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Abstract. We compared the C storage of two nitrogen-fixing trees in mixed and monospecific plantations to investigate the C sequestration potential after 10 years of their establishment. The study was carried out in three types of plantation, *Dalbergia sissoo* Roxb. ex DC. pure (P₁DS), *Leucaena leucocephala* (Lam.) de Wit pure (P₂LL) and mixed plantation of *D. sissoo* and *L. leucocephala* (P₃DS.LL). The results of the study indicated that, P₃DS.LL sequestered 34.30 \pm 0.24 t yr⁻¹ha⁻¹ CO₂ compared to 27.35 \pm 0.19 t yr⁻¹ ha⁻¹ in P₁DS and 19.81 \pm 0.44 t yr⁻¹ ha⁻¹ in P₂LL. Total carbon storage was also maximum in P₃DS.LL (93.47 \pm 0.67 t ha⁻¹) followed by P₁DS (74.54 \pm 0.53 t ha⁻¹) and P₂LL (53.98 \pm 1.21 t ha⁻¹). This indicates that *L. leucocephala* has synergetic effect with *D. sissoo* to enhance the carbon sequestration potential when interplanted together. The study revealed that mixed plantation of N-fixer trees have potential to sequester more carbon than same species in monoculture. The study concluded that in reforestation or afforestation program the synergic effect of N-fixer trees can be helpful projects to offset more C emissions.

Keywords: carbon sequestration, *Dalbergia sissoo, Leucaena leucocephala*, nitrogen-fixing, pure, mixed.

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Introduction

Since the beginning of industrial revolution, carbon dioxide concentration in the atmosphere has been rising alarmingly. Prior to the industrial revolution carbon dioxide concentration in the atmosphere was around 270 ppm (Sage, 1995), which has now increased approximate to 398 ppm (ESRI, 2013). If the pace of increase in carbon dioxide concentration remains constant and efforts are not made to reduce it, carbon dioxide concentration in the atmosphere may go up to 800–1000 ppm by the turn of this century (Whipps, 1990). The increasing concentration of greenhouse

gases (e.g., CO_2 , CH_4 , N_2O , O_3) have led to changes in the earth's climate and a warming of the earth's surface although, forestry and afforestation in particular, is regarded as an important means to offset greenhouse gas emissions (Miehle et al., 2006), particularly by reducing the concentration of atmospheric carbon dioxide by increasing carbon sequestration in tree biomass and soils (Turner et al., 2005; Nsabimana et al., 2008). Forest soils are also one of the major carbon sinks on earth, because of their higher organic matter content (Dey, 2005), where soils play a key role in the global carbon budget (Jha et al., 2003; Sheikh et al., 2009)

Projects that increase the area of plantations have been suggested for inclusion under the clean development mechanism (CDM) as defined in Article 12 of the Kyoto-Protocol (van Vliet et al., 2003). The UN framework convention on climate change (UNFCCC) followed by the Kyoto Protocol were the first steps taken by the international community in this direction. Afforestation is one of the viable options of C sequestration in terrestrial ecosystems (IPCC, 1999; Lamb et al., 2005). The potential of C sequestration through afforestation is estimated, for example, at 3 Tg C yr⁻¹ in Norway, 6 TG C yr⁻¹ in New Zealand, 9 TG C yr⁻¹ in Sweden, 107 TG C yr⁻¹ is in Russia and 117 TG C yr⁻¹ in USA (IPCC, 1999). Forest plantations in 2000 occupied 116 Mha (million hectare) in Asia, 32 Mha in Europe, 28 Mha in America and 8 Mha in Africa (FAO, 2001a). These 187 million ha of tree plantation worldwide represent approximately 5% of the global forest area (FAO, 2001b; Alvaro & Florencia, 2006). Proper design and management of plantations can increase biomass accumulation rates making them more effective carbon sinks. The vast majority of tropical tree plantations are monocultures (Binkely et al., 2003). However, mixtures that contain N-fixing species may have an additional potential to increase overall biomass C sequestration. Estimates of the effects of nitrogen deposition on forest carbon sequestration vary from 0.1 to 2.3 Pg carbon yr⁻¹ (Peterson & Melillo, 1985; Townsend et al., 1996; Holland, 1997). If the higher estimates of the effects of nitrogen deposition on forest carbon uptake are accurate, then the terrestrial carbon sink could persist well into the coming century as nitrogen deposition increases. The role that nitrogen deposition plays in determining sink strengths of forests for CO₂ depends on where nitrogen inputs to forests ultimately reside (Rastetter et al., 1992; Houghton et al., 1998). If the primary recipients are trees with woody tissues, high carbon-tonitrogen (C:N) mass ratios (of between 200 and > 500) and long turnover times, then the effects of nitrogen deposition on forest carbon uptake are relatively large (Nadelhoffer *et al.*, 1999).

Mixed-species plantations have the potential to improve nutrient cycling, soil fertility (Montagnini, 2000), biomass production (DeBell et al., 1985; Parrotta, 1999) and carbon sequestration (Kaye et al., 2000). Forests with nitrogen-fixing trees typically accumulate more carbon than similar forests without nitrogen-fixing trees (Resh et al., 2002). To understand the role of nitrogen-fixing trees in carbon sequestration, two nitrogen fixing tree species, Dalbergia sissoo Roxb. ex DC. and Leucaena leucocephala (Lam.) de Wit were selected for the study, because both species have wide representation in India. D. sissoo is native to India and is found in most parts of the country whereas L. leucocephala is exotic to India and was introduced in the last century in Andhra Pradesh, Karnataka, Tamil Nadu, Himachal Pradesh and Uttar Pradesh (Lohani, 1979; Luna, 2005), and have almost naturalized in some parts. However, it received attention recently in 1980s, when it was re-introduced in Maharashtra, Gujarat, Punjab, Haryana and West Bengal because of its fast growing nature. Previous researchers (Bi & Turvey, 1994; Parrotta et al., 1996; Khanna, 1997; Kaye et al., 2000; Resh et al., 2002, Piotto et al., 2003; Petit & Montagnini, 2004; Bristow et al., 2006) have compared above ground biomass production (and subsequently carbon sequestration potential) between different plantations composed of non Nfixing trees with N-fixing tree species in pure and mixed-plots and their synergetic effect on non N-fixing tree species in mixed plantation. But in the present study we have tried to examine the hypothesis that:

• If both the tree species having nitrogen fixing ability are planted in pure and in mixed stands, will their synergistic effect on each other in mixture results in higher biomass production than pure stands.

For this study we selected the plantation which was established on land of the Forestry Department, HNB Garhwal University, Srinagar Garhwal, Tehri district of Uttarakhand state. The plantations were not originally established with the view to address the hypothesis but keeping in view the homogeneity of soil and the combination of plantations, where three types of plantations i.e., *D. sissoo* pure (P_1DS), *L. leucocephala* pure (P_2LL) and mixed plantation of *D. sissoo* and *L. leucocephala* (P_3DS . LL) already been planted.

Material and Methods

Study site

The study area is located in district Tehri Garhwal (lat. 30° 3′ N, long. 78° 48′ E) at an elevation of 530 m asl. Mean temperature ranges from 12.8 °C in December–January to 32 °C in April-June. The mean annual precipitation of the area reported of 363 mm. The soils of the area is well drained, stone free and acidic in nature, the area is flat, and of uniform terrain.

Sampling

For estimating above ground biomass sampling was done by the nested plot design method for each hectare (Hairiah *et al.*, 2001). A nested sampling approach was followed, assessing large diameter trees (with a stem diameter above 30 cm) in rectangular plots of 20×100 m = 2000 m², smaller trees (stem diameter 5–30 cm) in sub plots of 5×40 m = 200 m².

Volume and biomass estimation

We used a different approach for volume estimation instead of allometric equations, to avoid the felling of trees. We measured diameter at breast height (dbh) of each tree in the 20×100 m sample plot as per sampling procedure mentioned above. The diameter at breast height (dbh) was measured with caliper and height with Ravi Multimeter, form factor was calculated

with Spiegel Relaskop to find out tree volume (Pressler, 1895; Bitterlich, 1984).

$$F = 2 h_1 / 3 h_2$$

where, F is the form factor, h_1 is the height at which diameter is of half dbh and h is the total height. Volume (V) was calculated by using the Pressler formula (Pressler, 1895).

$$V = F \times h \times g_{\prime}$$

where F is the form factor, h is the total height and g is the basal area and calculated as:

 $G = (dbh / 2)^2$

Bole volume was combined with measured wood density to estimate bole biomass. Branch, twig and foliar biomass were calculated using the fresh mass and water content. We calculated the total number of branches of the sample trees irrespective of size. These branches were categorized on the basis of basal diameter into three groups i.e., < 6 cm, 6–10 cm and > 10 cm. Fresh weight of two branches from each size group was recorded separately. Dry weight of branches was estimated by using following equation (Chidumaya, 1990).

$$B_{dwi} = B_{fwi} / 1 + M_{edbi}$$

where B_{dwi} is the oven dry weight of branches, B_{fwi} the fresh / green weight of branches and M_{edbi} the moisture content of branches on dry weight basis. Total branch biomass (fresh / dry) per sample tree was determined as follows

$$B_{bt} = n_1 bw_1 + n_2 bw_2 + n_3 bw_3 = \sum_{i=1}^n n_i bw_{i'}$$

where B_{bt} is the branch biomass per tree, n_i is the number of branches in the ith branch group and I = 1, 2, 3 ... the branch groups.

Leaves from five branches of individual trees were removed. Five trees per quadrant were taken randomly for observation. The leaves were weighed and oven dried separately to a constant weight at 80 ± 5 °C. The average leaf biomass was then derived by multiplying the average biomass of the leaves per branch with the number of branches in a single tree and then the number of trees in a quadrant (Chidumaya, 1990). The carbon content of vegetation is surprisingly constant across a wide variety of species. Most of the information for carbon estimation described in the literature suggests that carbon constitutes between 45 to 50 percent of dry matter (Chan, 1982; Schlesinger, 1991). We assumed carbon to equal 45% of tree's biomass. The estimates are based on the assumption of common carbon content per biomass unit as in many other similar studies (Woomer, 1999; Koul & Panwar, 2008). Total CO₂ accumulated per hectare and average rate of CO₂ (t yr⁻¹ ha⁻¹) was estimated by combining the carbon storage values with the molecular weight of carbon dioxide. SPSS programme was used to determine the statistical significance for differences in aboveground biomass, and other parameters within the species and between the plantations.

Results

At ten years of age, tree stands having different tree composition showed a significant difference in total aboveground biomass. Total aboveground biomass accumulation and its allocation to different tree components i.e., bole, branch, twig and foliage is given in Table 1. Tree biomass was highest in P₂DS.LL mixed plantation plot and lowest in P₂LL monoculture. The biomass was in order of bole > branch > twig > foliage. The biomass of each component in P₂LL was 88.12 ± 0.76 t ha⁻¹ for bole followed by $19.17 \pm$ 1.40 t ha⁻¹ in branch, 9.43 \pm 0.97 t ha⁻¹ in twigs and 3.24 ± 1.19 t ha⁻¹ in foliage. Similar to P₂LL, the biomass of each component in P₁DS was also recorded in decreasing trend as 126.28 ± 0.14 t ha⁻¹, 25.10 \pm 0.49 t ha⁻¹, 9.65 \pm 0.29 t ha⁻¹ and 4.62 \pm 0.50 t ha⁻¹ for bole, branch, twig and foliage, respectively. In P₃DS.LL similar trend was also recorded in biomass i.e., 157.16 ± 0.66 t ha⁻¹ in bole, 28.19 ± 0.35 t ha⁻¹ in branch, 11.24 ± 0.47 t ha⁻¹ in twig and 11.13 ± 0.49 t ha⁻¹ in foliage. The values of bole biomass were significant (p < 0.05) between the plantations. In the present study, we observed that the maximum (207.27 ± 1.49 t ha⁻¹) total biomass was in P₃DS.LL followed by P₁DS (165.55 ± 1.19 t ha⁻¹) and P₂LL (119.96 ± 2.70 t ha⁻¹).

Above ground tree biomass in each plantation was calculated separately for different components i.e., bole, branch, twig and foliage. In P_1DS the biomass allocation for different components was 76%, 15%, 6% and 3% for bole, branch, twig and foliage respectively. In P_2LL bole contributed 73% biomass in comparison to 16% (branch), 8% (twig) and 3% (foliage). Similarly in P_3DS . LL the maximum biomass was stored in bole (76%) followed by branch (14%), twig (5%) and foliage (5%) (Figure 1).

Converting biomass into carbon stock revealed significant difference in total C stock (p < 0.05) between the plantations. The carbon stock stored and total atmospheric carbon dioxide sequestered by different components during the ten year age of different plantations is given in Table 1. The carbon stock in each component of P₂LL was 39.65 ± 0.34 , 8.62 ± 0.63 , 4.24 ± 0.43 and 1.45 ± 0.53 t ha⁻¹ in bole, branch, twig and foliage respectively. Similarly in P₁DS and $P_3DS.LL$ the C stock was found in reducing order from bole > branch > twig > foliage with the values of 56.82 ± 0.06 , 11.29 ± 0.22 , 4.34 ± 0.13 and 2.08 ± 0.22 in P₁LL and 70.72 ± 0.30 , 12.68 ± 0.16 , 5.05 ± 0.21 and 5.00 ± 0.22 in P₃DS.LL., respectively. The maximum carbon stock stored was again reported in $P_3DS.LL$ 93.47 ± 0.67 t ha⁻¹ followed by P_1DS (74.54 ± 0.53 t ha⁻¹) and P_2LL $(53.98 \pm 1.21 \text{ t ha}^{-1}).$

The monoculture plantations P_1DS and P_2LL accumulated atmospheric CO_2 with an annual rate of 27.35 ± 0.19 t ha⁻¹ and 19.81 ± 0.44 t ha⁻¹ (Table 2). However, in $P_3DS.LL$, the accumulation of CO_2 from at-

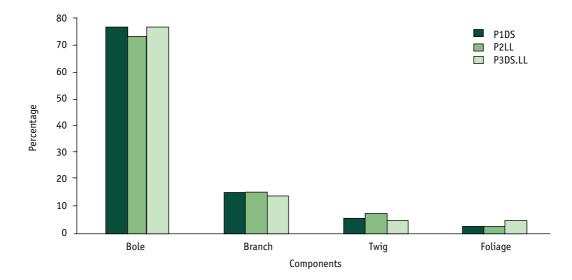


Figure 1. Percentage of biomass accumulation and its allocation to different tree components.

Parameter	Component	Plantation								
		P ₁ DS			P ₂ LL			P ₃ DS.LL		
		Mean	SE	Sig	Mean	SE	Sig	Mean	SE	Sig
Biomass	Bole	126.28	0.14	(b) B	88.12	0.76	(b) C	157.16	0.66	(b) A
	Branch	25.10	0.49	(c) B	19.17	1.40	(c) B	28.19	0.35	(c) A
	Twig	9.65	0.29	(d) A	9.43	0.97	(d) A	11.24	0.47	(d) A
	Foliage	4.62	0.50	(e) B	3.24	1.19	(d) B	11.13	0.49	(d) A
	Total	165.66	1.19	(a) B	119.96	2.70	(a) C	207.72	1.49	(a) A
Carbon	Bole	56.82	0.06	(b) B	39.65	0.34	(b) C	70.72	0.30	(b) A
	Branch	11.29	0.22	(c) B	8.62	0.63	(c) C	12.68	0.16	(c) A
	Twig	4.34	0.13	(d) A	4.24	0.43	(d) B	5.05	0.21	(d) A
	Foliage	2.08	0.22	(e) B	1.45	0.53	(e) C	5.00	0.22	(d) A
	Total	74.54	0.53	(a) B	53.98	1.21	(a) C	93.47	0.67	(a) A

Table 1. Component wise biomass t ha⁻¹ and carbon t ha⁻¹ in different plantations after 10 years.

Difference within a species (a) and between the species (A) for a given issue are statistically significant (p < 0.05) when means are followed by different letters.

Parameter	Component	Plantation								
		P ₁ DS			P ₂ LL			P ₃ DS.LL		
		Mean	SE	Sig	Mean	SE	Sig	Mean	SE	Sig
CO ₂ (t yr ⁻¹ ha ⁻¹)	Bole	20.85	0.02	(b) B	14.55	0.12	(b) C	25.95	0.11	(b) A
	Branch	4.14	0.08	(c) B	3.16	0.23	(c) C	4.65	0.05	(c) A
	Twig	1.59	0.04	(d) A	1.55	0.15	(d) A	1.85	0.07	(d) A
	Foliage	0.76	0.08	(e) B	0.53	0.19	(e) C	1.83	0.08	(d) A
	Total	27.35	0.19	(a) B	19.81	0.44	(a) C	34.30	0.24	(a) A

Table 2. Rate of CO₂ sequestration (t yr⁻¹ ha⁻¹) in different components and plantations.

Difference within a species (a) and between the species (A) for a given issue are statistically significant (p < 0.05) when means are followed by different letters.

mosphere was maximum that of monocultures, with an annual rate of 34.30 ± 0.24 t ha⁻¹ (Table 2).

Discussion

In the present study we observed that maximum above ground biomass and carbon stock was present in mixed plantation P₃DS.LL followed by P₁DS and P₂LL. P₁DS stored more carbon than P₂LL. However, the P₃DS.LL mixed stand stored 18 % more carbon than P_2LL and 9% more than P_1DS . The maximum carbon stock in P₃DS.LL might be due to the high nitrogen fixing capacities of *L. leucocephala* and its combined effect with D. sissoo. Kaye et al. (2000) observed that carbon sequestration was significantly boosted when Eucalyptus plantations included nitrogen-fixing trees. Resh et al. (2002) also found that the forests with nitrogen-fixing trees typically accumulate more carbon in soils than similar forests without N-fixing trees. However, the present study showed that the mixture of nitrogen-fixing trees act synergistically too. Nitrogen-fixing tree species have larger effects on forest soils than other species, and these effects include consistent increases in soil organic matter and carbon. Across 19 case studies, an increase in 1 g N was associated with an increase of 12 to 15 g C (Binkley & Menyailo, 2005). Nitrogen, fixing trees change soils more rapidly than other species. The changes in soil nitrogen begin with the fixation of N by symbiotic bacteria in root nodules and the incorporation of this N into tree tissue (Binkley & Menyailo, 2005).

It is interesting to note that foliage biomass nearly doubled in the mixed species P₃DS.LL compared to the single species stands 5.4% vs 2.87% and 2.8%. This greater investment in assimilating leaf area may explain the increased rate of carbon sequestration in P₃DS.LL. Increased N availability may also increase leaf area, which increases light capture and canopy photosynthesis, and hence gross primary production (Landsberg, 1997). For example Cromer et al. (1993) and Smethurst et al. (2003) found that N and P fertiliser increased leaf area and biomass productions in plantations of *Eucalyptus grandis* W. Hill ex Maiden and E. nitens (H. Deane et Maiden) Maiden, respectively. However, increases in leaf area in response to fertiliser may also be associated with changes in the allocation of C from belowground to aboveground growth (Cannell, 1985). Increase in nutrient availability can shift allocation of C from roots and mycorrhizae (for nutrient uptake) to above ground plant parts, to increase the capture of light and CO₂ (Cannell, 1985; Raich, 1998; McConnaughay & Coleman, 1999). However, few studies have examined whether increase in aboveground growth associated with a higher nutrient availability results from an increase in total productivity (both aboveand belowground) or a shift in biomass partitioning, or both (Ryan *et al.*, 1996; Keith *et al.*, 1997). Furthermore, the results of such studies have been variable, documenting increase, decrease and no change in belowground C fluxes with increasing nutrient supply (Haynes & Gower, 1995; Ryan *et al.*, 1996; Keith *et al.*, 1997; Raich, 1998; Zak & Pregitzer, 1998; Pongracic, 2001; Giardina & Ryan, 2002; Giardina *et al.*, 2003).

Changes in the composition of tree species which result from land use or climate change may have important feedbacks to terrestrial carbon sequestration (Kaye et al., 2000). Results from the previous research have indicated the capacity of mixed-species stands to produce relatively high levels of biomass (Montagnini & Porras, 1998). The idea of tree plantations as a sink for carbon dioxide has gained momentum over the last decade. The use of tree plantations can be multifunctional; soil rehabilitation, direct economic rewards and carbon sequestration. Pure and mixed-species plantations at La Selva have shown that mixed plantations grow well, with productivities either similar or larger than the same species grown in pure plantations (Piotto et al., 2003; Petit & Montagnini, 2004). As a consequence, mixed plantations also accumulate more aboveground biomass and sequester carbon at high rates as compared to pure plantations (Montagnini & Porras, 1998; Stanley & Montagnini, 1999). The mixed plantations also contribute to recovery of soil fertility (Montagnini & Porras, 1998). Mixed-species plantations have the potential for out producing monocultures, but actual yields depend on soil, silviculture, and species (Binkley et al., 2003).

Conclusion

Earlier studies have revealed the synergistic effect of nitrogen fixing trees for nonnitrogen fixing trees species. Similar increase in plant productivity and soil C was seen in the current study when two N-fixing tree species were intermixed in a single stand. Today, the earth's forests are shrinking; we are loosing a major CO_2 sink. Hence the goal is to expand the earth's tree cover, growing more trees to soak up CO_2 .

The study concluded that mixed plantation of N-fixing tree species has potential to sequester more carbon and have higher synergetic effect to enhance the carbon sequestration potential. The mean CO_2 sequestration rate increases significantly in mixed plantation than in monoculture plantations.

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