## Quantification of carbon stocks and sequestration potential through existing agroforestry systems in the hilly Kupwara district of Kashmir valley in India

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The dynamic carbon accounting model CO2FIX was used for evaluating carbon stocks and estimate greenhouse gas mitigation through tree-based systems, outside the forest area, in Kupwara district of Kashmir valley India. Primary survey results revealed that on an average, there were about 135 trees per hectare, existing on farmers' field. Malus (33.75%), populus (29.91%), salix (14.32%), juglans (6.68%) and robinia (4.7%) were dominant tree species. Paddy and maize are the dominant kharif crops, whereas rabi season is dominated by oilseeds and fodder crops. The carbon sequestration potential, all the three pools simultaneously (viz. tree, crop and soil), of existing agroforestry systems (AFS) has been predicted as 0.88 Mg C ha<sup>-1</sup> yr<sup>-1</sup> AFS at district level are estimated to sequester 146,996 tonnes of CO<sub>2</sub> equivalent annually, which may offset completely the greenhouse gas emissions from agriculture/irrigation sector on account of electricity consumption throughout the state of Jammu and Kashmir.

**Keywords:** Agroforestry systems, carbon sequestration potential, GHG mitigation, soil carbon, tree biomass.

DIVERSIFICATION opportunities, improved soil use efficiency, microclimate changes, provision of permanent cover, etc. are significant elements of agroforestry systems (AFS) that play a substantial role in adaptation to climate change<sup>1,2</sup>. It has been projected<sup>3</sup> that the present worldwide area under agroforestry is 400 million ha. Agroforestry has been recognized as a promising tool for reducing atmospheric CO<sub>2</sub> concentration through fossil fuel substitution in the Kyoto Protocol<sup>4</sup>.

Tree-based systems are commonly observed throughout Kashmir valley. However, no estimates of carbon sequestered under these existing systems on farmers' fields at the district level are yet available for the state of J&K. This study aims at evaluating carbon stocks and carbon sequestration potential through tree-based systems, outside the forest area, in the Kupwara district of Kashmir valley in India.

Many recent studies have reported carbon stocks under different tree species of Kashmir valley in India<sup>5-7</sup>. However these studies are limited to few selected species like *Cedrus deodara*, *Fraxinus floribunda* and *Ulmus wallichiana*. Moreover, these studies do not consider the contribution of crop, soil, pasture, etc. in totality. This study, perhaps, is the first attempt in Kashmir valley to assess the carbon sequestered through existing AFS at farmers' field. The objective was to evaluate carbon stocks in all the three pools, viz. tree, crop and soil of AFS on farmers' field simultaneously and to simulate the carbon sequestration potential (CSP) of existing systems. It further aims to estimate green house gas (GHG) mitigation potential of existing AFS at farmers' field in the Kupwara district of Kashmir.

The study was initiated in 2014, utilizing a combination of extensive village level field surveys, soil samples, district level climatic data and a process-based simulation model. Extensive primary survey work for enumerating the different tree species being grown by the farmers on fields, their number, DBH (diameter at breast height), total tree height, etc. was done by the team of AICRPAF-Srinagar-Centre (All India Coordinated Research Project on Agroforestry, SKUAST-K Srinagar Centre, Jammu & Kashmir) following standard methodology. The trees were enumerated outside the natural forests in all the 11 blocks of the district.

The dynamic carbon accounting model CO2FIX v3.1 has been used to assess the baseline carbon and simulating the CSP of AFS. CO2FIX is a C computing model<sup>8-10</sup> evolved under CASFOR II project (dataservices.efi.int/ casfor/project.htm). In the CO2FIX model, the carbon credits and biomass are simulated at hectare level with one-year steps. The volumetric growth of the tree in terms of Stem-CAI (Current Annual Increment in m<sup>3</sup>/ha/ year) is the basic input to the model. The other components of the tree, viz. branches, leaves and roots are input as percentage of stem growth. The stem volumetric growth is converted into biomass annual stocks through biomass-module. The flow of biomass to soil is driven through harvest parameters and turnover rates. This model was employed in numerous studies and different ecosystems for evaluating carbon stocks under Kyoto-Protocol<sup>11</sup>. CO2FIX has been used to estimate the carbon storage and sequestration potential of selected trees species<sup>12</sup>, for AFS in Indo-gangetic-regions<sup>13</sup> and existing AFS in India14.

This model can be downloaded free of cost from <a href="http://www.efi.int/projects/casfor/CO2FIX/register32.php">http://www.efi.int/projects/casfor/CO2FIX/register32.php</a>. Detailed information about the model can be obtained at <a href="http://www.efi.int/projects/casfor">http://www.efi.int/projects/casfor</a>. CO2FIX can be used to deal with multiple species concurrently, compared to other available models like Century, Roth and Procomap

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which generally deal with single species system. Readers may refer refs 13 and 14 for detailed methodology, input parameters and parameterization of CO2FIX cohorts, viz. tree, crop and soil. To estimate the agricultural GHG mitigation potential, the total number of estimated trees has to be divided by the total district crop sown area. Then the estimated value of CSP of trees ha<sup>-1</sup> was expressed in terms of carbon dioxide equivalent to represent the mitigation potential.

Kupwara is a backward frontier district of Kashmir valley located between 34.17 and 34.12N and 73.16E at an attitude of 5300 m above mean sea level. The Kupwara district has 11 blocks, consisting of 363 villages and about 90,418 households. The average annual rainfall of Kupwara district is about 1061 mm. The driest month is November with 28 mm rainfall. Most of the rainfall occurs in April, and the average precipitation is 147 mm. The warmest month of the year is July with an average temperature of 23.9°C. In January, the average temperature is 2.9°C, which is the lowest average temperature of the whole year. The variation in rainfall between the driest and wettest month is 119 mm.

The data collected during the survey estimated that a total number of approximately 6,198,349 trees were found growing in different agroforestry systems, field boundaries, trees in patches, horticultural gardens, waste lands, nalas, trees inside the fields, etc. An aggregate of 26 different tree species were recorded as being grown in Kupwara district. The category wise spread was 9 in 'fast', 10 in 'medium' and 7 in 'slow' growing species.

A total of 472,863 trees were recorded in the slow growing category. In the case of slow growing trees, walnut trees were recorded in highest number (414,074) followed by *celtis*, deodar, kail, fig, chinar and oak. The survey recorded a maximum average height of 22.28 m in *Plantinus* spp. with the average highest DBH of 1.53 m followed by *Juglans* spp.

The total number of medium growing trees was 2,402,420 with the highest number being apple (2,092,262) followed by *morus*, pear, cypress, *aesculus*, elm, *fraxinus*, lemon, orange and almond. The data further revealed that *Ulmus* spp. recorded a maximum average height of 13.79 m and *morus* recoded the highest DBH of 0.34 m. Among the medium growing species, lemon and oranges were found growing only in one block (Teetwal), almond in 6 blocks and the rest of the medium growing species were common in all blocks throughout the district.

A total number of 3,323,065 trees under fast growing category were recorded in the district with maximum number of poplar (1,854,288) followed by *salix*, *robinia*, *ailanthus*, peach, pomegranate, wild apricot and cherry. The average maximum height and DBH were 22.65 and 0.33 m recoded for poplar species. Interestingly, poplar was the most preferred fast growing tree species existing in the district for all the blocks.

The estimated number of trees existing in farmers' field on per hectare basis was 135.76. Category-wise distribution of trees was 72.79, 52.62 and 10.35 trees/ha in fast, medium and slow growing categories respectively. Among the fast growing species, *poplus* was the dominant species (29.91%) followed by *salix* (14.32%) and *robinia* (4.7%). *Malus* (33.75%) was the dominant medium growing species followed by *morus* (2.33%), whereas *Juglans* (6.68%) was the dominant slow growing species (Table 1).

The crop productivity secondary data at district level was acquired from NIC (National Informatics Centre) and the district administrative office, Kupwara. The four cohorts considered in the study include three tree cohorts, viz. 'fast', 'medium' and 'slow' in addition to the fourth intercrop cohort. Paddy and maize were the common and important *kharif* crops in Kupwara district with average productivity of 6.2 and 2.0 t ha<sup>-1</sup> respectively. Oilseeds and fodder crops are grown in *rabi* season and the average productivity is 0.6 and 17.5 t ha<sup>-1</sup> respectively. Apple and walnut are the two most preferred horticultural crops with average fruit production of 8.5 and 2.3 t ha<sup>-1</sup> respectively.

CO2FIX model was parameterized with the primary tree survey data/secondary data of the crop productivity. The district level monthly climatic data (minimum and maximum temperature, precipitation, evapotranspiration, etc.) were fed in the soil decomposition YASOO<sup>15</sup> subroutine of the dynamic simulation model.

The model results revealed that the tree biomass (above and below ground) in the base line (2014) accumulated in the existing trees on farmers' field at the district level is estimated to be 99.59 Mg DM ha<sup>-1</sup> and is expected to increase from 99.59 to 147.19 Mg DM ha<sup>-1</sup> (Table 2) during the thirty years' simulation period. The amount of annual increase in tree biomass at district level is estimated to be 1.58 Mg DM ha<sup>-1</sup> yr<sup>-1</sup>. Our results are in line with Dar and Sundarapandian<sup>16</sup>, who measured biomass and carbon stocks of tree (above and below ground biomass), understorey (shrubs and herbaceous), dead-wood (standing and fallen trees and stumps), floor litter, and soil from 111 plots in temperate forests of Kashmir valley and reported that the tree biomass ranged from 100.8 to 294.8 Mg DM ha<sup>-1</sup>.

The total biomass (tree and crop) in the base line was  $102.18 \text{ Mg DM ha}^{-1}$  and is likely to increase to  $149.85 \text{ Mg DM ha}^{-1}$ . The thirty year simulation results of CO2FIX model predict that biomass carbon would enhance to the tune of  $71.78 \text{ Mg C ha}^{-1}$  from the baseline of  $48.91 \text{ Mg C ha}^{-1}$  (Table 2).

The base line (2014) soil carbon was 22.28 Mg C ha<sup>-1</sup> and it is estimated to increase to the tune of 25.99 Mg C ha<sup>-1</sup> during the simulated period of 30 years. The estimated rate of soil carbon sequestration at district level was 0.1236 Mg C ha<sup>-1</sup> yr<sup>-1</sup>. Ajit *et al.*<sup>13</sup> reported that the estimated rate of soil carbon sequestration at district level

Table 1. District-wise dominant tree species observed in the primary survey and their per cent contribution

Slow	Medium	Fast
Juglans spp (6.68)	Malus spp (33.75)	Poplus spp. (29.91)
Cedrus deodara (0.23)	<i>Morus</i> spp (2.33)	Ailanthus excelsa (2.87)
<i>Ficus</i> spp (0.04)	Prunus spp (1.68)	<i>Prunus</i> spp. (0.26)
Quercus spp (0.0005)	Cupressus torlusa (0.75)	<i>Prunus</i> spp. (0.18)
Celtis austrails (0.37)	Aesculus indica (0.17)	Salix spp. (14.32)
Pinus wallichana (0.21)	<i>Ulmus</i> spp. (0.03)	Robinia pseudoacacia (4.7)
Plantinus spp (0.02)	Fraxinus spp. (0.011)	Prunus spp. (0.65)
	Citrus spp. (0.004)	Punica granatum (0.56)
	Citrus spp. (0.0038)	<i>Prunus</i> spp. (0.77)
	Prunus amygdalus (0.003)	

Table 2. Biomass accumulated in the tree/crop components and carbon sequestered under existing AFS in Kupwara district of Kashmir valley (simulated using CO2FIX model)

Tree biomass (above and below ground) Mg DM ha <sup>-1</sup>	Baseline	Biomass	99.59
	Simulated		147.19
Total biomass (tree + crop) Mg DM ha <sup>-1</sup>	Baseline		102.18
	Simulated		149.85
Soil carbon (Mg C ha <sup>-1</sup> )	Baseline	Carbon	22.28
	Simulated		25.99
Biomass carbon (Mg C ha <sup>-1</sup> )	Baseline		48.91
	Simulated		71.78
Total carbon (biomass + soil) (Mg C ha <sup>-1</sup> )	Baseline		71.19
	Simulated		97.77
Net carbon sequestered in agroforestry systems over the simulated period of thirty years (Mg C ha <sup>-1</sup> )		Carbon sequestered	26.58
Estimated annual carbon sequestration potential of agroforestry system (Mg C ha <sup>-1</sup> yr <sup>-1</sup> )		_	0.88
Estimated CO <sub>2</sub> mitigation potential of agroforestry system (Tonnes of CO <sub>2</sub> equivalent ha <sup>-1</sup> yr <sup>-1</sup> )		CO <sub>2</sub> equivalent mitigated	3.22
Estimated CO <sub>2</sub> mitigation potential of agroforestry system at district level (Million tonne of CO <sub>2</sub> equivalent annually)	s		0.15

was 0.016 Mg C ha<sup>-1</sup> yr<sup>-1</sup> for Sultanpur (with 13 tree/ha) and 0.037 Mg C ha<sup>-1</sup> yr<sup>-1</sup> for Dinajpur (with 24 tree/ha) in Indo-Gangetic Plains and ranged from 0.003 to 0.51 Mg C ha<sup>-1</sup> yr<sup>-1</sup> for different districts at the country level<sup>14</sup>. In fact, soil carbon sequestration depends upon a number of factors, viz. existing tree/ha, rainfall, temperature, sunshine hours and other local climatic parameters as well as on management practices.

The CSP of existing agroforestry systems at Kupwara district has been estimated to be 0.88 Mg C ha<sup>-1</sup> yr<sup>-1</sup> (Table 2) which is quite high when compared to 0.21 Mg C ha<sup>-1</sup> yr<sup>-1</sup> at country level<sup>14</sup>, but lower than reported for *Cedrus deodara* in Kashmir valley<sup>7</sup>. However, the current findings are well in agreement with the findings of Jana *et al.*<sup>17</sup> and Yadava<sup>18</sup>. Our results are also in line with Sheikh *et al.*<sup>3</sup>, who estimated that the current worldwide area under agroforestry is 400 million ha, which results in a carbon gain of 0.72 Mg ha<sup>-1</sup> year<sup>-1</sup>.

In J&K, greenhouse gases (GHG) are emitted mainly from burning of fossil fuels. Generation of electricity, which is mostly from conventional power plants in the state, results in considerable amount of GHG emission. An assessment was made by MoEF (Ministry of Environment and Forests, New Delhi) to account for the contribution of GHG from consumption of electrical energy

in the state of J&K (http://www.moef.nic.in/sites/default/ files/sapcc/Jammu-Kashmir.pdf), which reported that GHG emission from electricity consumption in agriculture/irrigation sector were 132,229.80 tonnes of CO<sub>2</sub> equivalent. As per the current study the CSP of existing AFS (considering 30 years simulation) is to the tune of  $0.88 \text{ Mg C ha}^{-1} \text{ yr}^{-1}$  for Kupwara district and accordingly, the CO<sub>2</sub> sequestration from the atmosphere is estimated to be 3.23 t ha<sup>-1</sup> annually. With the net sown area of 45,651 ha in the Kupwara district, the existing AFS at district level are estimated to sequester 146,996.22 tonnes of CO<sub>2</sub> equivalent annually, i.e. mitigation potential of 0.15 million tonnes of CO<sub>2</sub> equivalent annually at the district level. Thus the existing agroforestry systems at farmers' field in Kupwara district are estimated to offset completely the GHG emissions from agriculture/irrigation sector on account of electricity consumption throughout the state of J&K. Thus, the results of this study quantitatively reaffirm the secondary role of ecosystem services (in atmospheric GHG mitigation) rendered through the agroforestry systems at farmers' field in addition to their primary role of providing food, fuel, fruit and fodder.

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## Fusion of ginseng farnesyl diphosphate synthase and *Centella asciatica* squalene synthase involved in triterpenoid biosynthesis

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Farnesyl diphosphate synthase (FPS) is a key enzyme in isoprenoid biosynthesis, generating farnesyl diphosphate as the central precursor for the broad classes of sesquiterpenoids and triterpenoids. On the one hand, cyclization of farnesyl diphosphate catalysed by various sesquiterpene synthases leads to structurally diverse sesquiterpenoids, while on the other, dimerization catalysed by squalene synthase (SQS) yields squalene as the first intermediate in the production of triterpenoids. To optimize triterpenoid production, the activities of an FPS generating farnesyl diphosphate and an SQS converting it should be coupled. Here, we constructed a fusion protein combining a ginseng FPS and a Centella asiatica SQS via a short peptide (Gly-Ser-Gly) linker. Heterologous expression in Escherichia coli resulted in a soluble fusion protein detected by SDS-PAGE. The fusion protein had both FPS and SQS activities, at approximately 94% and 71% of the single enzyme levels respectively. This novel fusion protein will serve as a valuable tool for genetic engineering of triterpenoid compounds, including saponins.

**Keywords:** Farnesyl diphosphate synthase, fusion protein, squalene synthase, triterpenoids.

THE isoprenoid biosynthetic pathways produce a number of important primary as well as secondary metabolites in many plants<sup>1-3</sup>. Sesquiterpenoids and triterpenoids are two major groups of isoprenoid compounds with important biological functions and medicinal properties. In particular, triterpenoid saponins have a variety of interesting pharmaceutical activities, including anti-inflammatory, anti-cancerogenic and anti-bacterial effects, depending on their chemical structures<sup>4</sup>.

Sesquiterpenoid and triterpenoid biosynthesis starts by the conversion of three units of acetyl-coenzyme A (CoA) to 3-hydroxy-3-methylglutaryl-coenzyme A (HMG–CoA),

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