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Evaluation of the relation between soil biomass of arbuscular mycorrhizal fungi and glomalin-related soil protein in conservation agriculture

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) are indicators of soil health and are associated with various soil benefits, primarily linked to glomalin accumulation from hyphal turnover. However, the direct connection between glomalin-related soil protein (GRSP) and AMF has been questioned. The study aimed to investigate the correlation between different fractions of GRSP and fatty acid fractions in soil, as well as the impact of conservation agriculture practices on AMF biomass and GRSP content. Findings revealed a positive correlation between easily extractable (EE) GRSP and phospholipid fatty acid (PLFA) 16:1ω5, while no significant correlations were found for difficultly extractable (DE) or total GRSP fractions. These results highlight the complexity of GRSP dynamics and the need for further research on different fractions and their relation to AMF biomass. Additionally, the study demonstrated that mechanical soil management had a greater impact on AMF hyphal biomass and EE-GRSP compared to residue management. Direct seeding, a reduced tillage approach, led to higher hyphal biomass and EE-GRSP, indicating AMF sensitivity to tillage intensity. This suggests that tillage practices exert a stronger influence on AMF abundance and GRSP content than residue management.

1. Introduction

The increasing demand for food, feed, and fuel has raised concerns about the sustainability of agricultural practices and their impact on the environment and climate change, along with its resulting consequences (FAO, 2018). Notably, the importance of field management practices has been highlighted, and emphasis has been focused on exploring ways to avoid soil degradation in agricultural fields (Jia et al., 2019; Lal, 2015; Keesstra et al., 2018). Conservation agriculture (CA) is defined as a plant production system that embodies three main principles, namely, minimal soil disturbance, diverse crop rotation and residue retention; and the contribution of these managements to agricultural sustainability and to improve soil quality has been thoroughly discussed (Hobbs et al., 2008; Palm et al., 2014; Cárceles Rodríguez et al., 2022). In particular, the combination of no-tillage with residue retention has been connected with higher microbial biomass carbon (Li et al., 2018), better soil structural stability (Abdollahi et al., 2017) and increased total soil porosity (Abdollahi and Munkholm, 2017).

Arbuscular mycorrhizal fungi (AMF) represent a pivotal soil biota group that can promote sustainable plant production strategies (Gianinazzi et al., 2010). AMF form symbiotic relationships with around 72% of all land plant species, and this association supplies the fungal symbiont with C in exchange for soil-derived nutrients (Brundrett and Tedersoo, 2018). The AMF-induced C demand generates a flow of photosynthates to the fungi, reaching up to 30% of the total C fixed by the plants (Drigo et al., 2010). Carbon enters fungi through arbuscules – short-lived fungal structures developed inside the root cortex – in the form of hexoses, some of which are then converted into neutral lipids and phospholipids or directly through lipid transfer from the plant hosts (Pfeffer et al., 1999; Bonfante and Genre, 2010; Keymer et al., 2017). The neutral lipids are subsequently translocated to the extraradical hyphae to maintain C flow in the mycelium, and they are correlated with storage organs such as spores and vesicles, while phospholipids are membrane segments associated with arbuscules and hyphal length (Olsson et al., 1997).

Both phospholipid fatty acids (PLFA) and neutral lipid fatty acids (NLFA) have been applied to estimate AMF biomass in the soil (Olsson et al., 1995, 1999). However, there is an ongoing debate regarding using PLFAs as an indicator of AMF biomass. This debate is based on the observation that the AMF-specific PLFA 16:1ω5 can also be found in certain Gram-negative bacteria (Nichols et al., 1986), and therefore it has been seen as an inaccurate biomarker for AMF (Hydbom and Olsson,

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2021). In a recent review, the use of PLFA 16:1ω5 for quantification of AMF biomass in field studies was not discouraged, but it was strongly recommended that it is supported by the estimation of NLFA 16:1ω5, which is considered more sensitive to AMF biomass fluctuations (Olsson and Lekberg, 2022; Lekberg et al., 2022). On the contrary, Joergensen (2021) encourages using PLFA 16:1ω5 to estimate AMF biomass in the soil as he concluded that there was no experimental evidence that PLFA 16:1ω5 occurs in marked amounts in Gram-negative bacteria.

AMF biomass has been estimated by applying direct saponification of soil samples and quantifying the 16:1ω5 in whole cell or whole soil fatty acids (WCFA-WSFA) of the soil (Larsen et al., 2009; Welc et al., 2010). Due to the absence of lipid fractionation in this method, it is not suitable for determining whether the fatty acids are linked to storage organs or membrane compounds, which can be achieved by measuring NLFA and PLFA separately (Olsson et al., 1997). Still, the WCFA-WSFA methodology has been successfully employed to explore AMF biomass response to different agricultural practices (Ferrari et al., 2018).

The contribution of AMF to soil C sequestration and soil aggregation has been associated with a soil glycoprotein initially termed 'glomalin' that is released to the soil during AMF hyphae turnover (Wright and Upadhyaya, 1996, 1998; Rillig et al., 2001; Driver et al., 2005). The importance of this protein in soil organic carbon (SOC) distribution and storage has been well documented (Singh et al., 2016, 2017; Kumar et al., 2018; Agnihotri et al., 2021). Since its discovery by Wright et al., in 1996, a growing controversy has been raised regarding terminology, quantification, and origin of 'glomalin' (Rillig, 2004; Singh et al., 2013). For this study, we will follow the nomenclature proposed by Irving et al. (2021), where glomalin is considered as an unidentified putative gene product, potentially HSP60 of AMF origin, and glomalin-related soil protein (GRSP) is a proteinous soil glomalin fraction that is operationally defined using citrate buffers and autoclaving for its extraction from soil. Total GRSP (TG) can then be divided into two different groups; easily extractable (EE) and difficultly extractable (DE), based on the conditions of autoclaving and the molarity and pH of the buffer used.

After the extraction, GRSP can be quantified by utilizing an indirect enzyme-linked immunosorbent assay (ELISA) or, more commonly, with the Bradford protein assay (Bradford, 1976); results from the two methods show a high positive correlation (Wright and Upadhyaya, 1999; Rillig, 2004). However, other compounds like lipids, phenols, or humic substances can be found in the extracts (Rosier et al., 2006; Gillespie et al., 2011), which can cause interference with the Bradford assay (Jorge-Araújo et al., 2015). This is the main reason why the AMF origin of GRSP has been questioned (Irving et al., 2021), and approaches have been adopted where this group of soil extracts has been defined more as a general soil health indicator rather than a specific AMF-produced protein (Hurisso et al., 2018). Nevertheless, GRSP is reported to decline when no AMF hyphae are grown (Steinberg and Rillig, 2003; Rillig, 2004), while numerous studies indicate a significant correlation between GRPS fractions and AMF abundance or hyphal length (Li et al., 2020; Agnihotri et al., 2021; Wang et al., 2022). At the same time, the estimates of Bradford-detected compounds are aligned with the expected behaviour of glomalin, bolstering the assumption that the Bradford assay is suitable for identifying GRSP (Koide and Peoples, 2013).

This study seeks to enhance our understanding of the relation between AMF biomass, measured by different fatty acid fractions, and GRSP content while also exploring the effects of CA practices on these variables.

2. Materials and methods

2.1. Field experiment conditions

The field experiment was conducted in Flakkebjerg, Denmark (55°32′ N and 11°39' E) during the growing season of 2021. The soil is a sandy loam formed from mixed glacial deposits, and it classifies as Glossic Phaeozem according to the World Reference Base (FAO). The subsoil is typically clayey, but sandy lenses can occur. The soil at Flakkebjerg contains 14.7% clay, 13.7% silt, 42.6% fine sand, 27% coarse sand, and 2% organic matter (Munkholm et al., 2008). Total organic carbon content in 2021 was 13.2 g kg^{-1} dry weight. The experiment followed a split-plot design with four replicates, having residue management as the main plot factor and soil mechanical treatment as the subplot factor. The main plot factor included two levels; one where residues were removed from the field (R3) and one where they were retained (R4), while the subplot factor had three levels; moldboard ploughing to 20 cm (MP), harrowing to 10 cm (H) and direct drilling (D). The field had been treated with the same methods since 2003, while the crop rotation included cereal and legume crops. In the year of sampling, winter wheat was sown as the main crop, and fodder radish was used as cover crop. Sampling took place in September 2021 by randomly taking ten subsamples of 0–20 cm depth for each plot. The subsamples were then pooled, mixed, freeze-dried, and ground before the analysis.

2.2. AMF biomass quantification

AMF biomass was quantified by measuring signature fatty acids from soil samples. To extract PLFA/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. After extraction, the lipids were separated into neutral, polar and glycol-lipids. This was done by dissolving the samples in 100 μl of chloroform. NLFA were eluted with 1.5 ml chloroform, whereas PLFA were eluted with 1.5 mAl of methanol. The increase in lipid polarity in this step separates the lipids into PLFA and NLFA pools, which are collected and evaporated to dryness with N at 40 °C in a heat block. Next, 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 then incubated for 15 min at 37 °C in a water bath before adding hexane: chloroform (4:1), acetic acid and water and centrifuged at 2000 g for 5 min. Then the top phase was transferred to a 4 ml vial and evaporated to dryness with N_2.

For the extraction of WCFA, 1 g of freeze-dried and ground soil was mixed with 45 g of NaOH, 150 ml of methanol and 150 ml of MilliQ water and placed in a water bath at 100 °C for 30 min. The strong methanolic base and the high temperature separate the fatty acids from the cell lipids and convert them to sodium salts, which were later transformed into fatty acid methyl esters by adding 325 ml of 6 M HCl and 275 ml of methanol and placing the mixture in a water bath at 80 °C for 10 min. Next, 200 ml of hexane and 200 ml of methyl tert-butyl ether were added, and the samples were centrifuged at 1500 g for 5 min. The upper phase was transferred to a new tube with 10.8 g of NaOH and 900 ml of MilliQ water and centrifuged at 1500 g for 5 min. Finally, the supernatant was transferred to a 4 ml vial and evaporated to dryness with N_2 and resuspended in 100 μL of hexane.

GC analysis, with a flame ionization detector, was used to estimate each sample's signature fatty acid concentration using nonadecanoate fatty acid marker (C 19:0) as internal standard. The GC oven temperature was raised gradually from 170 to 260 °C at a rate of 5 °C per minute and then increased further (at a faster rate of 40 °C per minute) until reaching a final temperature of 310 °C. Hydrogen and nitrogen were employed as carrier and make-up gases, respectively. A phenyl-siloxane (2,5%) column was used (25 m long, 200 μm ID, 0,33 μm film). A hydrogen-air mixture was used to supply a flame ionization detector. The analysis of fatty acids was conducted using the MIDI microbial identification protocol (specifically, Sherlock version 4.5 MIDI, Microbial ID, Newark, DE, USA) and the software library TSBA41. Results were expressed as nmol g^{-1} soil on dry weight basis.

2.3. GRSP extraction and quantification

Two fractions of GRSP, EE and DE, were extracted from the soil samples based on the method suggested by Wright and Upadhyaya

(1998). Before the extraction, technical replicates of the samples were generated.

First, EE was extracted from 1 g soil in 8 ml 20 mM sodium citrate (pH = 7) by autoclaving for 30 min at 120 °C in 50 ml Teflon tubes. Subsequently, samples were centrifuged at 5000 g for 10 min, and 6 ml of the supernatant was transferred to 15 ml Falcon tubes. These supernatant samples were centrifuged again at 5000g for 10 min, and 4 ml of the resulting supernatant were transferred to new 15 ml falcon tubes, which were stored for one day at 4 °C until quantification.

The DE fraction was extracted by adding 6 ml of 50 mM sodium citrate (pH = 8) to the Teflon tubes, where the pellet from the autoclave cycle of EE process was, and vortexed until homogeneous. Subsequently, the resuspended mixture was autoclaved for 1h at 120 °C, before centrifugation (5000g). Finally, 6 ml of the supernatant were removed and stored for one day at 4 °C until quantification. It should be noted that in this study only the quasi-total DE fraction was obtained. This differs from other studies where a variable number of extractions are typically conducted until the solution shows none of the red brown colour. This approach is based on the unconfirmed assumption that the coloration is attributed to proteins and not humic substances.

The content of GRSP was evaluated using the Bradford assay (Bradford, 1976). For this procedure, 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 A595. Bovine serum albumin (BSA) was used as the standard. GRSP content was expressed as mg protein g^{-1} soil on dry weight basis.

2.4. Statistical analysis

All statistical data analysis was performed using R version 4.2.1 (R Core Team, 2022). Linear mixed models were created to evaluate the correlation between the different fatty acid and glomalin fractions using the lme4 package (Bates et al., 2015). The fatty acid fractions were set as response variables, whereas the glomalin pools (i.e., EE, DE, and TG) were the fixed effect predictors. Blocks in the field experiment were set as random effect variables. Homoscedasticity and normality were tested by visual examination of residual plots. Conditional R-squared (R^2C) values described by Nakagawa and Schielzeth (2013) were calculated to evaluate the proportion of the variation explained by the fixed effect and the total model using the MuMIn package (Bartoń, 2023). The Pearson correlation coefficient (r) was used to assess the linear correlation between the variables. For exploring the impact of soil and residue management treatments on fatty acids and glomalin fractions, linear mixed models were developed again using the lme4 package. Here soil and residue management were considered fixed effect variables, and blocks were set as random effects. The fixed variables' interaction, additivity, and single effects 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. For comparing the treatments, the Tukey multiple comparison method with a significance

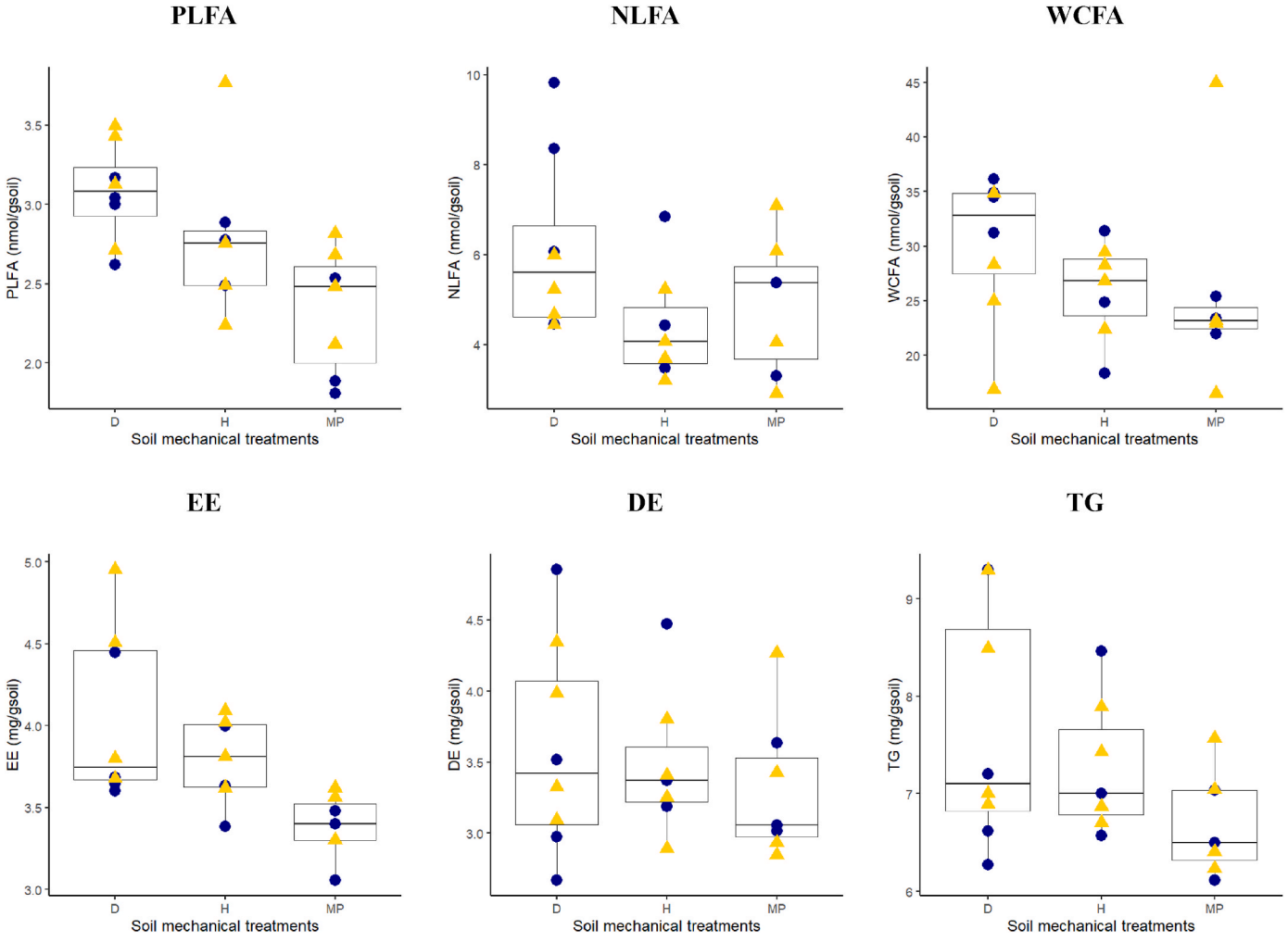

Fig. 1. Soil mechanical and residue management treatment effects on phospholipid fatty acid (PLFA), neutral lipid fatty acid (NLFA), whole cell fatty acid (WCFA), easily extractable (EE), difficultly extractable (DE), and total (TG) GRSP content. Soil mechanical treatments represent ploughing (MP), harrowing (H), and direct sowing (D). Symbols illustrate residue management treatments, i.e., with straw removed (blue-circles) or straw retained (yellow-triangles).

level of 0.05 was used.

3. Results

3.1. Effect of soil and residue management treatments on fatty acid and GRSP pools

The effect of residue treatments was not found to be significant for any of the variables examined.

The average concentration of PLFA 16:1ω5 showed a decrease as tillage intensity increased, going from 3.07 nmol g^{-1} soil in directly sown plots to 2.83 nmol g^{-1} soil in harrowed plots and 2.36 nmol g^{-1} soil in ploughed plots (Fig. 1). The impact of soil management on PLFA content was statistically significant ($F_{(2, 20)} = 9.337$, $p = 0.001$). Similarly, EE exhibited a similar trend, decreasing with increasing tillage intensity. The average EE content in directly sown plots was 4.04 mg g^{-1} soil, while it was 3.81 mg g^{-1} soil in harrowed plots and 3.43 mg g^{-1} soil in ploughed plots (Fig. 1). The effect of soil management on EE content was also significant ($F_{(2, 20)} = 7.66$, $p = 0.003$).

No significant treatment effect was revealed in NLFA concentration which ranged from an average of 4.89 nmol g^{-1} soil in ploughed plots to 4.56 nmol g^{-1} soil in harrowed plots and 6.13 nmol g^{-1} soil in direct sown plots. This was also the case in WCFA content, which had a minimal increase from 25.47 nmol g^{-1} soil in ploughing treatment to 30.21 nmol g^{-1} soil in direct sowing on average (Fig. 1).

No significant impact of the soil treatment was found on DE and TG content (Fig. 1). The average DE content only showed a slight increase from 3.3 mg g^{-1} soil in ploughing treatment to 3.47 mg g^{-1} soil in harrowing treatment and 3.59 mg g^{-1} soil in direct sowing treatment. Similarly, the average TG content varied from 6.69 mg g^{-1} soil in ploughed to 7.26 mg g^{-1} soil in harrowed plots and 7.64 mg g^{-1} soil in direct sown plots.

The NLFA/PLFA ratio was greater than 1 in 23 out of 24 samples.

3.2. Relationship between signature fatty acid for AMF (16:1ω5) and GRPS fractions

Changes in EE were associated with changes in PLFA 16:1ω5 ($p=0.006$), and EE explained a significant proportion of the variation in PLFA 16:1ω5 ($R^2C=0.44$), and the two variables exhibited a moderate positive correlation ($r=0.42$). EE did not predict NLFA 16:1ω5 and WCFA 16:1ω5 accurately, with p-value being 0.61 and 0.25, respectively. It did not explain a considerable level of their variation as they had a very low R^2C, and no significant correlation was present according to the Pearson correlation coefficient.

DE was a weak predictor of any of the signature fatty acid 16:1ω5 fractions, and it did not account for the variation observed, as indicated by R^2C values ranging from less than 0.001 to 0.03. No correlation between DE and PLFA 16:1ω5 or WCFA 16:1ω5 was found, while a weak negative correlation ($r= -0.35$) with NLFA 16:1ω5 was detected.

No association between changes in TG and changes in signature fatty acid 16:1ω5 was observed as the relative p-values varied from 0.15 to 0.53. TG did not explain the variation in fatty acids, as evidenced by R^2C values of 0.02 for PLFA 16:1ω5, 0.08 for NLFA 16:1ω5 and 0.02 in WCFA 16:1ω5. The Pearson correlation coefficient did not reveal any correlation between TG and fatty acid fractions.

4. Discussion

4.1. Relationship between fatty acid and GRPS fractions

The positive impacts of AMF on soil structure and soil C sequestration have been associated with GRSP (Wright and Upadhyaya, 1998; Rillig et al., 2001; Singh et al., 2020). However, the utilization of GRSP for evaluating substances derived from AMF has faced criticism, particularly when employing the Bradford method to measure this proteinaceous pool (Rosier et al., 2006; Irving et al., 2021). Consequently, efforts have been made to explore ways of improving the methodology (Redmile-Gordon et al., 2013; Moragues-Saitua et al., 2019; Cissé et al., 2020). However, recent studies utilizing the Bradford assay to determine GRSP have reported very strong positive correlations between AMF biomass indicators and EE or DE GRSP (Li et al., 2020; Agnihotri et al., 2021; Wang et al., 2022). Our study was able to detect a moderate positive correlation only between EE and PLFA (Table 1).

The connection between EE and PLFA is reasonable, taking into account that EE is released from AMF hyphae (Wright and Upadhyaya, 1998), and PLFA are indicative of the hyphal biomass (Olsson et al., 1995). However, GRSP accumulates in the soil after extraradical hyphae turnover (Driver et al., 2005), and that increases the complexity around GRSP temporal dynamics in soil, as it becomes challenging to account for the coexistence of substantial quantities of living hyphae and high amounts of EE – a substance that accumulates after the hyphae degradation. A possible answer may be hidden in the intraradical GRSP content, which was not included in the present study. (Rosier et al., 2008) discovered significant amounts of GRSP, identified through the Bradford assay, that were produced by the intraradical mycelium. They also found good correlations between GRSP and root colonization. As a result, it is not unreasonable to assume that, although the main mode of delivery for GRSP into the soil is through the turnover of extraradical mycelium (Driver et al., 2005), a portion of the GRSP present in the soil may have originated from the intraradical mycelium. Consequently, it is plausible that a substantial amount of GRPS coexists with living extraradical hyphae.

However, there is a potential alternative explanation that may stem from the ambiguity surrounding the exact lifespan of the recently produced GRPS. In other words, EE is considered a younger GRSP fraction compared to DE, but its exact age is unknown (Rillig, 2004). In addition, by utilizing the Bradford assay, it has been estimated that the GRSP has a turnover rate of roughly 35 years (Harner et al., 2004). Thus, if we assume that the detection of EE, produced at least one year ago, is feasible using the same method, it may elucidate the coexistence of both EE and living hyphae.

No correlation was observed between DE or TG and any of the measured fatty acids. In addition, these two GRSP fractions were poor predictors of AMF living biomass (Table 1). DE is presumed to be the firmly attached to soil particles fractions and it constitutes the older component of the GRSP present in the soil. Therefore, it can be more challenging to discover a direct connection between long-persistent DE and the PLFA 16:1ω5, which are dynamically associated with the living microbial biomass and have a high turnover rate in soil (Frostegård et al., 2011; Zhang et al., 2019).

4.2. Effect of soil and residue management treatments

The findings of our study suggest that mechanical soil management had a greater impact on biomass of AMF hyphae and EE than residue management. Specifically, the direct seeding of crops resulted in a significantly higher amount of hyphal biomass and EE than the ploughing treatment. This result was anticipated as the negative impact of tillage intensity on AMF abundance is well-documented (Bowles et al., 2017; Agnihotri et al., 2021; Mhlanga et al., 2022). AMF is known to be a tillage-sensitive organism, and shifts in soil disturbance can adversely affect its hyphal network (Kabir et al., 1999). Tillage can detach AMF hyphae from the host plant, while deep ploughing can disperse fungal propagules to greater depths in the soil, thereby reducing the infection levels on host plants (Kabir, 2005). In addition, tillage-induced changes in soil aggregation can also negatively affect AMF abundance resulting in a lower hyphal network (Helgason et al., 2010).

Consequently, it is reasonable to assume that the favourable impact of conservational tillage on AMF hyphae (Fig. 1) led to a higher accumulation of GRSP in soil. However, this was only observed in EE as no distinct trend was found in DE, even though the plots had undergone the

Table 1
P-value, Pearson correlation coefficient(r), and conditional R^2 as measured from the regression models performed using easily extractable (EE), difficultly extractable (DE), and total (TG) glomalin as predictor variables and signature fatty acid for arbuscular mycorrhizal fungi 16:1ω5 quantified by phospholipid fatty acids (PLFA), neutral-lipid fatty acids (NLFA), and whole-cell fatty acids (WCFA), as response variables.

	EE			DE			TG		
	P-value	r	R^2C	P-value	r	R^2C	P-value	r	R^2C
PLFA	0.006	0.42	0.44	0.8	−0.06	0.03	0.15	0.16	0.25
NLFA	0.61	−0.11	0.01	0.09	−0.35	0.12	0.18	−0.27	0.08
WCFA	0.25	0.25	0.06	0.89	0.06	<0.001	0.53	0.16	0.02

same tillage practices for a period of twenty years. Since DE represents the older GRSP fraction, this management period was expected to result in noteworthy differences in DE content, but our findings did not support this assumption. The study's outcome, however, agrees with Rillig (2004), who found that due to the GRSP turnover time, shifts in soil GRSP stocks in response to tillage practices may occur at a relatively slow rate. Besides soil management impacts on AMF hyphal network, previous studies have also associated conservation tillage with richer AMF spore density and higher NLFA values compared to ploughing (Wetzel et al., 2014; Säle et al., 2015; Hydbom and Olsson, 2021). Interestingly, our study did not detect significant differences in NLFA 16:1ω5 or WCFA 16:1ω5 among soil or residue management treatments, but only a trend for higher fatty acid content in response to reduced tillage intensity.

The study findings contribute to the ongoing debate regarding the suitability of using PLFA 16:1ω5 as an indicator of AMF biomass. In a recent work by Olsson and Lekberg (2022), they proposed that NLFA 16:1ω5 might be a more accurate measure of AMF biomass in field studies, provided that bacterial biomass is assessed using signature PLFAs and the NLFA/PLFA ratio exceeds 1 indicating that a substantial proportion of the NLFA 16:1ω5 corresponds to AMF. Although our study adhered to both of these conditions, the expected changes in AMF biomass in response to tillage intensity were not detected using NLFA 16:1ω5. On the contrary, the shifts were evident when using PLFA 16:1ω5, despite the absence of non-mycorrhizal control plants or mycelium-free compartments in our investigation.

In this study, the impact of residue management on AMF biomass and GRSP content was investigated. By retaining residues from the previous crop on the field, the organic carbon input into the soil is enhanced, and although AMF cannot directly benefit from soil carbon sources, previous research has suggested that changes in AMF growth can occur in the presence of organic compounds (Ravnskov et al., 1999). Gryndler et al. (2009) described two main ways that AMF mycelia could be affected by organic matter decomposition, either through the compounds released during the decomposition or, additionally, by the presence of secondary metabolites produced by the microorganisms involved in decomposition. Our study did not observe any significant effect of crop residue retention on AMF signature fatty acids or on GRSP content. Likewise, Duan et al. (2011) could not detect a clear impact of wheat or medic residue application in hyphal length or AMF colonization, while on the contrary, more recent studies report a significant increase of AMF root colonization and GRPS concentration under wheat and rice residue retention (Yang et al., 2020). Our findings align with the study conducted by Gu et al. (2020), which also indicated that residue management did not have a significant impact on AMF biomass. In addition, their research suggested that tillage practices exerted a stronger influence on AMF compared to residue treatments (Gu et al., 2020).

In conclusion, the objective of this study was to advance our comprehension of the relationship between AMF, assessed through various fatty acid fractions, and GRSP, utilizing a long-term conservation agriculture experiment as an example. Findings suggest that a moderate positive correlation between EE-GRSP and PLFA 16:1ω5 was observed. However, no correlation was found between DE- or TG-GRSP fractions and any measured fatty acids fractions. The complex temporal dynamics of GRSP in soil and the ambiguity surrounding the lifespan of GRSP pools highlight the challenges in understanding this correlation. Further research is needed to clarify the temporal dynamics of different GRSP fractions and their connections to AMF biomass. In addition, mechanical soil management had a greater impact on the biomass of AMF hyphae and EE-GRSP than residue management. Direct seeding resulted in higher hyphal biomass and EE-GRSP, confirming the sensitivity of AMF to tillage intensity. The findings suggest that the CA principle of no tillage may have a more pronounced effect in promoting AMF abundance and GRSP content when compared to the CA practice of maintaining a permanent soil cover.

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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.

Data availability

Data will be made available on request.

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