Tree species traits and soil biochemical properties drive carbon stability and temperature sensitivity of soil aggregates in agroforestry systems of subtropical northeast India

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Abstract

Agroforestry systems play a critical role in enhancing soil organic carbon (SOC) stability and mitigating climate change by integrating trees and crops to improve soil fertility and carbon sequestration. This study investigates the SOC stability, aggregate dynamics, and temperature sensitivity of SOC mineralization across four agroforestry systems (*Michelia oblonga, Parkia roxburghii, Alnus nepalensis*, and *Pinus kesiya*). Tree traits, soil properties, and aggregate characteristics were analyzed alongside a 60-day incubation experiment under three temperature regimes (25°C, 30°C, and 35°C). The results revealed the SOC mineralization significantly varied amongst the agroforestry systems with highest value in *M. oblonga* (25.59 mg CO_2 g^{-1}) and lowest in *A. nepalensis* (20.39 mg CO_2 g^{-1}). Macroaggregates consistently showed higher SOC concentrations and biochemical indicators, such as polysaccharides and total glomalin-related soil proteins (TG-RSP), compared to microaggregates and bulk soil. The temperature and aggregate sizes statistically influenced the SOC mineralization rates, with noticeable interaction effect. SOC mineralization rates increased with temperature, but *Alnus nepalensis* exhibited the highest temperature sensitivity (Q_{10} = 0.955 and activation energy = 24.25 kJ mol^{-1}), highlighting its resilience to thermal stress. Strong positive correlations were observed between soil aggregate stability and soil biochemical indicators such as SOC, polysaccharides and TG-RSP of bulk soil and aggregates. Temporal trends indicated that carbon mineralization peaked at 30 days before stabilizing, reflecting the decomposition of labile carbon pools. These findings highlight the critical role of tree traits, soil aggregates, and thermal stability in driving SOC retention in agroforestry systems.

1. Introduction

Soil organic carbon (SOC) is a cornerstone of soil health and ecosystem sustainability, playing a critical role in maintaining soil fertility, supporting biological activity, and regulating the global carbon cycle. SOC stability, defined as its resistance to decomposition and loss, is pivotal for ensuring long-term carbon sequestration and mitigating climate change impacts (Lorenz and Lal 2014; Das 2023). Among the mechanisms contributing to SOC stability, soil aggregation stands out as a protective barrier against microbial decomposition, enhancing nutrient retention and soil physical properties (Keller and Phillips 2019; Augusto and Boca 2022). Studies suggest that soil texture significantly affects SOC accumulation, with finer-textured soils, such as clays, exhibiting a greater capacity for SOC storage due to their ability to stabilize organic matter through physical and chemical interactions (Castellano et al. 2015; Zhou et al. 2019; Rasmussen et al. 2018; Ribbons et al. 2018). Aggregate dynamics further influence SOC stability, as evidenced by the higher SOC concentrations often found in larger macroaggregates (Schmidt et al. 2011; Russell et al. 2018). Liu et al. emphasized that organic carbon enhances aggregate formation and stability, creating microenvironments that protect carbon from decomposition. Moreover, management practices, such as biochar application, have been recognized for their potential to modify soil physicochemical properties and enhance SOC stabilization (Cheng et al. 2018; Mayer et al. 2020). Conversely, land-use changes, such as deforestation and agricultural expansion, disrupt soil structure and reduce organic matter inputs, leading to declines in SOC (Li et al. 2015; Maes et al. 2019; Rytter and Rytter 2020). Zhou et al. (2019) noted that agricultural practices could lead to modifications in soil organic carbon content, directly affecting soil stability and productivity. The impact of land use on SOC is further illustrated by studies showing that different cropping systems can alter aggregate stability and SOC storage (Zhou et al. 2020; Zhang et al. 2022).

Agroforestry systems have emerged as a viable solution for improving SOC storage and stability (Cardinael et al. 2015; Feliciano et al. 2018). By integrating trees with agricultural crops or pastures, these systems offer a multifunctional approach that enhances carbon sequestration, soil fertility, and microbial activity while fostering resilient ecosystems (Pardon et al. 2017). The higher organic matter input from tree litter, including leaves, branches, and roots, contributes significantly to SOC accumulation (Palma et al. 2017). Studies have shown that agroforestry systems can maintain SOC levels exceeding those of conventional agricultural systems, with storage values reaching up to 24.91 tonnes per hectare in some tropical regions (Narender et al. 2021; An et al. 2023). Further, the dynamics of SOC in agroforestry systems are influenced by various factors, including vegetation characteristics, climate, and soil management practices. The interplay between these factors determines how carbon is stored, stabilized, and decomposed over time (Lorenz and Lal 2014). Temperature, in particular, plays a critical role in SOC dynamics by affecting microbial activity and organic matter decomposition rates. Temperature sensitivity indices, such as Q10 and activation energy, are increasingly used to assess the resilience of SOC under varying thermal regimes (Jenkins and Adams 2011; Blagodatskaya et al. 2016; Heskel et al. 2016).

Land-use changes, such as transitions from monoculture to agroforestry, have been shown to enhance SOC pools through improved organic matter inputs and enhanced soil structure (Augusto and Boca 2022). However, the effectiveness of agroforestry systems in stabilizing SOC depends on site-specific factors, such as soil type, climate, and management practices. Despite the advancements in understanding these dynamics, knowledge gaps remain in quantifying the effects of specific agroforestry systems on SOC stability across soil depths, thermal conditions, and management regimes (Lorenz and Lal 2014, Das 2023). This study addresses these gaps by exploring the stability of SOC pools under varying land-use scenarios and thermal conditions in agroforestry systems. Thus, the SOC stability at the aggregate level in soils under four agroforestry systems: *Michelia oblonga, Parkia roxburghii, Alnus nepalensis*, and *Pinus kesiya*. SOC stability and carbon mineralization were assessed across varying temperature regimes (25°C, 30°C, and 35°C) and durations (15, 30, and 60 days) to capture decomposition dynamics and temperature sensitivity. To complement this, parameters such as SOC content, polysaccharides, and glomalin were analyzed, providing a cumulative assessment of stabilization mechanisms. By examining the interplay between temperature, land use, and SOC stabilization

processes, the findings aim to provide actionable insights for optimizing land management practices to enhance SOC stability, improve soil health, and mitigate climate change. The objectives of the present study are (i) to investigate how the biochemical indicators (polysaccharides and total glomalin) varies among the aggregates and bulk soil under different agroforestry systems and (ii) to assess the temperature sensitivity of SOC mineralization using Q10 and Arrhenius activation energy under different agroforestry systems od subtropical northeast India.

2. Materials and methods

2.1 Site description

The study was conducted with four various multipurpose tree species planted under agroforestry systems planted in 1983 at Indian Council of Agricultural Research- Complex for North-east Hill Region, Umiam, Meghalaya. The experimental institute is located in the central part of Meghalaya in the East Khasi Hills of Northeast India at an elevation of 980 m above mean sea level with a slope of 32 to 53% ($25^{o}41'21"$ N latitude and $91^{o}55'25"$ longitude). The region experiences a humid subtropical climate characterised by distinctive warm-wet and cold-dry seasons annually. The annual mean rainfall of the region is about 2210 mm of which 85-90% is received from May to October. The average yearly minimum and maximum air temperature are 6.8 ^{o}C (February) and 29.3 ^{o}C (April), respectively. Relative humidity varies from 40% in winter and 88% during summer. The initial soil properties are *Typic Hapludalf* type, highly acid (pH 4.36-4.76), high in organic carbon (1.91-3.12%), available nitrogen (403-584 kg ha^{-1}), high in available phosphorus (19.3-47 kg ha^{-1}) and available potassium (248-361 kg ha^{-1}). The detailed informations about experimental site, agroforestry tree species and crop yields were described in Ramesh et al. (2013 and 2015).

2.2 Soil sampling

Surface soil samples (0-15 cm) were collected from the four agroforestry systems blocks following the method described by Dhyani and Tripathi (1999). By using 8 cm corer samplers, soil samples were collected from different agroforestry systems including control (natural fallow). The collected soil samples were brought to the research laboratory, shade dried at ambient temperature and pulverized to pass through 2-mm sieve for subsequent analysis. The mean-weight diameter (MWD) was calculated using air-dried samples that were pre-sieved through a 4 mm mesh using the method established by Kemper and Chepil (1965). A 250 µm sieve was used to separate 500g of air-dried soil samples into macro aggregates (>250 µm) and micro aggregates (<250 µm) in order to characterise the soil aggregates. The separated macro (>250 µm) and micro aggregates (<250 µm) were analysed to study the carbon stability by using the procedure established by Six et al. (1998). For the analysis of the total polysaccharides (TP), dilute acid-extractable polysaccharides (DAEP) and incubation experiment, the soil samples were stored at 4 ± 1 oC in the laboratory. TP and DAEP were estimated with the procedure adapted from Whistler and Wolfrom (1962) by Lowe (1994). The total glomalin was estimated using 1 g soil sample with 100 mM sodium pyrophosphate as outlined by Wright et al. (1996) and Rillig (2004).

2.3 Incubation experiment

A laboratory experiment was conducted for A 60 days to investigate the variations in the temperature sensitivity of SOC mineralization of macro- and micro- aggregates of various agroforestry systems. The stored moist soil samples of 0-15 cm depth equivalent to 100 g (oven dry basis) were added into Schott jars, deionized water was cautiously mixed and 30% water holding capacity was maintained through the experiment. A glass vial containing 25 mL of 1 mol L^{-1} NaOH solution was placed into the jars to capture the released CO_2 from the soil. Additionally, a series of blank jars containing vial with NaOH were kept for each temperature as control to account for the CO_2 captured from the air inside the jar. The incubation experiment was conducted up to 60 days with three temperature viz. 25, 30 and 35 ^{o}C using BOD incubators. The three temperatures were selected based on minimum and maximum temperatures experienced of the experimental farm of the region. Soil moisture was adjusted to 30% water holding capacity periodically as it is generally contemplated to impersonate the field moisture conditions. By weighing each sample once in a week, the soil moisture content was adjusted to the desired moisture content (30% field capacity) to maintain the soil moisture. Three replications were set for each treatment of the experiment. The released CO_2 from each sample including blank was estimated at 15, 30 and 60 days after the incubation by the titration with 0.1 mol L^{-1} NaOH solution after adding BaCl2. The generated CO_2 data for various temperatures was used for the soil aggregate carbon content, carbon mineralization and temperature dependency of CO_2 efflux by Arrhenius activation energy (k = A exp (-E/RT)) (Knorr et al. 2005)

Where, k is the rate constant; A is the pre-exponential factor; E is the activation energy; R is the universal constant and T is the absolute temperature.

2.4 Statistical analysis

The data on the impact of agroforestry systems on carbon related parameters and carbon mineralization of soil aggregates were analysed statistically by using the software SPSS for Windows (SPSS Inc. USA). A two-way analysis of variance (ANOVA) was utilized for the statistical evaluation of the measured parameters. The Duncan multiple range test (DMRT) at 5% level of probability was performed to find out the

significance of the difference between the means for different agroforestry systems. Correlation analyses were also performed for the identification of the functional relationship among the carbon related parameters of the aggregates from the agroforestry systems.

3. Results

3.1 General characteristics of agroforestry systems

The morphological and biomass characteristics of tree species varied significantly across the agroforestry systems. *Parkia roxburghii* exhibited the tallest trees (24.99 ± 1.21 cm) and largest diameter at breast height (DBH) (27.95 ± 2.82 cm), while *Pinus kesiya* had the shortest trees (15.60 ± 3.80 cm) and smallest DBH (23.32 ± 8.24 cm). Timber volume was highest for *Michelia oblonga* (231.31 m^3 ha^{-1}), surpassing *Pinus kesiya* (117.77 m^3 ha^{-1}) by 49%, indicating superior timber productivity. Fine root biomass was greatest in *Pinus kesiya* (496.75 g m^{-2}) but closely followed by *Alnus nepalensis* (435.50 g m^{-2}), highlighting their roles in below-ground carbon contributions. Annual litter biomass was highest in *Pinus kesiya* (625.30 ± 3.83 g m^{-2}), followed by *Michelia oblonga* (512.45 ± 9.51 g m^{-2}) (Table 1). Similarly, Soil nutrient content and physical properties varied widely among the systems. *Alnus nepalensis* soils exhibited the highest total nitrogen (TN, 584.3 ± 10.4 kg ha^{-1}), total phosphorus (TP, 47.2 ± 1.6 kg ha^{-1}), and organic carbon content (35.6 ± 2.1 g kg^{-1}), alongside the lowest bulk density (1.07 ± 0.05 Mg m^{-3}). These results reflect its ability to enhance soil fertility and nutrient availability. Conversely, *Pinus kesiya* soils had the lowest TN (370.8 ± 8.9 kg ha^{-1}), TP (31.6 ± 1.4 kg ha^{-1}), and organic carbon content (25.8 ± 1.7 g kg^{-1}), alongside the highest bulk density (1.26 ± 0.03 Mg m^{-3}) and lowest EC (0.41 ± 0.03 dS m^{-1}). Potassium (K) concentrations were highest in *Michelia oblonga* (420.0 ± 15.6 kg ha^{-1}) and lowest in *Pinus kesiya* (330.5 ± 14.2 kg ha^{-1}), underscoring differences in nutrient cycling across systems. Crop yields were highest in systems with *Alnus nepalensis*, producing grain yields of 3.8 ± 0.2 t ha^{-1} and straw yields of 4.6 ± 0.3 t ha^{-1}, which were 25% and 31% higher, respectively, than in systems with *Pinus kesiya*. *Michelia oblonga* and *Parkia roxburghii* exhibited intermediate grain yields (3.5 ± 0.2 t ha^{-1} and 3.4 ± 0.2 t ha^{-1}) and straw yields (4.2 ± 0.2 t ha^{-1} and 4.0 ± 0.2 t ha^{-1}) (Table 2).

3.2 Macro- and microaggregate carbon stability parameters

The data on distribution of soil organic carbon (SOC), polysaccharides, glomalin, and aggregate stability across macro- and microaggregates in the four agroforestry systems is presented in figures 1-5. SOC concentrations (Figure 1) were consistently higher in macroaggregates than in microaggregates across all systems ($p < 0.05$). Thus, *Alnus nepalensis* soils exhibited the highest SOC concentrations in macroaggregates (27.1 ± 1.4 g kg^{-1}), approximately 1.8-fold higher than microaggregates (15.2 ± 1.1 g kg^{-1}). Conversely, *Pinus kesiya* had the lowest SOC in both macroaggregates (19.5 ± 1.2 g kg^{-1}) and microaggregates (11.8 ± 0.9 g kg^{-1}), reflecting its lower contribution to carbon storage. Across all systems, macroaggregates accounted for 60–70% of the total SOC pool. Similarly, the estimation of total polysaccharide (TP) concentrations (Fig. 2) were remarkably higher in macroaggregates compared to microaggregates ($p < 0.05$), with the highest values observed in *Alnus nepalensis* soils (1.74 g $100g^{-1}$ in bulk soil and 1.53 g $100g^{-1}$ in microaggregates). With respect to the macroaggregates, the total polysaccharides content was at par with the *Michalia oblonga*. These levels were 14-23% higher in bulk soil, 1-13% higher in macroaggregates and 8-13% greater in microaggregates as compared to other agroforestry systems. Agroforestry systems enhanced the total polysaccharides about 1.21-1.48 times in bulk soils, 1.17-1.32 times in macroaggregates and 1.20-1.35 times in microaggregates as compared to fallow lands. Polysaccharides were positively correlated with SOC stability ($R^2 = 0.78$, $p < 0.01$) across all systems, underscoring their role in aggregate formation and carbon stabilization. The dilute acid-extractable polysaccharides (DAEP) (Fig. 3) showed a similar trend as total polysaccharides and significant variations across agroforestry systems and aggregate fractions ($p < 0.05$). *Alnus nepalensis* exhibited the highest concentrations of total polysaccharides in macroaggregates (1.53 g $100g^{-1}$ soil), which were 1.21 times greater than those in microaggregates (1.24 g $100g^{-1}$ soil) and 1.14 times greater than bulk soil (1.32 g $100g^{-1}$). In contrast, *Pinus kesiya* exhibited the lowest values for total polysaccharides with bulk soil (1.41 g $100g^{-1}$ soil), macroaggregates containing only 1.74 g $100g^{-1}$ soil and microaggregates containing only 1.38 g $100g^{-1}$ soil. Similarly, for dilute acid extractable polysaccharides, *Pinus kesiya* recorded the lowest value in bulk soil (1.41 g $100g^{-1}$ soil), macroaggregates (1.74 g $100g^{-1}$ soil) and microaggregates (1.38 g $100g^{-1}$ soil). Adaption of agroforestry systems in the fallow lands, increased the dilute acid extractable polysaccharides about 5-28% in bulk soil, 5-21% in macroaggregates and 18-36% in microaggregates (Fig. 3). The TP in microaggregates had a significant correlation with SOC ($r = 0.666^{**}$), macroaggregates carbon ($r = 0.514^{*}$) and microaggregates carbon ($r = 0.587^{*}$). The total Glomalin-related soil protein (TG-RSP) significantly differed among the agroforestry systems irrespective of the soil aggregates. The TG-RSP was highest in *Alnus nepalensis* of bulk soil, macroaggregates and microaggregates, with the value reaching 0.31, 0.48 and 0.32 g/100g soil, respectively (Figure 4). These values were 1.20-1.41, 1.23-1.54 and 1.03-1.83 times greater than those observed in other agroforestry systems' aggregates. *Pinus kesiya* had the lowest TGRSP concentrations in bulk soil, macroaggregates and microaggregates, reflecting reduced biochemical protection of SOC. However, converting fallow/barren land to agroforestry systems increased the TG-RSP content in bulk soil (16-60%), macroaggregates (4-60%) and microaggregates (4-33%). GRSP levels were significantly associated with SOC content ($R^2 = 0.85$, $p < 0.01$), emphasizing their contribution to aggregate stability. The mean contribution of agroforestry systems TG-RSP to SOC is 1.08, 1.13 and 1.33-fold times in bul soil, macroaggregates and microaggregates, respectively compared to barren land (Fig. 5). Further, the stability of aggregates, as measured by the mean weight diameter (MWD), was statistically higher in macroaggregates compared to microaggregates ($p < 0.05$, Figure 5). Macroaggregates under *Alnus nepalensis* soils exhibited the greatest stability, with an MWD of 2.5 ± 0.2

mm, which was 40% higher than the MWD of *Pinus kesiya* macroaggregates (1.8 ± 0.1 mm). Microaggregate stability followed a similar trend, with *Pinus kesiya* consistently showing lower values across all systems.

3.3 Temperature and time effects on SOC mineralization

SOC mineralization rates varied significantly with temperature and tree species ($p < 0.05$, Table 3). The average macroaggregates C mineralization rate ranged from 1.88-7.71, 5.65-11.52 and 11.45-23.7 mg CO_2-C g $soil^{-1}$ at 15, 30 and 60 days of incubation, correspondingly. On the other hand, the microaggregates C mineralization rate ranged from 1.47-5.36, 2.86-8.66 and 5.21-16.59 mg CO_2-C g $soil^{-1}$ at 15, 30 and 60 days of incubation, respectively (Table 3). The C mineralization rate of different agroforestry systems was 52% higher in macroaggregates at 15 days, 77% higher at 30 days and 68% higher at 60 days of incubation as compared to microaggregates, irrespective of the incubation temperatures. The cumulative C mineralization ranged from 22.4 to 24.6 in macroaggregates while in microaggregates it ranged from 13.95 to 17.98 mg CO_2-C g $soil^{-1}$ among the agroforestry systems. Agroforestry systems, on an average, showed 1.07 times lesser cumulative C mineralization rate while in microaggregates it was 1.57 times lower than the control. *Alnus nepalensis* soils exhibited the lowest cumulative carbon mineralization across all temperatures, with emissions of 22.4 mg CO_2-C/g SOC at 35°C. In contrast, *Michelia oblonga* soils had the highest rates (24.6 mg CO_2-C/g SOC), reflecting greater carbon loss. Carbon mineralization increased by 2.13-4.80 times in between 25°C and 35°C, with the highest increments observed in *Pinus kesiya*. Microaggregates consistently showed 43% lower mineralization rates than microaggregates at 35 ^oC across all the agroforestry systems, emphasizing their role in SOC protection. Overall, increase in temperature from 25 to 35 ^oC augmented the rate of C mineralization of both aggregates, on average, by 100, 62 and 63% at 15, 30 and 60 days of incubation, correspondingly. Further, temperature sensitivity indices (Q_{10}) highlighted significant differences in SOC stability among agroforestry systems (Table 4). *Alnus nepalensis* soils exhibited the highest Q_{10} values (0.955 ± 0.1), indicating higher thermal stability compared to *Pinus kesiya* (0.862 ± 0.2). *Parkia roxburghii* and *Michelia oblonga* displayed intermediate Q_{10} values (0.905 ± 0.1 and 0.932 ± 0.2, respectively). Thus, the activation energy (Ea) for SOC mineralization varied significantly across systems, with *Alnus nepalensis* soils requiring the lowest energy (24.25 ± 2.3 kJ mol^{-1}) to initiate decomposition, indicating the presence of more thermally stable carbon pools (Table 4). *Pinus kesiya* had the highest Ea (74.97 ± 3.1 kJ mol^{-1}), suggesting less stable carbon pools. The study demonstrated that the macro and microaggregates in agroforestry systems recorded a relatively lower rate constant values than that of control throughout all study temperatures (Table 4). The mean rate constant macroaggregate varied between 0.0005 to 0.0016 in agroforestry systems whereas, it varied between 0.0008 to 0.0022 in control. Conversely, the mean rate constant of microaggregates ranged from 0.0007 to 0.0014 in agroforestry systems while in control plot it ranged from 0.0004 to 0.0017. The increase in incubation temperature from 25 to 30 ^oC increased the rate constant by 2.42 times whereas, from 30 to 35 ^oC showed 1.41 times increase in macroaggregate C mineralization. But, in microaggregates C mineralization, increase in temperature from 25 to 30 and 30 to 35 ^oC recorded 1.38 and 1.35 times increase in rate constant, respectively.

4. Discussion

Research has also emphasized the importance of aggregate-level SOC stabilization in protecting carbon from microbial decomposition (Orgill et al. 2016; Pan 2011; Singh et al. 2018). Macroaggregates and microaggregates within the soil matrix provide physical and biochemical protection to SOC, thus influencing its long-term stability. Furthermore, the interactions between land use and environmental conditions can significantly impact aggregate dynamics and SOC stabilization mechanisms (Augusto and Baco 2022). The tree species demonstrated distinct contributions to soil properties and SOC dynamics, with *Alnus nepalensis* outperforming others due to its balanced above- and below-ground biomass inputs. Its high nitrogen-fixing capability and litter quality enhanced soil fertility, as evidenced by the highest total nitrogen (TN), phosphorus (TP), and SOC concentrations. These findings align with studies emphasizing the role of nitrogen-fixing trees like *Alnus* spp. in boosting soil nutrient pools and SOC stabilization (Lorenz and Lal 2014; Augusto and Baco 2022). Moreover, the incorporation of organic materials into the soil has been shown to improve aggregate stability and increase SOC content. For example, it is reported that organic material treatments significantly enhanced the mean weight diameter (MWD) of soil aggregates, indicating improved stability (Pan et al. 2015; Wang et al. 2020). This finding corroborates with results from Cheng et al. (2018) who emphasized the importance of understanding SOC fractions and their dynamics in relation to soil properties. In contrast, *Pinus kesiya* exhibited the lowest SOC concentrations and nutrient content despite its high litter biomass, suggesting limited nutrient cycling efficiency. Similar trends have been reported in systems dominated by conifers, where slow litter decomposition impedes nutrient availability and SOC accrual (Yang et al. 2022; Zhang et al. 2021).

SOC concentrations were significantly higher in macroaggregates than in microaggregates across all systems, underscoring the role of aggregate dynamics in carbon stabilization. *Alnus nepalensis* macroaggregates had the highest SOC, supported by elevated polysaccharide and glomalin-related soil protein (GRSP) levels. These findings are consistent with studies showing that biochemical inputs, such as polysaccharides and GRSP, strengthen soil structure and protect SOC from microbial decomposition (Schlecht-Pietsch et al. 1994; Wang et al. 2021). Conversely, *Pinus kesiya* had the lowest biochemical indicators and aggregate stability, reflecting its reduced capacity to stabilize SOC. This variability highlights the influence of tree traits on aggregate formation and stability, which are critical for long-term carbon sequestration (Pardon et al. 2017). Further, this relationship is mediated through several mechanisms, including root traits, litter quality, and microbial interactions. For instance, broadleaf trees have been shown to enhance soil aggregate stability compared to coniferous species, with studies indicating

improvements in stability by 57–103% in mixed forest stands dominated by broadleaf trees (Zheng 2023). The presence of fine roots and their associated organic matter contributes to the binding of soil particles, which is essential for the formation of stable aggregates (Zheng 2023; Kemner et al. 2020). Additionally, the biomass of various tree species and the litter they produce are key indicators affecting aggregate stability, as they influence the organic matter content and microbial activity within the soil (Wang 2023). The decomposition of litter contributes organic materials that serve as binding agents for soil particles, promoting the formation of aggregates (Su et al. 2021). The quality of litter, particularly its carbon content, affects the microbial community structure and activity, which are vital for the stabilization of soil aggregates (Jing et al. 2023). For example, higher organic carbon inputs from tree litter can enhance microbial metabolism and lead to increased stability of soil aggregates through the production of extracellular polysaccharides and other binding agents (Su et al. 2021; Jing et al. 2023). Changes in tree species composition due to land-use changes can lead to alterations in soil pH, which has been linked to the dynamics of soil macroaggregates (Russell et al. 2018; Russell et al. 2017). The ratio of TG-RSP to SOC in agroforestry systems ranged from 1.08-1.33-times higher than barren land. The constituents of TG-RSP consisted of a diverse amalgamation of proteins, lipids, humus, and inorganic components, exhibiting remarkable stability in nature (Wang et al. 2017). Thus, the elevated ratio of glomalin to SOC with agroforestry systems may offer additional evidence that microbial-derived C facilitates more SOC accumulation in soils of agroforestry systems. Our results suggest that the increased contribution of glomalin to SOC in agroforestry systems may enhance the stability of aggregates in these systems. Further, the high and significant correlations between TG-RSP and aggregates SOC support the increase in the aggregates stability in the present investigation.

SOC mineralization is significantly influenced by incubation temperature, with both the mineralization ratio and cumulative SOC mineralization rates increasing as temperatures rise (Qin et al. 2016). In the present study, temperature significantly influenced SOC mineralization rates, with the highest rates observed at 35°C. The concept of temperature sensitivity, often quantified using the Q_{10} value, reflects the rate of increase in SOC mineralization for every 10°C rise in temperature (Duan et al., 2023). *Alnus nepalensis* exhibited the lowest temperature sensitivity (higher Q_{10} value: 0.955) and activation energy, indicating greater thermal stability of its SOC pools. This finding is corroborated by He et al. (2022) who found that higher substrate carbon levels resulted in increased SOC mineralization rates under elevated temperatures, suggesting that substrate quality also modulates temperature sensitivity. Similarly, Wang et al. (2013) reported that SOC mineralization rates significantly increased with temperature across a range of incubation conditions, reinforcing the notion that temperature is a critical driver of SOC dynamics (Wang et al. 2013). These results align with findings that species with efficient nutrient cycling and root contributions exhibit better resilience to temperature-induced carbon loss (Bai et al. 2023). Temporal trends revealed peak SOC mineralization at 30 days, driven by the rapid decomposition of labile carbon pools. This pattern, followed by stabilization at 60 days, reflects the transition from labile to recalcitrant carbon decomposition phases, consistent with previous incubation studies (Augusto and Baco 2022; Das 2023). Palma et al. (2017) highlight that the accumulation of carbon in agroforestry systems can be influenced by the ratio of root biomass to decomposed plant matter, suggesting that a higher proportion of root biomass can lead to greater carbon retention in the soil. However, as temperatures increase, the decomposition of this organic matter may accelerate, potentially leading to a reduction in SOC levels (Kay et al. 2019). This is supported by findings from Silva et al. (2014) which indicate that biological activity in agroforestry systems can be significantly affected by temperature fluctuations, impacting carbon storage. In the present study, the activation energy was highest in *Pinus kesiya* and lowest in *Alnus nepalensis*, however, control plot had the highest Ea than agroforestry systems (132%), irrespective of the aggregates. According to Davidson and Janssens (2006), the mineralization of poor-quality C substrates requires highest Ea than the high-quality C substrates. The C:N ratio, chemical compositions and their complexity, SOC content, etc. influence the substrate quality (Bhattacharyya et al 2020). In contradiction of activation energy, the Q_{10} values remained 2–11% more in agroforestry systems than control plot. Xu et al (2012) stated that the Ea and Q_{10} are inversely related with respect to SOC mineralization which is in consistent with the present study. The high-quality organic substrates require less Ea and more sensitive to temperature than the poor-quality organic substrates. The association of aggregate stability to SOC was perhaps attributed to binding agents including fungal exudates like polysaccharides and glomalin (Wright et al., 1999) as well as hyphae and roots which improve stability of aggregates. According to Eynard et al. (2004), these polysaccharides and glomalin improves the stability soil aggregates through the changes in the degree of water repellency. The examination of first order rate constant or kinetics indicated that aggregates in the agroforestry systems provided a superior protection of the SOC compared to control in both aggregate types. The reaction rate was more rapid in macroaggregates than microaggregates regardless of the agroforestry systems including control. The present investigation indicated a steady rise in reaction rate with temperature in both aggregates. The elevated rate constants indicate the comparatively lesser C stability in the macroaggregates over microaggregates. This is due to the variations in C availability among and within the aggregates across the agroforestry systems. Aggregates provides physical protection to soil organic matter by creating physical barricades that separate microbes and enzymes and their substrates. The variations in aggregates stability within the agroforestry systems including control, are mostly attributed to variations in organic inputs from crop residues, litter and root biomass, and the severity of soil physical disturbance (Zhou et al 2022). Consequently, the improved protection of soil organic matter by aggregates in agroforestry systems leads to greater accumulation of carbon compared to control system. Hence, effective management strategies that enhance soil health and organic matter inputs can mitigate the impacts of temperature on carbon dynamics. For instance, practices that maintain soil cover and reduce soil disturbance can help preserve SOC levels even in the face of rising temperatures (Niguse et al. 2022).

Conclusions

This study provides a comprehensive evaluation of soil organic carbon (SOC) stability across four agroforestry systems, emphasizing the critical roles of tree traits, aggregate dynamics, and temperature sensitivity in enhancing soil health and carbon sequestration. The results demonstrated significant variability among the agroforestry systems, with *Alnus nepalensis* emerging as the most effective in enhancing SOC stability. The balanced above- and below-ground biomass contributions of *Alnus nepalensis*, including moderate litter inputs and substantial root biomass, played a key role in improving nutrient cycling and aggregate stability. In contrast, *Pinus kesiya*, despite its high litter biomass, showed lower SOC concentrations and weaker biochemical contributions, suggesting limited nutrient enrichment and carbon stabilization potential. Macroaggregates were identified as critical to SOC stabilization, with consistently higher SOC concentrations, polysaccharides, and glomalin-related soil proteins (GRSP) compared to microaggregates. Among the systems, *Alnus nepalensis* macroaggregates exhibited the greatest stability, supported by high biochemical indicators, such as polysaccharides and GRSP than other agroforestry systems. The correlation between SOC stability and biochemical properties underscores the role of organic matter inputs in strengthening soil structure and enhancing carbon retention. Temperature sensitivity of SOC mineralization varied significantly across systems, with *Alnus nepalensis* showing the highest Q_{10} values and lower activation energy. Temporal trends revealed peak carbon mineralization at 30 days, followed by stabilization, reflecting the decomposition of labile carbon pools and the persistence of more recalcitrant fractions. The reduced mineralization rates in *Alnus nepalensis* soils further affirm its ability to sequester carbon effectively over time. Overall, the findings demonstrate that agroforestry systems have immense potential to enhance SOC stability, mitigate climate change, and improve soil health.

Declarations

Author Contribution

Dr. Ramesh Thangavel and Dr. KM Manjaiah prepared the main manuscript textA. Arunachalam and JMS Tomar provided the details of the study siteS Hazarika, BU Choudhury, and A Balusamy prepared the figures and edited the manuscriptVK Mishra is the overall co-ordinator and the Director of the institute

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Tables

Table 1 Characteristics of the experimental tree species of different agroforestry systems

Tree species	Local name	Height (cm)	DBH (cm)[a]	Timber vol. (m^3 ha^{-1})	Total fine root biomass[b] (g m^{-2})	Annual litter biomass (g m^{-2})
Michelia oblonga	Champak	23.88±3.34	27.45±7.28	231.31	462.00	512.45±9.51
Parkia roxburghii	Tree bean	24.99±1.21	27.95±2.82	204.62	415.50	353.92±14.4
Alnus nepalensis	Alder	19.77±3.91	26.81±2.26	195.01	435.50	476.75±5.57
Pinus kesiya	*Khasi* pine	15.60±3.80	23.32±8.24	117.77	496.75	625.30±3.83

[a]Mean tree bole diameter at brest height (cm); [b]Fine root biomass recorded in 0-30 cm soil depth (Adapted from Ramesh et al. 2013)

Table 2 Average yield of crops under tree canopy of different agroforestry systems

Tree species	Average Yield of crop (q ha^{-1})			
	Soybean (*Glycine max*)	Pineapple (*Ananas comosus*)	Ginger (*Zingiber officinale*)	Turmeric (*Curcuma longa*)
Michelia oblonga	14.08±2.91[a]	138.89±6.506b	87.42±11.50ab	149.33±8.46a
Parkia roxburghii	14.73±2.68a	156.50±10.476a	97.67±7.78a	153.63±7.23a
Alnus nepalensis	16.25±3.11a	148.89±7.095ab	99.25±11.89a	145.67±4.25a
Pinus kesiya	14.55±2.73a	124.10±4.726c	72.17±13.84b	97.40±7.65b

(Adapted from Ramesh et al. 2013)

Table 3 Effect of agroforestry systems, duration and temperature on soil macro-and micro-aggregates carbon mineralization (mg CO_2 g^{-1})

Agroforestry systems	15 days			30 days			60 days		
	25 °C	30 °C	35 °C	25 °C	30 °C	35 °C	25 °C	30 °C	35 °C
Macroaggregate									
Michelia oblonga	1.84ab	4.77b	7.34b	5.51b	8.07b	10.64bc	12.48b	15.88b	25.59a
Parkia roxburghii	1.47b	4.40c	6.97c	4.77bc	7.71b	10.28bc	10.64c	13.95c	23.49b
Alnus nepalensis	2.57a	5.14ab	10.64a	9.18a	11.01a	14.68a	15.05a	17.35a	20.39bc
Pinus kesiya	0.73c	4.77b	6.24d	4.40c	6.61c	11.74b	9.91cd	17.98ab	23.12b
Control (Barren)	2.79a	5.51a	7.34b	4.43c	8.09b	12.31bc	9.18d	15.78b	26.96a
Mean	1.88	4.92	7.71	5.65	8.29	11.93	11.45	16.37	24.11
Microaggregate									
Michelia oblonga	1.47b	4.04a	6.61a	2.24bc	6.81ab	7.34bc	4.04bc	9.91b	14.68bc
Parkia roxburghii	2.57a	2.20bc	6.24a	2.94b	6.29b	6.61c	6.24ab	11.74ab	16.88b
Alnus nepalensis	1.10c	2.57b	4.81b	1.84c	6.24b	8.81b	3.67bc	11.01ab	13.95c
Pinus kesiya	0.73c	1.84c	4.77b	2.20bc	6.45b	9.54b	4.77b	11.94ab	17.98ab
Control (Barren)	1.47b	2.57b	4.40c	5.14a	8.07a	11.01a	7.34a	13.21a	19.45a
Mean	1.47	2.64	5.36	2.86	6.68	8.66	5.21	11.52	16.59

Table 4 Effect of agroforestry systems and temperature on rate constant, activation energy and Q_{10} values of aggregates carbon loss

Agroforestry systems	Rate constant (k)			AE	Q_{10}
	Temperature (°C)			(kJ mol^{-1})	
	25	30	35		
Macroaggregates					
Michelia oblonga	0.0003	0.0009	0.0015	37.00d	0.932ab
Parkia roxburghii	0.0006	0.0013	0.0018	54.27c	0.905b
Alnus nepalensis	0.0005	0.0011	0.0013	24.25e	0.955a
Pinus kesiya	0.0005	0.0013	0.0019	74.97b	0.868c
Control (Barren)	0.0008	0.0014	0.0022	110.51a	0.855d
Mean	0.0005	0.0012	0.0017	60.2	0.902
Microaggregates					
Michelia oblonga	0.0008	0.0010	0.0013	92.99a	0.802a
Parkia roxburghii	0.0007	0.0009	0.0014	83.97b	0.852a
Alnus nepalensis	0.0008	0.0009	0.0011	73.13b	0.874a
Pinus kesiya	0.0006	0.0012	0.0016	102.06ab	0.824a
Control (Barren)	0.0004	0.0010	0.0017	107.19b	0.858a
Mean	0.0007	0.0010	0.0014	91.87	0.842

Table 5. Pearson's correlation matrix between MWD, macro and microaggregate carbon, and soil properties

Properties	MWD	Soil C	MAC-C	MIC-C	Soil TP	MAC-TP	MAC-TP	Soil DAEP	MAC-DAEP	MIC-DAEP	Soil TG	MAC-TG	MIC-TG
MWD	1												
Soil C	0.637*	1											
MAC- C	0.531*	0.943**	1										
MIC-C	0.241	0.437	0.400	1									
Soil TP	0.310	0.641*	0.476	0.457	1								
MAC- TP	0.580*	0.423	0.266	0.355	0.511	1							
MIC- TP	0.417	0.666**	0.514*	0.587*	0.750**	0.363	1						
Soil DAEP	0.625*	0.554*	0.546*	0.321	0.439	0.491	0.348	1					
MAC-DAEP	0.723**	0.422	0.346	0.311	0.227	0.597*	0.308	0.314	1				
MIC-DAEP	0.745**	0.747**	0.628*	0.498	0.625*	0.783**	0.501	0.562*	0.667**	1			
Soil TG	0.406*	0.419	0.438	0.450	0.694**	0.344	0.590*	0.490	0.205	0.424	1		
MAC- TG	0.634**	0.695**	0.558*	0.582*	0.817**	0.695**	0.627*	0.556*	0.418	0.693**	0.637*	1	
MIC-TG	0.537*	0.614*	0.476	0.293	0.658**	0.465	0.689**	0.342	0.296	0.658**	0.631*	0.629*	1

** Correlation is significant at the 0.01 level (2-tailed); *. Correlation is significant at the 0.05 level (2-tailed); MWD-mean weight diameter; MAC: macroaggregate; MIC: microaggregate; C: carbon; TP: total polysaccharides; DAEP: dilute acid extractable polysaccharides; TG: total glomalin

Figures

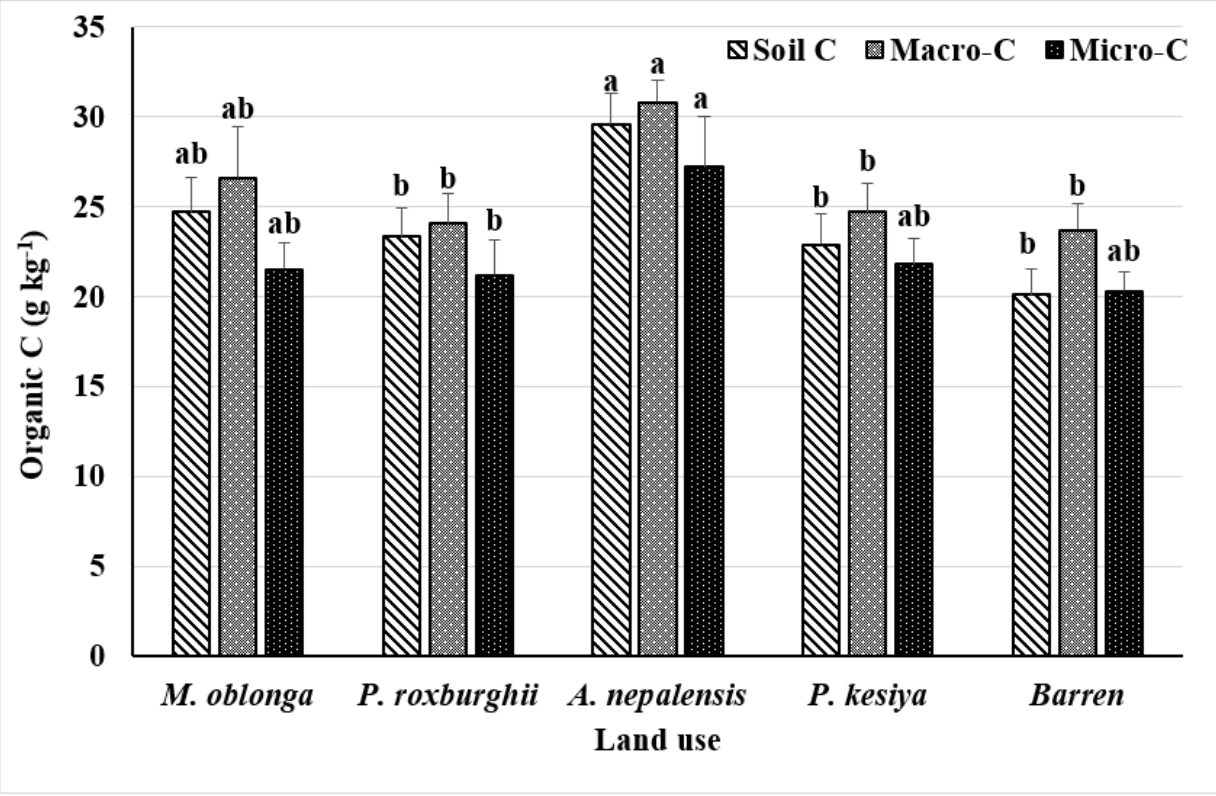

Figure 1

Effect of agroforestry systems on organic carbon (g kg^{-1}) content of bulk soil, macro- and microaggregates

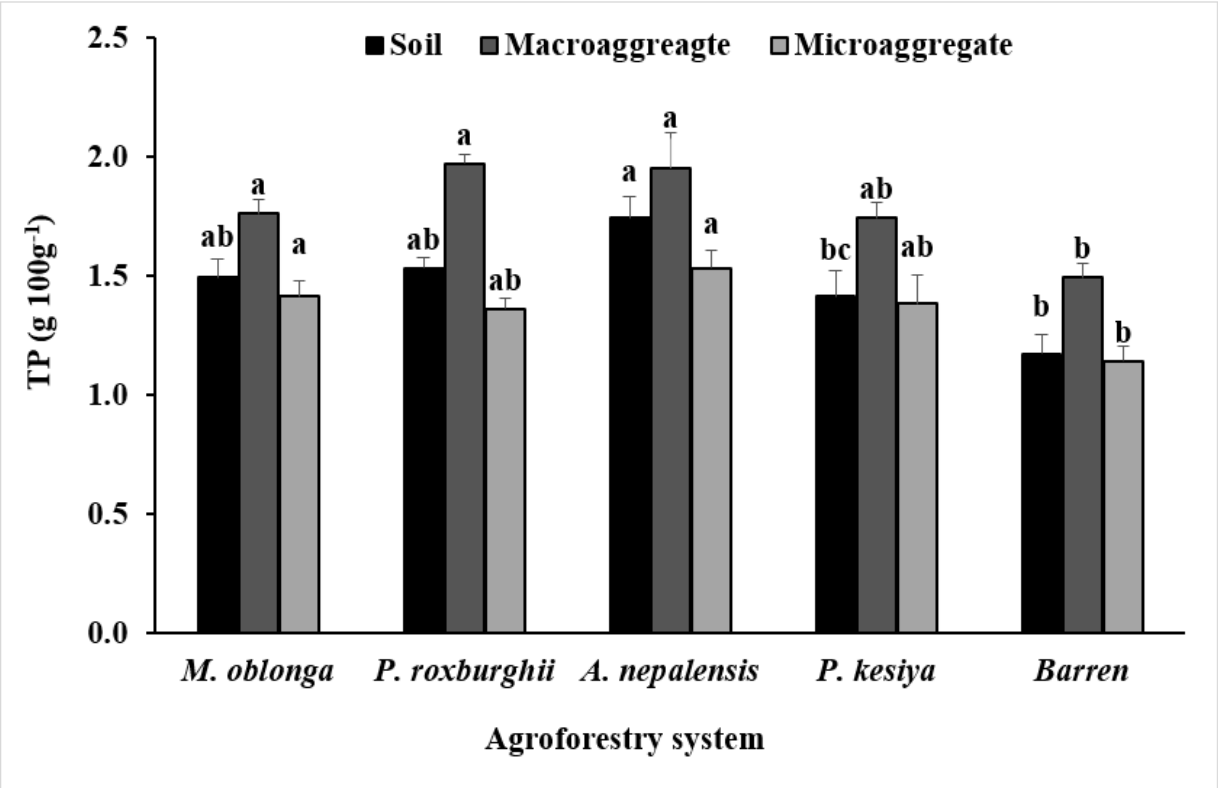

Figure 2

Effect of agroforestry systems on total polysaccharides (TP) (g $100g^{-1}$) of bulk soil, macro- and microaggregates

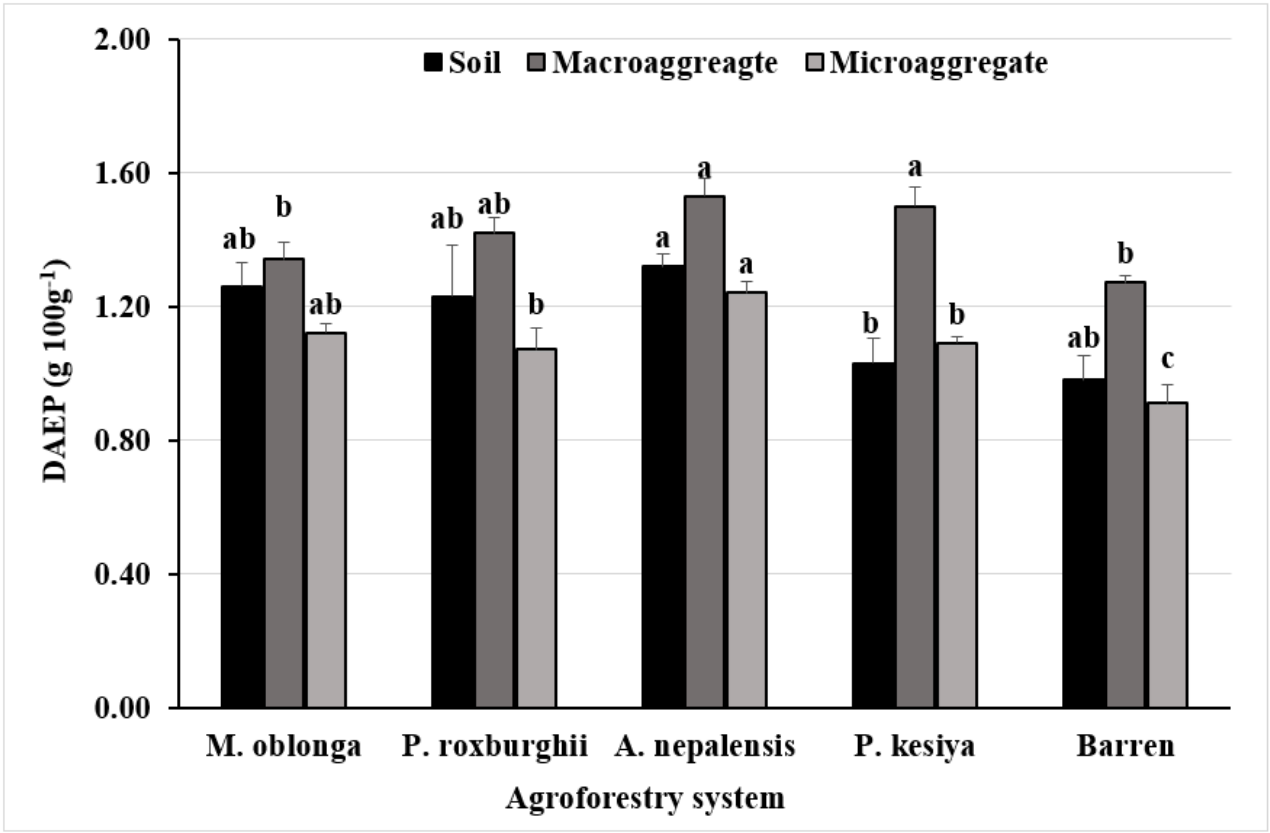

Figure 3

Effect of agroforestry systems on dilute acid extractable polysaccharides (DAEP) (g $100g^{-1}$) of bulk soil, macro- and microaggregates

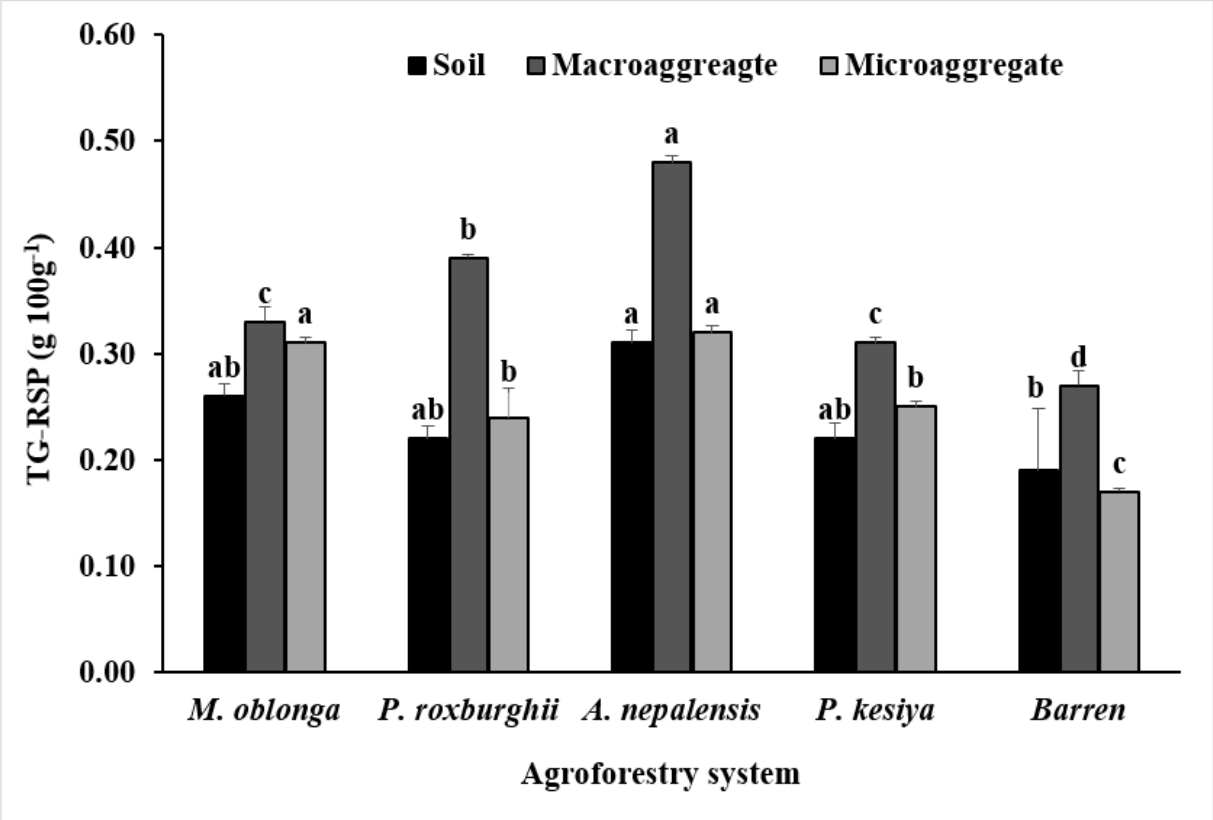

Figure 4

Effect of agroforestry systems on total glomalin (TG) (g $100g^{-1}$) of bulk soil, macro- and microaggregates

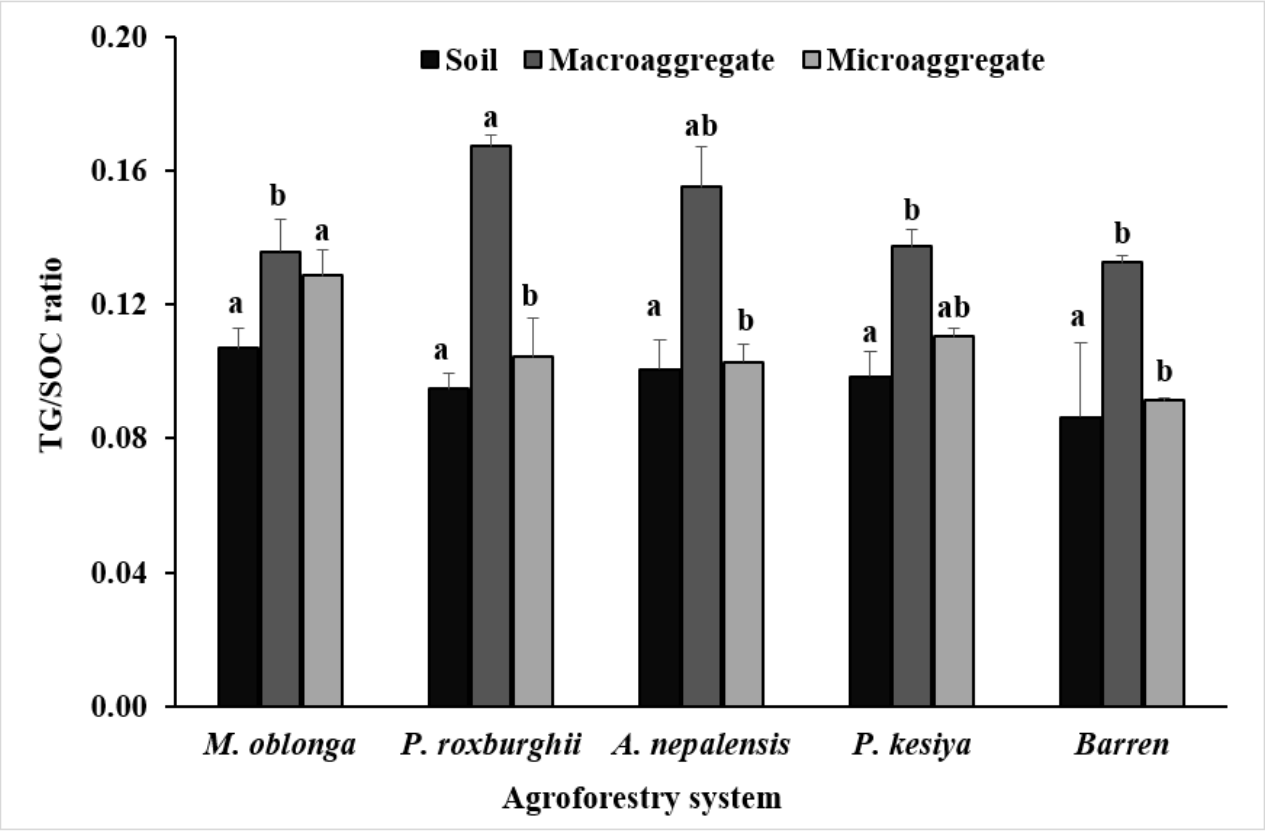

Figure 5

Effect of agroforestry systems on total glomalin (TG) to soil organic carbon (SOC) ratio of bulk soil, macro- and microaggregates