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Glomalin related soil protein, soil aggregate stability and soil aggregate-associated organic carbon under agroforestry practices in southern Ethiopia

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Abstract
Land degradation in Ethiopia is escalating due to high population density and the shift from tree-based agricultural systems, like agroforestry practices (AFP), to monoculture farming. These land use changes, compounded by climate change, threaten biodiversity and soil resources. Key soil health parameters, such as glomalin, soil aggregation, and aggregate stability, are negatively impacted by such practices. Agroforestry is proposed as a sustainable alternative to address these challenges. This study aimed to evaluate the effects of AFPs on soil glomalin, soil aggregate stability (mean weight diameter, MWD), and the relationship between soil aggregates and soil organic carbon (SOC). Undisturbed soil samples were collected from 0 to 30 cm and 30–60 cm depths in four land use types: home garden (HAFP), cropland (ClAFP), woodlot (WlAFP), and trees on soil and water conservation-based agroforestry (TSWAFP). Results showed significantly higher glomalin-related soil protein (GRSP) in HAFP and WlAFP compared to ClAFP and TSWAFP ($p<0.05$). HAFP also exhibited the highest soil aggregate stability (SAS) and MWD, followed by WlAFP. These findings suggest that agroforestry practices can significantly enhance soil health, ecosystem stability, and long-term sustainability, contributing to land restoration efforts.

Keywords Agroforestry practices, Glomalin related soil protein, Soil aggregate stability, Soil organic carbon

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Introduction

Globally, since the 20th century, land degradation has escalated and aggravated due to ecological degradation, increasing of population pressure, and unwise use of land resources [1]. The conversion of natural ecosystems to agriculture-based systems is known to cause large losses of soil organic carbon (SOC), and biodiversity losses including arbuscular mycorrhizal fungi diversity [2, 3]. Land degradation have impacted soil aggregation and aggregate stabilizing compounds [4]. Consequently, the glomalin-related soil protein (GRSP), SOC, mean weight diameter (MWD), and soil aggregate stability (SAS) distributions are expected to be influenced by land degradation.

Among the soil organisms, arbuscular mycorrhizal fungi (AMF) are widely distributed plant symbiotic soil microorganisms that form symbiotic relationships with the roots of more than 80% of higher plants [5]. The glomalin-related soil protein (GRSP), a glycoprotein organic compound produced from AMF [6] is a persistent organic substance with a strong cementing effect [7]. This compound is the source of soil organic carbon [8] and contains 30–40% carbon [9] and 3–5% nitrogen, 4–6% hydrogen, and 33–49% oxygen [10]. The GRSP plays a fundamental role in the formation of soil aggregate structure [11], which binds soil particles to form aggregates [12]. They also form soil aggregates and improves soil structure and stability against erosion [13]. These soil aggregates are the fundamental structural units that govern the dynamics of soil organic matter and nutrient cycling [14]. Soil with good structure can improve the soil microbial community, enrich biodiversity, promote nutrient cycling, and reduce soil carbon emissions [15]. Besides, soil aggregate stability also prevents soil erosion and other environmental problems caused by soil degradation [16].

The AMF composition, GRSP, SOC, and mean weight diameter (MWD) contribute to enhance soil fertility and productivity. However, the conversion of land use systems coupled with intensive agricultural practices is the most direct anthropogenic factor that affects soil carbon storage, and soil structure [17, 18] and AMF community structure [19]. Moreover, studies indicated that agricultural practices have negatively impacted vegetative cover [20], SOC [21], soil aggregation [22] and the distribution of soil aggregate-associated soil organic carbon (SAASOC) [23]. It has also been reported that land management practices affect GRSP production significantly [24], and its production could be hampered by agricultural practices that can destroy microbial habitat and decrease AMF growth [25]. On the other hand, the concentration of GRSP in soil depends on different factors like AMF richness, plant community composition, land use types, and soil properties [26].

Agroforestry (AF) is the combinations of trees with crops or pasture systems, being a sustainable and ecological way of using land [16]. This system enhances organic matter accumulation in soils through the inclusion of cover crops and permanent vegetation, also promotes soil microbial populations and SOC [27]. Research has shown that AF systems produce a higher spore and better distribution of AMF spores than monoculture [28]. Those, in turn, may improve GRSP [8], SOC [29], soil structure, and soil aggregate size distribution (SASD) compared to monoculture [30]. Moreover [29] and [31], also found a higher amount of soil aggregates and MWD under rubber-based AF systems than sole rubber practices. This is because AF systems can leave more plant residues on the soil surface [32]. It was also reported that the continuous input of organic matter from AF systems can provide a favorable environment to biological activity [33, 34] and [35] identified the increased contents of aggregate associated SOC under conserved fallow land as these systems contributes large carbon inputs. Conversely [29, 3], reported decreased SOC content on land which has been converted from forest to arable. Similarly, the conversion of natural ecosystems for agricultural purposes leads to changes in the content of SOC and other binders in the soil, adversely affecting soil aggregation [4]. The change in SOC may affect agricultural resilience against the exhibit risk of climate change [36].

Several studies have been conducted in southern Ethiopia, among which, the effects of indigenous AFS on biodiversity, carbon stocks, and litter fall [37, 38] and the diversity and composition under enset-coffee based homegarden agroforestry practices HAFP [39], and coffee based AF [40] could be mentioned. Additionally, the composition and abundance of AMF under different land use types were studied [41]. In the same region, the AMF community under the savannah ecosystem of Nachi Sar National Park was also investigated and reported higher richness and diversity from un-encroached plots [42]. The impacts of different land use types on phosphorus in acidic agricultural soil [43] and the physiographic features of agricultural lands and soil fertility management [44] have been carried out. This elucidates that no studies have been made on the effects of different AFP on GRSP, MWD, SASD, and SAASOC in the drylands of southern Ethiopia. This study aimed: (1) to compare the differences in GRSP (EGRSP and TGRSP) contents, MWD, SASD, and SAASOC across different AFPs in two soil depth layers and (2) to ascertain the presence or absence of relationship between bulk soil carbon and GRSP (EEGRS and TGRSP), and MWD and SASD with SAASOC. The research also hypothesized that the soil stability, particle size distribution, GRSP contents and fraction of SAASOC would be improved in tree based AFPs and different according the differences in land management practices.

Methods

Study area

The study was carried out in the Southern Nations, Nationalities, and Peoples' Region (SNNPR) and located in the southwestern parts of Ethiopia (5° 50′ 26″– 6° 12′ 48″ N, 38° 03′ 02″–38° 18′ 59″ E) [44]. Different agro-climatic zones exist in the region but the moist to sub-humid warm subtropical climate areas are situated between1500-2300 m elevation are the most important in terms of agricultural productivity. The AFS/practices are predominant practices in the region. In this region of the country, the mixed framing system is the most predominant land management practice which encompasses the combination of annual crops, perennial crops, livestock, and forestry practices at farm level. In the region, most of the areas have potential for perennial crops like enset (*Ensete ventricosum*), coffee (*Coffea arabica*), roots, and tuber crops. The annual crops, trees, and rearing of livestock under homestead AFP are common agricultural practices [45]. There are different zones and special districts within in the SNNPR, of which Wolaita and Kembata Tembaro are zones where the AFP has been widely Practiced. The Wolaita and Kembata Tembaro zones were selected to conduct the current study (Fig. 1). This is because of the presence of different types of AFP in these zones. Agroforestry practices are the different types of specific land management practices that consists of the three most important components namely tree, livestock and annual crops at farm level. The area receives bimodal rainfall: the small and medium rainfall seasons usually occur between March to May, whereas the highest rainfall is in July and August, and the mean annual temperature is 20.1 °C. Agricultural activities in this region heavily depend on the bimodal rainfall seasons. However, these rainy seasons are often unpredictable, leading to periods of drought and food insecurity for local populations during certain month. The dominant soil of the area is nitosol [46] with an acidity level ranges from moderately to strongly acidic [47] and such soils are sesquioxidic.

Though there are different types of AFPs in the SNNPR, the Wolaita and Kembata Tembaro zones share almost the same types of AFPs. In the areas, the home garden based agroforestry practice (HAFP) characterized with more than 75% covered by different woody species like: *Ensete ventricosum*, *Persea americana*, *Coffee arabica*, *Grevillea robusta*, *Cordia africana*, *Ficus vasta*, *Mangifera indica*, and different types of vegetables, roots, tuber crops and herbs. This practice is located near to residence home; consequently the application of organic

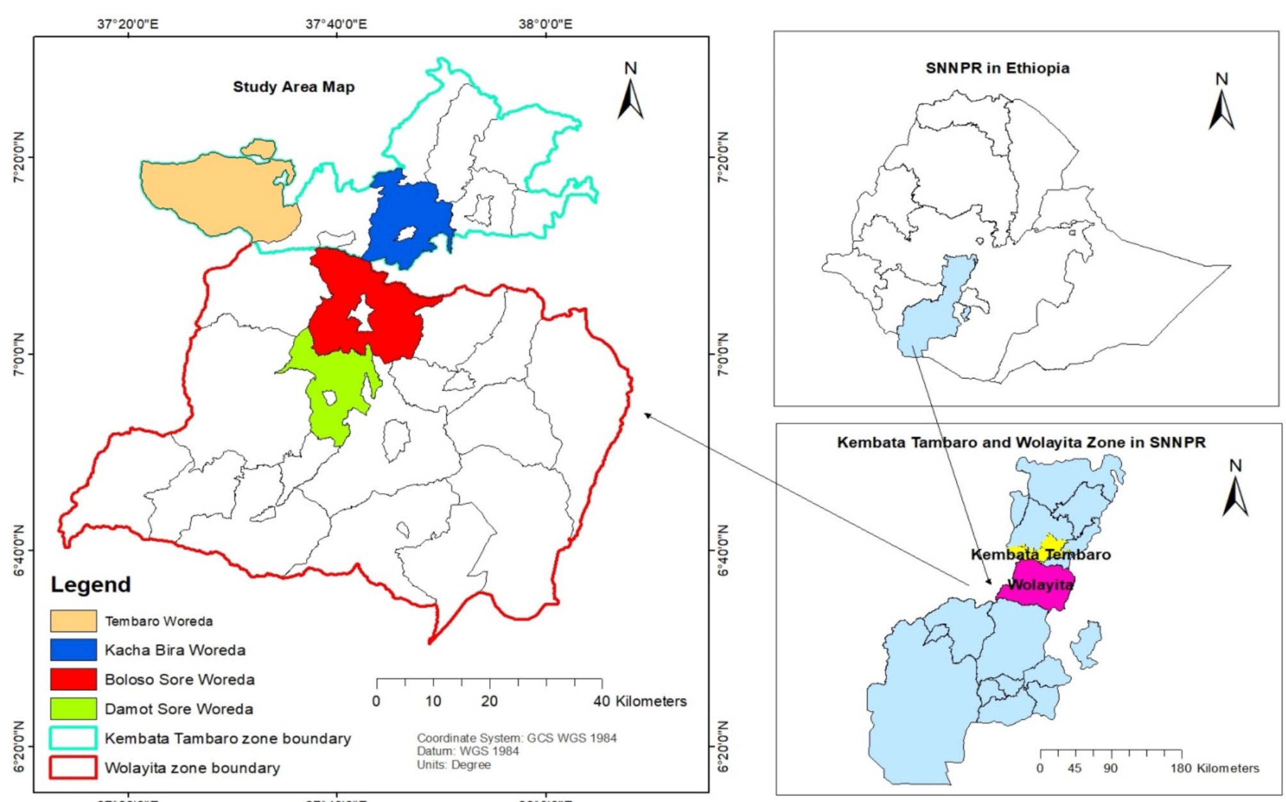

Fig. 1 Map of the study area showing the location of the study districts in Wolaita and Kembata Tembaro zones in south nations and nationalities peoples regional state in Ethiopia

inputs is widely known, less disturbed land use practice, while minimum or zero application of inorganic fertilizers. The other commonly practicing AFP is woodlot based agroforestry practices (WlAFP), in this practices, the eucalyptus species are the predominant plant species and acacia species, *Croton macrostachyus*, and *Juniperus procern* are also the most common farmers 'growing woodlot species. Likewise the HAFP, WlAFP is the less disturbed and hardly usage of inorganic fertilizers to such type of practice. In the case of cropland-based agroforestry practices (ClAFP) and trees on soil and water conservation-based agroforestry practices (TSWAFP), the scattered different types of woody species such as *Persea americana*, *Mangifera indica*, *Cordia africana*, *Ficus vasta*, *Musa*, grass species, and *Croton macrostachyus* grow commonly. On the other hand, using high inorganic fertilizer in ClAFP and TSWAFP types is a common trend due to the fact that they are highly disturbed and cultivated agricultural fields.

Sampling design

The zones, districts, and villages were selected from the SNNPR region using a multistage sampling design. The sample districts were selected by using stratified sampling techniques which means the districts were classified based on agroecology (highland, midland and lowland) and the presence of different agroforestry practices, while the villages and sampling plots were selected by using random sampling techniques, taking into account the slope, AFP type, altitude, agroecology, and land management practices, while at the village level, the AFPs were selected by using random sampling methods. The soil samples were collected from sampling units of (four different types of AFPs): HAFP, ClAFP, WlAFP and TSWAFP types. To collect data from different AFPs, different-sized plots were established namely: a 40 m x 40 m plot was established, for both ClAFP and TSWAFP types; a 10 m x 10 m plot for WlAFP; and a 20 m x 20 m plot for HAFP. In this study, we have used different plot size for different agroforestry practices (four types) this is because vegetation types and coverage varies as land management practices varies in these agroforestry practices. For instances, high vegetation coverage in homegarden and woodlot based agroforestry practices as compared to cropland/parkland and trees on soil and water conservation based agroforestry practices. Soil samples were collected from 128 soil samples (4 districts x 4 different AFPs x 2 villages x 2 farms x 2 depth layers) to analyse GRSP, MWD, SASD, and SAASOC content. Of 128 soil samples (64 soil samples from upper soil depth & 64 soil samples from the lower soil depth), 32 samples were collected from each of sample districts which implies that 8 soil samples were also collected from each of AFP types.

Extraction and determination of glomalin related soil protein (GRSP)

The extraction and determination of EEGRSP and TGRSP were performed according to Wright and Upadhyaya [7], and Janos et al. [48]. To extract the EEGRSP, one gram of air-dried soil was placed in centrifugates tubes of 15 mL with 8 mL of 20 mM sodium citrate at pH 7.0, autoclaved for 30 min at 121 ºC. Then, samples were centrifuged for 30 min (10,000 g); the supernatant was poured off and stored it at 4 °C until analysis. For TGRSP, the soil pellet was resuspended in 8 mL of 50 mM sodium citrate at pH 8.0 and autoclaving at 121 ºC for 60 min. Extractions were repeated until the supernatant became straw- colored, indicating that the reddish brown glomalin had been removed. All the supernatants from the TGRSP extractions were put together, brought up to a known volume and stored at 4 ºC until analysis. Then, Bradford-reactive substances (BRS) were measured to determine the GRSP content in the extracts (1 mL) by measuring absorbance at 595 nm of Bio-Rad protein dye reagent (Bio-Rad 500-0006) in 96-well flat, microplates with bovine serum albumin as the standard solution.

Dry and wet soil aggregate stability and aggregate size distribution

To estimate the MWD and SASD (dry and wet), the undistributed soil sample was collected using a shovel from two soil depth layers (0–30 and 30–60 cm). In the dry sieving method, the MWD_d and $SASD_d$ were determined by using the procedures of [49]. In this procedure, 100 g of air dried soil that passed through 8 mm sieve was sieved by using 5, 2, 1, 0.5, 0.25, and 0.053 mm sieves that were nested each other and mounted on a vibratory sieve-shaker, adjusted to a 3 mm shaking amplitude, with a sieving time of 2 min.

Then, the percentage of soil aggregates left on the sieve after dry sieving measured to estimate the dry soil aggregate of size distribution ($SASD_d$) and dry mean weight diameter (MWD_d). Subsequently, the aggregates with different sizes of SASD were stored at room temperature for analysis of soil macroaggregate and microaggregate associated SOC of the fraction. In case the wet sieving method, the soil aggregate size distribution ($SASD_w$) and mean weight diameter (MWD_w) were estimated according to the procedure of [50]. In this method, 100 g of air-dried soil that passed through 8 mm sieve was sieved using 5, 2, 1, 0.5, 0.25, and 0.053 mm sieve types. The soil sample was pre-wetted for 30 min in deionized water before the sieving process had been started and then transferred to the top of nested sieves. The nested sieves were then arranged in columns that have been dipped in water for 5 min carefully during up and down process. The remaining aggregate particle on each sieve was oven dried at 105 ºC for 24 h that resisted break down, and then the dry mass was used to estimate the MWD wet sieved fraction and SASD. Additionally, the resistant soil particles from each sieve were transferred into beakers and stored for analysis of the associated SOC of macro-aggregate and microaggregate soil fraction.

Based on the weights of these soil aggregates, the following variables were estimated:

$$\mathrm{SASD_d} = \frac{\mathrm{M_{di}}}{\mathrm{M_t}}\ \mathrm{x}\ 100 \quad (1)$$

Where $SASD_d$ is dry soil aggregate stable size distribution, M_{di} is the mass of dry aggregates on each size, and M_t is the total mass of dry-sieved soil

$$\mathrm{MWD_d} = \sum{}_{\mathrm{i=1}}^{\mathrm{n}} \mathrm{X_i W_i} \quad (2)$$

where MWD_d the mean weight diameter of dry soil aggregates, X_i is the mean diameter of each sieve fraction (mm), and W_i is the proportion of the total sample mass in the corresponding size fraction,

$$\mathrm{SAS_w} = \frac{\mathrm{M_{wi}}}{\mathrm{M_t}}\ \mathrm{x}\ 100 \quad (3)$$

Where $SASD_w$ is the wet soil aggregate stable size distribution, M_{wi} is the mass of wet aggregates on each size and M_t is total mass of wet-sieved soil.

$$\mathrm{MWD_w} = \sum{}_{\mathrm{i=1}}^{\mathrm{n}} \mathrm{X_i W_i} \quad (4)$$

where MWD_w is the mean weight diameter of wet soil aggregates, X_i is the mean diameter of each sieve fraction (mm), and W_i is the proportion of the total sample mass in the corresponding size fraction.

Determination of soil physicochemical properties

Soil particle size was determined using the hydrometer method [51]. Organic carbon content was analyzed using the [52], while total nitrogen was measured according to [53]. Soil pH was determined by suspending soil in deionized water at a 1:2.5 soil-to-water ratio [54]. Available phosphorus was measured using the Olsen method [55].

Data analysis

The normality of the data was assessed using histogram and the Shapiro-Wilk test before analysis. A two-way analysis of variance (ANOVA) with a general linear model (GLM) was used to examine variations in GRSP (EEGRSP and TGRSP), MWD (dry and wet soil aggregate stability), soil aggregate stability distribution (SASD), and the soil macro- and microaggregate-associated SOC across different AFP types and soil depth layers. Tukey's Honestly Significant Difference (HSD) post-hoc test was applied for pairwise mean comparisons between the AFPs and the studied variables. All statistical analyses were performed using R programming version 4.2.1, with a significance level set at $p < 0.05$.

Results

Glomalin related soil protein (GRSP)

In this study, the mean concentrations of EEGRSP and TGRSP varied significantly ($P < 0.05$) among the different AFP types (Fig. 2). The soils from the HAFP had the highest EEGRSP and TGRSP content, followed by the WlAFP, TSAWFP, and ClAFP types. The EEGRSP and TGRSP content in HAFP and WlAFP increased significantly in both soil depth layers as compared to ClAFP and TSWAFP. In the study, on the topsoil (0–30 cm), the EEGRSP content in HAFP and WlAFP increased by 211.78% and 163.38%, respectively, compared to that in the ClAFP type. Similarly, the TGRSP content on topsoil in HAFP and WlAFP increased by 120.18% and 121.09%, respectively, compared to that in the ClAFP type. Conversely, in the subsurface soil (30–60 cm), the EEGRSP and TGRSP content in HAFP and WlAFP increased by 209.26% and 121.07%, respectively, compared to that in the ClAFP. The EEGRSP and TGRSP content showed a decreasing trend with the increasing intensity of agricultural activities, use of inorganic fertilizers, and increasing soil depth (Fig. 2).

Dry and wet soil aggregates

The soil aggregate stability was significantly affected by different types of AFPs and soil depth layers. Dry mean weight diameter (MWD_d) and wet mean weight diameter (MWD_w) were significantly higher in HAFP compared with ClAFP types and increased by 218.99% (MWD_d) and 219.05% (MWD_w). The mean of the MWD_d in the different AFPs was 5.04, 4.29, 1.99, and 1.58 for HAFP, WlAFP, TSWAFP, and ClAFP, respectively, while the mean of the MWD_w was 4.02, 3.57, 1.84, and 1.26 for HAFP, WlAFP, ClAFP, and TSWAFP, respectively (Fig. 3). This shows that the cropland-based AFP types (ClAFP and TSWAFP) had lower MWD_{dry} and MWd_w in the soil than the other types of AFP. On the other hand, the AFP with tree-based management practices (HAFP and WlAFP) showed significant superiority over the rest of the AFP (ClAFP and TSWAFP) in both MWD_d and MWD_w.

The amount of MWD_d and MWD_w was greater in the top soil layer (0–30 cm) and decreased with soil depth in HAFP and WlAFP as compared to other AFP types (TSWAFP and ClAFP). However, in the case of subsurface (30–60 cm), the high remarkable values of MWD_d and MWD_w were registered under TSWAFP and ClAFP

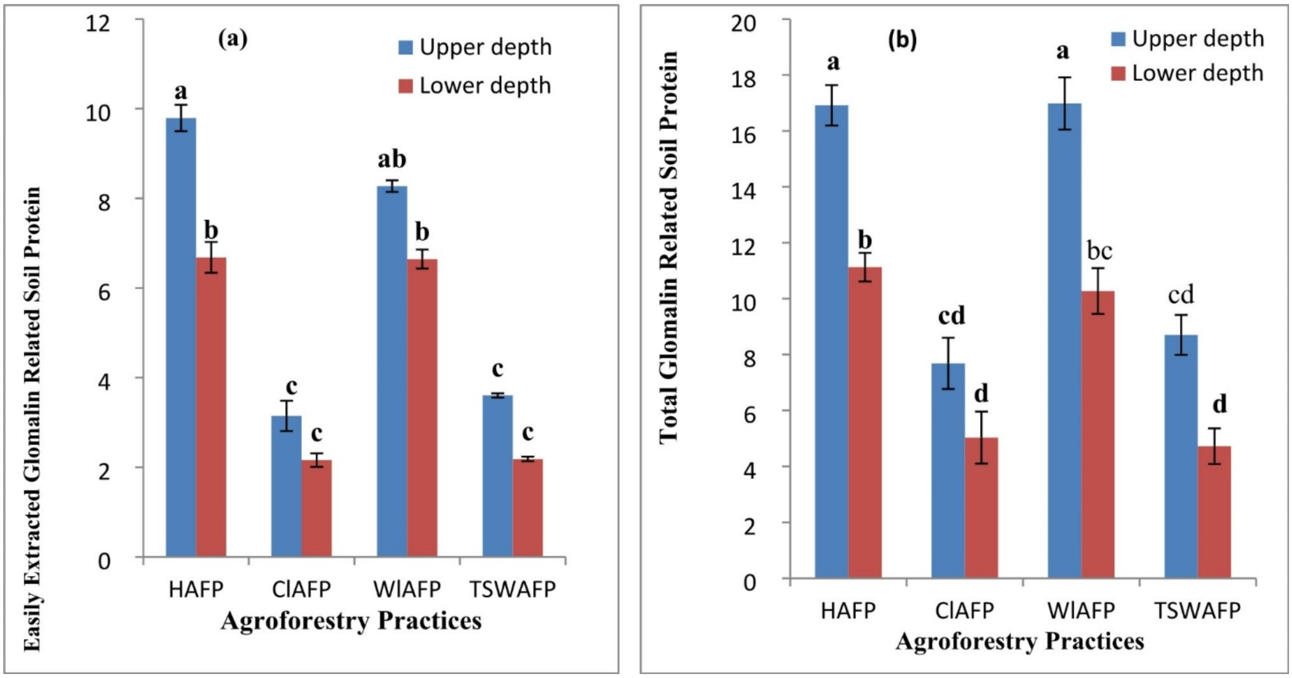

Fig. 2 Mean of glomalin related soil protein (a = easily extractable glomalin related soil protein and b = total glomalin related soil protein) under different agroforestry practices in drylands of southern Ethiopia; HAFP = homegarden agroforestry practices, ClAFP = cropland based agroforestry practices, WlAFP = woodlot based agroforestry practices, TSWAFP = trees on soil and water conservation measures based agroforestry practices. Bars with the same small letters are not significant at $p < 0.05$

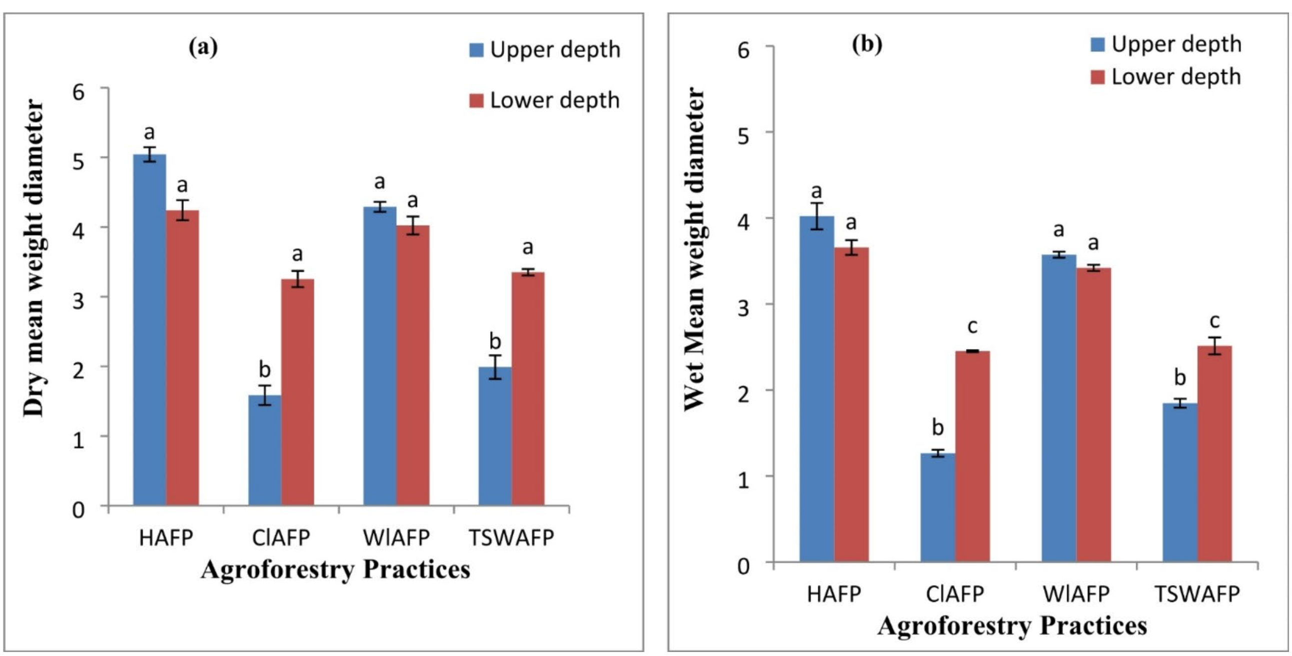

Fig. 3 Mean weight diameter ((a) = dry mean weight diameter, (b) = wet mean weight diameter) under different agroforestry practices in drylands of southern Ethiopia; HAFP = homegarden agroforestry practices, ClAFP = cropland based agroforestry practices, WlAFP = woodlot based agroforestry practices, TSWAFP = trees on soil and water conservation measures based agroforestry practices. Bars with the same small letters are not significant at $p < 0.05$

types. The results elucidated that the mean of MWD_d in TSWAFP and ClAFP was 60.539% and 68.570% lower than HAFP at 0–30 cm of depth, respectively, while the mean of MWD_d in TSWAFP and ClAFP was 54.091% and 68.540% lower than HAFP at subsurface soil layer (30–60 cm), respectively (Fig. 3).

The soil stable aggregate size class distributions

Soil stable aggregate stability class distribution (SASD) considerably varied with AFP type and soil depth ranges. The effect of different land management through AFPs on dry and wet SASD indicated that HAFP had a significantly higher percentage of aggregate types, followed by WlAFP, compared to the intensive agriculture based AFPs types (ClAFP and TSWAFP) which had significantly lower soil aggregates in all size classes (Fig. 4). In > 2 mm dry sieving, on topsoil layer (0–30 cm), the HAFP (31.51 g 100 g^{-1} dry soil) and WlAFP (24.74 g 100 g^{-1} dry soil) had significantly higher aggregate fractions than TSWAFP (7.82 g 100 g^{-1} dry soil) and ClAFP (5.68 g 100 g-1 dry soil), while in wet sieving, on the same soil layer, the HAFP (25.49 g 100 g^{-1} dry soil) and WlAFP (17.19 g 50 g^{-1} dry soil) had significantly larger aggregate fractions, followed by TSWAFP (8.36 g 50 g^{-1} dry soil), and the lowest was recorded in ClAFP type (4.89 g 100 g^{-1} dry soil). Additionally, in both dry and wet sieving and soil depth layers, the other aggregates (1–2, 0.5-1, 0.25–0.5, 0.053–0.25, and < 0.053 mm) were also relatively higher for the topsoil layer (0–30 cm) than the subsurface soil (30–60 cm) among the different AFP types. In contrast, the soil aggregates under ClAFP and TSWAFP types, however, didn't show a consistent trend with depth increment in the subsurface soil layer (Fig. 4).

Dry and wet soil aggregate associated with soil organic carbon

The dry and wet sieved aggregates and the SOC associated with macroaggregates were significantly different

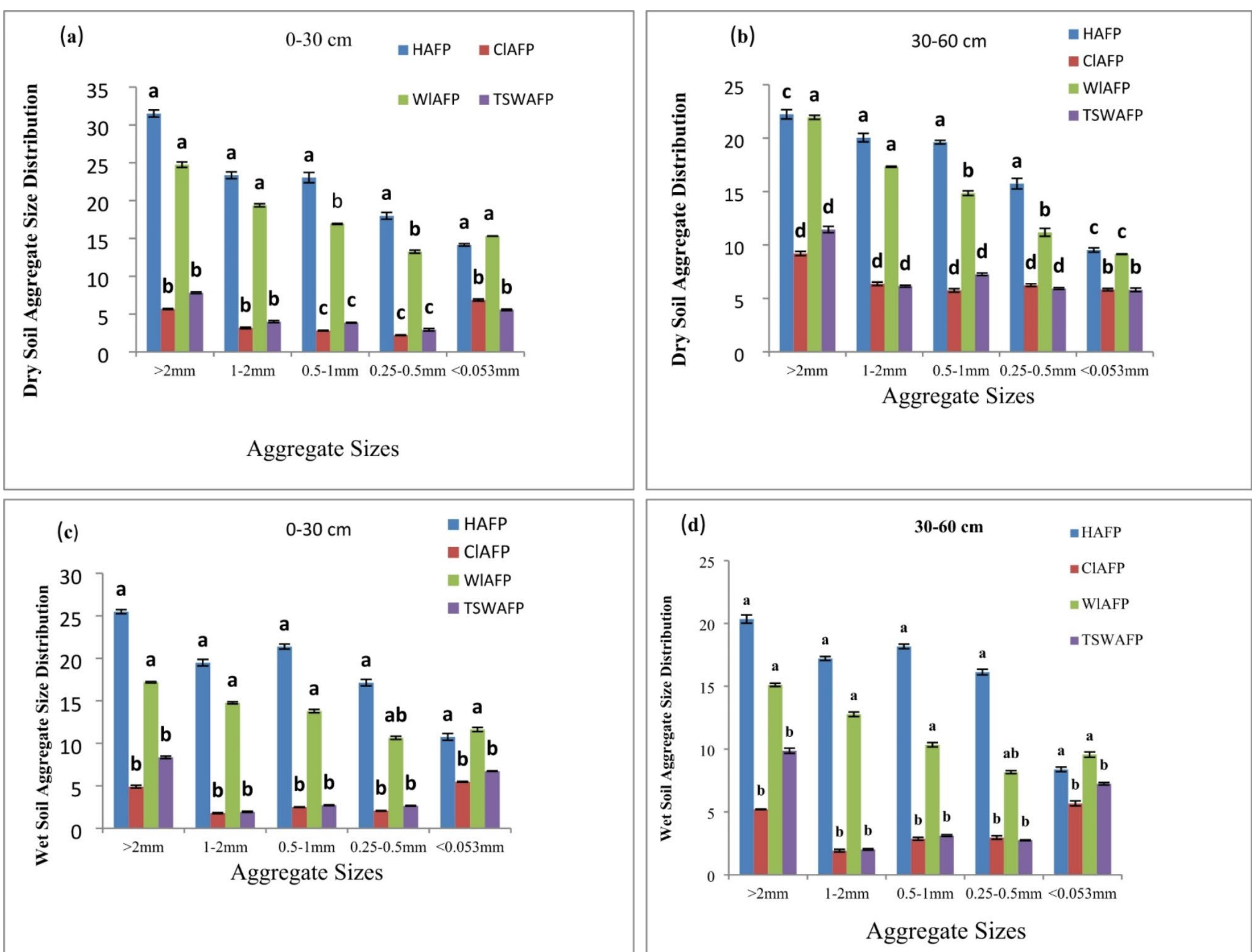

Fig. 4 The characteristics of dry (**a** and **b**) and wet (**c** and **d**) soil stable aggregate size distribution under different AFPs and soil depth variations, HAFP = homegarden based agroforestry, ClAFP = cropland based agroforestry practices, WlAFP = woodlot based agroforestry practices and TSWAFP = trees on soil and water conservation measures based agroforestry practices, bars with the same small case letters are not significant at $P < 0.05$

under different AFP types and depth variations. However, no significant variation was found in those associated with both macroaggregates and microaggregates by soil depth variation under the ClAFP type. The contents of SOC associated with macroaggregates of all AFP types in both dry and wet decreased with the decrease in particle sizes, while the contents of SOC associated with microaggregates didn't follow the same regular trends as macroaggregates in subsurface soil depth. Additionally, the concentration of SOC associated with macroaggregates (>0.25 mm) was higher than the amount of SOC associated with that of microaggregates (<0.25 mm) in both dry and wet aggregates on surface soil depth in all AFP types (Fig. 5).

There were comparable concentrations of SOC in HAFP and WlAFP in macroaggregates as well as microaggregates, and they were considerably increased in these AFP types as compared to TSWAFP and ClAFP. In dry

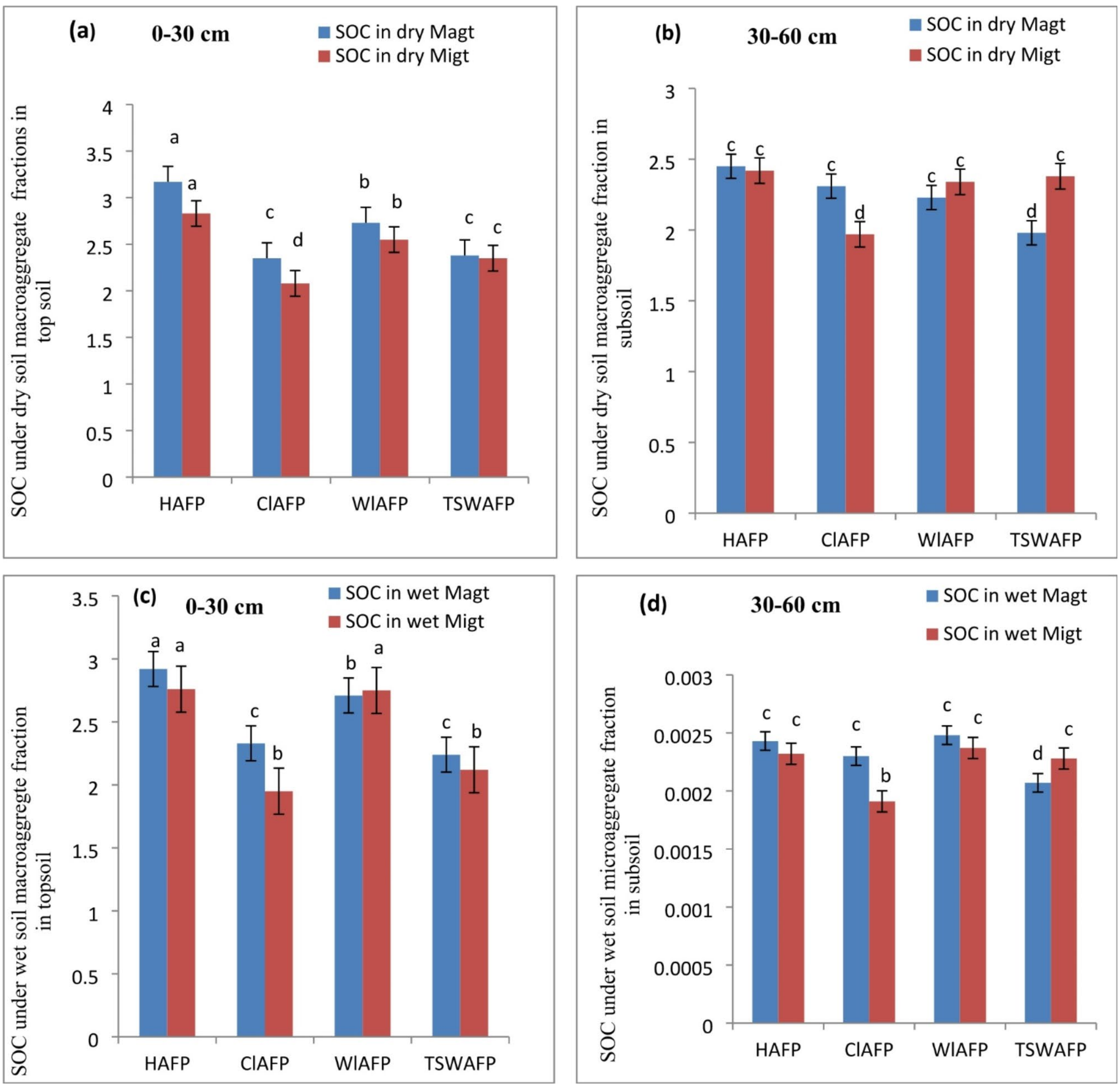

Fig. 5 The characteristics of dry (**a** and **b**) and wet (**c** and **d**) soil organic carbon (SOC) under soil macroaggregate and micraggregate fraction under different AFPs and soil depth variations, HAFP = homegarden based agroforestry, ClAFP = cropland based agroforestry practices, WlAFP = woodlot based agroforestry practices and TSWAFP = trees on soil and water conservation measures based agroforestry practices, bars with the same small case letters are not significant at $P < 0.05$. *SOC = soil organic carbon, magt = macroaggreagte soil fraction, migt = microaggregate soil fraction, 0–30 = topsoil, 30–60 = subsoil

Table 1 The mean of soil properties under different agroforestry practices in drylands of Southern Ethiopia

Soil Parameters	Depth (cm)	Agroforestry Practices			
		HAFP	ClAFP	WlAFP	TSWAFP
pH (1:2.5 soil: water)	0–30	7.07 ± 0.23^{a}	6.20 ± 0.19^{c}	6.34 ± 0.21^{bc}	6.39 ± 0.12^{abc}
	30–60	7.02 ± 0.07^{ab}	6.42 ± 0.11^{abc}	6.50 ±$^{a0.22bc}$	6.25 ± 0.06^{c}
Organic carbon (%)	0–30	3.62 ± 0.22^{a}	2.77 ± 0.22^{ab}	3.16 ± 0.13^{ab}	2.69 ± 0.13^{b}
	30–60	3.23 ± 0.16^{ab}	2.46 ± 0.07^{b}	2.87 ± 0.29^{ab}	2.77 ± 0.19^{ab}
Total nitrogen (%)	0–30	0.46 ± 0.03^{a}	0.23 ± 0.01 ^{ab}	0.31 ± 0.02^{ab}	0.20 ± 0.01^{ab}
	30–60	0.29 ± 0.01^{ab}	0.19 ± 0.02^{ab}	0.24 ± 0.02^{b}	0.22 ± 0.02^{ab}
Available phosphorus (mg/kg)	0–30	12.63 ± 0.57^{a}	13.84 ± 0.90^{a}	11.65 ± 0.25^{a}	12.07 ± 1.33^{a}
	30–60	11.06 ± 0.23^{a}	12.79 ± 0.66^{a}	10.66 ± 0.37^{a}	11.91 ± 1.01^{a}
Sand (%)	0–30	73.25 ± 1.09^{a}	67.25 ± 2.07^{a}	67.50 ± 2.67^{a}	65.25 ± 1.72^{a}
	30–60	69.88 ± 1.07^{a}	66.75 ± 2.13^{a}	67.63 + 2.75^{a}	68.81 ± 0.95^{a}
Clay (%)	0–30	9.31 ± 1.49^{a}	10.00 ± 0.35^{a}	8.75 ± 0.66^{a}	8.00 ± 0.81^{a}
	30–60	8.69 ± 1.27^{a}	9.75 ± 1.22^{a}	9.31 ± 1.29^{a}	8.19 ± 1.12^{a}
Silt (%)	0–30	17.44 ± 1.61^{b}	22.75 ± 1.90^{ab}	23.75 ± 2.80^{ab}	26.75 ± 2.49^{a}
	30–60	21.44 ± 0.96^{ab}	23.50 ± 1.92^{ab}	23.69 ± 1.92^{ab}	23.00 ± 1.81^{ab}

*Units within a rows followed by the same letter/s are not significantly different at $p < 0.05$. Homegarden based agroforestry practices (HAFP), cropland based agroforestry practices (CLAFP), woodlot based agroforestry practices (WlAFP) and trees on soil and water conservation based agroforestry practices (TSWAFP)

sieved aggregates on surface soil (0–30 cm), the HAFP (34.89%) increase in SOC associated with macroaggregates was followed by WlAFP (16.17%) and TSWAFP (1.33%) as compared to ClAFP type and decreased as depth increased. In the case of microaggregates in dry sieved aggregates on the surface soil layer, the highest (2.83%) SOC associated with microaggregates was found in HAFP, whereas the lowest (2.08%) was found in ClAFP. On the other hand, in wet sieving on surface soil depth, the distribution trends of SAASOC concentrations were also high in HAFP, followed by WlAFP and TSWAFP and the lowest was found under ClAFP and decreased as depth increased (Fig. 5).

Soil properties

In this study, the soil was slightly acidic with mean pH values ranges from 6.20 to 7.07 in ClAFP and HAFP respectively. The highest SOC content was found in the HAFP type being followed by the WlAFP in both soil depth layers, while the lowest was obtained in ClAFP where the intensive agriculture is the common trends in the study area. On the other hand, the soil total nitrogen concentration varied from 0.20 to 0.35%. pH, carbon, nitrogen and silt concentrations were significantly different between the AFP types ($p < 0.05$) (Table 1).

Discussion

Glomalin related soil protein (GRSP)

The current study confirmed that EEGRSP and TGRSP concentration varied significantly ($P < 0.05$) among different AFP types. The concentrations of GRSP in this study are consistent with those reported by [56], who reported values ranging from 2.0 to 14.8 g/kg in TGRSP in different ecosystems, Welemariam et al., [57] who reported values between 3.21 and 18.16 g/kg for various land uses, and Singh et al., [26], who found 3.6 to 12.7 g/kg of GRSP. However, the GRSP in this study was significantly higher than the findings of Bai et al., [58], who observed values of 0.68–1.18 mg g^{-1} in deserts and 0.25 to 1.8 mg g^{-1} in semiarid grasslands [59]. The type of land management practices could bring variations in abundance and diversity of arbuscular mycorrhizal fungi (AMF) [60], that influences the concentrations of GRSP. Moreover, in this study, the findings indicated the clear differences in the concentration of GRSP among different AFPs types. It has also been reported that land management practices affect glomalin production [24]. This is because glomalin production is hampered by agricultural practices that destroy microbial habitat and decrease AMF growth [25]. In line our findings, higher glomalin concentration was found under conserved tree based land use systems as compared to the purely cultivated agricultural fields [61, 62]. This is likely due to the more extensive root networks in soils dominated by trees, which contribute to higher AMF and glomalin concentrations compared to agricultural soils, where roots are typically smaller and shorter-lived. Tree based system sustains the sustaibility of soil productivity in improving the soil microganisms and this in turn improves the glomalin contents and ecosystem sustainability [63–65]. This implies that this could be one of the possible reasons for the higher glomalin concentrations under the HAFP and WlAFP land use types as compared less tree based fields (ClAFP and TSWAFP).

The contents of EEGRSP and TGRSP were highest among HAFP, followed by WlAFP, and lowest among cultivated agricultural practices (ClAFP and TSWAFP). This is because the HAFP and WlAFP soils are largely undisturbed compared to agricultural soils (ClAFP and

TSWAFP). This results are similar with previous findings of [12] found a 2.35–2.56 fold higher GRSP amount in forest land compared to farmland. It was also indicated that the soils from the forest had the highest TGRSP, while the cultivated field showed the lowest values [66]. Other studies, [67–68, 57] have also reported a higher amount of TGRSP under conserved areas compared to non-conserved lands, and Singh et al., [26] documented significantly higher GRSP under woody vegetation lands than soils of cropland. The presence of higher EEGRSP and TGRSP in HAFP and WlAFP could be due to the presence of high AMF root colonization in these systems [60] as glomalin is the product of AMF [69], and higher glomalin concentrations where AMF is more abundant. Furthermore, Ji et al., [64] and Yang et al., [65] reported higher glomalin concentration from the tree based land use systems as compared to monculre based agricultural systems. On the other hand, both EEGRSP and TGRSP concentrations were strongly affected by agricultural practices. The decrease in EEGRSP and TGRSP concentrations due to agricultural practices, which is in line with studies of Singh et al., [26] and Spohn and Giani [4]. This reduction of GRSP in croplands might have been the result of agricultural practices that resulted in changes in physical and chemical conditions of the soil that could have negative effects on GRSP content [10, 70]. Additionally, the EEGRSP and TGRSP concentrations were found to decrease while going down the soil depth layers. This finding agreed with the reports of Wang et al., [71], Wang et al., [6], who reported a higher glomalin levels in surface soils compared to the subsurface layers. This trend is likely due to AMF morphotypes being primarily limited to the upper soil surfaces, where soils are richer in SOC [60, 65, 72]. A similar trend was observed in a study of *Posidonia oceanica* from the Western Mediterranean, where agricultural activities negatively impacted AMF compositions and GRSP production [73].

The dry and wet soil stable aggregates

This study demonstrated the significant impact of different AFPs on mean weight diameter (MWD). It was observed that the decrease in MWDd and MWDw at the surface layer (0–30 cm) was 68.57% and 68.54%, respectively, in the ClAFP, while in TSWAFP, the decrease was 60.54% and 54.09%, following agricultural practices when compared to HAFP. The MWD of the different AFP types followed the order: HAFP > WlAFP > TSWAFP > ClAFP, with the maximum value observed in HAFP. This findings are consistent with previous research reports of Chittamart et al. [74], Pan et al. [75]. Baranian Kabir et al., [76], who reported the highest MWD value in grassland compared to agricultural cropland, and Cheng et al., [77], Jia et al., [78] demonstrated a significantly higher MWD value in places with vegetation-based land systems. Similarly, Xu et al., [79], Dou et al., [80] who found the highest MWD values in natural shrubland compared to farmland. Gupta et al., [29] also reported higher MWD in agroforestry (AF) systems compared to monoculture systems. The higher MWD in vegetation-based agricultural practices (HAFP and WlAFP) can be attributed to the presence of high woody species litter fall and SOC concentration [60, 81], which contribute to soil aggregate formation [82, 83]. These results align with those of Lawal et al., [84], Ashagrie et al., [85], who reported a decrease in soil aggregate values under agricultural practices. Moreover, MWD was significantly lower in cropland than in forestland [16, 86]. The lower values of MWD in the agricultural cropland (ClAFP and TSWAFP) compared to the less disturbed lands might be the lack of soil cover, less SOC, and increased erosion, which have been investigated previously Cates et al., [87]. Regarding depth variation, under all AFP types, the mean MWDd and MWDw were lower in subsurface layers (30–60 cm) compared to the surface layer (0–30 cm). This finding is agreed with studies by Dai et al., [88], Li et al., [89] and Bougma et al., [90], who reported higher MWD in surface soil layers, while Meena et al., [91] observed decreased MWD values in subsurface soils. The lower SOC content and root biomass in deeper soil layers likely result in less MWD formation [92, 93]. However, this finding contradicts the results of [94], who found lower MWD in forest soils compared to agricultural soils, possibly due to the clay and silt contents of the soil [95].

The soil stable aggregate size class distributions

The HAFP fields exhibited the highest macroaggregate size (> 2 mm) distribution, followed by WlAFP and TSWAFP, with ClAFP having the lowest macroaggregate size distribution. This finding agrees with studies by Gupta et al., [29] and Gama-Rodrigues et al., [31], who reported a higher macroaggregate size class under AF systems compared to cropland. These results are also consistent with studies by Okolo et al., [96], Zeng et al., [93], Welemariam et al., [57] who reported higher macroaggregates in vegetated areas than bare land., who found higher macroaggregate concentrations in vegetated areas than in bare land. The higher organic carbon content in HAFP, resulting from the presence of high woody species, may promote the formation of larger macroaggregates. Additionally, AFPs are known to enhance organic matter accumulation through plant biomass inputs, which promote macroaggregate formation [27]. The presence of high organic carbon and low disturbances in HAFP soils would have higher proportions of macroaggregates than those subjected to agricultural disturbance [97]. Higher carbon concentrations that acts as a cementing agent for macroaggregates formation [98]. In the least disturbed AFP types, the highest percentage of all aggregate size class distributions were found in the upper surface layer (0–30 cm) in both dry and wet sieving, which aligns with the findings of [99], who found that surface soils from perennial tree-based systems are influenced by plant residues.

Continuous agricultural practices have degraded macroaggregates, and soil erosion may further contribute to the decline in large macroaggregates in disturbed AFP types (ClAFP and TSWAFP). The decrease in macroaggregate content in these agricultural fields can also be attributed to the shortage of binding agents required for macroaggregate formation [100]. This suggests that land use change and varying land management practices significantly alter soil structure and aggregate size distribution. In northern Ethiopia, Okolo et al., [96] reported higher microaggregates (< 0.25 mm) than macroaggregates, and Gupta et al., [29] also found a higher proportion of microaggregates in maize crops compared to other perennials. This difference could be attributed to the mechanical breakdown of macroaggregates into microaggregates due to agricultural activities [101].

The dry and wet soil aggregate associated soil organic carbon

The different AFP and depth variations significantly affected the SOC associated with macroaggregates (> 0.25 mm size) and microaggregates (< 0.25 mm) both in dry and wet sieved fractions (Fig. 5**).** In the upper surface (0–30 cm), the HAFP type had significantly **(**$p < 0.05$) higher (3.17%) macroaggregate-associated SOC, followed by WlAFP (2.73%), TSWAFP (2.38%) and the lowest (2.35%) was measured from ClAFP. These results align with the findings of Singh et al., [29], who reported higher macroaggregate-associated SOC in forest land compared to croplands. Similarly, Welemariam et al., [26], Xiao et al., [102] reported higher amounts of macroaggregate-associated SOC under exlosures and undisturbed sites. It was also reported that SOC concentrations in aggregates considerably increased with vegetation coverage [103, 104]. The increase in SOC content in macroaggregates may result from the integration of smaller aggregates into larger ones [105] and the high input of organic matter under less disturbed lands [96]. Howevre, Novara et al., [106] found that SOC concentration in microaggregates (< 0.25 mm) was significantly higher compared to that of macroaggregates (> 0.25 mm), and Hu and Lan [107], reported the highest aggregate associated SOC concentration in microaggregates compared to macroaggregates. These observed differences might be the inherent soil variability [108], and can also be attributed to differences in the sequestration potentials of different tree species [109].

In all four types of AFP, the SOC content associated with aggregates with various particle sizes was highest at the surface depth layer (0–30 cm); this was comparable with the results of Tang et al., [110]. The increased amount of macroaggregates and microaggregates-associated with SOC in surface layers is due to the presence of plant residues, root exudates, and soil organisms, which contribute to the increase in SOC contents [88–89, 111]. In the case of agricultural fields (from ClAFP and TSWAFP fields), the lower macroaggregates and microaggregate-associated SOC were measured (Fig. 5). Similar trends were observed by Welemariam et al., [57] found a lower (2.2%) amount of macroaggregate-associated SOC from non-conserved grazing lands. Weidhuner et al., [112] also observed lower SOC associated with macroaggregates in agricultural fields. The low macroaggregate and microaggregate-associated SOC in ClAFP and TSWAFP could be due to low biomass input caused by agricultural disturbance [102, 107]. On the other hand, the SOC associated with macroaggregates was higher than the SOC associated with microaggregates under both dry and wet sieved fractions, except in wet sieved under WlAFP type. In line with this, Welemariam et al., [57] and Gelaw et al., [113] observed higher SOC in macroaggregates than in microaggregates in northern Ethiopia.

Conclusion

The present study results showed that soil aggregation and distribution of size fractions, glomalin related soil protein and aggregate fraction of associated soil organic carbon content significantly modified under different agroforestry practices. Tree based practices as homegarden agroforestry practices and woodlot agroforestry practices were less detrimental compared to scattered tree-based croplands on studied variables. Consequently, homegarden agroforestry practices followed by woodlot agroforestry practices lead to higher values of mean weight diameter, soil aggregate size distribution (dry and wet sieved fraction), glomalin related soil protein content (easily extractable glomalin related soil protein and total glomalin related soil protein) and soil aggregate associated soil organic carbon. Macroaggregate fraction also reached the highest values in soils under homegraden agroforestry practices and the lowest was in cropland based agroforestry practices. In case of microaggregate fraction, the highest values were recorded in woodlot agroforestry practices and the lowest was in trees on soil and water conservation based agroforestry practices. This indicates the homegarden agroforestry practices and woodlot agroforestry practices promoted the accumulation of soil organic carbon and glomalin related soil protein as result of formation and stability of soil aggregates. From the ecosystem sustainability point of view, homegarden agroforestry practices followed by woodlot agroforestry practices are the agroforestry practices types that could improve glomalin related soil protein, mean weight diameter, soil aggregate stability distribution and its associated soil organic carbon. This implies that how the managed agroforestry practices can improve the ecosystem stability through the increasing of soil glomalin concentration, soil organic carbon and soil aggregate stability.

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Author contributions
Nebiyou Masebo-did the research design, data analysis, compilation and wrote the main manuscript, Emiru Brihane-did the research design, data analysis and supervision, Serekebrehane Takele-did the research design and supervision, Araceli Pérez-Sanz -did the research design and supervision, Juan J.Lucena-did the research design and supervision, Zerihun Belay-did the research design and supervision, Agena Anjulo-did the research design and supervision and Felipe Yunta-did the research design and supervision.

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Data availability
Data will be provided upon the reasonable request.

Declarations

Competing interests
The authors declare no competing interests.

Ethics approval and consent to participate
Ethics approval and consent to participate isn't applicable this is because this manuscript does not report on or involve any animals, humans, human data, human tissue and plants as well.

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