International Journal of Agriculture & Biology
ISSN Print: 1560–8530; ISSN Online: 1814–9596
25–259/2025/34:340407
http://www.fspublishers.org

Full Length Article

Soil Carbon Sequestration Potential and Microbial Biomass Activity in Different Agro-eco Systems: A Comparative Study of Agricultural, Forest, Shrub and Grassland Land Uses

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Received 18 March 2025; Accepted 31 May 2025; Published online 29 June 2025

Editor: Muhammad Farooq

Abstract

The rise in atmospheric CO_2 due to global temperature rise necessitates a targeted strategy to reduce CO_2 concentration. The current study aimed to understand how C sequestration occurs in soils from various agro-ecosystems by using MBC and key soil physicochemical properties. Soil samples from various land types in the Pothwar Plateau, Punjab, Pakistan, were collected at two depths —0–15 cm and 15–30 cm. Physicochemical parameters analyzed included electrical conductivity, pH, TOC, nitrogen, phosphorus, organic matter, soil moisture, bulk density, and MBC. Results indicated significant variations in MBC across agro-ecosystems, with forest soils exhibiting the highest values (181.6 μg/g in summer and 176.18 μg/g in winter), followed by shrub land (143.57 μg/g), agricultural land (137.83 μg/g) and grassland (96.07 μg/g). Seasonal differences were observed, with MBC levels higher in summer compared to winter. C sequestration was more pronounced in the upper soil layer (0–15 cm) than in the subsurface (15–30 cm). Forest ecosystems significantly contribute to C sequestration, a crucial aspect of climate change mitigation, and are recommended for large-scale reforestation and sustainable land management practices.

Keywords: Carbon sequestration; Agro-ecosystem; Climate change; Microbial biomass carbon

Introduction

Carbon dioxide (CO_2) acts as an essential part in global carbon (C) cycles while serving as an essential requirement for photosynthetic processes. However, human activities, particularly the excessive combustion of fossil fuels, have led to a significant rise in atmospheric CO_2 levels—from approximately 280 parts per million (mg. kg^{-1}) to 418 mg. kg^{-1} over the past 150 years. This increase has intensified the greenhouse effect, contributing to climate change and global temperature rise. CO_2 is the most dominant anthropogenic greenhouse gas, accounting for nearly 80–82% of total emissions, alongside methane, nitrous oxide, and fluorinated gases. The ongoing rise in CO_2 levels is expected to cause a global temperature increase of 3–5°C over the next century, with significant environmental consequences (Gayathri *et al.* 2021). These activities are the main source of greenhouse gas emissions, particularly CO_2, both directly and indirectly, which are increasing global temperatures.

To mitigate climate change, strategies are required to reduce CO_2 emissions and enhance C capture (Ahmed *et al.* 2019). Current approaches include energy conservation, the development of low-C fuels, and direct C sequestration methods. Among these, C sequestration—capturing and storing atmospheric CO_2 in natural or artificial reservoirs—offers a sustainable and cost-effective solution. Agro-ecosystems, particularly forests, shrub lands, grasslands and agricultural lands, have significant potential for C storage through microbial biomass carbon (MBC) and soil organic matter accumulation (Kaur *et al.* 2016). The method for the transport and subsequent accumulation of CO_2 in long term C reservoirs, which may be existing naturally or artificially set up is C sequestration. To retain and mitigate the levels of atmospheric CO_2, multiple approaches need both the biotic and abiotic components (Anjum *et al.* 2022). Despite various efforts to reduce greenhouse gas emissions, limited attention has been given to enhancing C sequestration in agro-ecosystems as a complementary strategy to offset emissions.

To cite this paper: Abrar K, T Mahmood, A Khalid, AR Saleem, MI Ashraf (2025). Soil carbon sequestration potential and microbial biomass activity in different agro-eco systems: a comparative study of agricultural, forest, shrub and grassland land uses. *Intl J Agric Biol* 34:340407. https://doi.org/10.17957/IJAB/15.2379

Globally, increasing the quantity of C accumulated in soil is one suggested strategy to lower atmospheric CO_2 levels. With C content greater than that of both the atmosphere and vegetation combined, soil is regarded as the greatest C repository. Over two-thirds of the organic C deposited in terrestrial ecosystems is found in soil organic matter (SOM). The transformation of C in soil is facilitated by both fungi and soil bacteria. Microbial activity plays a key role in soil C dynamics by regulating organic C decomposition, transformation, and storage. The microbial biomass's uptake of organic C is distributed among respiration, metabolite excretion, and the creation of new microbial cells (Ahmed *et al.* 2019). Soil C sequestration slows down the rate at which atmospheric CO_2concentrations enrich, which might lessen the consequences of climate change. The ability of different land-use systems to sequester C varies depending on soil characteristics, land management practices, and climatic conditions. Increasing soil C storage not only mitigates climate change but also improves soil health, enhances biodiversity, and supports sustainable agriculture (Teskey *et al.* 2015).

Despite growing recognition of the role of soil MBC in C sequestration, limited comparative data exist on the seasonal and depth-wise variations in MBC and associated soil physicochemical properties across multiple agro-ecosystems (forest, shrub land, grassland, and agricultural land) in semi-arid regions like the Pothwar Plateau of Pakistan. Most existing studies focus either on single land use types or lack integration of seasonal and depth-specific dynamics, leading to an incomplete understanding of how land use affects soil C storage potential. Therefore, this study aimed to evaluate C sequestration potential and microbial biomass activity across different agro-ecosystems in the Pothwar Plateau, Pakistan. By analyzing key soil properties at two depths (0–15 cm and 15–30 cm), this study helped in understanding the relationship between land use and C sequestration dynamics. Understanding these processes is essential for developing land management strategies that enhance C storage and contribute to climate change mitigation.

Materials and Methods

Site description

This study conducted in the Pothwar Plateau in Pakistan's Punjab province covers the districts of Rawalpindi and Attock during year 2020-22. This plateau lies adjacent to the Jhelum and Indus rivers, with the Salt Range to the south and the Hazara Hills to the north. The sampling sites were selected based on variation in environmental conditions and soil properties. Its diverse terrain is perpetually impacted by various types of erosion *i.e.*, wind erosion, water erosion, gravity erosion etc. The area is made up of a series of leftover hills and small mounds created from glacial deposits, which are remnants from the Ice Age. Its altitude ranges from 300 to 600 m. Rainfall is a major factor in agriculture, It is typically between 15 and 20 inches (380 and 510 mm) per year (Sarwar *et al.* 2016). Selected from each region were the various agro-ecosystems, such as woods, grasslands, shrub lands, and agricultural land.

Sampling procedure

During the months of October 2020 to April 2022, soil samples were taken from various agro-ecosystems (forests, shrubs, agricultural land, and grassland) in four districts of Punjab province: Rawalpindi and Attock to capture seasonal variability. A two-year sampling was completed. Samples were gathered using a stratified random sampling approach to obtain a representative sample of soil from a large area by dividing the area into subgroups (strata) and then randomly sampling was done within each strata. With the use of a soil auger, samples were taken in marked and polythene bags from four randomly chosen locations in each district that included shrubs, woodlands, farmland, and grassland. Based on depth, two subsamples of soil samples were separated: 0-15 cm and 15-30 cm. Homogeneity was ensured by dividing area into strata based on soil properties and by using the same sampling methods and protocols across all sites with each agro-ecosystem category. To eliminate the animal or plant waste screening was followed by sifting (less than 0.002 m). Samples of soil were broken down into smaller parts for examination after letting them to air-dry. Soil samples were stored at 4°C and sterile equipment and gloves were used to avoid contamination and consistent storage conditions were maintained.

Soil physicochemical attributes

Soil pH and electrical conductivity (EC) were measured using a 1:10 suspension of soil and distilled water, with a pH meter and an EC meter model MM 40 plus, respectively (Nautiyal *et al.* 2019). For the determination of soil bulk density (BD), the volumetric ring method was used, collecting soil cores of 100 cm^3 for the analysis (Rodriguez *et al.* 2025). For the water content, a 20 g moist soil sample was weighed and then oven-dried at 105°C for 48 h in a Gallenkamp OV-165 oven before being weighed again. The water content was calculated using the gravimetric method, which is the ratio of the mass of water to the mass of the dried soil (Spasić *et al.* 2023). Total organic carbon (TOC) was measured using the Walkley and Black method. First, one gram of air-dried soil was placed in a 50 mL beaker, and then 20 mL of concentrated H_2SO_4 was added using a dispenser. Next, a 10 mL aliquot of 1 N $K_2Cr_2O_7$ solution was added with a pipette. The mixture was swirled to combine and allowed to sit for 30 min. A Teflon magnetic stirrer bar was then added, along with 10-15 drops of phenanthrene-amine $(C_6H_5)_2NH$ indicator. The beaker was placed on a magnetic stirrer, and the mixture was titrated with a 0.5M $(NH_4)_2SO_4.FeSO_4.6H_2O$ solution until the color changed from violet-blue to green. To ensure accuracy, two blanks were prepared without the soil sample and processed in the same way (Poudel 2020). The chloroform fumigation-extraction method was used for extraction of MBC (Nautiyal *et al.* 2019). For this, 10 g of soil (fresh weight) were fumigated with chloroform in a glass desiccator for 24 h. Following fumigation, both fumigated and non-fumigated soil samples were shaken for 1 h at 200 strokes per minute with 0.5 *M* K_2SO_4, and then filtered through Whatman filter paper No. 42. The resulting extract was treated with 1 mL of 0.667 *M* $K_2Cr_2O_7$ in 5 mL of concentrated H_2SO_4, along with 2-3 drops of phenanthroline amine indicator, and titrated with a ferrous ammonium sulfate solution. A blank sample underwent the same procedure for calibration. Total nitrogen (TN) was determined using the Kjeldahl method (Li *et al.* 2018). Available phosphorus (Avb P) was quantified by the Olsen method using an ultraviolet spectrophotometer.

Statistical analysis

Three-way analysis of variance (ANOVA) was used throughout the analysis as three factors; seasons, depth and soil physical-chemical variables were compared across different soil depths under different land use systems. The experimental data were generated from three replicate tests performed independently multiple times. The least significant differences (LSD) test at a 5% probability level was used for mean value comparison. Statistix version 8.1 was employed for conducting all statistical computations.

Results

The study found that bulk density (BD) in the Rawalpindi region increased during summer (0.52 - 0.58 g/cm^3) than winter in 2020 (Fig. 1). BD ranged from 0.52 to 0.57 g/cm^3 at topsoil (0-15 cm), with maximum BD in agriculture land, followed by grass land and forest land area. Shrubs land had the lowest BD at the topsoil layer. Subsurface soil BD decreased from agriculture land to grass, shrubs, and forest land areas.

Soil pH levels vary across land uses, with a consistent pattern during summer and winter. In 2020, higher pH values were observed at depths of 15-30 cm, with grass land having the highest values (8.47) during winter, followed by shrubs and agriculture land (8.42). Forest land had the lowest pH value (8.25) during summer (Fig. 2).

The highest recorded EC values were observed in the summer and winter seasons across all land use types. The soil top layer had the highest values, with forest land having the highest value (0-15 cm). Agriculture and grass land also had the highest values. The subsurface layer showed fluctuations, with forest land having the highest values 0.81 dS/m, followed by agriculture and grass land (Fig. 3).

Soil Moisture (SM) percentage varies between 9.67 - 14.28% during summer and 9.61 - 13.40% in winter across all land use types. Surface layer soil has higher moisture percentages (Fig. 4). Forest land has the highest moisture

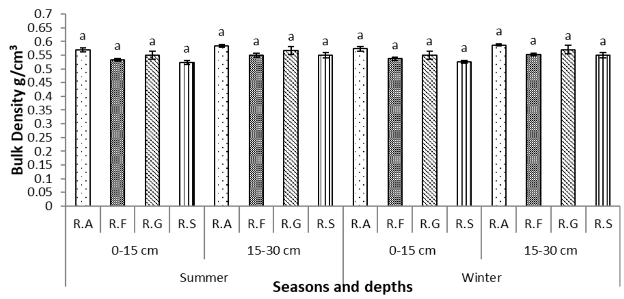

Fig. 1: Soil bulk density (g/cm^3) under different land use systems R.A (Rawalpindi agriculture land), R.F (Rawalpindi Forest Land), R.G (Rawalpindi grass land) and R.S (Rawalpindi shrub land) between 0-15 and 15-30 cm soil strata during summer and winter season from Rawalpindi region. Histogram with distinct alphabets within each category differ greatly from one another at LSD (*P < 0.05*)

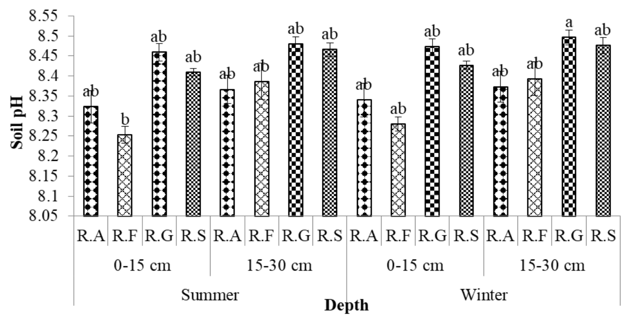

Fig. 2: Soil pH under different land use systems R.A (Rawalpindi agriculture land), R.F (Rawalpindi forest land), R.G (Rawalpindi grass land) and R.S (Rawalpindi Shrub Land) between 0-15 and15-30 cm soil strata during summer and winter season from Rawalpindi region. Histgram with distinct alphabets within each category differ greatly from one another at LSD (*P < 0.05*)

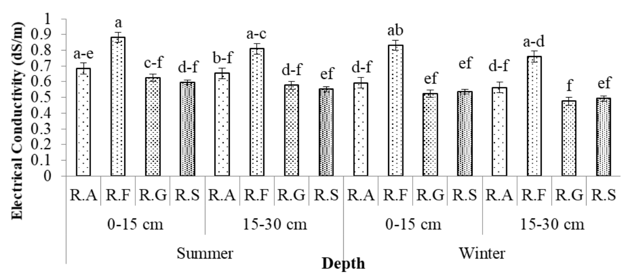

Fig. 3: Soil EC under different land use systems R.A (Rawalpindi agriculture land), R.F (Rawalpindi forest land), R.G (Rawalpindi grass land) and R.S (Rawalpindi Shrub Land) between 0-15 and 15-30 cm soil strata during summer and winter season from Rawalpindi region. Histgram with distinct alphabets within each category differ greatly from one another at LSD (*P < 0.05*)

(14.28%), followed by agriculture and shrub land (12.49%) and 10.80% in summer. Grass land has the lowest moisture (9.99%) during winter. SM percentage varies between 9.61-13.28% at sub surface layer, with higher percentages in forest, agriculture, and shrub land during summer and lowest in grass land during winter.

Total Organic Carbon (TOC) in the Rawalpindi region increased from 2.90 - 9.11 g/kg during the summer season, higher than in winter. The highest TOC values were found in forest areas (9.11 g/kg), followed by shrub and agriculture land (7.9 g/kg). The lowest TOC value was found in grass land (3.48 g/kg) during winter. Soil TOC ranged from 2.77 - 8.08 g/kg at the subsurface layer (15-30 cm) during both seasons (Fig. 5). Total Nitrogen (TN) levels varied across all land use types, with the highest values recorded in summer at depths of 0-15 cm and the lowest in winter at depths of 15-30 cm. Forest land had the highest TN values 0.78 g/kg, followed by shrub and agriculture land 0.68 g/kg. The lowest TN value was found in grass land during winter (Fig. 6).

Soil avaliable phosphorus levels were higher in summer than winter across all land use types in Rawalpindi. Forest land had the highest phosphorous value (5.20 mg/kg), followed by shrub and agriculture land areas (4.36 mg/kg). The lowest phosphorus value (2.47 mg/kg) was recorded in grass land (2.56 mg/kg) during the winter season. Significant differences were observed at 0-15 cm depth in all land areas (Fig. 7).

Microbial Biomass (MBC) values varied with seasons and soil depth, with summer having higher values (81.3 - 181.6 μg/kg) and higher than winter (74.78 - 176.18 μg/kg). The top soil layer had more MBC values than the sub-surface layer (15-30 cm). Forest land had higher MBC values, followed by shrub and agriculture land. Grass land had the lowest MBC value (89.89 μg/kg) during winter (Fig. 8).

Organic Matter (OM) was higher in summer than in winter in 2020, with the highest values in forest areas (15.70 g/kg), followed by shrubs land (13.67 g/kg) and agriculture land (10.68 g/kg). The lowest values were found in shrub land during winter. The subsurface layer values varied from 4.54 - 13.89 g/kg (Fig. 9).

In 2020, Bulk Density (BD) in the Attock region ranged from 0.53 to 0.62 g/cm^3 in summer and 0.53 to 0.63 g/cm^3 in winter across all land types. Shrubs land had lower BD, with higher BD in the sub surface layer. Maximum BD was in grass land, followed by agriculture and forest land (Fig. 10).

The TOC concentration varies among land uses, with summer having the highest concentration in forest, agriculture, and shrubs land, and lowest in grass land in winter. As depth increases, TOC decreases due to litter fall and residue removal (Fig. 11). The concentration of TN varies significantly across soil depths and seasons, with the highest concentration in summer (0-15 cm) at topsoil, followed by forest land (0.66 g/kg), agriculture, and shrubs land (0.66 g/kg) and the minimum in winter (0.66 - 0.19 g/kg) at subsurface layer (15-30 cm) (Fig. 12).

Soil pH values are alkaline, ranging from 7.63-8.52 in summer and slightly increasing in winter. Maximum values were observed at 15-30 cm depth during 2020 growing years. Forest land had the highest pH value (8.53) during winter, followed by shrubs, grass and agriculture land (Fig. 13).

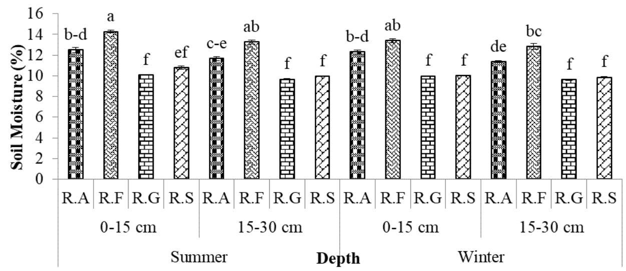

Fig. 4: Soil moisture percentage under different land use systems R.A (Rawalpindi agriculture land), R.F (Rawalpindi forest land), R.G (Rawalpindi grass land) and R.S (Rawalpindi shrub land) between 0-15 and 15-30 cm soil strata during summer and winter season (2020) from Rawalpindi region. Histgram with distinct alphabets within each category differ greatly from one another at LSD (*P < 0.05*)

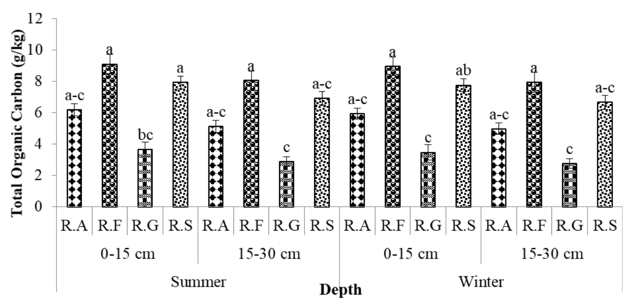

Fig. 5: Total organic carbon under different land use systems R.A (Rawalpindi agriculture land), R.F (Rawalpindi forest land), R.G (Rawalpindi grass land) and R.S (Rawalpindi Shrub land) between 0-15 and 5-30 cm soil strata during summer and winter season from Rawalpindi region. Histgram with distinct alphabets within each category differ greatly from one another at LSD (*P < 0.05*)

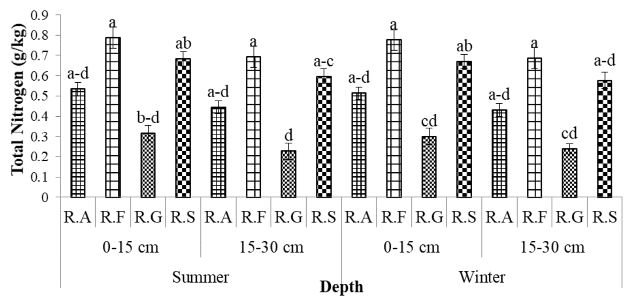

Fig. 6: Total nitrogen under different land use systems R.A (Rawalpindi Agriculture land), R.F (Rawalpindi forest land), R.G (Rawalpindi grass land) and R.S (Rawalpindi shrub land) between 0-15 and 15-30 cm soil strata during summer and winter season (2020) from Rawalpindi region. Histgram with distinct alphabets within each category differ greatly from one another at LSD (*P < 0.05*)

EC in the Attock region decreased in winter from 0.6 - 1.11 dS/m, with higher values in the surface layer (0-15 cm) than in the subsurface layer (15-30 cm). Higher conductivity was observed in shrubs, forest and grass land during summer, while lowest conductivity was found in agriculture (Fig. 14).

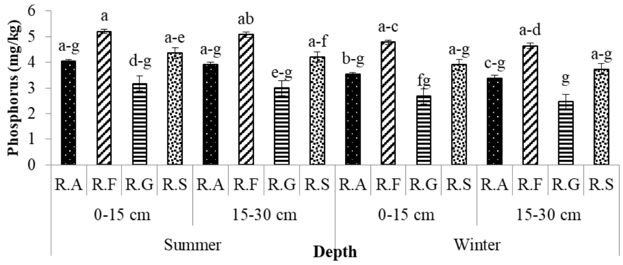

Fig. 7: Avaliable phosphours under different land use systems R.A (Rawalpindi agriculture land), R.F (Rawalpindi forest land), R.G (Rawalpindi grass land) and R.S (Rawalpindi Shrub Land) between 0-15 and 15-30 cm soil strata during summer and winter season from Rawalpindi region. Histgram with distinct alphabets within each category differ greatly from one another at LSD (*P < 0.05*)

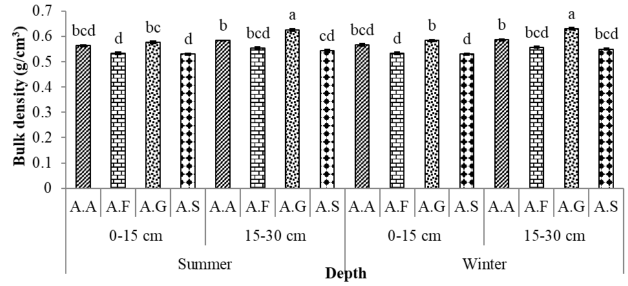

Fig. 10: Soil BD (g/cm^3) under different land use systems A.A (Attock agriculture land), A.F (Attock forest land), A.G (Attock grass land) and A.S (Attock shrub land) between 0-15 and 15-30 cm soil strata during summer and winter season from Attock region. Histgram with distinct alphabets within each category differ greatly from one another at LSD (*P < 0.05*)

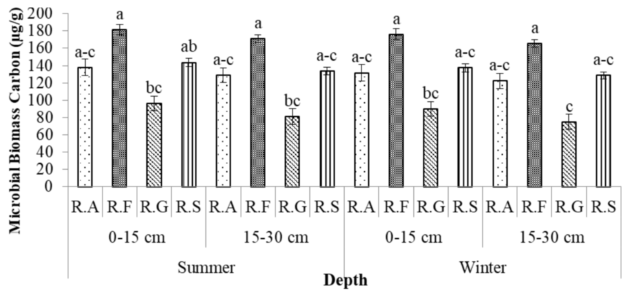

Fig. 8: Microbial biomass carbon under different land use systems R.A (Rawalpindi agriculture land), R.F (Rawalpindi forest land), R.G (Rawalpindi grass land) and R.S (Rawalpindi shrub land) between 0-15 and 15-30 cm soil strata during summer and winter season (2020) from Rawalpindi region. Histgram with distinct alphabets within each category differ greatly from one another at LSD (*P < 0.05*)

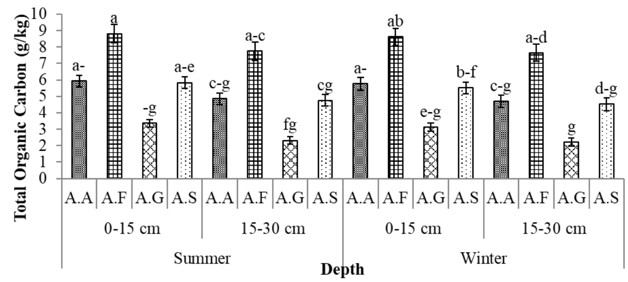

Fig. 11: Total organic carbon under different land use systems A.A (Attock agriculture land), A.F (Attock forest land), A.G (Attock grass land) and A.S (Attock Shrub Land) between 0-15 and 15-30 cm soil strata during summer and winter season from Attock region. Histgram with distinct alphabets within each category differ greatly from one another at LSD (*P < 0.05*)

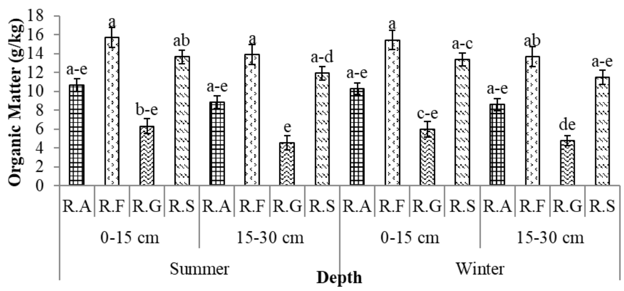

Fig. 9: Organic matter under different land use systems R.A (Rawalpindi agriculture land), R.F (Rawalpindi forest land), R.G (Rawalpindi grass land) and R.S (Rawalpindi shrub land) between 0-15 and 15-30 cm soil strata during summer and winter season from Rawalpindi region. Histgram with distinct alphabets within each category differ greatly from one another at LSD (*P < 0.05*)

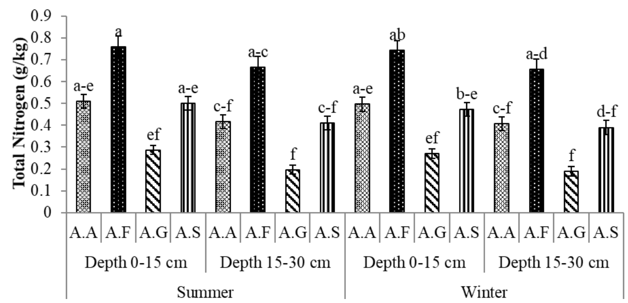

Fig. 12: Soil total nitrogen under different land use systems A.A (Attock agriculture land), A.F (Attock forest land), A.G (Attock grass land) and A.S (Attock shrub land) between 0-15 and 15-30 cm soil strata during summer and winter season (2020) from Attock region. Histgrams with distinct alphabets within each category differ greatly from one another at LSD (*P < 0.05*)

Forest land had the highest SM (13.84%) during summer, followed by agriculture (11.32%) and shrub land (10.05%). In winter, the minimum SM was found in grass land (9.71). During the winter season, shrub land had the least SM (9.50) (Fig. 15).

Soil phosphorous levels were higher during the summer season than in winter, with the highest values in top soil (0-15 cm) and subsurface layer (15-30 cm) across all land use types. Forest areas had the highest P values, followed by shrubs and agriculture land (Fig. 16).

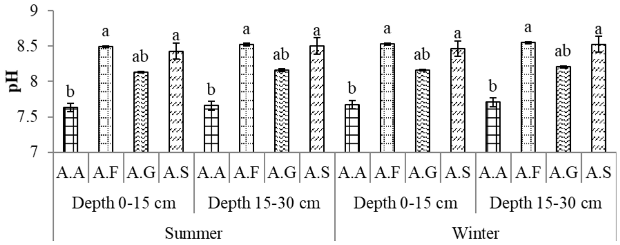

Fig. 13: pH of the soil under various land use systems A.A (Attock agriculture land), A.F (Attock forest land), A.G (Attock grass land) and A.S (Attock shrub land) between 0-15 and 15-30 cm soil strata during summer and winter season from Attock region. Histgram with distinct alphabets within each category differ greatly from one another at LSD (*P < 0.05*)

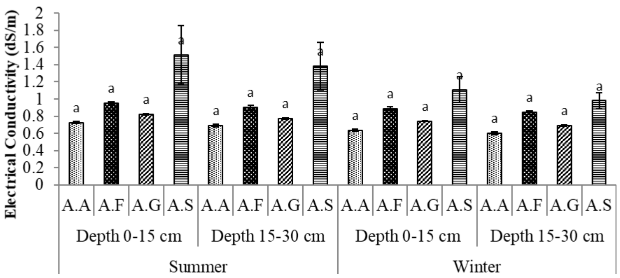

Fig. 14: EC of soil under various land utilization systems A.A (Attock agriculture land), A.F (Attock forest land), A.G (Attock grass land) and A.S (Attock shrub land) between 0-15 and 15-30 cm soil strata during summer and winter season from Attock region. Histgram with distinct alphabets within each category differ greatly from one another at LSD (*P < 0.05*)

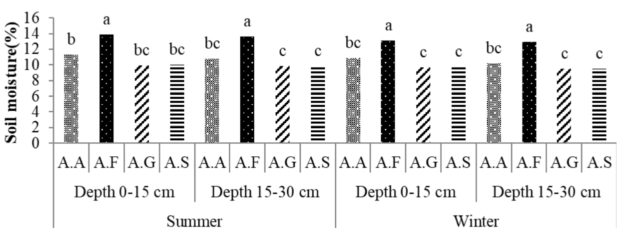

Fig. 15: Soil Moisture % under different land use systems A.A (Attock agriculture land), A.F (Attock forest land), A.G (Attock grass land) and A.S (Attock shrub land) between 0-15 and 15-30 cm soil strata during summer and winter season from Attock region. Histgram with distinct alphabets within each category differ greatly from one another at LSD (*P < 0.05*)

MBC values varied across land use types, with the highest values recorded in summer at depths of 0-15 cm and lowest in winter at depths of 15-30 cm. Forest land had the highest MBC values (166.3 μg/kg), followed by shrubs and agriculture land (122.33 μg/kg) and the lowest values were found in grass land during winter (Fig. 17).

OM properties vary significantly during summer and winter seasons. Summer has higher OM values, with forest and agriculture land areas having the highest OM contents. Winter has the lowest OM content. Soil depth and season significantly influence OM contents (Fig. 18).

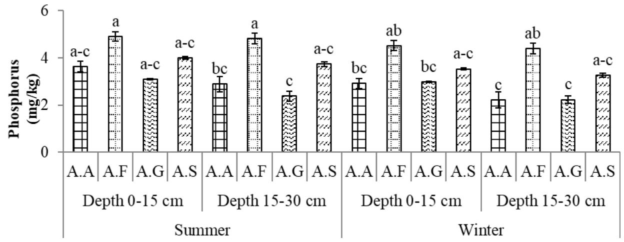

Fig. 16: Soil avaliable phosphours under different land use systems A.A (Attock agriculture land), A.F (Attock forest land), A.G (Attock grass land) and A.S (Attock Shrub Land) between 0-15 and 15-30 cm soil strata during summer and winter seasonfrom Attock region. Histgram with distinct alphabets within each category differ greatly from one another at LSD (*P < 0.05*)

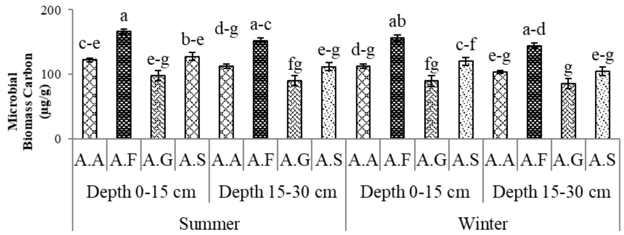

Fig. 17: Soil microbial biomass carbon under different land use systems A.A (Attock agriculture land), A.F (Attock forest land), A.G (Attock grass land) and A.S (Attock shrub land) between 0-15 and 15-30 cm soil strata during summer and winter season from Attock region. Histgram with distinct alphabets within each category differ greatly from one another at LSD (*P < 0.05*)

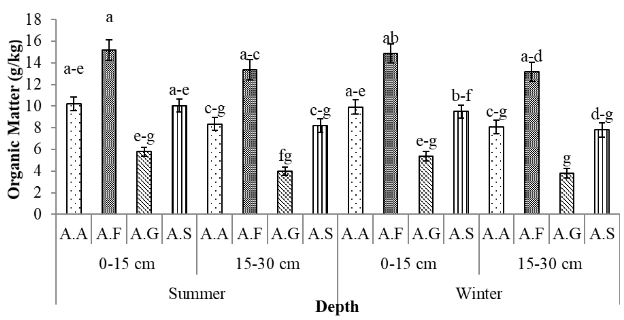

Fig. 18: Soil organic matter under different land use systems A.A (Attock agriculture land), A.F (Attock forest land), A.G (Attock grass land) and A.S (Attock shrub land) between 0-15 and 15-30 cm soil strata during summer and winter season from Attock region. Histgram with distinct alphabets within each category differ greatly from one another at LSD (*P < 0.05*)

Discussion

MBC influences nutrient cycling by decomposing organic matter, releasing nutrients available to plants and can also immobilize nutrients by making them less available to plants. MBC can influence soil resilience to climate stress by improving soil structure, increasing its water holding capacity and reducing erosion. Higher MBC can help soil tolerate drought by increasing water retention and reducing evaporation. MBC is an indicator for soil health as micro-organism decomposes organic matter influencing soil C storage and contributes to improved soil fertility and structure (Saad *et al.* 2020). MBC was used to evaluate C sequestration potential of different land use systems (grass land, agricultural land, shrub land and forest land). The distinctive nutrient changes occurring in the soil ecosystem have been significantly impacted by global climate change. Land usage has a significant impact on how much C is stored in the soil. The observed pattern of high MBC and C storage in forest soil can be attributed to a combination of factors including organic matter input, soil; structure and aggregation, microbial community composition and plant-microbe interactions. These factors interact and influence one another, creating complex mechanisms that promote C storage in forest (Cortijos *et al.* 2024). Grasslands exhibited higher BD than agricultural and forest lands, with variations across soil depths but no significant differences between seasons or years. This was likely due to factors such as poor aggregation, increased sand content, overgrazing, and organic matter loss (Chauhan *et al.* 2019). Worku and Bedadi (2016) also found that higher TOC improves porosity but reduces BD, while deeper soils tend to have higher BD due to lower organic matter and aggregate stability, leading to compaction. The BD was lower in shrub lands by the constant addition of organic matter intake from stem litter and leaf. Some of the essential factors such as productivity in agriculture, nutrient content and fundamental soil health are included in quality of soil (Smith and Doran 1997). Availability of nutrients and microbial diversity are influenced by pH of the soil (Saad *et al.* 2020). The study found no significant pH variations across agro-ecosystems, seasons, soil depth, or years, but forests and shrub lands had higher pH levels due to increased ion solubility and organic matter content (Kidanemariam *et al.* 2012; Cortijos *et al.* 2024). Poor management practices, erosion as well as the use of ammonium-based fertilizers, are some of the factors that can lead to degradation of soil that is why, agricultural soils have decreased pH levels (Chandel *et al.* 2018; Kaushik *et al.* 2018). Due to leaching downwards of salts and bases, pH of the soil showed an increasing trend the increase in soil depth in all the systems that were evaluated (Mandal *et al.* 2018; Fayyaz *et al.* 2025a).

No apparent differences were observed in soil EC of various agro-ecosystems as well as depths of the soil, seasons and years. The largest EC was shown by shrub land as a result of deposition of salt along with the enhanced evaporation Dhaliwal *et al.* (2020), while reduction in nutrient ions along with depth lowered EC (Kaur and Bhat 2017). Soil moisture trends vary across depths and seasons, with forested areas showing higher value due to organic matter accumulation. Top-of-soil variation affects nutrient availability, soil structure, and plant production.

The outcome is in accordance with the previous studies indicating that leaf litter, biomass of the root and residues of plant influence the level of organic C in the soil surface (Cao *et al.* 2021). Reduced TOC at deeper depths in core is explained by the decreased organic inputs and slower rate of C turnover on account of reduced microbial activity (Shahid *et al.* 2022). The higher levels of TOC levels were observed in the summer season as compared to winter likely due to seasonal variations. The reason behind this is probably due to low rate of microbial degradation in the winter (Yang *et al.* 2023) while, stabilization of organic matter is enhanced by increased moisture retention (Trevathan *et al.* 2024). Grasses degraded organic matter fast and received fewer organic inputs which resulted in less organic inputs, therefore, grasslands showed lower levels of TOC (Panettieri *et al.* 2017). These results demonstrate that such seasonal variations, depth of soil and land usage are crucial in determining TOC levels. It also proposed the most efficient approaches to reduce soil organic C and also overall health of soil (Sarkar *et al.* 2024).

SOM is crucial for soil health, varying among agroecosystems, seasons, and soil depths. Forest soils have high SOM levels due to constant plant litter storage (Prescott and Vesterdal 2021; Panchal *et al.* 2022). Higher moisture levels further support plant growth and organic matter production (Paul *et al.* 2022), reinforcing the importance of managing soil conditions for ecosystem resilience.

The maximum amount of organic matter in the subsoil across different habitats was found as a result of constant inflow of nutrients and degradation of plant (Knebl *et al.* 2017). Higher temperatures are capable of nurturing the activity of microbes to strengthen soil organic matter, decomposition rates and so on. But too high temperatures could also threaten the stability of that organic matter (Takoutsing *et al.* 2018; Antony *et al.* 2022). These results are in accordance with the earlier studies (Tautges *et al.* 2019; Suleymanov *et al.* 2023; Garrett *et al*. 2024). Due to faster decay of grasses and lower contribution to OM as compared to trees, grasslands showed lower levels of organic matter (Subhatu *et al.* 2017). Similarly, deeper soil layers contained less organic matter due to lower organic inputs and nutrient loss (Kahsay *et al.* 2024).

The highest values of TN were found in the upper layers of the soil in all the districts during the summer season. The enzyme that causes this is because of elevated nitrogen mineralization, a greater content of organic matter and stable surroundings that does not result in loss of TN by volatilization (Wang *et al.* 2016; Fayyaz *et al.* 2025b). These findings are in consistent with Assefa *et al.* (2020) who observed that addition of plant waste and erosion of soil which allocated nutrients affected storage of TN in forests. TN levels varied based on soil organic matter across land uses (Kidanemariam *et al.* 2012), with grasslands showing lower TN due to decreased organic inputs, surface runoff, and nitrate seepage (Bizuhoraho *et al.* 2018). Additionally, high summer rainfall contributed to nitrogen leaching and reduced organic input (Jemal and Tesfaye 2020).

The Avb P is not influenced by seasons or soil depth but increases during summer due to organic phosphorus in forested areas (Liu *et al.* 2018). Organic matter breaks down contributed to the higher levels of Avb P in the topsoil among all agro-ecosystems (Uthappa *et al.* 2015; Mandal *et al.* 2018). The decreased amount of Avb P levels was found in grassland, likely due to the immobilization and fixation brought about by lower pH.

Forest soil, however, showed highest levels of MBC when compared to that of grassland, shrub land and agricultural land. The powerful rhizosphere effect was rooted exudates, properties of soil and a unique rhizoflora (Raiesi and Salek-Gilani 2020). Deep root systems and production of litter in forests also promoted the increased microbial activity (Zhu *et al.* 2022; Zhou *et al.* 2024). MBC was affected by fluctuations in season, with the greater counts in summer as a result of changes in temperature and precipitation, in accordance with Bowd *et al.* (2022). As a result of limited availability of resources, lower organic matter content and properties of soil, the lowest MBC levels were observed in the grassland (Leeuwen *et al.* 2017; Chen *et al.* 2021).

To overcome deforestation and sustainable agriculture, deforested forests should be restored to maximize C sequestration and MBC benefits. Tree species that are well suited to the local climate and soil conditions should be chosen to ensure successful reforestation effort. Agro-forestation practices should be integrated to promote eco-system services including C sequestration and MBC. Furthermore, conservation agriculture practices such as reduced cover cropping should be implemented to promote soil health and reduce erosion.

Long-term monitoring studies should be conducted to track changes in MBC soil C sequestration and eco-system services on time. Long term stability of microbial derived C interaction between soil microbes and C sequestration should be studied. Also, studies should be conducted to investigate the mechanism of C sequestration including the composition and structure of microbial communities in different eco-systems.

The limitation of this research is that it didn’t cover soil texture, land-use history and vegetation cover which could give bias results.

Conclusion

The study explores the impact of agroecosystems on soil C sequestration and microbial biomass C turnover in Pakistan's Pothwar Plateau. Forest ecosystems sequestered the most C, followed by agroforestry and pastures. Variations in microbial biomass C and soil properties were found across agro-ecosystems, season, and soil depths. Summer MBC values suggest higher C sequestration capacity on forest lands. The research underscores the importance of targeted land management strategies for soil C sequestration, climate change mitigation, and ecosystem resilience.

Acknowledgements

Authors are thankful to all who have contributed in research, data analysis, write-up and publication of this manuscript.

Author Contributions

Kiran Abrar designed and planned the research, performed statistical analyses, interpreted the results, and prepared the manuscript. Tariq Mahmood supervised the entire study and provided overall guidance. Azeem Khalid assisted in research planning and methodology development and reviewing manuscripts. Aaynsa Rukya contributed to the interpretation of results. Muhammad Irfan Ashraf carried out proofreading to ensure accuracy and clarity.

Conflict of Interest

The authors assert that they have no conflicts of interest to declare.

Data Availability

The data can be obtained from the corresponding author upon a reasonable request.

Ethics Approval

Not applicable to this paper.

Funding Source

No funding was received for this study.

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