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A comprehensive analysis of soil health indicators in a long-term conservation tillage experiment

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Abstract

Conservation tillage (CT) is a ploughless tillage with a reduced number of operations, and its positive effect on soil functions and health is widely known. Multivariate analyses are required to choose indicators that adequately characterize the changes in soil health. However, there is little research on the comprehensive analysis of the full spectrum of soil physical, chemical and biological properties. Therefore, we examined 21 soil parameters in a long-term CT experiment conducted in Hungary. Four pairs of similarly sized CT and conventional ploughing tillage (PT) plots were set up in 2003 on Luvisols. The soil samples were collected after 17 years. The total organic carbon (TOC) increased significantly in the 0–15 cm layer at CT sites compared to those in PT, indicating a total increase of 5.22 t ha^{-1} TOC stock. In addition, the increasing biological activity and improved soil structure were the most important processes at the CT sites. Furthermore, more complex humic substances with higher molecular weights are characteristic of water-extractable organic matter (WEOM) as a result of CT. The potentially available nitrogen, phosphorus and potassium were also measured with a relatively high response ratio. Slowly changing parameters, such as cation exchange capacity and base saturation, are important soil physical and chemical parameters, but are not good indicators of the impact of tillage practices. Based on principal component analysis, we suggest the use of water-extractable organic C, amino-nitrogen, water-stable aggregates, available P and K and photometric analysis of WEOM to identify the soil improving processes.

KEYWORDS
aggregate stability, available nutrient, organic matter composition, soil biological activity

1 | INTRODUCTION

Land-use change and intensive agriculture have significant impacts on soil health. Soil health refers to the capacity of soil to function as a living ecosystem that sustains plants, animals and humans and supports ecosystem services including agricultural production (Karlen et al., 2019; Kertész & Křeček, 2019). Improving and sustaining soil health through proper agricultural management practices is key to sustainable crop production. Soil health is reflecting actual soil conditions, that is, the condition of a given soil for a specific moment of time, which can deviate from achievable conditions of the soil because of, for example, past management (Rinot et al., 2019; Ros et al., 2022).

It is widely known that mouldboard ploughing leads to severe soil degradation (Eleftheriadis & Turrión, 2014; Kosmas et al., 2001; Terzudi et al., 2006). Conservation tillage (CT) practices improve sustainability via better water management and cost efficiency. Moreover, they provide parallelly increased soil health, food security and natural resource protection as a holistic response to climate change challenges (Busari et al., 2015; Derpsch et al., 2010; FAO, 2015). Several variations have been applied worldwide depending on the depth and width of the CT, including no-till, ridge-till, strip-till, mulch-till and reduced-till. In the European Union, the proportion of CT technologies has slowly increased (Kertész & Madarász, 2014) and reached 26.5% in 2016 (Eurostat, 2016). Reduce tillage is a simpler and somewhat less innovative but more widespread practice of CT in East-Central Europe, which is a summarizing name for cultivation practices applying shallow, non-inversion tillage instead of deep ploughing. It is also often characterized by lower number of yearly tillage operations (Van Balen et al., 2023).

The effects of the CT methods on many soil properties have already been investigated. For example, tillage depth and intensity significantly affect the carbon sequestration rate of soils (Aguilera et al., 2013; Francaviglia et al., 2017; Kopittke et al., 2016; Trigalet et al., 2017). There is considerable evidence that small changes in total soil organic carbon (SOC) content because of CT have disproportionately large impacts on soil physical properties, such as aggregate stability and water infiltration rate, and that microbial activity is crucial for the formation of stable aggregates (Bai et al., 2018; Eleftheriadis & Turrión, 2014; Hannula et al., 2021; Madarász et al., 2021; Powlson et al., 2011; Zhao et al., 2017).

Soil biological properties are also sensitive indicators of changes in tillage methods. Previous investigations in European soils have demonstrated that earthworms (Dekemati et al., 2019; Rasmussen, 1999) and nematodes (Amossé et al., 2016; Bongiorno, Bodenhausen, et al., 2019) are significantly affected by tillage and the abundance of organic matter. CT, particularly in green cover crops, is highly associated with the appearance of arbuscular mycorrhizal fungi (AMF). AMF are more abundant in the soil of CT than in PT (Alguacil et al., 2008; Säle et al., 2015). Gajda and Przewłoka (2012) measured the soil biological activity under ploughing tillage (PT) and CT (reduce and no-tillage) and found that the activities of dehydrogenase, phosphatases and potentially mineralizable nitrogen were significantly higher (20%–30%) in CT than in PT.

Replacing PT (inversion ploughing tillage) with CT contributes to the accumulation of carbon and nutrients in the near-surface soil layers after 5–8 years (Abdollahi & Munkholm, 2014; Shao et al., 2016). In the short term (1–5 years), an increase in SOC is not usually accompanied by an improvement in soil chemical properties, such as total nitrogen, available phosphorous, exchangeable calcium and magnesium (Cooper et al., 2020; Haruna & Nkongolo, 2019; Sokolowski et al., 2020; Veenstra et al., 2007). Long-term experiments mainly focus on total nitrogen, and limited data are available on the concentration of other elements (Kopittke et al., 2016).

Recognizing that the impact of land use and tillage on soil health is dependent not only on management but also on a number of environmental factors, several authors have performed meta-analyses by processing data from several long-term experiments (Hannula et al., 2021; Lehtinen et al., 2014; Pittelkow et al., 2015; Poeplau et al., 2016). However, meta-analyses based on large databases have been limited to examining only a few soil parameters.

If the effects on soil measurements are examined separately, a detailed understanding of how tillage affects soil health cannot be achieved. Soil health is difficult to quantify because the complicated interrelations between soil measurements and the different temporal dynamics of the indicators must be considered (Rinot et al., 2019; Ros et al., 2022). Decision-makers still require exact metrics. In order to be able to choose the indicators that adequately characterize the soil functions and improvement or degradation processes, multivariate studies must be carried out (Nandan et al., 2019; Sekaran et al., 2020; Zhao et al., 2017).

Therefore, to evaluate and compare the effects of CT and PT practices on soil health, we carried out a comprehensive soil physical, chemical and biological assessment for a 17-year long-term experiment established in Central Europe. According to our hypothesis, 17 years is sufficient time to examine which are the slowly and rapidly changing soil parameters and their relationships. According to our hypothesis, measurements that change more rapidly under the influence of management and are characterized by a higher response ratio are the most suitable for monitoring soil health. Therefore, we measured and evaluated 21 different soil indicators that quantify the soil ecosystem service characterized by main soil functions. Our aim was to provide suggestions for the temporal and spatial monitoring of different soil indicators and to identify the key soil health indicators (a minimum data set) for evaluating the sustainability of tillage practices.

2 | MATERIALS AND METHODS

2.1 | Site description

This study used an experimental field originally established in 2003 to study PT and CT (Bádonyi et al., 2008;

Kertész et al., 2010; Madarász et al., 2016). The plots are located in western Hungary, in a hilly region near the village of Dióskál (46°42′15″ N, 17°02′50″ E, 176–206 m a.s.l.) (Figure 1). The regional climate is warm-summer humid continental (Köppen, 1936), with a mean annual temperature of 11°C and mean annual precipitation of 600–700 mm. The area is located on a 0%–12% east-facing slope. Soil profiles are eroded on the convex upper part of the slopes, while thick soil sections are typical on the lower concave slopes because of sedimentation. The parent material was loess, and the soil was classified as a haplic Luvisol (loamic, humic) (WRB, 2014). The soil is slightly acidic, and the average pH (KCl) is 5.46 in the 0–15 cm layer. The experimental area well represents the region's (Central Europe) most typical soil type, topography, climatic conditions and crop rotation.

2.2 | Experimental design and tillage systems

The 32 ha study area was divided into four pairs of CT and PT plots of similar size (ca. 4 ha) (Figure 1). The plot design was arranged in 2003 in congruence with the requirements of agro-ecological and ornithological studies (Field et al., 2007). Before the experiment, PT was applied for decades to the entire area with the same maize-wheat crop rotation. The plots were tilled across the slope. The PT consists of mouldboard ploughing (25–30 cm depth), harrowing and seed-bed preparation every year. However, in some years (2006, 2008 and 2010), owing to weather conditions and (extremely dry or wet) soil conditions, ploughing was left behind. Characteristic features of the CT were as follows: non-inversion, ploughless tillage with a reduced number of tillage operations. Besides, ca. 30% of the soil surface is left covered by crop residues. Shallow discing was used throughout the first 4 years of CT. However, as this method left space for repeated weed problems, this method was replaced by the use of a cultivator (20–25 cm) (Madarász et al., 2016). In addition, medium-deep (35–45 cm) subsoiling was carried out in 3 years on PT plots (2007, 2011 and 2013) and two times on CT plots (2011 and 2018). The only difference between the plot pairs was the tillage type, and they received the same treatment in every other aspect (crop rotation and crop type, fertilizer application and plant protection). During the 17 years of study research, winter wheat (5×), maize (5×), oilseed rape (4×), spring barley (2×) and winter barley (1×) were produced in crop rotation (2004–2020). Yield data were collected from each plot and the results were published by Madarász et al. (2016). Exact crop rotation and yield data by year are given in Table S1.

2.3 | Soil sampling and measurements

This study examined soil samples collected in 2020 after 17 years of shifting to CT. Seven soil samples (0–15 cm depth) were collected evenly distributed along the length of the plots from each plot after harvesting winter barley. Each sample was collected as a mixture of seven subsamples taken randomly from 1 m^2. Samples were sieved <2 mm after drying at air temperature. Before measurements, all plant fragments were eliminated using tweezers. We chose relevant measurements that quantify the soil ecosystem service characterized by main soil functions (Rinot et al., 2019) (Table 1).

An SSM 5000A-equipped TOC-L analyser (Shimadzu, Japan) was used to determine total and inorganic carbon contents by dry combustion. In brief, the aliquot was heated to 200°C with a phosphoric acid application. At this temperature, the carbonates are released as CO_2, which was measured by an NDIR detector. Another aliquot from the same sample was heated to 900°C, where the total C content was released as CO_2 and measured by the NDIR detector. Total organic carbon (TOC) concentration was then calculated as the difference between total and inorganic carbon content (Jakab et al., 2016, 2019).

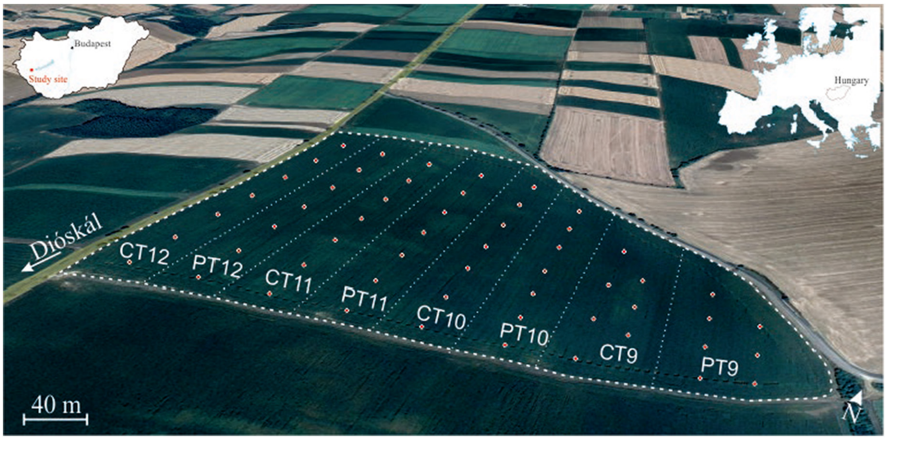

FIGURE 1 The geographical location of the research sites and the experimental design. Red dots indicate the sampling locations. CT, conservation tillage; PT, ploughing tillage.

TABLE 1 The selected measurements that quantify the soil ecosystem service characterized by main soil functions.

Ecosystem service	Main soil functions	Selected relevant measurements	References
Regulating	Climate and water regulation Erosion and flood control	Water-stable aggregates (infiltration)	Jakab et al. (2016); Madarász et al. (2021); Terzudi et al. (2006); Wu et al. (2014)
	Carbon sequestration	Total organic carbon, photometric and fluorescent humus quality indices	Aguilera et al. (2013); Abdollahi and Munkholm (2014); Busari et al. (2015); Pittelkow et al. (2015); Kopittke et al. (2016); Francaviglia et al. (2017); Trigalet et al. (2017); Zhao et al. (2017); Jakab et al. (2022)
Provisioning	Water retention	Water-stable aggregates (infiltration)	Bartlová et al. (2015); Hernández-Hernández and López-Hernández (2002); Zotarelli et al. (2007); Jacobs et al. (2009)
	Weathering and soil formation	Cation exchange capacity, exchangeable cations and base saturation	Shao et al. (2016); Nandan et al., 2019; Li et al. (2020); Cooper et al. (2020); Sokolowski et al. (2020)
Supporting	Nutrient cycling	Amino-nitrogen, available and total phosphorous, water-extractable organic carbon and permanganate oxidizable organic carbon	Gajda and Przewłoka (2012); Haney et al. (2012); Yun et al. (2012); Li, Wen, et al. (2018), Haruna and Nkongolo (2019); Li et al. (2020); Bankó et al. (2021)
	Provision of habitat	Abundance of earthworms, easy extractable glomalin-related soil proteins and microbial biomass carbon	Curaqueo et al. (2010); Balota et al. (2014); Capowiez et al. (2014); Bertrand et al. (2015); Dai et al. (2015); Säle et al. (2015); Li, Chang, et al. (2018); Dekemati et al. (2019); Hannula et al. (2021)

To compare the TOC concentrations of soils with different cultivation and compaction, TOC stocks ($t\,ha^{-1}$) were calculated using Equation (1):

$$\text{TOC stocks}\left(\text{Mg ha}^{-1}\right) = \text{TOC}\left(\text{g kg}^{-1}\right) \times \text{Bulk Density}\left(\text{Mg m}^{-3}\right) \times \text{soil depth (m)} \times 10 \quad (1)$$

The stock change rate (SCR) was calculated, which corresponds to the differences between the TOC stock for the two management practices expressed as a rate ($kg\,C\,ha^{-1}year^{-1}$) over a certain period of time ((CT − PT)/17 years) (Bolinder et al., 2020).

Permanganate oxidizable C (POXC) was measured according to Weil et al. (2003), with the change in the concentration of $KMnO_4$ used to estimate the amount of carbon oxidized. The method was modified by shaking 1 g of air-dried soil in 10 mL 0.02 M $KMnO_4$ solution for 5 min, followed by centrifugation (805 g). The absorbance was measured at 565 nm using a Biochrom Libra S22 spectrophotometer. Alkali-extractable soil labile nitrogen (Amino-N) was determined using the Labile Amino-Nitrogen gel probe system (Woods End Laboratories, Inc.) (Moore et al., 2019). Water-extractable organic carbon (WEOC) was measured using the procedure described by Sharma et al. (2017), but we used a 1:10 suspension to avoid further dilution. The samples were mixed with ultrapure deionized water and shaken for 2 h on a horizontal shaker at 140 rpm. After 15 min of centrifugation, the samples were filtered through 0.45 μm glass filters to recover the water extract. The WEOC concentrations of the solutions were measured using Shimadzu TOC-L device.

A Shimadzu 3600 spectrophotometer was used to determine the UV–VIS absorbance spectra of the WEOC extracts, from 180 to 1000 nm, with 0.5 nm resolution. The fluorescence characteristics were measured with a Shimadzu RF-6000 spectrofluorometer on an excitation emission matrix (EEM) (Coble, 1996). The EEMs were recorded over the emission wavelength range from 260 to 590 nm, increasing sequentially in 5 nm steps the excitation wavelength from 200 to 465 nm. The measured absorbance values and indices were used as proxies for estimating the degree of humification (E4/E6, absorbance at 460 and 660 nm), aromaticity (E2/E3, 254/280 nm) and ultraviolet absorbance ratio index (URI) (210/254 nm) (Chen et al., 1977; Her et al., 2008; Weishaar et al., 2003). The fluorescence peak intensities and indices (Coble, 1996) were calculated to characterize the water-extractable organic matter (WEOM) composition. Because the peak height was a function of the WEOC concentration in the sample, we calculated the indices of relative peak intensity (A/T and (A+C)/(B+T)) (Baker et al., 2008).

The glomalin-related soil proteins (GRSP) were extracted for the bulk soil according to the method of Wright and Upadhyaya (1998). The easily extractable GRSP (EE-GRSP) was obtained from 1 g of air-dried soil in 8 mL of citrate buffer (20 mM, pH 7.0) and autoclaved at 121°C for 30 min. The samples were centrifuged to remove soil particles, and the protein in the supernatant was determined using Thermo Pierce™ bicinchoninic acid (BCA) protein assay with bovine serum albumin as the standard (Sekaran et al., 2021). The absorbance was measured at 562 nm using a Biochrom Libra S22 spectrophotometer.

Exchangeable bases (Ca, Mg, Na and K) and base saturation (BS) were determined by buffered 1 M ammonium acetate (pH 7.0) extraction. The full potential cation exchange capacity (CEC) was determined using the Na-acetate/ethanol/NH_4-acetate replacement method (Chapman, 1965).

For the tests of the carbon content in the microbial biomass (MBC), the samples were stored under refrigerated conditions until use (4°C). The chloroform fumigation–extraction method (Paul et al., 1999; Vance et al., 1987) was applied for the determination MBC. This revealed the organic matter content of the organisms in the soil samples by disrupting their cell walls and membranes. The organic carbon content of the prepared fumigated and control samples were extracted with 0.5 M K_2SO_4 and determined using a Shimadzu TOC-L apparatus equipped with an OCT-L liquid unit. The C content of MBC was determined as the difference between the C content of the non-fumigated and fumigated samples, from which the biomass was calculated. To assess the organic matter dynamics and mineralization process in the soil, the metabolic quotient (qMic) was calculated as the ratio of MBC to TOC, expressed as a percentage (Li, Chang, et al., 2018).

The number and biomass of earthworms was determined twice, in spring and autumn of 2020, and averaged per sampling point (Harper Adams University College, 2003). Each plot was sampled at seven points. Both the height and diameter of the cylinder used for sampling was 10 cm. The samples were checked for earthworms manually, which were weighted after their removal from the soil.

Seven samples were collected of each plot (0–10 cm, 28 per tillage type) for water-stable aggregate measurement (WSA). WSA was determined using an Eijkelkamp wet sieving apparatus to measure the ratio of soil aggregates >250 μm (Kemper & Koch, 1966). The bulk density (BD) was determined on undisturbed core samples of known volume using a drying oven and an exicator (McKenzie et al., 2002). To determine the BD of the 0–15 cm layer, soil samples were taken in 7–7 replicates in the spring and late summer of 2020 and averaged per sampling point.

2.4 | Data processing

For all measured variables, the mean relative response ratios (RR) were calculated, which represent the percentage change (%) based on the comparison of results between a management practice and the reference treatment ((CT − PT)/PT × 100). To determine the effect of tillage systems on the soil physical, chemical and biological properties, a one-way ANOVA was conducted for each measured indicator. The normality of the data was analysed using the Shapiro–Wilk test. The variance homogeneities for the variables were tested using Levene's test. We did not apply any data transformation. The paired relationships between variables were examined using the Pearson correlation coefficient (r). Based on the correlation coefficient of the indicators principal component analysis (PCA) was performed using R version 4.2.1. The principal components with eigenvalues greater than 1 were evaluated using one-way MANOVA (principal component variance analysis). When selecting the minimum data set, the final conclusion was drawn based on the response ratio (Bai et al., 2018) and the PCA structure matrix (Juhos et al., 2019; Nandan et al., 2019; Rinot et al., 2019; Sekaran et al., 2020).

3 | RESULTS AND DISCUSSION

3.1 | Change in TOC and composition of soil organic matter

In our study area, the TOC content significantly ($p < .001$) increased in the 15 cm layer at CT sites (mean TOC: 10.29 g kg^{-1}) over 17 years as compared to that in PT (mean TOC: 7.74 g kg^{-1}) (Table 2). The average RR was close to 33% (Figure 2). Considering the slight difference in BD (1.27 on the PT plots and 1.25 g cm^{-3} in the CT plots), this indicated a total increase of 5.223 t ha^{-1} TOC stock in the CT soil and an annual SCR of 326 kg ha^{-1} $year^{-1}$ for the upper 0–15 cm layer of the soil compared to the PT plots. The lower TOC content of PT soils is because ploughing improves the oxygen and water supply in the soil, thus accelerating the rate of microbial decomposition and mineralization of organic matter (Li, Chang, et al., 2018). Higher TOC content in the soil under long-term zero tillage practice has been reported by many researchers worldwide (Aguilera et al., 2013; Busari et al., 2015; Francaviglia et al., 2017; Pittelkow et al., 2015; Yang et al., 2008; Zhao et al., 2017). However, much less data are available on the

TABLE 2 Effect of tillage on soil indicators at 0–15 cm layer after 17 years of treatment (CT: conservation tillage; PT: ploughing tillage) in Dióskál, Hungary.

Parameters		Unit	PT		CT		ANOVA	
			Mean	SD	Mean	SD	*F*-value	Sig. level
TOC	Total organic carbon	$g\,kg^{-1}$	7.74	0.93	10.29	1.06	91.21	0.000
MBC	Microbial biomass carbon	$mg\,kg^{-1}$	49.41	38.87	137.05	59.07	32.99	0.000
WEOC	Water extractable organic carbon	$mg\,kg^{-1}$	207.32	18.26	289.06	28.96	59.63	0.000
POXC	Permanganate oxidizable organic carbon	$mg\,kg^{-1}$	288.17	60.94	369.78	55.80	11.71	0.002
Amino-N	Amino-nitrogen	$mg\,kg^{-1}$	120.10	8.91	136.88	9.60	19.67	0.000
URI	Photometric humus quality indices (in water extractable organic matter)	—	1.52	0.13	1.27	0.26	21.54	0.000
E_2/E_3		—	2.87	0.04	2.87	0.03	0.34	0.561
E_4/E_6		—	5.57	0.30	5.40	0.18	7.02	0.011
A/T	Fluorescent humus quality indices (in water extractable organic matter)	—	5.29	1.36	4.93	1.18	0.49	0.492
(A+C)/(B+T)		—	4.66	1.20	4.23	1.28	0.69	0.414
Earthworms	Abundance of earthworms	no m^{-2}	27.42	25.57	66.35	39.60	10.91	0.000
EE-GRSP	Easy extractable glomalin-related soil proteins	$mg\,g^{-1}$	2.14	0.33	3.00	0.74	15.99	0.000
WSA	Water-stable aggregates	%	28.13	9.47	46.75	8.32	59.80	0.000
Ca	Exch. Ca++	cmol(+) kg^{-1}	9.03	1.21	9.33	2.04	0.20	0.659
K	Exch. K+	cmol(+) kg^{-1}	0.55	0.07	0.63	0.10	5.59	0.027
Na	Exch Na+	cmol(+) kg^{-1}	0.19	0.08	0.20	0.07	0.23	0.637
Mg	Exch Mg++	cmol(+) kg^{-1}	2.99	0.26	2.70	0.24	7.86	0.010
CEC	Cation exchange capacity	cmol(+) kg^{-1}	16.24	1.38	16.88	1.88	0.88	0.359
BS	Base saturation	%	78.40	3.32	76.02	3.92	2.59	0.122
Total P	Total phosphorous	$mg\,kg^{-1}$	580.40	83.44	614.59	73.13	1.14	0.297
Bray-P	Potential available phosphorous	$mg\,kg^{-1}$	32.23	9.97	38.62	10.11	2.43	0.133

Note: The result of the ANOVA test for the means in the two treatments.

Abbreviation: SD, standard deviation.

effects of reduced or minimum tillage. On a sandy loam soil in Denmark, Abdollahi and Munkholm (2014) measured a 6.6% SOC increase compared to PT in the 0–10 cm layer of the soil as a result of 5 years of reduced tillage. Jacobs et al. (2009) reported a result from Germany that after 37–40 years, TOC increased by 35%–50% under minimum tillage than under PT, which is very similar to our results. Approximately 20 km from our experimental area, Bankó et al. (2021) analysed the effects of three farmyard manure (FYM) doses with and without ploughed-in plant residues on the PT field. After four decades, the increase in TOC was only 9.57%–15.10% for the FYM input and 14.66%–26.20% for the residue management. Based on this, tillage appears to have a much more significant effect on TOC than FYM on the silty loam Luvisol.

Microbial biomass significantly ($p < .001$) increased by 177% in the soil under CT (Table 2 and Figure 2). This result is consistent with that previous study by Franchini et al. (2007), who found an increase of 80% in MBC in the fifth year of soil with no-tillage with crop residue. The microbial biomass plays a major role in the carbon cycle. The qMic was 1.19% in the CT soils on average, which was significantly ($p < .001$) higher than in the PT, where the mean qMic was 0.65%. Less than 1% means the activities of the soil microbial community are limited by certain factors, such as frequent and deep tillage, removal of

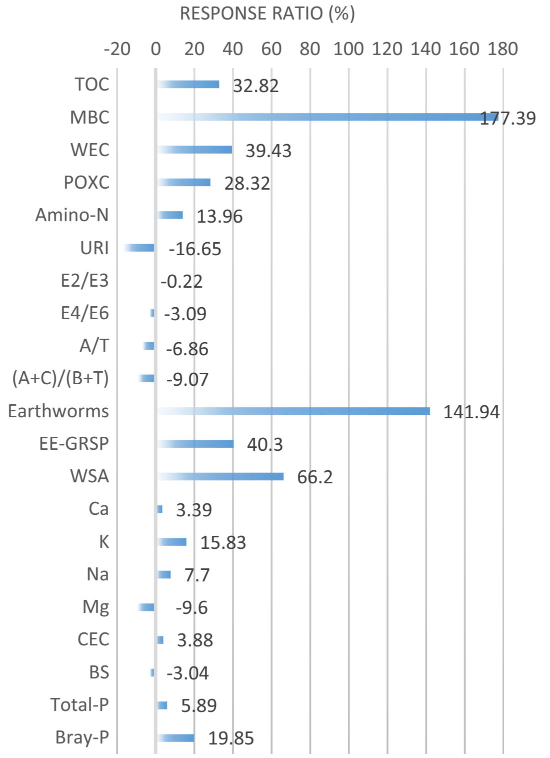

FIGURE 2 Mean relative response ratios (RR) which are representing the percentage change (%) based on the comparison of results between the conservation tillage (CT) against the ploughing tillage (P) (RR = (CT − PT)/PT × 100).

residues and application of large amounts of pesticides (Insam, 1990; Sparling, 1992). However, our results above 1% indicate that CT provided the appropriate soil conditions for microbial biomass growth and activities, which in turn improved the mineralization of organic reserve nutrients. This seems to contradict previous findings that ploughing increases the microbial decomposition and mineralization of organic matter (Li, Chang, et al., 2018). However, this is true only in the short term, that is, immediately after ploughing. Our MBC and qMic results measured in spring probably already show the long-term effects of tillage changes. This statement agrees with a global meta-analysis study by Li, Chang, et al. (2018), which showed a greater qMic of 57% in no-tillage than in PT. However, since MBC is a highly variable indicator over time, it can be completely different before or after cultivation, or the weather also has a great influence, so it is not enough to examine at a single time. For example, Madarász et al. (2021), in a similar long-term experiment, also in the case of spring sampling, did not show a significant difference in MBC between PT and CT plots. The higher qMic found in CT was linked to the occurrence of significantly ($p < .001$) higher POXC and WEOC in CT soil (Table 2), which meant an increase of 28.32% and 39.43%, respectively (Figure 2). Higher labile carbon provides sufficient energy to stimulate soil microorganism activities, including decomposition. Labile carbon fractions also function as a food source for earthworms and other soil fauna (Bongiorno, Bünemann, et al., 2019; Haynes, 2005; Li, Wen, et al., 2018; Nandan et al., 2019). MBC showed a strong positive linear correlation with TOC ($r = .737$) and WEOC ($r = .776$) but a moderate linear correlation with POXC ($r = .483$) (Table S2).

Of the photometric organic matter composition indices, URI and E_4/E_6 were 16.65% ($p < .001$) and 3.09% ($p < .05$) lower in CT than in PT soils indicating more aromatic and humified humus quality in the CT sites (Table 2, Figure 2). Our results are consistent with previous observations by Jakab et al. (2022) in a similar experiment. However, we found no significant differences in the case of E2/E3, A/T and (A + C)/(B + T) between the two tillage systems. Of the humus quality indices, only the URI showed a significant linear correlation with TOC ($r = -.514$) (Table S2). Our results suggest that CT can stabilize the recalcitrant form of carbon that persists longer in the soil. WEOC, the most reactive organic carbon in the soil, reflects both SOM composition and MBC. The ratio of the two main sources of WEOM composition may change rapidly owing to varying environmental conditions. Therefore, monitoring WEOM composition during the growing season would better describe the differences between PT and CT than a single measurement.

3.2 | Change in quality of soil structure and biological activity

Water-stable aggregate measurement refers to soil structure. A positive impact of CT on the WSA was also observed in this study. The stability of aggregates in CT (on average 46.75%) was significantly higher ($p < .001$) than in PT sites (on average 28.13%) (Table 2). This represents an increase of 66.20% in WSA (Figure 2). According to the classification of structural quality based on the percentage of water stability of soil aggregates published by Bartlová et al. (2015), our long-term CT practice implies an improvement in soil structure quality from low to medium levels. The organic substance content in the soil is a prominent factor that determines soil aggregate stability, as the WSA showed a moderately significant correlation with TOC ($r = .527$) (Table S2). A higher WSA in soil under long-term CT practice has been reported by several authors (Hernández-Hernández & López-Hernández, 2002; Jacobs et al., 2009; Zotarelli et al., 2007).

The abundance of earthworms in the CT was growing more than two times compared in the PT (RR = 141.94%) (Table 2 and Figure 2). A moderately significant correlation was observed between the abundance of earthworms and WSA ($r=.441$) (Table S2). This is because the activities of earthworms from the topsoil to the subsoil create channels that improve the soil structure, aeration and infiltration rate (Bertrand et al., 2015; Capowiez et al., 2014; Madarász et al., 2021). A significant correlation was observed between the abundance of earthworms and MBC ($r=.339$) (Table S2), suggesting that earthworms also transport organic material (including their secretion), which is utilized as the source of energy by smaller soil fauna and microorganisms (Bertrand et al., 2015).

Glomalin is a glycoprotein produced in the hyphae of arbuscular mycorrhizal fungi (AMF) which forms mutualistic associations with the roots of plants. In our study, EE-GRSP significantly ($p<.001$) increased by 40.3% in the soil under CT (Table 2 and Figure 2). This corroborates previous studies which found that CT practice significantly improves AMF hyphal length and GRSP, resulting in more water-stable aggregates over traditional tillage practices (Balota et al., 2014; Borie et al., 2006; Curaqueo et al., 2010; Dai et al., 2015).

3.3 | Changes in soil chemical properties and nutrient supply capacity

Cation exchange capacity is predominantly influenced by the type of clay mineral and soil texture. However, the contribution of organic matter is also important (Parfitt et al., 2008). Despite the significant increase in TOC of CT soils in our research, the difference of CEC between CT and PT (16.88 ± 1.88 and 16.24 ± 1.38 cmol(+) kg^{-1}, respectively) was not significant (Table 2 and Figure 2). The amounts of exchangeable Na, Ca and K in the CT soils were higher than those in the PT sites, but a significant difference ($p<.05$) was only found in the concentration of exchangeable K. Exchangeable Mg was significantly ($p<.01$) lower in the CT.

Changes in CEC have not been studied in detail. However, several studies have found significant changes in exchangeable bases in the surface soil because of non-inversion zero-tillage, leaving more residues in the surface layers (Ali et al., 2006; Ismail et al., 1994; Nandan et al., 2019; Rahman et al., 2008; Shao et al., 2016). However, we cannot completely explain our results because the cations were examined in the 0–25 cm layer and the residue input was the same in the CT and PT plots. It is likely that with increasing soil infiltration and stabilizing soil aggregates, nutrient losses will be diminished in the case of CT (Madarász et al., 2021).

The BS decreased in the CT soil, but the difference was not significant (Table 2 and Figure 2). This phenomenon may be related to the decomposition of organic matter and more intensive mineralization in the CT which increased the potential acidity. This result was consistent with the findings of Limousin and Tessier (2007), Rahman et al. (2008) and Cookson et al. (2008), who reported that the lower pH in no-tillage soil was because of acidification of organic residues. Based on the average BS (78.4% and 76.02% in PT and CT) in our study, we can state that the tillage practices did not directly affect soil pH.

It is likely that the increase in exchangeable bases was related to an increase in other nutrients. Higher concentrations of Bray-P and Total-P were recorded in the CT plots than in the PT plots, but the difference was not significant (Table 2). The Increases in Bray-P and Total-P were +19.85% and +5.89%, respectively (Figure 2). Amino-N in the CT soils was significantly ($p<.001$) higher compared to the PT sites, indicating an increase of 13.96% (Figure 2). In relation to the amount of organic matter and biological activity, amino-N showed a strong positive linear correlation with WEOC ($r=.785$), POXC ($r=.703$), exchangeable K ($r=.765$) and a moderately significant correlation with MBC ($r=.533$) and WSA ($r=.449$) (Table S2). In addition to carbon, nitrogen is an essential element for the energy of microorganisms. Amino-N is a derivative of organic matter that provides N that is ready for use by microorganisms (Moore et al., 2019). Soil microbial activity is highly related to soil organic C and N. Several authors have concluded that the water-extractable or amino-N is a more sensitive measurement of the soil substrate and the impact of the tillage system which drives soil microbial activity (Bankó et al., 2021; Haney et al., 2012). Theoretically, an adequate amount of C and N stimulates the decomposition process, releasing high amounts of organic carbon and nutrients in the soil. However, the active nitrogen content increased less than the carbon content because of CT. The C/N ratio was likely to increase, contributing to C stabilization (Yun et al., 2012) in the CT plots. However, microbial activity was still higher in CT soils than in PT plots, which is not a limiting factor in plant nutrient uptake and yield. The same amount of N fertilizer was applied everywhere in the investigated area, and the plant residues were usually straw and stem residues with a high C/N ratio. The excess nitrogen in the CT plots was probably also because of lower erosion and leaching losses. However, the higher organic C content temporarily immobilized mineral N and was preserved in organic N form in the soil. Shao et al. (2016) reported that 8 years of conservation tillage treatments (no tillage, subsoiling and ridge planting) resulted in a significant increase in available phosphorus in topsoil, by 3.8%, 37.8% and 36.9%, respectively, compared to PT. They explained the higher availability of P because of the effects of returning straw over several years, and also to the degree of decomposition of the returned straw in different treatments.

3.4 | The role of single soil properties/indicators in tillage system evaluation

Principal component analysis (PCA) was performed based on the correlation coefficients of all measured indicators. PCA yielded five principal components (PCs) with eigenvalues greater than 1, explaining a total of 76.5% of the variance for the entire set of variables (Table 3). PC1, PC2 and PC5 differed significantly between the two tillage methods (Table 4; Figure 3). According to the component matrix obtained using varimax rotation, PCs can be interpreted as independent, non-correlated indices of soil processes or soil functions. The first principal component (PC1) can be considered an index of organic matter composition, closely related microbiological activity and soil structure quality. The high component loadings of WEOC, TOC, Amino-N, POXC, MBC, abundance of Earthworms, WSA, URI and EE-GRSP show that all of these variables are closely related and indicate the same process. All variables were significantly different between the CT and PT plots. PC2 and PC5 can be labelled as indicators of humus quality. Although only E_4/E_6 differed significantly between the CT and PT plots among the variables with high loadings, PC2 and PC5 showed significantly different ($p < .05$) humus quality between the two tillage treatments (Table 4). In PC3, the concentration of macronutrients was expressed based on the high loadings of exchangeable Mg and K, and the total and Bray-P variables (Table 3). Mg and K differed significantly between the CT and PT plots. However, a relatively high RR was also found for Bray-P. Overall, PC3 did not differ significantly between the two tillage methods, and the changes in macronutrients were relatively high but not significant (Table 4). PC4 expresses the variables that were least affected by tillage and that changed very slowly, such as CEC, exchangeable Ca and BS. Based on the effect size values (Table 4), PC1 was the most important indicator of the long-term effect of tillage practice (Figure 3).

TABLE 3 The results of the principal component analysis.

Indicators	Communalities	Principal components PC1 (32.2%)	PC2 (15.5%)	PC3 (12.5%)	PC4 (9.0%)	PC5 (7.3%)
WEOC	0.897	**<u>0.875</u>**	−0.013	0.355	−0.025	−0.066
TOC	0.877	**0.824**	0.067	0.426	0.108	0.014
Amino-N	0.871	**<u>0.815</u>**	0.055	0.292	−0.288	0.188
POXC	0.777	**0.767**	0.124	0.191	0.191	0.317
MBC	0.643	**0.692**	0.072	0.338	−0.063	−0.202
Earthworms	0.591	**<u>0.687</u>**	0.072	−0.273	0.195	−0.032
WSA	0.687	**0.679**	−0.347	0.004	0.078	−0.316
URI	0.617	**−0.617**	−0.202	−0.041	0.207	0.389
EE-GRSP	0.477	**0.606**	0.086	0.065	0.057	−0.308
A/T	0.920	0.039	**0.939**	0.045	0.065	−0.176
(A + C)/(B + T)	0.900	0.045	**0.933**	0.034	0.102	−0.125
Na	0.711	−0.118	**−0.704**	−0.195	−0.067	−0.399
E2/E3	0.840	−0.096	**−0.656**	−0.078	0.078	0.623
Bray-P	0.839	0.119	0.009	**<u>0.872</u>**	0.212	−0.139
Total P	0.907	0.139	0.213	**0.868**	0.269	0.129
Mg	0.585	−0.345	−0.021	**−0.609**	0.087	0.296
K	0.769	0.512	0.150	**<u>0.589</u>**	−0.310	0.204
Ca	0.977	0.084	0.076	0.138	**0.972**	−0.006
CEC	0.881	0.230	−0.032	0.030	**0.909**	0.012
BS	0.713	−0.361	0.188	0.111	**0.722**	0.116
E4/E6	0.594	−0.104	−0.063	−0.079	0.014	**<u>0.757</u>**

Note: Boldface loadings are considered highly weighted within the PCs; and underlined loadings correspond to the indicators included in the suggested minimum data set. Rotation method: Varimax with Kaiser normalization. Rotation converged in six iterations.

TABLE 4 The effect of tillage on the principal components (PC) as complex soil health indicators.

Predictor	Response indicators	*F*-value	Sig. level	Effect size (η^2)
Tillage	PC1	144.687	0.000	0.728
	PC2	4.007	0.050	0.069
	PC3	3.194	0.080	0.056
	PC4	0.023	0.880	0.000
	PC5	4.161	0.046	0.072

Note: Tests of between-subjects effects (MANOVA). Wilk's lambda = 0.075; $p < 0.001$.

4 | CONCLUSION

In our study area, in the longer term, organic matter accumulation and the closely related increasing biological activity and improved soil structure were by far the most important processes in response to changes in conservation tillage. Because there is a very strong interrelation among the response indicators of these processes, it is sufficient to measure a few parameters that have a high response ratio and do not change quickly seasonally. For example, in case of similar environmental conditions (well-developed Luvisols in Central European hills), we

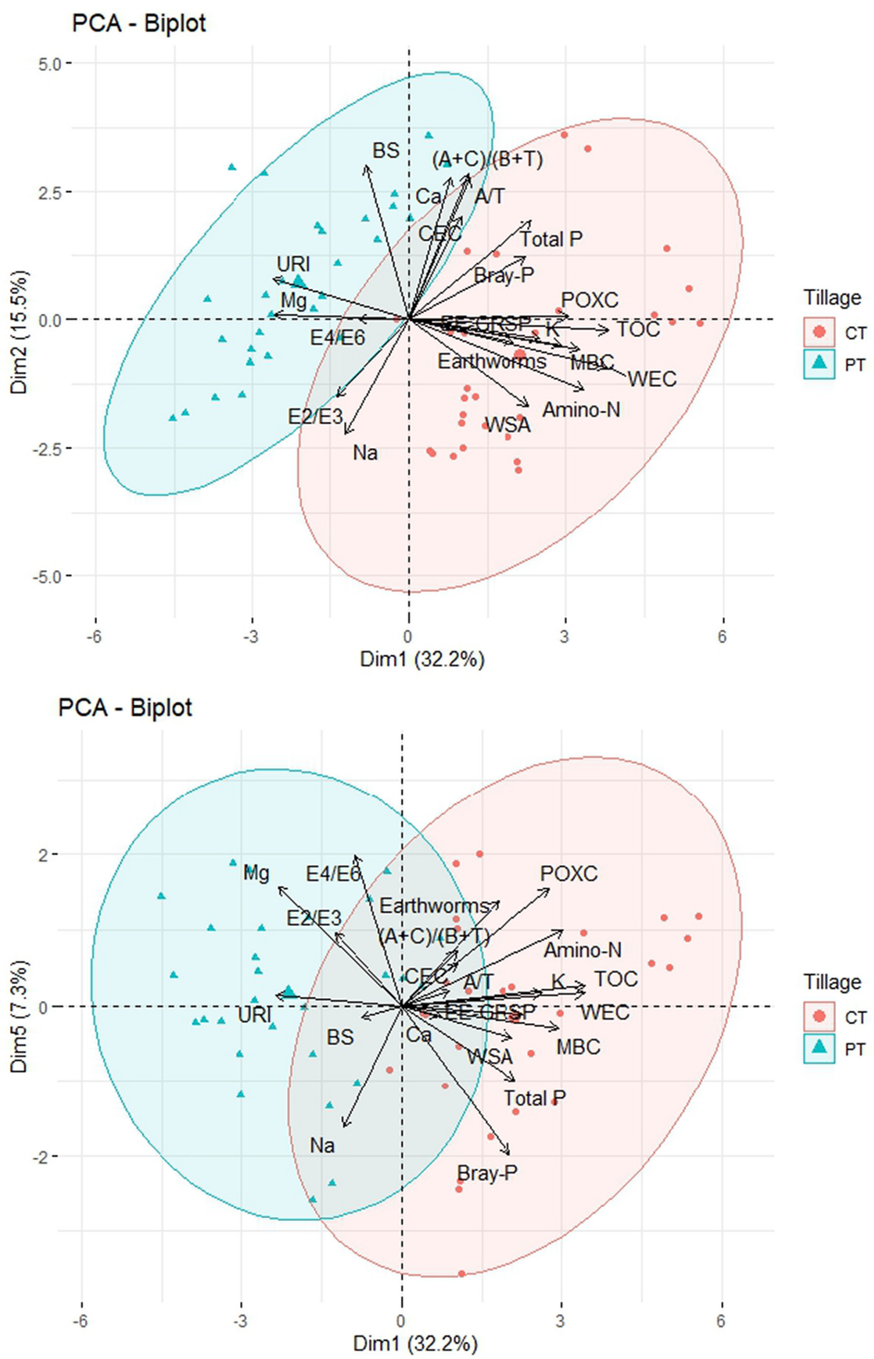

FIGURE 3 The three principal components with eigenvalues greater than 1 and which were significantly different between the two tillage methods (Dim1: PC1; Dim2: PC2; Dim5: PC5). The biplots plot points representing the observations (soil samples) and vectors representing the variables (soil indicators). CT, conservation tillage; PT, ploughing tillage.

suggest using the WEOC, Amino-N and WSA to indicate the soil-improving processes. Furthermore, we found that more complex humic substances with higher molecular weights are characteristic of water-soluble organic matter as a result of conservation tillage. Among these indicators, the URI can be measured with the highest response ratio. However, since the URI was significantly correlated with other parameters characterizing organic matter composition and biological activity, it is worth choosing another indicator (e.g. A/T or E_4/E_6) in order to carry as much new information as possible in the range of selected variables. Nutrient management properties that are somewhat less related to soil organic matter, such as potential available phosphorus and potassium, could also be measured with a relatively high response ratio. From the perspective of Central European growers, this is an important change in the soil function. Slowly changing parameters, such as CEC, exchangeable Ca and BS, are important indicators of general soil physical and chemical properties, thus determining the organic carbon storage capacity. However, they are not good indicators of the impact of tillage practices.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors.

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