Geoderma 454 (2025) 117204

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Tillage and residue management modulate the links between soil physical signatures and arbuscular mycorrhizal fungal biomarkers

Stamatios Thomopoulos [a], Lars Juhl Munkholm [b], Lars Elsgaard [b], Sabine Ravnskov [a,*]

[a] Department of Agroecology, Aarhus University, Forsøgsvej 1, 4200 Slagelse, Denmark
[b] Department of Agroecology, Aarhus University, Blichers Alle 20, 8830 Tjele, Denmark

ARTICLE INFO

Handling Editor: Y. Capowiez

Keywords:
Arbuscular mycorrhizal fungi
Conservation agriculture
Soil aggregation
Soil pore system
Soil organic carbon

ABSTRACT

Conservation practices such as direct sowing and residue incorporation are crucial for enhancing soil health. This study investigated the long-term effects of different tillage practices and crop residue management on soil biological and physical health indices to elucidate their interconnections. The impact of tillage intensity (direct sowing, harrowing, moldboard ploughing) was assessed in combination with residue management (retention or removal) across two soil depths (0–10 and 10–20 cm) and two experimental sites. Measurements included three soil biological indicators—two fatty-acid biomarkers of arbuscular mycorrhizal fungi (AMF) and easily extractable glomalin-related soil protein (EE) – as well as two soil physical indices (water-stable aggregates (WSA), and clay dispersibility), five soil pore characteristics (air permeability, gas diffusivity, tortuosity, total porosity, air-filled porosity, and volumetric water content) and soil organic carbon (SOC). Conservation agriculture practices increased the presence of AMF, while the importance of considering soil depth in AMF biomass measurements was underscored. Harrowing and direct sowing treatments resulted in a vertical stratification of SOC. Residue retention increased SOC levels by 5 % and 15 % at the two sites and only significantly at the latter. Minimal soil disturbance enhanced wet aggregate stability by 14 % on average but negatively affected pore characteristics. AMF played a critical role in soil aggregate stability, evidenced by a strong correlation ($r = 0.68$ and $r = 0.86$ in the two sites) between hyphal networks and WSA. The study also demonstrated that direct sowing strengthened the relationship between EE and AMF ($r = 0.52$ and $r = 0.64$ for the two sites). In minimally disturbed soils, AMF contributed to a complex pore structure, with this effect being more closely related to EE than to the hyphal network. These findings underscore the significant role of AMF in maintaining soil health under various tillage practices and residue management strategies.

1. Introduction

Soil health is a multifaceted concept that evolved from work on soil quality in the early 2020 s (Lehmann et al., 2020). One definition of soil health is the ability of the soil to sustain the productivity, diversity, and environmental services of terrestrial ecosystems (FAO & ITPS, 2020). Improving soil health is a key sustainability goal, and recent attention has focused on developing agricultural management practices that can contribute to this goal (FAO, 2018; Walder et al., 2023). One strategy to promote soil health is the adaptation of conservation agriculture, a crop production system that includes three main management practices, namely minimal soil disturbance, crop residue retention, and crop rotations (FAO & ITPS, 2020; Hobbs et al., 2008). Soil health encompasses the physical, chemical, and biological properties of the soil, and conservation agriculture has the potential to affect each of these (Cárceles Rodríguez et al., 2022; Das et al., 2024; Indoria et al., 2017).

Direct sowing has shown positive effects on soil structural stability and water retention in the surface layer (0–10 cm) compared to traditional methods such as moldboard ploughing (Blanco-Canqui & Ruis, 2018). However, direct sowing could cause reductions in total porosity and air permeability, which is also considered to be the reason for the low adaptation rate of conservation agriculture in northern European

Abbreviations: AMF, arbuscular mycorrhizal fungi; WSA, water-stable aggregates; SOC, soil organic carbon; NLFA, neutral-lipid fatty acid; PLFA, phospholipid fatty acid; GRSP, glomalin-related soil protein; EE, easily extractable; DE, difficultly extractable; MP, moldboard ploughing; H, harrowing; D, direct sowing; ε_a, air-filled porosity; D_s/D_o, gas diffusivity.

* Corresponding author.
E-mail address: sabine.ravnskov@agro.au.dk (S. Ravnskov).

https://doi.org/10.1016/j.geoderma.2025.117204
Received 22 August 2024; Received in revised form 25 November 2024; Accepted 3 February 2025
Available online 9 February 2025

countries (Abdollahi et al., 2017; Abdollahi & Munkholm, 2017). Still, the retention of crop residues is reported to counteract the negative effects of direct sowing on soil structure by increasing aggregate stability and total porosity and reducing bulk density (Abdollahi & Munkholm, 2017; Gómez-Muñoz et al., 2021; Kumar et al., 2019).

Incorporation of crop residues is also reported to increase soil organic carbon (SOC) stocks under both direct sowing and moldboard ploughing practices (Aditi et al., 2023), at least until a new equilibrium is reached (Jensen et al., 2022). The observed increase in SOC content is one contributing factor linking no-till to improved soil physical properties, particularly structural stability (Blanco-Canqui & Ruis, 2018). Higher SOC can positively affect physical soil health indicators such as clay dispersibility or the Ontario Soil Health Assessment index (Chebet et al., 2023; Wepruk et al., 2023). However, direct sowing appears to affect the distribution of SOC across different soil layers (i.e., a vertical SOC stratification), rather than changing SOC stocks (Blanco-Canqui & Ruis, 2018). Therefore, the general improvement of surface-layer soil structure under no-till may be attributed to factors beyond just the SOC level (Rocco et al., 2024).

Arbuscular mycorrhizal fungi (AMF) are an important soil biota, that play a central role in supporting sustainable crop production and serve as indicators of soil health (Gianinazzi et al., 2010; Gupta, 2020). AMF form symbiotic relationships with approximately 72 % of all land plant species, where the fungi receive reduced organic carbon (C) compounds from the plants in exchange for soil-derived nutrients and water (Brundrett & Tedersoo, 2018). This mutualistic relationship results in the transfer of up to 30 % of the total C fixed by plants in the form of photosynthates to the fungi (Drigo et al., 2010). In a recent study, it was estimated that global plant communities allocate 3.93 Gt CO_2 equivalents annually to AMF mycelia (Hawkins et al., 2023). To estimate the living biomass of AMF, signature neutral lipid fatty acids (NLFA) and phospholipid fatty acids (PLFA) have been extensively employed (Giray et al., 2024; Hydbom & Olsson, 2021; Joergensen, 2021; Thomopoulos et al., 2023). Neutral lipids are associated with storage organs, such as spores and vesicles, while phospholipids are components of membranes related to arbuscules and hyphae (Olsson et al., 1997).

AMF play an important role in shaping soil structure and physical properties. Research indicates a positive relationship between AMF biomass and soil aggregation (Lin et al., 2023; Wilson et al., 2009). Moreover, AMF have been shown to enhance the stability of macroaggregates and enlarge soil macropores, thereby contributing to physical carbon sequestration (Morris et al., 2019; Wilson et al., 2009). The involvement of AMF in aggregate formation and stabilization is attributed to (i) biological factors, such as interactions within the soil food web and other microbial communities, (ii) biophysical aspects, i.e. the physical binding of soil particles by fungal hyphae (Degens, 1997), and (iii) biochemical effects, including the influence of glomalin-related soil proteins (GRSP) (Rillig & Mummey, 2006). The latter represents at least a temporary effect of AMF on soil stabilization, as the turnover time of GRSP in soil has been estimated to be 6 to 42 years (Rillig et al., 2001). Several studies report a correlation between AMF hyphae indicators and GRSP (Agnihotri et al., 2021; Thomopoulos et al., 2023); however, the extent of the association between AMF, GRSP, and the soil structural and pore characteristics remains controversial (Irving et al., 2021).

The overall objective of this study was to investigate the collective impacts of important components of long-term conservation agriculture, specifically soil mechanical practices and residue management, on soil health as indicated by AMF biomass and soil physical properties in two topsoil layers (0–10 and 10–20 cm). Another objective was to explore whether changes in AMF biomass were associated with variations in the soil structural stability and pore characteristics. It was hypothesized that (i) the long-term adoption of conservation agriculture practices, such as minimal soil disturbance and retention of crop residues, will promote soil health as indicated by AMF biomass, soil structural stability and soil pore characteristics, (ii) the AMF biomarkers will be related to changes in soil structural stability and pore characteristics, and (iii) this relationship will be more pronounced at lower tillage intensities.

2. Materials and methods

2.1. Experimental setup

The two experimental sites are located in Foulum (56°30′N, 9°35′E) and Flakkebjerg (55°19′ N and 11°23′ E), Denmark. The soil in Foulum is a sandy loam based on ground morainic deposits from the last glaciation (Munkholm et al., 2008), and it is classified as a Mollic Luvisol and a Typic Hapludalf. The topsoil (0 to 25 cm depth) contains 9.2 % clay (<2 mm), 12.6 % silt (2–20 mm), 44.4 % fine sand, 30.7 % coarse sand, and 3.1 % organic matter, where percent (%) refers to g 100 g^{-1} dry soil (Munkholm et al., 2008). The soil in Flakkebjerg is a sandy loam formed from mixed glacial deposits, and it is classified as Glossic Phaeozem. The topsoil at Flakkebjerg contains 14.7 % clay, 13.7 % silt, 42.6 % fine sand, 27 % coarse sand, and 2.0 % organic matter (Munkholm et al., 2008).

The experiment had a similar setup at both sites, i.e., a split-plot design with four replicates (blocks) and plot sizes of 12 by 6 m. Residue management was the main plot factor, and tillage was the subplot factor. The main plot factor included two levels: one where residues were removed from the field and one where they were retained, while the subplot factor had three levels: moldboard ploughing to 20 cm (MP), harrowing to 10 cm (H) and direct sowing (D). The resulting number of plots was 24 at each site. The fields had been subjected to the same management practices since 2003, with the crop rotations including cereal and legume crops. In the study year (2021), winter wheat (*Triticum aestivum* L.) was sown as the main crop, and fodder radish (*Raphanus sativus* L.) was used as a cover crop. At Flakkebjerg, the MP plots were ploughed in the late autumn prior to the sowing of spring crops and approximately one year before sampling. The MP plots at Foulum were ploughed in the spring, approximately nine months before sampling.

2.2. Soil sampling

Sampling took place at both sites during November 2021. Cubic soil samples were collected at two depths (0–10 and 10–20 cm) using a spade, summing up to 96 samples (2 experimental sites x 3 tillage systems x 2 crop residue management x 2 depths x 4 blocks). Samples were stored in sealed plastic boxes at −20 °C until analysed. These cubic soil samples were used for measurement of AMF biomass, GRSP content, wet stability of aggregates (WSA), clay dispersibility, SOC, phosphorus (P) content, and pH.

In addition, metal rings (6.1 cm in diameter, 3.4 cm in height, 100 cm^3) were used to sample undisturbed soil from the 0–10 and 10–20 cm soil layers, with six rings sampled per depth in each plot. The resulting 576 soil cores in metal rings were stored at 2 °C until used for estimating soil pore characteristics.

2.3. AMF biomass and GRSP content quantification

The signature fatty acid C16:1ω5 in PLFA and NLFA was extracted from soil samples to quantify AMF biomass (Olsson et al., 1995). To extract PLFA and NLFA, 3 g of freeze-dried and ground soil were mixed with 1.5 mL citrate buffer, 1.9 mL chloroform, and 3.75 mL methanol. Lipids were then separated into neutral, polar and glycolipids on silicic acid columns. NLFA were eluted with 1.5 mL chloroform, whereas PLFA were eluted with 1.5 mL of methanol. The extracted fractions were collected and evaporated to dryness with N_2 at 40 °C in a heating block. Mild alkaline methanolysis was completed by dissolving the sample in 1 mL of toluene/methanol (1:1) and adding 1 mL of freshly prepared 0.2 M KOH in methanol. The samples were equilibrated for 15 min at 37 °C in a water bath before adding hexane: chloroform (4:1), acetic acid and water. Finally, the samples were centrifuged (2000 g, 5 min), and the top phase was placed in a 4 mL vial and evaporated to dryness with N_2.

The concentration of the signature fatty acid was estimated using gas chromatography analysis with a flame ionization detector, with the nonadecanoate fatty acid (C19:0) employed as the internal standard. The oven temperature was increased from 170 to 260 ∘C at a rate of 5 ∘C per min and then raised at a rate of 40 ∘C per min until it reached a final temperature of 310 ∘C. Hydrogen and nitrogen were employed as carrier and make-up gases, respectively, and fatty acids were separated on a phenyl-siloxane (2.5 %) column (25 m long, 200 µm ID, 0.33 µm film). The identification and quantification of fatty acids was conducted using the MIDI microbial identification protocol (Sherlock version 4.5 MIDI, Microbial ID, Newark, DE, USA) and the software library TSBA41. Results were expressed as nmol/g soil on a dry weight basis.

The easily-extractable fraction of GRSP (EE) was measured in this study as it seems that EE is more sensitive to CA practices compared to other fractions of GRSP under our experimental conditions (Thomopoulos et al., 2023). EE was extracted based on the method suggested by Wright and Upadhyaya (1998) using 1 g soil in 8 mL 20 mM sodium citrate (pH = 7) and autoclaving 30 min at 120 ∘C in 50 mL Teflon tubes. Subsequently, samples were centrifuged (5000 g, 10 min), and 6 mL of the supernatant were transferred into 15 mL Falcon tubes. These supernatants were centrifuged again (5000 g, 10 min), and 4 mL of the resulting supernatant were transferred to new 15 mL Falcon tubes, which were kept for one day at 4 ∘C until quantification.

The content of GRSP was measured using the Bradford assay (Bradford, 1976), where 120 µL of the extracts were mixed with 200 µL of Bio-Rad Bradford dye (Coomassie brilliant blue G-250), and after 10 min of incubation, absorbance was measured at 595 nm. Bovine serum albumin was used as the standard. GRSP content was expressed as mg protein g^{-1} soil on a dry weight basis.

2.4. SOC, water stable aggregates, and clay dispersibility

For the measurement of SOC, approximately 200 g of the soil cubes were left to air-dry, crushed, and passed through a 2-mm sieve. A subsample of ca. 1 g was used for the determination of total C by high-temperature dry combustion at 950 °C using a Vario Max Cube (Elementar Analysensysteme GmbH, Hanau, Germany). Droplets of 10 % HCl were used to verify the absence of carbonates, and total C was rated as SOC. The SOC content was expressed as g 100 g^{-1} oven-dry soil (105 °C for 24 h).

For measurement of WSA, 4 g soil were placed on a sieve with 250 µm openings. Subsequently, the aggregates were rewetted by using a vaporizer with artificial rainwater (0.012 mM $CaCl_2$, 0.150 mM $MgCl_2$ and 0.121 mM NaCl; pH 7.82; EC 2.24×10^{-3} S m^{-1}). The sieve, positioned in aluminum cans filled with 100 mL of artificial rainwater, was moved up and down for 3 min at a rate of 34 cycles per minute with a stroke length of 13 mm. After 30 s, aggregates larger than 250 µm were transferred from the sieves to 100 ml beakers. Following oven-drying at 105 °C for a minimum of 24 h, the samples were transferred to 50 mL tubes. The sediment in the tube was adjusted for particles larger than 250 µm, which were isolated through chemical dispersion. A comprehensive description of the method is provided by Kemper and Rosenau (1986). This adjustment was made to express WSA based on soil free of particles larger than 250 µm, such as sand and stones.

For measurement of clay dispersibility, 10 g soil were placed in 100 mL plastic tubes, and artificial rainwater was gently added along the tube wall to achieve a soil-to-water ratio of 1:8 by weight. The suspension was shaken end-over-end for 2 min at 40 rpm (Stuart Tube Rotator model SB3 with a 25-cm diameter rotation) and was then allowed to settle for 3 h and 50 min, based on Stokes' Law. Particles smaller than 2 µm (clay) were siphoned off, and the weight of the dispersed clay was determined after oven-drying at 105 °C for a minimum of 24 h. The methodology is thoroughly detailed in Jensen et al. (2019). Similar to the WSA procedure, the sediment in the tube was corrected for particles larger than 250 µm.

2.5. Soil pore characteristics

To assess soil pore characteristics, the undisturbed 100 cm^3 soil core samples were carefully trimmed using a sharp-edged knife and then placed on a sandbox to be saturated from beneath. The samples were drained to matric potentials of −1, −3, −6, −10, and −30 kPa using tension tables and ceramic plates. The weight of each sample was recorded at every matric potential. After oven-drying at 105 °C for 24 h, soil porosity was determined based on bulk density and particle densities of 2.61 g cm^{-3} for the Foulum soil and 2.65 g cm^{-3} for the Flakkebjerg soil (Eden et al., 2011). Volumetric water content at −10 kPa was computed from weight loss during oven drying. The air-filled porosity (ε_a) at a specific matric potential was derived as the difference between total porosity and water retention at that potential. Air permeability was measured on the 100 cm^3 samples at a matric potential of −10 kPa, according to the steady-state method described by Iversen et al. (2001).

Relative gas diffusivity (D_s/D_o) measurements were conducted at matric potentials of −10 kPa using the one-chamber technique outlined by Schjønning et al. (2013), which is rooted in the non-steady-state method proposed by Taylor (1950). In essence, the O_2 concentration in the diffusion chamber was recorded at two-minute intervals for approximately 2 h following the flushing of the chamber with O_2-free N_2. Fick's second law of diffusion and the soil gas diffusion coefficient (D_s) were employed for calculations, which were subsequently converted to gas-independent diffusivity or relative diffusivity by establishing a relationship with the diffusion of O_2 in air (D_o), which is 0.205 cm^2 s^{-1} at 20 °C and atmospheric pressure (Smithsonian Physical Tables; Forsyth, 2003).

Soil tortuosity (pore length to sample length) was calculated from D_s/D_o and air-filled porosity at −10 kPa based on the equation introduced by Ball (1981):

$$Tortuosity = sqrt[\varepsilon_a / (D_s/D_o)]$$

2.6. Statistical analysis

Statistical data analysis was performed using R version 4.2.1 (R Core Team, 2022). Linear mixed models were used to explore the impact of soil and residue management treatments on soil structural parameters, soil pore characteristics, AMF biomass and EE content using the *lmer4* package (Bates et al., 2015). Homoscedasticity and normality were tested by visual examination of residual plots. Soil depth, soil tillage, and residue management were considered fixed effect variables, and blocks were set as random effects. The analysis was performed separately for the two experimental sites. Interaction, additivity, and single effects of the fixed variables were tested using *p* values, computed through likelihood ratio tests, that compared the full model, which included the effect in question, to a model without the effect in question. Logarithmic transformations were applied to the raw data for air permeability. For comparing the treatments, the Tukey multiple comparison method with a significance level of $\alpha = 0.05$ was used. To assess the relationship between the various measured parameters, we employed the Pearson correlation coefficient, and *p* values were calculated to determine the significance of each correlation. The correlation analysis was performed per tillage treatment since the soil mechanical disturbance was found to have a stronger effect on biological soil health indicators compared to residue management in Denmark (Thomopoulos et al., 2023).

3. Results

3.1. Tillage and residue effects on AMF biomass and EE

At Flakkebjerg, there was a significant interaction between tillage and residue management on PLFA 16:1ω5 (Fig. 1a). Conservation management – direct sowing and retaining crop residues – resulted in higher PLFA 16:1ω5 levels than ploughed plots (Fig. 1a). Tillage as a single factor had a significant effect on the AMF NLFA 16:1ω5 with direct drilling and harrowing resulting in higher levels than ploughing (Fig. 1c). The effect of soil depth on NLFA 16:1ω5 was also significant, with higher values for the 0–10 cm compared to the 10–20 cm layer (Fig. 1c).

At Foulum, for PLFA16:1ω5, there was a significant interaction between soil depth and tillage treatment. Soil in harrowed and direct sown plots had higher contents of PLFA 16:1ω5 than ploughed plots in the top layer (Fig. 1b). In the 10–20 cm layer, ploughed soil had a marginally higher amount of PLFA 16:1ω5 than directly drilled soil (Fig. 1b). The interaction between soil depth and residue treatment was also significant, with residue removal yielding lower PLFA 16:1ω5 in the top layer compared to retaining residues (Fig. 1b).No significant treatment effects were found for soil NLFA 16:1ω5, but the effect of depth was significant with higher values for the 0–10 cm than the 10–20 cm layer (Fig. 1d).

No significant differences between treatments were observed at Flakkebjerg for concentrations of soil EE (Fig. 1e), while at Foulum, residue retainment significantly yielded higher EE compared to removal of crop residues (Fig. 1f).

3.2. Tillage and residue effects on SOC

The SOC content was significantly higher in the 0–10 cm layer compared to the 10–20 cm layer in both sites (Fig. 2). At the Foulum site, the residue treatment significantly affected SOC, as higher SOC content was measured when residues were retained (Fig. 2b). The residue effect was not significant at the Flakkebjerg site. Tillage did not significantly affect SOC levels at any site. There was, however, a significant interaction between soil depth and tillage across sites. Harrowed and directly sown plots had a higher SOC content compared to ploughed plots only in the top layer. Additionally, in harrowed and directly sown plots, the 0–10 cm layer had higher SOC content than the 10–20 cm layer, while in ploughed plots, this difference was not significant (Fig. 2).

3.3. Tillage and residue effects on WSA and clay dispersibility

Tillage treatment significantly affected WSA and clay dispersibility at Flakkebjerg (Fig. 3a, c). Higher WSA and lower clay dispersibility were identified in harrowed soil and soil with direct sowing as compared to ploughed soil (Fig. 3a,c).

Similarly, at Foulum, WSA was affected by tillage as higher WSA was identified in harrowed soil and soil with direct sowing as compared to ploughed soil (Fig. 3b). Clay dispersibility was affected by the interaction between tillage and residue as moldboard ploughing resulted in significantly higher dispersible clay than harrowing and direct sowing only when straw was removed (Fig. 3d).

3.4. Tillage and residue effects on soil pore characteristics

The main effect of tillage at Flakkebjerg was significant for total porosity, macroporosity (air-filled pores at −10 kPa corresponding to pores with equivalent diameter > 30 μm), and VWC at −10 kPa (Fig. 4a, c, e). It also affected soil tortuosity ($F_{(2,43)} = 4.66, p = 0.014$). Ploughing resulted in lower soil tortuosity across depths in moldboard ploughing (2.86 m/m) compared to harrowing and direct sowing (3.17 and 3.30 m/m, respectively). Relative gas diffusivity was affected by tillage ($F_{(2,42)} = 13.13$, $p < 0.001$) with higher levels in soil with moldboard ploughing (0.027) compared to in soil with harrowing (0.019) and direct sowing (0.017) across depths (Supplementary Table 1). At Foulum, there was no main effect of tillage on total porosity, microporosity and VWC (Fig. 4b, d, f). Yet, there was a significant effect of tillage on pore characteristics for gas diffusivity ($F_{(2,42)} = 3.81$, $p = 0.0302$), with higher levels in moldboard ploughing (0.0394) compared to direct sowing (0.028) across depths (Supplementary Table 2).

The residue treatment effect was significant for total porosity at both sites and for macroporosity at Foulum (Fig. 4 a, b, d).

Soil depth significantly influenced total porosity and volumetric water content (−10 kPa) at both sites and macroporosity at Flakkebjerg (Fig. 4). It also affected air permeability at Flakkebjerg ($F_{(1,37)} = 8.58$, $p = 0.006$) with higher values in the 0–10 cm layer (4.43 μm^2) compared to the 10–20 cm layer (3.91 μm^2) (Supplementary Table 1).

The interaction of tillage and soil depth was significant for total porosity at both sites and for volumetric water content (−10 kPa) at Flakkebjerg (Fig. 4 a, b, e). At Foulum, the interaction between residue management and depth was significant for volumetric water content (−10 kPa), while at Flakkebjerg, the same interaction was significant for air permeability (Supplementary Table 3).

3.5. Correlation among soil health indicators

At Flakkebjerg, 14 out of 114 Pearson correlation coefficients showed significant correlations across the tillage treatments (Fig. 5). For the ploughing treatment, four significant correlates were found and included SOC with VWC ($r = 0.76$), NLFA with PLFA ($r = 0.57$), and PLFA with total porosity ($r = 0.50$). In the harrowing treatment, there were five significant correlations (Fig. 5b), with the most notable being EE with SOC ($r = -0.67$) and EE with WSA ($r = 0.53$).

Finally, under direct sowing treatment, five significant correlations were observed (Fig. 5c). PLFA showed correlations with EE ($r = 0.52$), WSA ($r = 0.68$), tortuosity ($r = 0.54$), and volumetric water content at −10 kPa ($r = 0.50$). Additionally, WSA was associated with EE ($r = 0.71$).

At Foulum, 24 out of 114 Pearson correlation coefficients were significant across the tillage treatments. For moldboard ploughing, 7 of 38 pairs were significantly correlated (Fig. 6a). High correlations were noted between PLFA and WSA ($r = 0.86$) and EE with SOC ($r = 0.92$), clay dispersibility ($r = -0.62$) and total porosity ($r = 0.64$). SOC was further correlated with WSA ($r = 0.52$), clay dispersibility ($r = -0.72$) and volumetric water content at −10 kPa ($r = 0.60$). For the harrowing treatment, there were five significant correlations (Fig. 6b). High correlations included PLFA with WSA ($r = 0.73$) and EE with SOC ($r = 0.78$).

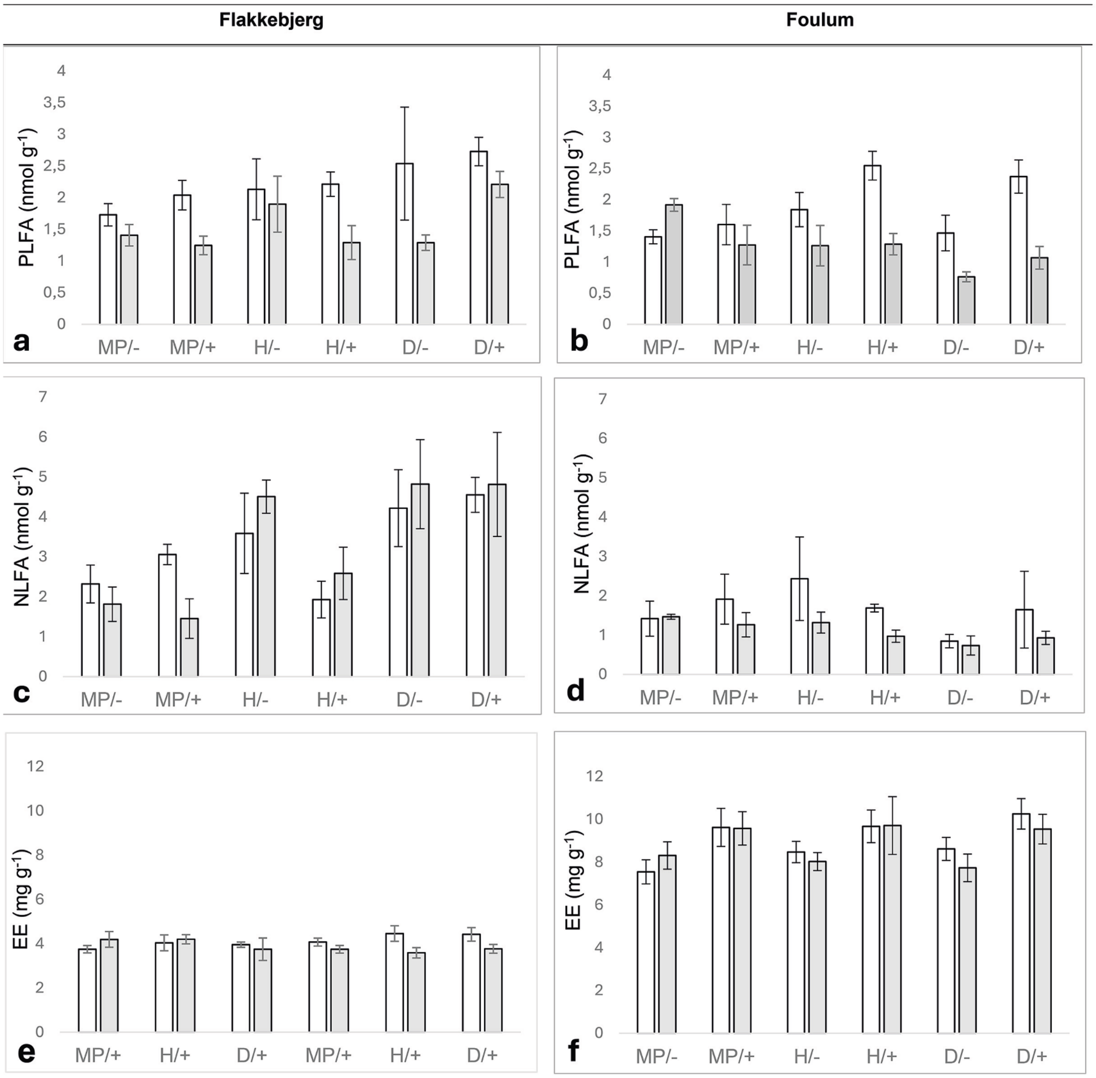
In direct sowing, 12 significant correlations were observed (Fig. 6c). Noteworthy correlations encompassing biological indicators included NLFA with tortuosity ($r = 0.78$), PLFA with WSA ($r = 0.85$) and EE ($r = 0.64$), EE with SOC ($r = 0.90$), WSA ($r = 0.75$), and total porosity ($r = 0.77$). SOC was further associated with WSA ($r = 0.83$), clay dispersibility ($r = -0.76$), and total porosity ($r = 0.69$).

4. Discussion

4.1. Correlation between biological soil health indicators and soil structural stability parameters

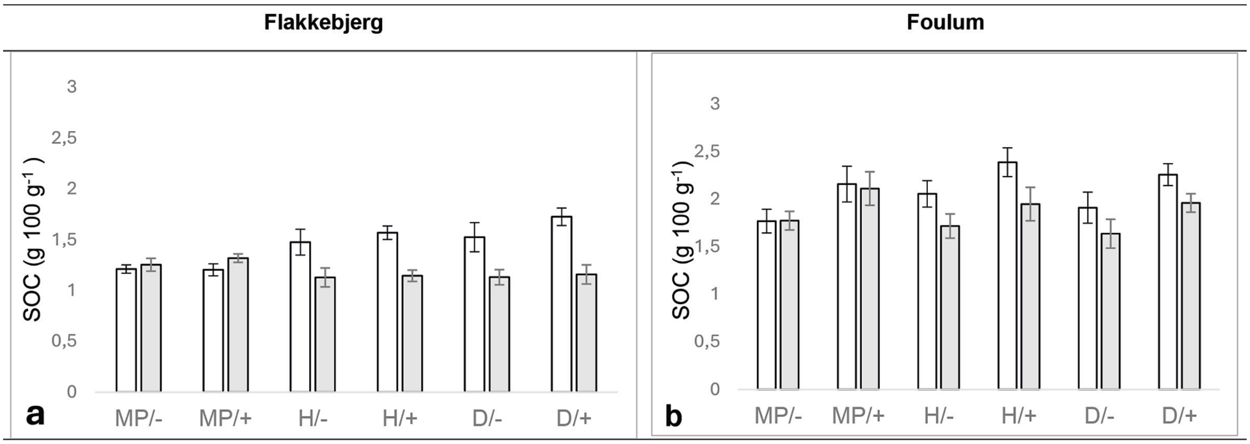
AMF, serving as a soil health indicator, plays a pivotal role in fostering the formation and resilience of soil aggregates (Morris et al., 2019; Wilson et al., 2009). This is supported by the present results from Foulum, where the strong correlation between the AMF hyphal network and WSA was independent of tillage methods. Conversely, at Flakkebjerg, where the soil has a higher clay content (14.7 and 9.2 g 100 g^{-1} for Flakkebjerg and Foulum, respectively), this correlation was not evident across the tillage practices, implying that soil texture might influence the impact of AMF. Degens et al. (1997) highlight that the binding mechanisms of fungal hyphae are more likely to enhance aggregation in soils with a higher proportion of sand. Still, the significant correlation between the presence of AMF hyphae and WSA in direct sowing treatment at Flakkebjerg indicates that the beneficial impact of AMF on soil stability exists also in more loamy soils.

Nunes et al. (2018) reported a very high correlation between autoclaved-citrate extractable proteins (referred to as EE in the current



Factor	PLFA	NLFA	EE	PLFA	NLFA	EE
Tillage (T)	F(2,37)=1.49 *p*=0.24	**F(2,41) = 8.49 *p*<0.001**	F(2,38)=0.45 *p*=0.28	F(2,40)=1.31 *p*=0.28	*NI*	*NI*
Residue (R)	F(1,37)=2.29 *p*=0.14	*NI*	F(1,38)=0.29 *p*=0.59	F(1,40)=2.39 *p*=0.13	*NI*	**F(1,43)=21.51 *p*<0.001**
Depth (DP)	**F(1,37)=8.58 *p*=0.006**	*NI*	F(1,38)=1.99 *p*=0.17	**F(1,40)=14.38 *p*<0.001**	*NI*	*NI*
TxRxDP	*NI*	*NI*	*NI*	*NI*	*NI*	*NI*
TxR	**F(2,37)=4.35 *p*=0.019**	*NI*	*NI*	*NI*	*NI*	*NI*
TxDP	*NI*	*NI*	F(2,38)=3.23 *p*=0.0508	**F(2,40)=4.79 *p*=0.014**	*NI*	*NI*
RxDP	*NI*	*NI*	*NI*	**F(1,40)= 4.84 *p*=0.034**	*NI*	*NI*

Fig. 1. Effects of soil management treatments (moldboard ploughing [MP], harrowing [H], and direct sowing [D]) and crop residue management (removed [-] or retained [+]) on phospholipid-lipid fatty acid 16:1ω5 (PLFA) [a, b], neutral-lipid fatty acid 16:1ω5 (NLFA) [c, d], and easily-extractable glomalin related soil protein (EE) [e, f] in soil layers 0–10 cm (white bars) and 10–20 cm (grey bars) for the Flakkebjerg [a, c, e] and Foulum [b, d, f] sites. Data are shown as mean with standard error bars (n = 4). Test statistic (F) and p values were extracted from the best-fitted mixed model using biological soil health indicators (PLFA, NLFA, and EE as the response variables and soil management (T), residue management (R) and depth (DP) as fixed effects. Values in bold indicate significant effects at the level of $p < 0.05$, and values not shown (NI) correspond to effects not included in the best-fitted models.



Factor	SOC (Flakkebjerg)	SOC (Foulum)
Tillage (T)	F(2,38)=2.29 *p*=0.11	F(2,38)=0.81 *p*=0.45
Residue (R)	F(1,38)=2.56 *p*=0.12	**F(1,38)=29.7 *p*<0.001**
Depth (DP)	**F(1,38)=38.16 *p*<0.001**	**F(1,38)=14.97 *p*<0.001**
TxRxDP	*NI*	*NI*
TxR	*NI*	*NI*
TxDP	**F(2,38)=17.07 *p*<0.001**	**F(2,38)=3.34 *p*=0.046**
RxDP	*NI*	*NI*

Fig. 2. Effects of soil management treatments (moldboard ploughing [MP], harrowing [H], and direct sowing [D],) and crop residue management (removed [-] or retained [+]) on soil organic carbon (SOC) in soil layers 0–10 cm (white bars) and 10–20 cm (grey bars) for the Flakkebjerg [a] and Foulum [b] sites. Data are shown as mean with standard error bars (n = 4). Test statistic (F) and p values were extracted from the best-fitted mixed model using SOC as the response variable and soil mechanical management (T), residue management (R) and depth (DP) as fixed effects. Values in bold indicate significant effects at the level of $p < 0.05$, and values not shown (NI) correspond to effects not included in the best-fitted models.

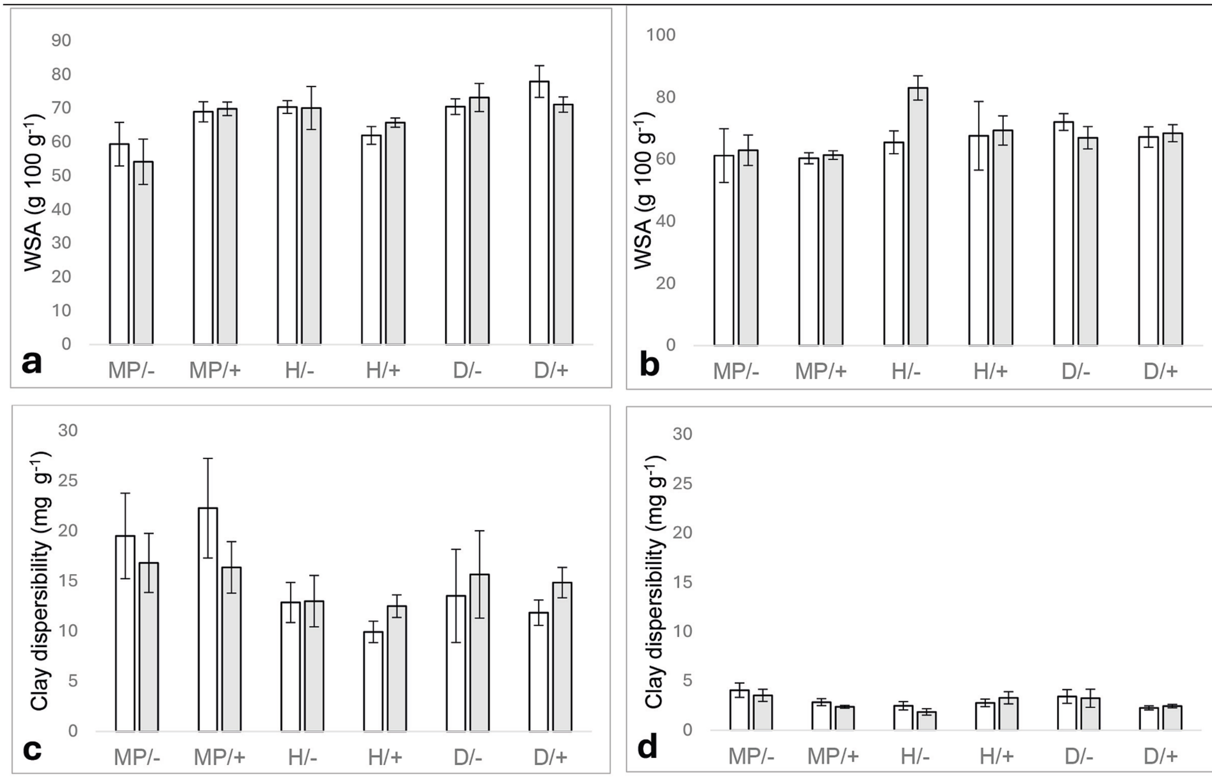
study) and WSA in different soil textures. Building on these results, the present study reveals that direct sowing enhances the connection between EE and WSA at both sites, suggesting that the role of EE in WSA becomes more significant as tillage intensity decreases.

The clay dispersibility method involves a more aggressive physical disturbance than the WSA method. It is plausible, therefore, that the enmeshing effects of AMF hyphae might have been disrupted during the laboratory procedure, resulting in the absence of any significant correlation at Flakkebjerg. On the contrary, at Foulum, EE was linked with clay dispersibility, supporting the effect of GRSP on aggregate stability reported by Rillig (2004). Contrary to our third hypothesis, this relationship was not influenced by tillage intensity, as significant correlations were found for both ploughed and directly sown treatments. This is likely related to the non-significant overall effect of tillage on EE, while there was a significant effect of residue retention (Fig. 1).

AMF biomass in soil with reduced or no tillage from the Foulum site was significantly correlated with SOC. When taking the strong connection between PLFA 16:1ω5 and WSA at Foulum plots into account, the results indicate an effect of hyphae on improved physical protection of C by soil aggregates. The strong association between EE and SOC for the Foulum soil supports the putative link between GRSP fractions and SOC storage (Agnihotri et al., 2022; Singh et al., 2017). On the other hand, it could partially be attributed to the co-extraction of organic compounds during the EE extraction process. Previous studies have indicated such interference from organic compounds (Gillespie et al., 2011; Irving et al., 2021). EE was also expected to be significantly correlated with PLFA 16:1ω5, as shown in previous research (Agnihotri et al., 2021; Thomopoulos et al., 2023). The results of the present study suggest that this relationship is strongly influenced by soil tillage, as the correlation was significant only under direct sowing at both sites. Therefore, soil tillage should be factored in when evaluating the connection between the AMF hyphal network and GRSP fractions. Additionally, the observed results should be interpreted with consideration of the temporal variation, as the sampling occurred at a single time point after the growing season.

4.2. Correlation between biological soil health indicators and soil pore characteristics

Low levels of air permeability and high levels of tortuosity at the same or higher total porosity indicate a more complex pore system with smaller and more tortuous pores. The significant positive correlation between AMF storage lipids and tortuosity and their negative correlation with air permeability, as observed for direct sowing at Foulum, suggests that AMF may play a substantial role in generating a more complex, sponge-like pore structure with smaller and more tortuous pores for soils with minimal disturbance. This correlation might also indicate a trade-off, where the fungal symbiont takes advantage of a more porous soil habitat to facilitate its growth. This mechanism of AMF hyphae to induce changes in soil pore characteristics has also been described in earlier studies (Bitterlich et al., 2018). Still, the strong positive correlation between EE and total porosity suggests that the effect of AMF on soil porosity is more linked to the role of GRSP fractions as binding agents and less to the enmeshing properties of hyphae, in contrast to the link between AMF and soil structural stability where the hyphal network also appears to be important. Therefore, the second hypothesis can be



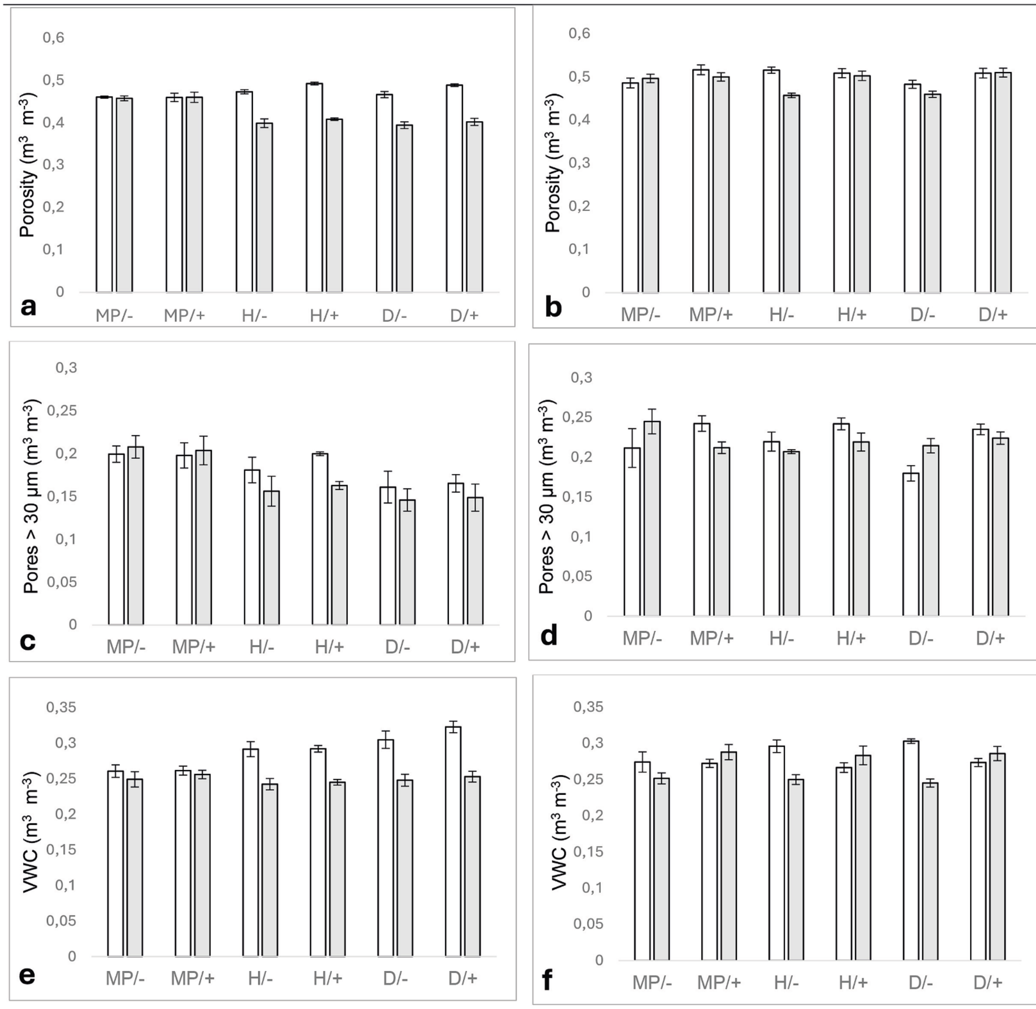
Factor	Flakkebjerg		Foulum	
	WSA	Clay dispersibility	WSA	Clay dispersibility
Tillage (T)	**F(1,43)=8.98, *p*=0.0045**	**F(2,42)=5.19, *p*=0.0096**	**F(2,44)=4.69 *p*=0.0142**	F(2,38)=1.43 *p*=0.25
Residue (R)	*NI*	*NI*	*NI*	F(1,38)=2.19 *p*=0.15
Depth (DP)	*NI*	*NI*	*NI*	F(1,38)=0.39 *p*=0.53
TxRxDP	*NI*	*NI*	*NI*	*NI*
TxR	*NI*	*NI*	*NI*	**F(2,38)=4.93 *p*=0.013**
TxDP	*NI*	*NI*	*NI*	*NI*
RxDP	*NI*	*NI*	*NI*	*NI*

Fig. 3. Effects of soil management treatments (moldboard ploughing [MP], harrowing [H], and direct sowing [D]) and crop residue management (removed [-] or retained [+]) on wet stability of aggregates (WSA) [a, b] and clay dispersibility [c, d] in soil layers 0–10 cm (white bars) and 10–20 cm (grey bars) for the Flakkebjerg [a, c] and Foulum [b, d] sites. Data are shown as mean with standard error bars (n = 4). Test statistic (F) and p values were extracted from the best-fitted mixed model using WSA and clay dispersibility as the response variables and soil mechanical management (T), residue management (R) and depth (DP) as fixed effects. Values in bold indicate significant effects at the level of $p < 0.05$, and values not shown (NI) correspond to effects not included in the best-fitted models.

confirmed, but only to some extent, since this relationship does not seem to be consistent across sites, as in Flakkebjerg, AMF storage lipids or EE were not significantly correlated with any of the measured soil pore parameters. The differences between the two sites can be attributed to differences in soil textures, climate, and tillage timing, but further research is needed to fully understand these factors.

4.3. Effect of CA practices on biological soil health indicators

The present data documented the beneficial effects of conservation management on AMF biomarkers, substantiating that tillage practices can significantly impact AMF, which are known to be sensitive to soil disturbance (Kabir et al., 1999). When soil is tilled, AMF hyphae can become detached from their host plants, while ploughing may result in the dispersal of fungal propagules to deeper soil layers, potentially reducing infection levels in host plants (Kabir, 2005; Säle et al., 2015). Moreover, tillage-induced changes in soil aggregation can negatively affect AMF abundance, leading to a decrease in the hyphal network (Bowles et al., 2017; Helgason et al., 2010). While the main effect of tillage on AMF hyphae, as indicated by PLFA 16:1ω5, was not significant at the two sites, there were significant interactions of tillage with residue management in Flakkebjerg and with depth at Foulum. Besides its effects on the AMF hyphal network, direct seeding resulted in higher levels of spores and vesicles, as indicated by NLFA 16:1ω5, at Flakkebjerg (Fig. 1c). This finding is consistent with the results reported by Hydbom and Olsson (2021) and by Säle et al.(2015), highlighting the benefits of conservation tillage to the abundance and survival of AMF, and confirming our first hypothesis.



Factor	Porosity	Pores > 30μm	VWC	Porosity	Pores > 30μm	VWC
Tillage (T)	**F(2,278)=23.63 p<0.001**	**F(2,38)=17.15 p<0.001**	**F(1,38)=17.06 p<0.001**	F(2,273)=1.74 p=0.1772	F(2,39)=1.115 p=0.3255	F(2,39)=0.42 p=0.6583
Residue (R)	**F(1,278)=11.79 p<0.001**	F(1,38)=0.47 p=0.4964	F(1,38)=2.49 p=0.1229	**F(1,273)=35.17 p<0.001**	**F(1,39)=4.34 p=0.0439**	F(1,39)=2.96 p=0.0931
Depth (DP)	**F(1,278)=350.96 p<0.001**	**F(1,38)=4.01 p=0.0523**	**F(1,38)=127.10 p<0.001**	**F(1,273)=13.65 p<0.001**	F(2,39)=0.036 p=0.8513	**F(1,39)=8.11 p=0.0069**
TxRxDP	*NI*	*NI*	*NI*	**F(2,273)=7.86 p<0.001**	*NI*	*NI*
TxR	*NI*	*NI*	*NI*	F(2,273)=2.53 p=0.0814	*NI*	*NI*
TxDP	**F(2,278)=82.22 p<0.001**	F(2,38)=2.81 p=0.7261	**F(2,38)=21.30 p<0.001**	**F(2,273)=4.42 p=0.0129**	*NI*	*NI*
RxDP	*NI*	*NI*	*NI*	F(2,273)=3.87 p=0.0502	*NI*	**F(2,39)=35.39 p<0.001**

Fig. 4. Effects of soil management treatments (moldboard ploughing [MP], harrowing [H], and direct sowing [D]) and crop residue management (removed [-] or retained [+]) on total porosity [a, b], the fraction of soil volume represented by pores > 30 μm [c, d], and volumetric water content (VWC) at −10 kPa [e, f] in soil layers 0–10 cm (white bars) and 10–20 cm (grey bars) for the Flakkebjerg [a, c, e] and Foulum [b, d, f] sites. Data are shown as mean with standard error bars (n = 4). Test statistic (F) and p values were extracted from the best-fitted mixed model using total porosity, pores > 30 μm and VWC as the response variables and soil mechanical management (T), residue management (R) and depth (DP) as fixed effects. Values in bold indicate significant effects at the level of $p < 0.05$, and values not shown (NI) correspond to effects not included in the best-fitted models.

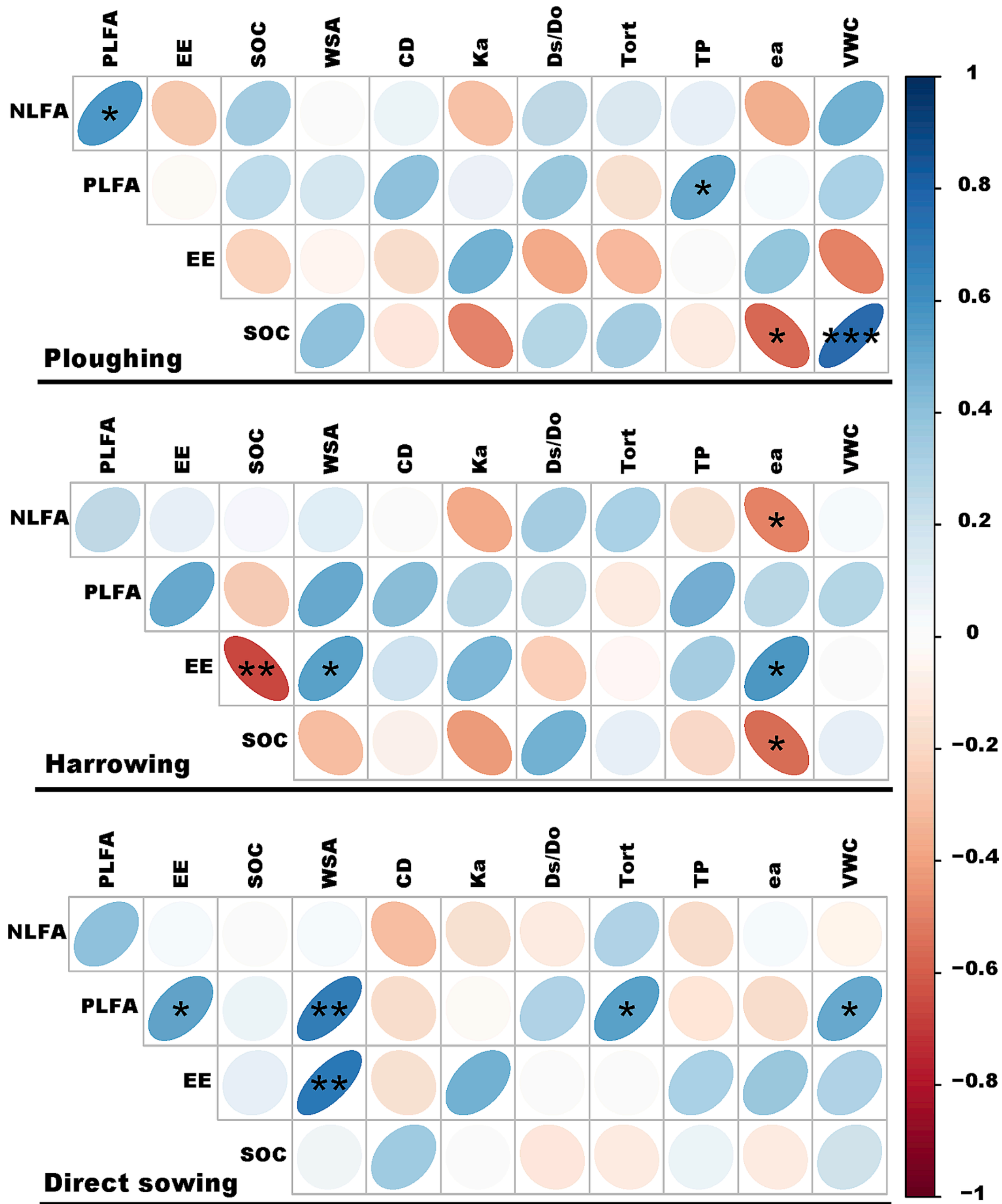


Fig. 5. Correlation matrices including Pearson correlation coefficient for the soil health indicators for the Flakkebjerg site across depths and residue management and for the different tillage treatments. * Significant at α = 0.05, ** significant at α = 0.01, *** significant at α = 0.001. NLFA: Neutral-lipid fatty acid 16:1ω5, PLFA: Phospholipid fatty acid 16:1ω5, EE: Easily extractable glomalin-related soil protein, SOC: Soil organic carbon, WSA: Water stable aggregates, CD: Clay dispersibility, Ka: Air permeability, Ds/Do: Relative gas diffusivity, Tort: Tortuosity, TP: Total porosity, ea: Air-filled porosity (at −10 kPa), VWC: Volumetric water content (at −10 kPa).

Our results also support the beneficial effect of residue management on AMF biomass. While this aligns with previous studies (Martínez-García et al., 2018), it appears inconsistent with other studies that could not document a clear impact of residues on AMF biomass (Duan et al., 2011; Gu et al., 2020). Retaining residues from the previous crop in the fields boosts the input of organic matter into the soil. Although AMF cannot directly utilize soil C substrates, their growth and activity can be influenced by the presence of secondary metabolites produced by the microorganisms involved in organic matter decomposition and by the nutrients released during the decomposition (Gryndler et al., 2009).

The influence of soil depth as a primary factor affecting the distribution of AMF is a topic that has been discussed in previous studies (Shukla et al., 2013). In the present study, an expected decrease in the AMF biomarker levels in the 10–20 cm layer was observed at both sites and can be attributed to the naturally lower occurrence of roots and propagules compared to the surface 0–10 cm layer. A similar vertical stratification was reported by Säle et al.(2015), who noted a significant decrease in spore density below the 0–10 cm depth, and by Kabir et al. (1998) who reported similar findings for hyphal density. Wang et al. (2024) also observed the highest AMF root colonization in the topsoil. These results underscore the importance of considering soil depth in understanding the ecological distribution patterns of AMF.

An important aspect of AMF's contribution to soil carbon pools and ecosystem services may be linked to GRSP (Rillig et al., 2002; Rillig et al., 2001). The prevailing theory about the pathway through which GRSP are deposited in the soil suggests that GRSP accumulate through

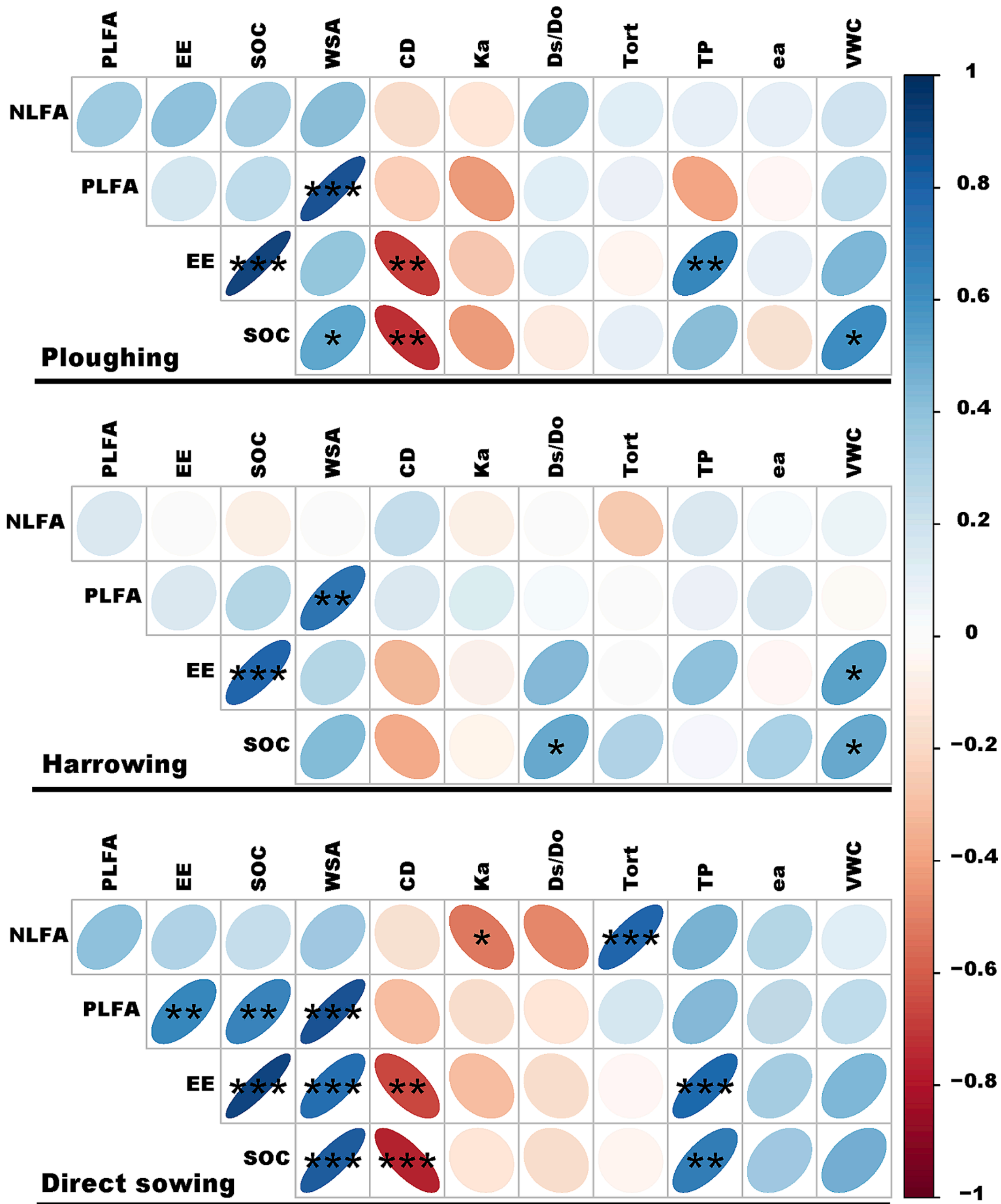


Fig. 6. Correlation matrices including Pearson correlation coefficient for the soil health indicators for the Foulum site across depths and residue management and for the different tillage treatments. * Significant at α = 0.05, ** significant at α = 0.01, *** significant at α = 0.001. NLFA: Neutral-lipid fatty acid 16:1ω5, PLFA: Phospholipid fatty acid 16:1ω5, EE: Easily extractable glomalin-related soil protein, SOC: Soil organic carbon, WSA: Water stable aggregates, CD: Clay dispersibility, Ka: Air permeability, Ds/Do: Relative gas diffusivity, Tort: Tortuosity, TP: Total porosity, ea: Air-filled porosity (at −10 kPa), VWC: Volumetric water content (at −10 kPa).

hyphal turnover and release from dead mycelia (Driver et al., 2005). Although the increased levels of AMF hyphae indicators in conservation-managed plots could potentially lead to higher EE content, this was not evident in Flakkebjerg. This finding aligns with previous results from the same site (Thomopoulos et al., 2023) and may be due to the significant legacy effect of management practices on GRSP fractions, which makes changes harder to detect (Rillig, 2004). However, this is in contrast to Nunes et al. (2018), who observed a 49 % increase in autoclaved-citrate extractable protein content (equivalent to EE in the present study) after long-term no-till in loamy fine sand soil. Residue retention significantly increased EE content in Foulum, suggesting that the application of residues might promote the buildup of stable carbon pools. Similar findings were reported in a recent study (Li et al., 2024).

4.4. Effect of CA practices on soil organic carbon

Although SOC levels were consistent across tillage treatments at both sites, a vertical stratification was observed in harrowing and direct sowing treatments. This SOC stratification for reduced and no-tillage is in line with previous studies (Abdollahi et al., 2017; Krauss et al., 2022; Weidhuner et al., 2021). In a recent study conducted by Schjønning (2023) across three different sites in Denmark, it was reported that incorporating residues into the soil for 35 years caused a nearly 13 % increase in SOC content compared to residue removal. Our findings in Foulum align with this observation, demonstrating a significant approximately 15 % increase across 0–20 cm after 20 years of residue incorporation. However, at Flakkebjerg, residue retention showed a much lower and non-significant effect (~5 % increase in SOC content). Higher SOC levels with residue incorporation can be ascribed to an increase in carbon input into the soil (Bolinder et al., 2020). The observed contrast between the two sites is consistent with previous research conducted at the same sites, and the underlying reasons could be related to straw biomass, initial soil properties, and different environmental conditions (Abdollahi et al., 2017; Gómez-Muñoz et al., 2021).

4.5. Effect of CA practices on soil structural stability and pore characteristics

Previous studies have noted a decrease in wet aggregate stability with increased tillage intensity (Abdollahi et al., 2017; Mondal & Chakraborty, 2022; Nunes et al., 2018), and the present findings were consistent with this. Further, at Foulum, the negative impact of ploughing on clay dispersibility was apparent only when residues were removed, suggesting that retaining crop residues was critical for intensively tilled soil that can be sensitive to water and wind erosion. These findings align with our first hypothesis that conservation practices can enhance soil health as indicated by soil structural stability.

While minimal soil disturbance had positive effects on soil structural stability, it may lead to deteriorated soil pore characteristics compared to conventional tillage practices. In the present study, ploughing led to improved soil porosity indices (i.e., tortuosity, gas diffusivity, macroporosity), consistent with earlier findings from the same long-term experiment and elsewhere (Mondal & Chakraborty, 2022; Rocco et al., 2024; Skaalsveen et al., 2019). Results from the previous sampling of the same experiment in 2013 and 2014 (Abdollahi & Munkholm, 2017) are similar to the present study, suggesting that an extra seven years of treatment time did not have a clear effect on soil porosity indices.

Schjønning (2023) did not observe significant effects of residue incorporation on the volume of pores >30 μm but found notably higher air permeability, in agreement with the present findings. Likewise, residue retention led to a significant increase in total porosity at both sites, implying that the adverse effects of reduced or no-till on pore characteristics can be alleviated through residue retention (Abdollahi & Munkholm, 2017).

5. Conclusion

This study examined the effects of long-term tillage practices and crop residue management on soil biological and physical health indices, aiming to reveal their connections. Results indicate that conservation management increased the presence of AMF, as indicated by fatty-acid biomarkers, with soil depth playing a critical role in AMF biomass distribution. Harrowing and direct sowing treatments induced vertical stratification of SOC. The results show that SOC levels increased under residue retention – although only significantly at Foulum. Minimal soil disturbance improved soil wet stability, although it negatively impacted pore characteristics. AMF appeared to play a significant role in soil aggregate stability, primarily through the hyphal network, as evidenced by a strong correlation between PLFA biomarkers and WSA. Conversely, the role of AMF on pore structure seemed more closely associated with EE than with the hyphal network. The findings also indicate that direct sowing enhanced the relationship between the presence of AMF and EE. Overall, the present study provides insights into the soil bio-physical effects of conservation agriculture practices, such as minimal soil disturbance and crop residue retention, highlighting their potential to enhance soil health while acknowledging that their impacts may vary depending on specific conditions.

CRediT authorship contribution statement

Stamatios Thomopoulos: Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Lars Juhl Munkholm:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Lars Elsgaard:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Sabine Ravnskov:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Funding

This work was supported by GUDP – Grønt Udviklings-og Demonstrationsprogramthe of the Danish Agricultural Agency of the Ministry of Food, Agriculture and Fisheries of Denmark via the project CarbonFarm 2 – bæredygtige dyrkningssystemer i landbruget (34009-20-1723).

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.

Acknowledgements

The authors are also very grateful to Bente Birgitte Laursen, Stig Rasmussen and Jørgen Munksgaard Nielsen for their invaluable technical support in the field and the laboratory and to Maarit Mäenpää for her assistance with the statistical analysis.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2025.117204.

Data availability

Data will be made available on request.

References

Abdollahi, L., Getahun, G.T., Munkholm, L.J., 2017. Eleven years' effect of conservation practices for temperate sandy loams: I. Soil physical properties and topsoil carbon content. *Soil Sci. Soc. Am. J.* 81 (2), 380–391. https://doi.org/10.2136/sssaj2016.06.0161.

Abdollahi, L., Munkholm, L.J., 2017. Eleven years' effect of conservation practices for temperate sandy loams: II. Soil pore characteristics. *Soil Sci. Soc. Am. J.* 81 (2), 392–403. https://doi.org/10.2136/sssaj2016.07.0221.

Aditi, K., Abbhishek, K., Chander, G., Singh, A., Falk, T., Mequanint, M.B., Cuba, P., Anupama, G., Mandapati, R., Nagaraji, S., 2023. Assessing residue and tillage management options for carbon sequestration in future climate change scenarios. *Curr. Res. Environ. Sustainability* 5, 100210. https://doi.org/10.1016/j.crsust.2023.100210.

Agnihotri, R., Bharti, A., Ramesh, A., Prakash, A., Sharma, M.P., 2021. Glomalin-related protein and C16:1ω5 PLFA associated with AM fungi as potential signatures for assessing the soil C sequestration under contrasting soil management practices. *Eur. J. Soil Biol.* 103, 103286. https://doi.org/10.1016/j.ejsobi.2021.103286.

Agnihotri, R., Sharma, M.P., Prakash, A., Ramesh, A., Bhattacharjya, S., Patra, A.K., Manna, M.C., Kurganova, I., Kuzyakov, Y., 2022. Glycoproteins of arbuscular mycorrhiza for soil carbon sequestration: review of mechanisms and controls. *Sci. Total Environ.* 806 (Pt 2), 150571. https://doi.org/10.1016/j.scitotenv.2021.150571.

Ball, B.C., 1981. Modelling of soil pores as tubes using gas permeabilities, gas diffusivities and water release. *J. Soil Sci.* 32 (4), 465–480. https://doi.org/10.1111/j.1365-2389.1981.tb01723.x.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Bitterlich, M., Franken, P., Graefe, J., 2018. Arbuscular mycorrhiza improves substrate hydraulic conductivity in the plant available moisture range under root growth exclusion. *Front. Plant Sci.* 9. https://doi.org/10.3389/fpls.2018.00301.

Blanco-Canqui, H., Ruis, S.J., 2018. No-tillage and soil physical environment. *Geoderma* 326, 164–200. https://doi.org/10.1016/j.geoderma.2018.03.011.

Bolinder, M.A., Crotty, F., Elsen, A., Frac, M., Kismányoky, T., Lipiec, J., Tits, M., Tóth, Z., Kätterer, T., 2020. The effect of crop residues, cover crops, manures and nitrogen fertilization on soil organic carbon changes in agroecosystems: a synthesis of reviews. *Mitig. Adapt. Strat. Glob. Chang.* 25 (6), 929–952. https://doi.org/10.1007/s11027-020-09916-3.

Bowles, T.M., Jackson, L.E., Loeher, M., Cavagnaro, T.R., 2017. Ecological intensification and arbuscular mycorrhizas: a meta-analysis of tillage and cover crop effects. *J. Appl. Ecol.* 54 (6), 1785–1793. https://doi.org/10.1111/1365-2664.12815.

Brundrett, M.C., Tedersoo, L., 2018. Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytol.* 220 (4), 1108–1115. https://doi.org/10.1111/nph.14976.

Cárceles Rodríguez, B., Durán-Zuazo, V.H., Soriano Rodríguez, M., García-Tejero, I.F., Gálvez Ruiz, B., Cuadros Tavira, S., 2022. Conservation agriculture as a sustainable system for soil health: A review. *Soil Systems* 6 (4), 87. https://doi.org/10.3390/soilsystems6040087.

Chebet, S., Munkholm, L.J., Jensen, J.L., 2023. Rapid increase in soil organic carbon and structural stability in a sandy loam soil following conversion from long-term arable to semi-natural grassland irrespective of initial soil conditions. *Geoderma* 438, 116646. https://doi.org/10.1016/j.geoderma.2023.116646.

Das, S., Biswas, S., Ramakrishnan, B., Das, T.K., Purakayastha, T.J., Gawade, B.H., Singh, P., Ghorai, P.S., Tripathy, S., Sinha, K., 2024. Biological soil health with conventional and qPCR based indicators under conservation agriculture based rice-wheat cropping system in Indo-Gangetic Plain. *Appl. Soil Ecol.* 193, 105128. https://doi.org/10.1016/j.apsoil.2023.105128.

Degens, B.P., 1997. Macro-aggregation of soils by biological bonding and binding mechanisms and the factors affecting these: a review. *Soil Res.* 35 (3), 431–460. https://doi.org/10.1071/s96016.

Drigo, B., Pijl, A.S., Duyts, H., Kielak, A.M., Gamper, H.A., Houtekamer, M.J., Boschker, H.T., Bodelier, P.L., Whiteley, A.S., van Veen, J.A., Kowalchuk, G.A., 2010. Shifting carbon flow from roots into associated microbial communities in response to elevated atmospheric CO2. *Proc. Natl. Acad. Sci. U S A* 107 (24), 10938–10942. https://doi.org/10.1073/pnas.0912421107.

Driver, J.D., Holben, W.E., Rillig, M.C., 2005. Characterization of glomalin as a hyphal wall component of arbuscular mycorrhizal fungi [Article]. *Soil Biol. Biochem.* 37 (1), 101–106. https://doi.org/10.1016/j.soilbio.2004.06.011.

Duan, T., Facelli, E., Smith, S.E., Smith, F.A., Nan, Z., 2011. Differential effects of soil disturbance and plant residue retention on function of arbuscular mycorrhizal (AM) symbiosis are not reflected in colonization of roots or hyphal development in soil. *Soil Biol. Biochem.* 43 (3), 571–578. https://doi.org/10.1016/j.soilbio.2010.11.024.

Eden, M., Schjonning, P., Moldrup, P., De Jonge, L.W., 2011. Compaction and rotovation effects on soil pore characteristics of a loamy sand soil with contrasting organic matter content. *Soil Use Manag.* 27 (3), 340–349. https://doi.org/10.1111/j.1475-2743.2011.00344.x.

Fao, 2018. Transforming food and agriculture to achieve SDGs. *Food and Agriculture Organization of the United Nations*.

FAO, & ITPS. (2020). Towards a definition of soil health. *ITPS Soil Letters 1*. https://openknowledge.fao.org/server/api/core/bitstreams/ffb5feaf-8388-4e2f-b319-2260a9a6f5a2/content.

Gianinazzi, S., Gollotte, A., Binet, M.-N., Van Tuinen, D., Redecker, D., Wipf, D., 2010. Agroecology: the key role of arbuscular mycorrhizas in ecosystem services. *Mycorrhiza* 20 (8), 519–530. https://doi.org/10.1007/s00572-010-0333-3.

Gillespie, A.W., Farrell, R.E., Walley, F.L., Ross, A.R.S., Leinweber, P., Eckhardt, K.U., Regier, T.Z., Blyth, R.I.R., 2011. Glomalin-related soil protein contains non-mycorrhizal-related heat-stable proteins, lipids and humic materials. *Soil Biol. Biochem.* 43 (4), 766–777. https://doi.org/10.1016/j.soilbio.2010.12.010.

Giray, K., Banfield, C., Piepho, H.-P., Joergensen, R.G., Dippold, M., Wachendorf, C., 2024. Main soil microbial groups assessed by phospholipid fatty acid analysis of temperate alley agroforestry systems on crop- and grassland. *Appl. Soil Ecol.* 195, 105277. https://doi.org/10.1016/j.apsoil.2024.105277.

Gómez-Muñoz, B., Jensen, L.S., Munkholm, L., Olesen, J.E., Møller Hansen, E., Bruun, S., 2021. Long-term effect of tillage and straw retention in conservation agriculture systems on soil carbon storage. *Soil Sci. Soc. Am. J.* 85 (5), 1465–1478. https://doi.org/10.1002/saj2.20312.

Gryndler, M., Hršelová, H., Cajthaml, T., Havránková, M., Řezáčová, V., Gryndlerová, H., Larsen, J., 2009. Influence of soil organic matter decomposition on arbuscular mycorrhizal fungi in terms of asymbiotic hyphal growth and root colonization. *Mycorrhiza* 19 (4), 255–266. https://doi.org/10.1007/s00572-008-0217-y.

Gu, S., Wu, S., Guan, Y., Zhai, C., Zhang, Z., Bello, A., Guo, X., Yang, W., 2020. Arbuscular mycorrhizal fungal community was affected by tillage practices rather than residue management in black soil of northeast China. *Soil Tillage Res.* 198, 104552. https://doi.org/10.1016/j.still.2019.104552.

Gupta, M.M., 2020. Arbuscular Mycorrhizal Fungi: The Potential Soil Health Indicators. In: *Soil Health*. Springer International Publishing, pp. 183–195. https://doi.org/10.1007/978-3-030-44364-1_11.

Hawkins, H.J., Cargill, R.I.M., Van Nuland, M.E., Hagen, S.C., Field, K.J., Sheldrake, M., Soudzilovskaia, N.A., Kiers, E.T., 2023. Mycorrhizal mycelium as a global carbon pool. *Curr. Biol.* 33 (11), R560–R573. https://doi.org/10.1016/j.cub.2023.02.027.

Helgason, B.L., Walley, F.L., Germida, J.J., 2010. No-till soil management increases microbial biomass and alters community profiles in soil aggregates. *Appl. Soil Ecol.* 46 (3), 390–397. https://doi.org/10.1016/j.apsoil.2010.10.002.

Hobbs, P.R., Sayre, K., Gupta, R., 2008. The role of conservation agriculture in sustainable agriculture. *Philos. Trans. R. Soc., B* 363 (1491), 543–555. https://doi.org/10.1098/rstb.2007.2169.

Hydbom, S., Olsson, P.A., 2021. Biochemical signatures reveal positive effects of conservation tillage on arbuscular mycorrhizal fungi but not on saprotrophic fungi and bacteria. *Appl. Soil Ecol.* 157, 103765. https://doi.org/10.1016/j.apsoil.2020.103765.

Indoria, A.K., Rao, C.S., Sharma, K.L., Reddy, K.S., 2017. Conservation agriculture - a panacea to improve soil physical health. *Curr. Sci.* 112 (1), 52–61. https://doi.org/10.18520/cs/v112/i01/52-61.

Irving, T.B., Alptekin, B., Kleven, B., Ané, J.M., 2021. A critical review of 25 years of glomalin research: a better mechanical understanding and robust quantification techniques are required. *New Phytol.* 232 (4), 1572–1581. https://doi.org/10.1111/nph.17713.

Iversen, B.V., Schjønning, P., Poulsen, T.G., Moldrup, P., 2001. In situ, on-site and laboratory measurements of soil air permeability: boundary conditions and measurement scale. *Soil Sci.* 166 (2), 97–106. https://journals.lww.com/soilsci/fulltext/2001/02000/in_situ,_on_site_and_laboratory_measurements_of.3.aspx.

Jensen, J.L., Eriksen, J., Thomsen, I.K., Munkholm, L.J., Christensen, B.T., 2022. Cereal straw incorporation and ryegrass cover crops: The path to equilibrium in soil carbon storage is short. *Eur. J. Soil Sci.* 73 (1), e13173. https://doi.org/10.1111/ejss.13173.

Jensen, J.L., Schjønning, P., Watts, C.W., Christensen, B.T., Peltre, C., Munkholm, L.J., 2019. Relating soil C and organic matter fractions to soil structural stability. *Geoderma* 337, 834–843. https://doi.org/10.1016/j.geoderma.2018.10.034.

Joergensen, R.G., 2021. Phospholipid fatty acids in soil—drawbacks and future prospects. *Biol. Fertil. Soils* 58 (1), 1–6. https://doi.org/10.1007/s00374-021-01613-w.

Kabir, Z., 2005. Tillage or no-tillage: Impact on mycorrhizae. *Can. J. Plant Sci.* 85 (1), 23–29. https://doi.org/10.4141/p03-160.

Kabir, Z., O'Halloran, I.P., Hamel, C., 1999. Combined effects of soil disturbance and fallowing on plant and fungal components of mycorrhizal corn (*Zea mays* L.). *Soil Biol. Biochem.* 13, 307–314. https://doi.org/10.1016/S0038-0717(98)00124-2.

Kabir, Z., O'Halloran, I.P., Widden, P., Hamel, C., 1998. Vertical distribution of arbuscular mycorrhizal fungi under corn (*Zea mays* L.) in no-till and conventional tillage systems. *Mycorrhiza* 8 (1), 53–55. https://doi.org/10.1007/s005720050211.

Kemper, W.D., Rosenau, R.C., 1986. Aggregate Stability and Size Distribution. In: Klute, A. (Ed.), *Methods of Soil Analysis. Part 1 - Physical and Mineralogical Methods*. American Society of Agronomy Inc, Madison, Wisconsin, USA. https://doi.org/10.2136/sssabookser5.1.2ed.c17.

Krauss, M., Wiesmeier, M., Don, A., Cuperus, F., Gattinger, A., Gruber, S., Haagsma, W. K., Peigné, J., Palazzoli, M.C., Schulz, F., van der Heijden, M.G.A., Vincent-Caboud, L., Wittwer, R.A., Zikeli, S., Steffens, M., 2022. Reduced tillage in organic farming affects soil organic carbon stocks in temperate Europe. *Soil Tillage Res.* 216, 105262. https://doi.org/10.1016/j.still.2021.105262.

Kumar, N., Nath, C.P., Hazra, K.K., Das, K., Venkatesh, M.S., Singh, M.K., Singh, S.S., Praharaj, C.S., Singh, N.P., 2019. Impact of zero-till residue management and crop diversification with legumes on soil aggregation and carbon sequestration. *Soil Tillage Res.* 189, 158–167. https://doi.org/10.1016/j.still.2019.02.001.

Lehmann, J., Bossio, D., Kögel-Knabner, I., Rillig, M., 2020. The concept and future prospects of soil health. *Nat. Rev. Earth Environ.* 1 (10), 544–553. https://doi.org/10.1038/s43017-020-0080-8.

Li, J., Zhao, J., Liao, X., Hu, P., Wang, W., Ling, Q., Xie, L., Xiao, J., Zhang, W., Wang, K., 2024. Pathways of soil organic carbon accumulation are related to microbial life history strategies in fertilized agroecosystems. *Sci. Total Environ.* 927, 172191. https://doi.org/10.1016/j.scitotenv.2024.172191.

Lin, J.S., Sarto, M.V.M., Carter, T.L., Peterson, D.E., Gura, C., Mino, L., Rohrs, M., Lucas, H., Clark, J., Rice, C.W., 2023. Soil organic carbon, aggregation and fungi community after 44 years of no-till and cropping systems in the Central Great Plains, USA. *Arch. Microbiol.* 205 (3), 84. https://doi.org/10.1007/s00203-023-03421-2.

Martínez-García, L.B., Korthals, G., Brussaard, L., Jørgensen, H.B., De Deyn, G.B., 2018. Organic management and cover crop species steer soil microbial community structure and functionality along with soil organic matter properties. *Agr Ecosyst Environ* 263, 7–17. https://doi.org/10.1016/j.agee.2018.04.018.

Mondal, S., Chakraborty, D., 2022. Global meta-analysis suggests that no-tillage favourably changes soil structure and porosity. *Geoderma* 405, 115443. https://doi.org/10.1016/j.geoderma.2021.115443.

Morris, E.K., Morris, D.J.P., Vogt, S., Gleber, S.C., Bigalke, M., Wilcke, W., Rillig, M.C., 2019. Visualizing the dynamics of soil aggregation as affected by arbuscular mycorrhizal fungi. *ISME J.* 13 (7), 1639–1646. https://doi.org/10.1038/s41396-019-0369-0.

Munkholm, L.J., Hansen, E.M., Olesen, J.E., 2008. The effect of tillage intensity on soil structure and winter wheat root/shoot growth. *Soil Use Manag.* 24 (4), 392–400. https://doi.org/10.1111/j.1475-2743.2008.00179.x.

Nunes, M.R., van Es, H.M., Schindelbeck, R., Ristow, A.J., Ryan, M., 2018. No-till and cropping system diversification improve soil health and crop yield. *Geoderma* 328, 30–43. https://doi.org/10.1016/j.geoderma.2018.04.031.

Olsson, P.A., Baath, E., Jakobsen, I., 1997. Phosphorus effects on the mycelium and storage structures of an arbuscular mycorrhizal fungus as studied in the soil and roots by analysis of Fatty Acid signatures. *Appl. Environ. Microbiol.* 63 (9), 3531–3538. https://doi.org/10.1128/aem.63.9.3531-3538.1997.

Rillig, M.C., 2004. Arbuscular mycorrhizae, glomalin, and soil aggregation. *Can. J. Soil Sci.* 84, 355–363.

Rillig, M.C., Mummey, D.L., 2006. Mycorrhizas and soil structure. *New Phytol.* 171 (1), 41–53. https://doi.org/10.1111/j.1469-8137.2006.01750.x.

Rillig, M.C., Wright, S.F., Eviner, V.T., 2002. The role of arbuscular mycorrhizal fungi and glomalin in soil aggregation:comparing effects of five plant species. *Plant and Soil* 238 (2), 325–333. https://doi.org/10.1023/a:1014483303813.

Rillig, M.C., Wright, S.F., Nichols, K.A., Schmidt, W.F., Torn, M.S., 2001. Large contribution of arbuscular mycorrhizal fungi to soil carbon pools intropical forest soils. *Plant and Soil* 233 (2), 167–177. https://doi.org/10.1023/a:1010364221169.

Rocco, S., Munkholm, L.J., Jensen, J.L., 2024. Long-term soil quality and C stock effects of tillage and cover cropping in a conservation agriculture system. *Soil Tillage Res.* 241, 106129. https://doi.org/10.1016/j.still.2024.106129.

Schjønning, P., 2023. Straw management in small grain cereal crop production-The long-term effects on soil carbon and soil pore characteristics. *Geoderma* 435, 116499. https://doi.org/10.1016/j.geoderma.2023.116499.

Shukla, A., Vyas, D., Anuradha, J., 2013. Soil depth: an overriding factor for distribution of arbuscular mycorrhizal fungi. *J. Soil Sci. Plant Nutr.* 13, 23–33. https://doi.org/10.4067/s0718-95162013005000003.

Singh, A.K., Rai, A., Pandey, V., Singh, N., 2017. Contribution of glomalin to dissolve organic carbon under different land uses and seasonality in dry tropics. *J. Environ. Manage.* 192, 142–149. https://doi.org/10.1016/j.jenvman.2017.01.041.

Skaalsveen, K., Ingram, J., Clarke, L.E., 2019. The effect of no-till farming on the soil functions of water purification and retention in north-western Europe: A literature review. *Soil Tillage Res.* 189, 98–109. https://doi.org/10.1016/j.still.2019.01.004.

Säle, V., Aguilera, P., Laczko, E., Mäder, P., Berner, A., Zihlmann, U., Van Der Heijden, M.G.A., Oehl, F., 2015. Impact of conservation tillage and organic farming on the diversity of arbuscular mycorrhizal fungi. *Soil Biol. Biochem.* 84, 38–52. https://doi.org/10.1016/j.soilbio.2015.02.005.

Taylor, S.A., 1950. Oxygen diffusion in porous media as a measure of soil aeration. *Soil Sci. Soc. Am. J.* 14 (C), 55–61. https://doi.org/10.2136/sssaj1950.036159950014000c0013x.

Thomopoulos, S., Elsgaard, L., Munkholm, L.J., Ravnskov, S., 2023. Evaluation of the relation between soil biomass of arbuscular mycorrhizal fungi and glomalin-related soil protein in conservation agriculture. *Soil Biol. Biochem.* 187, 109222. https://doi.org/10.1016/j.soilbio.2023.109222.

Walder, F., Büchi, L., Wagg, C., Colombi, T., Banerjee, S., Hirte, J., Mayer, J., Six, J., Keller, T., Charles, R., Van Der Heijden, M.G.A., 2023. Synergism between production and soil health through crop diversification, organic amendments and crop protection in wheat-based systems. *J. Appl. Ecol.* 60 (10), 2091–2104. https://doi.org/10.1111/1365-2664.14484.

Wang, Z., Zhao, J., Xiao, D., Chen, M., He, X., 2024. Higher colonization but lower diversity of root-associated arbuscular mycorrhizal fungi in the topsoil than in deep soil [Article]. *Appl. Soil Ecol.* 194, 105195. https://doi.org/10.1016/j.apsoil.2023.105195.

Weidhuner, A., Hanauer, A., Krausz, R., Crittenden, S.J., Gage, K., Sadeghpour, A., 2021. Tillage impacts on soil aggregation and aggregate-associated carbon and nitrogen after 49 years. *Soil Tillage Res.* 208, 104878. https://doi.org/10.1016/j.still.2020.104878.

Wepruk, E., Diochon, A., Van Eerd, L.L., Gregorich, E., Deen, B., Hooker, D., 2023. Identifying rotation and tillage practices that maintain or enhance soil carbon and its relation to soil health. *Can. J. Soil Sci.* 103 (1), 191–199. https://doi.org/10.1139/cjss-2021-0161.

Wilson, G.W., Rice, C.W., Rillig, M.C., Springer, A., Hartnett, D.C., 2009. Soil aggregation and carbon sequestration are tightly correlated with the abundance of arbuscular mycorrhizal fungi: results from long-term field experiments. *Ecol. Lett.* 12 (5), 452–461. https://doi.org/10.1111/j.1461-0248.2009.01303.x.