

Fine root biomass and production in a *Salix viminalis* and *Salix dasyclados* plantation

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Received 18 September 2008, revised 15 December 2008

Abstract. The biomass, production, and spatial distribution of fine roots were studied in an Estonian short rotation willow (*Salix viminalis* and *S. dasyclados*) plantation by ingrowth cores and soil coring during the first rotation. Fine root biomass was vertically concentrated (39–54%) in the uppermost 10 cm soil layer. This pattern was not principally affected by fertilization or species. Fertilization significantly reduced the biomass and annual production of fine roots. Large differences in the ratio of fine root to aboveground production were detected between fertilized and control plots. High production of harvestable biomass in the fertilized *Salix* plots was thus associated with a fertilization-related large positive effect on wood production and a negative effect on belowground allocation.

Key words: biomass allocation, fine roots, production, short rotation forest, willow.

INTRODUCTION

Short rotation forest (SRF) plantations are used as a renewable source of energy. In temperate conditions, the annual wood biomass production of *Salix* species may approach 20 tonnes of dry wood matter per hectare (Heinsoo et al., 2002; Stolarski et al., 2008). In recent decades, applications of SRF have combined biomass production for energy and pollutant management (Mirck et al., 2005). In vegetation filters, roots take up wastewater nutrients and plant rhizosphere provides an aerobic environment to microbes that enhance nitrification of waste compounds (Mitch & Gosselink, 2000). Moreover, willows are effective in phyto-extraction of heavy metals such as Cd and Zn from contaminated soils due to their high metal concentrations and high biomass (Keller et al., 2003).

Data on belowground biomass in SRF, root distribution in the soil, and allocation patterns are important, but relatively scarce compared to abundant reports on aboveground growth and yield. Since fine roots grow, die, and decompose rapidly, the amounts of carbon and nitrogen that cycle through them

are high (Ruess et al., 2003). However, low fine root decomposition rates were found in an Estonian SRF plantation (Püttsepp et al., 2007). Roots function as important storage sites for carbohydrates and nitrogen in SRF species (Bollmark et al., 1999) and re-growth after coppicing is completely dependent on (coarse) root reserves. Improved knowledge on root biomass and production will enable to evaluate the total productivity of a SRF stand. Information on below-ground processes is also needed for a proper establishment of *Salix* plantations in order to purify wastewater and remediate contaminated soils. Moreover, root studies will help to improve the management of plantations and promote their long-term vigour.

Rytter (2001) studied the biomass partitioning in young lysimeter-grown *Salix viminalis* plants and found that the ratio of belowground to aboveground biomass decreases from 0.3–0.4 in the first growing year to 0.1 in subsequent years. The reduction of the root to shoot biomass ratio during ageing was reported for different SRF trees (Dickmann & Pregitzer, 1992). According to Rytter (2001), the fine root to aboveground production is variable ranging from 0.4 to 1.2, and depends on soil and year. The proportion of aboveground biomass and therefore also annual wood yield increase during the first years in conventional forests (Cannell, 1989). After canopy closure the proportion partitioned to wood remains almost constant (Cannell, 1989).

This study aimed to analyse the biomass and spatial distribution of fine roots in a *Salix viminalis* and *S. dasyclados* SRF plantation. The effect of fertilization on the annual fine root production was also studied. Allocation of annual production into different plant parts was determined to improve our understanding of different options for increasing the harvestable yield in SRF.

MATERIAL AND METHODS

Plant material

The experiment was conducted in a SRF plantation of Saare, eastern Estonia (58°42' N and 26°55' E). The plantation was established on a mineral soil of brown gleyic podzoluvisol type with a sandy loam texture in May 1993 with cuttings of six *S. viminalis* clones and one *S. dasyclados* clone using a randomized block design. Each clone was planted in double rows (distance between the rows 1.25 and 0.75 m) into four plots (plot size 16 m × 16 m or 8 m × 16 m). The planting density was 2 cuttings per m². In May 1994, shoots were cut at 5 cm above the ground to promote denser sprouting. Two plots per clone were annually fertilized with 60–168 kg N ha⁻¹, 0–37 kg P ha⁻¹, and 0–70 kg K ha⁻¹ during the first rotation. The exact scheme of fertilization is presented in Heinsoo et al. (2002). Mechanical and chemical weed control was carried out in 1993 and 1994.

Fine root biomass and production were estimated in one fertilized and one control plot of *S. viminalis* clone 78183 (further abbreviated as SvF and SvC, respectively) and in one fertilized and one control plot of *S. dasyclados* clone 81090 (further SdF and SdC). Clone numbers correspond to the Swedish clone numbering system. Although the initial planting density was 2 plants per m², it decreased due to stool mortality and was 1.94, 1.80, 1.84, and 1.63 plants per m² for SvC, SvF, SdC, and SdF, respectively, in 1997.

Soil cores

For soil core sampling the approach of Vogt & Persson (1991) was followed. Ten soil cores were sampled randomly from the four studied plots in June 1996 avoiding two border rows. In June 1997, soil cores were taken only from SvF and SvC plots, 10 cores per plot. A metal cylinder with a sharpened tip (diameter of cutting edge 48 mm) was used to extract soil cores of 40 cm in depth. The distance from the core centre to the nearest plant was determined during the procedure. Each core was divided into four 10 cm layers. Samples were packed in plastic bags, marked, and frozen until washing.

Roots were washed out from soil manually using sieves and fine brushes. As the understory vegetation was scarce, all the observed roots were assumed to be from *Salix* species. All roots (both living and dead) with diameter less than 2 mm were sorted out, cleaned from soil particles, dried at 80°C until constant weight, and weighed (accuracy ± 1 mg). In order to evaluate the contamination of roots with soil particles, the ash content of subsamples was determined by burning the roots at 450°C in a furnace oven. The average ash content of subsamples was 31.5% (SE = 0.5). All ash was assumed to originate from mineral soil particles and was subtracted from the dry weight of root samples. All data in the Results section are presented as ash-free dry mass of fine roots.

Ingrowth cores

Ingrowth cores consisted of a nylon netting (8 × 8 mm) pulled on a plastic tube of 54 mm diameter and inserted vertically into the hole (40 cm deep) drilled with a metal corer. Ingrowth cores were filled with sieved (nylon net 4 × 4 mm) root-free soil, taken from two different horizontal layers of the same plantation area. A wooden bat was used to repack the ingrowth core to achieve a soil bulk density similar to the natural one. The plastic tube was gradually removed during the course of core filling. Five ingrowth cores per plot were installed in spring 1996. In autumn, the ingrowth cores were extracted with a corer (inner diameter of cutting edge 120 mm). The outer edges of the nylon net were cleaned carefully and the fine roots on the outside of the netting were cut with a sharp knife. The samples taken from the netting were divided into four 10 cm layers, separated from the soil, washed with sieves and brushes, and the ash-free root dry mass was

calculated as described above. Fine root production was estimated as the total fine root ingrowth during a season. The same procedure was repeated in 1997 with five ingrowth cores per plot.

Leaf and wood production

Annual leaf and wood production were estimated using allometric relations between shoot diameter and its leaf or wood (stem + branches) biomass. To estimate leaf production, the parameters of the power relation ($LM = a \cdot D55^b$) between shoot diameter at 55 cm height (D55) and leaf dry mass (LM) were calculated for both species in August 1996 and 1997, when the leaf area had reached its seasonal maximum. The procedure and data used were published earlier (Merilo et al., 2004). The calculation procedure and data of annual wood production were also presented earlier (Heinsoo et al., 2002).

Statistical analyses

Statistical analyses were carried out using the computer software SAS GLM package. The significance of various parameters and their interactions were evaluated with analysis of variance. The multiple comparisons of average values were performed with the Least Square Means (LSM) method. The comparison between two different methods used (soil cores and ingrowth cores) was carried out with Student's *t*-test Sample for Means. The level of significance $\alpha = 0.05$ was accepted.

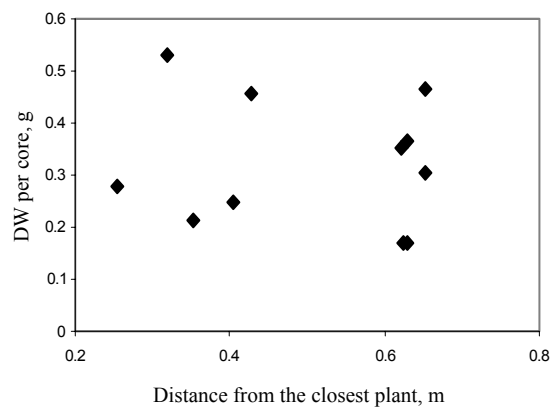
RESULTS

The average values of fine root biomass recovered per core varied between 0.323 g and 0.691 g (Table 1). The fine root biomass ranged from 1.8 to 3.8 t ha⁻¹. Comparison of corresponding pairs revealed no difference between control and fertilized Sv plots in 1996. However, fertilization reduced fine root biomass in Sd in 1996 and in Sv in 1997 (Sd plots were not estimated in 1997). In none of the plots, the amount of fine roots in soil cores depended significantly on the distance from the closest plant (see Fig. 1 for SvC), regardless of different stool density per plot.

Our results showed that fine roots of willow were concentrated near the soil surface. In 1996, 46–54% of the fine roots were found in the uppermost 10 cm layer, and the fine root biomass decreased gradually in lower soil layers (Fig. 2). The result was similar in 1997, although the proportion of the fine root biomass in the uppermost layer decreased to 39–42%. In most cases, the vertical distribution pattern of fine roots was not significantly influenced by fertilization in either species, except the significant difference in the fine root biomass between control

Table 1. Average fine root biomass (\pm SE) of *Salix viminalis* (Sv) and *S. dasyclados* (Sd) control (C) and fertilized (F) plots in 1996 and 1997. ne = not estimated

Plot	1996		1997	
	g core ⁻¹	t ha ⁻¹	g core ⁻¹	t ha ⁻¹
SvC	0.323 (\pm 0.039)	1.8 (\pm 0.22)	0.627 (\pm 0.026)	3.5 (\pm 0.15)
SvF	0.323 (\pm 0.037)	1.8 (\pm 0.21)	0.476 (\pm 0.061)	2.6 (\pm 0.33)
SdC	0.691 (\pm 0.056)	3.8 (\pm 0.31)	ne	ne
SdF	0.473 (\pm 0.034)	2.6 (\pm 0.19)	ne	ne

**Fig. 1.** Horizontal distribution of fine root dry weight (DW) in the *Salix viminalis* control plot in 1996.

and fertilized plots found for the uppermost layer of Sd plots. The control plot of Sd had significantly more roots than the control plot of Sv in the two upper layers and in the lowest layer (Fig. 2). In fertilized plots, no species differences in the vertical distribution of fine roots were detected in 1996.

The annual fine root production in the ingrowth cores ranged from 1.2 to 1.7 t ha⁻¹ in the fertilized plots and from 2.6 to 6.8 t ha⁻¹ in the control plots (Fig. 3). Because of the smaller sample size in the case of ingrowth cores, the standard error was higher (9–26% of the mean value) compared to the soil core data (Table 1). The vertical distribution of fine roots in the ingrowth cores (data not shown) did not differ significantly from that in the soil cores. The amount of fine roots in the ingrowth cores was significantly higher in the control plots compared to the fertilized plots, if corresponding pairs were compared. In the fertilized plots, the annual leaf and wood production were higher than those in the corresponding control plot (Fig. 3). This resulted in considerable differences in the ratio of fine root to aboveground (leaf + wood) production between the control and fertilized plots (Table 2). In the control plots, the fine root production in the ingrowth cores was significantly higher in 1997 than in 1996 for both species (Fig. 3). This difference was not detected in the fertilized plots.

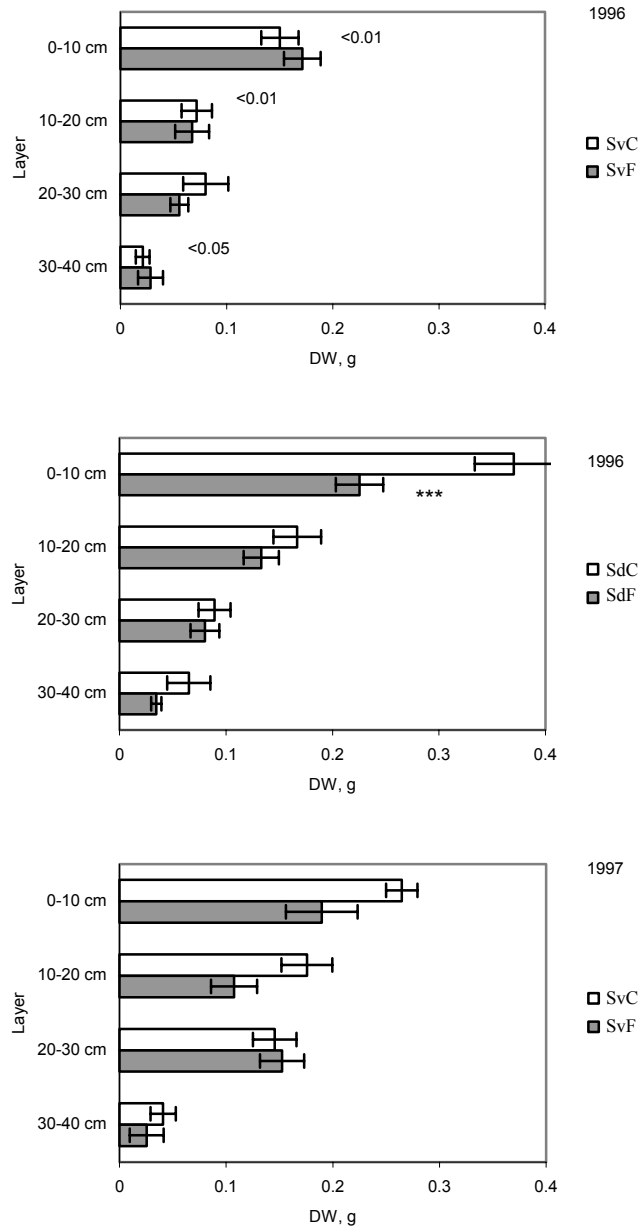


Fig. 2. Vertical distribution of fine root dry weight (DW) in 1996 and 1997. Sv – *Salix viminalis*; Sd – *Salix dasyclados*; C – control plot; F – fertilized plot. Asterisks mark significant differences between corresponding fertilized and control plots ($\alpha = 0.05$). Numbers denote significant differences in the fine root biomass between SvC and SdC plots.

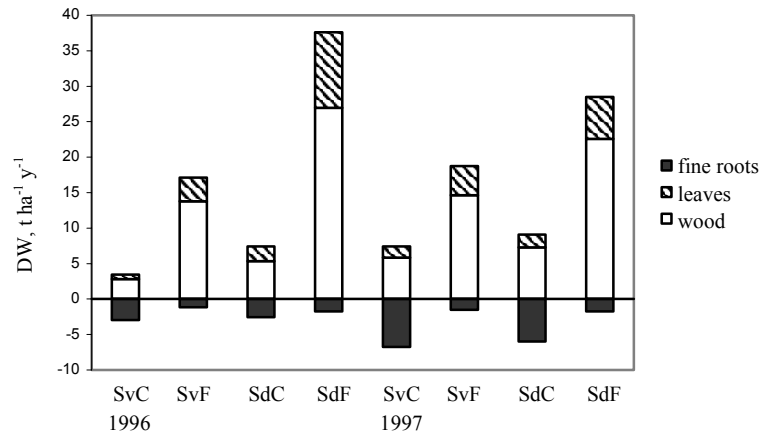


Fig. 3. Annual leaf, wood, and fine root production ($t\ ha^{-1}\ y^{-1}$). Sv – *Salix viminalis*; Sd – *Salix dasyclados*; C – control plot; F – fertilized plot; DW – dry weight.

Table 2. Total production ($t\ ha^{-1}\ y^{-1}$) and the distribution of annual production in *Salix viminalis* (Sv) and *S. dasyclados* (Sd) plots in 1996 and 1997. C – control plot; F – fertilized plot

	1996				1997			
	SvC	SvF	SdC	SdF	SvC	SvF	SdC	SdF
Total production	6.40	18.26	9.97	39.32	14.19	19.70	15.05	30.23
Fine root/aboveground	0.86	0.07	0.35	0.05	0.91	0.08	0.66	0.06
Leaf/wood	0.23	0.24	0.39	0.39	0.27	0.24	0.24	0.26

DISCUSSION

The fine root biomass obtained using soil cores ranged from 1.8 to 3.8 $t\ ha^{-1}$ and tended to be reduced by fertilization. Compared with literature data for other SRF species, our fine root biomass estimates are relatively high. In a 1–2-year-old *Populus* plantation, similar or even lower numbers were found for total (fine + coarse roots) root biomass (Pallardy et al., 2003). Fine root biomass of Sv in our plots was higher than found for the same species in Uppsala, Sweden (0.83–1.7 $t\ ha^{-1}$) (Rytter & Hansson, 1996). This difference could be explained by daily irrigation and fertilization in the Swedish experiment to create near optimum conditions, which probably decreased allocation to fine roots. High stool density and plantation age of 3–4 years probably also contributed to the high estimates of fine root biomass in our SRF plantation.

The vertical distribution of fine roots in SRF plantations depends on water availability (Dickmann et al., 1996), nutrient availability, and plant density (Dickmann & Pregitzer, 1992). Most of the fine roots of different young broad-

leaf trees are found near the soil surface (0–10 cm) (Elowson & Rytter, 1993; Al Afas et al., 2008). Fertility and temperature are higher in upper soil layers and fine root growth is correlated with soil temperature (Steele et al., 1997). The vertical distribution pattern of fine roots in our study was principally not affected by fertilization or species – about 40–50% of the fine roots were recovered in the 0–10 cm soil layer consistent with previous results for *Salix* plantations (Rytter & Hansson, 1996; Heaton et al., 2002). In the plots where fine root biomass was lower due to fertilization (Sd plots in 1996 and Sv plots in 1997), control plots tended to have more fine roots in the 0–20 cm soil layer. We did not find differences in the fine root vertical distribution between the ingrowth core and soil coring data, previously found in other tree stands (Makkonen & Helmisaari, 1999). This indicates that in fast growing SRF species ingrowth core experiments may give production and root vertical distribution data faster than in species characterized by a lower growth rate. A study from Finnish Scots pine forest showed that roots in the ingrowth cores can be compared with those in the surrounding soil only after more than three seasons (Makkonen & Helmisaari, 1999).

The horizontal distribution of fine roots was fairly uniform within the plantation (Fig. 1). Similar uniform distribution of fine roots was shown for a 4-year-old *Alnus* stand (Elowson & Rytter, 1993). Extended fine root growth and overlapping were also reported in *Populus* plantations (Dickmann & Pregitzer, 1992). We found roots longer than 6 m in our plots (data not shown). Root overlapping and horizontally uniform distribution indicate high competition for belowground resources. High planting density increases also aboveground competition for light. In 1997, the stool survival was 92–97% in the studied control plots, but 90% in the SvF and only 82% in the SdF plots. Stool mortality could thus be associated with strong above- and belowground competition and the promotive effect of high density on the spreading of various pathogens (Cambours, 2004).

The fine root production ranged from 1.2 to 3.0 t ha⁻¹ y⁻¹ in 1996 and from 1.5 to 6.8 t ha⁻¹ y⁻¹ in 1997. The finding that fine root production estimates from ingrowth cores are lower compared to production estimates from other methods, e.g. minirhizotrons or sequential soil coring (Steele et al., 1997; Makkonen & Helmisaari, 1999), was not supported by our data. The error of root production estimates resulting from the slow recolonization rate into ingrowth cores was probably small in our willow plantation, which showed a high growth rate, horizontally uniform root distribution, and high level of competition. In fact, the amount of roots recovered from ingrowth cores was sometimes even higher than that recovered from soil cores in our experiment. Establishment of ingrowth cores enabled roots to grow into a space with lower competition than in the surrounding soil and free space was quickly filled. Moreover, wounding during the installation of ingrowth cores may have triggered a compensatory fine root proliferation response.

Decreased fine root production due to fertilization was observed in our SRF plantation. In the literature, both positive and negative effects of improved N nutrition on fine root production have been reported (Burton et al., 2000; Nadelhoffer, 2000; Lee & Jose, 2003; Kern et al., 2004) and the result depends on the method used to estimate fine root production (Nadelhoffer, 2000). Higher root

production of control plots in 1997 than in 1996 may be associated with the exacerbation of nutrient limitation during the rotation in these unfertilized plots.

One way to improve wood yield in SRF could be lower assimilate allocation to roots, particularly to fine roots. Generally, the ratio of wood to foliage partitioning is rather insensitive to fertilization (Cannell, 1989), as was also found in our experiment (Table 2). The ratio of root to aboveground production was very variable, ranging from 0.05 to 0.91 in different plots and years in our experiment. The decrease in the root to aboveground biomass ratio was limited because of adverse consequences for nutrient acquisition and, thus, overall production. Literature data on root to shoot ratios are difficult to compare due to different methods used. In a lysimeter experiment with 3-year-old daily irrigated and fertilized *Salix viminalis* the ratio of fine root ($D < 2$ mm) to aboveground production was about 0.6 (Rytter, 2001). In poplar plantations, the root to shoot biomass ratios range from 0.17 to 0.36 (Scarascia-Mugnozza et al., 1997; Pallardy et al., 2003), with both positive and negative effects of fertilization detected (Dickmann & Pregitzer, 1992; Rytter, 2001). The effect of fertilization on root to shoot production ratio was drastic (about 10 times) in our experiment if corresponding plots were compared. The differences in annual fine root production between corresponding fertilized and control plots ranged from 0.85 to 5.26 t ha⁻¹, indicating a considerable potential for increasing aboveground production in some cases, but not in others.

To conclude, our *Salix* fine root biomass estimates from soil coring generally agreed with previous results on SRF species, as did also the patterns of horizontal fine root distribution, showing extended root growth and overlapping, and of vertical root distribution, showing fine root concentration into upper soil layers. Fertilization decreased fine root production and the root to aboveground production ratio. Thus, larger harvestable yield due to fertilization was associated with a considerable positive effect of fertilization on wood production and differences in production allocation, with reduced belowground allocation detected in fertilized plots.

ACKNOWLEDGEMENTS

This study was partly supported by the Estonian Science Foundation (grant No. 4831). We thank Dr Krista Lõhmus for advice and discussions and two anonymous reviewers for valuable comments.

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Peenjuurte biomass ja produktsioon paju (*Salix viminalis* ning *Salix dasyclados*) energiavõlas

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Aastatel 1996–1997 määrati Saare pajuistanduse neljal katseruudul (*Salix viminalis*'e ja *Salix dasyclados*'e väetatud ning väetamata ruudud) peenjuurte biomass, produktsioon ja ruumiline paigutus sissekasvu- ning mullasilindrite abil. Vertikaalselt kontsentreerus peenjuurte biomass ülemisse 10 cm mullakihti, sõltumata liigist ja väetustasemest. Väetamisel oli oluline negatiivne mõju peenjuurte biomassile ja produktsioonile. Peenjuurte produktsiooni suhe maapealsesse produktsiooni oli väetatud ruutudel oluliselt väiksem, võrreldes väetamata ruutudega. Seega oli väetatud ruutude kõrge saak seotud väetamise positiivse mõjuga puidu- produktsioonile ja negatiivse mõjuga maa-alusele allokatsioonile.