European Journal of Soil Biology 103 (2021) 103286

Contents lists available at ScienceDirect

European Journal of Soil Biology

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

Original article

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

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ARTICLE INFO

Handling editor: X Xu

Keywords:
AMF signature Fatty acid biomarker
Glomalin related protein, soil management practices
Raised-beds
Soil carbon stocks

ABSTRACT

Arbuscular mycorrhizal fungi (AMF) contribute to the sequestration of soil organic carbon (SOC) by glomalin production through their hyphal network which helps to bind soil aggregates and improve other physical and biological properties of soil. The current study was aimed to assess (i) AMF biomass, glomalin related protein (GRP), SOC stocks and soil quality parameters such as microbial biomass carbon (MBC) and β-glucosidase activity, and (ii) to find out whether GRP production and PLFA C16:1ω5 can be used as consistent indicators of soil quality across seven different rhizosphere soil niches such as zero-tillage with *Cenchrus ciliaris* and minimum-tillage with *Chloris barbata*; conventional tillage with soybean-wheat system from soybean rhizosphere and raised beds with four mycorrhizal host plants (Fenugreek, maize, marigold and sorghum). Among all the soil niches, AMF biomass, the content of SOC, MBC, soil and root GRP, the activity of β-glucosidase were significantly higher under zero tillage. The AMF biomass, SOC-sequestration and soil quality parameters established a common trend across all the soil management systems and hosts examined. PLFA C16:1ω5 was positively correlated with microscopic estimates of AMF biomass, MBC, β-glucosidase activity and both the fractions of total (T) GRP (the easily extractable and difficulty extractable) in soil and roots. A significant positive correlation of both the fractions of soil-GRP with MBC (r = 0.78**, 0.83**) and β-glucosidase activity (r = 0.86**, 0.76**) was also found. In general, soil T-GRP (r = 0.93**), soil T-GRP stocks (0.94**) and PLFA C16:1ω5 (r = 0.68**) were highly related to SOC stocks. These findings confirm that zero tillage and raised beds favour AMF activity thus improving SOC sequestration potential and soil quality which can be assessed using GRP and PLFA C16:1ω5 as potential indicators.

1. Introduction

Soils are the largest organic carbon sink recognized in the terrestrial ecosystems and are of significant interest because of their potential in mitigating atmospheric carbon dioxide (CO_2). As a result, any changes in soil C stocks may influence the concentration of CO_2 in the atmosphere [1]. Several studies have indicated that besides mining of C, the change in land-use of an agroecosystem adversely affects the belowground soil microbiota, and associated ecological functions [1,2]. Hence, the adoption of appropriate agricultural management practices for sequestering high soil organic carbon (SOC) is an important strategy to improve agroecosystem's carbon storage capacity, soil biological health and to mitigate atmospheric CO_2 emissions [3].

Soil microbes are known to contribute to soil C sequestration through various processes primarily mediated by plants and management practices. Arbuscular mycorrhizal fungi (AMF) are an important group of soil microbes present in most agroecosystems and colonize majority of land plants including agricultural crops [4] and are also involved in carbon cycling. These fungi have the potential to increase the plant-root absorptive surface area which enables plants to access soil resources which are otherwise beyond the reach of plants. Thus, AMF help plants in nutrient acquisition, improve plant growth and protect plants from biotic and abiotic stresses [4]. In return, the AMF acquire plant-assimilates which are necessary for their growth. Hence, AMF are significant in regulating C transport from host plants to their hyphae and thus may, directly or indirectly, influence the soil C sequestration [1,

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https://doi.org/10.1016/j.ejsobi.2021.103286
Received 13 January 2020; Received in revised form 23 December 2020; Accepted 9 January 2021

5–7].

During symbiosis, the extended network of AMF hyphae in the soil (extraradical) binds the soil particles together and aid in soil aggregation by the glueing action by producing a glycoprotein called glomalin on hyphal walls [6–9]. Glomalin, has been characterized as a recalcitrant glycoprotein having C as an important moiety and is involved in the stabilization of soil carbon [5,7,8,10]. Glomalin also makes a substantial contribution to SOC storage measured in terms of the ratio of total glomalin stocks to SOC stocks [11].

Studies under taken during the past 40 years have shown that the common agricultural management practices like the cultivation of non-mycorrhizal crops, fertilizer applications, crop rotation, crop protection and intensive tillage may affect AMF symbiosis, particularly in arable lands [12]. Similarly, glomalin content may also vary among different ecological habitats, tillage systems [13–15] and across the plant species colonized by one or different AMF species [16]. Conventional tillage can lead to disruption of AMF extraradical hyphal network, thereby contributing to low levels of glomalin, active AMF biomass, other beneficial soil microorganisms [1,15]. On the other hand, conservation tillage and sustainable farming practices (low tillage, intercropping, cover crops) enhance AMF biomass, SOC which in turn improve soil structure and aggregation [12,15,17]. Therefore, to aptly elucidate the effect of particular agricultural management practice on AMF biomass and glomalin content, the assessment techniques plays a critical role. The studies on the comprehensive assessment of glomalin (root and soil) and AMF live-biomass through microscopic and biochemical methods (AMF signature fatty acid biomarkers) under different soil and crop management practices are scarce. The typical methods used to measure AMF biomass include conventional microscopic measurement of AMF spores density in soil [18] and colonization in roots [19,20]. The biochemical methods where analysis of AMF signature fatty acid biomarkers such as AMF specific C16:1ω5 PLFA (phospholipid fatty acid) and NLFA (neutral lipid fatty acid) have gained popularity over conventional methods in recent years [21]. C16:1ω5 PLFA and NLFA have been used as signatures to represent hyphal biomass and storage lipids in spores respectively [22]. Glomalin production also takes place inside the roots by intraradical hyphae [23]. Thus, by quantifying root glomalin, mycorrhizal colonization inside the plant roots can be detected [24]. Upon its release into the soil, glomalin associates with organic matter and therefore, it was more specifically termed as 'glomalin related soil protein (GRSP)' [23]. Depending upon the turnover in soil, glomalin can be divided into different fractions. The recently produced glomalin is quantified as easily extractable glomalin related soil protein (EE-GRSP) and another fraction called difficulty extractable- GRSP (DE-GRSP) is tightly bound to soil colloids and is considered recalcitrant fraction [3,25]. However, the precise quantification of GRSP pools using Bradford reagent is bit difficult due to the co-extraction of organic matter present in the soil [26]. Glomalin content and β-glucosidase activity are important soil quality indicators [27,28]. β-glucosidase is crucial to C-cycling [27] as it induces the release of glucose into the soil [29]. Glomalin and β-glucosidase could perform a similar function as the activity of β-glucosidase correlates with GRSP and is affected by its spatial distribution in soil [30]. The maintenance of active microbial biomass in the soil is attributed to the activity of β-glucosidase [29]. Besides, microbial biomass carbon (MBC) is also an important predictor of soil quality indicator for the identification of preliminary changes in soil C stabilization and nutrient dynamics resulting from soil disturbance [31]. Hence, glomalin content is expected to correlate with microbial biomass carbon and the activity of β-glucosidase and since glomalin is produced on AMF hyphae, the higher content of glomalin, microbial biomass carbon and the activity of β-glucosidase are expected to be influenced under the system with minimum soil disturbance.

Apart from the soil, glomalin produced inside the AMF colonizing roots may correlate with the mycorrhizal colonization percentage in roots and PLFA C16:1ω5 in the soil thus acting as a potential indicator for assessing the impact of agricultural management systems on AMF biomass. As far as we know, there are no reports to indicate the glomalin content in soil and roots and AMF biomass as a measure of soil C sequestration and associated pertinent soil quality parameters in different agriculture management systems. Therefore, the hypothesis of the current study is to examine if AMF biomass (microscopic estimates and signature fatty acid biomarkers) and GRP (glomalin related protein) can act as consistent indices for soil C sequestration potential and soil quality under contrasting soil management practices. Hence, the present study was carried out to (i) assess the AMF biomass, soil C-sequestration through glomalin production and pertinent changes in important soil quality parameters in different soil management systems and host plants and, (ii) examine the relationships between AMF biomass, GRP, SOC, soil GRP stocks, and associated soil quality parameters.

2. Materials and methods

2.1. Study site and experimental setup

The study was conducted during 2016 at ICAR- Indian Institute of Soybean Research, Indore, Madhya Pradesh, India, which is located at 22°8′N latitude and 75°4′E longitude. The climatic parameters are subtropical (semi-arid), mean temperature of 25 °C (5–45 °C), and mean precipitation of 800 mm; principally sustained by the monsoon (June to September). The edaphic characteristics (across collection sites) exhibited Sarol series soils (Fine, iso-hyperthermic, montmorillonitic, typic haplusterts) [32]; pH (1:2.5, soil: water) 8.0, clay content 56.2%, and organic carbon 0.5%, bulk density 1.30 Mg m^{-3} (all measured at zero time). The β-glucosidase activity, microbial biomass carbon and soil T-GRP were 135.00 μg p-nitrophenol g^{-1} soil, 180.00 mg C kg^{-1} soil and 0.80 g kg^{-1} soil respectively.

The treatments comprised of seven soil management systems that included raised bed with four host plants including maize (*Zea mays*) (RB-M), sorghum (*Sorghum bicolor*) (RB-SV), fenugreek (*Trigonella foenum-graceum*) (RB-F) and marigold (*Tagetes erecta*) (RB-MG); zero tillage with *Cenchrus ciliaris* (ZT-CC), minimum tillage with *Chloris barbata* (MT-CB) and conventional tillage with mineral fertilization from soybean rhizosphere under soybean-wheat rotation (CT-S).

The conventional tillage system under soybean-wheat rotation was managed with mineral fertilization (nitrogen: phosphorus: potassium (NPK) for soybean- 20:26:20 kg ha^{-1}; wheat-120:26:20 kg ha^{-1}). The conventional tillage practice comprised one-time ploughing + two times cultivator + one-time planking and performed before sowing. These systems were maintained for the past 10 years. Organic manures (farmyard manure) at the rate of 10 tonnes ha^{-1} were applied to the permanent raised bed, in addition to zero and minimum tillage systems. The plants were irrigated to requisite field capacity and managed with recommended agronomic practices. The study was laid out in a completely randomized block design with three replications.

2.2. Rhizosphere soil and root sampling

The established methodology for rhizosphere soil and root sampling was adopted [32]. Sampling was undertaken during September 2016 at the reproductive stages of the plants (soybean: 65 days after sowing (DAS); marigold: 55 DAS; fenugreek: 50 DAS; maize: 65 DAS; sorghum: 65 DAS). In *Cenchrus ciliaris* and *Chloris barbata* sampling was done when grasses started showing dried flowers. Soil samples from the rhizosphere of all nine plants were collected from each niches and subsequently mixed, homogenized, and constituted into three composite samples to serve as three replicates per site. The root samples (three replicates) were washed gently under tap water, removed excess moisture and stored at 4 °C and process for root colonization and glomalin.

All the three replicates of each rhizosphere host soil were divided in to three parts, one part was stored at 4 °C and used for the analysis of β-glucosidase activity, MBC and AMF spore extraction, second part was stored at −20 °C for phospholipids analysis and the remaining part was

air dried and used for glomalin related protein pools, soil organic carbon and their stocks.

2.3. Quantification of AMF biomass, soil C and glomalin stock and pertinent soil quality parameters

2.3.1. Assessment of mycorrhizal colonization percentage (MCP) and spore density

About 1.5–2.0 g (fresh weight) of fine roots were used for staining and the assessment of AMF colonization. Roots were cleared and stained as per the method described by Phillips and Hayman [19] and estimated the root colonization under a compound microscope at 20 × (Motic digital microscope DMWB series) using the grid line-intersect method [20]. The extraction of AMF spores was carried out by wet sieving and decanting method [18], quantified in the suspension under a stereo zoom microscope (Motic SMZ-168 series) and expressed as spores g^{-1} soil.

2.3.2. Glomalin extraction and quantification

The extraction of GRP pools from the soil samples (1 g) was carried out by the method described by Wright and Upadhyaya [25] and for root samples (10 mg) as per the protocol of Rosier et al. [24]. The two pools of GRP viz., the easily extractable (EE-GRP) and difficulty extractable (DE-GRP) were sequentially extracted and the sum of the two pools represented the total GRP (T-GRP) in both soil and roots. EE-GRP was extracted with 20 mM sodium citrate (pH 7) at 121 °C for 30 min. The supernatants were kept at 4 °C till quantification. Post EE-GRP extraction, 50 mM sodium citrate (pH 8) at 121 °C for 60 min was used to extract DE-GRP (till a colourless supernatant was obtained) from the remaining soil sample. using. The supernatants from each extraction cycle were centrifuged at 15,000 rpm for 15 min and pooled. Samples from zero tillage (ZT-CC) and raised beds required 5–6 extraction cycles, whereas, samples from MT-CB and CT-S required 4–5 extraction cycles. As a precautionary measure, the pooled supernatants were concentrated by evaporation in a water bath at 90 °C, to prevent glomalin overestimation that may result from dilution of samples during the repeated extraction cycles. Samples were purified by precipitation with 20% trichloroacetic acid (TCA) and stored at 4 °C until further analysis. The content of glomalin related protein pool in soil and root samples was determined spectrophotometrically (λ = 595 nm) by the Bradford protein assay [33] using Bovine serum albumin as standard. 2000 μl of the extract diluted with phosphate buffered saline (PBS) (500 μl: 1500 μl extract: PBS) was mixed with 500 μl of Bio-Rad Bradford dye (Coomassie brilliant blue G-250). After 5 min of incubation time, absorbance was measured at A_{595}. Prior to the procedure, the supernatants were centrifuged at 10,000xg for 5 min and the results were expressed in g kg^{-1} soil.

2.3.3. SOC, calculation of SOC and soil GRP stocks, MBC and β-glucosidase activity

SOC was analysed by adopting the standard dichromate oxidation technique [34]. The stocks of SOC was calculated by using the following equation: SOCs = h × d × c, where SOCs was SOC stock in the 0–15 cm soil layer (Mg ha^{-1}), h was the thickness (m), d was the bulk density (Mg m^{-3}), and c was the amount of SOC (g kg^{-1}) [35]. The calculations based on Bai et al. [11] aided in assessing the contribution of soil T-GRP stocks to SOC stocks. In addition, soil T-GRP (sum of soil EE-GRP and DE-GRP pools) was also calculated from the procedure described for SOC stocks.

The β-glucosidase activity was determined using 4-nitrophenyl β-D-glucopyranoside (PNG, 0.05 M) as a substrate and the amount of *p*-nitrophenol consequently released was determined spectrophotometrically at A_{420} and expressed as μg *p*-nitrophenol g^{-1} soil [36]. The standard fumigation-extraction method was applied for MBC estimation calculated as MBC = EC/KC, where EC is the difference of the extractable carbon between fumigated and non-fumigated soil samples and KC conversion factor (0.45) [37] and results were expressed in mg C kg^{-1} soil.

2.3.4. Quantification of AMF biomarker phospholipids fatty acids in soil

Phospholipid fatty acid analysis (PLFA) was performed following the high throughput method [21,38]. The whole process comprised of four steps that is drying, extraction, lipid separation and transesterification. From approximately 1.5 g of lyophilized soil sample post Bligh dyer extraction, lipids were separated and extracted on a 96 well solid phase extraction (SPE) column. The phospholipids obtained by elution with 5:5:1 methanol: chloroform: H_2O fraction from the SPE column were transesterified to fatty acid methyl esters (FAMEs), dissolved in 75 μl hexane and transferred to 2 ml GC vials with conical glass inserts. Gas chromatography was performed as described by Buyer and Sasser [38] on an Agilent 7890A GC (Agilent Technologies, Wilmington, DE, USA) equipped with an autosampler, split-splitless injector and flame ionization detector. The system was controlled with Agilent Chemstation and MIDI Sherlock software (Microbial ID, Inc., Newark, DE, USA). FAMEs were separated on an Agilent HP-Ultra 2 column, 25 m long × 0.2 mm internal diameter × 0.33 μm film thickness. A split ratio of 30:1 was used with hydrogen as a carrier gas and samples were analysed as per the programme described by Buyer and Sasser [38]. FAME profiles were identified using the MIDI PLFAD1 calibration mix and peak naming table (MIDI, Inc., DE, USA). The signature fatty acid PLFA C16:1ω5 which is a biomarker of AMF biomass was assessed and values were reported in PLFA nanomoles g^{-1} soil.

2.4. Statistical analysis/data analysis

All data were analysed using the one factor analysis of variance (ANOVA) (SAS Institute Inc., 1991). The data are mean values of three replicates ± standard deviation. Means with different letters differ significantly at $p = 0.05$ according to Fisher LSD. The least significant difference (LSD) of Duncan's Multiple Range Test (DMRT) was used to separate the treatment means. The analysis of Pearson correlation coefficients was done to determine the relationship of crop and soil management systems with AMF and soil related parameters.

3. Results

3.1. MCP, spore density and AMF signature phospholipid fatty acid analysis

AMF biomass assessed either microscopically or biochemically through signature phospholipid fatty acid analysis was found to be significantly higher in the ZT-CC and RB-M systems in comparison to the CT-S system (Fig. 1).

A significantly higher MCP was recorded in RB-M (76.83%) and ZT-CC (76.43%) over the other systems examined. MCP was lowest in CT-S plots (24%) (Fig. 1a). The spore density was highest in ZT-CC plots (36.29 spores g^{-1} soil) which was followed by RB-M (34.34 spores g^{-1} soil) and lowest count was observed in CT-S plots (3.20 spores g^{-1} soil). Nonetheless, among the raised beds, significantly higher spore densities were recorded with maize (34.34 spores g^{-1} soil) and sorghum (32.34 spores g^{-1}) over the other hosts examined (fenugreek: 18.91 spores g^{-1} soil and marigold: 14.80 spores g^{-1} soil) (Fig. 1b).

AMF signature PLFA C16:1ω5 was found to be significantly higher in ZT-CC (3.13 nmol g^{-1} soil) and did not significantly vary with raised beds, RB-M (2.86 nmol g^{-1} soil), RB-SV (2.18 nmol g^{-1} soil) and also with the adoption of MT-CB (2.06 nmol g^{-1} soil). A significantly lower content of PLFA C16:1ω5 was observed with RB-MG (1.08 nmol g^{-1} soil) and RB-F (1.98 nmol g^{-1} soil) and CT-S (1.65 nmol g^{-1} soil) (Fig. 1c).

3.2. Root and soil GRP

The perusal of the data on GRP pools (Fig. 2a) in roots revealed significantly higher root T-GRP under ZT-CC (3.15 g kg^{-1} root) as

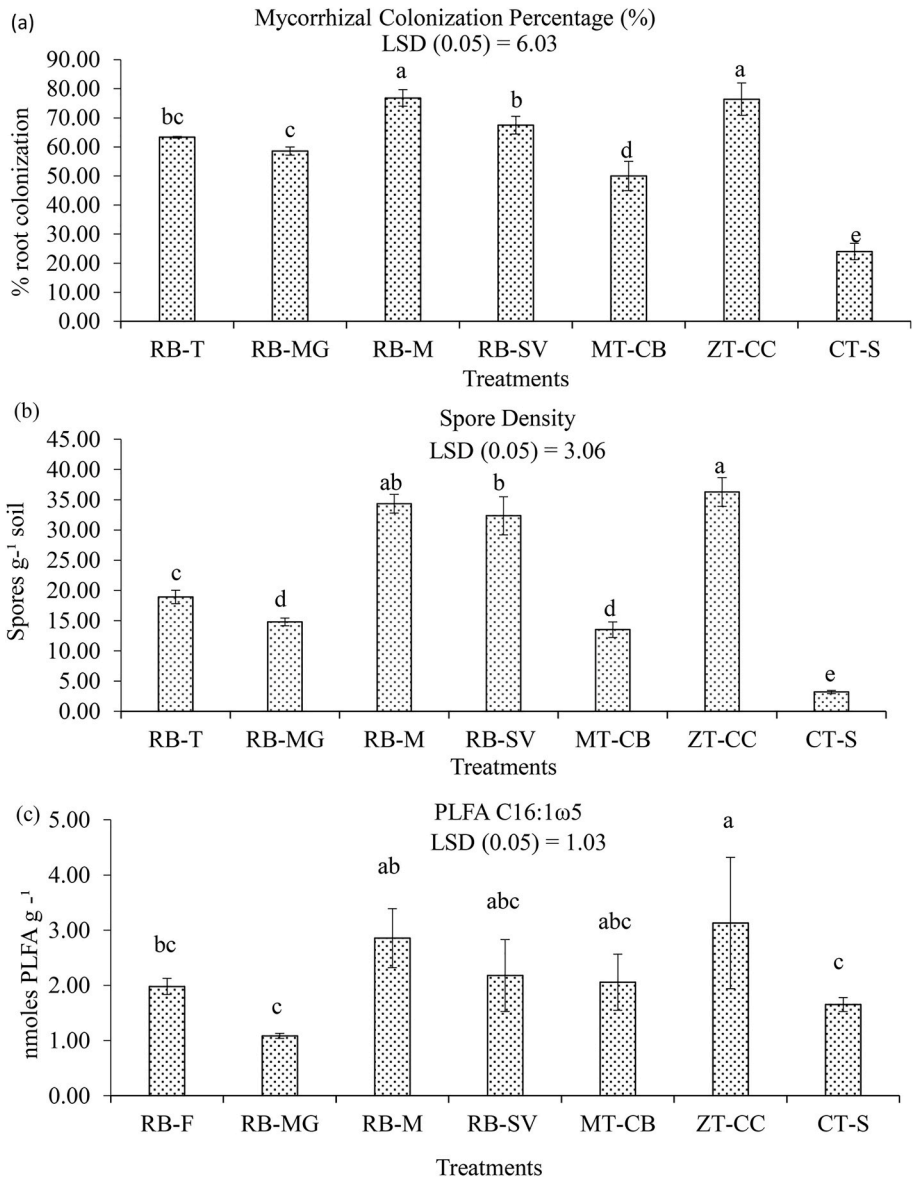

Fig. 1. Root and soil associated AMF parameters assessed under different soil management systems (a) Mycorrhizal colonization percentage (MCP) (b) Spore density (c) Phospholipid fatty acid (PLFA) C16:1ω5. Data are mean of three replicates ± standard deviation. The bars of treatment followed by the same letter did not differ significantly by least significant difference (LSD) of Duncan's multiple range test (DMRT; $P = 0.05$). RB-F: raised bed fenugreek; RB-MG: raised bed marigold; RB-M: raised bed maize; RB-SV: raised bed sorghum; MT-CB: minimum tillage with *Chloris barabata*; ZT-CC: zero tillage with *Cenchrus ciliaris*; CT-S: conventional tillage with soybean.

compared to the other management systems examined. This was followed by raised beds (all the hosts) wherein significantly higher content of root T-GRP was observed as compared to minimum and conventional tillage systems. Crops grown under raised bed systems significantly increased root T-GRP with higher content in RB-M (3.01 g kg^{-1} root) and RB-SV (2.85 g kg^{-1} root) compared to RB-F (2.76 g kg^{-1} root) and RB-MG (2.70 g kg^{-1} root) (Fig. 2a). The raised bed system, irrespective of hosts had significantly higher T-GRP in roots than the minimum tillage (2.18 g kg^{-1} root) and conventional management systems (0.86 g kg^{-1} root). Across all the systems, the root EE and DE-GRP pools followed a similar trend (Fig. 2a).

Regarding the soil GRP pools, ZT-CC registered significantly higher easily extractable (0.33 g kg^{-1} soil), difficulty extractable (3.68 g kg^{-1} soil) and T-GRP (4.01 g kg^{-1} soil) compared to other management paradigm tried (Fig. 2b). Among the raised bed undisturbed ecosystems, RB-M recorded higher soil T-GRP in comparison to other crops tried. The soil T-GRP content in RB ranged from 2.37 to 3.05 g kg^{-1} soil and significantly higher content was observed with the maize (3.05 g kg^{-1} soil) than the other host plants maintained in the raised beds. The lowest soil T-GRP (0.57 g kg^{-1} soil) was observed in CT-S. Irrespective of hosts, raised beds showed a higher soil T-GRP than minimum tillage (MT-CB) and conventional tillage (CT-S) (Fig. 2b).

3.3. Soil C-sequestration parameters (SOC and soil T-GRP stocks)

Among all the soil management systems examined, significantly higher soil T-GRP stocks and SOC stocks were recorded under ZT-CC (Fig. 3a). The ZT-CC system had significantly higher SOC-stocks (15.01 Mg C ha^{-1} $year^{-1}$) and soil T-GRP stocks (7.78 Mg GRP ha^{-1} $year^{-1}$) against CT-S (SOC stocks: 11.05 Mg C ha^{-1} $year^{-1}$; soil T-GRP: 1.19 Mg GRP ha^{-1} $year^{-1}$) and MT-CB (SOC stocks:12.11 Mg C $ha^{-1}year^{-1}$ and soil T-GRP stocks: 4.39 Mg GRP ha^{-1} $year^{-1}$) (Fig. 3a). The contribution of soil T-GRP stocks to SOC stocks was 51.84%, and 36.22% in ZT-CC, and MT-CB systems, respectively. The contribution of soil T-GRP stocks to SOC stocks in the CT-S system was the lowest (10.78%). Among the raised bed systems, RB-M showed significantly higher stocks of SOC (13.86 Mg C $ha^{-1}year^{-1}$) and soil T-GRP (5.93 Mg GRP ha^{-1} $year^{-1}$) over RB-F (SOC stocks: 12.37 Mg C ha^{-1} $year^{-1}$ and soil T-GRP stocks: 4.66 Mg GRP ha^{-1} $year^{-1}$), RB-MG (SOC stocks: 12.57 Mg C ha^{-1} $year^{-1}$ and soil T-GRP stocks: 4.72 Mg GRP ha^{-1} $year^{-1}$) and RB-SV (SOC stocks: 12.64 Mg C ha^{-1} $year^{-1}$ and soil T-GRP stocks: 4.80 Mg GRP ha^{-1} $year^{-1}$).Among the raised beds, RB-F had the

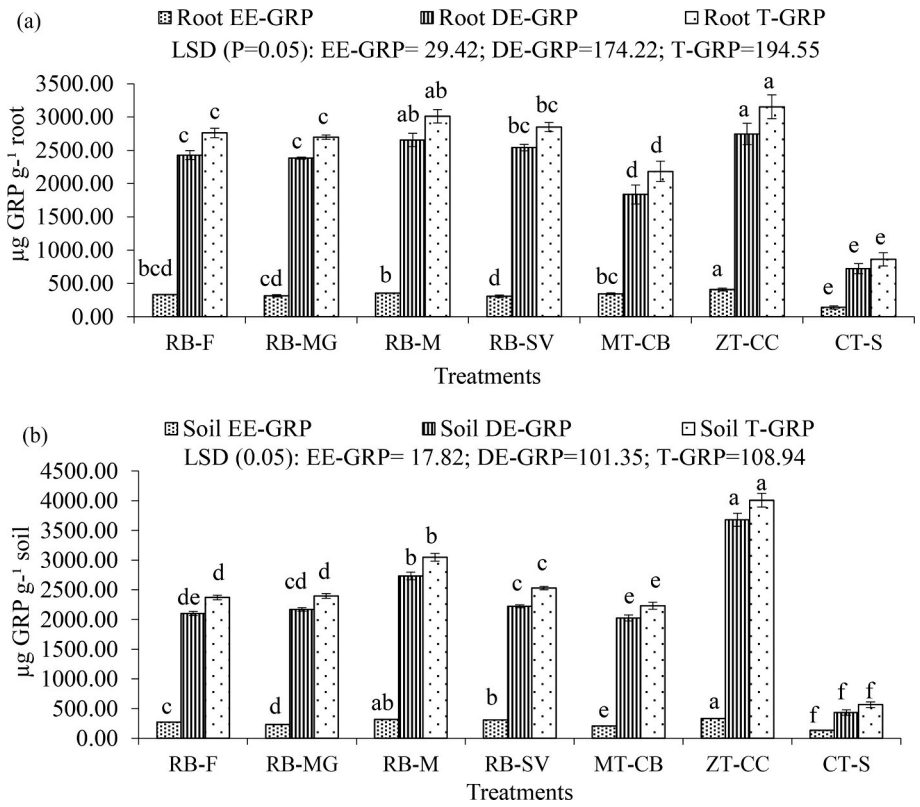

Fig. 2. Easily extractable (EE), difficulty extractable (DE) and total (T) glomalin related protein (GRP) assessed in (a) Root (b) Soil. Data are mean of three replicates ± standard deviation. The bars of treatment followed by the same letter did not differ significantly by least significant difference (LSD) of Duncan's multiple range test (DMRT; $P = 0.05$). RB-F: raised bed fenugreek; RB-MG: raised bed marigold; RB-M: raised bed maize; RB-SV: raised bed sorghum; MT-CB: minimum tillage with *Chloris barabata*; ZT-CC: zero tillage with *Cenchrus ciliaris*; CT-S: conventional tillage with soybean.

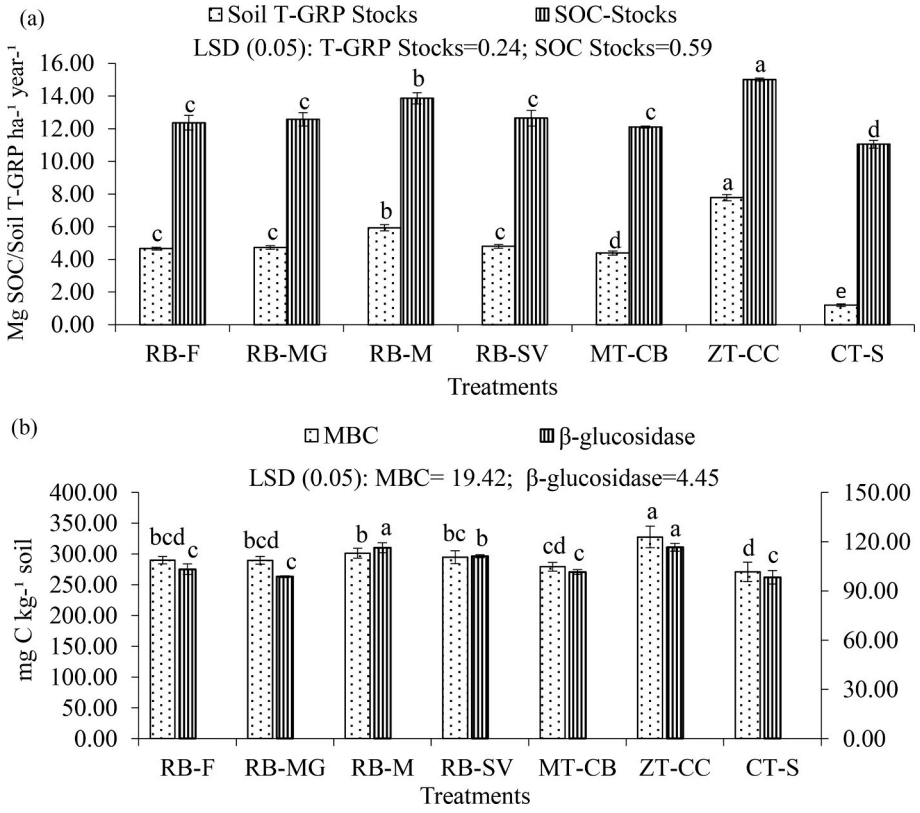

Fig. 3. Soil quality and carbon sequestration parameters assessed under different soil management systems (a) Soil total glomalin related protein (soil T-GRP) stocks and soil organic carbon (SOC) stocks (b) Microbial biomass carbon (MBC) and β-glucosidase activity. Data are mean of three replicates ± standard deviation. The bars of treatment followed by the same letter did not differ significantly by least significant difference (LSD) of Duncan's multiple range test (DMRT; $P = 0.05$). RB-F: raised bed fenugreek; RB-MG: raised bed marigold; RB-M: raised bed maize; RB-SV: raised bed sorghum; MT-CB: minimum tillage with *Chloris barabata*; ZT-CC: zero tillage with *Cenchrus ciliaris*; CT-S: conventional tillage with soybean.

lowest SOC stocks (12.37 Mg C ha^{-1} year^{-1}) and soil T-GRP stocks (4.66 Mg GRP ha^{-1} year^{-1}) (Fig. 3a). Among the different hosts used in the raised beds, a higher contribution of soil T-GRP stocks to SOC stocks was observed in RB-M (42.79%) than RB-F (37.69%), RB-MG (37.60%) and RB-SV (37.98%).

3.4. Soil quality parameters (MBC and β-glucosidase activity)

MBC also followed the same trend as observed in the case of AMF biomass and significantly higher content was observed with ZT-CC (327.67 mg C kg^{-1} soil) as compared to other agricultural management systems assessed in this study. The CT-S and MT-CB system had the lowest MBC (270.95 mg C kg^{-1} soil) (Fig. 3b). Among the raised beds systems, RB-SV (294.82 mg C kg^{-1} soil) and RB-M (301.33 mg C kg^{-1} soil) had significantly higher MBC than RB-F (289.82 mg C kg^{-1} soil) and RB-MG (289.66 mg C kg^{-1} soil) (Fig. 3b).

The ZT-CC and RB-M system showed significantly higher β-glucosidase activity, i.e., 116.60 mg p-nitrophenol kg^{-1} soil and 116.27 mg p-nitrophenol kg^{-1} soil, respectively compared to other soil management systems examined (Fig. 3b). Conversely, the activity of β-glucosidase in raised beds of sorghum fenugreek and marigold hosts was higher than CT-S and MT-CB, but, the content did not significantly vary except for sorghum beds which showed significantly higher activity than the CT-S and MT-CB system.

3.5. Relationship between the determinants of AMF biomass, measures of C-sequestration and soil quality parameters

The relationship among the microscopic (MCP and spore count), and biochemical parameters (C16:1ω5 PLFA, and soil and root GRP, SOC stocks, MBC and β-glucosidase activity) was significant and positive (Table 1). AMF signature PLFA C16:1ω5 was significantly and positively correlated with MCP (r = 0.55**) and spore count (r = 0.65**). PLFA C16:1ω5 was also correlated both with root T-GRP (r = 0.44*) soil T-GRP (r = 0.58**).

The correlation of PLFA C16:1ω5 with EE-GRP fractions of soil and root was higher as compared to T-GRP fractions. A significant positive correlation of PLFA C16:1ω5 with SOC stocks (r = 0.68**) and soil T-GRP stocks (r = 0.57**) was also observed. T-GRP (soil and root) had a significant and positive association with SOC stocks. The soil and root glomalin pools, both individually (soil EE-GRP, r = 0.85**; soil DE-GRP, r = 0.93**; root EE-GRP, r = 0.80**; root DE-GRP, r = 0.78**) and collectively (soil T-GRP, r = 0.93**; root T-GRP, r = 0.79**) were positively and significantly correlated with SOC stocks. Soil T-GRP stocks of all the systems were found to have a significant and positive association with SOC stocks (r = 0.94**). A significant positive correlation of MBC with T- GRP (soil and roots, r = 0.83**& 0.72**, respectively) and PLFA C16:1ω5 (0.75**) was obtained. T-GRP stocks and PLFA C16:1ω5 were significantly correlated with β-glucosidase activity (r = 0.76** and 0.78**, respectively) (Table 1 and supplementary Tables).

4. Discussion

In general, a conservation tillage practice supports resident AMF and concomitantly increases carbon-associated parameters (SOC-stocks, MBC and β glucosidase). A less disturbed soil ecosystem such as zero tillage, permanent raised beds and minimum tillage examined in the current study revealed a significantly higher spore density, root colonization and PLFA C16:1ω5 than the soils under conventional tillage. Higher AMF biomass (PLFA C16:1ω5, mycorrhizal colonization) is attributed to the intact hyphal network and shift in the community structure of AMF under undisturbed or minimum tillage system which supports higher hyphal density [39]. Being a product of the AMF hyphal walls, GRP fractions followed the similar trend as AMF biomass significantly higher GRP content (soil and root) was observed with zero tillage and permanent raised beds with maize as a host plant. The soils under the current study have a dominant population of *Glomus* species. The reason for a higher AMF biomass in maize is that *Glomus* species are the dominant colonizers of maize roots [40] and AMF biomarker C16:1ω5 has also been detected in maize [41]. Apart from AMF biomass, the stocks of soil T-GRP and SOC followed the similar trend and were significantly higher in zero tillage followed by raised bed maize.

Through rhizodeposition, about 40% of the photosynthetically fixed C as rhizodeposits are transferred into the soil [42]. This could be attributed that AMF colonized roots can make a substantial contribution to the carbon stored in the form of glomalin. Besides, C contained in soil sugars makes a substantial contribution to the soil organic matter [43]. Similarly, glomalin which is a C containing glycoprotein is also expected to substantially contribute to the soil organic matter. This in turn justifies the presence of higher stocks of SOC and soil GRP in the plots that harboured abundant AMF biomass. This also corroborates with the earlier findings, wherein the higher GRSP was observed with undisturbed soils such as no-tillage plots [15] or forest ecosystems [13]. Similarly, the pertinent soil quality parameters such as MBC and β-glucosidase activity were also significantly higher in the plots of zero-tillage and raised bed maize and lowest in case of conventionally tilled plots. The plausible explanation for the similar trend exhibited by soil GRP, MBC and activity of β-glucosidase is the release of carbohydrates by GRP that maintained active microbial biomass in soil [29,30]. It has also been speculated that the glomalin functions like an insoluble biofilm, wherein by the virtue of the sticking action it traps microorganisms and organic matter and the subsequent formation of extracellular polysaccharides that stabilizes soil aggregates [8,44].

In the case of glomalin, both easily extractable and difficulty extractable pools were extracted to get maximum recovery (total glomalin) because extraction conditions required may vary with the soil types [25]. Since, hyphae are the site of glomalin production

Table 1
Correlation matrix of AMF related parameters with soil carbon sequestration and associated important soil quality indicators assessed under contrasting soil management practices in various host plants. The data are means of three replications for which relationship was computed.

	Spore Count	MCP	PLFA C16:1ω5	Root EE-GRP	Root DE-GRP	Root T-GRP	Soil EE-GRP	Soil DE-GRP	Soil T-GRP	Soil T-GRP-stocks	MBC	β-glucosidase
MCP	0.92**											
PLFA C16:1ω5	0.65**	0.55**										
Root EE-GRP	0.73**	0.88**	0.52*									
Root DE-GRP	0.85**	0.97**	0.43^{NS}	0.88**								
Root T-GRP	0.85**	0.97**	0.44*	0.91**	0.99**							
Soil EE-GRP	0.96**	0.97**	0.61**	0.81**	0.93**	0.93**						
Soil DE-GRP	0.86**	0.91**	0.57**	0.94**	0.89**	0.91**	0.88**					
Soil T-GRP	0.87**	0.92**	0.58**	0.93**	0.90**	0.91**	0.90**	0.99**				
Soil T-GRP stocks	0.86**	0.92**	0.57**	0.94**	0.90**	0.91**	0.89**	0.99**	0.99**			
MBC	0.76**	0.75**	0.75**	0.72**	0.71**	0.72**	0.78**	0.83**	0.83**	0.83**		
β-glucosidase	0.92**	0.78**	0.78**	0.61**	0.66**	0.67**	0.86**	0.76**	0.77**	0.76**	0.79**	
SOC-Stocks	0.85**	0.84**	0.68**	0.80**	0.78**	0.79**	0.85**	0.93**	0.93**	0.94**	0.88**	0.84**

*, significant ($p < 0.01$); **, highly significant ($p < 0.05$); ^{NS}, non-significant.

(extraradical and intraradical) likely, some part of glomalin may also reside inside the colonizing roots which could remain unquantified [10,24,45]. The intraradical glomalin concentration has been shown to estimate the presence or absence of AMF colonization in roots [24]. Therefore, glomalin was also extracted from roots in addition to soil.

In the current study the AMF biomass assessed both microscopically and biochemically were significantly related and showed similar trends (Table 1). Across all the systems examined, glomalin related protein was positively correlated with microscopic and biochemical estimates of AMF biomass A major portion of glomalin (about 80%) is present on spores and hyphae [9] which explains the positive correlation of GRP with mycorrhizal spore density and PLFA C16:1ω5 observed in the present study. A significant correlation between EE-GRP and PLFA envisages that the easily extractable glomalin is recently produced and hyphal derived [8,25,44] and as PLFA 16:1ω5cis depicts its hyphal biomass [22]. When compared to the direct C – exudation by plant roots, the AMF mediated pathway is responsible for the transfer of a significant proportion of photosynthates into the soil, where the intraradical hyphae could be particularly important [46] which in turn is the site for GRP production [9]. The significant positive correlation observed between the two pools of glomalin related protein is in agreement with earlier studies [47]. The easily extractable soil glomalin pool is a part of the recalcitrant or difficulty extractable soil glomalin pool and therefore a strong correlation is expected between them [8].

C16:1ω5 PLFA and microscopic estimates of AMF biomass were significantly and positively correlated with both carbon and glomalin stocks. A positive correlation between AMF biomarker 16:1ω5cis and soil organic carbon was also observed in the experiments of Mathew et al. [48] In the same study, the PLFAs indicating that AMF, bacteria, fungi and actinobacteria were higher in the long term no-tillage practice and no-tillage system was also associated with higher contents of soil C and N [48].

The significant and positive correlation between SOC stocks and soil GRP pools (Table 1) concurs with the experiments of Singh et al. [3], wherein Bradford reactive soil protein pools (glomalin) maintained a significant and positive correlation with soil edaphic factors that include SOC. A positive correlation between SOC and easily extractable glomalin [13] and a significant positive relationship between GRSP and SOC ($r^2 = 0.96$) and also between the two pools of GRSP ($r^2 = 0.97$) [49] have been reported. Studies have identified glomalin as an important component of SOC [5,10]. Glomalin production depends upon the allocation of photosynthates to AMF from the plant and a higher allocation of carbon is a determinant for higher glomalin production [50] as observed in the present study, wherein besides soil GRP, root GRP was also positively correlated with SOC stocks which has not been reported earlier. A significant positive correlation of GRSP with root soluble sugar and soil hydrolysable and hot water extractable carbohydrate indicating the contribution of glomalin to soil and root C was observed by Wu et al. [30].

The high correlation of AMF biomass assessed biochemically (PLFA C16:1ω5 and GRP) and microscopically (spore density, mycorrhizal colonization percentage) with SOC stocks, MBC and β-glucosidase activity (Table 1) provides a strong basis that these AMF markers especially the biochemical ones can act as potential indicators for assessing soil carbon sequestration and soil quality of an agroecosystem.

5. Conclusions

The use of biochemical estimates of AMF biomass such as PLFA C16:1ω5 and GRP (in soil and root) as the indices of AMF activity, soil quality and SOC sequestration of a soil management system is a notable finding of this study. The long-term stabilized system, such as ZT-CC proved better for increasing AMF biomass and GRP production which resulted in better soil quality and higher soil carbon sequestration. GRP which is commonly produced on hyphae is significantly affected as a result of soil disturbance, which was evident from the lower content of GRP and AMF biomass levels quantified by PLFA C16:1ω5 and traditional methods in conventional tillage soybean-wheat system observed in the current study.

GRP content in both soil and roots and PLFA C16:1ω5 were also correlated with SOC-stocks, soil microbial biomass carbon and β glucosidase activity. Thus, it can be concluded that GRP and PLFA C16:1ω5 can serve as potential signatures to classify the best soil management system in terms of AMF activity, soil quality and C-sequestration. The results obtained in the study can also be used as a basis for further advancement in the understanding GRP production in various soil management practices eventually to be used as a convenient, robust proxy for AMF activity, in monitoring the soil quality and carbon mitigation strategies.

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

This research work was a part of the National Programme on Carbon Sequestration Research granted to MPS by Department of Science and Technology, Govt. of India, New Delhi, India under award no. DST/IS-STAC/CO2-SR-11612 (G) is gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejsobi.2021.103286.

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