Development of root biomass in an *Eucalyptus globulus* plantation under different water and nutrient regimes

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Abstract

The distribution along the soil profile of *Eucalyptus globulus* root biomass was followed in a plantation in central Portugal at 1, 2 and 6 years after planting, using an excavation technique. The experimental design consisted of a control (C) and 3 treatments: application of solid fertilizers twice a year (F), irrigation without the application of fertilizers (I) and irrigation combined with liquid fertilizers (IL). Below- and above-ground biomass decreased as follows: IL>I>F>C. So, water stress limited growth more severely than nutrient stress. The roots rapidly colonized the top soil volume (0–20 cm depth) during the first year after planting. Fine root biomass 6 years after planting was 2.2, 1.8 and 1.6 times higher in IL treatment than it was respectively in control, and in F and I treatments. The distribution of fine roots along the soil profile 6 years after planting was more even in IL compared to the other treatments. However, fine roots in the top soil were more concentrated along the tree rows in the irrigated treatments than in the others. The proportion of below-ground biomass relative to the total tree biomass and the root/shoot ratio were higher in C than in the treatments at early growth stages. This pattern was not so clear 6 years after planting, due to the increased proportion of the tap root relative to total biomass, especially in the IL treatment.

Introduction

Partitioning of biomass and nutrients between the different parts of a tree depends on genotype, site characteristics and silvicultural techniques (Cromer, 1984; Keyes and Grier, 1981; Miller, 1986, 1989; Santantonio, 1989). Moreover, the allocation of carbon to roots is probably altered by the water and nutrient status of the soil (Axelsson and Axelsson, 1986; Keyes and Grier, 1981; Persson, 1983).

Plantations of blue gum (*Eucalyptus globulus* Labill.) have been established in Portugal on a wide range of site conditions. Their above-ground production is usually high when compared with other forest tree plantations in temperate regions (Fabião, 1986), in spite of being limited by water stress during summer. Irrigation during the dry season increased above-ground biomass production more than nutrient supply without irrigation, at least in early growth stages

(Madeira and Pereira, 1990/91; Pereira et al., 1987, 1989; Pereira and Pallardy, 1989).

Root growth and distribution within the soil profile, have already been studied in rainfed and unfertilized *E. globulus* plantations growing in different soil types in central Portugal (Fabião et al., 1985, 1987). It was concluded that the amount and distribution of root biomass within the soil profile were strongly dependent on soil texture and on the availability of water and nutrients. Stands growing in a coarse soil had higher total- and fine-root biomass than stands in a finer soil (Fabião et al., 1987). The fraction of the total stand biomass corresponding to roots was higher at drier than at wetter sites. Measurements of the seasonal growth of fine-roots indicated that the growth rate was highest between late spring and early summer, when soil moisture was still high enough to support root growth and mean monthly air temperatures ranged between 15 and 20°C (Fabião et al., 1994).

Root growth was found to be also affected by soil preparation techniques. Madeira et al. (1989) compared superficial disc harrowing with deep ploughing in a Cambisol, with respect to their effects on below- and above-ground biomass production, as well as soil properties. Although significant differences between treatments in above-ground biomass were not found at early growth stages (14 months after planting), it was observed a more even distribution of roots along the vertical soil profile in the deep ploughed plots than in those with superficial disc harrowing. On the other hand, in the deeply ploughed plots, the highest proportions of both coarse and fine roots were found below 20 cm depth.

The influence of site characteristics and silvicultural factors on root growth along the soil profile are very difficult to assess without a well controlled experimental approach (Santantonio, 1989). Therefore, the influence of water and nutrient availability on *E. globulus* production was investigated on a large scale experiment in Portugal (Pereira et al., 1989). Studies concerned with the growth of root systems and the partitioning of biomass between roots and shoots were also carried on. This paper reports on the amount of root biomass and its distribution along the soil profile, and on the biomass partitioning, both measured at early growth stages (1 and 2 years after planting) and at the end of the experiment (6 years after planting). Preliminary results were published elsewhere (Fabião et al., 1990/91, 1994).

Materials and methods

Site description

The experiment was established in a new plantation at Quinta do Furadouro, Óbidos, central Portugal (39° 20' N, 9° 13' W, 30 m elev.). The climate is of the Mediterranean type, tempered by an oceanic influence. The annual mean temperature and precipitation at Caldas da Raíña, 12 km from the site and 70 m a. s. l., are respectively 15.2°C and 607 mm. Precipitation during the dry season (usually from May to September) is in average less than 10% of the annual. However, the frequent occurrence of fogs throughout the year may smooth the summer drought. The mean monthly temperature range between 10.4°C (January) and 19.8°C (August). Temperatures below 0°C rarely occur (Reis and Gonçalves, 1981).

The soil is sandy, with a clay content lower than 4% throughout the whole profile (0–170 cm), and is classified as a Spodosol (Madeira and Pereira, 1990/91). Exchangeable bases, organic matter and available P occur in low to very low concentration in the 0–90 cm soil layer. A uniform pH of approximately 6.25 (in water) was measured throughout the soil profile (Madeira and Pereira, 1990/91; Pereira et al., 1989). The main soil characteristics after ploughing but before planting were described in Madeira and Pereira (1990/91) and are summarized in Table 1.

Experimental design

Three month old *E. globulus* seedling trees were planted at 3×3 m spacing in March 1986, after ploughing down to 80 cm depth. The seedlings were obtained from a commercial seed source. Before planting dolomitic limestone (66.5% of CaCO₃, 32.5% of MgCO₃) was applied to the experimental area at a rate of 1.5 t ha⁻¹. Each seedling was supplied at planting with 200 g of a commercial fertilizer (14.0 g of N : 18.3 g of K : 11.6 g of P).

The experimental design consisted of a control and three treatments replicated in 2 blocks and was described in detail by Madeira and Pereira (1990/91) and Pereira et al. (1989). The treatments were: daily irrigation from April to October (I); broadcast application of a pelleted fertilizer twice each year (in March and October), (F); daily irrigation combined once a week with a simultaneous application of liquid fertilizer during the dry season (IL). The control (C) consisted of rainfed plots where the application of fertilizer was limited to the initial amount supplied to all seedlings. The weekly amounts of fertilizer were lower in spring and late autumn than in summer, following the seasonality of biomass production. Approximately the same amount of water was supplied to I and IL treatments, using drip tubes placed along the tree rows. Similar amounts of nutrients were supplied to F and IL treatments. An additional amount of dolomitic limestone (4 t ha⁻¹) was applied to the plots that received fertilizer as treatment (F and IL). For more details see Pereira et al. (1989).

Field sampling

In February 1987 and 1988 six trees per treatment (three in each treatment plot of each block) were selected for root measurements from a group of trees randomly harvested for above-ground biomass measure-

Table 1. Main soil characteristics of the experimental area after ploughing, but before planting

Depth (cm)	Particle size (g kg ⁻¹)			Carbon (g kg ⁻¹)	pH	Sum of bases ----- (cmol _c kg ⁻¹) -----	Exchan. calcium	Aver. nutrients (μg g ⁻¹)	
	sand	silt	clay					K	P
0–20	933	37	30	2.8	6.2	0.75	0.56	24.7	7.8
20–40	925	41	34	2.3	6.2	0.63	0.48	26.0	8.9
40–70	931	43	26	2.6	6.3	0.48	0.38	19.0	8.3
70–90	938	33	29	1.9	6.3	0.45	0.30	20.8	8.0

ments (Pereira et al., 1989). The selection in 1987 was based on the height distribution of the trees, and in 1988 on the DBH (diameter at breast height) distribution. In each treatment plot the trees were selected as follows: one tree with height or DBH equal to the plot mean, one with height or DBH equal to the plot mean plus one standard deviation (SD), and one equal to the plot mean minus one SD. For collection of root samples a soil monolith centered around each tree stump and with a surface area of 0.5×0.5 m was excavated down to 60 cm depth. The monoliths were cut horizontally in situ in layers 10 cm thick. The soil of each layer was sifted in the field through a 2-mm mesh sieve to remove most of the mineral particles. The discarded soil fraction was examined and sifted again through a 1-mm mesh sieve to recover fine-root fragments.

In 1992 (i.e. six years after planting) only block 2 was available for the last sampling. Seven trees were randomly selected in each treatment plot for above-ground biomass measurement, as follows: 3 with DBH equal to the plot mean, 2 equal to the plot mean plus 1 SD, 2 equal to the plot mean minus 1 SD. The trees were felled, divided into tree biomass components (stem with bark, branches and leaves) and weighed. Subsamples were collected for dry weight determination. Due to the large tree size, a different method was used to obtain the root mass in 1992. One tree with a DBH representing the treatment mean was chosen in each plot, taking care to avoid trees near clearings or close to other trees exceptionally large or small. After felling the chosen trees about 10 cm above the ground, a soil volume with a surface area of 3×3 m, i.e. the average area per tree, centered on the tree stump, was excavated down to 80 cm depth. The whole monolith was divided into 8 subsamples. The depth stratum 0–20 cm was divided into 4 equal subsamples (0.75×3 m, the larger side parallel to the drip tubes along the tree rows); the depth strata 20–50 cm and 50–80 cm were divided

into two subsamples each (1.5×3 m). The tap root with the remaining stump was taken independently of the described excavation. The excavated soil with roots was sifted through a 5-mm mesh sieve. After homogenization of sifted soil, 8 subsamples per stratum were taken to quantify the amount of fine root fragments passed through the sieve. All samples were sealed in plastic bags and kept frozen until laboratory handling.

Laboratory procedures

The subsamples from the above-ground tree components (1992) were oven dried at 80–85°C and weighed. The dry weight of the components was calculated from moisture content of those subsamples. The biomass of the components and of the whole above-ground was calculated on a tree basis by averaging the sampled trees.

The root samples were dry cleaned using a brush and, when needed, sifted through a 2-mm sieve followed by a 1-mm mesh sieve. Roots were divided into three diameter classes: ≤ 2 mm (fine roots), 2–30 mm (intermediate roots) and ≥ 30 mm (coarse roots). They were oven dried at 80–85°C and weighed. Roots and rhizomes from species other than *Eucalyptus*, which were found in small amounts, were discarded. Dead roots were found in proportions roughly similar in all treatments and were not considered in this study.

The subsamples obtained from the soil fraction discarded during the field procedures in the 1992 root excavation were dry-sifted through a 1-mm mesh sieve and fine root fragments were recovered. They were oven-dried at 80–85°C and weighed. An average correction factor for fine-root biomass was calculated for each treatment. The ash content of all root samples was determined by combustion for 6 h at 450°C. All below-ground biomass data presented here are expressed as

ash-free dry mass, to eliminate the effect of remaining soil particles (Kelly and Joslin, 1989; Persson, 1990).

Statistical analysis

Treatments were compared in terms of their biomass using analysis of variance and Duncan's new multiple range test for mean separation analysis (Steel and Torrie, 1980). Since in 1992 only one tree per treatment was selected for below-ground sampling, a statistical analysis of this fraction was not possible. Nevertheless, the corresponding above-ground data were subjected to an analysis of variance considering each tree has a replicate, since no marked trends in topography or soil properties were observed within block 2. The 0.05 probability level was used for all comparisons.

Results

Below- and above-ground biomass are shown in Table 2 for measurements made at 1 and 2 years after planting and in Table 3 at 6 years after planting. The total biomass (above- plus below-ground) for all treatments, also presented in the same tables, was always ranked as $IL > I > F > C$. The biomass per tree in the IL treatment was 2.35, 2.48 and 1.67 times larger than in the control (C) at 1, 2 and 6 years after planting, respectively. The biomass obtained 1 year after planting was significantly higher in both irrigated treatments (I and IL) than in the rainfed ones (C and F). In the second year, the total amount in IL was significantly higher than in any other treatment, but I and F were not significantly different (cf. Table 2). Differences between treatments appear to decrease with the age of the trees.

The below-ground biomass was also higher in the irrigated and fertilized treatments than in the control. Both irrigated treatments (I and IL) had significantly higher amounts of root biomass than C and F 1 year after planting, whereas in the second year only IL and C were significantly different. The ratio between below-ground biomass in the IL treatment and in the control (C) six years after planting was of the same magnitude as that for the above-ground biomass. The difference between C, F and I were smaller for total root biomass than for the above-ground total.

The proportion of below-ground biomass relative to the total was higher in C than in the treatments: 19.7, 16.1 and 22.3% of the total were found in the below-ground organs respectively 1, 2 and 6 years after planting. The lowest proportions were observed

in IL one and two years after planting (respectively 15.1 and 10.2%), and in I six years after planting (17.3%). The differences between F and I in the proportions of below-ground relative to the total were small in the first and second years (cf. Table 2).

The root/shoot ratio, i. e., the ratio between below- and above-ground totals, was highest in the control (C) and lowest in the IL treatment one and two years after planting (cf. Table 2). Six years after planting the highest values were found in IL and C (respectively 0.28 and 0.29), (cf. Table 3). Differences in root/shoot ratio between F and I treatments were negligible along the experimental period.

The biomass of coarse, intermediate and fine roots is also presented in Tables 2 and 3. The coarse root fraction was usually heavier in both irrigated treatments than in F treatment and in control, with the difference between IL and C being significant 2 years after planting. The difference between irrigated (I and IL) and rainfed treatments (C and F) was significant for intermediate diameter roots 1 year after planting. The proportion of coarse plus intermediate roots (including the tap root) relative to the total biomass was highest in the control (17.3 and 15.7%) and lowest in IL (14.3 and 10.0%) 1 and 2 years after planting, respectively. At harvest such a proportion was highest in C and lowest in I; however, if the tap root was not included, the differences between F, I and IL were very small (5.9% in I to 6.9% in F), with C still keeping an higher proportion (10.2%).

The amount of fine root biomass was not significantly different between treatments at early growth stages, but 6 years after planting it was much higher in IL than in the other treatments. Its proportion relative to the total tree biomass was very low at the three sampling occasions (cf. Tables 2 and 3).

More than 50% of the root biomass other than the tap root in the IL treatment was concentrated in the 0–20 cm soil layer 6 years after planting (Fig. 1), while in the other treatments the proportion ranged between 35 and 40% only. In the I treatment and in the control, more than 40% of this root biomass was in the 20–50 cm layer. For all treatments, the biomass of these roots per unit soil depth decreased with increasing depth, but faster in IL than in the others. In contrast, the fine root biomass was more evenly distributed along the soil profile in IL treatment than in the other treatments (Fig. 2). For example, the proportion of the total fine root biomass in the top soil was between 44.2 and 47.4% in C, F and I, while in IL, was only about 35%.

Table 2. Average above-ground total (abgt), below-ground total (blgt) and partitioning of root biomass in the treatments and in the control 1 and 2 years after planting. The tap root is included in the coarse and/or intermediate diameter fractions. In each year, values in the same column followed by different letters are significantly different ($p=0.05$). (C: control; F: application of fertilizers; I: irrigation; IL: irrigation plus application of liquid fertilizers)

Treatment	Biomass components (kg per tree)						Root/ /Shoot Ratio
	abgt ^w	Coarse	Interm.	Fine	blgt	Average total per tree	
		roots (d≥30mm)	roots (2–30mm)	roots (d≤2mm)			
1st. year							
C	1.77a	0.24	0.14a	0.06	0.43a	2.20a	0.245
F	2.36ab	0.26	0.14a	0.04	0.45a	2.80a	0.190
I	3.71bc	0.42	0.24b	0.07	0.72b	4.44b	0.195
IL	4.39c	0.46	0.28b	0.05	0.78b	5.18b	0.178
2nd. year							
C	9.70a	1.57a	0.25	0.04	1.86a	11.56a	0.192
F	14.46ab	1.88ab	0.35	0.05	2.27ab	16.73ab	0.157
I	15.47b	2.16ab	0.29	0.10	2.55ab	18.02b	0.165
IL	25.75c	2.56b	0.30	0.07	2.93b	28.68c	0.114

^w Madeira and Pereira (1990/91); Pereira et al. (1989).

Table 3. Average above-ground total (abgt), below-ground total (blgt) and partitioning of root biomass in the treatments and in the control 6 years after planting. Units and treatments are the same as in Table 2. Values of above-ground biomass were not significantly different ($p=0.05$)

Treatment	Stem	Branch.	Leaves	abgt	Tap root	Biomass components (kg per tree)				Average total per tree	Root/ Shoot Ratio
						Coarse roots ($d \geq 30\text{mm}$)	Interm. roots ($2-30\text{mm}$)	Fine roots ($d \leq 2\text{mm}$)	blgt		
C	65.83	8.31	10.15	84.29	11.66	6.59	4.52	1.37	24.13	108.42	0.286
F	79.80	8.52	10.41	98.73	13.19	2.10	6.29	1.69	23.27	122.01	0.236
I	109.11	9.25	11.99	130.35	16.19	4.23	5.01	1.93	27.36	157.71	0.210
IL	120.46	10.80	10.42	141.68	24.74	7.97	3.98	3.04	39.74	181.42	0.280

The fine root biomass in the 0–20 cm top soil, 6 years after planting, was concentrated along the tree rows corresponding to the irrigation tubes in I and IL treatments (Table 4). The area close to the irrigation tubes had 63.9% (I) to 80.1% (IL) of fine root biomass in the top soil. In C and F the proportions found in a similar area were about 50%, corresponding to a regular horizontal distribution of fine root biomass in the top soil. In contrast, the coarse plus intermediate roots (tap root not included) in all treatments were concentrated along the tree rows.

Discussion

The rate at which water and nutrients become available in the root medium has a decisive effect on plant nutrition and growth rate (Ericsson, 1981; Ingestad, 1988; Vogt and Bloomfield, 1991). Therefore, irrigation during the dry season combined with the weekly application of liquid fertilizers was expected to have the greatest effect on biomass production. Such an effect was observed on both above- and below-ground production at early growth stages and at the end of the

Table 4. Relative distribution of root biomass in the 0–20 cm depth top soil within and between tree rows 6 years after planting. The root amounts correspond to ash free biomass. The treatment symbols are as in Table 2

	Treatment	Within tree rows			Between tree rows		
		Fine roots	Other roots	Total in rows	Fine roots	Other roots	Total bet. rows
C	kg/tree	0.30	3.50	3.80	0.35	0.24	0.59
	%	46.6	93.5	86.6	53.4	6.5	13.4
F	kg/tree	0.38	2.53	2.91	0.38	0.72	1.09
	%	50.4	78.0	72.8	49.6	22.0	27.2
I	kg/tree	0.55	2.79	3.34	0.31	0.41	0.72
	%	63.9	87.2	82.3	36.1	12.8	17.7
IL	kg/tree	0.85	5.84	6.69	0.21	0.76	0.97
	%	80.1	88.6	87.4	19.9	11.4	12.6

Fig. 1. Distribution of total root biomass within the 0–80 cm soil profile at 6 years after planting, in relation to treatment (C-control; F-application of fertilizers; I-irrigation; IL-irrigation plus application of liquid fertilizers).

experiment, i.e. 6 years after planting. Furthermore, the results obtained confirm that irrigation only was more effective in promoting below-ground biomass production than the application of fertilizers without irrigation, as found for the above-ground production (Pereira et al., 1989). The effect of treatments on total biomass production decreased with tree age, as report-

Fig. 2. Distribution of fine roots within the 0–80 cm soil profile at 6 years after planting, in relation to treatment. Legend as in Figure 1.

ed by Cromer and Williams (1982) for above-ground biomass production in an *E. globulus* fertilizer trial (cf. Tables 2 and 3).

The total root biomass (tap root not included) down to 80 cm depth obtained in the control 6 years after planting (12.48 kg per tree) was lower than that obtained down to 70 cm depth by Fabião et al. (1987, 1994) in an unfertilized and rainfed 12-year-old stand

in a neighbour area and in a similar type of soil (19.64 kg per tree). The latter, obtained by wet-sieving with tap water, was not on an ash free basis. In contrast, in an 18-year-old stand established in a sandy loam to clay loam Eutric Cambisol also in a neighbour area (Fabião et al., 1987, 1994), the total amount of root mass (11.11 kg per tree) was slightly lower than that in the above mentioned control.

The seasonal periodicity of fine root growth may influence considerably its quantification (Santantonio et al., 1977). The amount of fine roots found in the control 6 years after planting (1.37 kg per tree at early spring) was much lower than those found in the above mentioned 12- and 18-year-old stands (respectively 9.18 and 5.76 kg per tree). In the 12-year-old stand, Fabião et al. (1985, 1994), using a mesh-bag technique, found a peak of fine root growth at late spring 2.8 times higher than that in winter. Therefore, in the present experiment roots were harvested before the season when fine root biomass would be at a maximum. In the experiment reported here, a minirhizotron technique was used to study the fine root growth and also found it to be 2.6 to 3.2 times lower in March than at its maximum.

Fertilizers and water supply also influenced the biomass partitioning. Irrigation, combined or not with fertilizers, induced a lower biomass partitioning to below-ground organs and a lower root/shoot ratio in relation to the control (C) at early growth stages. A similar tendency was reported by Axelsson and Axelsson (1986) for irrigated treatments of coniferous species in Sweden. This may be mainly due to an effect of irrigation water in the improvement of tree nutrition during the dry season, leading to reduced allocation into roots compared to non-irrigated treatments (Nilsson and Wiklund, 1992; Rytter, 1989). The application of fertilizers not combined with irrigation induced a biomass partitioning pattern similar to that of the irrigation only.

Six years after planting, the proportion of below-ground biomass relative to the total was also lower in the I and F treatments than in the control (C). In contrast, such a proportion in the IL treatment was similar to that in the control. This may be due to the high proportion of biomass partitioning to the tap root in IL. In fact, the proportion of root biomass other than tap root was higher in the control (11.5%) than in the treatments (7.1–8.3%), similar to the pattern at the early growth stages (cf. Table 2). The amount of the tap root biomass also influenced the root/shoot ratio. Six years after planting, the IL treatment and the con-

trol (C) showed similar high ratio values relative to the other treatments (cf. Table 3). The ratio calculated without taking into account the tap root biomass gave a considerably higher value in the control (0.15) than in the treatments (0.09–0.11), as found at early growth stages.

The coarse plus intermediate roots were concentrated along tree rows both in the treatments and in the control. Such a concentration may be due to the root crown, which usually has a large proportion of the biomass of those roots. As an example, similar findings were reported by Rytter (1989) in an experiment with grey alder (*Alnus incana*) in a peat bog in Sweden. The application of fertilizer at planting at both sides of the seedlings within the tree row may be another factor for the above mentioned root system distribution in rainfed conditions.

The supply of water and nutrients affected the distribution of fine roots. In the 0–20 cm top soil layer, a large proportion of fine roots was close to the drip tubes in IL, six years after planting (Table 4). Such a proportion was lower in I than in IL, but still higher than in both rainfed treatments (C and F). This was apparently an adaptive response to an improved soil nutrient and water availability: fine roots were most frequent where water was available. A high density of fine roots along the drip tubes was already observed during the second and the third year of the experiment (Fabião et al., 1990/91). Ahlström et al. (1988), Axelsson and Axelsson (1986) and Persson (1980, 1983) reported similar results in boreal forests. The evenness of the horizontal distribution in the control (C) and in the F treatment was already observed in the first and second years after planting (Fabião et al., 1994). Rytter (1989) reported a similar fine root distribution in a grey-alder experiment where irrigation combined with the application of fertilizers was regular over the whole soil surface.

The distribution of fine roots with depth was more regular in IL than in the other treatments (Fig. 2). Probably, the nutrients followed the irrigation water down through the soil profile, resulting in a generally deeper root distribution along the irrigation tubes. In I, on the other hand, no fertilizer was supplied, and nutrient mineralization was concentrated in the top soil, where the content of organic material was high (Kätterer et al., 1994).

The combination of excavation and minirhizotron techniques in the same field experiment was found to be a promising approach for the estimation of root biomass and its seasonal dynamics. The interrelation

between fine root turnover and soil organic matter was confirmed at 6 years after planting: fine root biomass, the main soil carbon source during one rotation, was highest in IL, intermediate in I and F and lowest in C.

Conclusions

- Water and fertilizers supply increased both above- and below-ground biomass production. Such an effect was especially pronounced when irrigation was combined with fertilizers.
- The supply of water and fertilizers reduced the proportion of biomass partitioning to roots other than tap root. Nevertheless, differences between treatments were generally small.
- A very high proportion of the below-ground biomass production was allocated to tap root, especially in irrigated treatments.
- The irrigation design strongly influences the distribution of fine roots, but not that of the coarser root fractions, within soil depth.

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