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Ten-year effects of perennial cropping systems on soil organic carbon stock and stability in sandy soils: Mechanisms and biochemical drivers

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ABSTRACT

Perennial crops can be a sustainable alternative to annual crops due to plant traits and management practices that improve productivity and may contribute to soil organic carbon (SOC) sequestration. However, our understanding of the mechanisms behind the potential differences in SOC pools between perennials and annuals is incomplete, particularly in the sandy soils that dominate Danish croplands. Based on a 10-year field experiment on a temperate sandy soil with perennials (tall fescue, grass/legume mixture) and annuals (triticale monoculture, triticale in a rotation), we investigated SOC pools at depths of 0–20 cm (topsoil) and 20–50 cm (subsoil) through (i) physical fractionation into pools of particulate organic carbon (POC) and mineral-associated organic carbon (MAOC) and (ii) chemical analysis of aliphatic-to-aromatic/carboxylic ratios via Diffuse Reflectance Infrared Fourier Transform Mid-Infrared Spectroscopy. These analyses were complemented with measurements of extracellular enzyme activities, microbial biomass, root biomass, and aboveground biomass C/N ratio. We found that tall fescue had significantly higher activities of nutrient-releasing enzymes (e.g., N-acetylglucosaminidase and acid phosphatase), and lower oxidase activities (peroxidase and phenol oxidase) in the topsoil compared to annuals. The grass/legume mixture had higher activities of C-, N-, and P-acquiring enzymes than annuals at both soil depths. Soil fractionation analyses showed no significant differences between tall fescue and annuals in POC and MAOC stocks in the topsoil. However, tall fescue exhibited a lower aliphatic to aromatic/carboxylic ratio in the topsoil compared to annuals, which correlated negatively with root biomass and phenol oxidase activity. In the subsoil, the MAOC stock in the tall fescue system tended to accumulate at a rate of 0.35 Mg ha^{-1} yr^{-1} compared to annual triticale, and was positively correlated to microbial biomass carbon. In contrast, the grass/legume mixture, without N fertilization, had limited potential for SOC stock increases at both soil depths. Altogether, the results emphasize the role of microbial processes in SOC dynamics and the importance of perennial cropping systems, such as tall fescue, in enhancing SOC stability during the transition from annual to perennial crops for biorefining on sandy soils.

1. Introduction

Producing sufficient plant biomass for food, materials and bioenergy for the growing global population, without depleting soil organic carbon (SOC) stocks, remains a key challenge for future land use (Paustian et al., 2016; Vogt and Weckhuysen, 2024). One potential strategy is the cultivation of perennial crops, which can increase biomass production compared to annual crops due to a longer period of photosynthesis (Manevski et al., 2017). Perennial systems can also increase SOC compared to annual cropping systems (Ledo et al., 2020; Chen et al., 2022; Rui et al., 2022). Such effects may be related to increased belowground organic carbon (C) inputs from root biomass (Rasse et al., 2005), and possibly to reduced SOC mineralization due to the absence of tillage (Ledo et al., 2020). However, to explore the long-term benefits on SOC stocks, it is essential to examine the contribution of perennial cropping systems to different SOC pools that vary in their long-term stability at different soil depths (Kim et al., 2022).

Physical fractionation can divide soil organic matter into two fractions — particulate organic matter (POM) and mineral-associated organic matter (MAOM) — that vary in their stability in the soil (Angst

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et al., 2023). POM primarily consists of partially decomposed plant residues, formed through the fragmentation and initial decomposition of organic inputs. This fraction typically has relatively rapid turnover, as it remains accessible to microbial attack (Lavallee et al., 2020). In contrast, MAOM forms when microbial residues and small organic molecules bind to soil minerals and aggregates, providing physical and chemical protection that reduces microbial access. This stabilization mechanism slows decomposition and significantly contributes to the long-term SOC reservoir, essential for climate change mitigation (Lavallee et al., 2020, Liang et al., 2017). Perennial cropping systems have been shown to increase SOC inputs due to their extensive roots and rhizodeposition (Kim et al., 2022). However, the understanding of SOC pool changes in perennial systems, particularly in sandy soils, remains uncertain. Sandy soils cover large areas and are widely used for agricultural production, such as in the post-glacial regions of Northern Europe (Farooq et al., 2024). Their widespread presence and vulnerability to SOC loss make it essential to understand long-term changes in SOC pools and develop effective management strategies. Sandy soils often have poor nutrient retention and a low nitrogen (N) mineralization capacity, necessitating higher N fertilization for frequently harvested perennial crops compared to annual crops. This high N input may stimulate microbial mineralization of fresh plant residues or existing SOC (Kuzyakov et al., 2000, Wang et al., 2018), resulting in low SOC sequestration potential for perennial systems in sandy soils. However, Carter and Gregorich, (2010) reported significant SOC accumulation in sandy soils under tall fescue cultivation, with rates of 1.8 Mg SOC ha^{-1} yr^{-1} in the top 0–60 cm over 7 years. This contrasting evidence illustrates that the effectiveness of perennials to increase SOC stocks is inconclusive, particularly because most existing studies are short-term and do not capture the long-term SOC dynamics in perennial versus annual cropping systems (Siddique et al., 2023).

The stability of SOC can be reflected not only by the physical fractionation but also by its chemical composition. Characterizing the chemical composition is challenging due to the complexity of soil organic matter, but it represents an important complement to physical fractionation methods (Lehmann and Kleber, 2015; von Lützow et al., 2007). Diffuse reflectance infrared Fourier-transform mid-infrared spectroscopy (DRIFT-MIR) offers a valuable and rapid method for identifying various SOC functional groups, such as aliphatic and aromatic/carboxylic compounds (Calderón et al., 2013; Margenot et al., 2023). The ratio of these compounds may indicate the degree of decomposition and stability of SOC by reflecting the proportion of fast (i.e., aliphatic) to slowly (i.e., aromatic/carboxylic) decomposable compounds (Demyan et al., 2012; Laub et al., 2020). Perennials, with their extensive root systems and continuous organic inputs, may increase the proportion of slow cycling compounds (derived from chemical recalcitrance) compared to annual crops (Rasse et al., 2005) thereby contributing to the stability of SOC in perennial cropping systems. Combining information on SOC chemical composition with physical fractionation can provide a more comprehensive understanding of the processes driving SOC stability in different cropping systems (Jagadamma and Lal, 2010).

Extracellular enzyme activity is another potential indicator of SOC cycling (Cenini et al., 2016). Differences in root characteristics, nutrient turnover, and microbial activity between perennial and annual crops result in variations in soil enzyme activities (Cattaneo et al., 2014), which may regulate SOC dynamics (Sinsabaugh, 2010). Key extracellular enzymes, such as oxidases and hydrolases, are vital for mediating the transformation and mineralization of soil organic matter. For example, glucosidases decompose cellulose into simple sugars, indicating labile plant residue breakdown (Stott et al., 2010), while phenol oxidases (PHO) are important for lignin depolymerization (Sinsabaugh, 2010). The varying characteristics and fertilization regimes of annual and perennial crops could lead to differences in extracellular enzyme activity. For example, some studies have shown that N addition inhibits phenol oxidase activity by causing soil N saturation or altering microbial community composition (Matocha et al., 2004; Waldrop et al., 2004). Exploring the links between soil extracellular enzymes, SOC pools, and SOC quality across cropping systems can help clarify the ecological processes that shape SOC dynamics.

This study investigated SOC stabilization pathways in annual and perennial cropping systems designed for feedstock production for future biorefineries (Manevski et al., 2017; Vogt and Weckhuysen, 2024). Soils were collected from long-term cropping systems (10 years), including annuals (triticale in monoculture and in crop rotation) and perennials (tall fescue and grass/legume mixture) in order to measure soil extracellular enzyme activities, SOC stocks and its distribution in coarse and fine particle size fractions (POC and MAOC stocks), and SOC composition by DRIFT-MIR. Furthermore, aboveground biomass C/N ratio, root biomass, and microbial biomass were quantified. Our hypotheses were that: 1) perennials can increase SOC stock in topsoil and subsoil, and in both POM and MAOM fractions, compared to annuals due to higher root biomass input and microbial biomass, 2) higher root input and turnover in perennial systems promote the accumulation of slow-cycling aromatic and carboxylic SOC, resulting in a lower aliphatic-to-aromatic/carboxylic ratio, and 3) perennials increase enzyme activity associated with SOC cycling due to increased belowground C allocation, with the pattern of enzymes targeting labile and more stable C compounds reflecting the state of SOC stocks and composition.

2. Material and methods

2.1. Site description and experimental design

The study was performed within a long-term cropping experiment (LTE) initiated in 2012 at Foulumgaard Experimental Station, Aarhus University, Denmark (9°35'E, 56°30'N, elevation 53 m a.s.l.) (Fig. S1). The study area has a temperate and wet climate with mean annual temperature of 7.8 °C and precipitation of 740 mm; the potential annual evapotranspiration is approximately 600 mm (Zong et al., 2024). The soil is a sandy loam classified as Mollic Luvisol (Krogh and Greve, 1999) and Typic Hapludalf (Soil Survey Staff, 2014), with approximately 46 % fine sand, 34 % coarse sand, 10 % silt, 7 % clay and 3 % organic matter, and a pH(H_2O) of 6.5 at a depth of 0–20 cm (Heidmann, 1989). Prior to establishing the LTE, the land was cultivated with annual crops, primarily winter wheat (*Triticum aestivum* L.), winter rye (*Secale cereale* L.), potato (*Solanum tuberosum* L.), and spring barley (*Hordeum vulgare* L.), and received mineral and organic fertilizers with an average annual N input of 150 kg ha^{-1}.

The LTE area (2.2 ha) was initially established with a wide range of annual and perennial crops to investigate their productivity, environmental impact, and quality for biorefining (Zong et al., 2024). The present study comprised four treatments (Fig. S2), including 1) annual triticale (*Triticosecale*) in monoculture, 2) annual triticale in crop rotation with hemp (*Cannabis sativa* L.) (replaced by faba beans (*Vicia faba* L.) in 2020), maize (*Zea mays* L.) and beet (*Beta vulgaris* L.) as main crops and winter rye and winter rape (*Brassica napus* L.) as secondary crops also for harvest, 3) perennial tall fescue (*Festuca arundinacea* Schreb.), and 4) a perennial grass/legume mixture (*Trifolium repens* cv. Silvester, *Festuca arundinacea* cv. Tower, *Lolium multiflorum* cv. Humbi, *Phleum pratense* cv. Winnetou, and *Festuca arundinacea* cv. Laura), with a composition of 90 % grass seeds and 10 % legume seeds by weight. The four treatments were based on an incomplete split-plot experimental design with four blocks (i.e., four replicates). The size of plots nested within blocks was 20 m × 3 m.

From 2012, triticale monoculture was sown in September and harvested in August, while triticale in rotation was sown after hemp (and later faba bean) in September/October and harvested in July before full maturity. Both annual systems had shallow tillage at 22–25 cm when seeding. Perennial treatments of tall fescue and grass/legume mixture were sown in May 2012 and aboveground biomass was harvested three

times annually. The tall fescue and grass/clover mixtures were renewed in 2018 due to decreasing yield. The proportion of grass seeds to legume seeds in the renewed mixture was approximately 80:20 by weight. These two perennial systems were not tilled, except in the years of renewal. The crops were fertilized with N, phosphorus (P) and potassium (K) at rates according to the existing Danish agro-legislation recommendations. On average from 2012 to 2021, 180 kg N ha^{-1} yr^{-1} and 192.2 kg N ha^{-1} yr^{-1} were applied for triticale monoculture and triticale in rotation, respectively, whereas the tall fescue was fertilized with an N rate higher than the recommendation (325.5 kg N ha^{-1} yr^{-1} on average) to sustain its high productivity. To exploit the natural N fixation of legume crops, no N was applied to the grass/legume mixture other than 30 kg N ha^{-1} in the first year. Regarding P fertilization, 26, 22, 43, 21 kg P ha^{-1} yr^{-1} were applied to the triticale, triticale in rotation, tall fescue and grass/legume systems, respectively. Triticale monoculture and triticale in rotation systems received K at a rate of 21 and 26 kg ha^{-1} yr^{-1}, while the tall fescue and grass/legume systems received 218 and 142 kg ha^{-1} yr^{-1}, respectively. Pesticides were sprayed when necessary to control weeds, pests and diseases, and 20–50 mm of irrigation was applied annually primarily from June to August to avoid drought spells.

2.2. Plant and soil samples

In August 2021, the above-ground biomass of all treatments was harvested in the center of each plot (1.5 m × 10 m) using a plot harvester (Haldrup F-55, Germany). Topsoil (0–20 cm) and subsoil (20–50 cm) were sampled from all plots before crop harvest by pooling two diagonally placed soil cores (diameter, 10 cm). The soil was sieved (<2 mm) and coarse roots and rock fragments remaining on the sieve were collected. Root samples were washed and oven-dried at 60ºC (to constant weight) to calculate dry root biomass. The rock fragments were weighed after oven-drying and used in subsequent calculations of SOC stock. The sieved soils were stored at 2°C for less than one week prior to analyses of microbial properties and mineral N. Soil subsamples were air-dried at room temperature for other soil analyses.

2.3. Physico-chemical and microbial biomass

Plant biomass fractions were oven-dried at 60ºC (to constant weight), and ball-milled for analyses of total C and N using a dry combustion method (CN analyzer, Elementar Analysensysteme AG, Langenselbold, Germany). Air-dried soil samples were treated with 1 M HCl to remove inorganic C before being analyzed for SOC and total N using the same method as plant samples. Soil total P was extracted with H_2SO_4-$HClO_4$ according to Kafkafi (1972) using 0.1 g ball-milled soil, and Olsen-P was extracted with 0.5 M sodium bicarbonate (Olsen, 1982). Both P fractions were measured colorimetrically using the molybdate blue method. Mineral N was extracted with 1 M KCl (30 min, rotary shaker) and filtered (1.6 μm glass microfibre filter; VWR Int., France) for nitrate (NO_3^-) and ammonium (NH_4^+) analysis by a continuous segmented flow auto-analyzer (SEAL AA500 AutoAnalyzer, Norderstedt, Germany). The soil pH was measured using a glass electrode in the soil suspension extracted with 1 M KCl solution (Van Reeuwijk, 2002). The soil water content (SWC) was determined gravimetrically by oven-drying the samples at 105°C for 24 h.

Microbial biomass C (MBC) and N (MBN) were quantified using the fumigation-extraction method (Brookes et al., 1985; Wu et al., 1990). Fresh sieved soil samples (10 g) were fumigated with alcohol-free chloroform ($CHCl_3$) vapor in a vacuum desiccator with NaOH for 24 h. Residual $CHCl_3$ was removed by repeated evacuations. Fumigated and non-fumigated soils were extracted with 40 mL of 0.5 M K_2SO_4 by shaking for 30 min. Total organic C and N of extracts were analyzed using a TOC analyzer (Shimadzu TOC-VCPH) and an autoanalyzer (SEAL AA500 AutoAnalyzer, Norderstedt, Germany), respectively. MBC and MBN were calculated using extraction coefficients of 0.45 and 0.54, respectively (Beck et al., 1997; Brookes et al., 1985). Microbial biomass P (MBP) was measured following Brookes et al. (1982). Fresh soil samples (2 g) were prepared for blank, P recovery, and fumigation sets. All samples were extracted with 0.5 M $NaHCO_3$ (1:20 soil/reagent) for 30 min. P concentrations were determined colorimetrically at 880 nm using the molybdate blue method with a UV–vis spectrophotometer. The conversion coefficient for MBP was 0.4 (Brookes et al., 1982).

2.4. Soil extracellular activities

Extracellular enzymes involved in the cycling of C, N, and P were measured with soil suspensions prepared by mixing 2 g of fresh soil with 125 mL of acetate buffer (50 mM, pH 5.0). The targeted enzymes were (i) three hydrolases for degrading labile C compounds such as cellulose and hemicellulose. i.e., α-1,4-glucosidase (AG), β-1,4-glucosidase (BG) and cellobiohydrolase (CBH), (ii) two oxidases for degrading recalcitrant and more complex C compounds such as lignin, i.e., phenol oxidase (PHO) and peroxidase (POD), (iii) two hydrolases for N acquisition, i.e., leucine amino peptidase (LAP) and β-1,4-N-acetylglucosaminidase (NAG), and (iv) one hydrolase for P acquisition, i.e., acid phosphatase (AP). The potential activity of the hydrolases was determined using a fluorometric method with 4-methylumbelliferone (MUB, Sigma-Aldrich Corporation, St. Louis, MO, USA) except for LAP where 7-amino-4-methylcoumarin (MUC, Sigma-Aldrich Corporation, St. Louis, MO, USA) was used (Bell et al., 2013). The assay involved combining 800 μL of soil suspension with 200 μL of enzyme-substrate solutions or standard solutions in 96-well microplates, followed by incubation at 25°C for 4 hours. After incubation, the plates were centrifuged for 3 min at a speed of 2900 rpm, and the fluorescence of the supernatant was measured at 365 nm excitation and 450 nm emission using a microplate reader (PerkinElmer Envision 2103 Multilabel Reader, Shelton, USA). The activities of the two oxidases were measured using a colorimetric method with L-3,4-dihydroxyphenylalanine (L-DOPA) as the substrate (DeForest, 2009). For POD assays, 40 μL of 3 % H_2O_2 was added prior to incubation. The microplates were incubated at 25°C for 4 hours for POD and 24 hours for PHO. Absorbance was measured at 450 nm using a microplate reader (PerkinElmer Envision 2103 Multilabel Reader, Shelton, USA). Enzyme activities were expressed as nmol (substrate transformed) h^{-1} g^{-1} (dry soil).

2.5. SOC fractionations

Wet sieving, using a 50 μm sieve, was applied to separate the soil into POM and MAOM fractions (Begill et al., 2023). In short, 10 g of soil was mixed with 150 mL distilled water and sonicated at 100 J/mL (Just et al., 2021). Then, wet sieving was performed by pouring the mixture over the sieve and washing with deionized water. The coarse fraction remaining on the sieve (>50 μm), considered as POM, was dried at 60°C. The smaller particles, passing through the sieve, were combined with a 0.8 g L^{-1} $CaCl_2$ solution, centrifuged at 4000 g for 15 minutes to extract the fine fraction as MAOM, and then dried at 60°C to constant weight. The soil mass recovery of the two fractions (combined) was typically between 99 % and 100 %. Both fractions were milled prior to SOC analysis using a CN analyzer (Elementar Analysensysteme AG, Langenselbold, Germany). The recovery of SOC in POM and MAOM (combined) was 96 % on average. If the recovery was outside 85–100 % the soil was retested.

2.6. Ratio of aliphatic to aromatic/carboxylic compounds

The ratio of the aliphatic to aromatic/carboxylic compounds was used as an indicator for the SOC partitioning in fast and slow cycling pools. Bulk soil samples were homogenized using ball milling, oven dried, and analyzed using a Nicolet iS50 spectrometer (Thermo Fisher) equipped with a Collector II assessor for DRIFT-MIR. Atmospheric interferences from CO_2 and H_2O were corrected using roughened gold (Plasmagold, Pleiger Laseroptik) for background spectra. Spectra were

collected at wavenumbers between 4000–400 cm^{-1} with a 2 cm^{-1} resolution, averaging 32 scans per sample. Backgrounds were collected for every 20 samples. All acquired spectra were then resampled in the 4000–450 cm^{-1} range with a resolution of 2 cm^{-1}, scatter corrected using a Savitzky-Golay filter with a window size of 27 and normalized using Standard Normal Variate. Local baseline corrections were applied to determine areas under the curve for aliphatic (3010–2800 cm^{-1}) and aromatic/carboxylic (1660–1580 cm^{-1}) bands (Margenot et al., 2023).

2.7. Calculations and statistical analysis

The total soil BD was calculated according to Grossman and Reinsch (2002):

$$BD_{total} \quad (g \quad cm^{-3}) = \frac{m_{sample} \times 100}{V_{sample} \times (100 + SWC)} \tag{1}$$

where m_{sample} is the total mass (g) consisting of moist soil and rock fragments. V_{sample} is the total volume (cm^3). SWC is the soil gravimetric water content (%).

The SOC stock was calculated according to Harbo et al. (2022):

$$SOC\ stock\left(Mgha^{-1}\right) = C \times BD_{total} \times \left(1 - \frac{RF}{100}\right) \times D \times 0.1 \tag{2}$$

where C is the SOC content (g kg^{-1}) (<2 mm), RF is the mass proportion (%) of rock fragments (>2 mm), D is the soil layer thickness (cm), 0.1 is the conversion factor, and BD_{total} is total soil bulk density (g cm^{-3}) of corresponding soil depth. To ensure precise SOC stock comparison between cropping systems, corrections for varying soil masses were made across the full soil profile (0–50 cm) and its subdivisions (0–20 and 20–50 cm), using the lightest single core from the annual crops, addressing soil BD differences between no-tillage perennial and tillage annual systems (Poeplau and Don, 2013):

$$SOCstock_{corr} = SOC\ stock - \left(\frac{CFSM - CFSM_{lightest}}{CV} \times C_{deepest}\right) \tag{3}$$

where $SOC\ stock_{corr}$ is the corrected SOC stock (Mg ha^{-1}); SOC stock is the uncorrected SOC stock of an individual core (Mg ha^{-1}); $CFSM$ is the fine soil mass of the individual core up to a certain depth (g); $CFSM_{lightest}$ is the cumulative fine soil mass of the lightest core of the site (g); CV is the cumulative volume of each soil core (cm^3); $C_{deepest}$ is the SOC concentration (%) in the deepest sampled depth increment.

All statistical analyses were performed in R 4.0.5 (R Development Core Team, 2020). Residuals for all parametric models were tested for homogeneity of variance using Levene's test and for normality using the Shapiro-Wilk test, with a significance level of $\alpha = 0.05$. If residuals did not meet these assumptions, data transformations (e.g., log or square root transformation) were applied to achieve normality. If transformations were insufficient, non-normal distribution models were used. For example, a Gamma distribution was chosen to analyze the effects of different cropping systems and depth on root biomass due to its suitability for positively skewed data. Linear mixed effects models were employed using the "lmer" function from the "lme4" package (Bates et al., 2015) to test the effects of different cropping systems and soil depth on the SOC stock (bulk and its fractions) and ratio of chemical compounds, plant properties (C/N ratio of aboveground biomass and root biomass), soil properties (BD, water content, pH, and C, N, P concentrations), soil extracellular enzymes, and soil microbial biomass indices (MBC, MBN, MBP). The cropping systems and soil depth were set as fixed factors, and the block was treated as a random factor. The Tukey test was applied from the "emmeans" package to compare the differences among cropping systems at the same soil depth across all variables with a significance level of $\alpha = 0.05$ (Lenth, 2019). Principal component analysis (PCA) was conducted using the "PCA" function from the "FactoMineR" package to assess the relationships among plant and soil properties across different cropping systems at two depths (0–20 cm and 20–50 cm). Pearson correlation analysis was conducted using the "corr.test" function from the "psych" package to examine pairwise relationships between different variables. All DRIFT spectra processing was performed using the "propectr" package (Stevens and Ramirez-Lopez, 2022). Areas under the curve were determined using the "DescTools" package (Signorell, 2023). Data reported as central tendency and dispersion represent mean ± standard error with $n = 4$ unless otherwise indicated.

3. Results

3.1. The effect of cropping systems on SOC, physical fractions and ratio of aliphatic to aromatic/carboxylic compounds

Topsoil SOC content ranged from 12.8 to 13.7 g kg^{-1}, while the subsoil SOC content was lower, ranging from 5.7 to 8.1 g kg^{-1} (Table 1). MAOC stocks represented approximately 90 % of the total SOC stocks across all treatments and depths, ranging from 19.3 to 28.0 Mg ha^{-1} in the topsoil (0–20 cm) and from 10.1 to 31.9 Mg ha^{-1} in the subsoil (20–50 cm). Corresponding total SOC stocks ranged from 21.7 to 31.4 Mg ha^{-1} in the topsoil and from 11.0 to 33.7 Mg ha^{-1} in the subsoil. In the topsoil, there were no statistically significant differences in SOC contents and stocks among the four treatments, neither in bulk soils nor in the POM and MAOM fractions (Table 1, Fig. 1A). In the subsoil, compared to monoculture triticale, tall fescue showed a tendency for higher bulk SOC and MAOC stocks, with increase rates of 0.35 Mg ha^{-1} yr^{-1} ($P = 0.8$, Fig. 1A). The other treatments had negligible effects relative to monoculture triticale. Tall fescue had a lower aliphatic to aromatic/carboxylic ratio than the other treatments in the topsoil ($P < 0.001$), while the ratio was similar between treatments in the subsoil ($P = 0.89$, Fig. 1B).

3.2. Aboveground and root biomass properties

The coarse roots biomass (remaining on the 2-mm sieve) in the topsoil was significantly higher in the perennial tall fescue (354 g m^{-2}) and grass/legume mixture (233 g m^{-2}) as compared to the annual cropping systems ($P = 0.003$, Fig. 2A). The same pattern was seen for the subsoil, where the root biomass in tall fescue was further significantly higher by 94 % than in the grass/legume mixture (Fig. 2A, $P = 0.001$). The C/N ratio of the aboveground biomass at the time of harvest was highest for the annuals, and 2-fold lower for tall fescue and the grass/legume mixture (Fig. 2B).

3.3. Soil physico-chemical properties and biochemical indicators

Soil BD in the perennial cropping systems was significantly higher (by up to 13 %) than in the annuals in the topsoil ($P < 0.001$), but not in the subsoil (Table 1). Tall fescue had higher topsoil NO_3^- concentration compared to grass/legume mixture system. The grass/legume system had lower pH than the other three cropping systems in both soil depths and higher Olsen-P concentration and NH_4^+ concentration in the topsoil. No significant differences in SWC, soil total N and SOC/N ratio were found among the four cropping systems (Table 1).

Microbial biomass C was highest in tall fescue in both topsoil and subsoil (Fig. 2C). In contrast, the grass/legume mixture had significantly lower MBC and MBN in the topsoil but maintained relatively high MBP in both soil layers compared to the other cropping systems (Fig. 2E). Enzyme activities in the topsoil were similar among the four cropping systems for the hydrolases AG, BG, and CBH ($P = 0.33$–0.73). However, in the subsoil, significantly higher activities of these three hydrolases were observed in the grass/legume mixture (Fig. 3A-C). In the topsoil, perennial crops had higher NAG and AP activities than annual crops ($P = 0.03$, $P = 0.003$). In the subsoil, nutrient-cycling enzyme activities (LAP, NAG, AP) were highest in the grass/legume mixture ($P \leq 0.001$,

Table 1
Topsoil and subsoil properties of the four cropping systems.

Cropping system	Soil depth cm	BD g cm^{-3}	pH(KCl) [-]	SWC %	SOC g kg^{-1}	Total N g kg^{-1}	SOC/N ratio [-]	Total P g kg^{-1}	Olsen P mg kg^{-1}	NH_4^+ mg kg^{-1}	NO_3^- mg kg^{-1}
Tall fescue	0–20	1.25^{A}	5.2^{A}	16.2^{A}	13.7^{A}	1.2^{A}	11.3^{A}	0.9^{A}	0.03^{B}	0.2^{B}	11.1^{A}
Grass/legume mix	0–20	1.20^{AB}	4.6^{B}	16.6^{A}	13.3^{A}	1.2^{A}	11.3^{A}	0.9^{A}	0.05^{A}	2.3^{A}	4.3^{B}
Triticale, mono	0–20	1.11^{B}	5.3^{A}	15.0^{A}	13.4^{A}	1.2^{A}	11.2^{A}	0.9^{A}	0.03^{B}	0.2^{B}	6.4^{AB}
Triticale, rotation	0–20	1.15^{B}	5.1^{A}	16.0^{A}	12.8^{A}	1.2^{A}	10.8^{B}	0.8^{A}	0.03^{B}	0.2^{B}	6.8^{AB}
Tall fescue	20–50	1.38^{a}	5.5^{a}	14.0^{a}	8.1^{a}	0.7^{a}	11.9^{a}	0.7^{a}	0.02^{a}	0.2^{a}	4.8^{a}
Grass/legume mix	20–50	1.26^{a}	5.1^{b}	13.2^{a}	6.7^{a}	0.5^{a}	12.3^{a}	0.6^{ab}	0.02^{a}	0.3^{a}	1.9^{a}
Triticale, mono	20–50	1.27^{a}	5.3^{ab}	13.0^{a}	7.5^{a}	0.6^{a}	12.3^{a}	0.7^{ab}	0.02^{a}	0.2^{a}	2.3^{a}
Triticale, rotation	20–50	1.27^{a}	5.3^{ab}	13.0^{a}	5.7^{a}	0.5^{a}	11.8^{a}	0.5^{b}	0.01^{a}	0.1^{a}	3.9^{a}
Cropping system		ns	* **	ns	ns	ns	ns	*	*	* *	*
	Depth	* *	* **	* **	* **	* **	* **	* **	* **	* *	* **

Different upper- and lowercase letters indicate significant difference for the variables among these four cropping systems at 0–20 and 20–50 cm, respectively. Asterisks indicate the overall significance of cropping system and depth (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). ns, not significant ($P > 0.05$). BD, bulk density; SWC, gravimetric water content at the time of soil sampling.

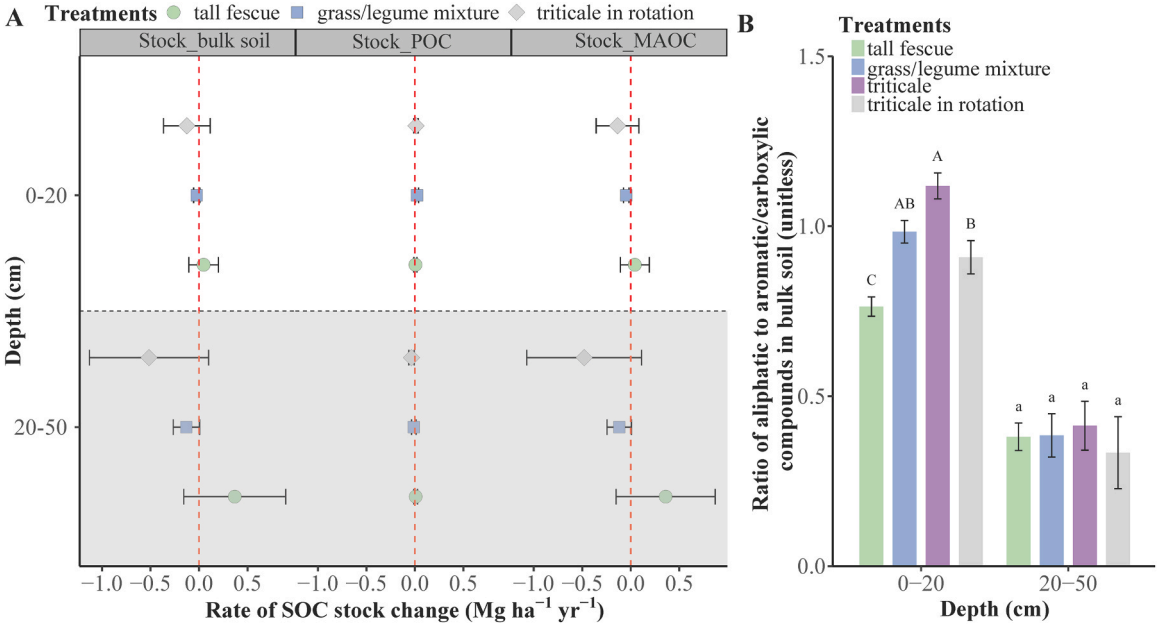

Fig. 1. Rate of corrected SOC stock change (A) and ratio of aliphatic to aromatic/carboxylic compounds (B) in perennial and annual cropping systems from the long-term experiment. SOC stock change data are shown for bulk SOC stock (Stock_bulk soil), particulate organic carbon stock (Stock_POC) and mineral associated organic carbon stock (Stock_MAOC). The dashed red line represents monoculture triticale as a baseline. The perennials were tall fescue and grass/legume mixture, and the annuals were triticale in monoculture and triticale in crop rotation. Data are means ± standard error ($n = 4$). Different upper- and lower-case letters indicate significant differences ($P < 0.05$) among cropping systems at 0–20 and 20–50 cm soil depths, respectively.

Fig. 3D-F). Tall fescue had the lowest PHO activity in the topsoil, 73 %-88 % lower than other cropping systems. Similarly, POD activity was lower in tall fescue and grass/legume mixture ($P = 0.02$, Fig. 3G). In the subsoil, POD and PHO activities were not significantly different among cropping systems (Fig. 3G-H).

3.4. Correlations between plant and soil properties

In the topsoil, root biomass was positively correlated with NAG and AP, and negatively with PHO (Fig. 4A, B). Plant C/N ratio was negatively correlated with NAG and AP and positively with PHO (Fig. 4B). The aliphatic to aromatic/carboxylic compounds ratio was significantly correlated with root biomass (negatively), PHO (positively) and plant C/N ratio (positively) in the topsoil, while these correlations were non-significant in the subsoil (Fig. 4C, D). In the subsoil, both bulk SOC stock and MAOC stock were positively correlated with MBC (Fig. 4D).

4. Discussion

4.1. SOC pools in perennial and annual cropping systems

4.1.1. Perennials did not increase topsoil SOC stocks in comparison to annual cropping systems

Previous studies suggest that perennial crops, due to their longer growing seasons and greater solar radiation capture, generally have higher aboveground and root biomass production than annual crops, contributing to higher SOC inputs (Friedman, 2020; Thorup-Kristensen et al., 2020). In alignment with this, we found that perennial tall fescue produced the highest average dry matter yield (~16.8 Mg ha^{-1}) and root biomass (354 g m^{-2}) between 2013 and 2021 (Table S1, Fig. 2A), surpassing triticale (~11.2 Mg ha^{-1}), triticale in rotation (~16.5 Mg ha^{-1}), and grass/legume mixtures (~10.2 Mg ha^{-1}). Similarly, the grass/legume mixtures also had higher root biomass (233 g m^{-2}, Fig. 2A) than annual crops. However, after a decade of cultivation, differences in topsoil SOC stocks between perennial and annual cropping systems were negligible. This trend was consistent for POC and MAOC

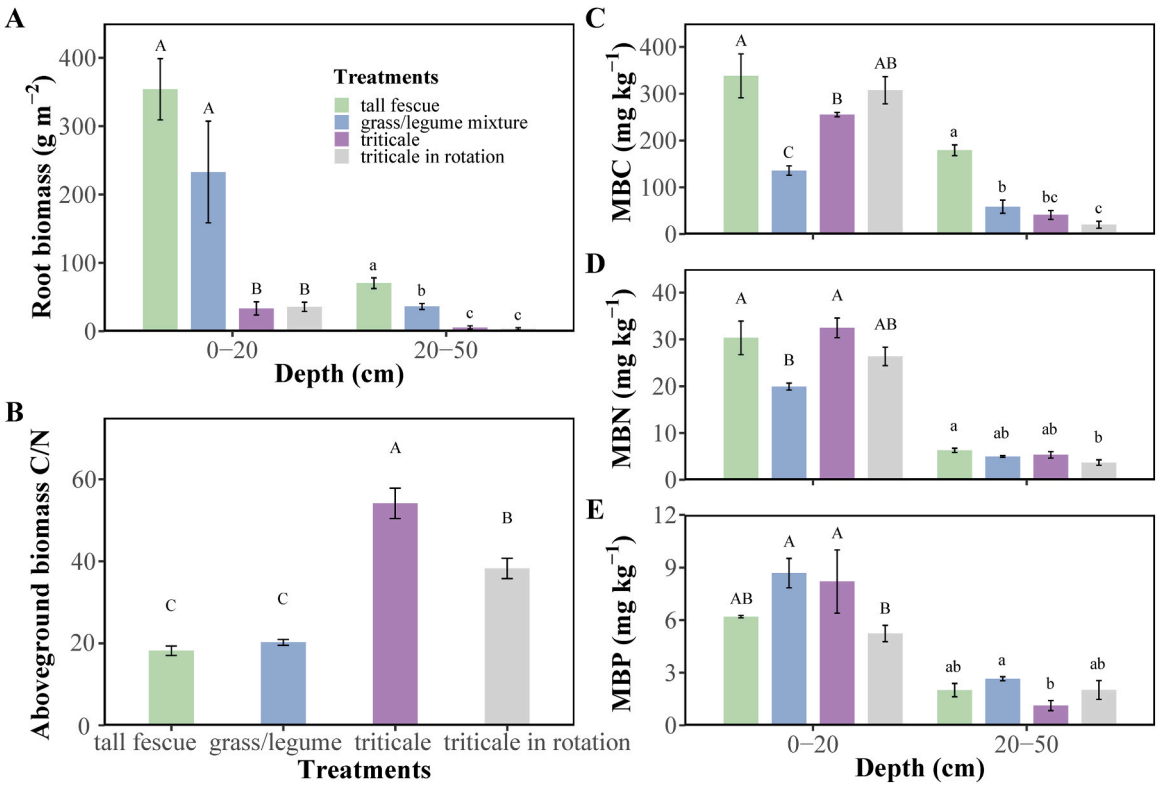

Fig. 2. Root biomass (A), C/N ratio of plant aboveground biomass (B) and microbial biomass indices (C-E) in perennial and annual cropping systems from the long-term experiment. The perennials were tall fescue and grass/legume mixture, and the annuals were triticale in monoculture and triticale in rotation. Indices of microbial biomass were microbial biomass carbon (MBC), nitrogen (MBN), and phosphorus (MBP). Data are means ± standard error ($n = 4$). Different upper- and lower-case letters indicate significant differences ($P < 0.05$) among cropping systems at 0–20 and 20–50 cm soil depths, respectively.

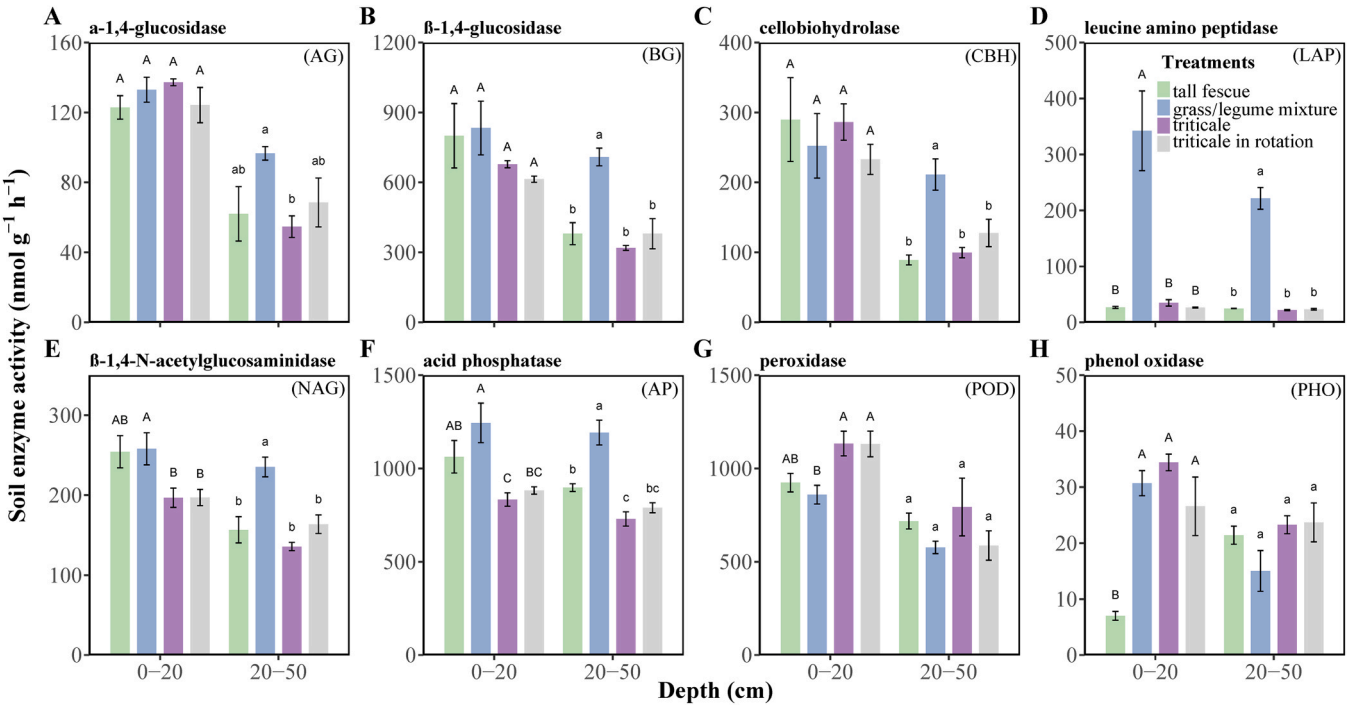

Fig. 3. Extracellular hydrolase (A-F) and oxidase (G, H) activity in perennial and annual cropping systems from the long-term experiment. The perennials were tall fescue and grass/legume mixture, and the annuals were triticale in monoculture and triticale in rotation. Data are means ± standard error ($n = 4$). Different upper- and lower-case letters indicate significant differences ($P < 0.05$) among cropping systems at 0–20 and 20–50 cm soil depths, respectively.

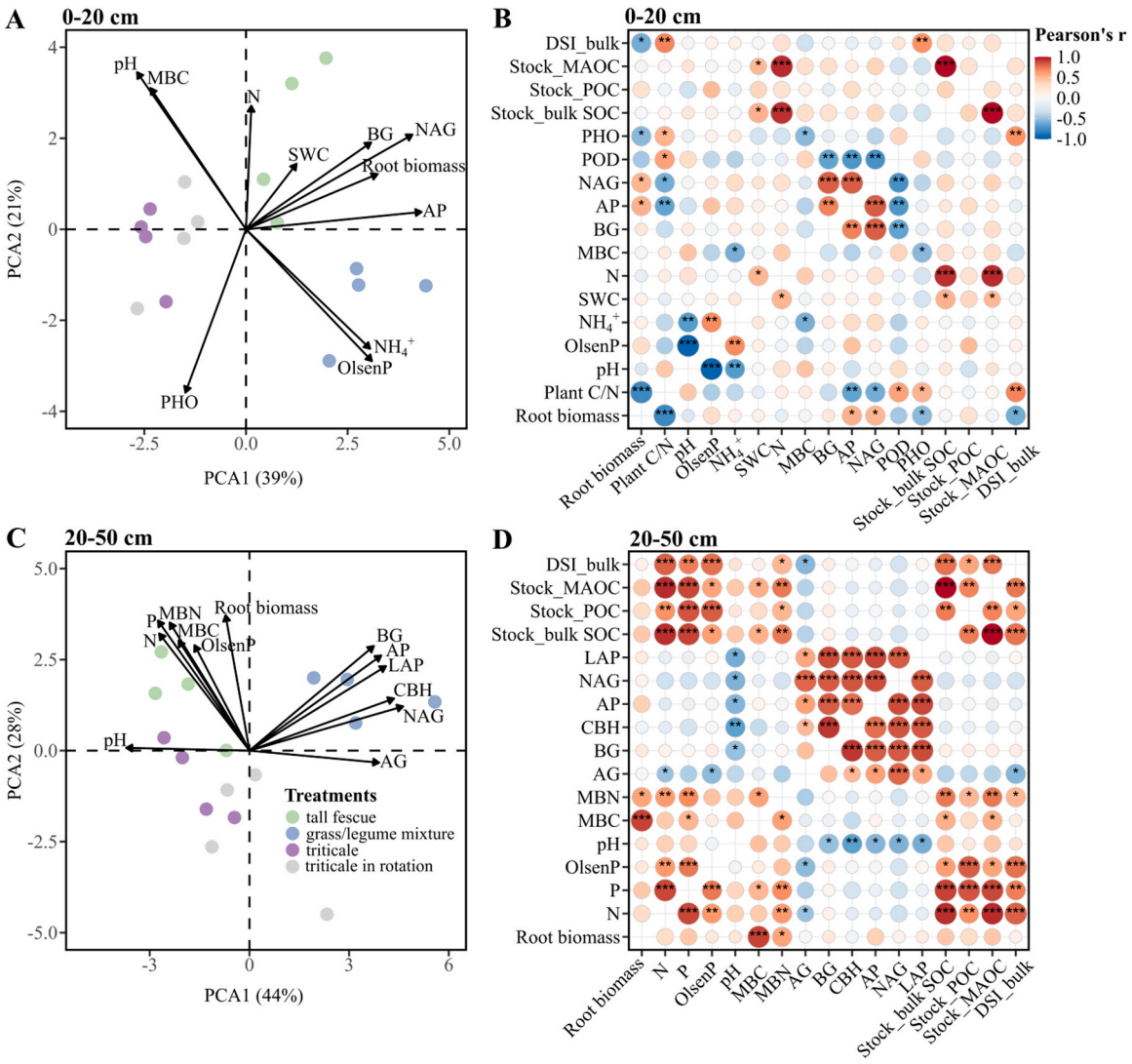

Fig. 4. Principal component analysis (PCA) and correlation heatmaps for plant and soil properties at two depths (0–20 cm and 20–50 cm). Red and blue color gradients represent the strength and direction of positive and negative correlations, respectively. Significant correlations are denoted by asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). The abbreviations are Plant C/N, the ratio of C and N of aboveground biomass; MBC, microbial biomass carbon; TN, total soil nitrogen; SWC, soil water content; AG, α-1,4-glucosidase; BG, β-1,4-glucosidase; CBH, cellobiohydrolase; LAP, leucine amino peptidase; NAG, N-acetyl-β-D-glucosaminidase; AP, acid phosphatase; PHO, phenol oxidase; POD, peroxidase; Stock_bulk SOC, SOC stocks in bulk soil; Stock_POC, particulate organic carbon stocks; Stock_MAOC, mineral associated organic carbon stocks; DSI_bulk, the ratio of aliphatic to aromatic/carboxylic compounds.

stock (Fig. 1A).

It is possible that the 10-year experimental period may still not capture the long-term effects of perennials on SOC accumulation. Nevertheless, in the present study, the potential SOC benefit from the higher biomass yields of perennials could also be limited by management practices in the combination of sandy soil conditions. All aboveground biomass was harvested annually for biorefinery purposes, so the belowground C was the primary C input to the soil. Even though perennial cropping systems generally involve less soil disturbance, tillage was applied once during the renewal year, which may have offset some of the expected benefits of reduced disturbance and contributed to SOC turnover. The absence of N fertilizer in the grass/legume mixture also likely restricted SOC inputs by limiting biomass productivity and nutrient cycling, as reflected by reduced MBN, lower MBC/SOC ratios in the topsoil (Fig. 2D, Fig. S3), and elevated C, N and P enzyme activities associated with nutrient acquisition (Fig. 3). Although tall fescue had the highest root biomass C inputs, higher MBC and soil enzyme activities (Figs. 2C, 3) could also accelerate SOC decomposition, preventing the accumulation of POM. We would have expected the improved microbial anabolism eventually resulted in enhanced MOAM (Liang et al., 2017). However, unlike clay-rich soils, our sandy soils offer weaker potential for physical and chemical protection of SOC (Cheng et al., 2015; Kim et al., 2022), making the accumulation of MAOM more challenging.

4.1.2. Tall fescue tended to increase subsoil SOC stocks in comparison to annual cropping systems

In the subsoil layers, MAOC stock under perennial tall fescue showed a tendency to accumulate by 0.35 Mg ha^{-1} yr^{-1} in comparison to triticale ($P = 0.8$, Fig. 1A). This should be interpreted with caution, but such MAOC accumulations would be important in sandy soils, which are prone to higher organic matter decomposition and loss (Witzgall et al., 2021). Accumulation in SOC stocks in the subsoil by tall fescue systems would align with previous research highlighting the potential for long-term stable SOC formation through deep root systems inputs (Kell, 2012). In our study, tall fescue had significantly higher subsoil root biomass than annual crops and grass/legume mixtures (Fig. 2A), which also likely increased organic matter inputs like root exudates, thereby boosting MBC activities (Figs. 2C, 4D) and necromass accumulation,

which are key precursors for MAOC formation (Fig. 4D; Ledo et al., 2020; Liang et al., 2017). More root exudates in tall fescue, composed of small molecular compounds, may also adsorb directly onto mineral surfaces, further stabilizing MAOM (Sokol and Bradford, 2019; Poeplau et al., 2021). Moreover, the deep root systems of tall fescue may also improve soil structure by forming stable aggregates through these exudates in the sandy soil (Zhang et al., 2023), protecting organic matter from decomposition and supporting stable SOC accumulation by limiting enzymatic activity in the subsoil (Fig. 3).

4.1.3. Tall fescue could have higher proportion of topsoil stable SOC than other systems

Despite the lack of effect on SOC quantity in the topsoil, the SOC composition assessed by DRIFT-MIR analysis showed significantly lower aliphatic to aromatic/carboxylic ratios for tall fescue compared to the other cropping systems (Fig. 1B). This indicates a higher depletion of easily decomposed organic C inputs from root exudates or low C/N ratio of aboveground biomass residues (Fig. 4B), likely due to enhanced microbial activity. This difference may also be associated with the higher root biomass of tall fescue (Fig. 4B). Dead root biomass tends to be richer in aromatic compounds like lignin, and living roots can release additional aromatic secondary metabolites, such as flavonoids and phenylpropanoids, into the rhizosphere (Bais et al., 2008). Although legumes are also generally rich in flavonoids, the relatively low proportion of legumes (20 %) in our grass-legume mixtures likely reduced their overall N-fixing capacity, thereby limiting their contribution to aromatic compounds compared to the grasses. Furthermore, in the rotation system, hemp was replaced by faba bean only in 2020. Given the short duration of legume cultivation and the specific management practices (full removal of aboveground biomass and shallow tillage after harvest), the influence of legumes on aromatic compounds in our study was likely minimal. Additionally, the higher aromatic content in tall fescue aboveground residues (compared to those of annuals during their growth) may make them less palatable to insects (Mastellar et al., 2024), thereby protecting the leaves. These processes can contribute to the accumulation of aromatic compounds in the soil. Furthermore, the positive relationship between phenol oxidase and aliphatic to aromatic/carboxylic ratio indicates that lower phenol oxidase activity in tall fescue limits the degradation of complex and aromatic compounds, resulting in their accumulation in the soil (Fig. 4B; Sinsabaugh, 2010). Therefore, the relative enrichment of aromatic compounds in combination with low phenol oxidase activity might represent a crucial mechanism for long-term SOM preservation in the perennial system of tall fescue. These findings collectively suggest that combining physical and SOC compound specific indicators provides a more comprehensive understanding of mechanisms and drivers of SOC dynamics and stability.

4.2. Increased enzyme activities and microbial adaptations in perennials

Perennial tall fescue stimulated enzyme activities in the topsoil, including NAG and AP, compared to annual crops (Fig. 3E, F), while the activity of carbon-acquiring enzymes, such as α-glucosidase, β-glucosidase and cellobiohydrolase, remained relatively stable across systems (Fig. 3A, B, C). This pattern suggests that compared to other systems, microbial communities in tall fescue soils faced greater demand for N and P. Tall fescue was harvested three times annually for biorefinery purposes, leading to substantial and repeated removal of nutrients from the system. Despite relatively high rates of N fertilizer input, the combination of frequent biomass exports and the nutrient demands of microbes and plant regrowth likely resulted in localized N and P limitations. Under these conditions, soil microbial communities appear to have responded by increasing the production of extracellular enzymes that facilitate the mineralization of organic N and P pools (Hargreaves and Hofmockel, 2014).

In the grass/legume mixture, LAP activity was highest in the topsoil, with NAG showing a similar trend, compared to other cropping systems (Fig. 3D, E). This increase in enzyme activity is likely influenced by the presence of legumes in the mixture, which add organic N to the soil through biological N fixation and subsequent exudation and via their N-rich residues (Drinkwater et al., 1998; Fustec et al., 2010). Specifically, LAP enzymes play a role in breaking down proteins into amino acids, releasing available N into the soil, while NAG targets N-rich polysaccharides like chitin, further increasing the available N pool (Table 1).

Compared to the other cropping systems, the grass/legume mixture without N fertilization also had higher activities of C- and P-acquiring enzymes (BG and AP), particularly in the subsoil (Fig. 3). This might be an adaptive response to C source and nutrient limitations experienced by both plants and microbes in this low-N environment. Generally, the reduced MBC and MBN levels in the grass/legume system (Table 1) indicate that microbial communities are under nutrient stress conditions, where microbes increase their metabolic activities to maximize nutrient release from organic matter via secretion of C-, N-, and P-acquiring enzymes (Mooshammer et al., 2014). Furthermore, microbes primarily mobilize nutrients like P to meet their own needs, which is often insufficient for plant demands (Tinker, 1980). To compensate, plant roots can release extracellular enzymes to decompose organic substrates and enhance nutrient uptake (George et al., 2008; Richardson et al., 2009). In our mixed cropping systems without N fertilization, a greater proportion of grasses than legume may result in enzyme release to increase nutrient acquisition to support grass growth.

Compared to triticale and triticale in rotation, tall fescue had lower PHO and POD activity in the topsoil (Fig. 3G-F). The positive correlation between aboveground plant C/N ratio and soil oxidase activity suggests that the input of high C/N ratio biomass from triticale during crop senescence and harvesting can stimulate soil oxidase activities (Fig. 4A, B). This agrees with the study by Tian and Shi. (2014), which showed that litter inputs with high C/N ratio promote soil oxidase production. Conversely, fresh harvested biomass of perennial tall fescue had a low C/N ratio and low structural complexity, which requires less oxidase enzymes to decompose (Sinsabaugh, 2010). The application of high N fertilizer rates in tall fescue may also be an important factor in contributing to reduction in extracellular oxidase activity. High N inputs favor copiotrophic bacteria that are efficient at using simple organic compounds in their metabolism. These bacteria outcompete fungi that produce complex enzymes such as phenol oxidase and peroxidase to decompose more complex organic matter (Wang and Kuzyakov, 2024).

4.3. Implications and uncertainties

This study highlights the challenges and opportunities of enhancing SOC storage in sandy soils, which have low C protection capacity and high organic matter turnover. Due to their weaker physical and chemical protection compared to clay-rich soils, sandy soils have an inherently limited potential for SOC storage potential, particularly under cropping systems for future biorefinery that involve high harvest frequency and rely solely on synthetic fertilizers. However, perennial cropping systems, such as tall fescue with its deep root systems, show promise for improving subsoil SOC storage. This underscores the need to further explore deep-rooted species for long-term stable SOC sequestration in sandy soils. Grass/legume mixtures are also increasingly adopted in Europe as a diverse and sustainable cropping practice to enhance soil fertility and reduce reliance on external inputs (Nyfeler et al., 2011). However, the limited SOC improvement in grass/legume mixtures emphasizes the importance of increasing legume proportions, optimizing grass-to-legume ratios, and supplementing N inputs to enhance productivity and C inputs. Thus, management strategies with focus on microbial activity and extracellular enzyme production could become important for balancing nutrient cycling and SOC stabilization.

A limitation of the present study is that some soil indicators, such as MBC, MBN, and enzyme activities, can vary significantly with time and season. Soil samples were taken only once (before harvest), which may

not fully capture system differences. Repeated measurements across several seasons would be needed to draw more robust conclusions. Future research should also focus on the temporal dynamics and interactions between plant and microbial processes to ensure C retention in diverse cropping systems. Nevertheless, this study assessed SOC stocks and stabilization under realistic agricultural management in sandy soils, where perennial crops often receive different fertilization regimes than annual crops. Thus, our results reflect the overall effects of the cropping system over a decade, and not only the isolated effect of the perennial crop species. Since a 10-year field experiment may not fully capture the long-term effects on SOC stability, particularly in perennial systems where the development of root-derived C and subsoil processes is slow, longer-term experiments are needed to confirm the trends and provide deeper insights into SOC stabilization mechanisms.

5. Conclusions

This study investigated the effects of annual and perennial cropping systems on SOC stocks and stability in sandy soils, with a focus on the underlying biochemical processes. Despite higher root biomass in perennial tall fescue compared to annual crops, negligible differences in SOC stocks and its fractions were observed in the topsoil, likely due to the higher SOC turnover driven by elevated nutrient-releasing enzyme activities. However, DRIFT-MIR analyses showed that the tall fescue system had a lower aliphatic to aromatic/carboxylic ratio in the topsoil compared to annual crop systems. This was associated with greater root biomass and reduced phenol oxidase activity, highlighting the role of chemical recalcitrance to SOC stabilization in this system. In the subsoil, tall fescue showed a higher potential for MAOC accrual due to higher MBC, compared to annual crops. In contrast, the grass/legume system without N fertilization did not increase SOC stock and stability compared to annual crops, likely due to nutrient limitation as evidenced by higher C, N, and P-acquiring enzyme activities as well as lower microbial biomass C and N contents. Altogether, our study indicates that adopting perennial cropping systems like tall fescue and optimizing N fertilization management in grass/legume systems may improve crop productivity and SOC stability in sandy soils, contributing to sustainable agriculture and climate change mitigation.

CRediT authorship contribution statement

Jørgensen Uffe: Writing – review & editing, Project administration, Funding acquisition. **Schiedung Marcus:** Writing – review & editing, Methodology. **Poeplau Christopher:** Writing – review & editing, Methodology. **Elsgaard Lars:** Writing – review & editing. **Li Yue:** Investigation. **Liang Zhi:** Writing – review & editing. **Chen Ji:** Writing – review & editing, Methodology, Funding acquisition. **Abalos Diego:** Writing – review & editing, Supervision, Methodology. **Zong Mingming:** Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.eja.2025.127639.

Data availability

Data will be made available on request.

References

Angst, G., Mueller, K.E., Castellano, M.J., Vogel, C., Wiesmeier, M., Mueller, C.W., 2023. Unlocking complex soil systems as carbon sinks: multi-pool management as the key. Nature Communications 14, 2967. https://doi.org/10.1038/s41467-023-38700-5.

Bais, H.P., Broeckling, C.D., Vivanco, J.M., 2008. Root exudates modulate plant-microbe interactions in the rhizosphere. Secondary metabolites in soil ecology. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 241–252.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Beck, T., Joergensen, R.G., Kandeler, E., Makeschin, F., Nuss, E., Oberholzer, H.R., Scheu, S., 1997. An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. Soil Biology and Biochemistry 29, 1023–1032. https://doi.org/10.1016/S0038-0717(97)00030-8.

Begill, N., Don, A., Poeplau, C., 2023. No detectable upper limit of mineral-associated organic carbon in temperate agricultural soils. Global Change Biology 29, 4662–4669. https://doi.org/10.1111/gcb.16804.

Bell, C.W., Fricks, B.E., Rocca, J.D., Steinweg, J.M., McMahon, S.K., Wallenstein, M.D., 2013. High-throughput fluorometric measurement of potential soil extracellular enzyme activities. Journal of Visualized Experiments (81), e50961. https://doi.org/10.3791/50961.

Brookes, P.C., Powlson, D.S., Jenkinson, D.S., 1982. Measurement of microbial biomass phosphorus in soil. Soil Biology and Biochemistry 14, 319–329. https://doi.org/10.1016/0038-0717(82)90001-3.

Brookes, P.C., Landman, A., Pruden, G., Jenkinson, D.S., 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry 17, 837–842. https://doi.org/10.1016/0038-0717(85)90144-0.

Calderón, F., Haddix, M., Conant, R., Magrini-Bair, K., Paul, E., 2013. Diffuse-Reflectance Fourier-Transform Mid-Infrared Spectroscopy as a method of characterizing changes in soil organic matter. Soil Science Society of America Journal 77, 1591–1600. https://doi.org/10.2136/sssaj2013.04.0131.

Carter, M.R., Gregorich, E.G., 2010. Carbon and nitrogen storage by deep-rooted tall fescue (*Lolium arundinaceum)* in the surface and subsurface soil of a fine sandy loam in eastern Canada. Agriculture, Ecosystems & Environment 136, 125–132. https://doi.org/10.1016/j.agee.2009.12.005.

Cattaneo, F., Di Gennaro, P., Barbanti, L., Giovannini, C., Labra, M., Moreno, B., Benitez, E., Marzadori, C., 2014. Perennial energy cropping systems affect soil enzyme activities and bacterial community structure in a South European agricultural area. Applied Soil Ecology 84, 213–222. https://doi.org/10.1016/j.apsoil.2014.08.003.

Cenini, V.L., Fornara, D.A., McMullan, G., Ternan, N., Carolan, R., Crawley, M.J., Clément, J.-C., Lavorel, S., 2016. Linkages between extracellular enzyme activities and the carbon and nitrogen content of grassland soils. Soil Biology and Biochemistry 96, 198–206. https://doi.org/10.1016/j.soilbio.2016.02.015.

Chen, J., Lærke, P.E., Jørgensen, U., 2022. Land conversion from annual to perennial crops: A win-win strategy for biomass yield and soil organic carbon and total nitrogen sequestration. Agriculture, Ecosystems and Environment 330, 107907. https://doi.org/10.1016/j.agee.2022.107907.

Cheng, M., Xiang, Y., Xue, Z., An, S., Darboux, F., 2015. Soil aggregation and intra-aggregate carbon fractions in relation to vegetation succession on the Loess Plateau, China. CATENA 124, 77–84. https://doi.org/10.1016/j.catena.2014.09.006.

DeForest, J.L., 2009. The influence of time, storage temperature, and substrate age on potential soil enzyme activity in acidic forest soils using MUB-linked substrates and l-DOPA. Soil Biology and Biochemistry 41, 1180–1186. https://doi.org/10.1016/j.soilbio.2009.02.029.

Demyan, M.S., Rasche, F., Schulz, E., Breulmann, M., Müller, T., Cadisch, G., 2012. Use of specific peaks obtained by diffuse reflectance Fourier transform mid-infrared spectroscopy to study the composition of organic matter in a Haplic Chernozem. European Journal of Soil Science 63, 189–199. https://doi.org/10.1111/j.1365-2389.2011.01420.x.

Drinkwater, L.E., Wagoner, P., Sarrantonio, M., 1998. Legume-based cropping systems have reduced carbon and nitrogen losses. Nature 396, 262–265. https://doi.org/10.1038/24376.

Farooq, U., Gorczewska-Langner, W., Szymkiewicz, A., 2024. Water retention curves of sandy soils obtained from direct measurements, particle size distribution, and infiltration experiments. Vadose Zone Journal 23, e20364. https://doi.org/10.1002/vzj2.20364.

Friedman, J., 2020. The evolution of annual and perennial plant life histories: ecological correlates and genetic mechanisms. Annual Review of Ecology, Evolution, and Systematics 51, 461–481. https://doi.org/10.1146/annurev-ecolsys-110218-024638.

Fustec, J., Lesuffleur, F., Mahieu, S., Cliquet, J.-B., 2010. Nitrogen rhizodeposition of legumes. A review. Agronomy for Sustainable Development 30, 57–66. https://doi.org/10.1051/agro/2009003.

George, T.S., Gregory, P.J., Hocking, P., Richardson, A.E., 2008. Variation in root-associated phosphatase activities in wheat contributes to the utilization of organic P substrates in vitro, but does not explain differences in the P-nutrition of plants when grown in soils. Environmental and Experimental Botany 64, 239–249. https://doi.org/10.1016/j.envexpbot.2008.05.002.

Grossman, R.B., Reinsch, T.G., 2002. 2.1 Bulk density and linear extensibility. In: methods of soil analysis. John Wiley & Sons, Ltd, pp. 201–228. https://doi.org/10.2136/sssabookser5.4.c9.

Harbo, L.S., Olesen, J.E., Liang, Z., Christensen, B.T., Elsgaard, L., 2022. Estimating organic carbon stocks of mineral soils in Denmark: Impact of bulk density and content of rock fragments. Geoderma Regional 30, e00560. https://doi.org/10.1016/j.geodrs.2022.e00560.

Hargreaves, S.K., Hofmockel, K.S., 2014. Physiological shifts in the microbial community drive changes in enzyme activity in a perennial agroecosystem. Biogeochemistry 117, 67–79. https://doi.org/10.1007/s10533-013-9893-6.

Heidmann, T., 1989. Startkarakterisering af arealer til systemforskning II. Resultater fra arealet ved. Foulum Tidsskrift for Planteavls Specialserie S 1–186.

Jagadamma, S., Lal, R., 2010. Integrating physical and chemical methods for isolating stable soil organic carbon. Geoderma 158, 322–330. https://doi.org/10.1016/j.geoderma.2010.05.014.

Just, C., Poeplau, C., Don, A., van Wesemael, B., Kögel-Knabner, I., Wiesmeier, M., 2021. A simple approach to isolate slow and fast cycling organic carbon fractions in central European soils—importance of dispersion method. Frontiers in Soil Science 1.

Kafkafi, U., 1972. Soil phosphorus. In: Halmann, M. (Ed.), Analytical chemistry of phosphorus compounds. Wiley, New York, pp. 727–774.

Kell, D.B., 2012. Large-scale sequestration of atmospheric carbon via plant roots in natural and agricultural ecosystems: why and how. Philosophical Transactions of the Royal Society B: Biological Sciences 367, 1589–1597. https://doi.org/10.1098/rstb.2011.0244.

Kim, K., Daly, E.J., Gorzelak, M., Hernandez-Ramirez, G., 2022. Soil organic matter pools response to perennial grain cropping and nitrogen fertilizer. Soil and Tillage Research 220, 105376. https://doi.org/10.1016/j.still.2022.105376.

Krogh, L., Greve, M.H., 1999. Evaluation of World Reference Base for Soil Resources and FAO Soil Map of the World using nationwide grid soil data from Denmark. Soil Use and Management 15, 157–166. https://doi.org/10.1111/j.1475-2743.1999.tb00082.x.

Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. Soil Biology and Biochemistry 32, 1485–1498. https://doi.org/10.1016/S0038-0717(00)00084-5.

Laub, M., Demyan, M.S., Nkwain, Y.F., Blagodatsky, S., Kätterer, T., Piepho, H.-P., Cadisch, G., 2020. DRIFTS band areas as measured pool size proxy to reduce parameter uncertainty in soil organic matter models. Biogeosciences 17, 1393–1413. https://doi.org/10.5194/bg-17-1393-2020.

Lavallee, J.M., Soong, J.L., Cotrufo, M.F., 2020. Conceptualizing soil organic matter into particulate and mineral-associated forms to address global change in the 21st century. Global Change Biology 26, 261–273. https://doi.org/10.1111/gcb.14859.

Ledo, A., Smith, P., Zerihun, A., Whitaker, J., Vicente-Vicente, J.L., Qin, Z., McNamara, N.P., Zinn, Y.L., Llorente, M., Liebig, M., Kuhnert, M., Dondini, M., Don, A., Diaz-Pines, E., Datta, A., Bakka, H., Aguilera, E., Hillier, J., 2020. Changes in soil organic carbon under perennial crops. Global Change Biology 26, 4158–4168. https://doi.org/10.1111/gcb.15120.

Lehmann, J., Kleber, M., 2015. The contentious nature of soil organic matter. Nature 528, 60–68. https://doi.org/10.1038/nature16069.

Lenth, R., 2019. emmeans: estimated marginal means, aka least-squares means. R package version 1.4. 3.01.

Liang, C., Schimel, J.P., Jastrow, J.D., 2017. The importance of anabolism in microbial control over soil carbon storage. Nature Microbiology 2, 1–6. https://doi.org/10.1038/nmicrobiol.2017.105.

Manevski, K., Lærke, P.E., Jiao, X., Santhome, S., Jørgensen, U., 2017. Biomass productivity and radiation utilisation of innovative cropping systems for biorefinery. Agricultural and Forest Meteorology 233, 250–264. https://doi.org/10.1016/j.agrformet.2016.11.245.

Margenot, A.J., Parikh, S.J., Calderón, F.J., 2023. Fourier-transform infrared spectroscopy for soil organic matter analysis. Soil Science Society of America Journal 87, 1503–1528. https://doi.org/10.1002/saj2.20583.

Mastellar, S.L., Lowe, B., Haden, V.R., Bennett-Wimbush, K., 2024. Equine relative preferences regarding selected cool-season grass hays. Animal Feed Science and Technology 315, 116011. https://doi.org/10.1016/j.anifeedsci.2024.116011.

Matocha, C.J., Haszler, G.R., Grove, J.H., 2004. Nitrogen fertilization suppresses soil phenol oxidase enzyme activity in no-tillage systems. Soil Science 169, 708. https://doi.org/10.1097/01.ss.0000146023.53936.6c.

Mooshammer, M., Wanek, W., Zechmeister-Boltenstern, S., Richter, A.A., 2014. Stoichiometric imbalances between terrestrial decomposer communities and their resources: mechanisms and implications of microbial adaptations to their resources. Frontiers in Microbiology 5. https://doi.org/10.3389/fmicb.2014.00022.

Nyfeler, D., Huguenin-Elie, O., Suter, M., Frossard, E., Lüscher, A., 2011. Grass–legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. Agriculture, Ecosystems & Environment 140, 155–163. https://doi.org/10.1016/j.agee.2010.11.022.

Olsen, S.R., 1982. Anion resin extractable phosphorus. Methods of Soil Analysis 2, 423–424.

Paustian, K., Lehmann, J., Ogle, S., Reay, D., Robertson, G.P., Smith, P., 2016. Climate-smart soils. Nature 532, 49–57. https://doi.org/10.1038/nature17174.

Poeplau, C., Don, A., 2013. Sensitivity of soil organic carbon stocks and fractions to different land-use changes across Europe. Geoderma 192, 189–201. https://doi.org/10.1016/j.geoderma.2012.08.003.

Poeplau, C., Don, A., Schneider, F., 2021. Roots are key to increasing the mean residence time of organic carbon entering temperate agricultural soils. Global Change Biology 27, 4921–4934. https://doi.org/10.1111/gcb.15787.

R Development Core Team, 2020. R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Rasse, D.P., Rumpel, C., Dignac, M.F., 2005. Is soil carbon mostly root carbon? Mechanisms for a specific stabilisation. Plant and Soil 269, 341–356. https://doi.org/10.1007/s11104-004-0907-y.

Richardson, A.E., Barea, J.-M., McNeill, A.M., Prigent-Combaret, C., 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant and Soil 321, 305–339. https://doi.org/10.1007/s11104-009-9895-2.

Rui, Y., Jackson, R.D., Cotrufo, M.F., Sanford, G.R., Spiesman, B.J., Deiss, L., Culman, S. W., Liang, C., Ruark, M.D., 2022. Persistent soil carbon enhanced in Mollisols by well-managed grasslands but not annual grain or dairy forage cropping systems. Proceedings of the National Academy of Sciences of the United States of America 119, e2118931119. https://doi.org/10.1073/pnas.2118931119.

Siddique, I.A., Grados, D., Chen, J., Lærke, P.E., Jørgensen, U., 2023. Soil organic carbon stock change following perennialization: a meta-analysis. Agronomy for Sustainable Development 43, 58. https://doi.org/10.1007/s13593-023-00912-w.

Signorell, A., 2023. DescTools: tools for descriptive statistics. R package version 0.99.51. ⟨https://andrisignorell.github.io/DescTools/⟩.

Sinsabaugh, R.L., 2010. Phenol oxidase, peroxidase and organic matter dynamics of soil. Soil Biology and Biochemistry 42, 391–404. https://doi.org/10.1016/j.soilbio.2009.10.014.

Soil Survey Staff, 2014. Keys to soil taxonomy. 12th edn (USDA-Natural Resources Conservation Service: Washington, DC).

Sokol, N.W., Bradford, M.A., 2019. Microbial formation of stable soil carbon is more efficient from belowground than aboveground input. Nature Geoscience 12, 46–53. https://doi.org/10.1038/s41561-018-0258-6.

Stevens, A., Ramirez-Lopez, L., 2022. Miscellaneous functions for processing and sample selection of spectroscopic data, Package Version 0.2.6.

Stott, D.E., Andrews, S.S., Liebig, M.A., Wienhold, B.J., Karlen, D.L., 2010. Evaluation of β-glucosidase activity as a soil quality indicator for the soil management assessment framework. Soil Science Society of America Journal 74, 107–119. https://doi.org/10.2136/sssaj2009.0029.

Thorup-Kristensen, K., Halberg, N., Nicolaisen, M., Olesen, J.E., Crews, T.E., Hinsinger, P., Kirkegaard, J., Pierret, A., Dresbøll, D.B., 2020. Digging deeper for agricultural resources, the value of deep rooting. Trends in Plant Science 25, 406–417. https://doi.org/10.1016/j.tplants.2019.12.007.

Tian, L., Shi, W., 2014. Short-term effects of plant litter on the dynamics, amount, and stoichiometry of soil enzyme activity in agroecosystems. European Journal of Soil Biology 65, 23–29. https://doi.org/10.1016/j.ejsobi.2014.08.004.

Tinker, P.B., 1980. Role of rhizosphere microorganisms in phosphorus uptake by plants. In: the role of phosphorus in agriculture. John Wiley & Sons, Ltd, pp. 617–654. https://doi.org/10.2134/1980.roleofphosphorus.c23.

Van Reeuwijk L.P., 2002. Procedures for Soil Analysis. Wageningen, International Soil Reference and Information Centre.

Vogt, E.T.C., Weckhuysen, B.M., 2024. The refinery of the future. Nature 629, 295–306. https://doi.org/10.1038/s41586-024-07322-2.

von Lützow, M., Kögel-Knabner, I., Ekschmitt, K., Flessa, H., Guggenberger, G., Matzner, E., Marschner, B., 2007. SOM fractionation methods: Relevance to functional pools and to stabilization mechanisms. Soil Biology and Biochemistry 39, 2183–2207. https://doi.org/10.1016/j.soilbio.2007.03.007.

Waldrop, M.P., Zak, D.R., Sinsabaugh, R.L., Gallo, M., Lauber, C., 2004. Nitrogen deposition modifies soil carbon storage through changes in microbial enzymatic activity. Ecological applications 14, 1172–1177. https://doi.org/10.1890/03-5120.

Wang, C., Kuzyakov, Y., 2024. Mechanisms and implications of bacterial-fungal competition for soil resources. The ISME Journal 18, wrae073. https://doi.org/10.1093/ismejo/wrae073.

Wang, H., Hu, G., Xu, W., Boutton, T.W., Zhuge, Y., Bai, E., 2018. Effects of nitrogen addition on soil organic carbon mineralization after maize stalk addition. European Journal of Soil Biology 89, 33–38. https://doi.org/10.1016/j.ejsobi.2018.10.002.

Witzgall, K., Vidal, A., Schubert, D.I., Höschen, C., Schweizer, S.A., Buegger, F., Pouteau, V., Chenu, C., Mueller, C.W., 2021. Particulate organic matter as a functional soil component for persistent soil organic carbon. Nature Communications 12, 4115. https://doi.org/10.1038/s41467-021-24192-8.

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 Biology and Biochemistry 22, 1167–1169. https://doi.org/10.1016/0038-0717(90)90046-3.

Zhang, W., Munkholm, L.J., Liu, X., An, T., Xu, Y., Ge, Z., Xie, N., Li, A., Dong, Y., Peng, C., Li, S., Wang, J., 2023. Soil aggregate microstructure and microbial community structure mediate soil organic carbon accumulation: Evidence from one-year field experiment. Geoderma 430, 116324. https://doi.org/10.1016/j.geoderma.2023.116324.

Zong, M., Manevski, K., Liang, Z., Abalos, D., Jabloun, M., Lærke, P.E., Jørgensen, U., 2024. Diversifying maize rotation with other industrial crops improves biomass yield and nitrogen uptake while showing variable effects on nitrate leaching. Agriculture, Ecosystems & Environment 371, 109091. https://doi.org/10.1016/j.agee.2024.109091.