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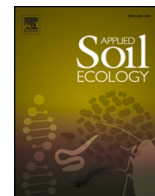


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Tillage and land use management effects on soil organic matter and soil microbial biomass in a field network of practical farms in France, Romania, and Sweden

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ABSTRACT

A reduction in tillage intensity, organic farming, and introduction of ley-grass periods into the crop rotation are means to improve the sustainable use of arable soils. The current study uses an on-farm approach to investigate soils from different practical farms in Northern France, Central Sweden, and Romania in comparison with previously published results from randomized field-experiments nearby. No-tillage generally increased the mean SOC and total N contents of arable fields in comparison with ploughing. However, this increase was only significant for the 70 % increase at 0–10 cm depth in France. No-tillage had no general effects on MBC or MBN and increased the ergosterol content at 0–10 cm depth in France and Sweden but not in Romania. Averaging depths and tillage systems, median MBC significantly increased in the order France (220 $\mu\text{g g}^{-1}$ soil), Romania (324 $\mu\text{g g}^{-1}$ soil), and Sweden (384 $\mu\text{g g}^{-1}$ soil), whereas the median MB-C/N ratios followed the order France (5.6), Sweden (8.3), and Romania (10.4). The study region-specific changes in soil pH significantly affected covariate MBC and MBN in the regression equations for equal slopes ANCOVA models. In Northern France, organic farming and ley-grass implementation into the crop rotation increased SOC and total N contents to the level of the no-tillage fields. On-farm research gives similar results to long-term field experiments in France, Romania, and Sweden. For this reason, on-farm research on practical fields is an important, less expensive alternative to randomized field experiments, with a high potential for investigating actual and relevant research objectives. This will intensify exchange in knowledge between scientists and farmers.

1. Introduction

Climate change, loss of biodiversity, and the demand for human nutrition exert considerable pressure on current conventional arable land use systems, mainly based on mouldboard ploughing and mineral fertilisation in Europe. In this context, soil organic carbon (SOC) is an important indicator of soil quality and agronomic sustainability, due to its positive effects on physical, chemical, and biological soil properties (Lal, 2015; Paul, 2016). While SOC contents change slowly, the soil microbial biomass responds more rapidly to disturbances (Powlson et al., 1987; Giray et al., 2024) and is, thus, an even more important indicator for soil fertility. Soil microbial biomass C (MBC) and nitrogen (MBN) as well as fungal ergosterol give information on the relationships between the plant residue input, carbon storage, and nutrient fluxes in

soil (Joergensen and Wichern, 2008; Khan et al., 2016).

However, as most soil microorganisms are dormant (Joergensen and Wichern, 2018), changes in MBC and MBN are slow and take many years to detect, e.g., in long-term field experiments (Engell et al., 2022). An alternative is the on-farm approach, investigating several practical farms in a defined study region conducting the specific management systems of interest (Koch et al., 2009; Jacobs et al., 2015; Wentzel et al., 2015; Cooper et al., 2020). Such on-farm approaches have several advantages, e.g., saving time to get results, increasing the scope of research issues, and intensifying the knowledge exchange between university research, extension services, and farmers.

Organic farming has usually positive effects on SOC and MBC, due to farmyard manure application and an extended crop rotation, including a period of grass/clover cropping without soil disturbance (Birkhofer

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et al., 2008; Gattinger et al., 2012; Seitz et al., 2018). Ley-grass systems have similar positive effects on SOC and soil organisms, due to extended grassland periods without soil disturbance (Hoeffner et al., 2021; Guest et al., 2022; Prendergast-Miller et al., 2021). No-tillage and minimum tillage systems have usually positive effects on MBC (Jacobs et al., 2009; Murugan et al., 2014) as well as soil animal abundance, particularly earthworms, and their biodiversity (van Capelle et al., 2012). In contrast, the effects on SOC are often small (Heinze et al., 2010a; Murugan et al., 2014; Haddaway et al., 2017) if not negligible (Kaiser et al., 2014; Skadell et al., 2023). Effects on SOC seem to depend on climate and soil texture as shown by Gocke et al. (2023), i.e., conservation tillage had smaller effects on clay soils, especially under continental climatic conditions (Engell et al., 2022).

The current study is based on investigating different arable study regions, being part of a European field-network in Northern France, Romania, and Sweden. The different fields were close to the long-term observatory SOERE-ACBB (Agro-écosystème, Cycle Biogéochimique et Biodiversité) in Lusignan, France (Hoeffner et al., 2021), and in the vicinity of long-term tillage experiments at Cluj, Romania, and Säby, Sweden (Engell et al., 2022). The three study regions differ in texture, climate, tillage, and management systems. The soils in France are silt loams, whereas those in Romania and Sweden are clay loams. Northern France has an oceanic climate, Romania and Central Sweden a summer warm humid continental climate. Conventional mouldboard ploughing was compared with chisel grubber tillage in Romania but with no-tillage in France and Sweden. In France, the tillage effects were additionally compared with organic farming and ley-grass management as alternative approaches for improving sustainability of land use management, despite using the mouldboard plough (Hoeffner et al., 2021).

The current study investigates the following four hypotheses: (I) A reduction in tillage intensity increases SOC and total N contents at 0–10 cm depth but does not affect their mean contents. (II) A reduction in tillage intensity increases the mean contents of MBC, MBN, and ergosterol. (III) In France, organic farming and ley-grass management has stronger positive effects than no-tillage, despite ploughing. (IV) On-farm research gives similar results to long-term field experiments in France, Romania, and Sweden published previously (Hoeffner et al., 2021; Engell et al., 2022).

2. Material and methods

2.1. Study sites

Within the framework of the BioDiversa project “SoilMan”, soils of practical farms in the European countries France, Romania, and Sweden were investigated with different conservational tillage systems, where a plough had not been used for at least five years. Soil samples were always taken under winter wheat (*Triticum aestivum* L.) at 0–10, 10–20, and 20–30 cm depth with a soil corer. Samples for bulk density were taken but a large number were sadly lost. This prevented statistical evaluation of this important soil property and also the calculation of SOC, total N, and microbial biomass stocks. Average data on mean annual precipitation, mean annual temperature, and mean sand, silt, and clay contents are presented in Table 1 for the three study regions. Data on fertilizer and pesticide application as well as on yields were not obtained from the farmers. The different practical fields were chosen

Table 1
Mean annual temperature (MAT), mean annual precipitation (MAP), and texture of the fields in the three study regions.

Study region	MAT (°C)	MAP (mm)	Sand	Silt (%)	Clay
France	11.0	737	29	54	17
Romania	9.2	597	28	26	46
Sweden	5.9	561	13	47	35

according to the use of identical tillage systems and the strong similarity in soil properties in comparison with the nearby long-term field experiments.

In Northern France, the practical fields were located near Lusignan (46° 26' 09" N, 0° 07' 22" E), Brittany (Supplementary-Table S1), comparing organic farming, ley-grass management, conventional mouldboard ploughing (0–25 cm depth), and no-tillage with direct seeding on six replicate practical fields for each treatment. On the ley-grass fields, grassland had been integrated into the crop rotation for at least three years. During the arable period, the ley-grass fields were mouldboard ploughed (0–25 cm depth). In contrast to ley-grass management, conventional ploughing, and no-tillage, organic farming fields did not use readily soluble mineral fertilisers but farmyard manure. The organic farming fields were mouldboard ploughed after harvest, except for the grass/clover period in the extended crop rotation. Further information on management details and yield level of crops in the study region are provided by Kunrath et al. (2015), Hoeffner et al. (2021), and Hu and Chabbi (2021). The predominant soil types are loamy Luvisols (IUSS Working Group WRB, 2022). The average bulk density at the neighbouring long-term observatory on environmental research (SOERE ACBB), managed by the French National Institute of Agricultural Research and Environment (INRAE) was approximately 1.51 kg dm⁻¹ (Hoeffner et al., 2021). The climate is classified as oceanic (Cfb) according to Köppen-Geiger (Beck et al., 2018). Soil samples were taken in spring 2018.

In Romania, the practical fields were located near Cluj-Napoca (46° 46' 16" N, 23° 37' 25" E), Transylvania (Supplementary-Table S2), comparing mouldboard ploughing (25–30 cm depth) and chisel grubber tillage (25–30 cm depth) on seven replicate practical fields for each treatment. Crop rotation of both tillage systems was soybean (*Glycine max* L.), winter wheat, and maize (*Zea mays* L.). Winter wheat and maize were fertilised with approximately 40 kg N ha⁻¹ and 40 kg P ha⁻¹ as complex fertilizer in autumn, while 30 kg N ha⁻¹ was added as NH₄NO₃ in spring (Engell et al., 2022). Further information on management details and yield level of crops in the study region are provided by Chetan et al. (2023) and Rusu et al. (2024). The predominant soil types are clayey Phaeozems (IUSS Working Group WRB, 2022). The average bulk density at the neighbouring SCDA Turda long-term field experiment was approximately 0.93 kg dm⁻¹ (Engell et al., 2022). The climate is classified as summer-warm humid continental (Dfb) according to Köppen-Geiger (Beck et al., 2018). Soil samples were taken in spring 2018.

In Sweden, the practical fields were located near Uppsala (59° 51' 31" N, 17° 38' 20" E), Uppland (Supplementary-Table S3), comparing mouldboard ploughing (0–23 cm depth) and no-tillage with direct seeding on eight replicate practical fields. The crop rotation consisted of winter wheat, oilseed rape (*Brassica napus* L.), and peas (*Pisum sativum* L.). Winter wheat and oilseed rape received approximately 120 kg N ha⁻¹ as mineral fertilizer (Engell et al., 2022). Further information on management details and yield level of crops in the study region are provided by Persson et al. (2008) and Etana et al. (2020). The predominant soil types are clayey Cambisols (IUSS Working Group WRB, 2022). The average bulk density at neighbouring Säby long-term field experiment was approximately 1.23 kg dm⁻¹ (Engell et al., 2022). The climate is classified as summer-warm humid continental (Dfb) according to Köppen-Geiger (Beck et al., 2018). Soil samples were taken in summer 2018.

2.2. Soil chemical analysis

Soil pH was determined with a soil to water ratio of 1 to 2.5 (Blume et al., 2010). The total amount of C and N was determined on dried (105 °C, 24 h) and ground samples using a high-temperature combustion elemental analyser (Vario MAX, Elementar, Hanau, Germany). Carbonate, if present, was gas volumetrically analysed with a Scheibler device (Blume et al., 2010). Soil organic C (SOC) was then calculated as

total C minus carbonate C.

2.3. Soil microbial analysis

Microbial biomass C (MBC) and N (MBN) were determined by fumigation extraction (Brookes et al., 1985; Vance et al., 1987), including pre-extraction (Mueller et al., 1992). For the pre-extraction, 30 g of a soil sample were weighed and mixed with 70 ml 0.05 M K_2SO_4 . This soil suspension was extracted for 30 min on the horizontal shaker at 200 rev min^{-1} . Samples were centrifuged for 10 min at 3000 g and then divided into two 12 g subsamples. One was fumigated for 24 h at 25 °C with ethanol-free chloroform. Fumigated and non-fumigated subsamples were extracted with 40 ml of 0.5 M K_2SO_4 , at 200 rev. min^{-1} for 30 min on a horizontal shaker and filtered. Organic C and total N were determined by infrared and electrochemical detection, respectively, using a multi N/C 2100S analyser (Analytik Jena, Germany). MBC was calculated as E_C/k_{EC} , where E_C = (organic C extracted from fumigated soils) - (organic C extracted from non-fumigated soils) and $k_{EC} = 0.45$ (Wu et al., 1990). MBN was calculated as E_N/k_{EN} , where E_N = (total N extracted from fumigated soils) - (total N extracted from unfumigated soils) and $k_{EN} = 0.54$ (Brookes et al., 1985).

The determination of fungal ergosterol was carried out according to Djajakirana et al. (1996). For each sample, 2 g of moist soil were extracted with 100 ml ethanol on a horizontal shaker for 30 min at 250 rev. min^{-1} and determined by high performance liquid chromatography and UV detection at 282 nm.

2.4. Statistical analysis

The results presented in tables and figures are expressed on an oven-dry basis (about 24 h at 105 °C). Normality was tested by the Shapiro-Wilk test and equal variance by the Levene test. Data were ln-transformed to match these two requirements if required. The significance of tillage and depth effects were tested by a study region-specific two-way repeated measures (RM) analysis of variance (ANOVA), using tillage as factor and depth as RM, followed by the Holm-Sidak post-hoc test. The significance of differences between the three study regions were tested by a two-way ANOVA, using study region as factor and depth as RM in a general linear model, followed by the Holm-Sidak post-hoc test. The effects of soil pH in the three study regions on the different soil properties analysed were tested by one-way analysis of covariance (ANCOVA), using region as factor and soil pH as covariate. The ANCOVA was performed on the mean of the three depths. French organic farming and ley-grass fields were compared separately with no-tillage and conventional land use management by a two-way RM ANOVA, using land-use management as factor and depth as RM. All statistical analyses were performed using SigmaPlot 14.0 (Systat, San José, USA).

3. Results

3.1. Tillage

No-tillage had no general effect on MBC and MBN in comparison with ploughing (Table 3). MBC and MBN were always lowest at 20–30 cm depth, but this decline was only significant in the no-tillage fields in France and particularly Sweden. No-tillage significantly increased the ergosterol content at 0–10 cm depth in France and Sweden but not in Romania. Also, the ergosterol content and the ergosterol/MBC ratio were always lowest at 20–30 cm depth, except at the ploughed conventional fields in France. The decline in ergosterol content and the ergosterol/MBC ratio with depth was strongest in Sweden, particularly in the no-tillage fields.

3.2. Study region

Median SOC contents (Fig. 1a), averaged over all depths and tillage

Table 3

Depth-specific microbial biomass C (MBC), MBN, and ergosterol contents of the fields in the three study regions.

Depth (cm)	France		Romania		Sweden		CV (± %)
	Plough	No-tillage	Plough	Grubber	Plough	No-tillage	
MBC ($\mu g g^{-1}$ soil)							
0–10	226 a	284 a	303 a	323 a	428 a	539 a	30
10–20	269 a	225 a	310 a	342 a	464 a	362 b	29
20–30	214 a	170 b	272 a	320 a	343 a	226 c	34
MBN							
0–10	42 a	56 a	31 a	33 a	50 a	68 a	37
10–20	49 a	45 a	31 a	34 a	54 a	46 b	36
20–30	36 b	32 b	26 a	32 a	41 a	28 c	36
Ergosterol ($\mu g g^{-1}$ soil)							
0–10	0.54 a	1.43 a*	1.56 a	1.33 a	1.62 a	2.62 a*	39
10–20	0.76 a	0.59 b	1.05 b	1.14 b	1.64 a	1.13 b	41
20–30	0.50 a	0.37 b	0.91 b	1.04 b	0.81 b	0.53 c	54
Ergosterol/MBC (%)							
0–10	0.25 a	0.54 a	0.49 a	0.42 a	0.40 a	0.53 a	31
10–20	0.29 ab	0.28 ab	0.35 b	0.32 b	0.37 b	0.32 b	32
20–30	0.23 b	0.24 b	0.34 b	0.30 b	0.24 c	0.22 c	45

CV = mean coefficient of variation between treatment- and study region-specific replicates (France: n = 6, Romania: n = 7; Sweden: n = 8); different letters within a study region-specific column indicate a significant difference ($P < 0.05$; Holm-Sidak test).

treatments, significantly increased in the order France (13.5 mg g^{-1} soil), Romania (20.8 mg g^{-1} soil), and Sweden (24.5 mg g^{-1} soil). Median total N contents increased in the same order, whereas the SOC/total N ratio (Fig. 1c) increased in the order France (9.4) < Sweden < (10.2), and Romania (10.6). Median MBC (Fig. 2a) significantly increased in the order France (220 $\mu g g^{-1}$ soil), Romania (324 $\mu g g^{-1}$ soil), and Sweden (384 $\mu g g^{-1}$ soil). Median MBN followed MBC with median MB-C/N ratios (Fig. 2b), significantly increasing in the order France (5.6), Sweden (8.3), and Romania (10.4). Median fungal ergosterol contents varied around 0.65 $\mu g g^{-1}$ soil in France and were at significantly higher median levels around 1.20 $\mu g g^{-1}$ soil in Romania and Sweden (Fig. 2c).

Soil pH varied around a median of 6.45 without significant tillage effects (Table 2), but with study region-specific differences. In Romania, soil pH was significantly lowest at 0–10 cm depth compared with the two bottom layers at 10–30 cm depth. In Sweden, the soil pH showed a significant increase with depth to maximum values at 20–30 cm depth (Table 2). The study region-specific changes in soil pH significantly affected as covariate the intercepts of the dependent variables MBC and MBN in the regression equations for the equal slopes ANCOVA model (Table 4). All intercepts of the parallel regression lines to soil pH showed highly significant differences for MBC, whereas those for MBN were on a lower level and did not differ comparing Sweden and France.

3.3. Land use management in France

In Northern France, organic farming and ley-grass implementation into the crop rotation increased SOC (Fig. 3a) and total N (Fig. 3b) contents to the level of the no-tillage fields. They exhibited significantly lower MBC/SOC ratios, with a median of 1.2 %, than the ploughed conventional fields, with a median of 1.6 % (Fig. 3c). This ratio was the only microbial index that was significantly affected throughout the whole 0–30 cm depth. The MBC/SOC ratios of the organic and ley-grass land fields, where similar to the ploughed conventional French fields.

4. Discussion

4.1. Tillage-induced depth gradients

No-tillage intensified the depth gradients for SOC, total N, MBC, MBN, and ergosterol in France and Sweden, but not in Romania.

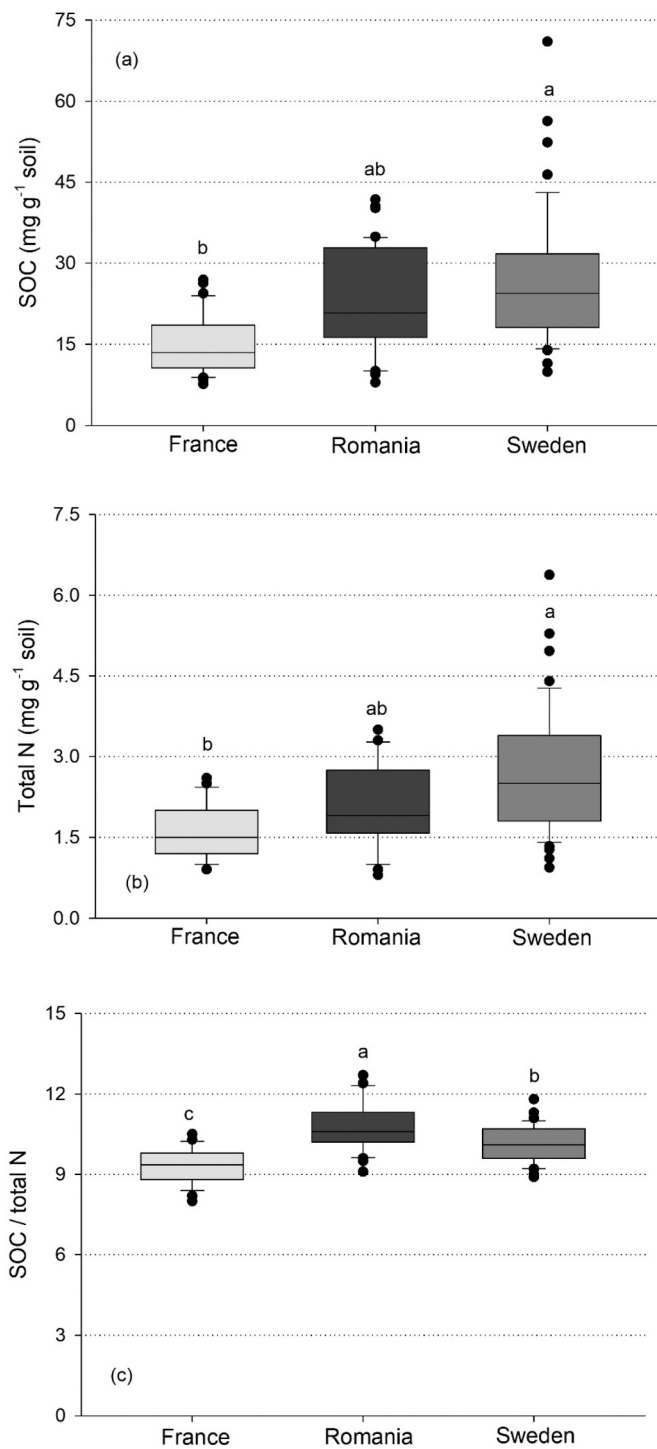


Fig. 1. Boxplots of the study region-specific medians of (a) SOC, (b) total N, and (c) the SOC/total N ratios; different letters on top of a bar or an outlier indicate a significant difference ($P < 0.05$; Holm-Sidak test).

However, the differences between no-tillage and ploughing were only significant at 0–10 cm depth for SOC and total N in France and for ergosterol in France and Sweden. In the other cases, the differences to the ploughed fields were too small for statistical validation, which was partly hampered by the fact that the ploughed fields were not always tilled down to 30 cm. This is obvious to some extent at the French fields, but is particularly evident at the Swedish fields, which exhibited significant depth gradients for SOC, total N, MBC, MBN, and particularly ergosterol in the no-tillage but also in the ploughed fields. In contrast to

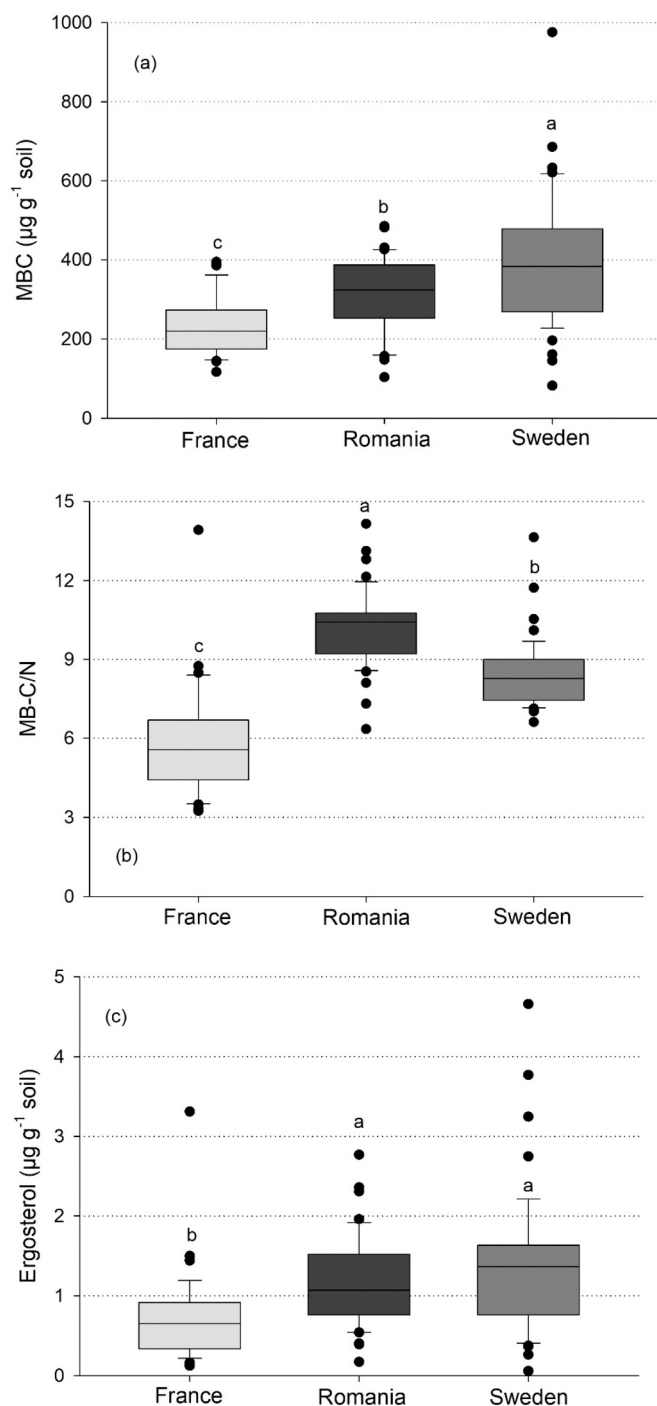


Fig. 2. Boxplots of the study region-specific medians of (a) MBC, (b) the MB-C/N ratio, and (c) the ergosterol contents; different letters on top of a bar or an outlier indicate a significant difference ($P < 0.05$; Holm-Sidak test).

the other soil properties analysed, the ergosterol content declined even in the Romanian no-tillage and ploughed fields.

The fungal cell-membrane component ergosterol generally revealed an even stronger response than SOC and total N at 0–10 cm depth. In the no-tillage systems, harvest residues, left as surface mulch layer on the fields, are incorporated into the soil by anecic earthworms (van Capelle et al., 2012; Bentley et al., 2024). In the soil, harvest residues are decomposed by saprotrophic fungi, which contain large amounts of ergosterol (Djajakirana et al., 1996). In the absence of ectomycorrhizal fungi, ergosterol is the most important biomass indicator for

Table 2

Depth-specific soil pH, contents of soil organic C (SOC) and total N as well as the SOC/total N ratios of the fields in the three study regions.

Depth (cm)	France		Romania		Sweden		CV (\pm %)
	Plough	No-tillage	Plough	Grubber	Plough	No-tillage	
Soil pH (H₂O)							
0–10	6.28 a	6.50 a	6.36 b	6.56 b	6.30 a	6.13 a	12
10–20	6.78 a	6.36 a	6.47 a	6.80 a	6.56 b	6.40 b	12
20–30	6.61 a	6.35 a	6.47 a	6.80 a	6.78 c	6.67 c	11
SOC (mg g⁻¹ soil)							
0–10	12.7 a	21.9 a*	21.8 a	24.2 a	27.9 a	33.6 a	37
10–20	13.8 a	15.5 b	21.8 a	24.2 a	27.7 a	28.8 a	40
20–30	12.6 a	13.5 c	21.3 b	23.5 b	21.9 b	20.9 b	41
Total N (mg g⁻¹ soil)							
0–10	1.35 a	2.24 a*	1.99 a	2.21 a	2.86 a	3.16 a	34
10–20	1.42 a	1.70 b	1.96 a	2.20 b	2.81 a	2.72 a	35
20–30	1.33 a	1.53 c	1.90 b	2.12 b	2.30 b	2.01 b	37
Soil C/N							
0–10	9.2 b	9.8 a	10.8 a	10.7 a	9.8 a	10.6 a	6
10–20	9.7 a	9.1 b	10.9 a	10.8 a	9.9 a	10.5 a	8
20–30	9.3 b	8.9 b	11.0 a	10.8 a	9.6 a	10.4 a	7

CV = mean coefficient of variation between treatment- and study region-specific replicates (France: $n = 6$, Romania: $n = 7$; Sweden: $n = 8$); different letters within a study region-specific column indicate a significant difference ($P < 0.05$; Holm-Sidak test).

Table 4

Pairwise multiple comparison procedures (Holm-Sidak method) and regression equations for the equal slopes model for MBC (region: $F = 12.42$ and $P < 0.001$; soil pH: $F = 6.13$ and $P = 0.018$) and MBN (region: $F = 6.92$ and $P = 0.003$; soil pH: $F = 8.09$ and $P = 0.007$), using region and soil pH as sources of variation; the interaction models were not significant.

Comparison	Difference of means	T	Probability value	Region	Regression equation
MBC					
Sweden / France	162.7	4.95	<0.001	France	$49.95 \times \text{pH} - 92.1$
Sweden / Romania	87.4	2.76	0.017	Romania	$49.95 \times \text{pH} - 16.8$
Romania / France	75.4	2.22	0.033	Sweden	$49.95 \times \text{pH} + 70.6$
MBN					
Sweden / France	4.3	0.87	0.390	France	$8.64 \times \text{pH} - 12.5$
Sweden / Romania	17.3	3.63	0.003	Romania	$8.64 \times \text{pH} - 25.4$
Romania / France	12.9	2.53	0.031	Sweden	$8.64 \times \text{pH} + 8.2$

saprotrophic fungi in arable and grassland soils (Joergensen and Wichern, 2008). The ergosterol content specifically responds to straw incorporation (Heinze et al., 2010b), leading to increased fungal biomass in field experiments (Faust et al., 2017; Hydbom et al., 2017).

4.2. Region-specific response to tillage and management

The stronger response of SOC and total N than MBC and MBN in France contradicts the view that the microbial biomass responds faster to changes in land-use management than soil organic matter (Powlson et al., 1987; Giray et al., 2024). The current results are also in strong contrast to those from German Luvisol fields, comparing no-tillage and conventional ploughing on practical farms (Murugan et al., 2014).

The positive effect of no-tillage on SOC is combined with lower MBC/SOC ratios, which is an indicator of SOC availability to soil microorganisms (Anderson and Domsch, 1989, 2010). An important reason might be soil compaction (Kaiser and Heinemeyer, 1993), combined

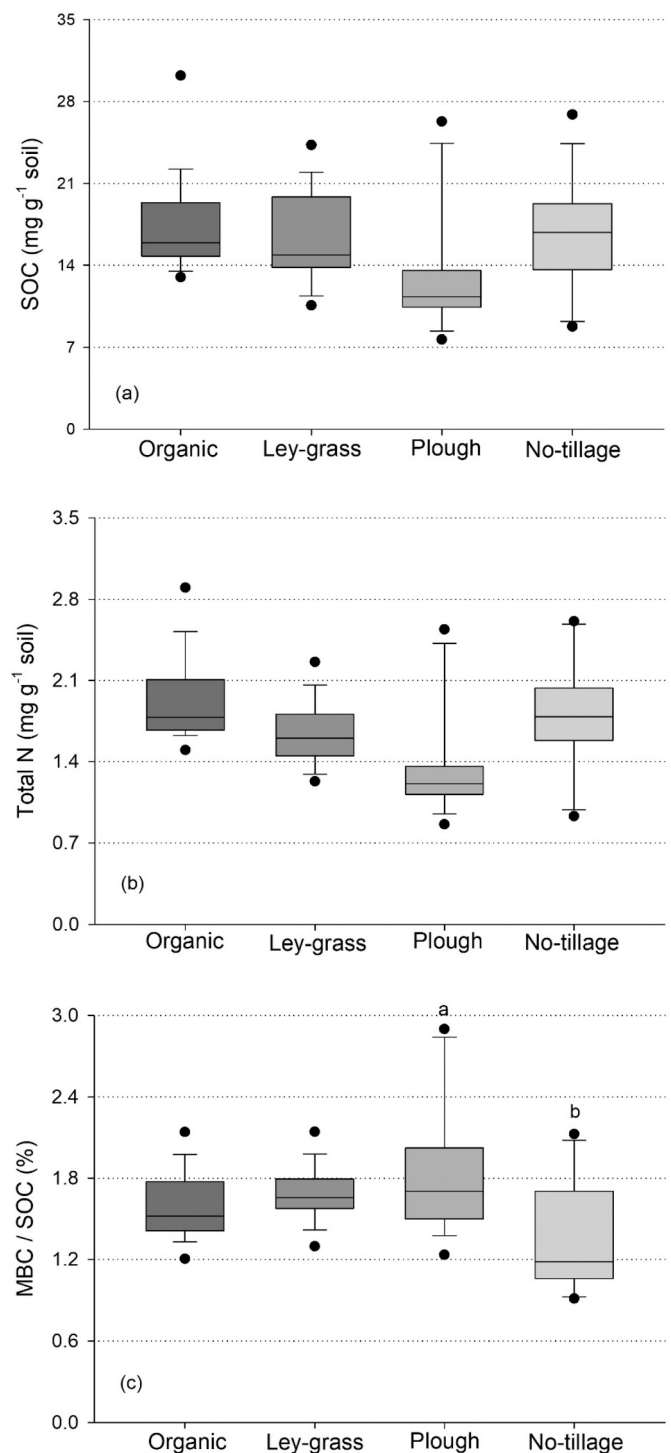


Fig. 3. Boxplots of the land use-specific medians of (a) the SOC contents, (b) the total N contents, and (c) the MBC/SOC ratios in France; different letters on top of a bar or an outlier indicate a significant difference ($P < 0.05$; Holm-Sidak test), obtained by comparing no-tillage and ploughing of the conventional fields.

with lower O₂ supply, due to high soil moisture of the Luvisols at oceanic French Brittany with a relatively high mean annual precipitation. This leads to reduction in microbial carbon turnover and ultimately to increased SOC contents (Keiluweit et al., 2016). In comparison with the French no-tillage fields, similar MBC/SOC ratios were measured in the ploughed fields of all three study regions as well as in the Romanian grubber fields and Swedish no-tillage fields. However, the MBC/SOC

ratios were generally at the lower end of the range observed in arable soils (Anderson and Domsch, 1989; Insam et al., 1989; Khan et al., 2016). Substrate availability to soil microorganisms might be reduced by high soil moisture in France (Faust et al., 2019), by low mean temperature in Sweden (Jenkinson et al., 1987, 2008), and by summer drought in Romania (Jenkinson et al., 1999; Goenster et al., 2017).

Low MBC/SOC ratios were often explained by low soil pH (Domsch et al., 1993, 2010), but this is not the case in the current study. However, the implementing of soil pH as a covariate into an ANCOVA showed that the proton concentration of the soil solution has a significant impact on MBC and MBN but not on SOC or total N. It is well documented that the soil pH is the most dominating factor controlling biomass, activity, diversity, and necromass of soil microorganisms (Anderson and Domsch, 1993; Fierer et al., 2009; Strickland and Rousk, 2010; Khan et al., 2016). Although the soil pH of the three study regions varied in a rather small range, the equal slope models of the current data reveal that the negative pH effects on soil microorganisms do not depend on study region, clay content, or microbial biomass level.

The differences in soil chemical and soil biological properties between the loamy French fields and the clayey Romanian and Swedish fields are obvious and certainly can be explained to a large degree by the high clay content (van Veen et al., 1986; Müller and Höper, 2004; Wentzel et al., 2015; Islam et al., 2022). The Romanian but also the Swedish fields indicate that soils with a good natural fertility, i.e., high clay and SOC contents, are generally less affected by differences in tillage and land-use management than sandy soils (Gocke et al., 2023). The significantly higher mean SOC/total N ratio in the Romanian fields indicate an increased presence of less decomposed plant residues (Jenkinson et al., 2008; Rumpel and Kögel-Knabner, 2011; Khan et al., 2016) in comparison with those in France and Sweden. The combination of high SOC/total N ratios with relatively high MB-C/N ratios, suggests that microbial decomposition might be not only retarded by summer drought in Romania but also by P deficiency to soil microorganisms (Hartmann and Richardson, 2013; Khan et al., 2016).

Ley-grass and particularly organic farming increased SOC contents to the same level as no-tillage, but resulted in a high MBC/SOC ratio similar to that of the ploughed conventional fields. This indicates that these two farming systems have positive effects on C sequestration, combined with the increased C input by grass and clover roots (Gattinger et al., 2012; Seitz et al., 2018; Le Guillou et al., 2019; Jacobs et al., 2020; Hu and Chabbi, 2022). Also, the regular application of farmyard manure usually increases the SOC concentration (Ludwig et al., 2007). However, no-tillage and minimum tillage systems without soil turning have the advantage of improving the habitat quality of the soil fauna, especially earthworms, carabids, and wild bees (van Capelle et al., 2012; Rowen et al., 2020; Müller et al., 2022; Tschanz et al., 2023).

4.3. On-farm approach advantages

The general contents of soil chemical and biological characteristics were similar to the data presented for Lusignan in Northern France (Hoeffner et al., 2021), for Turda in Romania (Engell et al. (2022)), for Lönnstorp in South Sweden (Hydbom et al., 2017), and Säby in Central Sweden (Engell et al., 2022). The data showed strong regional effects on all chemical and biological soil characteristic analysed in the three European study regions, caused by soil type and climate. However, just three study regions are not sufficient to separate climatic and soil type effects.

On-farm approaches, using practical fields, would help to increase the number of study regions, thus mitigating this problem. In addition, on-farm approaches of using practical fields with a management system of interest have a high potential for investigating actual and relevant research objectives. Practical farms have usually optimised their tillage system for the whole farm, whereas in field experiments a single technician usually performs all tillage treatments on small plots. This creates

a bias if this person is more familiar with plough than with no-tillage or reduced tillage systems. An on-farm approach supplies data much earlier and requires fewer financial expenses and organisational efforts than long-term field experiments. They need many years if not decades to provide effects on soil chemical and soil biological properties, with the risk that the initial research questions will lose their relevance. However, long-term field experiments are still highly valuable if research requires equilibrium of stocks and, particularly, exact knowledge on the history of land-use management, soil properties and climate data, e.g., for modelling purposes (Jenkinson et al., 1987, 2008).

Long-term treatments may lead to unintentional side effects, such as acidification (Birkhofer et al., 2008), often the soils are overly inhomogeneous, particularly in floodplains (Heinze et al., 2010b), and the initial conditions are usually insufficiently known on a plot basis. This is also true for the current approach. Particularly the information on fertilizer and pesticide application rates and crop yield levels is restricted and needs much more attention in future studies (Hydbom et al., 2020). However, important advantages are the higher number of large-scale replicates and the lower spatial autocorrelation (Wardle and Parkinson, 1991; Kim et al., 2016). Often results of long-term experiments are only valid for the direct surroundings, without the possibility of extrapolation (Heinze et al., 2010a). This generalization might be possible by integrating more study regions into the current approach using funds saved from maintaining long-term field experiments.

5. Conclusions

A reduction in tillage intensity increased the contents of soil organic C (SOC), total N, microbial biomass C (MBC), MBN, and fungal ergosterol at 0–10 cm depth but did not affect their mean contents at 0–30 cm depth. This increase at 0–10 cm depth was most pronounced for SOC, total N, and ergosterol in no-tillage fields on the Luvisols in Northern France, and for ergosterol also on clayey Cambisols in Central Sweden. No-tillage specifically promoted particularly saprotrophic fungi in the top layer of arable fields. The increase in MBC in the French no-tillage fields at 0–10 cm depth was combined with a reduced MBC/SOC ratio, indicating a decrease in C availability to soil microorganisms. In Northern France, organic farming and ley-grass management had similar positive effects on the contents of SOC and total N to no-tillage, despite ploughing. On-farm research gave similar results to long-term field experiments in France, Romania, and Sweden published previously. However, greater effort will be required to obtain information on land use management and crop yield levels in the future. On-farm research on practical fields is an important, less expensive alternative to randomized field experiments, with a high potential for investigating actual and relevant research objectives. This will intensify exchange in knowledge between scientists and farmers.

CRediT authorship contribution statement

Ilka Engell: Writing – original draft, Validation, Supervision, Formal analysis, Conceptualization. **Jacqueline Gerigk:** Writing – review & editing, Visualization, Validation, Methodology, Formal analysis, Data curation. **Deborah Linsler:** Writing – review & editing, Visualization, Supervision, Project administration, Formal analysis. **Rainer Georg Joergensen:** Writing – review & editing, Visualization, Supervision, Methodology. **Martin Potthoff:** Writing – review & editing, Resources, Methodology, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.apsoil.2024.105584>.

References

- Anderson, T.-H., Domsch, K.H., 1989. Ratios of microbial biomass carbon to total organic carbon in arable soils. *Soil Biol. Biochem.* 21, 471–479.
- Anderson, T.-H., Domsch, K.H., 1993. The metabolic quotient for CO₂ (q_{CO_2}) as a specific activity parameter to assess the effects of environmental conditions, such as pH, on the microbial biomass of forest soils. *Soil Biol. Biochem.* 25, 393–395.
- Beck, H.E., Zimmermann, N.E., McVicar, T.R., Vergopolan, N., Berg, A., Wood, E.F., 2018. Present and future Köppen-Geiger climate classification maps at 1-km resolution. *Scientific Data* 5, 180214.
- Bentley, P., Butt, K.R., Nuutinen, V., 2024. Two aspects of earthworm bioturbation: crop residue burial by foraging and surface casting in no-till management. *Eur. J. Soil Biol.* 120, 103575.
- Birkhofer, K., Bezemer, T.M., Bloem, J., Bonkowski, M., Christensen, S., Dubois, D., Ekelund, F., Fließbach, A., Gunst, L., Hedlund, K., Mäder, P., Mikola, J., Robin, C., Setälä, H., Tatin-Froux, F., Van der Putten, W.H., Scheu, S., 2008. Long-term organic farming fosters below and aboveground biota: implications for soil quality, biological control and productivity. *Soil Biol. Biochem.* 40, 2297–2308.
- Blume, H.-P., Stahr, K., Leinweber, P., 2010. *Bodenkundliches Praktikum. Spektrum Akademischer Verlag, Heidelberg.*
- Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method for measuring microbial biomass nitrogen in soil. *Soil Biol. Biochem.* 17, 837–842.
- van Capelle, C., Schrader, S., Brunotte, J., 2012. Tillage-induced changes in the functional diversity of soil biota – a review with a focus on German data. *Eur. J. Soil Biol.* 50, 165–181.
- Cheţan, F., Rusu, T., Cheţan, C., Simon, A., Vălean, A.-M., Ceclan, A.O., Bărdas, M., Tărau, A., 2023. Application of unconventional tillage systems to maize cultivation and measures for rational use of agricultural lands. *Land* 12, 2046.
- Cooper, R.J., Hama-Aziz, Z.Q., Hiscock, K.M., Lovett, A.A., Vrain, E., Dugdale, S.J., Sunnenberg, G., Dockerty, T., Hovsen, P., Noble, L., 2020. Conservation tillage and soil health: lessons from a 5-year UK farm trial (2013–2018). *Soil Till. Res.* 202, 104648.
- Djajakirana, G., Joergensen, R.G., Meyer, B., 1996. Ergosterol and microbial biomass relationship in soil. *Biol. Fertil. Soils* 22, 299–304.
- Engell, I., Linsler, D., Sandor, M., Joergensen, R.G., Meinen, C., Potthoff, M., 2022. The effects of conservation tillage on chemical and microbial soil parameters at four sites across Europe. *Plants* 11, 131747.
- Etana, A., Holm, L., Rydberg, T., Keller, T., 2020. Soil and crop responses to controlled traffic farming in reduced tillage and no-till: some experiences from field experiments and on-farm studies in Sweden. *Acta Agric. Scand. Section B – Soil Plant Sci.* 70, 333–340.
- Faust, S., Heinze, S., Ngosong, C., Sradnick, A., Oltmanns, M., Raupp, J., Geisseler, D., Joergensen, R.G., 2017. Effect of biodynamic soil amendments on microbial communities in comparison with inorganic fertilization. *Appl. Soil Ecol.* 114, 82–89.
- Faust, S., Koch, H.-J., Joergensen, R.G., 2019. Respiration response to different tillage intensities in transplanted soil columns. *Geoderma* 352, 289–297.
- Fierer, N., Strickland, M.S., Liptzin, D., Bradford, M.A., Cleveland, C.C., 2009. Global patterns in belowground communities. *Ecol. Lett.* 12, 1238e–1249.
- Gattinger, A., Muller, A., Haeni, M., Skinner, C., Fließbach, A., Buchmann, N., Mäder, P., Stolze, M., Smith, P., El-Hage Scialabba, N., Niggli, U., 2012. Enhanced top soil carbon stocks under organic farming. *PNAS* 109, 18226–18231.
- Giray, K., Banfield, C., Piepho, H.-P., Joergensen, R.G., Dippold, M., Wachendorf, C., 2024. Main microbial groups assessed by phospholipid fatty acid analysis of temperate alley agroforestry systems on crop- and grassland. *Appl. Soil Ecol.* 195, 105277.
- Gocke, M.I., Guigue, J., Bauke, S.L., Barkusky, D., Baumecker, M., Berns, A.E., Hobley, E., Honermeier, B., Kögel-Knabner, I., Koszinski, S., Sandhage-Hofmann, A., Schmidhalter, U., Schneider, F., Schweitzer, K., Seidel, S., Siebert, S., Skadell, L.E., Sommer, M., von Tucher, S., Don, A., Amelung, W., 2023. Interactive effects of agricultural management on soil organic carbon accrual: a synthesis of long-term field experiments in Germany. *Geoderma* 438, 116616.
- Goenster, S., Gründler, C., Buerkert, A., Joergensen, R.G., 2017. Soil microbial indicators across land use types in the river oasis Bulgan sum center, Western Mongolia. *Ecol. Indic.* 76, 111–118.
- Guest, E.J., Palfreeman, L.J., Joseph Holden, J., Chapman, P.J., Firbank, L.G., Lappage, M.G., Helgason, T., Leake, J.R., 2022. Soil macroaggregation drives sequestration of organic carbon and nitrogen with three-year grass-clover leys in arable rotations. *Sci. Tot. Environ.* 852, 158358.
- Haddaway, N.R., Hedlund, K., Jackson, L.E., Kätterer, T., Lugato, E., Thomsen, I.K., Jørgensen, H.B., Isberg, P.-E., 2017. How does tillage intensity affect soil organic carbon? A systematic review. *Environ. Evid.* 6, 77.
- Hartman, W.H., Richardson, C.J., 2013. Differential nutrient limitation of soil microbial biomass and metabolic quotients (q_{CO_2}): is there a biological stoichiometry of soil microbes? *PLoS One* 8, e57127.
- Heinze, S., Rauber, R., Joergensen, R.G., 2010a. Influence of mouldboard plough and rotary harrow tillage on microbial biomass and nutrient stocks in two long-term experiments on loess derived Luvisols. *Appl. Soil Ecol.* 46, 405–412.
- Heinze, S., Raupp, J., Joergensen, R.G., 2010b. Effects of fertilizer and spatial heterogeneity in soil pH on microbial biomass indices in a long-term field trial of organic agriculture. *Plant and Soil* 328, 203–215.
- Hoeffner, K., Beylich, A., Chabbi, A., Cluzeau, D., Dascalu, D., Graefe, U., Guzmán, G., Hallaire, V., Hanisch, J., Landa, B.B., Linsler, D., Menasseri, S., Öpik, M., Potthoff, M., Sandor, M., Scheu, S., Schmelz, R.M., Engell, I., Schrader, S., Vahter, T., Banse, M., Nicolai, A., Plaas, E., Runge, T., Roslin, T., Decau, M.-L., Sepp, S.-K., Arias-Giraldo, L.F., Busnot, S., Roucaute, M., Pérès, G., 2021. Legacy effects of temporary grassland in annual crop rotation on soil ecosystem services. *Sci. Tot. Environ.* 780, 146140.
- Hu, T., Chabbi, A., 2022. Grassland management and integration during crop rotation impact soil carbon changes and grass-crop production. *Agric. Ecosyst. Environ.* 324, 107703.
- Hydbom, S., Ernfors, M., Birgander, J., Hollander, J., Jensen, E.S., Olsson, P.A., 2017. Reduced tillage stimulated symbiotic fungi and microbial saprotrophs, but did not lead to a shift in the saprotrophic microorganism community structure. *Appl. Soil Ecol.* 119, 104–114.
- Hydbom, S., Olsson, J.A., Olsson, P.A., 2020. The use of conservation tillage in an agro-intensive region: results from a survey of farmers in Scania, Sweden. *Renew. Agric. Food Syst.* 35, 59–68.
- Insam, H., Parkinson, D., Domsch, K.H., 1989. Influence of macroclimate on soil microbial biomass. *Soil Biol. Biochem.* 21, 211–221.
- Islam, R., Singh, B., Dijkstra, F.-A., 2022. Stabilisation of soil organic matter: interactions between clay and microbes. *Biogeochem* 160, 145–158.
- IUSS Working Group WRB, 2022. World reference base for soil resources. International soil classification system for naming soils and creating legends for soil maps. In: International Union of Soil Sciences (IUSS), Vienna, Austria, 4th ed.
- Jacobs, A., Rauber, R., Ludwig, B., 2009. Impact of reduced tillage on carbon and nitrogen storage of two haplic Luvisols after 40 years. *Soil Till. Res.* 102, 158–164.
- Jacobs, A., Jungert, S., Koch, H.-J., 2015. Soil organic carbon as affected by direct drilling and mulching in sugar beet – wheat rotations. *Arch. Agron. Soil Sci.* 61, 1079–1087.
- Jacobs, A., Poeplau, C., Weiser, C., Fahrion-Nitschke, A., Don, A., 2020. Exports and inputs of organic carbon on agricultural soils in Germany. *Nutr. Cycl. Agroecosyst.* 118, 249–271.
- Jenkinson, D.S., Hart, P.B.S., Rayner, J.H., Parry, L.C., 1987. Modelling the turnover of organic matter in long-term experiments at Rothamsted. *INTECOL Bull.* 15, 1–8.
- Jenkinson, D.S., Harris, H.C., Ryan, J., McNeill, A.M., Pilbeam, C.J., 1999. Organic matter turnover in a calcareous clay soil from Syria under a two-course cereal rotation. *Soil Biol. Biochem.* 31, 687–693.
- Jenkinson, D.S., Poulton, P.R., Bryant, C., 2008. The turnover of organic carbon in subsoils. Part 1. Natural and bomb radiocarbon in soil profiles from the Rothamsted long-term field experiments. *Eur. J. Soil Sci.* 59, 391–399.
- Joergensen, R.G., Wichern, F., 2008. Quantitative assessment of the fungal contribution to microbial tissue in soil. *Soil Biol. Biochem.* 40, 2977–2991.
- Joergensen, R.G., Wichern, F., 2018. Alive and kicking: why dormant soil microorganisms matter. *Soil Biol. Biochem.* 116, 419–430.
- Kaiser, E.-A., Heinemeyer, O., 1993. Seasonal variations of soil microbial biomass carbon within the plough layer. *Soil Biol. Biochem.* 25, 1649–1655.
- Kaiser, M., Piegholdt, C., Andruschkewitsch, R., Linsler, D., Koch, H.-J., Ludwig, B., 2014. Impact of tillage intensity on carbon and nitrogen pools in surface and sub-surface soils of three long-term field experiments. *Eur. J. Soil Sci.* 65, 499–509.
- Keilueit, M., Nico, P.S., Kleber, M., Fendorf, S., 2016. Are oxygen limitations under recognized regulators of organic carbon turnover in upland soils? *Biogeochem* 127, 157–171.
- Khan, K.S., Mack, R., Castillo, X., Kaiser, M., Joergensen, R.G., 2016. Microbial biomass, fungal and bacterial residues, and their relationships to the soil organic matter C/N/P/S ratios. *Geoderma* 271, 115–123.
- Kim, D., Hirmas, D.R., McEwan, R.W., Mueller, T.G., Park, S.J., Šamonil, P., Thompson, J.A., Wendroth, O., 2016. Predicting the influence of multi-scale spatial autocorrelation on soil-landform modeling. *Soil Sci. Soc. Am. J.* 80, 409–419.
- Koch, H.-J., Dieckmann, J., Buchse, A., Merländer, B., 2009. Yield decrease in sugar beet caused by reduced tillage and direct drilling. *Eur. J. Agron.* 30, 101–109.
- Kunrath, T.R., de Berranger, C., Charrier, X., Gastal, F., de Faccio Carvalho, P.C., Lemaire, G., Emile, J.-C., Durand, J.-L., 2015. How much do soil-based rotations reduce nitrate leaching in a cereal cropping system? *Agric Water Manag* 150, 46–56.
- Lal, R., 2015. Restoring soil quality to mitigate soil degradation. *Sustainability* 7, 5875–5895.
- Le Guillou, C., Chemidlin Prévost-Bouré, N., Karimi, B., Akkal-Corfini, N., Dequiedt, S., Nowak, V., Terrat, S., Menasseri-Aubry, S., Viaud, V., Maron, P.-A., Ranjard, L., 2019. Tillage intensity and pasture in rotation effectively shape soil microbial communities at a landscape scale. *Microbiology Open* 8, e00676.
- Ludwig, B., Schulz, E., Merbach, I., Rethemeyer, J., Flessa, H., 2007. Predictive modelling of the C dynamics for eight variants of the long-term static fertilization experiment in Bad Lauchstädt using the Rothamsted carbon model. *Eur. J. Soil Sci.* 58, 1155–1163.

- Mueller, T., Joergensen, R.G., Meyer, B., 1992. Estimation of soil microbial biomass C in the presence of living roots by fumigation-extraction. *Soil Biol. Biochem.* 24, 179–181.
- Müller, T., Höper, H., 2004. Soil organic matter turnover as a function of the soil clay content: consequences for model applications. *Soil Biol. Biochem.* 36, 877–888.
- Müller, P., Neuhoﬀ, D., Nabel, M., Schiﬀers, K., Döring, T.F., 2022. Tillage eﬀects on ground beetles in temperate climates: a review. *Agron. Sust. Develop.* 42, 65.
- Murugan, R., Koch, H.-J., Joergensen, R.G., 2014. Long-term influence of diﬀerent tillage intensities on soil microbial biomass, residues and community structure at diﬀerent depths. *Biol. Fertil. Soils* 50, 487–498.
- Paul, E.A., 2016. The nature and dynamics of soil organic matter: plant inputs, microbial transformations, and organic matter stabilization. *Soil Biol. Biochem.* 98, 109e126.
- Persson, T., Bergkvist, G., Kätterer, T., 2008. Long-term eﬀects of crop rotations with and without perennial leys on soil carbon stocks and grain yields of winter wheat. *Nutr. Cycl. Agroecosyst.* 81, 193–202.
- Powlson, D.S., Brookes, P.C., Christensen, B.T., 1987. Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. *Soil Biol. Biochem.* 19, 159–164.
- Prendergast-Miller, M.T., Jones, D.T., Berdeni, D., Bird, S., Chapman, P.J., Firbank, L., Grayson, R., Helgason, T., Holden, J., Lappage, M., Leake, J., Hodson, M.E., 2021. Arable fields as potential reservoirs of biodiversity: earthworm populations increase in new leys. *Sci. Tot. Environ.* 789, 147880.
- Rowen, E.K., Regan, K.H., Barbercheck, M.E., Tooker, J.F., 2020. Is tillage beneficial or detrimental for insect and slug management? A meta-analysis. *Agric. Ecosyst. Environ.* 294, 106849.
- Rumpel, C., Kögel-Knabner, I., 2011. Deep soil organic matter - a key but poorly understood component of terrestrial C cycle. *Plant and Soil* 338, 143–158.
- Rusu, M., Mihai, M., Tritean, N., Mihai, V.C., Moldovan, L., Ceclan, A.O., Russu, F., Toader, C., 2024. Protection and modeling in the use of S, ca, and mg alternatives for long-term sustainable fertilization systems. *Agronomy* 14, 515.
- Seitz, S., Goebes, P., Puerta, V.L., Pereira, E.I.P., Wittwer, R., Six, J., van der Heijden, M. G.A., Scholten, T., 2018. Conservation tillage and organic farming reduce soil erosion. *Agron. Sustain. Dev.* 39, 4.
- Skadell, L.E., Schneider, F., Gocke, M.L., Guigue, J., Amelung, W., Bauke, S.L., Hobbey, E. U., Barkusky, D., Honermeier, B., Kögel-Knabner, I., Schmidhalter, U., Schweitzer, K., Seidel, S.J., Stefan Siebert, S., Sommer, M., Vaziritabar, Y., Don, A., 2023. Twenty percent of agricultural management eﬀects on organic carbon stocks occur in subsoils – results of ten long-term experiments. *Agric. Ecosyst. Environ.* 356, 108619.
- Strickland, M.S., Rousk, J., 2010. Considering fungal:bacterial dominance in soils – methods, controls, and ecosystem implications. *Soil Biol. Biochem.* 42, 1385–1395.
- Tschanz, P., Vogel, S., Walter, A., Keller, T., Albrecht, M., 2023. Nesting of ground-nesting bees in arable fields is not associated with tillage system per se, but with distance to field edge, crop cover, soil and landscape context. *J. Appl. Ecol.* 60, 158–169.
- Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19, 703–707.
- Wardle, D.A., Parkinson, D., 1991. A statistical evaluation of equations for predicting total microbial biomass carbon using physiological and biochemical methods. *Agric. Ecosyst. Environ.* 34, 75–86.
- Wentzel, S., Schmidt, R., Piepho, H.-P., Semmler-Busch, U., Joergensen, R.G., 2015. Response of soil fertility indices to long-term application of biogas and raw slurry under organic farming. *Appl. Soil Ecol.* 96, 99–107.
- Wu, J., Joergensen, R.G., Pommerening, B., Chaussod, R., Brookes, P.C., 1990. Measurement of soil microbial biomass C by fumigation-extraction - an automated procedure. *Soil Biol. Biochem.* 22, 1167–1169.