Tree Physiology 29, 229–238
doi:10.1093/treephys/tpn020

Adaptation of fine roots to annual fertilization and irrigation in a 13-year-old *Pinus pinaster* stand

M.R. BAKKER,[1,2] E. JOLICOEUR,[3] P. TRICHET,[4] L. AUGUSTO,[5] C. PLASSARD,[6] J. GUINBERTEAU[7] and D. LOUSTAU[4]

[1] *ENITA de Bordeaux, UMR 1220 TCEM, F-33883 Villenave d'Ornon, France*

[2] *Corresponding author (mark.bakker@bordeaux.inra.fr)*

[3] *Faculté de foresterie, Université de Moncton, Campus d'Edmundston, 165 boulevard Hébert, Edmundston, Nouveau-Brunswick, Canada*

[4] *INRA, UR 1263 EPHYSE, F-33612 Cestas, France*

[5] *INRA, UMR 1220 TCEM, F-33883 Villenave d'Ornon, France*

[6] *INRA, UMR 1222 BSR, F-34060 Montpellier, France*

[7] *INRA, UPR 1264 MYCSA, F-33883 Villenave d'Ornon, France*

Received June 26, 2008; accepted September 30, 2008; published online December 5, 2008

Summary Effects of fertilization and irrigation on fine roots and fungal hyphae were studied in 13-year-old maritime pine (*Pinus pinaster* Aït. in Soland), 7 years after the initiation of the treatments. The fertilization trials consisted of a phosphorus treatment, a complete fertilizer treatment (N, P, K, Ca and Mg), and an unfertilized treatment (control). Fertilizers were applied annually and were adjusted according to foliar target values. Two irrigation regimes (no irrigation and irrigation of a set amount each day) were applied from May to October. Root samples to depths of 120 cm were collected in summer of 2005, and the biomass of small roots (diameter 2–20 mm) and fine roots (diameter $\leq$ 2 mm) and fine root morphology were assessed. Biomass and length of hyphae were studied by a mesh ingrowth bag technique. Total fine root biomass in the litter and in the 0–120 cm soil profile ranged between 111 and 296 g m^{-2}. Results derived from the measurements of biomass and root length, or root area, showed that both fertilizer treatments reduced the size of the fine root system, especially in the top soil layers, but did not affect small roots. Compared with control treatments, fine root morphology was affected by both fertilizer treatments with the fine roots having increased specific root length/area, and irrigation tended to reinforce this finer morphology. The amount of hyphae in the mesh ingrowth bags was higher in the fertilization and irrigation treatments than in the controls, suggesting further extension of the root system (ectomycorrhizal infection) and thus of the uptake system. Irrigation had no significant effect on the size of the fine root system, but resulted in a shallower rooting system. Total root to shoot ratios were unaffected by the treatments, but fine root mass:needle mass and fine root area index:leaf area index ratios decreased with increasing nutrient supply. Overall, compared with the control fine roots, increased nutrient supply resulted in a lower fine root biomass but the dynamic fraction of the finest roots was greater. Irrigation had only limited effects on fine root size, distribution and morphology.

Keywords: annual resource optimization, fine root density, fine root morphology, hyphae, root to shoot ratios.

Introduction

Although elevated atmospheric CO_2 concentrations can potentially stimulate tree growth (DeLucia et al. 1999), this effect may be limited by low nutrient and water supplies (Oren et al. 2001, Loustau et al. 2005) and ultimately by the adaptability of the root system in facilitating adequate nutrient uptake. To provide sufficient nutrient uptake from low fertility soils, trees can display morphologic plasticity by selectively allocating biomass for growth within nutrient-rich patches (Hutchings and de Kroon 1994, George et al. 1997, Hodge 2004), or physiologic plasticity by increasing uptake rate per unit of root length (Göransson et al. 2006). The optimum morphology and physiology of fine roots depend on how a plant can best invest carbon to optimize benefits under given environmental conditions (Chapin et al. 1987, Eissenstat 1992, Ryser 2006). In addition, trees in forest ecosystems can form associations with mycorrhizal fungi (Smith and Read 1997). The growth of ectomycorrhizal hyphae can greatly enhance the absorption capacity of the root system, constituting up to 75% of the uptake potential of young pine systems (Rousseau et al. 1994). Therefore,

carbon used for soil exploration by the hyphae of ectomycorrhizal fungi can have a major effect on the nutrient uptake efficiency of forest trees.

Trials on *Picea abies* ((L.) H. Karst) and *Pinus sylvestris* (L.) in Sweden (Axelsson and Axelsson 1986), *Pinus taeda* (L.) in the southeastern USA (Albaugh et al. 1998), *Pinus radiata* (D. Don) in Australia (Waterworth et al. 2007), and *Pinus pinaster* (Aït. in Soland) in France at the site where our study was carried out (Trichet et al. 2008) have shown that increases in tree productivity can be much larger when nutrients or water, or both, are optimized continuously or added annually instead of only immediately after planting. These experiments highlighted the high responsiveness of trees to the addition of either nutrients or water, with some interactive effects of nutrients and water, depending on the local conditions. Trichet et al. (2008) showed that annual optimization of nutrients, but not irrigation, resulted in significant increases in aboveground productivity of maritime pine in the first five growing seasons after the beginning of the treatments.

As soils in the region of our study site are poor in phosphorus and water deficits in summer have occurred more frequently in recent years (Trichet et al. 2008), we investigated adaptations in the size and morphology of the maritime pine fine root system, along with associated hyphae, to annual fertilization and irrigation treatments. Previous studies on coniferous species showed a decline in fine root biomass in response to annual fertilization (Albaugh et al. 1998, Maier and Kress 2000), and overall fine root biomass of *P. pinaster* was lower at the most fertile sites in our study region compared with the least fertile sites (Achat et al. 2008). Consequently, we hypothesized that trees that are fertilized annually can meet their nutrient demands with a smaller fine root uptake system than unfertilized trees. As rooting depth is greater at drier sites than at more humid sites (Bakker et al. 2006) and as most nutrients are concentrated in the top 20 cm of the soil profile, we predicted that fine roots would be more concentrated in the upper layers of the soil (shallower rooting) in irrigated plots than in unirrigated plots.

Materials and methods

Site description

The study site is located in the Domaine de l'Hermitage experimental forest in Pierroton, 20 km southwest of Bordeaux, France (44°42′ N and 0°46′ W). Site elevation is 58 m a.s.l. and the topography is flat. Mean annual air temperature is 12.5 °C and annual precipitation averages 950 mm, with frequent prolonged droughts in summer. The soils are sandy podzols developed on an aeolian sandy deposit from the Quaternary era. Foliar nutrient concentrations in the control treatment indicated that the site is poor in phosphorus (Trichet et al. 2008). Water-table depth ranges from about 0.1 m in winter in wet years to 1.6 m in summer in dry years. In 1993, the site was ploughed to a depth of 0.3 m and planted with 1-year-old maritime pine (*P. pinaster* Aït. in Soland) seedlings at a spacing of 4.0×2.0 m. In 1998, when the stand was 6 years old, a randomized block design was established for the factorial combination of two irrigation regimes and three fertilization regimes: phosphorus only (P treatment), complete fertilization with N, P, K, Ca and Mg (F treatment), and no fertilization (C). Irrigation (I) was applied daily at a rate equivalent to the mean potential evapotranspiration (about 6 mm day^{-1}) during the summer (May–October). Fertilization was applied according to foliar analyses and target values for optimal foliar nutrition (13 mg g^{-1} for N and N/P, N/K, N/Ca and N/Mg ratios of 0.1, 0.37, 0.12 and 0.06, respectively; Trichet et al. 2008). Each of the six treatment combinations had four replicates, yielding 24 plots each measuring 60×36 m (0.216 ha). External border zones reduced the plot dimensions to 50×28 m (0.14 ha with 170 trees in each of the 24 plots). The fertilization and irrigation treatments have been applied annually since 1998, with the exception of 2000 when the trees were recovering from a storm event at the end of 1999 (see Trichet et al. 2008 for further information). In 1997, the understory was removed in the inter-tree lines with a disking device ($\sim$ 5–6 cm soil depth) resulting in some disturbance of the superficial soil. In 1998 and 1999, the understory was controlled with chemicals. From 2001 onward, a mowing machine was used and understory plant material as well as dead tree branches were crushed and left on the soil surface. Table 1 shows some stand characteristics for each treatment for the year 2005, when our study was conducted. Diameter at breast height (DBH at 130 cm) ranged from 18.9 to 24.0 cm, basal area from 25.5 to 30.5 m^2 ha^{-1} and leaf area index (LAI) from 2.6 to 3.1. Additional information on the site and treatments has been reported by Trichet et al. (2008).

Field data acquisition

Measurements (DBH, tree height, foliar nutrient concentrations and LAI) were performed every year, on all trees, or a subsample of trees, or in individual measurement plots (e.g., for LAI). Allometric relationships developed at the site were used to compute total tree needle biomass, total aboveground tree biomass (cf. Trichet et al. 2008), and the sum of total taproot + coarse root biomass (Bert and Danjon 2006). The allometric equations were: needle biomass = $1.4326(DBH^{1.9344})(AGE^{-1.4895})$, total aboveground biomass = $0.06575(DBH^{2.1469})(AGE^{0.322})$, and taproot + coarse root biomass = $84.7(C130^{2.37})$, where C130 is tree circumference at 130 cm and biomass is expressed in kg $tree^{-1}$, DBH in cm, AGE in years and C130 in m. The three years preceding our study (2002–2004) were dry years (17–21% less rain than the 30-year mean). Relative to the mean precipitation sums per season

Table 1. Treatment and stand characteristics in 2005 (stand age = 13 years).

Treatment code	C	P	F	I	IP	IF
Irrigation	None	None	None	Irrigation[a]	Irrigation[a]	Irrigation[a]
Fertilizer	Nil	P[b]	Complete F[c]	None	P[b]	Complete F[c]
Stocking (number ha^{-1})	914	832	798	870	777	680
DBH at 130 cm height[d,e,g] (cm)	18.9 (0.1)	20.8 (0.2)	21.9 (0.2)	20.5 (0.2)	22.3 (0.2)	24.0 (0.3)
Total aerial biomass[d,e] (kg $tree^{-1}$)	84.4 (1.4)	104.5 (2.2)	116.1 (2.7)	100.7 (1.9)	120.6 (2.8)	141.3 (4.0)
Basal area[d,f] (BA, m^2 ha^{-1})	25.5 (0.2)	28.3 (0.0)	30.1 (0.1)	28.5 (0.3)	30.5 (0.2)	30.5 (0.2)
Basal area increment[d,f] (BAI, m^2 ha^{-1} $year^{-1}$)	2.6 (0.0)	2.7 (0.0)	2.9 (0.0)	3.3 (0.0)	3.3 (0.0)	3.4 (0.0)
LAI[d,f] (m^2 m^{-2})	2.6 (0.0)	3.0 (0.0)	3.0 (0.0)	2.8 (0.0)	3.1 (0.0)	3.0 (0.0)

[a] Daily irrigation from May to October equivalent to about 6 mm day^{-1}.
[b] Mean rate in 1998–2005 of 32 kg P ha^{-1} $year^{-1}$.
[c] Mean rate (in kg ha^{-1} $year^{-1}$) for 1998–2005 of 84 N, 34 P, 56 K, 22 Ca, 7 Mg, 1.3 B, 2.9 Cu, 2.1 Mn and 0.6 Zn.
[d] Values refer to mean values and SE (between brackets).
[e] Mean values based on all trees present in the two blocks considered.
[f] Mean values based on sums for two blocks ($n = 2$).
[g] Using quadratic means for mean DBH.

for 1998–2007, autumn 2004 was dry (−22%), winter 2005 was very dry (−43%), spring 2005 was average (−9%), and summer 2005 was very dry (−38%).

Root samples were collected in early July 2005 in soil cores to a depth of 120 cm in two blocks (two plots per treatment). In each plot (0.14 ha), two sample zones (area around target trees; i.e., 8.0 × 4.0 m for a 4.0 × 2.0 m spacing) were selected from which four root samples were collected (yielding a total of 16 cores per treatment). The sample zones were chosen in relation to two subsequent target trees within the same tree line to enable comparisons between aboveground tree dimensions and belowground root data based on the dimensions of the trees and the root data corresponding to each zone. The target trees had to have diameters comparable to the mean of the treatment and had to be surrounded by all four neighboring trees in subsequent tree lines. The positions of the four root sample cores in each zone were aligned perpendicularly to the tree line at equal distances from both target trees. The distance between sample points was 1 m. The two central points were thus in the tree lines and the two outer points in interlines. For each core sample point, litter was sampled with a root corer device (internal diameter of core head 8 cm; maximum length 15 cm). Soil samples were taken with a percussion drill (internal diameter of 4 cm; maximum length 1 m) in two stages (0–60 and 60–120 cm) and were divided into 15-cm deep layers. One year later, in May 2006, 60 mesh bags (30 μm mesh size) were installed in the site as described by Wallander et al. (2004) to evaluate length and mass of hyphae. The mesh bags were filled with 120 g acid-washed quartz sand (passed through a 2-mm sieve) and buried in the C, P and IP treatments in the upper 5 cm of the mineral soil (20 bags per treatment). Bag positions were in tree lines at 1 m from the randomly selected trees. Half of the bags were left in the soil for 6 months (retrieved in November 2006) and the remainder for 12 months (retrieved in May 2007). Both years were slightly drier than average (−22% precipitation for 2006, −16% for 2007).

Root and mesh bag sample processing

Core samples were placed in plastic bags, transported to the laboratory the same day, and stored at 4 °C until they were processed (within 2 months). The moist samples were sieved through 4- and 2-mm sieves without water and the root fragments were transferred from the sieves to a floating basin, from which live fine roots (diameter < 2 mm) and live small roots (diameter 2–20 mm) of *P. pinaster* were collected manually using forceps. Despite the annual removal of understory vegetation, roots of understory shrubs and herbs were present in the core samples. Roots of these understory species and dead roots of *P. pinaster* were not retrieved. Species and vitality were distinguished by visible characteristics and root odor (Bakker et al. 2006). The lengths of the small roots were determined with a measuring rod. Root lengths, diameters and morphology of fine roots (specific root length, specific root area and number of root tips per unit of root length) were obtained by image analysis. The cleaned fine roots were submerged in 10% ethanol at 4 °C before being scanned. The images were analyzed using WinRHIZO Version 2005a (Regent Instruments Inc., Nepean, ON, Canada). The number of root tips counted during the analysis of these images (16 samples per treatment and soil layer) was compared with manual counts made with the aid of a stereo microscope ($n = 4$) while examining ectomycorrhizal morphotypes. Because the WinRhizo and manual counts were similar, we subsequently used the output value from WinRHIZO to estimate the number of ectomycorrhizal root tips per unit of root length (ECM-RL). Root dry mass was assessed by drying the root material at 105 °C to constant mass. Mesh bags containing quartz sand and the hyphal structures that had grown into them were kept at 4 °C until processed. They were then

opened and the sand was placed in a transparent basin to facilitate visual inspection of the sample with the aid of a binocular microscope. Hyphal length and hyphal mass were assessed as described by Wallander et al. (2004). Briefly, hyphal length was measured by placing a 15–20 g aliquot in a vial with ~ 80 ml of deionized water, shaking for 1 min, and then transferring 5 ml of the solution to a filter paper. The filter paper was sprayed with methylene blue to stain the hyphae. The quadrants of the filter paper were then observed with the aid of a stereomicroscope (100–200× magnification) and an optical monocular glass with a regular grid was placed on the parts of the filter paper where hyphal structures were detected. Intersections of the grid (interline distance of 0.625 mm) and the hyphal structures were used to calculate hyphal length as described by Tennant (1975). For hyphal mass (assessed after 12 months only), several 15–25 g samples were each placed in a sample vial containing ~ 80 ml of deionized water and shaken for 1 min. The entire solution was transferred to a filter paper in 5-ml steps. Observed hyphal structures were retrieved using forceps and transferred to petri dishes. Remaining small hyphal fragments that represented < 5% of the total amount of hyphae were evaluated visually (as a percentage of the recovered hyphal structures in the petri dishes). The retrieved hyphal structures in the petri dishes were observed with the aid of a binocular microscope and cleaned by removing adhering sand grains using forceps. Hyphal dry mass of the samples was expressed on an ash-free basis (the difference between the dry mass after 48 h at 60 °C and the ash content obtained by gradual ignition up to 550 °C for 5 h). To determine whether the hyphae were ectomycorrhizal or saprophytic species, 10 subsamples of hyphae (dried at 60 °C) as well as dried samples of carpophores collected in autumn 2006 (six ectomycorrhizal species, five saprophytic species) were analyzed for ^{13}C isotopic composition by mass spectrometry (Tracer Mass; Europa Scientific, Crewe, UK). Hyphal length and biomass were first expressed on the basis of 120 g sand and then converted to an area basis (m^2) by using 1.56 g cm^{-3} (cf. Wallander et al. 2004) as the mean density of both soil and sand and assuming that the bags were representative of the 0–10 cm soil layer. This value seems realistic as it is within the range of 1.58 ± 0.02 g cm^{-3} (mean ± SE) for the sandy soils of the study region that have a low C content (< 1 mg g^{-1}; $n = 19$, Augusto and Bakker unpublished data). Specific hyphal length was expressed as the ratio of hyphal length to hyphal mass.

Statistical analyses

Effects of treatments were evaluated separately for root distribution, root morphology, summed profile values of the roots, and for aboveground to belowground ratios. We used the SAS software Version 8.1 (SAS Institute, Cary, NC) to compute mean values and standard errors, and to test differences between treatments and soil depths. Two-way ANOVA was used to test the effects of phosphorus (P), irrigation (I) and their interaction on root and aerial parameters. Two-way ANOVA was also used to test the effects of complete fertilization (F), I and their interaction. One-way ANOVA was used to assess the general effects of the stand on hyphal parameters, followed by the Bonferroni t test to distinguish between the mean values per treatment. To assess the effects of both treatment and soil depth, mixed linear models were used with treatment, depth and treatment × depth effects as fixed effects and the root sample point as a random effect. The Bonferroni adjustment in the least squares means differences procedure was used to assess differences between treatments as a function of soil depth as well as differences between depth layers within treatments. Arc sine transformations were used for percentage values to meet model assumptions.

Results

The size of the fine root system was strongly dependent on soil depth ($P < 0.0001$). Fine root length densities were low in the shallow litter layer (0.19–0.57 cm cm^{-3}) and highest in the top 15 cm of soil (0.33–0.86 cm cm^{-3}). Fine root length densities decreased with soil depth in a similar manner in all treatments (Figure 1A and B), and no fine roots were recorded below 100 cm in cores of any of the treatments. Fine root area density, fine root biomass density and small root biomass density showed similar patterns with soil depth (data not shown separately). The effect of P on fine root length density was significant only in the 15–30 cm soil layer ($P = 0.018$), and I and the P × I interaction had no significant effects on fine root length density (Table 2) at any soil depth. The F treatment resulted in a significant decrease in fine root length density in the top 30 cm of the soil ($P < 0.01$), but I and F × I had no significant effects (Table 2). Fine root area density was affected by the treatments in a similar way as fine root length density (data not shown in detail). The morphological parameters of the fine root system, fine root diameter, specific root length, specific root area and number of ECM-RL, were all significantly affected by soil depth ($P < 0.0001$ in all cases). Overall, fine root diameter increased with soil depth (from 0.46–0.58 mm in litter to 0.76–0.90 mm at 45–60 cm) and specific root length (Figure 1C and D; Table 2); specific root area (from 286–485 cm^2 g^{-1} in litter to 144–245 cm^2 g^{-1} at 45–60 cm) and number of ECM-RL (Table 2) decreased with soil depth. Phosphorus application significantly increased specific root length and decreased the number of ECM-RL in several soil layers (Table 2; $P < 0.05$). In contrast, I had only one significant effect – it decreased the number of ECM-RL ($P < 0.046$) in the 15–30 cm soil layer – and the P × I interaction was never significant (Table 2). Complete fertilizer application resulted in a significant increase in specific root length ($P < 0.05$) and

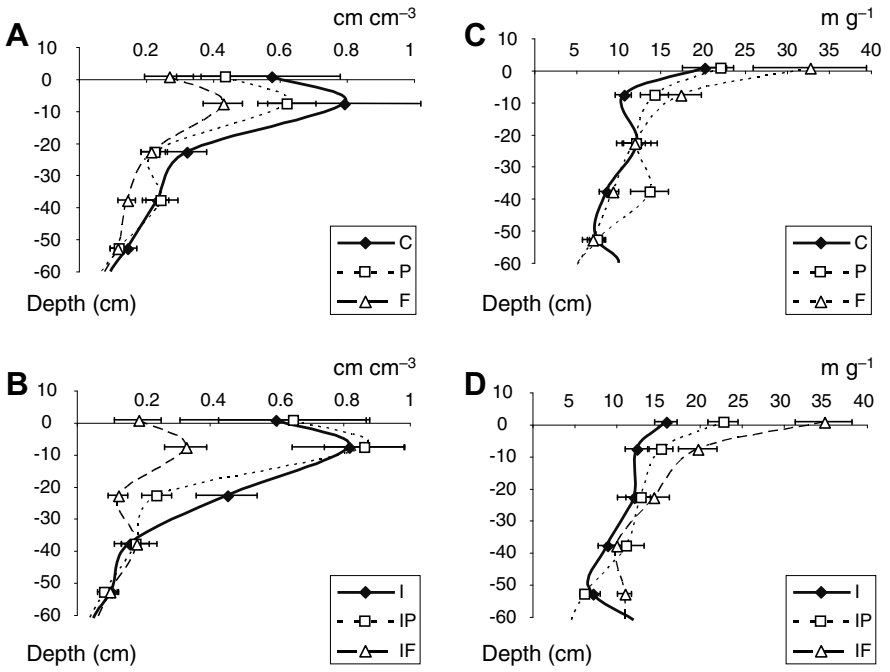

Figure 1. Fine root length density (cm cm^{-3}) in nonirrigated plots (A) and irrigated plots (B), and specific root length (m g^{-1}) in the nonirrigated (C) and irrigated plots (D). Mean values and SE are shown. Abbreviations: C, control treatment; P, phosphorus fertilization treatment; F, complete fertilization treatment; I, irrigation treatment; IP, irrigation + phosphorus fertilization treatment; and IF, irrigation + complete fertilization treatment.

Table 2. Treatment mean values (range of six treatment mean values) and statistical significance (*P* levels) for fine root length density (FRLD), specific root length (SRL), and ramifications (ECM-RL) at a stand age of 13 years, 7 years after treatment initiation for soil depths of 60 cm (n = 16 root cores per treatment).

	Treatment mean values	*P* values			*P* values		
		P	I	P × I	F	I	F × I
FRLD	cm cm^{-3}						
Litter	0.19–0.57	0.85	0.60	0.67	0.06	0.86	0.77
0–15 cm	0.33–0.86	0.70	0.41	0.50	**	0.79	0.67
15–30 cm	0.12–0.45	*	0.26	0.35	***	0.74	0.06
30–45 cm	0.14–0.24	0.74	0.08	0.91	0.38	0.60	0.18
45–60 cm	0.08–0.14	0.34	0.10	0.93	0.50	0.24	0.59
SRL	m g^{-1}						
Litter	15.9–34.8	*	0.38	0.22	**	0.61	0.96
0–15 cm	10.5–19.7	*	0.28	0.79	**	0.06	0.53
15–30 cm	11.8–14.4	0.86	0.85	0.83	0.76	0.39	0.62
30–45 cm	8.4–13.6	*	0.56	0.39	0.90	0.21	0.47
45–60 cm	6.1–11.0	0.57	0.41	0.53	*	0.14	**
ECM-RL	Number m^{-1}						
Litter	71–150	0.65	0.83	0.73	0.42	0.63	0.59
0–15 cm	174–242	0.46	0.76	0.48	*	0.53	0.79
15–30 cm	68–255	0.69	*	0.74	**	**	0.24
30–45 cm	87–216	*	0.09	0.69	0.10	0.38	0.84
45–60 cm	94–157	0.83	0.17	0.84	0.21	0.30	0.73

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

a significant decrease in the number of ECM-RL (Table 2; $P < 0.05$) in several soil layers, whereas I significantly reduced the number of ECM-RL in the 15–30 cm soil layer ($P = 0.003$), but had no effect on specific root length. The F × I interaction was only significant for specific root length at a depth of 45–60 cm ($P = 0.007$). The treatments affected specific root area in a similar way as specific root length, although specific root area showed a greater response to irrigation. Significant decreases in fine root diameter in response to the F and I treatments were recorded only in the litter layer (data not shown separately).

Hyphal growth was measured in ingrowth mesh bags 6–12 months after incubation in the C, P and IP treatments only (Figure 2). Hyphal length in ingrowth bags

was significantly greater in the IP treatment than in the P and C treatments (Figure 2A; $P < 0.0001$, Bonferroni t test), but values did not differ significantly between 6 and 12 months of incubation ($P = 0.12$). Hyphal lengths ranged between 0.6 and 10.8×10^2 km m^{-2} in the C and IP treatments after 6 months of incubation and tended to increase to 3.0 and 13.4×10^2 km m^{-2}, respectively, after 12 months of incubation (Figure 2A). After 12 months of incubation, hyphal mass was significantly higher in the IP treatment than in the C treatment ($P = 0.006$), and values in the P treatment were intermediate between those in the IP and C treatments (Figure 2B). Total hyphal mass ranged between 6 and 17 g m^{-2} (Figure 2B). Specific hyphal length was not significantly affected by the treatments ($P = 0.06$) and ranged between 44 and 89 km g^{-1} in the C and IP treatments (Figure 2C). Hyphae from the mesh ingrowth bags had a $\delta^{13}C$ value of $-26.95 \pm 0.15‰$ ($n = 10$) that was comparable with the value of $-26.83 \pm 0.32‰$ ($n = 6$) measured in the carpophores of species we identified as ectomycorrhizal species (data not shown in detail). Both values differed significantly from the $\delta^{13}C$ value of $-23.96 \pm 0.61‰$ ($n = 5$) obtained for carpophores of saprophytic fungal species ($P < 0.0001$).

Data in Table 3 summarize the treatment effects on the vertical depth distribution of fine roots. Based on biomass, length, or surface area, between 52% and 81% of all fine roots occurred in the top 30 cm of the soil. The P and P × I treatments did not affect this distribution significantly (Table 3). In contrast, I resulted in a significantly shallower vertical root distribution. The F treatment significantly affected the percentage of fine root biomass and fine root area in the top 30 cm (Table 3), whereas I had no significant effects on these measures but there were significant F × I interactions on the percentage of fine root length and fine root area in the top 30 cm of soil.

Aboveground growth was affected by the P, F and I treatments, but not their interactions (Table 4). Diameter at breast height, total needle mass and total aerial tree mass were all significantly increased by P, F or I (Table 4). Small root biomass in the entire 120-cm soil profile, including the litter layer, ranged between 112 and 562 g m^{-2} and was not significantly affected by the treatments (coefficient of variation 90–240%). Summed fine root biomass for the entire 120-cm soil profile, including the litter layer, ranged between 111 and 296 g m^{-2}. It decreased significantly in the P and F treatments, but was unaffected by I and the interactions between P and I and between F and I (Table 4). Fine root length varied from 1162 to 2486 m m^{-2} and the F treatment significantly decreased fine root length, whereas P, I and the interactions had no significant effects on fine root length (Table 4). Summed fine root area ranged between 2.40 and 4.79 m^2 m^{-2} and only the F treatment significantly reduced this value (Table 4). Total number of ectomycorrhizal tips ranged between 62 and 320×10^3 tips m^{-2} (Table 4). The F treatment significantly reduced this number whereas P, I, P × I and F × I did not significantly affect the total number of ectomycorrhizal tips (Table 4). Ratios of root biomass:shoot biomass were significantly affected only by

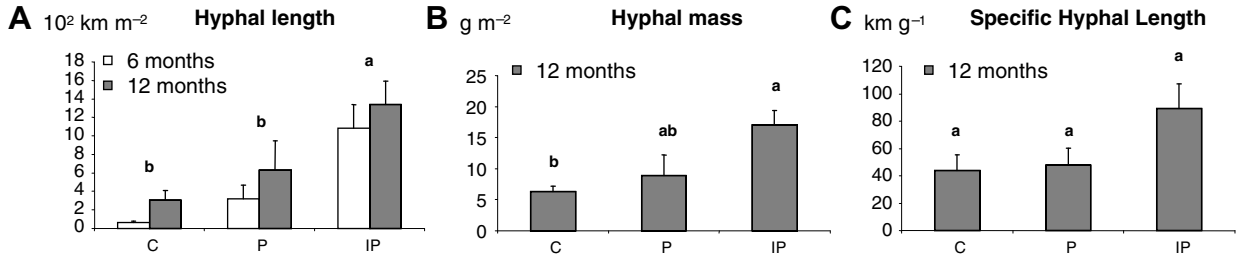

Figure 2. Hyphal length (10^2 km m^{-2}) after 6 and 12 months of incubation (A), hyphal mass (g m^{-2}) (B), and specific hyphal length (km g^{-1}) (C). Mean values for 10 ingrowth bags +1 SE are shown. Different letters indicate significant differences between treatments at $P < 0.05$ (Bonferroni t test). Differences between the 6- and 12-month incubation times were not significant ($P = 0.119$; Bonferroni t test).

Table 3. Summary of treatment mean values, SE, and statistical significance (P levels) on vertical root distribution at a stand age of 13 years, 7 years after treatment initiation ($n = 16$ root cores per treatment).

Variable (%)	Treatment mean values (SE)						P values			P values		
	C	P	F	I	IP	IF	P	I	P × I	F	I	F × I
FRB30	60 (4)	60 (4)	53 (4)	72 (5)	71 (5)	52 (6)	0.99	**	0.92	*	0.16	0.18
FRL30	68 (3)	67 (4)	70 (3)	79 (4)	81 (3)	62 (5)	0.77	**	0.55	0.05	0.50	*
FRA30	63 (3)	64 (4)	62 (4)	75 (5)	77 (4)	58 (5)	0.56	**	0.78	*	0.22	*

FRB, FRL or FRA30 = % of total fine root biomass, length, or surface in 0–120 cm confined to top 30 cm.
*$P < 0.05$; **$P < 0.01$.

Table 4. Summary of treatment mean values, SE and statistical significance (P levels) for aerial variables, summed root values for 0–120 cm, and root:aerial ratios at ages 13, 7 years after treatment initiation.

Variable (units)	Treatment mean values and SE						*P* values			*P* values		
	C	P	F	I	IP	IF	P	I	P × I	F	I	F × I
Aerial variables												
Number of trees[a]	256	233	165	226	165	140						
DBH (cm)	18.9 (0.1)	20.8 (0.2)	21.9 (0.2)	20.5 (0.2)	22.3 (0.2)	24.0 (0.3)	***	***	0.77	***	***	0.29
NB (kg tree^{-1})	8.2 (0.1)	10.0 (0.2)	11.0 (0.2)	9.7 (0.2)	11.4 (0.2)	13.1 (0.3)	***	***	0.92	***	***	0.08
TAB (kg tree^{-1})	84 (1)	105 (2)	116 (3)	101 (2)	121 (3)	141 (4)	***	***	0.98	***	***	0.06
Root variables												
Number of cores	16	16	16	16	16	16						
SRB (g m^{-2})	379 (161)	359 (74)	562 (336)	214 (48)	193 (48)	112 (27)	0.83	0.09	1.00	0.83	0.11	0.45
FRB (g m^{-2})	296 (44)	183 (14)	159 (17)	294 (53)	186 (17)	111 (20)	**	0.98	0.95	***	0.51	0.53
FRL (km m^{-2})	2.5 (0.4)	2.0 (0.2)	1.4 (0.1)	2.4 (0.3)	2.2 (0.2)	1.2 (0.2)	0.26	0.89	0.70	***	0.59	0.77
FRA (m^2 m^{-2})	4.8 (0.7)	3.8 (0.3)	2.8 (0.2)	4.7 (0.6)	4.0 (0.4)	2.4 (0.4)	0.12	0.82	0.76	***	0.66	0.73
T-ECM (N in 1000 m^{-2})	308 (51)	236 (41)	136 (19)	320 (52)	206 (41)	62 (10)	0.05	0.85	0.66	***	0.42	0.27
Root:aerial												
Number of ratios	16	16	16	16	16	16						
R:S	0.32 (0.01)	0.31 (0.00)	0.31 (0.00)	0.32 (0.01)	0.31 (0.00)	0.31 (0.00)	0.08	0.81	0.69	*	0.99	0.86
FR:N	0.32 (0.06)	0.15 (0.01)	0.12 (0.01)	0.28 (0.06)	0.14 (0.01)	0.07 (0.01)	***	0.50	0.72	***	0.25	0.89
RAI:LAI	1.88 (0.30)	1.24 (0.10)	0.93 (0.08)	1.68 (0.22)	1.31 (0.11)	0.81 (0.13)	*	0.75	0.53	***	0.43	0.86

[a] This is the sum of all trees in both replicated blocks that were in zones that had not been affected by the 1999 storm and could be included in the calculations. DBH, tree diameter at breast height (cm); NB, needle biomass per tree; TAB, total aerial biomass per tree; SRB, summed small root biomass in 0–120 cm soil and litter; FRB, summed fine root biomass in 0–120 cm and litter; FRL, summed fine root length in 0–120 cm and litter; FRA, summed fine root surface area in 0–120 cm and litter; T-ECM, total ectomycorrhizal root tips in 0–120 cm and litter in 1000 per m^2; R:S, total aboveground:total belowground biomass (fine roots + sum of tap and coarse roots); FR:N, summed fine root biomass in 0–120 cm and litter:mean needle biomass of the four closest trees; RAI:LAI, fine root area index as summed fine root area for 0–120 cm and litter in m^2 m^{-2}:LAI in m^2 m^{-2} for the corresponding treatment. Small roots, roots with diameter between 2 and 20 mm; fine roots, roots with diameter < 2 mm. *P < 0.05; **P < 0.01; ***P < 0.001.

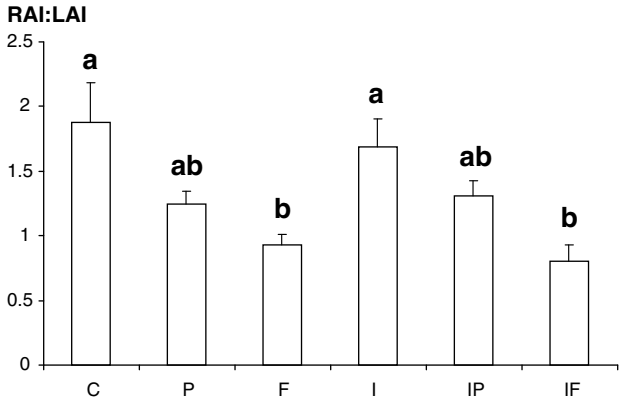

Figure 3. Root area index: leaf area index (RAI:LAI) ratios. Mean values (n = 16 per treatment) +1 SE are shown. Different letters indicate significant differences between treatments (P < 0.05; adjusted Bonferroni t test). Abbreviations: C, control treatment; P, phosphorus fertilization treatment; F, complete fertilization treatment; I, irrigation treatment; IP, irrigation + phosphorus fertilization treatment; and IF, irrigation + complete fertilization treatment.

F (Table 4). The fine root mass:needle mass ratio and the root area index:leaf area index (RAI:LAI) ratio showed similar patterns to those of fine root size with both ratios decreasing significantly in response to the P and F applications but not to I or the interactions including I (Table 4; Figure 3).

Discussion

Fine root size and morphology

Although the roots were sampled fairly close to the stems of the trees (< 2 m away), which is where highest rooting density should occur (Sudmeyer et al. 2004), our summed fine root biomass for the 0–120 cm soil and litter profile was lower (111–296 g m^{-2}) than the 180–720 g m^{-2} range previously recorded for *P. pinaster* in the region (Bakker et al. 2006, Achat et al. 2008). Sampling was performed in summer in the cited studies and should thus be comparable with our study. Values for the 0–15 cm soil layers appear to be lower in early spring and somewhat higher in autumn (n = 27 study sites; Bakker unpublished data) than values in midsummer, but this pattern may vary from year to year depending on climatic conditions. The proportion of fine root biomass or fine root length in the top 30 cm (relative to the total 0–120 cm soil profile) was 52–81% which is similar to the 52–80% reported by Bakker et al. (2006). Annual application of P or F significantly decreased the size of the fine root system, whereas irrigation resulted in a shallower rooting profile. Thus, under our experimental conditions, nutrient availability was more important in explaining differences in the size of the fine root system than water availability. Similar results were

obtained with soil cores in nutrient and water resource optimization experiments on *P. taeda* in USA (Albaugh et al. 1998, Maier and Kress 2000), and the relative but not absolute allocation to fine root biomass decreased in a fertilization and irrigation experiment on *P. sylvestris* in Sweden (Axelsson and Axelsson 1986). The absence of a strong effect of irrigation on the size of the fine root system in our study is likely because the soil water content of the unirrigated plots was often sufficient to meet the water demand of the trees, even though annual precipitation was 17–21% lower than normal in the 3 years preceding our sampling. The site is on humid moorland with fairly high water tables and a mean yearly precipitation of 950 mm, but prolonged summer droughts can lead to significant water deficits because rooting depth is limited to about 1 m by the high winter water tables and the presence of a discontinuous hard pan (Danjon et al. 1999, Bakker et al. 2006, Achat et al. 2008). Given the dry climatic conditions in recent years, the fine root system may exhibit a greater response to the combined effect of irrigation and fertilizers in the future because irrigation had a significant effect on the aerial dimensions of the trees (Table 4).

The specific root lengths that we had measured (6–35 m g^{-1}) were greater than those measured in 55-year-old *P. pinaster* stands in the region (4–12 m g^{-1}), but the ectomycorrhizal root tips per meter of fine root length (68–255) was comparable with the 90–350 ectomycorrhizal root tips per meter of fine root length measured in the 55-year-old *P. pinaster* stands (Bakker et al. 2006). The P and F treatments increased specific root lengths and specific root areas in both the irrigated and nonirrigated plots. The peak values of 22–35 m g^{-1} in the P and F treatments (Figure 1) accord with the values for the finest root diameter fractions in other studies: 12–24 m g^{-1} for 0–1 mm diameter roots measured in 38-year-old *P. sylvestris* in Sweden (Clemensson-Lindell and Persson 1993), 22–30 m g^{-1} in newly formed *P. sylvestris* roots in a greenhouse experiment (George et al. 1997), and 20–52 m g^{-1} for the finest order roots of *P. sylvestris* and *Pinus nigra* (Arnold) in Poland, Finland and Estonia (Withington et al. 2006, Ostonen et al. 2007*a*, 2007*b*). The high specific root lengths in the P and F treatments indicate that a larger proportion of the < 2 mm fraction commonly considered as fine roots comprises the finer root fraction (< 1 or < 0.5 mm diameter) in these treatments. This will have consequences on root uptake, respiration and turnover, because these processes differ among root diameter size fractions (Eissenstat and Yanai 1997, Pregitzer et al. 1998, King et al. 2002, Pierret et al. 2005). In addition, hyphal lengths and hyphal mass significantly increased when irrigation and phosphorus were applied simultaneously. These hyphae belonged to ectomycorrhizal species based on their $\delta^{13}C$ values which were comparable with those collected from ectomycorrhizal mushrooms (cf. Wallander et al. 2004). Overall, under our experimental conditions, increasing nutrient availability led to a nutrient uptake system comprising an increased proportion of fine roots of < 1 or even < 0.5 mm in diameter as well as increased extension of the ectomycorrhizal hyphal network.

A finer root system for increased nutrient availability is in accordance with the assumption that specific root lengths should be high at our productive study site (Delzon and Loustau 2005) because fast growth requires fast and efficient acquisition of resources (Ryser 2006). Our results are similar to studies in Estonia and Finland including 11 *Picea abies* stands, three *Betula pendula* (Roth) stands and three *P. sylvestris* stands (Ostonen et al. 1999, 2007*a*) where, irrespective of tree species, specific root areas increased with increasing site fertility. Assuming that fertilization affects the different diameter classes (mycorrhizal short roots, < 1, 1–2 and < 2 mm roots) differently (Ostonen et al. 2007*b*), this may explain the apparent discrepancies between core and minirhizotron methods (Albaugh et al. 1998, King et al. 2002). These two studies were carried out at the same experimental *P. taeda* site and showed less fine root standing biomass in soil cores after annual nutrient optimization (Albaugh et al. 1998) but more < 1 mm roots in minirhizotron images (King et al. 2002). Based on these results, we suggest that fine root production and turnover of the dynamic fraction are positively correlated with nutrient availability (Nadelhoffer et al. 1985, King et al. 2002, Maier et al. 2004), whereas this is not the case for the entire < 2 mm diameter fraction (Table 4). Increased root turnover is reported to be an efficient mechanism to increase phosphorus and potassium uptake (Steingrobe 2005). Under our conditions, the optimal carbon investment (Chapin et al. 1987, Eissenstat 1992, Ryser 2006) in response to increased nutrient availability is a higher specific root length and more ectomycorrhizal hyphae, and perhaps higher fine root turnover.

Root:shoot relationships

Total root:shoot ratios were not clearly affected by the treatments but the fine root mass:needle mass ratios, that ranged from 0.07 to 0.32, were significantly decreased by applications of P or F. Lower fine root mass:needle mass ratios with increasing P availability were also recorded by Zerihun and Montagu (2004) and by Helmisaari et al. (2007) at 16 sites where *Picea abies* and *P. sylvestris* grew on a natural fertility gradient. In our study, irrigation did not significantly affect fine root mass:needle mass ratios, although at sites with lower soil water content, higher root to shoot biomass ratios have been reported (Cairns et al. 1997). The fine RAI:LAI ratios in our study ranged from 0.81 to 1.88 and were lowest in the F treatment and highest in the control treatments; irrigation did not affect these ratios. Overall, fertilization treatments affected both fine RAI and LAI. For the fine RAI measurements, only fine root surface data were available from samples taken in 2005, whereas LAI was monitored on an annual basis. At our study site, LAI peaked in 2001 and then plateaued from

2002 onward, reaching 3.0 in the control treatment and up to 3.9 in the fertilized plots (Trichet et al. 2008). Values of 3.0 are higher than those typically found in mature stands of the highest fertility class in the region (Delzon and Loustau 2005). The LAI declined to 2.5–3.1 in 2004 and 2005 as a result of thinning and wind throw. Hence, both tree dimensions and LAI were lower in the control treatments than in the P and F treatments, whereas fine RAI was higher. This may be the direct result of increased nutrient availability (i.e., higher allocation to aboveground growth and crown biomass and a lower allocation to fine root standing biomass), or may be related to ontogeny- or size-related differences among the treatments (Ovington 1957, Helmisaari et al. 2002, Ritson and Sochacki 2003, Zerihun and Montagu 2004, Coyle and Coleman 2005, Coyle et al. 2008). The root:shoot ratios decreased from 0.87 at the age of 7 years to 0.29 at 55 years (Ovington 1957) in *P. sylvestris* in the UK, and from 0.33 at the age of 15 years to 0.15 at 100 years in *P. sylvestris* in Finland (Helmisaari et al. 2002). We obtained no clear indications that size-related differences explained any of the treatment effects. Overall root:shoot ratios did not differ significantly between treatments, but they decreased in the first decades after stand establishment in *P. pinaster* in Australia (Ritson and Sochacki 2003). Changes in *P. pinaster* tree or stand physiology are reported to occur somewhere between the ages of 10 and 32 years in the study region (Delzon and Loustau 2005), but LAI values were stable in all of our treatments. Given the age of our trees and the limited time span of the treatments, we believe that the observed shifts in allocation (toward more needle biomass relative to fine root biomass) together with an increase in the finest part of the fine root system (including ectomycorrhizal hyphae) are more likely to be the result of nutrient availability than of overall developmental effects.

We conclude that annual fertilization with either P or F for seven growing seasons led to a reduction in the size of the fine root system in the *P. pinaster* stands by the age of 13 years. This supports our hypothesis that allocation to fine roots decreases with increasing nutrient availability. So far, irrigation has had no significant effect on the size of the fine root system, but has resulted in a shallower rooting system. This finding supports our second hypothesis that roots are more concentrated in the top soil in irrigated stands. However, the P and F applications resulted in an increase in finer root morphology (higher specific root length/area) and irrigation tended to enhance this finer root morphology, which we had not predicted. The quantity of ectomycorrhizal hyphae that grew into the mesh bags was also higher in the P and IP treatments than in controls, suggesting a further extension of the finest part of the fine root system. Fine root to needle biomass or surface area ratios were highest in control treatments and decreased with increasing resources, which may be either a direct consequence of resource availability on fine roots or the result of size-dependent allocation differences, i.e., less allocation to fine root systems as trees increase in age or size. Intensive monitoring over a longer time span is needed to investigate whether, in the longer term, it is nutrient availability or tree size that best explains the differences in tree growth (Coyle et al. 2008). Our study suggests that the fine root class limit of 2 mm diameter may well be inappropriate for studying nutrient and fine root relationships and that fine root production or turnover in the finest and dynamic fraction should receive more attention (Withington et al. 2006, Ostonen et al. 2007*b*).

Acknowledgments

The authors thank Christian Barbot and Sylvie Niollet for their help in collecting the field samples and Cathérine Pernot for preparing the mesh ingrowth bags. Financial support was obtained from JPE (Jeunes Professionels à l'Etranger), enabling E. Jolicoeur's stay in France. The PN-ACI ECCO project 'Déterminants naturels et anthropiques de la biodiversité rhizosphérique et de la biodisponibilité du phosphore en forêt atlantique' funded the mesh bag study. The authors thank the INRA-Pierroton Experimental Unit, under the supervision of Patrick Pastuzska and Frédéric Bernier, for the installation and maintenance of the experimental site.

References

Achat, D.L., M.R. Bakker and P. Trichet. 2008. Rooting patterns and fine root biomass of *Pinus pinaster* assessed by trench wall and core methods. J. For. Res. 13:165–175.

Albaugh, T.J., H.L. Allen, P.M. Dougherty, L.W. Kress and J.S. King. 1998. Leaf area and above- and below-ground growth responses of loblolly pine to nutrient and water additions. For. Sci. 44:317–328.

Axelsson, B. and E. Axelsson. 1986. Changes in carbon allocation patterns of spruce and pine trees following irrigation and fertilisation. Tree Physiol. 2:189–204.

Bakker, M.R., L. Augusto and D.L. Achat. 2006. Fine root distribution of trees and understory in mature stands of maritime pine (*Pinus pinaster*) on dry and humid sites. Plant Soil 286:37–51.

Bert, D. and F. Danjon. 2006. Carbon concentration variations in the roots, stem and crown of mature *Pinus pinaster* (Ait.). For. Ecol. Manag. 222:279–295.

Cairns, M.A., S. Brown, E.H. Helmer and G.A. Baumgardner. 1997. Root biomass allocation in the world's upland forests. Oecologia 111:1–11.

Chapin, F.S., A.J. Bloom, C.B. Field and R.H. Waring. 1987. Plant responses to multiple environmental factors. Bioscience 37:49–57.

Clemensson-Lindell, A. and H. Persson. 1993. Long-term effects of liming on the fine-root standing crop of *Picea abies* and *Pinus sylvestris* in relation to chemical changes in the soil. Scand. J. For. Res. 8:384–394.

Coyle, D.R. and M.D. Coleman. 2005. Forest production responses to irrigation and fertilization are not explained by shifts in allocation. For. Ecol. Manag. 208:137–152.

Coyle, D.R., M.D. Coleman and D.P. Aubrey. 2008. Above- and below-ground biomass accumulation, production, and distribution of sweetgum and loblolly pine grown with irrigation and fertilization. Can. J. For. Res. 38:1335–1348.

Danjon, F., D. Bert, C. Godin and P. Trichet. 1999. Structural root architecture of 5-year-old *Pinus pinaster* measured by 3D digitising and analysed with AMAPmod. Plant Soil 217:49–63.

DeLucia, E.H., J.G Hamilton, S.L. Naidu, et al. 1999. Net primary production of a forest ecosystem with experimental CO_2 enrichment. Science 284:177–179.

Delzon, S. and D. Loustau. 2005. Age-related decline in stand water use: sap flow and transpiration in a pine forest chronosequence. Agric. For. Meteorol. 129:105–119.

Eissenstat, D.M. 1992. Costs and benefits of constructing roots of small diameter. J. Plant Nutr. 15:763–782.

Eissenstat, D.M. and R.D. Yanai. 1997. The ecology of root lifespan. Adv. Ecol. Res. 27:1–63.

George, E., B. Seith, C. Schaeffer and H. Marschner. 1997. Responses of *Picea*, *Pinus* and *Pseudotsuga* roots to heterogeneous nutrient distribution in soil. Tree Physiol. 17:39–45.

Göransson, H., H. Wallander, M. Ingerslev and U. Rosengren. 2006. Estimating potential nutrient uptake from different soil depths of *Quercus robur*, *Fagus sylvatica*, and *Picea abies*. Plant Soil 286:87–97.

Helmisaari, H.-S., K. Makkonen, S. Kellomäki, E. Valtonen and E. Mälkönen. 2002. Below- and aboveground biomass, production and nitrogen use in Scots pine stands in eastern Finland. For. Ecol. Manag. 165:317–326.

Helmisaari, H.-S., J. Derome, P. Nöjd and M. Kukkola. 2007. Fine root biomass in relation to site and stand characteristics in Norway spruce and Scots pine stands. Tree Physiol. 27:1493–1504.

Hodge, A. 2004. The plastic plant: root responses to heterogeneous supplies of nutrients. New Phytol. 162:9–24.

Hutchings, M.J. and H. de Kroon. 1994. Foraging in plants: the role of morphological plasticity in resource acquisition. Adv. Ecol. Res. 25:159–238.

King, J.S., T.J. Albaugh, H.L. Allen, M. Buford, B.R. Strain and P. Dougherty. 2002. Below-ground carbon input to soil is controlled by nutrient availability and fine root dynamics in loblolly pine. New Phytol. 154:389–398.

Loustau, D., A. Bosc, A. Colin, et al. 2005. Modeling climate change effects on the potential production of French plains forests at the sub-regional level. Tree Physiol. 25:813–823.

Maier, C.A. and L.W. Kress. 2000. Soil CO_2 evolution and root respiration in 11 year-old loblolly pine (*Pinus taeda* L.) plantations as affected by moisture and nutrient availability. Can. J. For. Res. 30:1093–1106.

Maier, C.A., T.J. Albaugh, H.L. Allen and P.M. Dougherty. 2004. Respiratory carbon use and carbon storage in mid-rotation loblolly pine (*Pinus taeda* L.) plantations: the effect of site resources on the stand carbon balance. Global Change Biol. 10:1335–1350.

Nadelhoffer, K.J., J.D. Aber and J.M. Melillo. 1985. Fine root production in relation to net primary production along a nitrogen availability gradient in temperate forests: a new hypothesis. Ecology 66:1377–1390.

Oren, R., D.S. Ellsworth, K.H. Johnsen, et al. 2001. Soil fertility limits carbon sequestration by forest ecosystems in a CO_2-enriched atmosphere. Nature 411:469–472.

Ostonen, I., K. Lõhmus and R. Lasn. 1999. The role of soil conditions in fine root ecomorphology in Norway spruce (*Picea abies* (L.) Karst.). Plant Soil 208:283–292.

Ostonen, I., K. Lõhmus, H.-S. Helmisaari, J. Truu and S. Meel. 2007*a*. Fine root morphological adaptations in Scots pine, Norway spruce and silver birch along a latitudinal gradient in boreal forests. Tree Physiol. 27:1627–1634.

Ostonen, I., Ü. Püttsepp, C. Biel, et al. 2007*b*. Specific root length as an indicator of environmental change. Plant Biosyst. 141:424–442.

Ovington, J.D. 1957. Dry-matter partitioning by *Pinus sylvestris* L. Ann. Bot. 21:287–314.

Pierret, A., C.J. Moran and C. Doussan. 2005. Conventional detection methodology is limiting our ability to understand the roles and functions of fine roots. New Phytol. 166:967–980.

Pregitzer, K.S., M.J. Laskowski, A.J. Burton, V.C. Lessard and D.R. Zak. 1998. Variations in sugar maple root respiration with root diameter and soil depth. Tree Physiol. 18:665–670.

Ritson, P. and S. Sochacki. 2003. Measurement and prediction of biomass and carbon content of *Pinus pinaster* trees in farm forestry plantations, south-western Australia. For. Ecol. Manag. 175:103–117.

Rousseau, J.V.D., D.M. Sylvia and A.J. Fox. 1994. Contribution of ectomycorrhiza to the potential nutriment-absorbing surface of pine. New Phytol. 128:639–644.

Ryser, P. 2006. The mysterious root length. Plant Soil 286:1–6.

Smith, S.E. and D.J. Read. 1997. Mycorrhizal symbiosis. 2nd Edn. Academic Press, London.

Steingrobe, B. 2005. A sensitivity analysis for assessing the relevant of fine-root turnover for P and K uptake. J. Plant Nutr. Soil Sci. 168:496–502.

Sudmeyer, R.A., J. Speijers and B.D. Nicholas. 2004. Root distribution of *Pinus pinaster*, *P. radiata*, *Eucalyptus globulus* and *E. kochii* and associated soil chemistry in agricultural land adjacent to tree lines. Tree Physiol. 24:1333–1346.

Tennant, D. 1975. A test of a modified line intersect method of estimating root length. J. Ecol. 63:995–1001.

Trichet, P., D. Loustau, C. Lambrot and S. Linder. 2008. Manipulating nutrient and water availability in a maritime pine plantation: effects on growth, production, and biomass allocation at canopy closure. Ann. For. Sci. (in press).

Wallander, H., H. Göransson and U. Rosengren. 2004. Production, standing biomass and natural abundance of ^{15}N and ^{13}C in ectomycorrhizal mycelia collected at different soil depths in two forest types. Oecologia 139:89–97.

Waterworth, R., R.J. Raison, C. Brack, M. Benson, P. Khanna and K. Paul. 2007. Effects of irrigation and N fertilization on growth and structure of *Pinus radiata* stands between 10 and 29 years of age. For. Ecol. Manag. 239:169–181.

Withington, J.M., P.B. Reich, J. Oleksyn and D.M. Eissenstat. 2006. Comparisons of structure and life span in roots and leaves among temperate trees. Ecol. Monogr. 76:381–397.

Zerihun, A. and K.D. Montagu. 2004. Belowground to aboveground biomass ratio and vertical root distribution responses to mature *Pinus radiata* stands to phosphorus fertilization at planting. Can. J. For. Res. 34:1883–1894.