

1 **Revised Version (Clean version)**

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3 **Soil organic carbon fractions, carbon stocks and microbial biomass carbon in**
4 **different agroforestry systems of the Indo-Gangetic Plains in Bihar (India)**

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13
14 **Abstract**

15 An investigation was undertaken in the Vaishali district (Bihar) during 2020 to assess the
16 effect of different agroforestry systems (AFS) on distribution of different pools of soil organic
17 carbon (fraction I-very labile, fraction II-labile, fraction III-less labile and fraction IV-non-labile
18 pools), carbon stocking and soil microbial activity. The mean (0-45 cm) total organic carbon
19 (TOC) in different AFS ranged from 5.55 to 6.64 Mg C ha⁻¹ with the highest under poplar based-
20 AFS (PB-AFS). Across the AFS studied, the C stocks (0-45 cm) varied from 36.24 (Mango
21 based-AFS) to 41.43 Mg C ha⁻¹ (PB-AFS). Overall, the magnitude of C fractions showed an
22 order of fraction I > fraction IV > fraction III > fraction II. Significantly higher soil microbial

23 biomass carbon (SMBC) was recorded under PB-AFS (219.36 $\mu\text{g g}^{-1}$) in 0-15 cm depth.
24 Similarly, basal respiration was also the highest under PB-AFS (0.54 $\mu\text{g CO}_2\text{-C g}^{-1} \text{hr}^{-1}$) followed
25 by TB-AFS (0.50 $\mu\text{g CO}_2\text{-C g}^{-1} \text{hr}^{-1}$) in 0-15 cm depth. Principal component analysis (PCA)
26 result showed that PC 1 & PC 2 represented about 97 % of the total variation. TOC and active
27 carbon pool have the maximum loading in PC 1 while microbial metabolic quotient ($q\text{CO}_2$) and
28 bulk density represent the maximum value in PC 2.

29 **Keywords:** Carbon stock; carbon fractions; carbon management index; poplar-based
30 agroforestry system

31

32 **Introduction**

33 The protection and enhancement of soil organic matter content is an essential element in
34 achieving sustainable agricultural production. However, continued intensive cultivation has led
35 to its depletion leading to a decline in soil fertility, crop productivity and increased atmospheric
36 CO_2 concentration¹. The combination of labile and recalcitrant fractions of carbon in the soil
37 denotes the total organic carbon (TOC) of soil. However, the measurement of TOC alone does
38 not truly reflect a clear picture of carbon dynamics in any cropping system, since the status of
39 labile fraction (LF) can be easily affected by land management practices while the recalcitrant
40 fraction (RF) in the soil cannot be changed easily due to alteration in land use practices². Soil
41 microbial biomass carbon (MBC) influences major nutrient cycling patterns in the ecosystem
42 vis-à-vis cycling of SOM³. In the light of the present climate change scenarios, there is a need for
43 identifying efficient and sustainable land use systems that could lead to long term storage and
44 sequestration of carbon.

45 On this contention, agroforestry, a practice of cultivating trees along with agricultural
46 crops or animal husbandry, is considered as one of the viable land use systems while delivering
47 the services of crop diversification, natural conservation as well as sequestering the atmospheric
48 carbon. By considering the importance of agroforestry practices in terms of carbon sequestration,
49 Intergovernmental Panel on Climate Change (IPCC) and other prominent organizations viz.,
50 Consultative Group on International Agricultural Research (CGIAR) have recognized the
51 services of agroforestry and incorporated this land use practice in many of their programmes.
52 Although preliminary studies on dry matter dynamics of poplar-based agroforestry system in
53 Indo-gangetic plains of Bihar (Samastipur) have been studied⁴, information regarding soil
54 organic fractions and carbon stocks across different agroforestry systems (AFS) of Bihar is
55 limited. Therefore, the present investigation was undertaken to evaluate the effect of different
56 AFS on distribution of different soil organic carbon fractions, carbon stocking and soil microbial
57 activity in different AFS.

58 **Materials and methods**

59 *Selection of agroforestry system*

60 Selection and identification of agroforestry system was carried out following a
61 preliminary reconnaissance survey in Vaishali district (Bihar) and these were classified
62 according to Nair⁵. In general, two types of AFS viz., agri-silvicultural and agri-horticultural are
63 widely practiced by farmers of this region (25° 41' to 25° 68' N latitude and 85° 13' to 85° 22' E
64 longitude). Teak (*Tectona grandis*) + agricultural crops, Poplar (*Populus spp* + agricultural crops
65 and Sissoo (*Dalbergia sissoo*) + agricultural crops represented agri-silvicultural system while
66 Mango (*Mangifera indica*) + agricultural crops denoted the agri-horticultural system (Table 1).
67 Under the Teak based agroforestry system (TB-AFS), farmers of this region generally grow

68 mustard (*Brassica spp*) and rice (*Oryza sativa*). Wheat (*Triticum aestivum*) and rice are
69 commonly grown as intercrop in poplar-based agroforestry system (PB-AFS). Similarly, under
70 the Sissoo based agroforestry system (SB-AFS) farmers favor the cultivation of mustard, potato
71 (*Solanum tuberosum*), maize (*Zea mays*) etc, while mustard, moong (*Phaseolus vulgaris*), and
72 potato are commonly cultivated under mango-based agroforestry system (MB-AFS). Vaishali
73 district of Bihar is a part of Indo-Gangetic plains where the average rainfall recorded is about
74 1400 mm. The study area belongs to tropical region having the characteristics of hot summers
75 from April to June followed by a brief autumn season and a mild, dry winter. Soil texture of this
76 region is mainly sandy, loam, and light clayey in nature.

77 ***Soil sampling and analysis***

78 During January 2020, ten soil samples were taken from each AFS at three depths (0-15,
79 15-30, and 30-45 cm) using power augers. After that, composite soil samples were prepared by
80 combining soil samples from each agroforestry system and transported to the laboratory in
81 polyethylene bags and stored at 4°C until analysis. Soil bulk density was measured by core
82 method⁶. The sub-samples of air-dried soil were used for analyzing the total organic carbon
83 (TOC) and different fractions of carbon (very liable, liable, less liable and non-liable). TOC was
84 analyzed following Haenes⁷. Different fractions of carbon were also analyzed as per method
85 suggested by Chan *et al.*⁸

86 Soil OC stock (t ha⁻¹) at different soil depths in different agroforestry systems were calculated
87 using the formula:

$$88 \text{ SOC stock (Mg ha}^{-1}\text{)} = \text{SOC (g kg}^{-1}\text{)} \times \text{bulk density (Mg m}^{-3}\text{)} \times \text{soil depth (m)} \times 10$$

89 Active pool of organic carbon was calculated by summing up fraction I and II together, while,
90 passive pool was derived by adding fractions III and IV together. Soil microbial biomass carbon

91 (MBC) was calculated using the method suggested by Nunan *et al.*⁹ with certain alteration
92 according with Parihara *et al.*¹⁰ Carbon dioxide (CO₂) evolution in terms of basal respiration was
93 calculated following the alkali absorption method¹¹. Microbial quotient (MQ) and microbial
94 metabolic quotient (qCO₂) were also calculated adopting the equation proposed by Anderson and
95 Domsch¹².

96 ***Carbon Management Index (CMI)***

97 The CMI was calculated by applying the formula proposed by Blair *et al.*¹³ -

$$98 \text{CMI} = \text{Carbon Pool Index (CPI)} \times \text{Liability Index (LI)} \times 100$$

99 Where CPI is calculated as

$$100 \text{CPI} = \frac{\text{Sample total organic carbon}}{\text{Reference total organic carbon}}$$

101 LI was calculated as

102

$$103 \text{LI} = \frac{\text{Fraction I}}{\text{Total organic carbon}} \times 3 + \frac{\text{Fraction II}}{\text{Total organic carbon}} \times 2 + \frac{\text{Fraction III}}{\text{Total organic carbon}} \times 1$$

104 ***Statistical analysis***

105 One way analysis of variance (ANOVA) was carried out to elucidate the effect of
106 different agroforestry systems and soil depths on different soil parameters. Tukey test was used
107 for multiple comparisons among the treatments at $p < 0.05$ using Indian NARS Statistical
108 Computing Portal <http://stat.iasri.res.in/sscnarsportal>. Principal component analysis biplot was
109 prepared using open software R.

110 **Results and Discussions**

111 ***Total organic carbon (TOC)***

112 TOC content was significantly ($p \leq 0.05$) influenced by both AFS and soil depths (Table
113 2). On an average, the TOC in different AFS ranged from 5.55 to 6.64 Mg C ha⁻¹. Amongst the
114 AFS, the top soil (0-15 cm) in PB-AFS had the highest (9.06 Mg C ha⁻¹) TOC followed by TB-
115 AFS (8.43 Mg C ha⁻¹), while, the MB-AFS recorded the lowest (7.44 Mg C ha⁻¹) TOC content
116 in soil. A variation in TOC under different AFS was strongly linked with the species
117 characteristics and their litter input patterns¹⁴. Carbon dynamics in any system can also be related
118 with adoption of different management practices along with their past cropping pattern¹⁵. Das
119 and Chaturvedi⁴ in Samastipur (Bihar) had reported that the annual litterfall accumulation by
120 poplar trees under poplar + wheat-based agroforestry system ranged from 2.46 (3 years) to 10.63
121 (9 years) Mg ha⁻¹yr⁻¹. Contrarily, Rathore *et al.*¹⁶ reported that 10 years old agri-horticultural
122 system (mango+cowpea-toria) accumulated around 1.46 t ha⁻¹ of dry leaves biomass on the floor.
123 This amount of litter biomass is far less than the litterfall added by poplar (2.46-10.63 Mg ha⁻¹yr⁻¹)
124 as earlier reported by Das and Chaturvedi⁴. Irrespective of AFS, the TOC content in the
125 surface soil (0-15 cm) was significantly higher than bottom layers (15-45 cm); registering a
126 decrease of ~ 28% and ~ 50% in 15-30 cm and 30-45 cm soil layers, respectively. This might
127 due to higher input of litter and dry biomass in surface soil as compared to the sub-surface
128 layer¹⁵.

129 ***Total organic carbon stock in soil (Mg C ha⁻¹)***

130 The TOC stocks varied from 12.08 to 13.81 Mg C ha⁻¹ across the different AFS. In 0-15
131 cm soil profile, PB-AFS contained the highest (18.18 Mg C ha⁻¹) TOC stocks followed by TB-
132 AFS based AFS (17.27 Mg/ha), while MB-AFS recorded the minimum (15.74 Mg C ha⁻¹) TOC
133 stock. The significant variation in TOC stocks across the different AFS is correlated with TOC
134 accumulation in the soil. Singh *et al.*¹⁷ have reported that spatial and temporal admixture of

135 various components in different AFS and their resulting *in-situ* interface with the abiotic
136 components favored carbon storage. Significantly higher TOCS content was recorded in upper
137 layer than lower layer of soil. On an average, the surface layer (0-15 cm) contained 35 % higher
138 carbon stocks than the lower layers (15-45 cm) of soil. The rhizo-deposition effects caused by
139 varied nature of different tree species have also significant influence on the distribution of SOC
140 across the soil depth¹⁸. The mean stratification ratio (SR) (ratio of C stocks in 0-15: C stocks in
141 15-30 cm) as proposed by Franzluebbers¹⁹, in our study sites of different AFS was 1.33, which is
142 comparable with the finding of Ramesh *et al.*¹⁴

143 ***Distribution of different carbon fractions***

144 Significant variations in different carbon fractions were observed across the different
145 AFS throughout the depth (0-45 cm) of the soil profile (Table 2). Our results revealed that the
146 very liable carbon fraction (FI) represented the highest proportion followed by recalcitrant
147 fraction (F IV) in all the AFS. The pattern of distribution of different carbon fraction (different
148 oxidability level) determines the permanency of SOC status of soil and this characteristic is
149 largely influenced by adoption of several management activities²⁰. The soil of PB-AFS had
150 consistently contained the highest carbon fractions of different oxidasibility than other
151 counterparts. On the other hand, the MB-AFS had the lowest carbon fractions of different
152 oxidisability. MBS-AFS and SB-AFS had recorded the minimum C pools and this could be
153 partly linked with the intercrops grown under these systems; maize and potato, which are
154 nutrient exhaustive crops, leading to more consumption of organic carbon from soil²¹. In this
155 study, the order of allocating different carbon fraction (fraction I > fraction IV > fraction III >
156 fraction II) was similar across AFS. Our result is consistent with the finding of Benbi *et al.*²²
157 where liable fraction represented the most dominating (56-60%) under PB-AFS in Punjab.

158 The percentage wise distribution of different carbon fractions indicated that labile form
159 (fraction I and II) represented 51.50. The active carbon pool and passive carbon pool also
160 showed significant difference due to variation in distribution of different forms of carbon
161 fractions across the AFS (Figure 1). The continuous addition of leaf litter and dry matter in
162 agroforestry floor enable to build up more labile carbon fraction across the different AFS and
163 thus, necessitating the continuation of the current land use system in order to protect soil organic
164 matter²³. Moreover, the proper management of systems by farmers through incorporation of an
165 adequate amount of farm yard manure as nutrient supplement for crop growth and development
166 will also have a significant result on formation of very labile carbon fraction in the soil²⁴.

167 ***Soil microbial biomass carbon (SMBC) and Basal respiration (BR)***

168 Overall, the SMBC in different AFS ranged from 103.08 and 131.65 $\mu\text{g g}^{-1}$ (Table 2).
169 Significantly higher value of SMBC was recorded in the surface layer than sub-surface layers in
170 all the AFS and which can be attributed to higher availability of substrate and easily
171 hydrolysable carbon in the upper soil layer²⁵. In the 0-15 cm depth, significantly the highest
172 SMBC was recorded in PB-AFS (219.36 $\mu\text{g g}^{-1}$) followed by TB-AFS (192.70 $\mu\text{g g}^{-1}$) while, the
173 SMB-AFS had the lowest (161.23 $\mu\text{g g}^{-1}$) SMBC. Similar trends were also observed in both the
174 sub-surface layers. Since, the TOC content in the soil was also the highest in the PB-AFS and
175 this is the indication that the level of TOC content in the soil was highly correlated with
176 SMBC²⁶. The overall result on basal respiration across the different AFS shows that TB-AFS
177 (0.41 $\mu\text{g CO}_2\text{-C g}^{-1} \text{hr}^{-1}$) registered the maximum BR but which is statistically at par with PB-
178 AFS (0.40 $\mu\text{g CO}_2\text{-C g}^{-1} \text{hr}^{-1}$). However, in the upper soil depth (0-15 cm), PB-AFS recorded the
179 highest (0.54 $\mu\text{g CO}_2\text{-C g}^{-1} \text{hr}^{-1}$) BR followed by TB-AFS (0.50 $\mu\text{g CO}_2\text{-C g}^{-1} \text{hr}^{-1}$) and the
180 minimum under MB-AFS (0.34 $\mu\text{g CO}_2\text{-C g}^{-1} \text{hr}^{-1}$). This is the indication of higher biomass in

181 PB-AFS and TB-AFS and followed by high mineralization of litter (includes dry biomass) and
182 root biomass and led to production of large CO₂ flux rates. Surface soil will have more CO₂
183 evolution rate than sub-surface soil due to higher inputs of litter and plant residues followed by
184 better mineralization process²⁷.

185 Microbial quotient (MQ) was the highest under PB-AFS while TB-AFS recorded the
186 highest microbial metabolic quotient (qCO₂) (Figure 2 and Figure 3). The microbial quotient
187 (MQ) value of different AFS varied from 1.67 to 1.87 % and our results are comparable with the
188 finding of Ramesh *et al.*¹⁴ The maximum MQ under PB-AFS indicating higher steadiness of
189 organic matter as MQ is generally related with the availability of C source for microbial
190 activities. In this study, PB-AFS showed the lowest qCO₂ indicating better substrate utilization
191 efficiency than other counterparts. The higher qCO₂ under TB-AFS and SB-AFS might be
192 associated with the higher demand and their consumption of SOC. Bastida *et al.*²⁸ had also
193 stressed that the disturbance caused by several factors primarily through intervention of different
194 cultural practices is likely to affect the abundance of organic C in the system which is also
195 responsible for producing varied qCO₂ under different land use systems.

196 ***Carbon Management Index (CMI)***

197 The calculated CMI of different AFS (Figure 4) indicated that it ranged from 201.54 to
198 186.47 in 0-15 cm soil depth. Significantly higher CMI value was attained by PB-AFS but was
199 statistically at par with TB-AFS and SB-AFS, while the least CMI was recorded under MB-AFS.
200 PB-AFS achieved the maximum CMI value than the rest of AFS, indicating a large potential to
201 have more C-stock. The dissimilarity in CMI value across the AFS was mainly attributed to the
202 level of litter accumulation as well as root decomposition activities exhibited by different tree
203 species, however, the effect of management practices also cannot be ruled out. The continuous

204 addition of annual litterfall helps in building the SOC under agroforestry systems as compared to
205 treeless farming and this is the indication of CMI value more than 100 in the present study.
206 Higher value (>100) of CMI in agroforestry practices as compared with treeless farming has
207 been reported by several workers^{29, 30}.

208 ***Principal Component Analysis (PCA)***

209 The principal component analysis result revealed that the first two PC 1 and PC 2
210 represented 91.5% and 2 5.5%, respectively of the total variation (Figure 5). In PC 1 TOC
211 followed by active carbon pool were found to be the most sensitive factor while microbial
212 metabolic quotient (qCO₂) and BD influence the most in PC 2. Across the soil depth, it was
213 found that all the studied soil parameters except qCO₂ and BD were found higher concentration
214 in the surface layer (0-15 cm). So, all soil variables are positively correlation between each other
215 except qCO₂ and BD as these two variables are negatively correlated with the remaining soil
216 variables. And qCO₂ and BD were found to be the most influential factor in the soil group of 30-
217 45 cm. In the present study, TOC was the most influential factor in PC1 as well in soil group of
218 0-15cm. TOC is heterogeneous, containing both liable and recalcitrant carbon forms. As a result,
219 changes in different fractions of organic carbon will have an impact on the total amount of TOC
220 in the soil. And more precisely, liable form of carbon can be easily influenced by changes in land
221 management practices³¹. The greater qCO₂ values in the subsurface layers indicated that the
222 microorganisms' substrate utilization efficiency decreases as soil depth increases.

223

224 **Conclusion**

225 Poplar based AFS had contained higher concentrations and greater amount of C as
226 compared to other counterparts. The choice of intercrops (mustard, rice, wheat, potato, maize,

227 moong, etc) and perennial components as well as management activities in different AFS largely
228 depend on farmer's interest. This has led to the variation in the distribution and stability of soil
229 organic carbon and its various forms in different AFS. Subsequently, this also has shown
230 significant impact on the microbial activity (biomass and basal respiration) and thus has
231 profound effect on the overall nutrient flux and dynamics of the system. Our study concludes that
232 tree-based farming system, especially poplar based AFS could be considered as one of the viable
233 options not only for carbon mitigation but also enhancing the profitability of the farmers
234 belonging to Indo-Gangetic plains of Bihar.

235 **Acknowledgement**

236 The authors are thankful to Dr. B.P. Bhatt, Director, ICAR Research Complex for Eastern
237 Region, Bihar, India for providing laboratory facilities. We are also grateful to Indian Council of
238 Agricultural Research for funding the research work. Thanks, are also due to farmers for sharing
239 their agricultural knowledge.

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338 Table 1: Details of different agroforestry systems investigated

| Agroforestry system (AFS) | Mean DBH* (cm) | Mean height (m) | Spacing (m) | Age of tree (years) |
|------------------------------|-------------------|--------------------|---------------------------------|---------------------|
| Teak based | 12.64 | 7.80 | 4m ×4m (boundary plantation) | 8-10 |
| Poplar based | 24.96 | 18.15 | 5m ×5m (boundary plantation) | 5-6 |
| Sissoo based | 6.74 | 3.15 | 4m ×4m | 5-6 |
| Mango based | 6.02 | 4.55 | 8m ×8m | 5-6 |

339 *DBH=diameter at breast height (1.3m)

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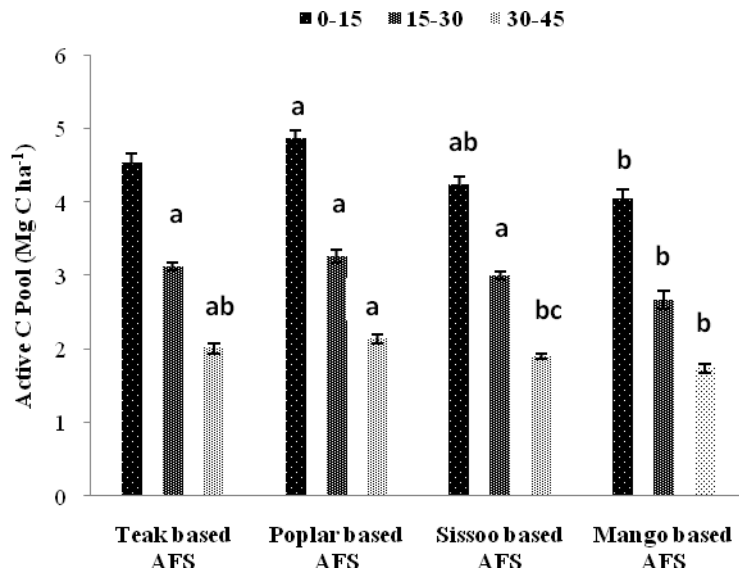
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350 Table 2: Distribution of soil total organic carbon (TOC) and TOC stocks (Mg C ha^{-1}), carbon fractions
 351 (Mg C ha^{-1}), SMBC ($\mu\text{g g}^{-1}$) and basal respiration ($\mu\text{g CO}_2\text{-C g}^{-1} \text{ hr}^{-1}$) at three depths in different
 352 agroforestry systems

| Soil depth (cm) | TOC | C Stock | F I (very labile carbon) | F II (labile carbon) | F III (less labile carbon) | F IV (non labile carbon) | Soil microbial biomass carbon | Basal respiration |
|-----------------|-------------------------|--------------------------|--------------------------|-------------------------|----------------------------|--------------------------|-------------------------------|-------------------------|
| TB-AFS | | | | | | | | |
| 0-15 | 8.43 ^a | 17.27 ^a | 3.13 ^a | 1.40 ^a | 1.44 ^a | 2.46 ^a | 192.70 ^a | 0.50 ^a |
| 15-30 | 6.06 ^b | 13.11 ^b | 2.05 ^b | 1.07 ^a | 1.13 ^b | 1.81 ^b | 111.64 ^b | 0.40 ^b |
| 30-45 | 4.29 ^c | 9.69 ^c | 1.20 ^c | 0.81 ^b | 1.01 ^c | 1.27 ^c | 49.65 ^c | 0.33 ^c |
| Mean | 6.26^B | 13.36^A | 2.13^A | 1.09^B | 1.19^A | 1.85^B | 118.00^B | 0.41^A |
| PB-AFS | | | | | | | | |
| 0-15 | 9.06 ^a | 18.18 ^a | 3.28 ^a | 1.59 ^a | 1.38 ^a | 2.81 ^a | 219.36 ^a | 0.54 ^a |
| 15-30 | 6.51 ^b | 13.71 ^b | 2.04 ^b | 1.22 ^b | 1.08 ^b | 2.17 ^b | 112.26 ^b | 0.47 ^b |
| 30-45 | 4.34 ^c | 9.54 ^c | 1.35 ^c | 0.79 ^c | 0.93 ^c | 1.27 ^c | 63.32 ^c | 0.18 ^c |
| Mean | 6.64^A | 13.81^A | 2.22^A | 1.20^A | 1.13^{AB} | 2.08^A | 131.65^A | 0.40^A |
| SB-AFS | | | | | | | | |
| 0-15 | 7.85 ^a | 16.21 ^a | 2.81 ^a | 1.43 ^a | 1.39 ^a | 2.22 ^a | 170.62 ^a | 0.45 ^a |
| 15-30 | 5.68 ^b | 12.20 ^b | 1.71 ^b | 1.29 ^a | 1.14 ^b | 1.54 ^b | 94.91 ^b | 0.41 ^a |
| 30-45 | 4.01 ^c | 8.87 ^c | 1.11 ^c | 0.79 ^b | 0.83 ^c | 1.28 ^c | 46.06 ^c | 0.25 ^b |
| Mean | 5.85^C | 12.43^B | 1.88^B | 1.17^A | 1.12^{BC} | 1.68^C | 103.87^C | 0.37^B |
| MB-AFS | | | | | | | | |
| 0-15 | 7.44 ^a | 15.74 ^a | 2.92 ^a | 1.13 ^a | 1.25 ^a | 2.14 ^a | 161.23 ^a | 0.34 ^a |
| 15-30 | 5.40 ^b | 11.93 ^b | 1.64 ^b | 1.03 ^a | 1.08 ^b | 1.65 ^b | 101.46 ^b | 0.34 ^a |
| 30-45 | 3.81 ^c | 8.57 ^c | 1.03 ^c | 0.71 ^b | 0.84 ^c | 1.23 ^c | 46.55 ^c | 0.23 ^b |
| Mean | 5.55^C | 12.08^B | 1.86^B | 0.96^C | 1.06^C | 1.67^C | 103.08^C | 0.30^C |

353 Different small letters denote significant difference ($p \leq 0.05$) among the soil depths within the agroforestry system.
 354 Different capital letters denote significant difference ($p \leq 0.05$) between the agroforestry systems.

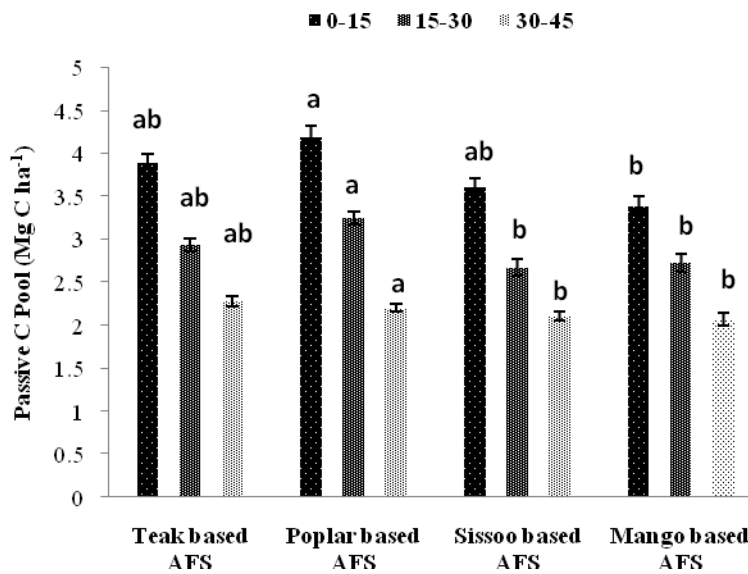
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Fig. 1a: Active soil organic carbon pool



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Fig. 1b: Passive soil organic carbon pool

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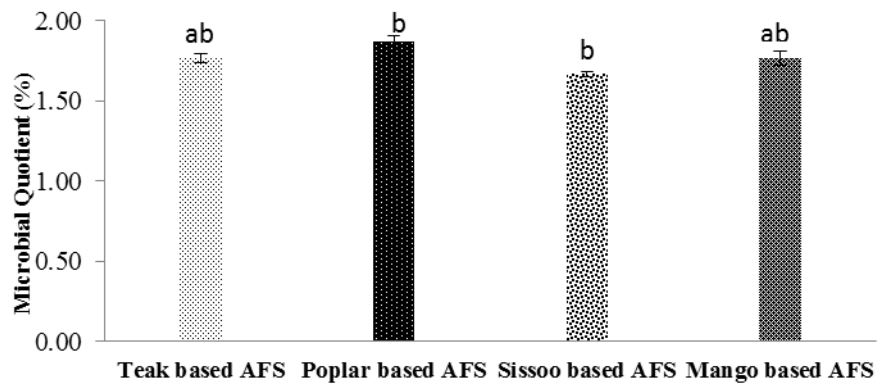
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Fig. 1: (a) Active and (b) Passive C pools in soil at three depths of different agroforestry systems
Each bar represents the mean and standard error (n = 3). Means not sharing a letter in common differ significantly ($p \leq 0.05$) between same soil layers of different systems; ns = non-significant

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Fig. 2: Microbial quotient (MQ) of different agroforestry systems.

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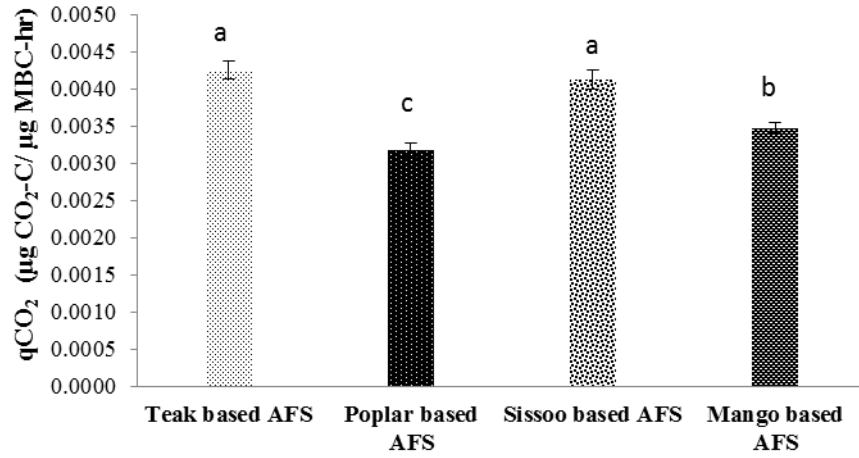
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Fig. 3: Microbial metabolic quotient (qCO₂) of different agroforestry systems.

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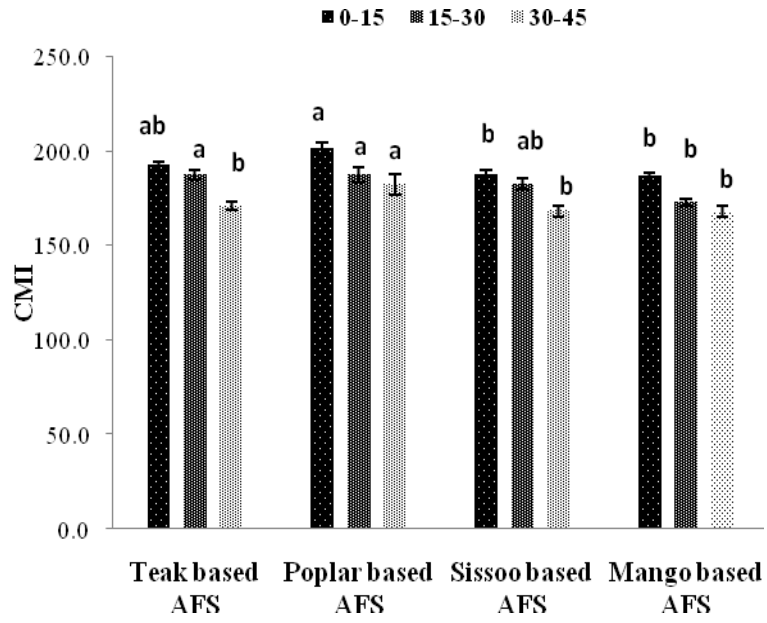
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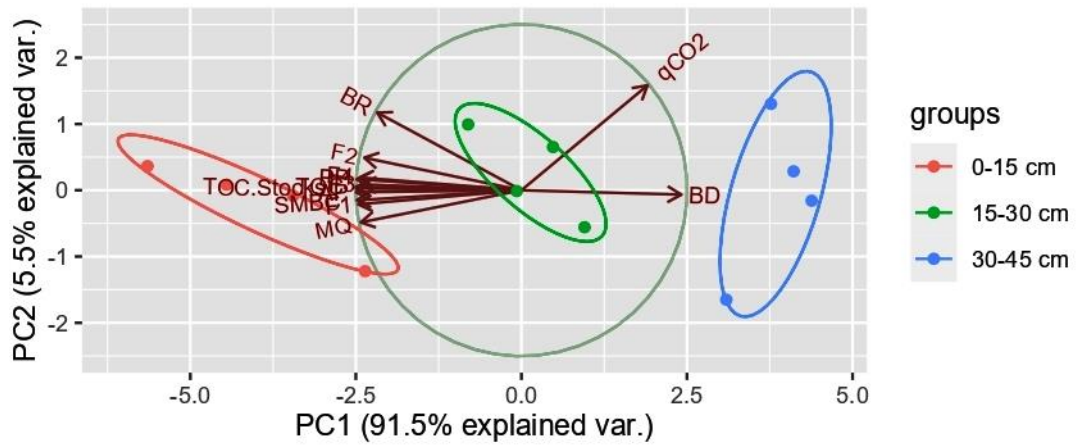


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Fig. 4: Carbon management index (CMI) of different agroforestry systems

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390 Fig. 5: Principal component analysis representing different soil parameters at three soil depths

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