Fine root biomass differs significantly across different forest types and soil depth in Central Himalaya, India

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Fine roots (diameter less than 2 mm) comprise a significant portion of the plant biomass. They are important for water absorption, cycling of nutrients and the carbon budget on a global scale. The aim of the present study was to quantify fine root biomass in the Nainital district, Central Himalaya, India, which has several dominant forest types. A total of 81 samples were collected from nine sample plots for each forest type in three distinct directions. The results showed that sal forest $(1.11 \pm 0.04 \text{ t ha}^{-1})$ had the largest fine root biomass, followed by oak forest $(0.72 \pm 0.06 \text{ t ha}^{-1})$ and pine forest $(0.61 \pm 0.06 \text{ t ha}^{-1})$. We observed that the trend in fine root biomass across different forest types was as follows: sal forest > oak forest > pine forest, significant at 0.05 level. Fine root biomass was also observed to decrease similarly with increasing soil depth in each forest type, following the trend: 0-20 cm > 20-40 cm > 40–60 cm, which was significant at 0.05 level. Researchers will benefit from this study since it will help them comprehend fine root biomass variation and offer baseline data for future research on nutrient cycling and the global carbon budget.

Keywords: Forest types, global carbon budget, nutrient cycling, plant biomass, soil depth.

FINE roots (diameter less than 2 mm) represent an essential part of the forest ecosystem. They are a minor but functionally essential component of the plant biomass. Fine roots are essential for water intake, nutrient cycling and the production of specific growth hormones¹⁻⁴. They significantly influence soil profile development and contribute substantially to soil organic carbon through root death and rhizodeposition⁵. Despite accounting for only a small proportion of the overall plant biomass, fine roots are estimated to account for 30% of above-ground biomass⁶ and one-third of yearly worldwide net primary output^{7,8}.

Fine roots have a lifespan of weeks to years, depending on the species and environmental conditions⁹. According to the site, the production of fine roots, their biomass and turnover might vary significantly over seasons and/or across years^{10,11}. Fine root biomass is influenced by the tree species, site and structure of the soil, how compact or airy it is, how far the roots can spread horizontally and vertically and how deep they can grow, as well as the competition among trees of the same species¹². In forests, the average yearly loss of the fine root system is estimated to range between 40% and 92%. Due to their high turnover, fine roots add 2–5 times more organic matter to the soil than the above-ground components¹³.

The international scientific community has made studying the responses of the terrestrial ecosystem to global change a priority. Estimating the fine root biomass of forests is becoming more important because of its role in the carbon budget of ecosystems, biogeochemical cycling, and a possible role of fine root vitality as an indicator of the physical and chemical state of the soil and how it changes over time¹⁴. Furthermore, difficulty in obtaining valid estimates of fine root growth has restricted the accuracy of nutrient and energy estimates for forest ecosystems^{15–17}. Despite their importance, sampling fine roots takes a longer time, and accurate estimates have been difficult to monitor by researchers. So, fine roots are one of the challenging and most useful features to study in terrestrial ecosystems.

The Himalayan region is expected to get warmer by 2.6°–4.6°C by the end of the 21st century, according to several CMIP5 models¹⁸. Climate change-related variations in fine root development might alter nutrient availability in forests, impacting the overall production. As a result, precise fine root biomass estimations are critical in enhancing carbon budgeting models¹⁹. Despite the valuable importance of fine roots in the growth of plants and the amount of tree biomass, there have been limited studies on fine roots biomass. The study aimed to quantify the fine root biomass across different selected forest types of the Nainital district, Central Himalaya, India.

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Figure 1. Map of the study area.

Materials and methods

Study site

This study was undertaken in three major dominant forests: pine (*Pinus roxburghii*, Sarg.), oak (*Quercus leucotricho-*

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phora, A. Camus) and sal (*Shorea robusta*, Gaertn. F) (Figure 1). These are located in Kailakhan (29°24'N; 79°28'E), Baldiyakhan (29°22'N; 79°26'E) and Ranibagh (29°17'N; 79°32'E) respectively, at different altitudes ranging from 500 to 2100 m amsl. All the sites fall in the Reserved Forest category of the Nainital Forest Division.

Each forest represents varied species composition and structure. These forests have a diverse terrain, soil characteristics and climatic conditions from a lower to a higher elevation, representing sal followed by pine and oak forests in Central Himalaya.

Method

The sequential soil core method was used in the present study^{12,20-22}. The method is based on volume metric samples excavated with a sharp-edged metallic tube called a soil core with a diameter of 7.62 cm (internal) and length of 90 cm (30 cm extra length kept to avoid hammering damage to the core). The soil core was divided into three depthwise sections: 0-20, 20-40 and 40-60 cm for each forest site. Altogether 81 samples were collected from nine plots for each forest type in three distinct directions during 2014-2015. Soil samples with roots were collected from each forest type at different depth intervals (0-20, 20-40 and 40-60 cm) using a 50 cm metallic rod with clear marked points at 20 and 40 cm. Soil coring was carried out randomly at 1, 2 and 4 m distances from a centrally chosen tree (reference point) in three distinct directions. The samples were tagged with unique identifiers and sent to the laboratory for additional processing.

Using a succession of sieves ranging from 1 to 5 mm, fine roots from each sample were sorted and separated from organic components contained in the soil. The fine roots were manually sifted and separated using forceps. The acquired roots were cleaned with flowing tap water. Next, fine roots (dead or alive) of 2 mm were air-dried for a few minutes before being separated individually using a ruler based on their colour and texture. For each sample, the fresh weight of the sorted fine root was determined (depthwise), packed in paper bags and oven-dried up to 60°C until it attained a constant temperature. Finally, the biomass was estimated by weighing the oven-dried fine roots using a volumetric sample size. The impact of soil depth and forest type on fine root biomass was studied using a one-way ANOVA.

Results

Descriptive analysis

Oak forest biomass for fine roots was estimated to be 0.72 ± 0.06 t ha⁻¹ on average. As shown in Figure 2, the mean biomass for fine roots in pine and sal forests was 0.61 ± 0.06 t ha⁻¹ and 1.11 ± 0.04 t ha⁻¹ respectively. Table 1 provides the descriptive statistics of fine root biomass in different forest types.

Fine root biomass

Fine root biomass was measured at three separate sites (hilltop, hill mid and hill base) at three different soil depth

intervals for each of the three forest types (oak, pine and sal-dominated forests) at 0-20, 20-40 and 40-60 cm.

Oak forest

Table 2 shows that the hilltop $(2.494 \pm 0.533 \text{ t ha}^{-1})$ had the highest total fine root biomass, followed by the hill base $(2.048 \pm 0.437 \text{ t ha}^{-1})$ and hill mid $(1.960 \pm 0.493 \text{ t ha}^{-1})$. The depthwise share of this total fine root biomass on the hilltop was 0.921 (36.9%), 1.185 (47.5%) and 0.388 t ha⁻¹ (15.6%) along 0-20, 20-40 and 40-60 cm respectively. Similarly, the depthwise share of total fine root biomass in the hill base was 0.709 (34.6%), 0.958 (46.8%) and 0.380 t ha⁻¹ (18.6%) and in hill mid was 1.002 (51.1%), 0.570 (29.1%) and 0.388 t ha⁻¹ (19.8%) along the same depth intervals respectively. The trend of root mass in different soil depth intervals showed that most of the roots were in 20-40 cm in the oak-dominated forest; however, for the hill mid, most of the roots were located at 0-20 cm depth. Majority of roots were found in the upper area of the soil, according to observations at 0-40 cm soil depth. Table 2 depicts the fine root biomass in oak forest estimated in each sample plot at different soil depth intervals.

Pine forest

Hill base $(1.894 \pm 0.502 \text{ t ha}^{-1})$ had the highest total fine root biomass, followed by hilltop $(1.894 \pm 0.502 \text{ t ha}^{-1})$ and hill mid $(1.499 \pm 0.350 \text{ t ha}^{-1})$ in the pine-dominated forest (Table 3). Depthwise share of this total fine root biomass at the hilltop was 0.980 (51.7%), 0.490 (24.9%) and 0.424 t ha^{-1} (22.4%), along with 0–20, 20–40 and 40– 60 cm respectively. Moreover, the depthwise share of total fine root biomass in the hill mid was 0.797 (53.2%), 0.395 (26.3%) and 0.307 t ha^{-1} (20.5%) and in the hill base was



Figure 2. Box plot showing fine root biomass (t ha^{-1}) in different forest types.

Particulars	Forest type	Descriptive analysis	Statistic	Standard error
Biomass	Oak	Mean	0.722	0.06
		95% Confidence interval for mean		
		Lower bound	0.57	
		Upper bound	0.87	
		5% Trimmed mean	0.73	
		Median	0.73	
		Variance	0.04	
		Standard deviation	0.19	
		Minimum	0.35	
		Maximum	1.00	
		Range	0.64	
		Interquartile range	0.26	
		Skewness	-0.74	0.72
		Kurtosis	0.55	1.40
	Pine	Mean	0.61	0.06
		95% Confidence interval for mean		
		Lower bound	0.46	
		Upper bound	0.75	
		5% Trimmed mean	0.61	
		Median	0.68	
		Variance	0.03	
		Standard deviation	0.18	
		Minimum	0.34	
		Maximum	0.83	
		Range	0.49	
		Interquartile range	0.35	
		Skewness	-0.55	0.72
		Kurtosis	-1.48	1.40
	Sal	Mean	1.11	0.04
		95% Confidence interval for mean		
		Lower bound	1.02	
		Upper bound	1.20	
		5% Trimmed mean	1.11	
		Median	1.09	
		Variance	0.013	
		Standard deviation	0.11	
		Minimum	0.94	
		Maximum	1.26	
		Range	0.32	
		Interquartile range	0.20	
		Skewness	-0.23	0.72
		Kurtosis	-1.48	1.40

 Table 1. Descriptive statistics for fine root biomass in different forest types

0.790 (38.3%), 0.863 (41.8%) and 0.410 t ha⁻¹ (19.9%), along the same depth intervals respectively. The maximum share of root mass was at 0–20 cm soil depth interval for most of the sample plots, except at the hill base, where root mass was maximum at 20–40 cm soil depth interval. Table 3 shows the fine root biomass measured in each sample plot at various depth intervals in the pine forest.

Sal forest

As indicated in Table 4, the overall fine root biomass in sal-dominated forest was highest at the hill base $(3.496 \pm 0.475 \text{ t ha}^{-1})$, followed by hill mid $(3.408 \pm 0.518 \text{ t ha}^{-1})$ and hill top $(3.108 \pm 0.349 \text{ t ha}^{-1})$. The depthwise share in total biomass of fine roots in different soil depth intervals, i.e. 0-20, 20-40 and 40-60 cm was 1.338 (43.1%), 1.309

(42.1%) and 0.461 t ha⁻¹ (14.8%) at the hilltop, 1.755 (51.5%), 1.112 (32.6%) and 0.541 t ha⁻¹ (15.9%) in the hill mid, and 1.580 (45.2%), 1.338 (38.3%) and 0.483 t ha⁻¹ (16.5%) at the hill base respectively. The largest root biomass in this forest was found in the top layer of the soil depth range, i.e. 0-20 cm in each study plot. Table 4 shows the fine root biomass measured in each sample plot at various depth intervals in the sal forest.

Statistical analysis

Before conducting ANOVA, the assumptions of normality of the data were tested. The kurtosis values and biomass skewness were within the range -1.96 to +1.96, indicating that the data are normally distributed. The test of normality for skewness and kurtosis is given in <u>Supplementary</u>

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Forest	Forest site	Tree girth (cm)	Soil depth (cm)	Fine root bi	omass (t ha ⁻¹) in		
				Ι	II	III	Mean value \pm SE (t ha ⁻¹)
Oak	Hilltop	151	0–20	0.483	1.624	0.658	0.921 (36.9) ± 0.355
			20-40	1.053	1.141	1.360	1.185 (47.5) ± 0.091
			40-60	0.505	0.219	0.439	0.388 (15.6) ± 0.086
			Total	2.040	2.984	2.457	$2.494(100.0) \pm 0.533$
			Mean	0.680	0.995	0.819	0.831 (33.3) ± 0.091
	Hill mid	85	0–20	1.097	0.548	1.360	1.002 (51.1) ± 0.239
			20-40	0.702	0.307	0.702	0.570 (29.1) ± 0.132
			40-60	0.351	0.197	0.614	0.388 (19.8) ± 0.122
			Total	2.150	1.053	2.677	$1.960(100.0) \pm 0.493$
			Mean	0.717	0.351	0.892	0.653 (33.3) ± 0.160
	Hill base	182	0-20	0.702	1.251	0.176	0.709 (34.6) ± 0.311
			20-40	1.141	0.812	0.921	$0.958(46.8) \pm 0.097$
			40-60	0.351	0.351	0.439	$0.380(18.6) \pm 0.029$
			Total	2.194	2.413	1.536	$2.048(100.0) \pm 0.437$
			Mean	0.731	0.804	0.512	$0.683(33.3) \pm 0.088$

Table 2. Fine root biomass in oak forest (values inside parenthesis are percentage of contribution)

Table 3. Fine root biomass in pine forest

			Fine root biomass (t ha ⁻¹) in each sample plot				
Forest site	Tree girth (cm)	Soil depth (cm)	Ι	II	III	Mean value \pm SE (t ha ⁻¹)	
Hilltop	155	0–20	1.316	0.483	1.141	0.980 (51.7) ± 0.254	
		20-40	0.548	0.351	0.570	$0.490(25.9) \pm 0.070$	
		40-60	0.329	0.176	0.768	$0.424(22.4) \pm 0.178$	
		Total	2.194	1.009	2.479	$1.894(100.0) \pm 0.502$	
		Mean	0.731	0.336	0.826	0.631 (33.3) ± 0.150	
Hill mid	76	0–20	0.548	1.141	0.702	0.797 (53.2) ± 0.178	
		20-40	0.395	0.527	0.263	$0.395(26.3) \pm 0.076$	
		40-60	0.329	0.461	0.132	$0.307(20.5) \pm 0.096$	
		Total	1.272	2.128	1.097	$1.499(100.0) \pm 0.350$	
		Mean	0.424	0.709	0.366	0.500 (33.3) ± 0.106	
Hill base	65	0–20	1.009	0.570	0.790	0.790 (38.3) ± 0.127	
		20-40	0.592	1.426	0.570	0.863 (41.8) ± 0.282	
		40-60	0.439	0.307	0.483	0.410 (19.9) ± 0.053	
		Total	2.040	2.304	1.843	$2.062(100.0) \pm 0.462$	
		Mean	0.680	0.768	0.614	$0.687(33.3) \pm 0.045$	
	Forest site Hilltop Hill mid Hill base	Forest siteTree girth (cm)Hilltop155Hill mid76Hill base65	Forest siteTree girth (cm)Soil depth (cm)Hilltop1550-20 20-40 40-60 Total MeanHill mid760-20 20-40 40-60 Total MeanHill base650-20 20-40 40-60 Total MeanHill base650-20 	Forest site Tree girth (cm) Soil depth (cm) I Hilltop 155 0–20 1.316 20–40 0.548 40–60 0.329 Total 2.194 Mean 0.731 Hill mid 76 0–20 0.548 20–40 0.395 40–60 0.329 Total 2.194 Mean 0.731 Hill mid 76 0–20 0.548 20–40 0.395 40–60 0.329 Total 1.272 Mean 0.424 Hill base 65 0–20 1.009 20–40 0.592 40–60 0.439 Total 2.040 0.592 40–60 0.439 Total 2.040 Mean 0.680 0.680	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	

<u>Table 1</u>. As shown in <u>Supplementary Table 2</u>, the Shapiro– Wilk test also suggests that the data are normally distributed when the significance value is less than 0.05.

Fine root biomass across different forest types

The one-way ANOVA revealed that the fine root biomass differed significantly between forest types. Multiple comparisons were analysed using a post-hoc test (Tukey HSD test). The findings revealed that fine root biomass varied significantly depending on forest type. The highest fine root biomass was found in the sal forest, followed by oak and pine forests. Thus, fine root biomass followed the pattern: sal forest > oak forest > pine forest, which was significant at 0.05 level. Table 5 presents ANOVA and Tukey HSD test for fine root biomass across different forest types.

Fine root biomass across different soil depth intervals

Fine root biomass was found to decrease with increasing soil depth. The highest fine root biomass was concentrated at a soil depth of 0–20 cm (1.10 t ha⁻¹), followed by 20–40 (0.91 t ha⁻¹) and 40–60 cm (0.43 t ha⁻¹) as shown in <u>Supplementary Table 3</u>. The decrease in fine root biomass with increase in soil depth was not the same for all plots of different forest types. However, statistical analysis showed that fine root biomass was maximum at 0–20 cm and decreased with increasing soil depth interval. The trend in fine root biomass was as follows: 0-20 > 20-40 > 40-60 cm, significant at 0.05 level. Table 6 presents the one-way ANOVA and Tukey HSD test for fine root biomass across different soil depth intervals.

			Table 4. Fir	ne root biomass in	sal forest		
Forest				Fine root biomass (t ha ⁻¹) in each sample plot			
	Forest sites	Tree girth (cm)	Soil depth (cm)	Ι	II	III	Mean value \pm SE (t ha ⁻¹)
Sal	Hilltop	82	0-20	1.492	1.053	1.470	1.338 (43.1) ± 0.143
	-		20-40	1.141	1.558	1.229	1.309 (42.1) ± 0.127
			40-60	0.505	0.307	0.570	$0.461(14.8) \pm 0.079$
			Total	3.137	2.918	3.269	3.108 (100.0) ± 0.349
			Mean	1.046	0.973	1.090	$1.036~(33.3)\pm 0.034$
	Hill mid	165	0-20	1.931	1.251	2.084	1.755 (51.5) ± 0.256
			20-40	1.448	0.878	1.009	1.112 (32.6) ± 0.173
			40-60	0.395	0.702	0.527	0.541 (15.9) ± 0.089
			Total	3.774	2.830	3.620	$3.408(100.0) \pm 0.518$
			Mean	1.258	0.943	1.207	1.136 (33.3) ± 0.098
	Hill base	65	0–20	1.667	1.755	1.316	1.580 (45.2) ± 0.134
			20-40	1.294	0.856	1.865	$1.338(38.3) \pm 0.293$
			40-60	0.636	0.614	0.483	$0.578(16.5) \pm 0.048$
			Total	3.598	3.225	3.664	$3.496(100.0) \pm 0.475$
			Mean	1.199	1.075	1.221	$1.165(33.3) \pm 0.046$

 Table 5.
 Statistical tests to determine the difference in fine root biomass among different forest types

	Sum of square	df	Mean square	F	Sigma	
One-way ANOVA (de	pendent variable = bio	omass)				
Between groups	1.267	2	0.633	22.448	0.0000003	
Within groups	0.677	24	0.028			
Total	1.944	26				
					95% Confider	nce interval
Forest type		Mean difference	Standard error	Sigma	Lower bound	Upper bound
Multiple comparison (Tukey HSD)					
Oak	Pine	0.11633	0.07918	0.323	-0.0814	0.3141
	Sal	-0.39011*	0.07918	0.000	-0.5878	-0.1924
Pine	Oak	-0.11633	0.07918	0.323	-0.3141	0.0814
	Sal	-0.50644*	0.07918	0.000	-0.7042	-0.3087
Sal	Oak	0.39011*	0.07918	0.000	0.1924	0.5878
	Pine	0.50644*	0.07918	0.000	0.3087	0.7042

*Mean difference is significant at 0.05 level.

Discussion

In oak, pine and sal forests, cumulative fine root biomass varied from 1.960 to 2.494, 1.499 to 2.062 and 3.108 to 3.496 t ha⁻¹ respectively. Fine root biomass estimations for oak and pine forests were greater than the reported values of 1.475 t ha⁻¹ for oak and 0.960 t ha⁻¹ for pine forests²¹. The present values are higher due to the marked difference in fine root diameter ≤ 1 mm. The fine root biomass of sal forests, on the other hand, was close to the values observed in sal forests of Nepal (3.200 and 3.700 t ha⁻¹)^{23,24}. Variation in fine root biomass was also observed for each forest at different sites (hilltop, hill mid and hill base). Comparatively, fine root biomass was higher in the hill base than in the other sites for each forest. This could be due to more fresh litter on the surface soil near the hill base. This provides the surface roots shelter and adds nutrients to the soil. It also creates a humid environment for new roots to grow and develop. In the study area, sal forest had substantially finer root biomass than oak and pine forests. Since sal and oak trees are deciduous, their fine root biomass is larger. According to studies, deciduous plants have more fine root organic matter than conifers^{2,24,25}. The concentration of nitrogen on the forest floor, varied soil types, and varying climate patterns in these diverse forest types could influence the fine root biomass.

The results of this study reveal considerable variance in fine root biomass across the soil at different depths. In each forest type, fine root biomass decreased as soil depth increased. These findings are consistent with those from studies across different forests of the world^{7,26,27}. Since the soil is porous and offers more nutrients at this depth, high fine root biomass in the upper soil layer is especially more frequent in Indian Himalayan forests^{28,29}. Fine root biomass was found to be concentrated in soil layers deeper

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	Sum of squares	df	Mean square	F	Sigma	
One way ANOVA (dependent variable =	biomass)				
Between Groups	2.131	2	1.065	11.675	0.00028	
Within Groups	2.190	24	0.091			
Total	4.321	26				
					95% Confide	nce interval
Soil depth (cm)		Mean difference	Standard error	Sigma	Lower bound	Upper bound
Multiple comparisor	ns (Tukey HSD)					
0-20	20-40	0.18356	0.14240	0.415	-0.1721	0.5392
	40-60	0.66611*	0.14240	0.000	0.3105	1.0217
20-40	0-20	-0.18356	0.14240	0.415	-0.5392	0.1721
	40-60	0.48256*	0.14240	0.007	0.1269	0.8382
40–60	0-20	-0.66611*	0.14240	0.000	-1.0217	-0.3105
	20-40	-0.48256*	0.14240	0.007	-0.8382	-0.1269

Table 6. Statistical tests to determine the difference in fine root biomass for different soil depths intervals

*Mean difference is significant at 0.05 level.

than 30 cm in various studies^{30–32}. This occurs when precipitation is higher in the study area and mostly depends upon the season of data collection. When nutrients are well accumulated and leached from the surface to deeper soil layers, fine root biomass is more concentrated in the deeper soil layers³⁰.

Fine root biomass contribution varied between 75% and 85% of total biomass within 0–40 cm soil depth for each forest in the study, which was lower than the contribution (90%) in pine and oak forests of Central Himalayas³³. The values observed in this study are higher, with Rawat³⁴ reporting 70.8% of total biomass within 0–40 cm soil depth and Noguchi *et al.*²⁶ reporting 74–93% fine root distribution within 0–40 cm soil depth along different elevation gradients in tropical moist forests of the Amazon. The reason for variation could be the seasonal changes in the soil profile, its physical and chemical composition, topography, forest types, species composition and forest floor conditions. Forest type, soil condition, stand age and sample method influence fine root organic matter, production, turnover and nutrients³⁵.

Conclusion

Fine roots are an essential component of forest carbon flow, contributing a considerable amount of net primary production of the forest ecosystem. They also play an important part in the nutrient cycle and carbon budgeting of the soil system. The aim of this study was to assess the fine root biomass in various forest types in Central Himalaya. The fine root biomass varied significantly across three dominant forest types, i.e. sal forest > oak forest > pine forest. Fine root biomass was also shown to decrease as soil depth increased, i.e. 0-20 > 20-40 > 40-60 cm. These findings provide baseline information that may aid future studies on nutrient availability as well as the global carbon budget.

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