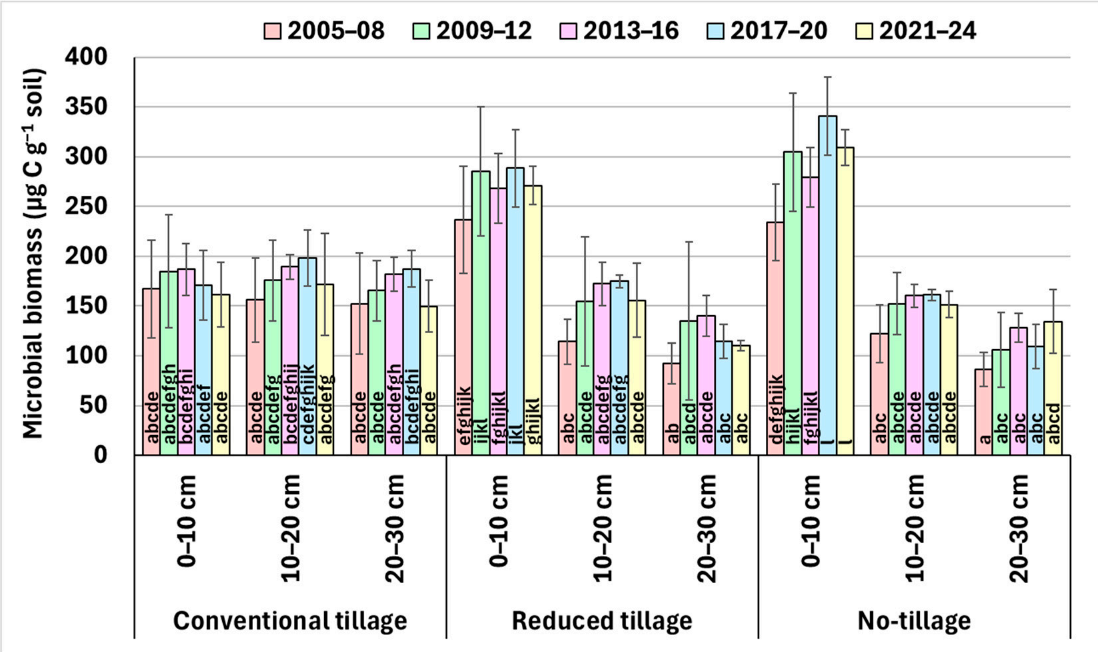

Figure 3. The microbial biomass content in the different soil layers under different soil tillage practices in the years of 2005–2024. The same letters at the bottom of the histograms represent statistically identical values of examined tillage practices according to factorial ANOVA Tukey's test ($p < 0.05$). Error bars represent the standard deviation.

Table 1. Correlations between SOC, C_{mic}, dehydrogenase, and urease activities (considering all soil depths studied).

Tillage	CT	RT	NT
Relationship			
SOC (%) × C_{mic}	ns	0.790 ***	0.909 ***
SOC (%) × Dehydrogenase activity	0.353 **	0.840 ***	0.843 ***
SOC (%) × Urease activity	ns	0.784 ***	0.276 *
C_{mic} × Dehydrogenase activity	ns	0.717 ***	0.828 ***
C_{mic} × Urease activity	ns	0.707 ***	0.295 **

(ns—non-significant; *—$p < 0.05$; **—$p < 0.01$; ***—$p < 0.001$).

3.3. Dehydrogenase and Urease Activities

Similarly to SOC and microbial biomass content, dehydrogenase activity (Figure 4) was evenly distributed among the soil layers under CT, whereas pronounced stratification was observed under RT and NT: a higher dehydrogenase activity in the 0–10 cm soil layer and lower values in the deeper soil layers of 10–20 and 20–30 cm were found under RT and NT than under CT. Dehydrogenase activities under RT and NT increased in the 0–10 cm soil layer in the fourth crop rotation, and after that, a slight non-significant decrease was noted. The same trend was also observed in the 10–20 cm layer under RT.

Similarly to the other biological and microbial parameters studied, the urease activity was more uniform under CT, whereas a substantial increase was observed under RT (by half) and particularly NT (up to twofold) in the top soil (Figure 5). The urease activity was notably stratified within soil layers under RT and especially NT, showing higher values in the 0–10 cm soil layer compared to deeper layers. Urease activities increased until the third crop rotation in the top soil layer, and after that, a decrease was noted.

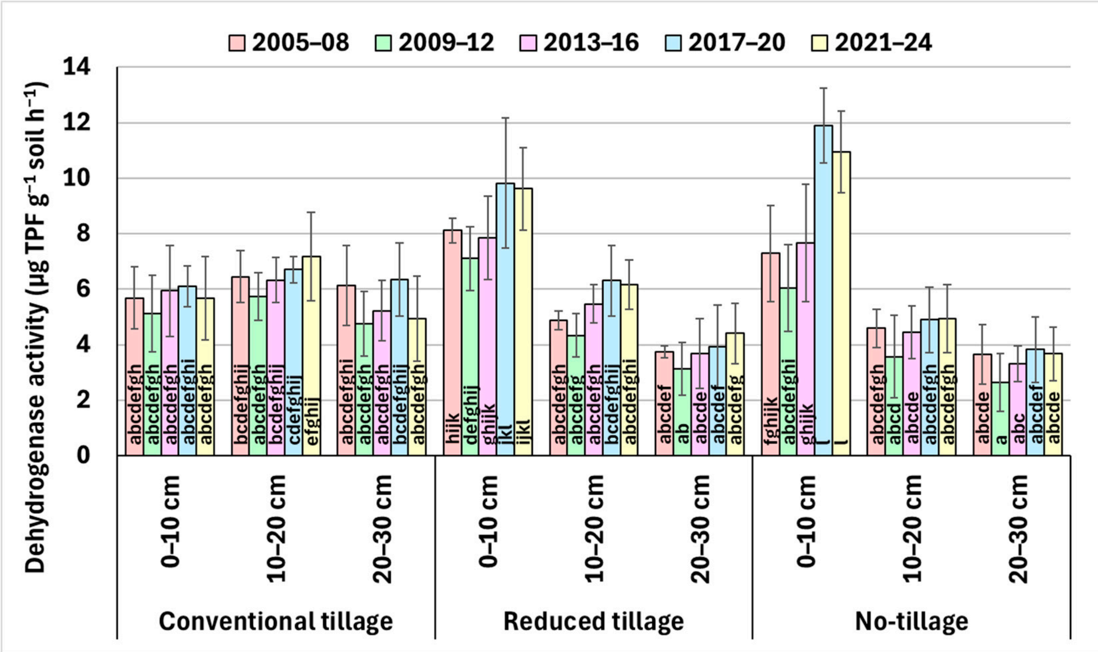

Figure 4. The dehydrogenase activity in the different soil layers under different soil tillage practices in the years of 2005–2024. The same letters at the bottom of the histograms represent statistically identical values of examined tillage practices according to factorial ANOVA Tukey's test ($p < 0.05$). Error bars represent the standard deviation.

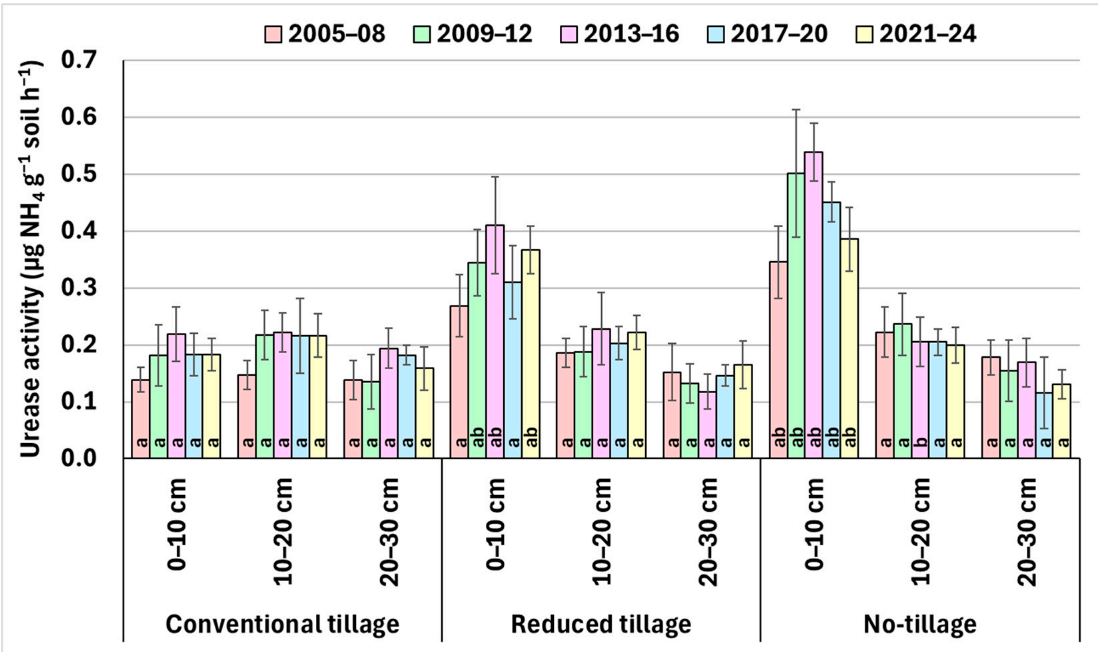

Figure 5. The urease activity in the different soil layers under different soil tillage practices in the years of 2005–2024. The same letters at the bottom of the histograms represent statistically identical values of examined tillage practices according to factorial ANOVA Tukey's test ($p < 0.05$). Error bars represent the standard deviation.

Positive significant correlations were generally found between SOC, dehydrogenase activity, and urease activity under RT and NT, showing mutual relationships among these characteristics (Table 1).

3.4. Air Temperatures and Precipitations

The increasing trend in air temperatures and, by contrast, a decrease in annual precipitation were observed during the studied period of 2005–2024 (Figure 6). The air temperature and precipitation totals were also observed over the whole studied period 7, 14, 28, 42, and 61 days before soil samplings (Table 2).

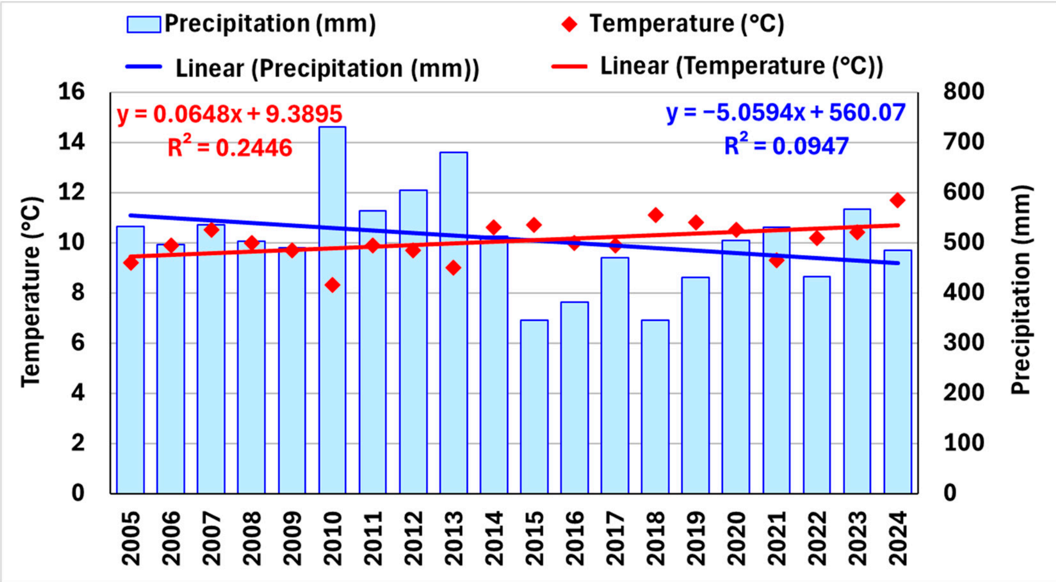

Figure 6. Annual temperature and precipitation averages at experimental site in Prague-Ruzyně.

Table 2. Average air temperatures and precipitation totals in Prague-Ruzyně in different periods before soil sampling.

	Temperature (°C)					Precipitation (mm)				
	Days Before Sampling									
Year	**7**	**14**	**28**	**42**	**61**	**7**	**14**	**28**	**42**	**61**
2005	12.2	12.0	11.5	11.3	9.3	21	63	67	75	79
2006	14.0	14.9	14.2	12.9	10.0	30	67	88	129	155
2007	21.3	16.7	15.6	14.7	11.6	0	36	37	39	48
2008	11.4	13.6	12.4	11.1	8.8	30	59	92	122	133
2009	17.1	14.4	14.1	14.1	11.2	26	59	69	83	102
2010	11.6	11.2	11.7	10.6	8.8	7	64	67	102	114
2011	15.9	13.3	13.3	12.5	10.5	9	30	38	49	84
2012	19.3	14.9	15.4	13.1	10.7	0	12	22	58	68
2013	11.2	13.6	13.3	12.4	8.7	16	46	69	84	93
2014	16.9	13.0	13.0	11.8	10.6	44	83	107	114	145
2015	13.2	14.1	12.9	11.7	9.3	10	25	52	56	84
2016	12.2	13.4	11.0	10.9	8.8	0	9	16	26	39
2017	16.0	15.1	12.6	11.2	9.6	1	20	49	69	97
2018	18.6	17.2	16.6	16.1	12.1	6	26	31	44	47
2019	14.3	11.7	10.9	13.5	12.1	20	40	66	66	68
2020	11.7	11.4	11.9	11.8	10.1	26	27	39	40	51
2021	11.9	13.0	11.4	9.6	8.8	2	63	101	108	116
2022	18.2	18.1	15.4	13.1	11.1	20	20	23	47	73
2023	14.7	14.1	12.5	11.4	9.7	0	8	13	44	77
2024	15.9	14.9	13.9	12.8	12.4	8	25	26	37	42

Positive correlations between SOC and temperature within different periods before sampling were found under CT when all soil depths within 0–30 cm were considered

(42 days: r = 0.352, $p < 0.01$; 61 days: r = 0.396, $p < 0.01$). Negative significant correlations between SOC and precipitation under CT were found on day 61 (r = −0.298, $p < 0.05$). No significant correlations were found between SOC and temperature or precipitation under RT and NT. Similarly, no correlations between temperature or precipitation and microbial biomass were found. Dehydrogenase activity was correlated under CT with temperature, mainly at longer time periods before sampling (42 days r = 0.352, $p < 0.01$; 61 days r = 0.396, $p < 0.01$). No correlations were found for urease activity.

3.5. Winter Wheat Yields and N Content in Grain

The main crop grown each second year in the long-term field experiment was winter wheat. The grain yields under CT and RT were usually comparable (Figure 7) with a slight tendency for higher yields under RT (average 2006–2024: CT—8.67 t ha^{-1}; RT—8.97 t ha^{-1}). Grain yields under NT were usually lower and reached an average of 8.23 t ha^{-1}. The differences in yields within years depending on weather conditions and type of previous crop were also observed. The highest average yield for the studied period was achieved under RT compared to CT, but not to such an extent that it would lead to an immobilization of nutrients and their reduced availability to plants as under NT. In addition, soil warming and water evaporation were limited due to the partial coverage of the soil surface with postharvest residues compared to CT. These factors had a greater influence in years with a deficit of precipitation (2018, 2022, 2024), when larger differences in yields were detected under CT and RT. In comparison with others, significantly lower yields were observed in 2012 and 2018. The grain yield in 2012 was affected by a dry spring and high precipitation in July (187 mm), whereas in the year of 2018, high temperatures and a lack of precipitation during the whole growing period were noted. Lower grain yields were also found in the years of 2022 and 2024, where in 2022 after dry spring, high precipitation within a few days in June (147 mm) was noted. Similarly, dry spring in the year of 2024 (4 mm in March, 13 mm in Aprile) negatively affected the dissolution of solid mineral fertilizers and nitrogen availability and uptake by wheat plants and their growth, resulting in lower yields.

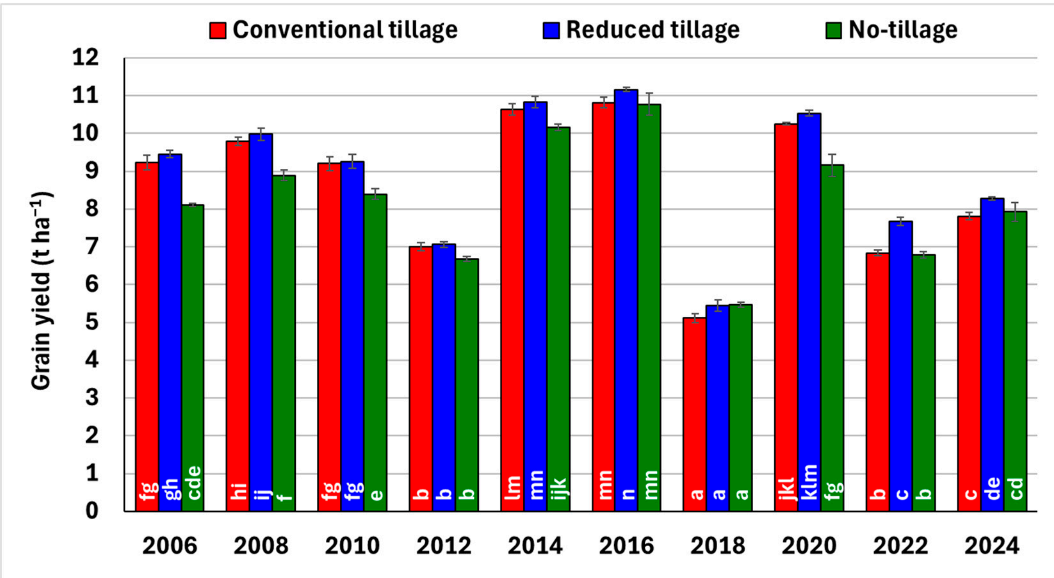

Figure 7. Winter wheat grain yields under different soil tillage practices. The same letters at the bottom of the histograms represent statistically identical values of examined tillage practices according to factorial ANOVA Tukey's test ($p < 0.05$). Error bars represent the standard deviation.

The nitrogen content in wheat grain under CT (2.16% N) and RT (2.13% N) was always at a similar level and higher than that under NT (2.03% N) (Figure 8). The winter wheat yields negatively correlated with the N content in grain across the three soil tillage practices (r = −0.665, $p < 0.001$).

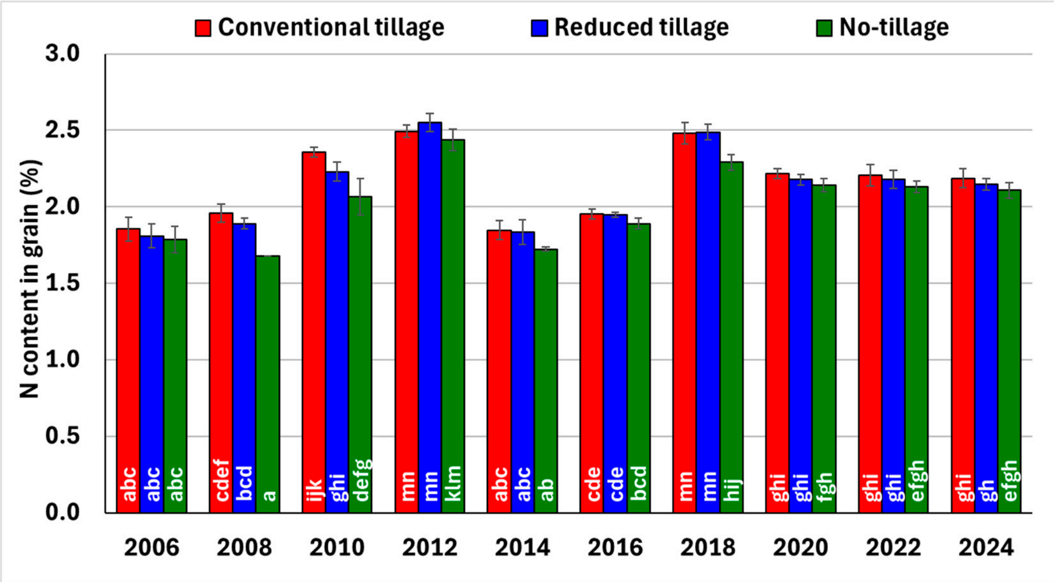

Figure 8. The N content in wheat grain under different soil tillage practices. The same letters at the bottom of the histograms represent statistically identical values of examined tillage practices according to factorial ANOVA Tukey's test ($p < 0.05$). Error bars represent the standard deviation.

4. Discussion

4.1. Soil Organic Carbon

A lower SOC content under CT compared to RT or NT in the surface layer of 0–10 cm (from 1.25 to 1.38%) was observed, thus confirming the results of other authors [4,18,19] who showed that moldboard plowing increased SOM mineralization processes and SOC losses. Similarly, Valkama et al. [40] reported that CT caused an SOC loss of 0.17–1.00 t ha^{-1} yr^{-1}. The increase in SOC in the layer of 0–10 cm under RT (1.55 to 1.70%) and NT (1.61 to 1.80%) was affected by low soil aeration as well as by postharvest residues that were only partly incorporated under RT and that remained on the soil surface under NT as a source of soil organic matter. Similarly, Topa et al. [41] found higher carbon fractions and total organic carbon (TOC) under no-till and chisel treatments at 0–10 cm depths. RT practice, with its shallow tillage accompanied by aeration and induced processes, also had a smaller increase in SOC in the surface layer compared to NT.

Under RT and, especially, NT, the increase in SOC in the top layer was accompanied by a stratification in the deeper soil layers. Our findings thus agree with Martínez et al. [42], who showed that the lack of mechanical incorporation of crop residues was the primary reason for the lower SOC in subsoils (10–20 and 20–30 cm layers) under RT and NT compared to CT. Despite the clear stratification in SOC in the soil profiles of RT and NT, the last two crop rotations in our field experiment showed an increase in SOC in the 20–30 cm layer. Martínez et al. [42] speculated that SOC under NT could increase in the subsoil as a result of colloid transport facilitated by continuous macropores, but their data did not support this hypothesis. However, this idea could be partly confirmed by our data as a result of the longer term of observation. We assume that the organic carbon in our long-term field experiment could be moved to deeper soil layers in several ways: (i) the activity of the macrofauna; (ii) the drainage of soluble C fractions facilitated, for instance, by drainage channels after oilseed rape roots, and (iii) the colloidal transport of SOC through macropores into deeper soil layers [42]. Minasny et al. [43] estimated the potential for C sequestration in the 1 m surface layer of agricultural soils at 2–3 Pg C per year on a global scale. On the other hand, Keel et al. [44] showed a decline in SOC in Swiss soils, irrespective of the type of soil tillage and organic inputs up to 0.29 t ha^{-1} $year^{-1}$. Our data show that an increase in SOC, microbial biomass, and enzymatic activities under RT and NT was noted up to the period of 2017–2020. However, the final years of 2021–2024 led to lower wheat yields (Figure 7), which resulted in a lower amount of postharvest residues left in the field. Lower yields were also found for other crops included in the crop rotation, and, especially, a very low yield of oilseed rape seeds as well as straw in 2021, the most important source of carbon in our experiment, played an important role in the stagnation (slight decrease) in SOC content during the last crop rotation (2021–2024).

The expression of SOC per land area also considering the bulk density showed that this expression is valuable for the determination of overall carbon content in the whole soil profile studied, mainly when the reduced or no-tillage practices are used. In fact, soil bulk density reflects the soil compaction according to the tillage practice used [41], and higher bulk densities are commonly found in deeper parts of the soil profile, as shown by Kumar et al. [45] and Topa et al. [41]. In addition, reduced and no-tillage practices can continue to be gradually compacted due to the influence of rainfall, particle resettlement, and cycles of wetting and drying [46].

The SOC increase was obtained by Topa et al. [41] after 10 years of their field experiment. In our field experiment, the SOC content increased during a study period in the whole soil profile (0–30 cm) from 51.0 t ha^{-1} in the period of 2005–2009 to 56.0 t ha^{-1} under CT in 2021–2024, and from 57.4 to 63.1 t ha^{-1} under RT and from 61.1 to 65.7 t ha^{-1} under NT up to the period of 2017–2020. After that, stagnation in SOC contents was noted in the years of 2021–2024. Higher SOC contents which are generally found under RT and NT are connected with crop residue retention, minimum mechanical disturbance, and restricted carbon oxidation [4,41].

4.2. Microbial Biomass (C_{mic})

Similarly to that of SOC, the content of soil microbial biomass was more evenly distributed within the soil layers under CT, whereas an increase in C_{mic} in the top layer was observed under RT and NT (Figure 3), as also found by Pandey et al. [47]. Generally, a decrease in soil microbial biomass was observed in the soil layers of 10–20 cm and particularly 20–30 cm under RT and NT, which was connected with greater soil compaction and the lack of mechanical incorporation of postharvest residues, causing energy deficiency for microorganisms [43,48] and thus reducing the microbial biomass content in RT and NT. However, our results showed that microbial biomass content can also increase in deeper soil layers and can become stabilized at a new level. Here, the hypothesis of the colloidal transport of SOC through macropores into deeper soil layers [43], thus giving more energy sources to the C_{mic}, could explain this finding.

A slight decrease in microbial biomass contents was noted in the period of 2021–2024, which is in line with the stagnation of SOC contents in the same period. The gradual increase in temperatures (Figure 6) and increasingly frequent periods of drought affect soil microbial abundance and functions—key parameters of plant–soil carbon allocation dynamics [49] which, in combination, lower the amount of crop residue left in the field and lower the organic sources for possible carbon sequestration in soil. Warming can also shift microorganisms toward utilizing soil organic matter as a C source instead of recently assimilated compounds [49].

4.3. Dehydrogenase and Urease Activity

Soil enzymes are good indicators of soil fertility, since they are involved in the cycling of the most important nutrients [50]. Enzymatic activities interact with the method of tillage used, soil depth, and other environmental factors such as temperature [47,51]. He et al. [3] reported that enzymatic activities were positively correlated with SOC fractions in soils under different tillage practices. The mostly greater dehydrogenase activity found in the top layer in RT and NT, in comparison to CT (Figure 4), corresponds to the results of Omidi et al. [52], who found the enzymatic activities to be significantly affected by NT in the top layer of soils. The stratification within soil layers and the decrease in dehydrogenase and urease activities in the deeper layers of the soil profile under RT and particularly NT that were observed are possibly due to not only a lower oxygen supply given by the higher bulk density, but also the decreased input of organic substrates from residues that are important for microbial activity [48]. Longer observation also showed, similarly to SOC and C_{mic}, that the dehydrogenase and urease activities can increase in deeper soil layers under RT and NT over time. All these soil characteristics showed up mainly in the 20–30 cm soil layer in RT and partly in NT, an increase which can be related to the colloidal transport of organic carbon in deeper soil layers [42].

Similarly to the SOC and C_{mic}, the enzymatic activity stagnation or slight decrease was detected in the period of 2021–2024, confirming close relationships among these soil characteristics. The conversion of C, N, and P sources into soluble compounds is a major process involving different soil enzymes. The microbial enzymes, such as dehydrogenase or urease, are also important for soil quality and can be used as biological soil quality indicators [53]. Lower enzymatic activities in the final studied period of 2021–2024 therefore reflect and confirm the SOC and C_{mic} dynamics in our soils under different soil tillage practices.

4.4. Air Temperatures and Precipitations

Sandor et al. (2011) [54] reported that short-term variations in climate had a significant effect on microbial biomass, with dry periods being distinguished by a reduced microbial biomass compared to wet periods. In addition, according to Parihar et al. [14], the favorable impact of rising temperatures for greater microbial activity and SOC turnover is a widely established fact.

Almost no direct correlations were found between SOC, C_{mic}, and dehydrogenase activity and precipitation under RT and NT. The positive correlations between SOC and temperature were significant under CT (r = 0.352–0.396, $p < 0.05$), which was more aerated than under RT and NT and therefore possibly more sensitive to weather changes. Soil organic carbon does not necessarily have to be directly affected by variations in temperature or rainfall [54], as other factors can also play an important role. Among these, the chemical composition of crop residues, the structure and composition of the microbial communities, and the C:N ratio in the soil can also all play an important role [55]. In addition, the wheat mulch remaining on the soil surface under NT, and only partial straw incorporation under RT, affects the mineralization of SOC by decreasing soil temperatures and maintaining higher soil humidity [56].

Soil enzymes play a vital role in nutrient mineralization and their activity is an excellent indicator for predicting the capacity of the nutrient supply for plants [57]. In this context, the lack of significant correlations between soil microbial characteristics and weather conditions under RT and NT suggest that soil microbial characteristics could possibly be able to resist better weather variability over the long term due to a greater number of organic sources laying on the soil surface (NT), or only partly incorporated (RT), when compared with CT.

A period of 20 years is still too short to determine all the effects of the weather conditions on significant changes in biological and microbiological activities in soils under different soil tillage practices, but the obtained results indicate that, in a changing climate noted also in Central Europe [8–11], these biological activities can be more vulnerable under CT than under the other less-disturbing soil tillage practices.

4.5. Winter Wheat Yields and N Content in Grain

Winter wheat yields were lower under NT in comparison with CT and RT, which could be ascribed to the lower aeration of the untreated soil [4], the consequently lower intensity of organic matter mineralization, and the release of nutrients accessible to plants. Therefore, higher dosages of nitrogen fertilizers should be applied to crops under NT to achieve the same plant yields as CT and RT. High organic carbon soil amendments like wheat straw promote microbial N immobilization by stimulating microbes to take up N from the soil [28,29]. In our field experiment, the crop straw represented an important source of soil organic matter and, depending on the tillage used, was incorporated into the soil. The SOC content increased in this field experiment mainly under NT, which was a result of the regular straw additions to the soil and decreased soil disturbance, which, in turn, led to a higher bulk density and greater soil microbial activity. In particular, the higher SOC in the NT top layer can be responsible for N immobilization [56] and would consequently contribute to lower yields as well as a lower N content in the wheat grain. Further, the microbial biomass newly synthesized due to the increased SOC can play an important role in N immobilization [28,58,59]. The competition of wheat plants with soil microbial biomass immobilizing N sources could be decisive for decreased wheat yields and N uptake in NT.

In our experiments, the years of 2012, 2022, 2024, and especially 2018, were characterized by the lowest grain yields under all tillage practices (Figure 7) in the whole period studied as a consequence of the deficiency in precipitation and high temperatures (Figure 6). In this case, the yield under CT was slightly lower compared to RT, where the ability of the soil to store water was greater [25]. The studies on the effect of tillage systems on wheat grain yield and quality are ambiguous. The results of some of these studies confirm our results, where CT and RT have no significant effect on grain yield and quality parameters. RT in comparison with CT improves the soil structure and bioactivity and also increases the water-holding capacity in the arable layer, positively affecting grain yields. The higher mineralization and nutrient availability for crops improve quality parameters such as N content in grain (Figure 8), which is also confirmed by the results of Buczek et al. [60]. In addition, Ahmadi et al. [61] achieved comparable yields and N content in winter wheat grain under CT and RT and significantly lower values under NT with the same fertilization. The surface retention of plant residues in the no-tillage treatment slowed down decomposition and mineralization, leading to reduced nutrient availability in the soil and subsequently lower nutrient concentrations in the plants. Sarker et al. [62] highlighted that the higher mineralization of SOC under CT and RT compared to NT resulted in lower net N and K availabilities. Higher nutrient rates are therefore required for NT to achieve crop yields at CT and RT levels, as also confirmed by Majrashi et al. [63], because nitrogen and other nutrient immobilization processes occur simultaneously with carbon storage in the soil. This can be disadvantageous for farmers. However, NT also provides the greatest benefits in terms of soil health and sustained soil fertility and water retention, which is important in a changing climate with increasingly frequent droughts.

Suitable genotypes for conservative tillage practices ensuring uniform crop establishment will be needed for the long-term sustainability of crop yields [7]. In the future, a selection of suitable wheat cultivars will be important for growth using reduced or no-tillage techniques, as the variability in hydrothermal conditions and their interactions with tillage systems have a decisive influence on grain yield [64]. The potential negative effects of NT on crop yields can also be reduced by adequate crop rotation [28], including other crops such as cereals which can ultimately maintain winter wheat yields [65].

Within the variability induced by climate and environmental factors, reduced tillage and the use of cover crops, or mixtures including legumes, can be identified as the most promising drivers of positive outcomes [66]. The more frequent use of cover crops shows from a global land-use perspective a great potential to replenish soil C stocks, reduce the use of fertilizer, and reduce intensive tillage. Conservation agriculture practices of crop rotation with permanent soil cover have been widely promoted for improving long-term agroecosystem resilience in the face of a changing climate [67]. Further research should be focused on further improvements in tillage practices to increase their potential for carbon storage in soils.

5. Conclusions

A long-term field experiment with different soil tillage practices was established in Prague-Ruzyně in 1995. The twenty-year period of 2005–2024, when the definitive crop rotation was stabilized, was evaluated in this research. Our long-term field experiment showed the sequestration of soil organic carbon and an increase in microbial activity in the top layer of 0–10 cm under RT and NT, accompanied by their stratification within soil layers, where the lower oxygen content and available substrate caused by the decrease in soil plowing did not allow soil microorganisms to maintain their activity. The SOC content increased during the studied period from 51.0 to 56.0 t ha^{-1} under CT, from 57.4 to 63.1 t ha^{-1} under RT, and from 61.1 to 65.7 t ha^{-1} under NT in the 0–30 cm layer. The annual SOC increase in the whole 0–30 cm layer reached 0.22, 0.46, and 0.57 t C ha^{-1} $year^{-1}$ under CT, RT, and NT, respectively, between 1995 and 2024. In recent years (2021–2024), this increase has slowed down, which can be partly explained by lower crop (and crop residues) yields in increasingly frequent dry years and the return of less C to the soil. The annual SOC increments decreased to values of 0.20, 0.32, and 0.44 t C ha^{-1} $year^{-1}$ under CT, RT, and NT, respectively, in the period of 2010–2024. The largest differences in all monitored parameters among the studied tillage practices were always found in the surface 0–10 cm layer, differing in soil cultivation intensity and the amount of postharvest residues incorporated. In deeper soil layers under RT and NT, the final years of the long-term field experiment showed an increase in SOC, C_{mic}, and enzymatic activities and indicated the capacity for the restoration of these soil characteristics in the long term due to the possible activity of the macroedaphon, the drainage of soluble C fractions, and colloidal transport.

Average winter wheat yields were mostly the lowest under NT, on average only 8.23 t ha^{-1} in comparison with CT and RT, which reached 8.67 and 8.97 t ha^{-1}, respectively. The highest average yield over the monitored period was achieved under RT, combining the beneficial effects of tillage on mineralization and nutrient availability for plants, higher water storage capacity, and carbon sequestration in the soil due to the conservation tillage practice without turning. NT required greater N fertilization than CT and RT for similar yields owing to higher C sequestration accompanied by nutrient immobilization.

This study indicates the importance of adjusting the tillage practice with regard to the soil and weather conditions in order to achieve optimal crop yields and soil fertility. We must assume that the average increase in temperatures and irregular precipitation will continue. Therefore, further study is necessary for improving soil tillage and growing practices for maintenance and replenishing the carbon stock in soils under conditions of a changing climate.

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Abbreviations

The following abbreviations are used in this manuscript:

CT	conventional tillage
RT	reduced tillage
NT	no tillage
SOC	soil organic carbon
SOM	soil organic matter
Cmic	soil microbial biomass
TTC	trichlortetrazolium chloride
TPF	triphenyl formazan
N content	nitrogen content

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