UNIVERSITA' DEGLI STUDI DELL'INSUBRIA

Facoltà di Scienze MM.FF.NN. - Como

Tesi di Dottorato di Ricerca
in Scienze Ambientali
XXIV ciclo

FINE-ROOT DYNAMICS, ANATOMY AND CARBON-NITROGEN CONCENTRATIONS IN RELATION TO FOREST MANAGEMENT AND SOIL WATER CONTENT. CASE STUDIES IN BEECH (*Fagus sylvatica* L.) AND TURKEY-OAK (*Quercus cerris* L.) FORESTS

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Anno 2011

"...We Are The Future Of Science..."

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(personal communication, September 5, 2009)

Contents

Abstract

Uncertainties in estimates of fine root dynamics prevent a proper quantification of net primary productivity and belowground C allocation. Moreover, model studies for estimating carbon budgets are biased by the lack of fine roots datasets at forest stand level. This study shed some lights on fine root dynamics in two different Italian forests. In particular fine-root systems was investigated: 1) in three beech forest stands (*Fagus sylvatica* L.) located in Southern-Alps in relation to different forest management practices and age 2) in a mature Turkey-oak stand (*Quercus cerris* L.) located in the Southern Apennines in relation to soil moisture seasonal changes.

Data from beech forests showed that conversion from coppice to high forest practice induced considerable variations in fine-root traits. Reduction of stand tree density induced a reduction of total fine-root mass and an increase of both production and turnover rate. Both fine-root production and turnover rate increased in converted stands. When fine-roots *Carbon* and *Nitrogen* contents were analyzed, their ratio was significantly lower in converted stands, supporting the finding of a higher turnover rate. A histological study was carried to assess if also anatomical changes occurred due to conversion practices. Anatomy on fine roots showed a higher percentage of xylem cells in conversion stands explaining the lowest carbon concentration.

Turkey-oak fine-root biomass and length showed a bimodal pattern with a peak in summer and a peak in autumn. SRL had only one peak in summer. All fine root traits increased during the transition from the wet to dry season. These results indicate a pulse in root growth in order to increase the soil exploitation when soil water content is low. Moreover, during the summer period, *Q. cerris* change fine-root morphology leading an increase of fine root length per unit mass.

Chapter I

Introduction

1.1 Global carbon cycle and carbon balance

Many efforts are being spent towards understand how much of the carbon emitted into the atmosphere remains there and how much is taken up by terrestrial ecosystems and world's oceans. Answers to these matters will provide at least a part of the scientific understanding necessary for establishing the amount and rate of CO_2 emissions that would meet a "safe" concentration (Houghton 2007).

The contemporary global carbon cycle (simplified form in Figure 1.1) refers to the exchanges of carbon within and between four major reservoirs: the atmosphere, the oceans, land, and fossil fuels. In terms of unit of time, the carbon exchanges may occur: -in seconds by the fixation of atmospheric CO_2 into sugar through photosynthesis; -over millennia by the accumulation of fossil carbon (coal, oil, gas) and processes such as weathering, vulcanism, seafloor spreading, and diagenesis. The amounts of carbon exchanged annually through the 'over millennia processes' are small and generally ignored in budgets of a century (see Sundquist and Visser 2004 for a review of the carbon cycle over longer time frames) (Houghton 2007).

Understanding how different mechanisms regulate carbon sinks is very important because they have different implications for the rate of future CO_2 increase as well as the rate of global warming. The most recent and comprehensive overview on the carbon cycle, particularly with respect to climate, is the fourth Intergovernmental Panel on Climate Change 2007 (IPCC 2007 www.ipcc.ch/). The basic aspects of the global carbon cycle have been

understood for ~35 years, but predictions of how sources and sinks of carbon will respond to a changing climate are actively debated.

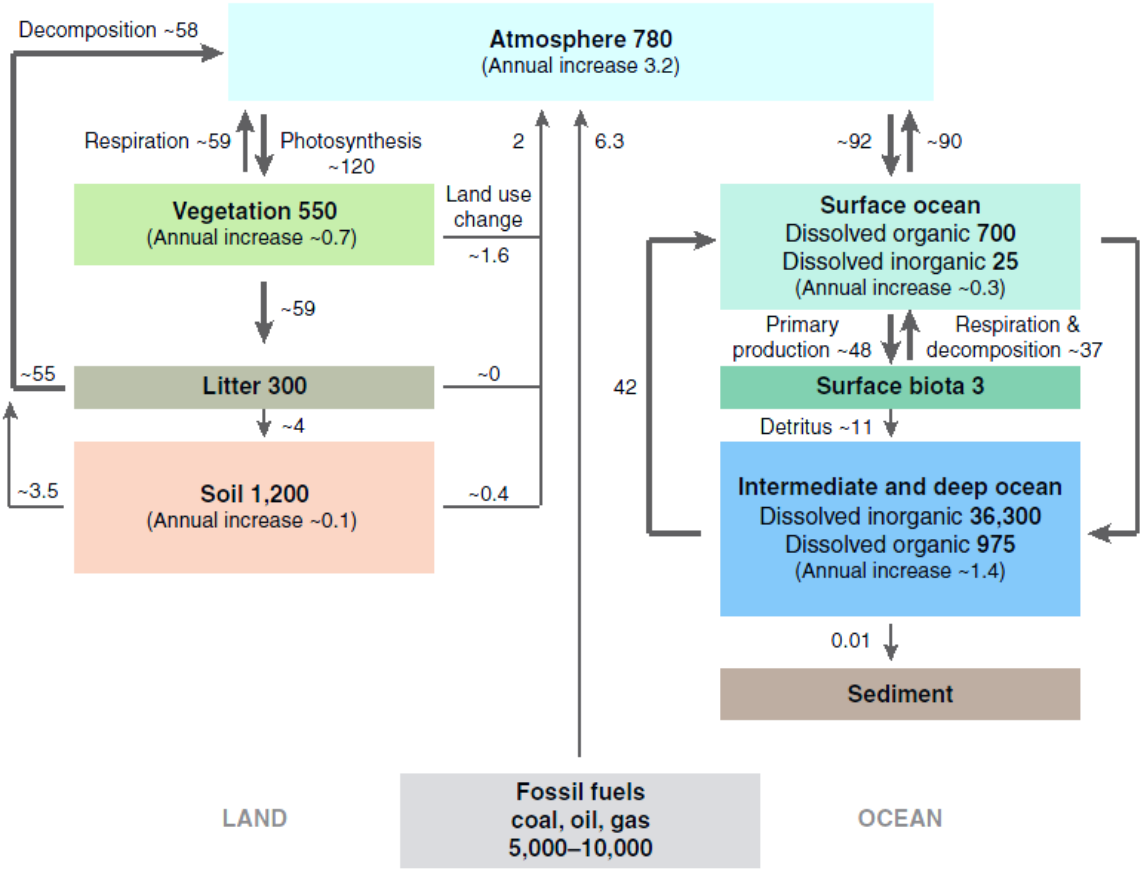

Figure 1.1 The global carbon cycle in the 1990s. Units are PgC or PgC year^{-1} (in Houghton 2007)

1.2 The carbon cycle and the human activities

Scientists have used a combination of data and models to reconstruct changes in the global carbon cycle over the past centuries. The historical information includes rates of fossil fuel use and rates of land-use change. Data on past CO_2 and CH_4 concentrations as well as temperature were obtained from Antartic ice cores (Houghton 2007). Approximately 300 PgC have been released since 1750, essentially all of it since 1860 (Keeling 1973, Andres et al. 1999). Today there are approximately 100 stations worldwide where weekly flask samples of

air are collected, analyzed for CO_2 and other constituents. Resulting data are integrated into a consistent global data set (Masarie and Tans 1995). In 2005, the concentration of atmospheric CO_2 reached nearly 380 ppm (388.92 ppm in October 2011), an increase of ~35% above the preindustrial concentration (275–285 ppm, Monnin et al. 2001).

The relatively recent increasing of CO_2 atmospheric concentration is attributable to the fossil fuel consumption by human activities:

1) Since 1850, the timing of the increase is coincident with the rising emissions of carbon from fossil fuel combustion and land-use change (e.g. deforestation and urbanization).

2) The latitudinal gradient in CO_2 concentrations is highest at northern midlatitudes and lower at higher and lower latitudes, consistent with the fact that most of the emissions of fossil fuel are located in northern mid-latitudes. Moreover, this latitudinal gradient has increased in proportion to emissions of carbon from fossil fuels (Keeling et al. 2005).

3) The distribution of carbon isotopes and other biogeochemical tracers are consistent with scientific understanding of the sources and sinks of carbon from fossil fuels, land, and the oceans. For example, the increase in concentrations over the period 1850 to 2000 was accompanied by a decrease in the ^{14}C content of CO_2. The decrease is what would be expected if the CO_2 added to the system were fossil carbon depleted in ^{14}C through radioactive decay. This dilution of $^{14}CO_2$ is called the Suess effect (Houghton 2007).

1.3 Carbon storage on land. The residual terrestrial sink.

The measured amount of carbon released by land use changes such as conversion of forests to agricultural lands (156-174 PgC) (Houghton 2003), is much larger than the amount calculated in the global carbon budget equation (39-40 PgC) (Sabine et al. 2004) (Tab. 1.1).

Table 1.1 The global carbon budget for two intervals (units are PgC) (in Houghton 2007)

	1800 to 1994	1850 to 2000
Emissions from fossil fuels and cement production	244±20[1]	275[3]
Atmospheric increase	−165±4[1]	−175[4]
Oceanic uptake	−118±19[1]	−140[5]
Calculated land-use change	39±28[1]	40
Measured land-use change	174[2]	156[2]
Residual terrestrial sink	−135	−116

[1] Sabine et al. 2004.
[2] Houghton 2003.
[3] Keeling 1973, Andres et al. 1999.
[4] Prentice et al. 2001.
[5] Joos et al. 1999

The difference between these two values (a residual sink of 116-135 PgC) may be due to errors in the analyses (either the ocean models or the land-use change calculations), or may indicate a terrestrial flux of carbon unrelated to land-use change (Houghton 2007). The release of carbon calculated from changes in land use includes only the sources and sinks of carbon resulting directly from human activity; ecosystems not directly modified by human activity are left out of the analysis. In contrast, the release computed by difference includes all ecosystems and processes. The mechanisms responsible for carbon sinks on land are not yet well known, therefore two competing factors have been proposed:

1) enhanced growth of forests from physiological or metabolic factors that affect rates of photosynthesis, respiration, growth, and decay.

2) regrowth from past disturbances, changes in land use, or management, affecting the mortality of forest stands, the age structure of forests, and hence their rates of carbon accumulation (Houghton 2007).

Carbon is taken up from the atmosphere through photosynthesis and released through respiration, including the respiration of plants, animals, and microbes (largely soil respiration), and fire. An imbalance between these two processes will cause ecosystems to be either carbon sinks or sources. Differently, if this two processes are balanced, an increase in productivity will lead to an increase in carbon storage until the carbon lost from the detritus pool comes into a new equilibrium..The longer the turnover time, the higher the storage (Houghton 2007).

1.4 Terrestrial ecosystems: vegetation and soils.

Carbon accounts for approximately 0.27% of the mass of elements in Earth's crust (Kempe 1979), yet accounts for approximately 50% of dry (water removed) organic matter The carbon exchanges between terrestrial ecosystems and the atmosphere are mainly the result of biological processes such as photosynthesis and respiration (~120 PgC $year^{-1}$ in each direction). Year-to-year variations in these fluxes owing to climatic variations, including variations in fires, may be as high as 5 PgC $year^{-1}$ (Peylin et al. 2005). The amount of carbon contained in terrestrial vegetation (550±100 Pg) is on the order of the amount in the atmosphere (800 Pg). Forests are particularly important as a carbon reservoir because trees hold much more carbon per unit area than other types of vegetation (Houghton 2007). The organic matter in soils is two to three times this amount [1500–2000 PgC in the top meter and as much as 2300 Pg in the top 3 m (Jobbàgy and Jackson 2000)].

1.5 The role of roots in carbon balance

Root system is an important part of the path for carbon and energy movement from plant canopy to soil. Root construction and maintenance influence carbon (C) and mineral nutrient consumption, while root death influences the partial return of these resources to soil (Eissenstat and Yanai 1997). Thus, root production and turnover directly impact the carbon cycle in terrestrial ecosystems (Matamala 2003). Net primary production (NPP) is greater below- then above-ground in a range of different ecosystems (Caldwell 1987). Even in forests with enormous above-ground biomass, below-ground NPP was consistently higher than above-ground, especially early in stand development (Gower et al. 1994). Roots, like other plant organs, have a life history: they are born, age and die (Harper 1977). The contribution of root C to the formation of soil organic matter depends on root productivity, turnover rates, exudation, mycorrhizal colonization, and soil characteristics, all of which vary with forest type (Matamala 2003). Current estimates have indicated that fine-root production contributes from 33 to 67% of the annual NPP in forest ecosystems. (Jackson et al. 1997; Grier et al. 1981; Santantonio et al. 1987). However, very little is known about below-ground systems dynamics.

So far scientists developed several models in order to analyze carbon budgets and fluxes at the forest stand level. These models range from very detailed ecophysiological models for climate impact assessment, to very general empirical/descriptive models for forest stand carbon budget (Mohren 1987; Dewar 1991; Mery and Kanninen 1999; Kirschbaum et al. 1998; Schlamadinger and Marland 1996; White et al. 2000; Karjalainen 1996, in Masera et al. 2003). None of these models have been widely disseminated or accepted as a possible standard for carbon crediting (Masera et al. 2003). These biomass estimates have the largest relative uncertainties because the lack of data related to fine roots. (Peltoniemi et al. 2004). Apart the type of model used, uncertainties in estimates of fine-root longevity prevent proper

quantification of net primary productivity and below-ground C allocation in forests. Thus, turnover rate has a large effect on carbon modeling. When the fine-root turnover rate was set to its lower or upper limit estimated, it alone changed the simulated soil carbon stock by 15% and carbon accumulation rate by 30% (Peltoniemi et al. 2004)

1.6 The research project

The function of forests in sequestering carbon is one of the most intensely investigated topics in forestr research. There is a need to better understand some of the aspects of root development and life cycle that might influence below-ground carbon stock turnover (Tobin et al. 2007). Forest root systems are mainly composed by – structural roots (diameter > 2 mm) and – fine roots (diameter < 2 mm). Given their simple anatomical organization, fine roots are the most sensitive component of the root system responding rapidly to variations in the rooting environment. Therefore, the fine-root compartment should be investigated when studying nutrient cycling and carbon accumulation in a forest ecosystem (Helmisaari et al. 2002).

1.6.1 Forest management

The Italian National Forest Inventory (www.sian.it/inventarioforestale/jsp/home_en.jsp 2011) indicates that more than 60 percent of Italian forests are maintained under a coppice regime. This situation stems from when there was a high demand for small timber, firewood and charcoal. Now, based on social and economic factors, there is a trend to convert traditional coppice management to high forest management (Ciancio et al. 2006). Coppice stands are usually characterised by dense distribution of stools each of which includes a number of stems. Only one stem in each stool is left during conversion to high forest management. At the same time, tree density per hectare usually decrease. Because the optimal

degree of tree density has not yet been established, converted stands may have different structures. Thus, high forest management results in a considerable change in the canopy structure, which in turn may alters important environmental factors such as light/shade distribution, soil-profile, soil-temperature, and nutrient distribution. Moreover roots belonging to the cut trees may die and decompose. Given this, is not unreasonable to speculate that the contemporaneous variation of so many important factors is not without consequences for the root life-cycle.

In this work, we studied three forest stands differing in use intensity and cutting age (an undisturbed 40-year-old coppice stand and two coppice stands converted to high forest in 1994 and 2004) in the Southern-Alps (see chapter II for site characterization). We analysed the impact of conversion practice in beech forest on fine-root traits. The cited order reflects the cutting age: the more recent the cutting operation, the more severe the forest use intensity and the effect of disturbance on the environmental factors that characterise a stand.

In chapther III, we evaluated the (a) monthly variation of the fine-root standing biomass and necromass during a growing season; (b) the annual fine-root production and turnover rate and (c) how the above-indicated fine-root traits vary in relation to the soil depth.

In chapter IV, we focused on carbon and nitrogen concentrations in beech fine roots because these two parameters could be used as indicators of the construction and maintenance costs respectively for fine-root biomass.

In chapter V, we present an histological analysis regarding beech fine roots. Our aim was to evaluate the occurrence of significant differences in anatomical characteristics among fine roots 1-2 mm thick sampled in the three different managed beech forests.

1.6.2 Soil water content

Simulations performed with atmospheric models over the Mediterranean Basin predict an overall warming in all seasons, a decrease in annual mean precipitation and the consequent reduction of soil water content (Gibelin and De´que 2003). In addition, data on fine roots of tree species growing in the Mediterranean Basin are scarce and their changes in biomass have been generally ignored (Finer et al. 2007; Jackson et al. 1997).

Even if oaks (*Quercus* ssp.) are major components of European temperate vegetation types (Bradshaw and Lindbladh 2005; Bolte and Löf 2010), very little is known about these species. Very few studies investigated the fine-root growth of *Q. cerris*. Manes et al. (2005) studied three-year old potted seedlings of *Quercus cerris* L. (Turkey-oak) under controlled conditions in relation to soil water stress. Claus and George (2005) investigated *Q. cerris* fine-root mass under natural conditions in central Italy in relation to chronosequence. Thus, there is a lack of data on the fine-root mass and morphology in forest stands growing under natural conditions and in relation to soil moisture seasonal variation. This lack of data hinders attempts to model accurately the behaviour of terrestrial ecosystems, and their potential responses to climate change.

In chapter VI was presente a study with objective the detection of adaptive responses of the fine-root systems in a Turkey-oak stand to changes in water availability. The study was carried on a *Quercus cerris* L. forest stand (see chapter II for site characterization) throughout an entire vegetative season. Our specific investigations were to: (i) quantify the seasonal variation of (a) live and dead fine-root as dynamic adaption of primary production to environmental changes, (b) length and (c) SRL; (ii) quantify these characteristics in the different diameter classes of the fine-root population. The effects of seasonal water deficits were assessed by comparing root mass and morphology between the wet and dry seasons.

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Chapter II

Study sites and stands characterization

Data were obtained from different study sites: three different managed beech stands (*Fagus sylvatica* L.) in Lombardy Alps and a Turkey-oak stand (*Quercus cerris* L.) in Molise Appennines.

2.1 *Fagus sylvatica* L. stands

2.1.1 Study site

The study area is located in the catchments of the Telo stream in the Lombardy Alps (Intelvi Valley, NW Italy, 45° 59' N, 9° 07' E) approximately from 1160 m to 1200 m above sea level between Lakes Como and Lugano. This area is characterised by a sub-continental climate, with a mean annual precipitation of 1600 mm, mainly concentrated in two main periods (April-May and October-November), and a mean annual temperature of 10-11°C. Rainfall (mm) and air temperature (°C) were recorded at 60 min interval. Sensors (Thermometer DMA572 and Rain gauge DQA030; LSI Latstem s.r.l.) were mounted on a 3-m high mast and set up on a hill (Alpe di Ponna) 0.8 km from the experimental site. An intense snow fall on 22 November 2008 prevented sampling until the following spring.

According to the World Reference Base (WRB) for Soil Resources (IUSS 2006), soil type is Leptosol 40-50 cm deep. Sampling plots were placed in three stands with different types of forest management. Specifically three beech stands were considered: a residual coppice stand, the only one left in the area, cut once 40 years ago and then allowed to re-grow from stumps and never recut; two conversions from coppice to high forest cut in 1994 and 2004, respectively (Fig. 2.1). This operation consisted in reducing the number of stems per stool to one per stool, and eliminating exceeding stools thereby reducing stand density, and

transforming the coppice to high forest. The three stands were located on the same slope facing south-west, slope average between 28-30 degrees, adjacent to each other.

Species and cover composition of the understory differed for each of the three stands. A vegetation survey in June 2008, showed that in the coppice stand, beech seedling cover was very low, herbaceous species covered 5% of the stand soil surface and mosses covered 35%.

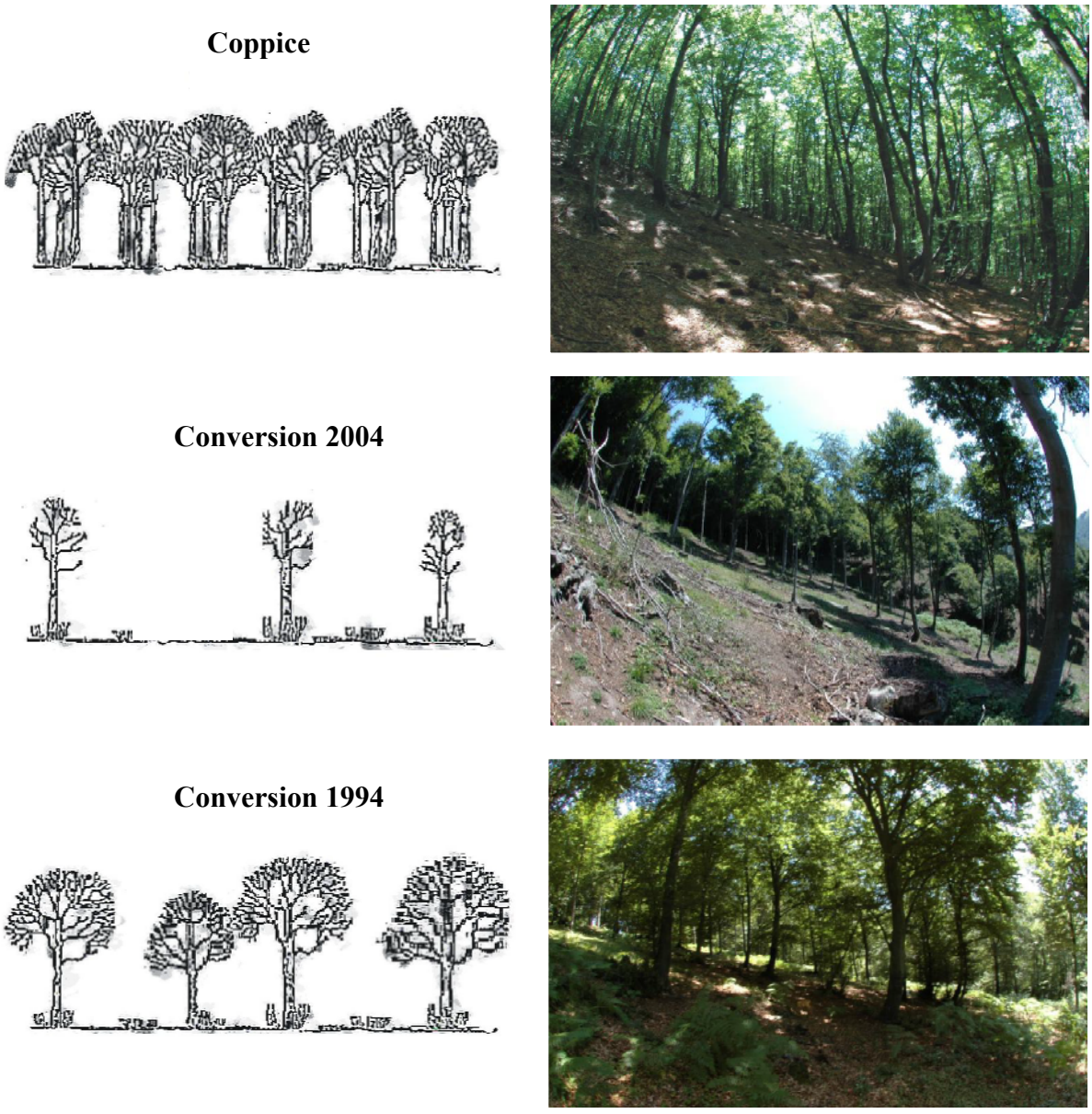

Figure 2.1 Three different managed beech (*Fagus sylvatica* L.) forests

Five herbaceous species were found and the most abundant species were *Luzula nivea* with a cover of 20% and *Maianthemum bifolium* (up to 4%). In the 1994 Conversion stand, beech seedlings covered up to 15% of the soil surface. The herbaceous species covered from 20% to 50% with 16 species the most abundant of which were *Pteridium aquilinum* (from 8% to 20%), *Maianthemum bifolium* (up to 20%) and *Silene rupestris* (up to 35%). Mosses covered only 5% of the soil surface. In the 2004 Conversion stand, beech seedlings covered up to 15% and seedlings of birch (*Betula pendula* Roth) covered 2%. Herbaceous species covered up to 85% and mosses only 1%. The number of herbaceous species was 19 and the most abundant were *Carex pallescens* (25%), *Veronica officinalis* (15%), *Rumex acetosella* (10%) and *Luzula pilosa* (10%).

2.1.2 Stand measurements (soil temperature, canopy cover, tree stocking density, above-ground biomass and leaf biomass)

Soil temperature was measured during the growing season. Measurements were taken next to the soil cores. On each sampling date, six measurements were taken at three soil depths: 5 cm, 15 cm and 25 cm. Soil temperature was measured using a high accuracy thermometer with a stainless steel probe (mod. CheckTemp 1). The probe utilizes a high-tech NTC thermistor sensor that makes it possible to obtain an extremely high accuracy (± 0.3°C) in a very short time.

In July 2008 canopy cover was measured by hemispherical photos (Rich 1990) analysed with the Can-eye freeware (www4.paca.inra.fr/can-eye 2011). 10 hemispherical photos per stand were taken at 7.5-meter intervals along a transect.

To determine the tree stocking density per stand, an area of 100x100 m was delimited within each stand and the number of trees was counted. In the Coppice stand, each multi-stemmed stool was counted as one tree.

.In November 2008, for each stand, three sample trees representing the range of tree sizes were selected for destructive harvesting, and the dbh was measured. Finally, a site-specific allometric relationship was developed to estimate the woody biomass from the tree dbh. The best fit (r^2= 0.97) was obtained by a unique power function suitable for all three stands (no Stand effect, ANCOVA P=0.74). The power equation (1) is:

$$W=aD^b \qquad (1)$$

where W = dry weight D = dbh a =1.0594 b = 1.8237

The above-ground biomass was surveyed on seven selected 20-m diameter circular-shaped sampling plots per stand (a total of 2198 m^2 per stand) with the site specific allometric relationship, estimating branch and stem biomass from tree diameter at breast heights (dbh).

In order to estimate leaf biomass, 10 litter traps where placed in summer 2008 in each stand at 7.5-meter intervals along a transect (Finotti et al. 2003). Leaves where sampled at the end of October 2008 after leaf shedding, dried and weighed.

2.1.3 Above-ground stand characteristics

Due to the different management intensities and age, the forest types differed clearly with respect to the above-ground stand structure. As shown in Table 2.1, stem density and above-ground biomass were higher in the Coppice stand than in the 2004 Conversion stand, whereas dbh and height were greater in the 2004 Conversion stand. In the 1994 Conversion stand, the values of all the parameters tested were intermediate between those of the Coppice

and the 2004 Conversion stands. The differences in canopy cover percentage measured by the hemispherical photo analysis reflected those recorded for tree density, namely canopy cover was highest in the Coppice stand and lowest in the 2004 Conversion stand (Tab. 2.1). Soil temperatures were invariably lower in the Coppice stand where the canopy cover was maximum and therefore the shading effect was higher whereas soil temperatures were highest in the 2004 Conversion stand where the percentage of canopy cover was lowest (Tab. 2.1). The soil temperature pattern was similar in the three stands (Fig. 2.2).

Table 2.1 Tree density, above-ground biomass, canopy cover and soil temperature of three forest management treatments. Canopy cover values are the means of 10 replicates; soil temperature (0 – 30 cm) is referred to the means of three soil depths (5 cm, 15 cm and 25 cm) and each value is the mean of 6 replicates for 7 sampling dates (May - October 2008). Above-ground biomass values are the mean of 7 replicates. All values are mean ±S.E.

Forest Management	No. stems hectares $^{-1}$	Above-ground Biomass (Mg ha^{-1})	Leaf biomass (Mg ha^{-1})	Mean dbh [a] (cm)	Mean tree height (m)	Canopy cover (%)	Soil temperature (°C)			
							0-30 cm	5 cm	15 cm	25 cm
Coppice	724 ±35	248.5 ±15.6	2.7 ±0.1	17.2 ± 0.7	12.1 ±0.3	94.2 ±0.6	10.24 ± 0.30 a	10.57 ± 0.55 a	10.22 ± 0.51 a	9.94 ± 0.51 a
Conversion 1994	279 ±24	123.7 ±7.3	3.3 ±0.1	22.6 ± 1.5	12.8 ±0.7	74.2 ±5.5	11.26 ±0.32 b	11.66 ± 0.58 ab	11.15 ± 0.54 ab	10.96 ± 0.53 b
Conversion 2004	167 ±20	91.8 ±20.2	1.6 ±0.2	31.9 ± 1.9	18.9 ±0.8	54.3 ±3.2	12.23 ± 0.36 c	12.75 ± 0.69 b	12.05 ± 0.59 b	11.90 ± 0.58 c

[a] dbh (diameter at breast height)

Soil temperature was measured with the Checktemp 1 thermometer with an NTC thermistor sensor (Hanna Instruments ®) (± 0.3°C). On each sampling date, measurements were taken at the soil core sampling point at three depths (5 cm, 15 cm and 25 cm). a, b and c indicate significant differences between forest management treatments within the same soil depth (Mann-Whitney U test, $p < 0.05$)

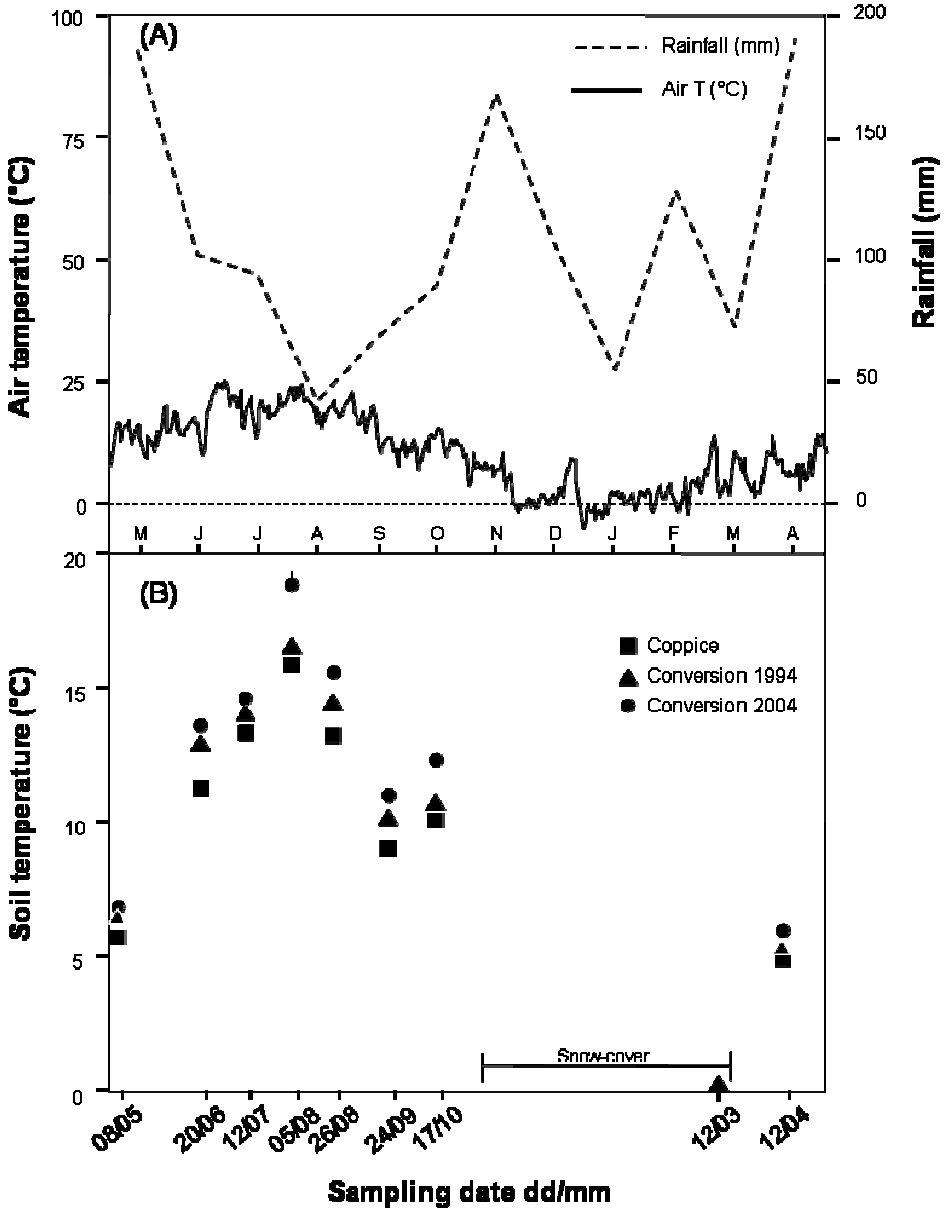

Figure 2.2 Upper panel (A) shows air temperature (°C) and monthly total rainfall (mm) characterizing the study site during the sampling period. Lower panel (B) shows soil temperatures (°C) measured in each stand for the whole 0-30 cm soil depth. During the snow cover period indicated on the X-axis, soil temperature was not measured. At the beginning of March 2009 in the 1994 Conversion stand, soil temperature was measured under snow cover. Data are the means of 12 measurements ± S.E.

2.2 *Quercus cerris* L. stand

2.2.1 Study site

The experimental site is located in the Trigno river basin near Trivento (Molise, Italy), on the east side of the southern Italian Apennine. The site is located on a north-facing hill slope, at an altitude of 600 m (latitude 41°43′ N, longitude 14°33′ E - shallow-slope conditions, 4°-5°). The climate is montane Mediterranean with summer drought generally spanning June, July and August. Rainfall is usually concentrated between late autumn and early spring (Fig. 2.3).

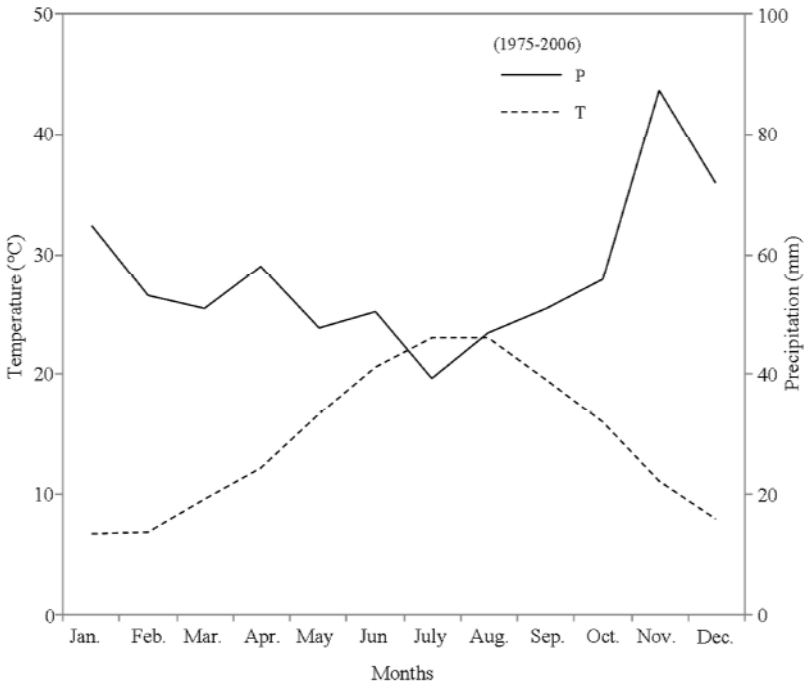

Figure 2.3 Climatic factors of the survey site from 1975 to 2006. The drought period was determined based on temperature and rainfall data according to Bagnolous and Gaussen (data from the Trivento weather station, Regione Molise)

The mean yearly precipitation is 656 mm, falling mostly as rain. The area under investigation is subject to seasonal and yearly variations in terms of drought and cold stress periods. Evapotranspiration is high during summer when rainfall is low thereby resulting in a considerable moisture deficit (Van Beek et al. 2001). The mean summer and winter temperatures are estimated to be 22 °C and 7 °C, respectively. Soil type is the widespread Typic Eutrudepts fine loamy mixed mesic (USDA, Keys to Soil Taxonomy, 1998) (see Table

1 for soil site features in Di Iorio et al. 2008) that reacts to the montane Mediterranean climate with high shrinkage and cracks forming to a maximum depth of 1.0 m in the summer. This soil type frequently becomes waterlogged during the winter months (Barij et al. 2007). Vegetation over-storey at the study site is dominated by European Turkey-oak (*Quercus cerris* L., 50-75% cover, Fig. 2.4). Turkey-oak extends from south-east Europe to south-west Asia. In Italy, it is distributed over all the territory, preferring clay, and deep sub-acid soils. In

Figure 2.4 *Quercus cerris* L. stand

the Apennines, this species forms pure or mixed forests together with other broadleaved species (i.e., *Quercus pubescens* Willd.) (Pignatti 1982).

The main understory species at the study site are *Crataegus monogyna* Jacq. (land cover percentage: 25-50%), *Euonymus europeus* L. (<25%), *Ligustrum vulgare* L. (25-50%), *Ruscus aculeatus* L. (25-50%), *Asparagus acutifolius* L. (<25%), *Lonicera caprifolium* L. (<25%), *Rosa canina* L. (<25%), Cornus mas L. (<5%) and *Prunus spinosa* L. (<5%). The

stand investigated is managed as coppice with standards, and the trees are felled at an average rate of once every 15-20 years. The mean age of the trees is between 28 and 30 years old with bigger seed origin trees almost 55 years old.

2.2.2 Stand measurements (soil moisture, canopy cover and above-ground biomass, tree stocking density and leaf biomass)

ThetaProbe type ML2 Delta-T Devices were used to record the volumetric soil moisture content (m^3 m^{-3}) by the well established method of responding to changes in the apparent dielectric constant. On each sampling date, measurements were taken at the soil core sampling point at three depths (10 cm, 20 cm and 30 cm; hereafter reported as mean 30 cm depth). Soil was classified according to the USDA keys to Soil Taxonomy (1988). Soil analyses were carried out according to the SISS (Italian Society of Soil Science) and USDA (United States Department of Agriculture) standard methods.

In July 2010 canopy cover was measured by hemispherical photos (Rich 1990) analysed with the Can-eye freeware (www4.paca.inra.fr/can-eye 2011). 10 hemispherical photos per stand were taken at 7.5-meter intervals along a transect.

Seven selected sampling plots per stand along a 140 m long transect were surveyed to obtain number of stems, and diameter at breast height (dbh) values. The plots were circular-shaped with a diameter of 20 m for a total area of 2199 m^2. To measure the above-ground biomass of the stand, in November 2010 we selected four sample trees that represented the range of tree size for destructive harvesting. The dbh and plant height of each tree were measured, and the trunks and branches were sliced into sections that measured almost one-tenth of their respective total length. We measured the total fresh weight of each tree using a forest skidding tractor and a portable dynamometer. For each tree, the dry weights of two sub-

samples of trunk and branch at small-end and large-end were determined after oven drying at 70 °C to a constant weight (2-3 weeks). A site-specific allometric relationship (r^2= 0.99) was developed to estimate the woody biomass from the tree dbh. The power equation (2) is:

$$W=aD^b \qquad (2)$$

where W = dry weight D = dbh a =0.193 b = 2.323

Finally, to determine the tree stocking density (number of trees ha^{-1}) of the stands, we counted the total number of trees in an area measuring 100x100 meters.

In order to estimate leaf biomass, 10 litter traps where placed in July 2010 at 7.5-meter intervals along a transect (Finotti et al. 2003). Leaves where sampled at the end of November 2010 after leaf shedding, dried and weighed.

2.2.3 Above-ground stand characteristics

As shown in Table 2.2, stem density was higher in the Turkey-oak stand than in the beech stands, whereas dbh and height were smaller. The above-ground biomass value was intermediate between those of the 1994 Conversion stand and the 2004 Conversion stand. There was a total canopy cover more than 95%.

Table 2.2 Vegetation and soil features of the survey site

Soil

Layer depth (cm)	Texture	Ph H2O	C/N	N (g kg^{-1})	P (ppm)	K (ppm)	Organic matter (g kg^{-1})
A (0-7)	Clay	8.4	8.2	0.9	2.9	180.4	13.0
C1 (7-30)	Silty clay	8.6	7.5	0.4	2.3	124.8	5.1

Vegetation

Tree number (stem per Ha^{-1})	Above-ground biomass (Mg ha^{-1})	Leaf biomass (Mg ha^{-1})	Canopy cover (%)	Mean dbh (cm)	Mean tree height (m)
1735 ±106	106.4 ±9.2	3.0 ±0.1	95.6 ±0.4	9.4 ±0.6	8.35 ±0.4

Vegetation data from all stems over 5 cm diameter in July 2010. All values are mean ±S.E.

Soil features data from Di Iorio et al. (2008)

2.3 References

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www4.paca.inra.fr/can-eye 2011

Chapter III

Effect of forest management on fine roots in *Fagus sylvatica* L. stands

There is an intrinsic difficulty in modelling carbon allocation in the below-ground compartment with respect to the above-ground compartment. Firstly, it is highly labour-intensive, and secondly, models must include a variety of internal (e.g., genotype of plant species) and external (e.g., temperature, precipitation, soil properties, nutrient availability and competition between plants) factors (Majdi et al. 2005). A major external factor is the effect of anthropological action on the forest including management practices (Rötzer et al. 2010). Interestingly, a recent review of root biomass data of the three main types of North-European beech ecosystems demonstrated that the fine-root biomass undergoes considerable fluctuations in relation to above-ground characteristics (Finer et al. 2007). Moreover, various studies have shown that forest use intensity and disturbance has a profound impact on fine-root standing mass, fine-root growth rate and fine-root turnover (Chertov et al. 2005; Leuschner et al. 2008).

In an attempt to shed light on this issue, we investigated how fine-root compartment reacts to management practices, which have intensified in recent years consequent to regional and governmental policy to convert most of these forests from a coppice to a high-standard condition.

3.1 Materials and methods

3.1.1 Fine-root measurements

Fine-root biomass (d < 2 mm) was determined in soil cores (Vogt and Persson 1991). In each stand, four permanent 10-m^2 plots were established. Each plot was the centre of a circular-shaped plot with a 20 m diameter where above-ground characteristics were also measured. Two soil cores (4 cm diameter × 30 cm deep) were randomly collected in each plot using a motor-driven portable core sampler (adapted from Ponder Jr. and Alley 1997). In this study, we established sampling times in relation to the growing seasons of beech forests and when the soil was free of snow. Therefore, the kinetics of biomass and necromass variation was investigated from May to October 2008 by collecting core samples approximately every 30 days. Because of snow cover, during winter period experimental site was very difficult to reach, therefore we couldn't sample. As already observed by other authors (Claus and George 2005; Crider F.J. 1928), we assumed that fine-root production and decomposition are low during winter season. Therefore in order to evaluate root biomass present throughout the winter and to conclude our annual experiment, in April 2009 after snowmelt core samples were collected. Each core sample was divided into three portions according to the depth from the soil surface: 0-10 cm (including the first 2/3 cm of humus layer), 10-20 cm and 20-30 cm. Mean distance between plots was 50 meters, six- to ten fold the distance between trees in all stands.

Samples were stored in plastic bags at 4°C until processed. For processing, each sample was placed in a nylon bag (300 μm mesh) that was contained in a plastic cylinder (6 mm mesh) and washed automatically using a washing machine. We distinguished beech roots from other understory roots by identifying morphological characteristics at the microscope. The morphological characteristics of beech fine roots were previously established from samples dug near the stem. Beech fine roots were reddish and stiffer than the understorey

roots (herbaceous). Moreover, the fine roots of *F. sylvatica* were classified "live" (relate dry weight hereafter termed biomass) or "dead" (relate dry weight hereafter termed necromass) depending on their colour, texture and shape (Vogt and Persson 1991). Live roots were resilient, translucent, and white to tan; dead roots fragmented easily, were dull, and gray to black. The reliability of the criteria we used was confirmed by observations at the binocular microscope. These visual and manual criteria were based on readily observable morphological features. These criteria yielded reproducible results providing a practical approach to classifying roots on the scale required by this study. The roots freed from soil were scanned at a resolution of 400 dpi with a calibrated flatbed scanner coupled to a lighting system for image acquisition (Epson Expression 10000 XL). Afterwards they were separately oven-dried and weighed. Fine-root images were analysed by the WinRhizo Pro V. 2007d software (Regent Instruments Inc., Quebec, Canada) to obtain length and diameter. The following root traits were determined for each stand: (1) annual mean live (LFRM g m^{-2}) and dead (DFRM; g m^{-2}) fine-root dry mass; (2) fine-root biomass and necromass seasonal pattern; (3) fine-root annual production (FRP, g m^{-2} yr^{-1}) and turnover rate (yr^{-1}); (4) fine-root depth distribution.

In a recent work carried in a *Fagus-Quercus* mixed forest, Hertel and Leuschner (2002) compared different methods and found that minimum-maximum would yield a more realistic result (25% overestimation). We estimated FRP using the 'minimum-maximum method' procedure (Edwards and Harris 1977; McClaugherty et al. 1982) considering only significant differences between maximum and minimum for each soil layer. Rates of biomass turnover were calculated as Annual Root Production divided by Maximum Standing Biomass (Gill and Jackson 2000).

3.1.2 *Statistical analysis*

To compare three beech stands with differing forest management practices, permanent plots were established within each stand, and considered independent replicates. This is a point comparison approach rather than a replicated experiment on the ecosystem scale. The effects of forest management on fine-root biomass and necromass were evaluated for the whole 0-30 cm soil depth and at three different soil depths (0–10 cm, 10–20 cm, and 20–30 cm). For the whole 0-30 cm soil depth, fine-root biomass, necromass and production were calculated by summing values obtained from all soil layers. Data were not normally distributed nor did they meet the assumption of homoschedaticity. A non parametric Mann-Whitney U two-sample test with a 5% rejection level was used to test for significant differences between stands (annual mean fine-root biomass and necromass, total mean fine-root mass per number of stems), soil layers and fine-root mass at two sampling dates. Statistical analysis was performed with SPSS software package version 12.0 (SPSS Inc, Chicago IL, USA).

3.2 Results

3.2.1 Fine-root standing mass and seasonal pattern

Figure 3.1 shows the seasonal variations of standing mass of live (LFRM) and dead (DFRM) fine roots in the three stands and at three different soil depths. In the case of LFRM, there was a bimodal pattern in almost all the conditions examined with the formation of two peaks that became more evident when standing mass was examined at greater depths. However, the timing of peak formation differed in the three stands in June-July and in September-October. In the case of DFRM, there was only one clearly evident peak, which occurred at the end of August (and at all the soil-depths examined) in the Coppice stand. Both 1994 and 2004 Conversion stands showed a slight increase, albeit not a peak, in July and

August respectively. This was more evident at a soil depth of 0-30 cm. For each single layer, a slight increase was evident only at 0-10 cm and at 20-30 cm in the case of the 2004 Conversion stand. Moreover, only in the case of the Coppice stand a second increase in DFRM values was found in October at a soil depth of 20-30 cm. The annual mean LFRM and DFRM, expressed as the sum of the three depths considered, was significantly higher in the Coppice stand (Mann-Whitney U test, $p < 0.05$; Table 2) than in either of the two Conversion stands. Biomass was significantly higher in the 1994 Conversion stand than the 2004 Conversion stand, while necromass did not show significant difference.

The fine-root biomass per tree was calculated by dividing the fine-root biomass per hectare by the stem number per hectare. The results showed a significantly higher value ($p < 0.05$) for both Conversion stands (2004, 7.15 ±0.6 kg stem^{-1}; 1994, 5.19 ±0.5 kg stem^{-1} – data not shown) than for the Coppice stand (3.18 ±0.2 kg stem^{-1} – data not shown). The fine-root biomass per tree did not differ between Conversion stands ($p = 0.06$). Moreover, when above-ground stand characteristics such as stem density per hectares were compared with fine-root biomass we found that fine-root biomass increased as stand stem density increased (Fig. 3.2).

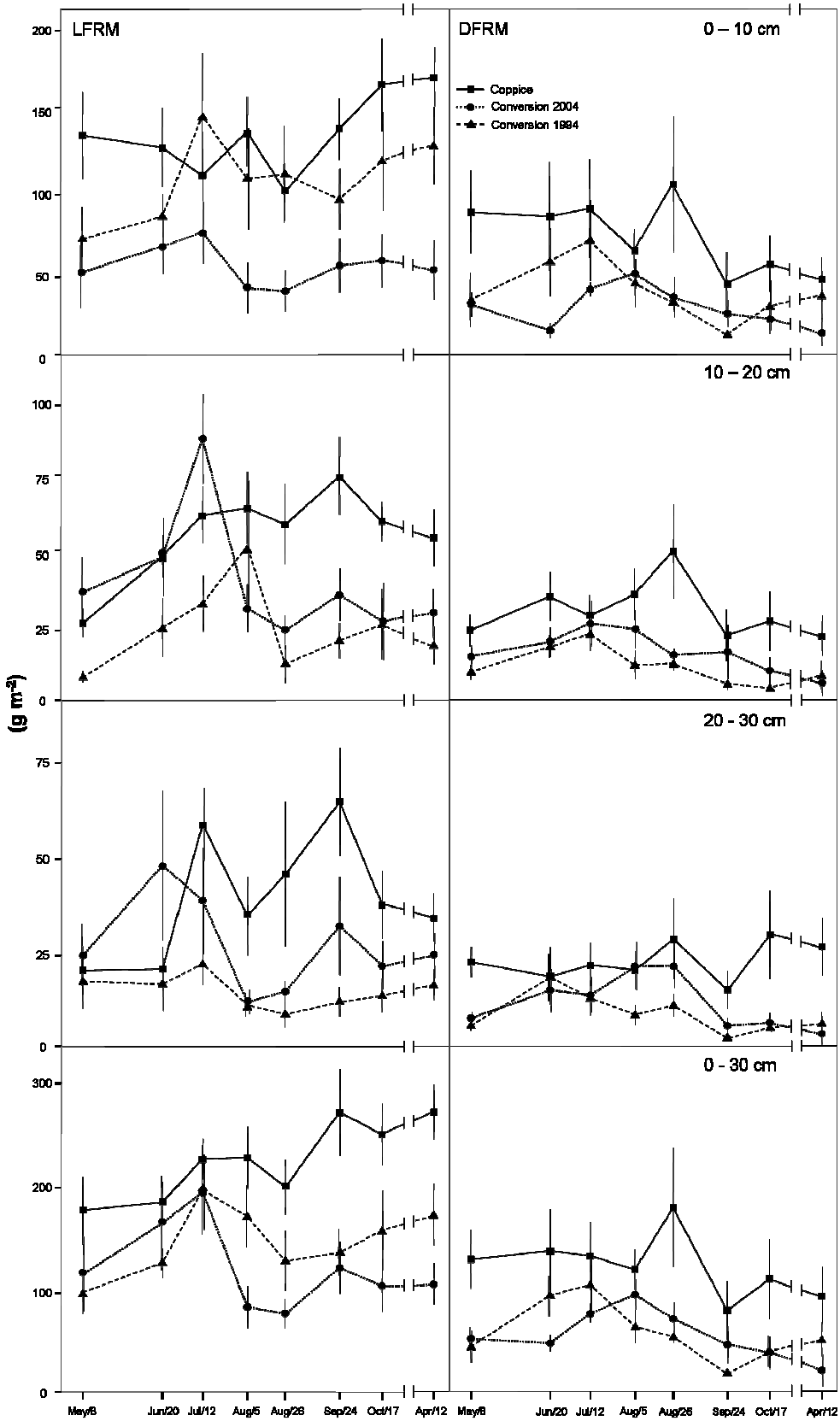

Figure 3.1 Seasonal pattern of live (LFRM) and dead (DFRM) fine-root mass (g m^{-2}) according to soil depth and type of forest management. Each value represents the mean of 8 samples, vertical bars indicate standard error. Scale ranges are not standardized between each panel in order to allow a more clear presentation

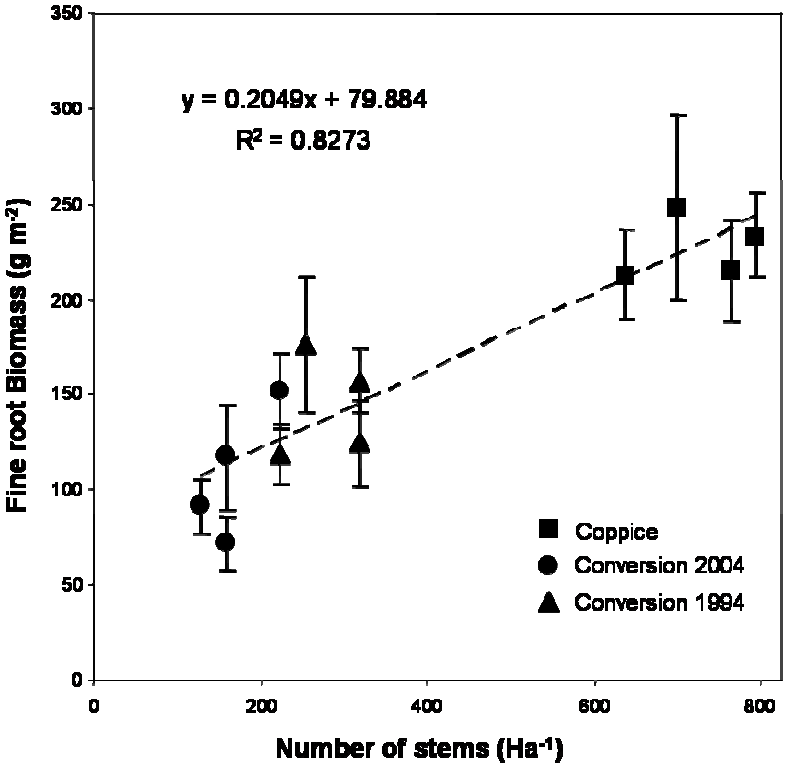

Figure 3.2 Relation between stem density (number of stems Ha^{-1}) and fine-root biomass (g m^{-2}). Each point represents a sampling plot. Above-ground characteristics were measured around each plot by circular-shaped area with 20-m diameter. Fine-root biomass for each sampling plot is the mean of 16 samples (from May 2008 to April 2009) ± S.E.

Analysed separately, the biomass of both live and dead fine roots decreased significantly with depth (Tab. 3.1). Indeed, a mean of 59% (biomass) and 58% (necromass) of the total value

Table 3.1 Annual mean fine-root biomass and necromass of three forest management treatments in the 0-10, 10-20 and 20-30 cm soil layer. Profile 0-30 values are sums of each soil layer. Values are means of 32 samples ±S.E.

	Coppice stand		**Conversion 1994 stand**		**Conversion 2004 stand**	
Soil depth	LFRM (g m^{-2})	DFRM (g m^{-2})	LFRM (g m^{-2})	DFRM (g m^{-2})	LFRM (g m^{-2})	DFRM (g m^{-2})
0-10	135.4 ±8.9 ax	71.5 ±8.9 ax	106.5 ±9.1 bx	38.7 ±5.3 bx	53.6 ±5.7 cx	28.9 ±3.0 bx
10-20	55.8 ±4.1 ay	30.1 ±3.2 ay	23.9 ±3.8 by	11.5 ±1.3 by	39.5 ±4.3 cx	16.4 ±2.0 by
20-30	38.8 ±4.1 az	22.2 ±2.5 az	14.4 ±1.7 by	8.6 ±1.2 bz	26.4 ±3.8 cy	11.4 ±1.6 bz
Profile 0-30	230.0 ±17.2 a	123.8 ±14.6 a	144.8 ±14.7 b	58.8 ±7.8 b	119.4 ±13.7 c	56.8 ±6.6 b

a, b and c indicate significant differences between forest management treatments within the same soil depth (Mann-Whitney U test, $p < 0.05$). x, y and z indicate significant differences between soil depth within the same forest management treatment (Mann-Whitney U test, $p < 0.05$)

was concentrated in the uppermost soil layer (0-10 cm). In the 1994 Conversion stand the remaining value was equally distributed between the other two soil layers, whereas in the 2004 Conversion stand and the Coppice stand, the values in the other two soil layers decreased by almost one third that of the upper layer. In the Coppice stand, both live and dead fine-root mass significantly differed between soil layers. In the 2004 Conversion stand, the live mass in the first layer (0-10 cm) did not differ significantly to the second layer (10-20 cm) and both were significantly higher compared to the deepest layer (20-30 cm). In the 1994 Conversion stand, LFRM were significantly higher in the first soil layer than in the two deeper layers while DFRM significantly differed over all the tree soil layers. Differently, the percentage of necromass on the total mass (biomass plus necromass) was higher in the deeper soil layer. In particular, in the 1994 Conversion stand, it increased from 27% in the upper soil layer to 32% and 60% respectively in the 10-20 cm and 20-30 cm layer. In the Coppice stand, the necromass percentage slightly increased from 34% in the upper soil layer to 36% in the deeper layer. Differently, in the 2004 Conversion stand (in which the soil had been recently disturbed cause the management practices) the necromass percentage was highest in the uppermost soil layer (35%) then in the lower (30%).

3.2.2 Fine-root production and turnover

The annual FRP in the uppermost soil layers (0-10 cm) was higher in both 1994 and 2004 Conversion stands than in the Coppice stand where zero production was found because minimum and maximum were not significantly different. Both 1994 and 2004 Conversion stands showed no significant difference, resulting in a zero production, in the lowest soil layer (20-30 cm). Where zero production was found, fine-root turnover rate was not given. In the Coppice stand and the 2004 Conversion stand FRP increased in the middle soil layer, decreasing at the lower one. The 1994 Conversion stand showed a clear FRP decrease along

the three soil depth. Fine-root turnover rate in both Coppice and 1994 Conversion stand did not vary in the two soil layers where values were given, while the 2004 Conversion stand showed a slight increase at depth. The annual FRP and fine-root turnover rate of the whole 0-30 cm depth was different in the three stands. Both FRP and turnover were lower in the Coppice stand and higher in the 1994 Conversion stand, with intermediate values for the 2004 Conversion stand (Table 3.2).

Table 3.2 Seasonal maximum and minimum of fine-root dry mass (biomass and necromass), net annual fine-root production (FRP) (according to minimum-maximum method) and turnover rate (production/seasonal maximum fine-root biomass) of three forest management treatments in the 0-10, 10-20 and 20-30 cm soil layers. Profile 0-30 FRP and seasonal maximum fine-root biomass are sums of each soil layer. Profile 0-30 turnover rate means are weighted means

Soil depth	Fine-root biomass and necromass (g m^{-2})		Significance of min-max difference[1]	FRP (g m^{-2} yr^{-1})	Seasonal maximum fine-root biomass (g m^{-2})	Turnover rate (yr^{-1})
	Seasonal minimum	Seasonal maximum				
Coppice						
0-10	180.9	224.0	n.s	0	178.6	–[2]
10-20	49.2	99.5	*	50.3	74.7	0.67
20-30	38.2	76.3	*	38.1	63.6	0.60
Profile 0-30				88.4	**316.9**	**0.64**
Conversion 1994						
0-10	87.7	244.6	*	157.0	145.2	1.08
10-20	17.2	71.7	*	54.4	51.4	1.06
20-30	13.5	34.1	n.s	0	21.4	–[2]
Profile 0-30				211.4	**218.0**	**1.08**
Conversion 2004						
0-10	62.1	114.0	*	51.9	74.1	0.70
10-20	35.0	112.8	*	77.8	87.1	0.89
20-30	27.1	61.6	n.s.	0	47.1	–[2]
Profile 0-30				129.7	**208.3**	**0.81**

[1] * marks significant maximum-minimum difference ($p < 0.05$) for a given soil layer.
[2] No data are given because none of the seasonal differences were significant

3.3 Discussion

There is a surge of interest in understanding how management practices affect below-ground events taking place in a forest. Apart from the obvious scientific interest in the topic, such studies can have a practical impact given the potential implication for modelling carbon sequestration. Furthermore, data about the effect of management practices on fine roots will lead to a more accurate evaluation of the forest carbon stock, which is an indicator of sustainable forest management (IPCC 2007 www.ipcc.ch; Bakker 1999).

Here we show that at the stand level the general decrease of the total root biomass observed in the Conversion stands can be related to the reduction of tree density that occurs during conversion. A coppice stand is usually characterised by a dense distribution of stools each of which includes a number of stems. During conversion to high forest management, only one stem in each stool is left to continue growing. This kind of management leads to the death of roots belonging to the eliminated stools. A higher fine-root biomass: stem number ratio was observed in both 1994 and 2004 Conversion stands. This suggested that fine-root biomass production per tree in these stands was higher than in Coppice stand. Management practices also affect biomass distribution along the soil profile. Indeed, a lack of significant differences in fine-root mass between soil layers was observed in the Converted stands with respect to Coppice stand. Therefore, from a practical point of view, derangements caused by conversion management practices (such as cutting, skid trailing and logging-operations) seem to result in a temporary stimulation of fine-root emission (as described by Helmisaari et al. 2002) as well as a high soil disturbance which is reflected by fine-root soil distribution (as described by Gondard et al. 2003, Hartanto et al. 2003). Moreover, it is reasonable to assume that the stimulation of biomass production observed in the 2004 Conversion stand will decrease after 10 years given the finding that root mass per tree was higher in 2004

Conversion stand than in the 1994 Conversion stand, which has not undergone management practices in the last 14 years.

When investigating the effect of conversion of a stand from coppice to high forest management it is necessary to consider that the consequent overall rejuvenation of the stand would also directly affect the life-cycle of the roots. In this context, both a decrease (Fujimaki et al. 2007) and an increase of fine-root biomass have been directly related to stand rejuvenation (Curt and Prevosto 2003) in coniferous and deciduous stands. Our data fits with the fine-root biomass successional pattern model throughout a forest's life cycle constructed by Claus and George (2005). Indeed, the more recent 2004 Conversion stand represents the first phase after cut with a rapid increase in fine-root production. The 1994 Conversion stand represents the second phase with a relatively slow decrease in FRP and the Coppice stand represents the third phase of the model where FRP reach equilibrium together with canopy closure.

In analogy with fine-root production also fine-root turnover was affected during conversion from coppice to high forest stand. In fact, there was an increase in fine-root turnover that probably reflects the need to construct a different type of root system when a single large stem remains on the stool. Moreover, soil temperature may play an important role in fine-root dynamics. In the stands of the present study canopy cover increased with tree density and both of these factors were inversely related to soil temperature. Jackson et al. (1996) found that within the same plant functional type fine-root turnover rate increased with increasing of temperature. Our results show that a lower mean soil temperature was associated with a lower fine-root turnover rate. The turnover rates we obtained are close to those obtained in other studies (Gill and Jackson 2000).

Majdi et al. (2005) postulated the occurrence of unimodal or bimodal seasonal patterns in the root life cycle of temperate forests, which could depend on seasonal variations of water

and nutrient availability (Coners and Leuschner, 2005). The relation between LFRM and DFRM peaks reported confirms the occurrence of a bimodal pattern in the life cycle of the fine-root component in a beech forest. It also suggests that this bimodal pattern is not affected by the conversion of the stands examined. Furthermore, as found by Mainiero and Kazda (2006), the match between the seasonal pattern of root mass and the seasonal pattern of soil temperature and rainfall suggests that both of the environmental factors affect root turnover. The general decrease of fine-root biomass we found in midsummer might derive from an increase of soil temperature. Moreover, during this period also rainfall reached the seasonal minimum with a consequent shortage in water availability. In summer it would have been too costly for the trees of our stand to continue their normal production and maintenance of root mass (see, Atkin et al. 2000). This is supported by the occurrence of a second small LFRM peak in late summer when the temperature decreases and rainfall increases. In addition values obtained in April 2009 were similar to those obtained at the end of the previous growing season (October 2008), thereby ruling out variability during the winter. This confirms that the decline and consequent arrest of new roots production and mortality in the fall coincides with cold temperature and leaf shedding.

Joslin et al. (2006) hypothesised that a tree's fine-root system consists of pools of fine roots of different ages. One pool is very dynamic and has a life span < 1 year ("short-lived"), and the other has a life span > 1 year ("long-lived") and consists of older fine roots. Andersson and Majdi (2005) suggested that the seasonal timing of production might influence fine-root age. In particular, fine-roots produced in spring have shorter life spans that those produced later. In our experiments, the difference between the initial and peak values of biomass is roughly due to a flush of new roots, and the fact that the peak was followed by a marked decrease suggests that most of these new fine roots are of the "short-lived" type. The second flush of new roots seems to be less abundant than the first one and is probably formed

mainly by "short-lived" fine roots because also this second peak lasts only a few weeks. If this hypothesis is valid, the amount of fine roots present during winter could represent the portion of the root system classifiable as "long-lived" fine roots. Consequently, the increment in the amount of fine roots between the beginning and the end of the vegetative season would represent the yearly production of "long-lived" fine roots.

3.4 Acknowledgements

I'm grateful to Dr. Antonio Montagnoli, Dr. Samanta Riva and Dr. Antonino Di Iorio for their valuable help in the field and laboratory work, to Professor Donato Chiatante for his valuable comments and suggestions. I thank Dr. Davide Beccarelli and Dr. Lorenzo Guerci from Consorzio Forestale "Lario Intelvese" for helping with the field work and data on forest management. This work was supported by the Italian Ministry of Environment as part of the research project "Trees and Italian forests, sinks of carbon and biodiversity, for the reduction of atmospheric CO_2 and improvement of environmental quality". The authors are also indebted to the Italian Botanic Society Onlus for supporting this research.

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Chapter IV

Forest management and its effect on carbon and nitrogen concentration in fine roots of beech (*Fagus sylvatica* L.)

Conversion to high forest results in considerable alteration of almost all micro-environmental factors that characterized the coppice stand. Various studies have shown that increase in canopy opening size causes an increase of both seasonal average soil temperatures and soil temperature extremes (Liechty et al. 1992, Hashimoto and Suzuky 2004). The seasonal and daytime–night-time differences in average maximum-minimum air temperatures are increased 15 cm above the soil surface consequent to canopy opening (Carlson and Groot 1997).

In the attempt to shed some light on the effects of conversion, we studied the effect of conversion of a coppice stand to high-standard management on the below-ground biomass, particularly the fine-root component (roots with a diameter between 0-2 mm). Our rationale was that fine roots represent the component of a root system that is most sensitive to climate and microclimate variations (Aussenac 2000, Fotelli et al. 2002), and to stressful conditions such as drought, competition and herbivory (Lopez et al. 1998, Glen and Robert 2006, Withington et al. 2006). Moreover, stand conversion induces a decrease in the fine-root standing biomass (Lopez et al. 2003, Tufekcioglu et al. 2005). In addition, Fotelli et al. (2002, 2004) reported both an increase and a decrease of fine-root biomass in thinned forests, depending on site exposure, whereas Lopez et al. (2003) confirmed that fine-root production is positively affected by management operations.

We found that the conversion of a beech stand from coppice to high forest induces, in the fine-root component, a decrease of total biomass and an increase of turnover rate. We also found that fine-root biomass production is transiently stimulated by conversion (see chapter III). Taken together, our earlier findings suggest that the fate of fine roots after conversion is factor to be considered in the measurement of a forest carbon stock that will be used as an indicator of sustainable forest management (http://www.sfmindicators.org/ 2011).

In this chapter we focus on carbon and nitrogen concentrations in fine roots. The *C* concentration of fine roots is associated with construction costs (Gordon and Jackson 2000, Guo et al. 2004) whereas *N* concentration is associated with their metabolic activity, respiration and root longevity (Ryan 1991, Pregitzer et al. 1998, Withington et al. 2006). Furthermore, the C:N ratio could provide an indication of the fine-root life-span (Withington et al. 2006).

4.1 Materials and methods

4.1.1 Fine-root measurements

Fine roots were collected at different soil depths using a motor-driven portable root soil core sampler (adapted from Ponder and Alley 1997) during the 2009 growing season (between May and October). In each stand, a permanent 10 m^2 plot was set. Two soil cores (4 cm diameter x 30 cm deep) were randomly collected in each plot. Samples were taken when the soil was free of snow cover. Fine roots were sampled on six dates approximately every 30 days for a total of 36 cores (2 cores x 3 stands x 6 collecting dates). The soil cores were separated into three soil layers: 0-10 cm including the humus layer (0- 2/3 cm), 10-20 cm and 20-30 cm from the soil surface. Samples were stored in plastic bags at 4°C until processed. Each sample was washed automatically in a filtering nylon bag (300 μm mesh) using a washing machine (adapted from Benjamin and Nielsen 2004).

Soil-free roots were sorted into colour, texture and shape under a 10x stereomicroscope (Vogt and Persson 1991). Subsamples of live fine roots were scanned at resolution of 400 dpi and divided in three diameter classes (0-0.5; 0.5-1; 1-2 mm) by using WinRhizo Pro V. 2007d (Regent Instruments Inc., Quebec). Fine roots of each diameter class were scanned again and images were analyzed by WinRhizo Pro to obtain morphological data, namely, total root length and mean diameter (Fig. 4.1). The subsamples were then separately oven-dried, weighed and stored in sealed vials

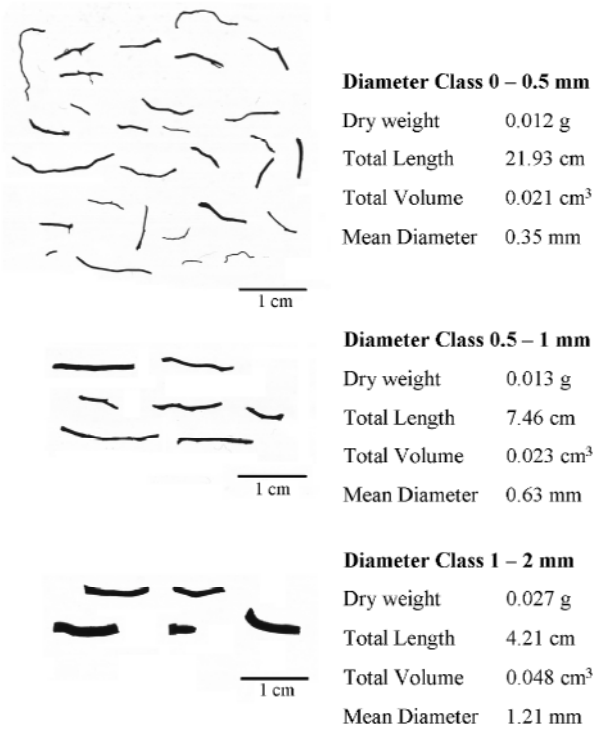

Figure 4.1 Examples of three fine root subsamples selected for CHN-analysis. The subsamples represent the three diameter classes investigated (0-0.5, 0.5-1 and 1-2 mm). The morphological data for each sub-sample were obtained with the WinRhizo Pro V. 2007d program

4.1.2 Fine-root nitrogen and carbon concentrations

The subsamples of fine roots were ground in liquid N_2 with mortar and pestle and analyzed for *C* and *N* concentrations with a CHN-analyzer (NA-2000 N-Protein; Fisons Instruments S.p.A., Rodano [MI], Italy). The analyzer was calibrated with an atropine standard, and every 10th sample with an atropine sample. The mean total *N* and *C* recovery rate for nutrient analysis of atropine was 100.48 % (1 SE = 0.6%) and 101.02 % (1 SE = 0.22%), respectively.

4.1.3 Statistical analysis

Statistical analyses were carried out using the SPSS software package version 12.0 (SPSS Inc, Chicago IL, USA). C:N ratio data did not meet the normal distribution and homoscedasticity. A logarithm transformation produced normal distributions and equal variances, and were therefore analysed using normal parametric statistics. It was not necessary to transform fine-root *N* and *C* concentration data. Analysis of variance (4-way ANOVA) was performed to assess the effects of diameter class (0-0.5; 0.5-1.0; 1.0-2.0 mm), depth (0-10; 10-20; 20-30 cm), management (Coppice; 1994 Conversion; 2004 Conversion) and time (six sampling times). A Bonferroni test with a 5% rejection level was used to detect significant differences between stands, soil depth and diameter class. Mean sub-sample root diameters were included as covariates into the 4-way ANOVA for each diameter class.

4.2 Results and discussion

We measured *N* and *C* concentrations in fine roots in three beech stands: one maintained as coppice; the other two had been converted to high forest (in 1994 and 2004, respectively) but had a different tree density. Conversion resulted in a decrease in tree density in the 2004 Conversion stand, whereas tree density in the 1994 Conversion stand was intermediate between that of Coppice and the 2004 Conversion stand. The decrease in tree density increased light and soil temperature within the stand (see Tab. 2.1), but we cannot exclude that the reduced tree density also affected other environmental factors. Given the amount of variations introduced in stands during conversion, it is conceivable these changes could have affected *N* and *C* concentrations in fine roots.

4.2.1 Fine-root nitrogen concentration

The concentration of *N* was affected by all the main factors investigated (Tab. 4.1). *N* concentration in pooled fine-root samples was significantly higher in the two Conversion stands than in the Coppice stand (Tab. 4.2). This finding coincides with studies showing that *N* concentration in fine roots is positively related to soil temperature (Gessler et al. 1998, Fotelli et al. 2004, Nahm et al. 2006). Our data also confirm that a reduction in tree density leads to an increase in *N* availability in the stand (Fotelli et al. 2002).

Table 4.1 Analysis of variance of nitrogen and carbon concentrations and the C:N ratios of fine roots. * P < 0.05; ** P < 0.01; *** P < 0.001

	ANOVA						
		Root N		Root C		Root C:N	
Source of variation	**df**	**F**	**P values**	**F**	**P values**	**F**	**P values**
Mean Diameter	1	-	-	10.293	0.001**	-	-
Management (Mng)	2	11.886	<0.001***	73.024	<0.001***	23.525	<0.001***
Soil Depth (Dep)	2	48.518	<0.001***	1.282	0.279	48.771	<0.001***
Diameter Class (Cls)	2	134.466	<0.001***	12.949	<0.001***	108.021	<0.001***
Sampling date (Dat)	5	9.694	<0.001***	14.096	<0.001***	12.212	<0.001***
Mng x Dep	4	2.976	0.020*	4.774	<0.001***	3.349	0.011*
Dep x Cls	4	2.884	0.023*	-	-	-	-
Mng x Dat	10	2.592	0.005**	2.723	0.003**	2.728	0.003**
Mng x Dep x Dat	30	1.724	0.13*	1.724	0.013*	1.785	0.009**

Table 4.2 Pooled values of fine-root N and C concentrations and the C:N ratio between stands. Values are the means of 108 replicates ± 1 SE. a, b and c denote comparison among three forest management differentiated stands (Bonferroni test, $P<0.05$)

Management	N (g kg^{-1})	C (g kg^{-1})	C:N ratio
Coppice	8.5 ± 0.1 a	537.9 ± 1.2 a	65.3 ± 1.0 a
Conversion 1994	8.9 ± 0.1 b	526.9 ± 1.1 b	60.9 ± 0.9 b
Conversion 2004	9.0 ± 0.1 b	528.5 ± 1.0 b	59.8 ± 0.8 b

Previous studies showed that the *N* concentration in fine roots is directly related to their metabolic activity and respiration, and inversely to their longevity (Ryan 1991, Pregitzer et al. 1998, Withington et al. 2006). Therefore, our finding that *N* concentrations were significantly higher in the two Conversion stands than in the Coppice stand suggests that the environmental variations introduced by conversion are responsible for the increased metabolic activities of fine roots, which in turn, would lead to an acceleration of their growth rate (Valverde-Barrantes et al. 2007) and a shorting of their life-span. This hypothesis is consistent with our previous findings (chapter three) that the fine-root turnover rate increases, and consequently the life-span decreases, as a result of conversion operations.

It has been suggested that *N* concentration is related to root diameter and it seems that the concentration is highest in the thinnest root branches (Gordon and Jackson 2000, Li et al. 2010) located in the uppermost soil layer (Pregitzer et al. 1998). We have limited our investigation to fine roots with a maximum diameter of 2 mm and have divided them into three diameter classes. Although we do not know whether functional differences exist between these three diameter classes, we cannot exclude that, also in our case, fine roots with a diameter between 0.0 and 0.5 mm could play a role in *N* uptake function as suggested by Hishi (2007) and Guo et al. (2008). If this is the case, we could speculate that fine roots

belonging to the two thicker diameter classes might play a role in transport and storage (Hishi 2007, Guo et al. 2008).

We evaluated *N* concentrations of fine roots belonging to the three different diameter classes excavated from the three different stands at three different soil depths (Tab. 4.3). The concentration of *N* was highest in fine roots with a diameter between 0.0 and 0.5 mm, which live in the most superficial (0-10 cm) soil layer. The fine-root *N* concentration in this superficial soil layer decreased as root diameter increased (Tab. 4.3). The decrease of *N* concentration with the increase of fine-root diameter was confirmed at the other two soil depths. Diameter class showed a statistical interaction with soil-depth (Tab. 4.1). Irrespective of stands, the fine roots of the 0.0-0.5-mm diameter class, but not those of the 1.0-2.0-mm class, had different *N* concentrations at different soil depths (Tab. 4.3). This result is in agreement with that reported by Pregitzer et al. (1998). The sensitivity of fine roots of the 0.0-0.5-mm-diameter class remains to be determined and could be related to their uptake function, which means fine roots are more sensitive to changes in soil features than thicker roots.

Table 4.3 Fine-root N concentration of three diameter classes. Values refer to three soil depths each 10-cm thick and three different forest management stands. Each value represents a mean of 12 samples ± 1 SE. a, b and c denote comparison between stands within the same diameter class and depth (Bonferroni test, $P<0.05$). x, y and z denote comparison among soil depths within the same management and diameter class (Bonferroni test, $P<0.05$)

	Diameter class 0 – 0.5 mm			**Diameter class 0.5 – 1 mm**			**Diameter class 1 – 2 mm**		
	Management			**Management**			**Management**		
Soil depth (cm)	**Coppice (g kg^{-1})**	**Conversion 1994 (g kg^{-1})**	**Conversion 2004 (g kg^{-1})**	**Coppice (g kg^{-1})**	**Conversion 1994 (g kg^{-1})**	**Conversion 2004 (g kg^{-1})**	**Coppice (g kg^{-1})**	**Conversion 1994 (g kg^{-1})**	**Conversion 2004 (g kg^{-1})**
0 - 10	10.3 ± 0.3 ax	10.6 ± 0.3 ax	11.2 ± 0.3 ax	9.7 ± 0.4 ax	9.0 ± 0.3 ax	9.4 ± 0.3 ax	8.2 ± 0.4 ax	8.4 ± 0.3 ax	8.1 ± 0.3 ax
10 - 20	8.9 ± 0.2 ay	10.2 ± 0.3 bxy	9.8 ± 0.1 by	8.0 ± 0.3 ay	8.8 ± 0.2 abxy	8.9 ± 0.3 bxy	7.2 ± 0.2 ax	7.9 ± 0.3 abx	8.2 ± 0.2 bx
20 - 30	8.6 ± 0.2 ay	9.3 ± 0.4 by	9.5 ± 0.2 by	8.1 ± 0.3 ay	7.9 ± 0.2 ay	8.3 ± 0.3 ay	7.3 ± 0.4 ax	7.7 ± 0.5 ax	7.8 ± 0.2 ax

Regarding management practices, fine roots living at a depth of 10-20 cm were more sensitive to forest management than roots living at the other two soil depths investigated (Tab. 4.3). Padula et al. (1987) found that, in aging coppiced stands, the soil features slowly improve so that the soil gradually assumes the typical profile of well-differentiated forest soil. Differently, management practices cause mixing of the uppermost soil, which consequently loses a clear profile (Gondard et al. 2003). Thus, we may speculate that at a depth of 10-20 cm fine roots in the two Conversion stands had access to more nitrogen than in the Coppice stand because of soil mixing.

In our experiments, the variations in the *N* concentration of fine roots were similar in the three beech stands (Fig. 4.2) during the vegetative season (from May to October). In all three stands, the *N* concentration decreased during spring, and returned to the same values (8.8-9.4 g kg^{-1}) at the end of the growing season. This pattern of *N* concentration variation is in line with the report that temperate forests are characterised by seasonal variations of *N* concentration (Cerasoli et al. 2004,

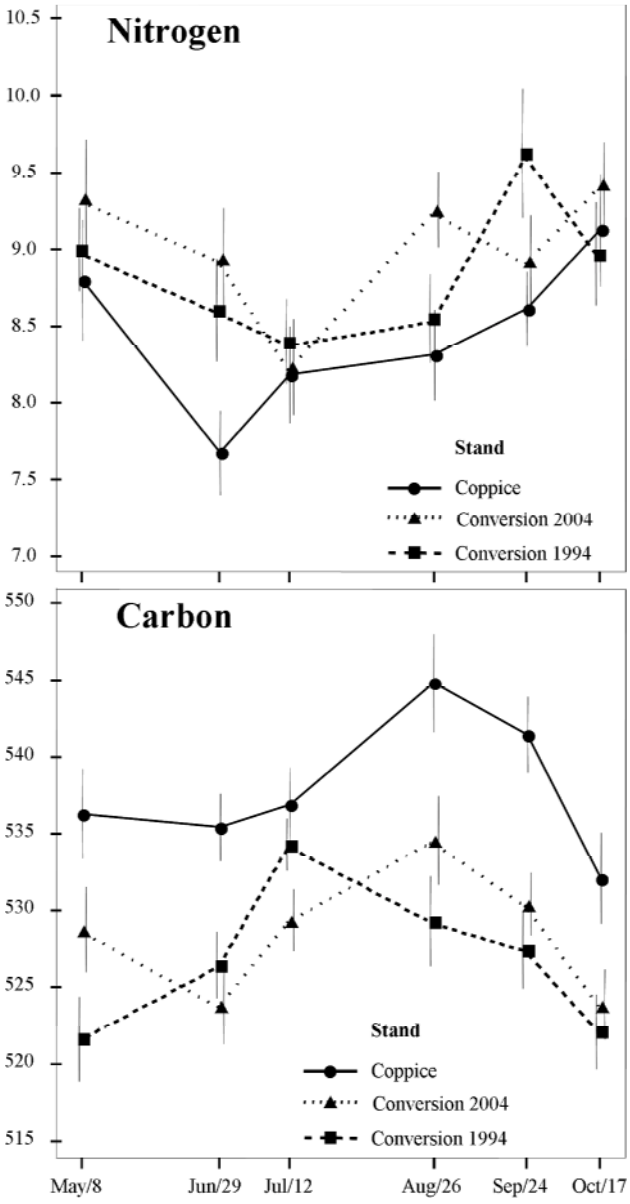

Figure 4.2 Fine root *N* and *C* concentrations (g kg^{-1} dry wt.) of three forest management differentiated stands measured during the 2008 growing season. Each value represents a mean of 18 samples. Vertical bars indicate one error standard

Nahm et al. 2006). Therefore, in analogy with Fotelli et al. (2002) and Millard (1989), we suggest that also in our beech stands the decrease in *N* could be associated to utilization of the *N* reserve in order to support newly developing tissues. Should this be the case, the increase observed by us could be associated to restoration of the N-depleted reserves.

4.2.2 Fine-root carbon concentration

Fine-root *C* concentration was affected by all the main factors investigated (management, diameter class, sampling date) except soil depth (Tab 4.1). The concentration of *C* was significantly higher in the Coppice stand than in either of the Conversion stands (Bonferroni, $P<0.05$; Table 2). However, these significant differences among the three stand managements were more pronounced as regards the thinnest root class (0-0.5 mm; Tab. 4.4).

Previous studies identified considerable differences in *C* concentration in the fine roots of different species, and showed that *C* concentration is related positively to root diameter (Gordon and Jackson 2000, Pregitzer et al. 2002). In contrast, another study reported that *C* concentration was highest in roots with the thinnest diameter (Goldfarb et al. 1990). We found that *C* concentration decreased significantly as root diameter increased (Tab. 4.4).

Our finding could be related to an increase in the secondary metabolite content (i.e., lignin and tannins) in the thinnest fine roots (Harborne 1980). In fact, secondary metabolites have a *C* content higher than compounds like cellulose and other sugars (Chua and Wayman 1979, Krässig, 1993), therefore an increase in secondary metabolites would result in an increase in total *C* concentrations. Alternatively, we cannot exclude that a higher *C* concentration in the thinnest fine root could derive from a lower cellulose and total-non-structural carbohydrate (TNC) concentration (Nguyen et al. 1990, Guo et al. 2004).

Table 4.4 Fine-root C concentration of three diameter classes. Values refer to three soil depths each 10-cm thick and three different forest management stands. Each value represents a mean of 12 samples ± 1 SE. a, b and c denote comparison between stands within the same diameter class and depth (Bonferroni test, P<0.05). h, j and k denote comparison among diameter class within the same management and soil depth (Bonferroni test, P<0.05)

	Diameter class 0 – 0.5 mm			**Diameter class 0.5 – 1 mm**			**Diameter class 1 – 2 mm**		
	Management			**Management**			**Management**		
Soil depth (cm)	**Coppice (g kg^{-1})**	**Conversion 1994 (g kg^{-1})**	**Conversion 2004 (g kg^{-1})**	**Coppice (g kg^{-1})**	**Conversion 1994 (g kg^{-1})**	**Conversion 2004 (g kg^{-1})**	**Coppice (g kg^{-1})**	**Conversion 1994 (g kg^{-1})**	**Conversion 2004 (g kg^{-1})**
0 - 10	551.4 ± 2.3 ah	536.8 ± 2.6 bh	533.3 ± 1.9 bh	540.4 ± 2.0 aj	528.3 ± 3.1 bj	525.2 ± 2.3 bj	527.3 ± 3.3 ak	519.4 ± 1.8 ak	519.7 ± 2.6 aj
10 - 20	548.8 ± 1.4 ah	536.6 ± 2.6 bh	536.8 ± 3.7 bh	538.8 ± 3.5 aj	526.6 ± 2.0 bj	528.2 ± 2.1 bhj	530.1 ± 3.0 aj	518.0 ± 2.6 bk	521.9 ± 1.8 abj
20 - 30	541.7 ± 2.5 ah	534.5 ± 2.0 bh	538.8 ± 3.3 abh	536.2 ± 2.2 ah	525.7 ± 2.0 bj	530.9 ± 2.4 abh	525.9 ± 2.3 aj	515.0 ± 2.7 bk	521.4 ± 2.4 abj

Goldfarb et al. (1990) suggested that *C* concentration in fine roots is higher in early summer than in spring or autumn. We confirm the significant (Tab. 4.2) variation of *C* concentration in fine roots during the year with a peak in July or August depending upon the type of management (Fig. 4.2). These variations differed with soil depth depending on management practice (significant management versus sampling date versus soil depth interaction, Tab. 4.2). The peak of *C* concentration found by us during summer could be related to the maximum vegetative activity which requires a reduction of investment in TNC. This possibility was suggested by Cerasoli et al. (2004) who reported, during the growing season, the highest *C* concentration in roots while TNC levels were the lowest. The rapid decrease of *C* concentration following the peak could be related to the end of the growing season and therefore to the need to restore the sugar reserve (Nguyen et al. 1990).

4.2.3 Fine-root C:N ratio

The C:N ratio is considered an indicator of fine-root life-span (Pregitzer et al. 2002, Tjoelker et al. 2005, Withington et al. 2006). It can also cast light on the relationship between

costs for fine-root biomass construction (in term of *C* concentration) and costs for biomass maintenance (in terms of *N* concentration) (Pregitzer et al. 1997, 2002). In our work, the C:N ratio was significantly higher in the Coppice stand than in the two Conversion stands (Bonferroni, $P<0.05$; Table 2), also in the case of fine roots excavated at different soil depths (Tab. 4.5).

Table 4.5 Fine-root C:N ratio of three diameter classes. Values refer to three soil depths each 10-cm thick and three different forest management stands. Each value represents a mean of 12 samples ± 1 SE. a, b and c denote comparison between stands within the same diameter class and depth (Bonferroni test, P<0.05). h, j and k denote comparison among diameter class within the same management and soil depth (Bonferroni test, P<0.05)

	Diameter class 0 – 0.5 mm			**Diameter class 0.5 – 1 mm**			**Diameter class 1 – 2 mm**		
	Management			**Management**			**Management**		
Soil depth (cm)	**Coppice (g kg^{-1})**	**Conversion 1994 (g kg^{-1})**	**Conversion 2004 (g kg^{-1})**	**Coppice (g kg^{-1})**	**Conversion 1994 (g kg^{-1})**	**Conversion 2004 (g kg^{-1})**	**Coppice (g kg^{-1})**	**Conversion 1994 (g kg^{-1})**	**Conversion 2004 (g kg^{-1})**
0 - 10	53.8 ± 1.4 ah	51.0 ± 1.2 abh	47.8 ± 1.1 bh	57.1 ± 2.4 ah	59.4 ± 2.3 aj	56.3 ± 1.7 aj	66.0 ± 2.6 aj	62.5 ± 2.0 aj	65.5 ± 2.3 ak
10 - 20	62.3 ± 1.5 ah	53.1 ± 1.3 bh	54.8 ± 0.9 bh	68.5 ± 2.4 ahj	60.2 ± 1.5 bj	59.8 ± 1.6 bj	73.9 ± 1.7 aj	66.9 ± 2.3 bk	64.1 ± 2.1 bj
20 - 30	63.6 ± 1.4 ah	58.1 ± 2.0 bh	57.3 ± 1.5 bh	67.6 ± 2.5 ahj	67.0 ± 1.5 aj	64.7 ± 2.2 aj	74.6 ± 3.6 aj	69.6 ± 3.4 aj	67.7 ± 2.0 aj

This suggests that when a stand is maintained under coppice management it is characterised by fine roots that have a longer life-span than those living in stands converted to high forest. Furthermore, the fact that the C:N ratio increased significantly with soil depth and diameter class (Table 5) suggests that fine roots living at greater depths have a longer life-span, and that an increase in their diameter induces a longer life-span irrespective of the soil-depth where they live and of the management practice that characterises the stand. The longer life-span associated with a high C:N ratio and a larger diameter suggests that these fine roots probably become perennial fine roots, which are more expensive to construct, but cheaper to maintain (Pregitzer et al. 2002). On the contrary, fine roots with a brief life-span associated

with a low C:N ratio and a low diameter probably become ephemeral fine roots, which are cheap to construct, but more expensive to maintain (Pregitzer et al. 2002).

4.3 Acknowledgements

This work was conducted within the framework of the research project "Trees and Italian forests, sinks of carbon and biodiversity, for the reduction of atmospheric CO_2 and improvement of environmental quality" funded by the Italian Ministry of Environment. I also acknowledge SBI (Italian Botanic Society Onlus) for supporting this research. I thank Dr. Alessandra Luzzaro for assistance with the CHN analysis. I also thank Dr. Antonio Montagnoli for his valuable help in the laboratory work. I'm grateful to Professor Donato Chiatante and Antonino Di Iorio for their valuable comments and suggestions.

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Chapter V

Effects of conversion of old coppice to high forest on anatomy *of Fagus sylvatica* L. fine roots

Fine roots (d < 2mm) play an important role in soil dynamics but they are usually treated all as physiologically identical units. On the contrary, it has been shown (Eissenstat and Yanai 1997) that fine roots belonging to a single root system show a wide range of variabilities in regard of seasonality, longevity and chemical composition. These variations seem to depend upon branching position, diameter size class, or functional role (water transport, nutrient absorption, mechanical stability) Moreover, it seems that such variability leads to morphological and anatomical variations (Coutts 1987). This great variability in fine roots has been described and named "heterorhizy" by von Alten (1909) and Noelle (1910, in Wilcox 1964) and has been reviewed more recently by Fitter (1991) and by Waisel and Eshel (1991). Differences among fine roots include also differences in decomposition rates and this leads to differences in the way fine roots contribute to organic matter accumulation in the soil (Ruess et al. 2003).

In previous chapters (III, IV) we have compared fine roots collected in various stands of *Fagus sylvatica* L. forests subjected to different management practices such as the conservation of coppicing or the conversion of coppice stand to high forest. The results presented demonstrate that fine-root dynamics is influenced by forest management; in particular we have shown that by after forest conversion, fine-root turnover, production and nitrogen concentration increased and fine-root carbon concentration decreased. The results presented in the present chapter refer to a histological approach regarding fine roots which

were sampled from all the three different beech forests considered in the previous chapters. In this case the aim was to evaluate the possible occurrence of anatomical differences which could be due to the type of forest management practice applied to a specific stand.

5.1 Material and methods

5.1.1 Fine-roots sampling

Fine roots were collected 19th of July 2010 at different soil depths by means of a hand auger in. In each stand, three soil cores (8 cm diameter x 30 cm deep) were collected. The soil cores were then divided into three fraction-layers depending upon the depth: the first (0-10 cm) layer included the humus (0- 2/3 cm); the second and third layer included respectively the fraction-layers comprised between 10-20 cm and 20-30 cm from the soil surface. Samples were stored in plastic bags at 4°C until processed. Each sample was washed automatically in a filtering nylon bag (300 μm mesh) using a washing machine (adapted from Benjamin and Nielsen 2004).

Soil-free roots were sorted under a 10x stereomicroscope depending upon their colour, texture and shape (Vogt and Persson 1991) and only the live roots were investigated. Furthermore, we considered only fine roots showing a diametr comprised between 1.0 and 2.0 mm which were measured by means of a digital calliper. After classification, root samples were fixed in FAA (Formalin-Acetic Acid-Alcohol, 5: 5: 90).

5.1.2 Histological analysis

Samples were dehydrated and embedded with Technovit 7100 resin system (Heraeus Kulzer, Wehrheim, Germany) based on 2-hydroxyethyl-methacrylate (GMA) for light-microscopy studies. Embedding protocol is listed in Table 5.1.

Samples (67) were sectioned by a sliding microtome and stained in Toluidine blue O (O'Brien et al. 1964, Parker et al 1982) for 2 minutes. Sections were photographed by Olympus BX63 light microscope and images were analysed by an open source analytical software (ImageJ, Abramoff et al. 2004, Rasband 1997-2011).

Table 5.1. Embedding steps using Technovit 7100 resin system

	Solution	Sample immersion time
Dehydratation		
	Ethanol 10%	15 m
	Ethanol 30%	15 m
	Ethanol 50%	15 m
	Ethanol 70%	15 m
	Ethanol 95%	15 m
	Ethanol 100%	12 h
Pre-infiltration		
	Etanolo 100% + Technovit 7100 (v/v ratio = 3:1)	1 h
	Etanolo 100% + Technovit 7100 (v/v ratio = 1:1)	1 h
	Etanolo 100% + Technovit 7100 (v/v ratio = 1:3)	1 h
Infiltration		
	100 ml Technovit 7100 + 1 g hardener I	12 h
Embedding		
	15 ml infiltration solution + 1 ml hardener II	2 h

For each section root traits measured were: Root Cross-section Area (Root CSA), Mean root diameter (obtained as a mean between 10 measurements), Xylem CSA, Central cylinder CSA,

Wood CSA (xylem area less central cylinder area), Number of vessels, Vessels CSA, Total Vessels CSA (Fig. 5.1)

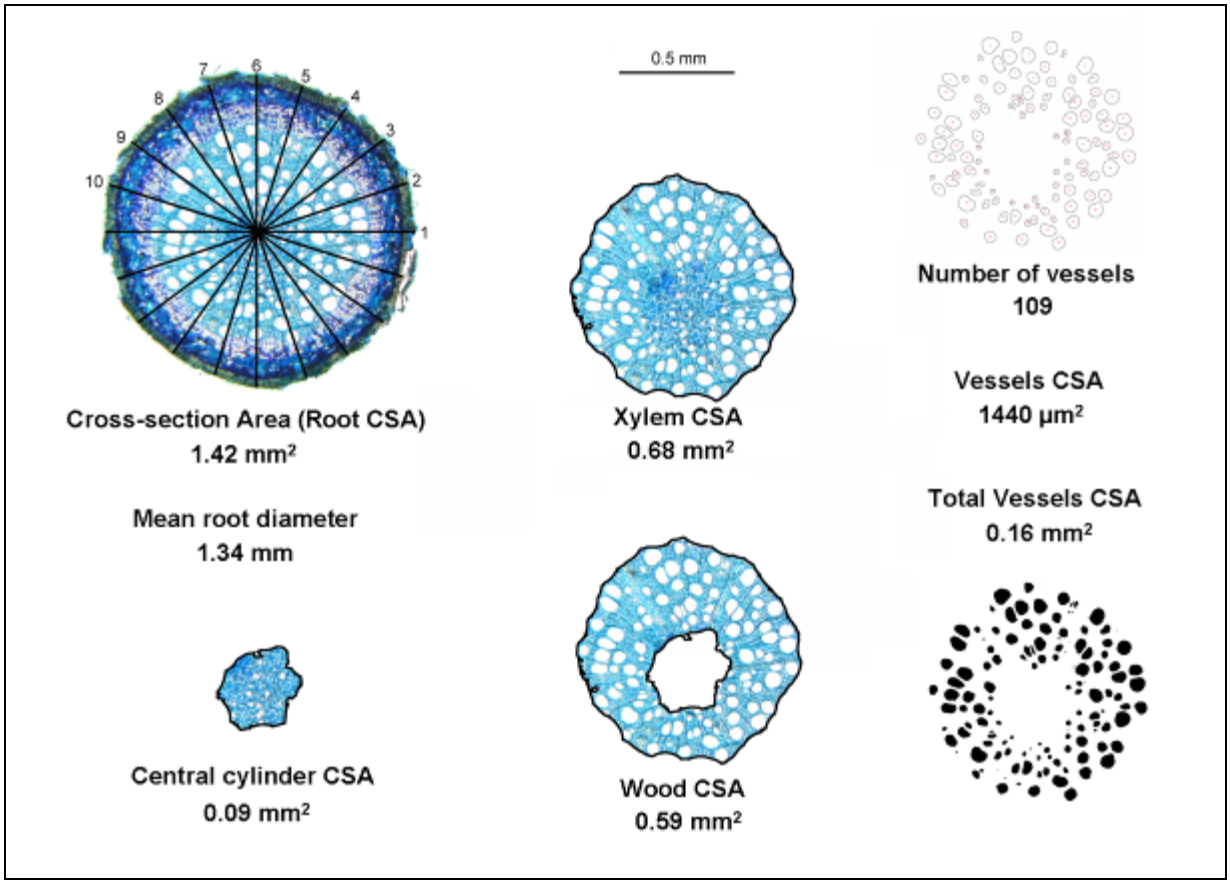

Figure 5.1 Example of section analysis using ImageJ software

5.1.3 Data analysis

Statistical analyses were carried out by using the SPSS software package version 12.0 (SPSS Inc, Chicago IL, USA). Data met the normal distribution and homoscedasticity. Because most of the measured anatomical traits showed a dependence on root diameter, the latter was used as a covariate in a two-way analysis of covariance (ANCOVA) that was performed to assess the effects of forest management (Coppice; 1994 and 2004 Conversion stands) and soil depth (0-10; 10-20; 20-30 cm). A Bonferroni test with a 5% rejection level was used to detect significant differences between stands and soil depths.

5.2 Results and Discussion

5.2.1 General fine-root histological traits in Fagus sylvatica L.

All roots investigated showed a secondary anatomical structure with a central stele surrounded by secondary xylem and phloem. A cork cambium resulted to be present which originated a thin periderm. (Fig. 5.2).

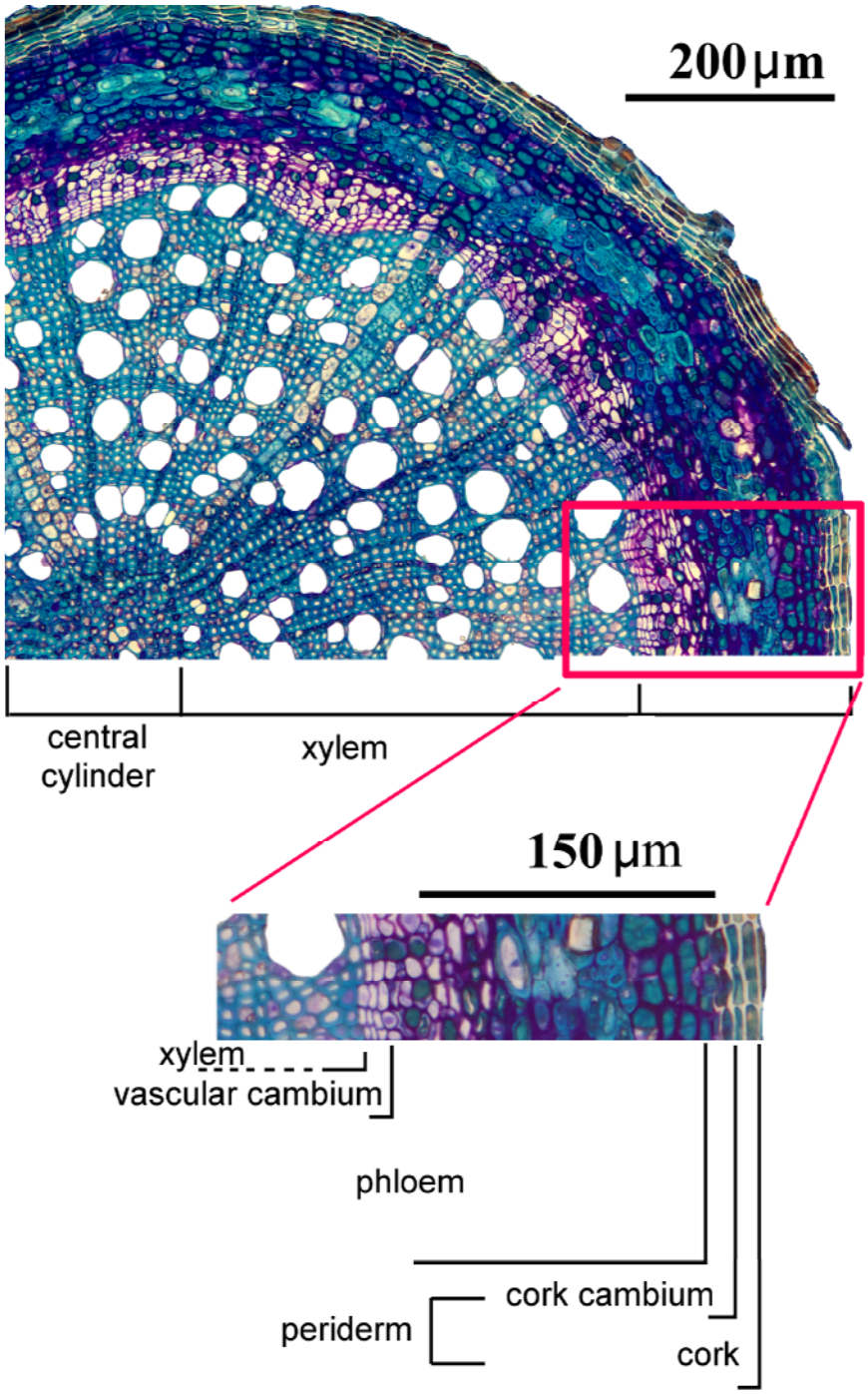

Figure 5.2 Light micrograph of a transverse section through a root of *Fagus sylvatica* L. Differentiations in cell types and tissue structures using Toluidine blue O

We observed in our sections that multiseriated rays originated from primary xylem poles, and their number coincided with the number of primary xylem poles. Therefore we adopted the easily detectable number of multiseriate rays as a way to detect rapidly the number of xylem poles present in each section (Fig. 5.3).

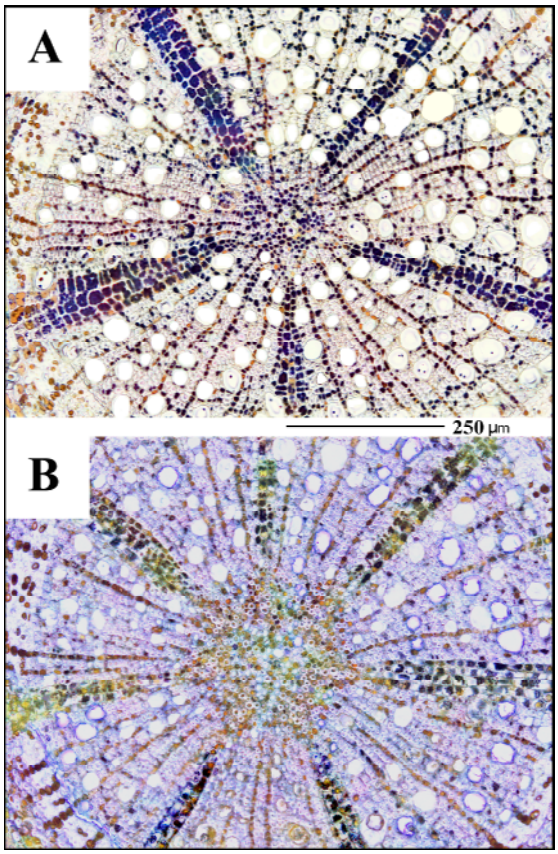

Figure 5.3 Light micrograph of a transverse section (stained with tincture of iodine) through a root of *Fagus sylvatica* L. 5 (A) and 7 (B) multiseriate rays radiate from central cylinder

We found that in *Fagus sylvatica* L. the number of protoxylem poles varied from 4 to 8 in relation with the size of the central stele (Fig. 5.4), but we didn't find any relation with type of forest management or soil depth.

Clowes (1951) investigated the structure of mycorrhizal roots of *Fagus sylvatica* L. and found that resistance to mycorrhizal infection increases proportionally with the increase of the number of protoxylem poles. In particular this author reported the presence of roots with a monarch, diarch and triarch (one, two and three protoxylem poles) xylem organization. In the present

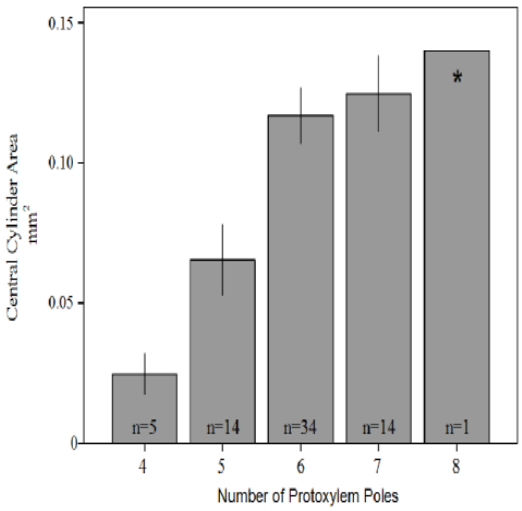

Figure 5.4 Relation between number of protoxylem poles and central cylinder area. n indicates number of sections analysed. Error bars are 1 S.E. * only one ottarch root was found

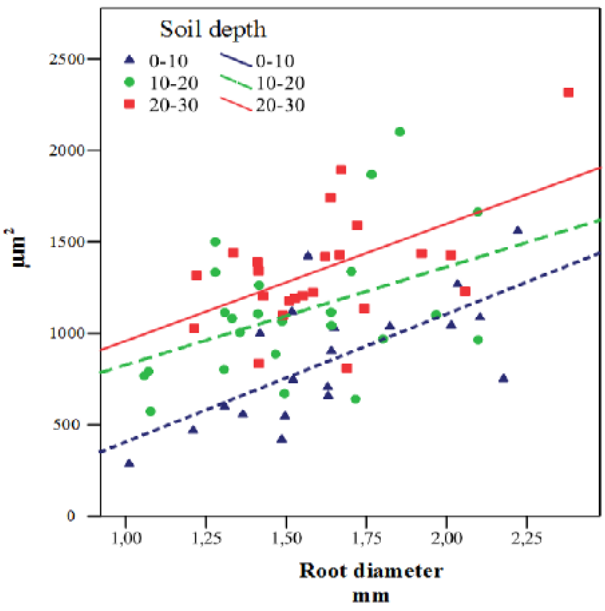

Figure 5.5 Relation between mean vessel area (µm^2) and fine root diameter (mm). Each point represents a fine root section. Different dots represent different soil depths

investigation a similar situation was absent and a possible explanation could be that in our study we investigated fine roots with a diameter comprised between 1.0-2.0 mm and showing a clear presence of secondary growth. On the contrary Clowes (1951) investigated thinner fine roots with a diameter comprised between 0.3-1.1 mm which did not present a secondary growth yet. The lack of a secondary structure in *Fagus* roots showing a small diameter and presence of 1,2 or 3 protoxylem poles suggest that these roots could play an hephemeral feeding function. This hypothesis is consistent with a suggestion by Hishi and Takeda (2005) according to which lower is the number of protoxylem poles present in a fine root and higher is its probability to die maintaining its primary anatomical organisation. In our roots mean vessel area was 1130 µm^2 but this value seemed to be strongly affected by root diameter as showed in Figure 5.5.

5.2.2 Fine-root histological traits and forest management

Figure 5.6 shows the percentage of secondary xylem (on total section area) found in each transverse root section analysed. Despite it is not surprising that we found that secondary xylem amount increased with root diameter it was unexpected to find that root from both Conversions stands showed a 7-9% increase of secondary xylem in respect to roots extracted from Coppice stand. The differences were significant difference when roots sampled at 5 cm soil depth were examined ($p<0.05$; Coppice, 43%; Conversion 1994, 52%; Conversion 2004,

50%; data not showed). A possible interpretation of these results could be that after a conversion cut, the roots apparatus of the remaining trees must arrange differently the anatomy of their roots in order to respond to a possible increase of tensile strengths (Gardiner et al. 1997). This hypothesis would explain well why is not rare within an early converted forest to find few trees fallen down because of snow or wind have affected their stability before the roots could adapt to the new mechanical forces which jeopardise their stability. A similar situation was also observed in this study with the 2004 Conversion stand (Fig. 5.7). If this is the case, then the increased percentage of

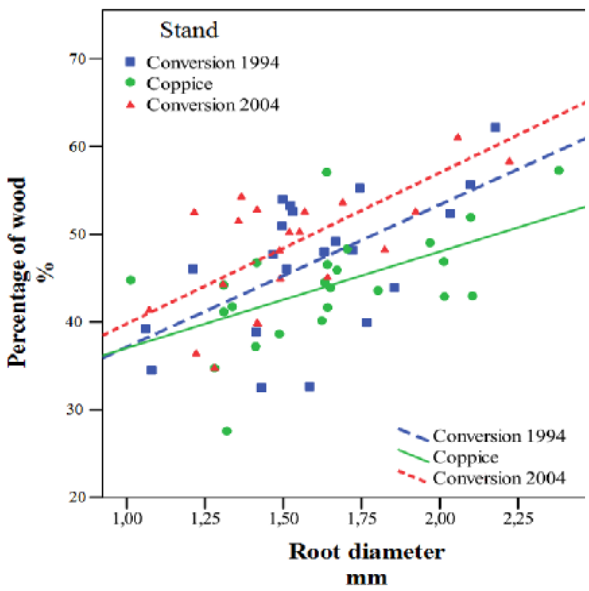

Figure 5.6 Relation between stem percentage of wood on total section area and fine root diameter (mm). Each point represents a fine root section. Different dots represent different stands

Figure 5.7 Fallen trees in Conversion 2004 stand

secondary xylem) observed in our experiment involves also an increase of capability of each single root for water transportation. This increase in water transportation could satisfy an increased demand of water by canopy, as a consequence of a more open canopy opening and an increased exposure to sunlight which would increase overall transpiration amount (Cochard et al. 1999).

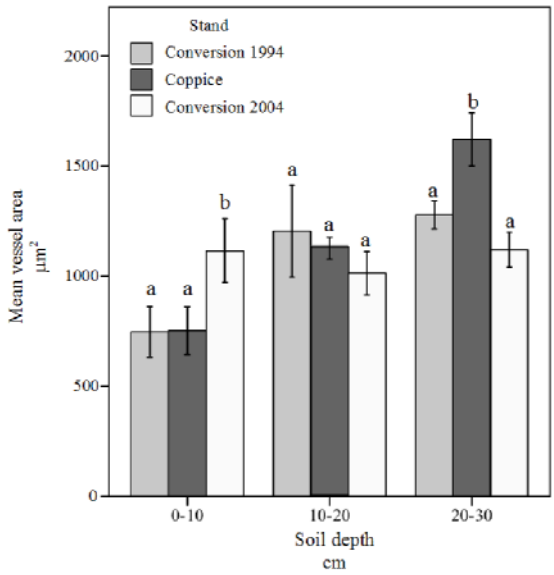

Figure 5.8 Mean Vessels CSA of fine roots (1-2 mm) from three different forest management stands. Values refer to three soil depths. Each value represents a mean of 7-8 samples ± 1 SE. a, b denote comparison between stands within the same depth (Bonferroni test, P<0.05)

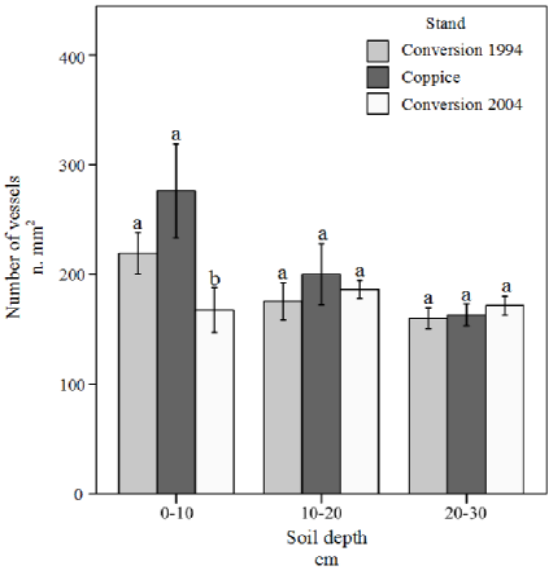

Figure 5.9 Number of vessels of fine roots (1-2 mm) from three different forest management stands. Values refer to three soil depths. Each value represents a mean of 7-8 samples ± 1 SE. a, b denote comparison between stands within the same depth (Bonferroni test, P<0.05)

Fine roots sampled from the most recently (2004) converted forest stand showed to be characterised at 5 cm of soil depth by vessels having a significantly larger lumen diameter (Fig. 5.8) despite their number decreased (Fig. 5.9) in respect to the other two stands considered in our experiments. In this stand root diameter values didn't change in deeper soil, unlikely the other two stands (Conversion 1994 and Coppice) where the increase of vessels diameter with depth coincided with a decrease of their number (Fig. 5.8, 5.9). In particular, fine roots growing at 25 cm soil depth in Coppice, stand showed the largest vessel diameter

value (Fig. 5.8, 5.9), so that the Total Vessels CSA resulted to be significantly higher in this stand than in both conversions ($p<0.005$, data not showed). In regard to root diameter it has been suggested (Sperry 1993) that despite larger vessels are more efficient in water transport they become more susceptible to embolism (vessels filled with air become unable to trasport water to the leaves). The two main factors responsible for inducing vessel embolism are dehydration and sap-freezing temperatures, and environmental conditions characterised by these two factors take place more frequently in shallow soil layers. This fact could explain why fine roots with larger vessels are developed in depth soil layer whereas those with smaller vessels are developed in shallow soil layers. The finding that in our experiment the most recently converted stand (Conversion 2004) didn't show such a typical pattern of diameter vessel distribution with depth could be the consequence of the primary need of a tree after conversion operations to restore an efficient fine-root network able to support an increased requirement of water absorbed from the entire soil profile.

5.3 Acknowledgements

This work was conducted within the framework of the research project “Trees and Italian forests, sinks of carbon and biodiversity, for the reduction of atmospheric CO_2 and improvement of environmental quality” funded by the Italian Ministry of Environment. I thank Dr. Di Iorio Antonino and Dr. Antonio Montagnoli for assistance with the field and laboratory work. I would like to express my gratitude to Professor Donato Chiatante for his valuable comments and suggestions.

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Chapter VI

Fine-root mass, length and specific root length in a Turkey-oak (*Quercus cerris* L.) stand in relation to seasonal changes in soil moisture

Fine-root dynamics is influenced by a variety of internal (e.g., genotype of plant species) and external (e.g., temperature, precipitation, soil properties, nutrient availability and competition between plants) factors (Majdi et al. 2005). During the summer, forest ecosystems in Mediterranean climate areas undergo natural soil moisture deficit coupled with elevated temperature. Thus, water deficit is subject to seasonal variability and causes mild and/or extreme drought. Soil moisture is a key factor that has been found to influence fine-root biomass and turnover of trees (Meier et al. 2008). Any distortion of the fine-root system by such a kind of stressors might affect carbon and nutrient cycling in the ecosystem.

Plants continuously adapt the growth of different organs to a dynamically hetereogeneous soil resources (Metcalfe et al. 2008). With regard to organ responses to stresses, roots use bio-adaptive processes as an ontogenic response to environmental conditions (Chiatante et al. 2005). Different tree species appear to use different adaptation strategies to optimize their mineral nutrition (Comas et al. 2002; Curt and Prevosto 2003; Comas and Eissenstat 2004). This species-specific adaptation in response to the local heterogeneity of the soil involves two possible strategies that depend on their ability to exclude or tolerate the stress (Manes et al 2006 and references therein). Briefly, a plant adopts an extensive strategy (Ostonen et al. 2007), namely it shifts its allocation of carbon towards roots, where photosynthate can be used to increase water uptake. This leads to an increase in root mass and length in the fine-root system as soil moisture declines (Manes et al. 2005;

Ostonen et al. 2007). Plants may also respond by closing their stomata thereby reducing CO_2 assimilation and diffusion into the plant (Metcalfe et al. 2008). The product of this change in the total amount of labile carbon available to the plant is probably a decline in root mass production as soil moisture falls (Manes et al. 2005). Alternatively, a plant may adopt an 'intensive' strategy, namely it may induce morphological adaptations of the fine roots that enable trees to grow even under harsh soil conditions (Ostonen et al. 2007).

Root length is assumed to be proportional to resource acquisition (benefit) and root mass to be proportional to construction and maintenance (cost) (Eissenstat and Yanai 1997). Specific root length (SRL m g^{-1}) is the length-to-mass ratio (L/M) of a root fragment. Fitter (1976, 1985, 1991) was one of the first authors to apply SRL and proposed the length/mass ratio as an index of root benefit to root cost. Long and thin roots (high SRL) are believed to be the below-ground equivalent of thin leaves, which are less expensive to produce (Withington et al. 2006; Ostonen et al. 2007). Water uptake may stimulate the production of finer roots, which results in a relatively greater length per unit mass thereby leading to an increase in SRL under drier conditions (Metcalfe 2009). Thus, a decline in soil moisture in some species may induce changes in the diameter of the root population (Ostonen et al. 2007).

6.1 Materials and methods

6.1.1 Fine-root measurements

The soil core sampling method (Vogt and Persson 1991) was used to quantify fine-root mass (diameter < 2 mm) during the 2008 growing season. Four permanent 10 m^2 square-shaped plots were set up; these were different from the plots set up for stand measurements. Sudmeyer et al. (2004) found that maximum lateral root spread is roughly 1.5-2.5 times the tree height. Although this value refers to an agroforestry system, we considered it valid for

our oak stands. Therefore, we set-up plots at a distance equal to 3.5-4.1 times the mean stand tree height and each plot was considered an independent replicate. At each sampling date, two soil cores (4 cm diameter x 30 cm deep) were randomly collected in each plot using a motor-driven portable core sampler (adapted from Ponder Jr. and Alley 1997). To investigate the kinetics of biomass and necromass, we collected soil samples on 12 days between April 4 and November 27. Samples were collected at an interval of not less than 15 days and not more than 26 days; eight cores were collected on each sampling date for a total of 96 cores. We did not sample in winter because we assumed that during this season fine-root production and decomposition are low (Claus and George 2005; Crider., 1928). Samples were stored in plastic bags at 4°C until processed. For processing, each sample was placed in a nylon bag (300 μm mesh) that was contained in a plastic cylinder (6 mm mesh) and washed automatically using a washing machine. Fine roots were examined at the microscope and were divided into two groups: oak and other understorey species. Fine roots from oak trees were classified "live" (dry weight hereafter termed "biomass" or live fine-root mass [LFRM]) or "dead" (necromass or DFRM) depending on their colour, texture and shape (Vogt and Persson 1991). The roots freed from soil were scanned at a resolution of 400 dpi with a calibrated flatbed scanner coupled to a lighting system for image acquisition (Epson Expression 10000 XL). They were then live and dead samples were oven-dried separately and weighed. Fine-root images were analyzed by WinRhizo Pro V. 2007d (Regent Instruments Inc., Quebec) to obtain root length and diameter. The following fine-root traits were determined for Oak trees: live and dead dry mass (g m^{-2}); length and SRL only for live roots; and diameter classes. We also estimated annual FRP using the 'minimum-maximum method' procedure (Edwards and Harris 1977; McClaugherty et al. 1982) considering only significant differences between maximum and minimum. Rates of biomass turnover were calculated as Annual Root Production divided by Maximum Standing Biomass (Gill and Jackson 2000).

6.1.2 Statistical analysis

The data were analyzed with the SPSS software package version 12.0 (SPSS Inc, Chicago IL, USA). The data were not normally distributed neither did they meet the assumption of homoscedasticity. They were square-root transformed or log-transformed to ensure normal distributions and equal variances to allow the use of normal parametric statistics. Analysis of variance (one-way ANOVA) was performed on fine-root biomass, length and mean forest fine-root SRL to assess the effect of time (seasonality). The effect of soil moisture on fine-root biomass, length and mean forest fine-root SRL was assessed using a power regression function.

6.2 Results

6.2.1 Soil moisture

As expected, the highest soil water content was recorded between April (40.3%) and June (38.9%) as a result of spring rainfall events (Fig. 6.1 a, b). It decreased to almost 30% at the beginning of July and to almost 18% in August. In fact, rainfall events were very rare in summer (5 in July and 2 in August, and rainfall was very scarce (in July, one rain event produced 10.4 mm and one produced 3 mm; all other rain events were under 1 mm). Soil moisture increased at the beginning of September and at the beginning October as result of early autumn rainfall. It decreased at the end of October in conjunction a decrease in rainfall, and then increased up to the end of November.

6.2.2 Fine-root mass, length and SRL

The annual mean LFRM was slightly lower than the annual mean DFRM (152.4 ± 8 g m^{-2} and 175.9 ± 7 g m^{-2}, respectively; data not shown). Time (seasonality) significantly affected both LFRM and DFRM, and both varied greatly during the vegetative season (Fig. 6.1c). LFRM progressively increased from the beginning of April to mid-June, and decreased slightly at the beginning of July. It peaked at the end of July and remained stable until the end of August, when it decreased until the beginning of October. There was a second small peak at the end of October. Then LFRM decreased until the end of November at which time it reached the same value measured at the beginning of the season. DFRM increased slightly during the study period. Small peaks occurred at the beginning of July and October, and at the end of November in conjunction with decreases in LFRM (Fig. 6.1c). Mean annual live fine-root length was 1.16 ± 0.80 km m^{-2} and mean annual live fine-root SRL was 7.68 ± 0.28 m g^{-1} (data not shown). Fine-root length had the same seasonal pattern as live dry mass with a peak

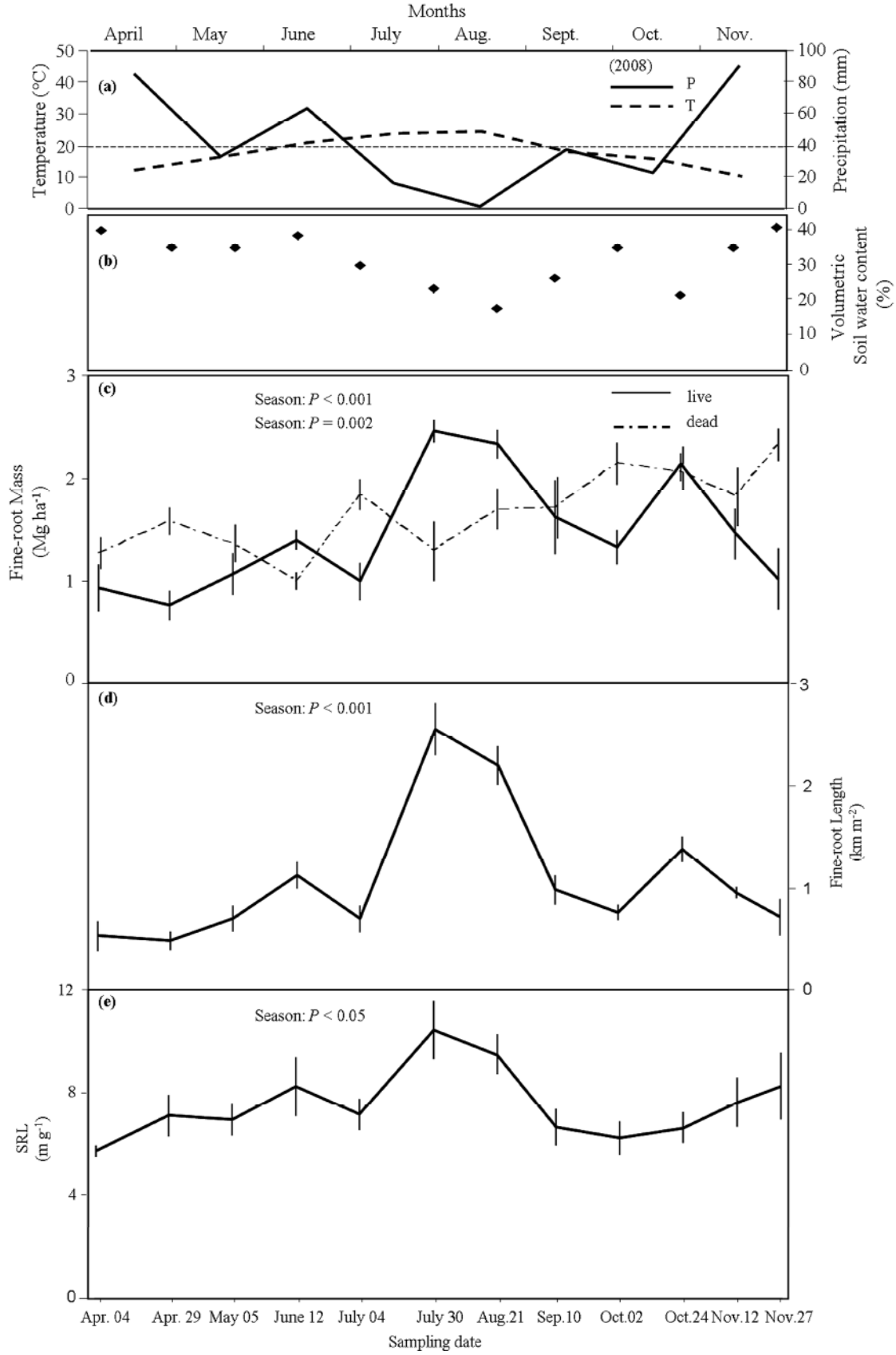

Figure 6.1 Monthly average temperature and rainfall from April to November 2008 (a). Seasonal variation of (b) soil moisture, (c) fine-root biomass and necromass, (d) length and (e) SRL. Data refer to each sampling date throughout the growing seasons. Data are the means of 8 measurements ±1 SE. (S.E. not shown if smaller than symbol). SRL, specific root length

in summer and a peak in autumn, whereas SRL had only one peak in summer, because the rate of increase was greater for length than that of mass. Thus, more root length per unit mass was produced under drier soil conditions. Both fine-root length and SRL showed a significant time effect (Fig 6.1 d, e). Fine-root mass, length and SRL peaked during the transition from the wet to dry season. In fact, fine-root dry mass, length and SRL showed an inverse power relationship with the soil moisture (Fig. 6.2 a, b, c).

Very fine roots (diameter < 0.5 mm) constituted a considerable proportion of total root length (79%), whereas they constituted only 21 % of biomass (Tab. 6.1). Analysis of mass and length seasonal variation per diameter class (Fig. 6.3) showed that very fine roots (diameter < 0.5 mm and 0.5 < diameter < 0.1) increased most when soil moisture was lowest.

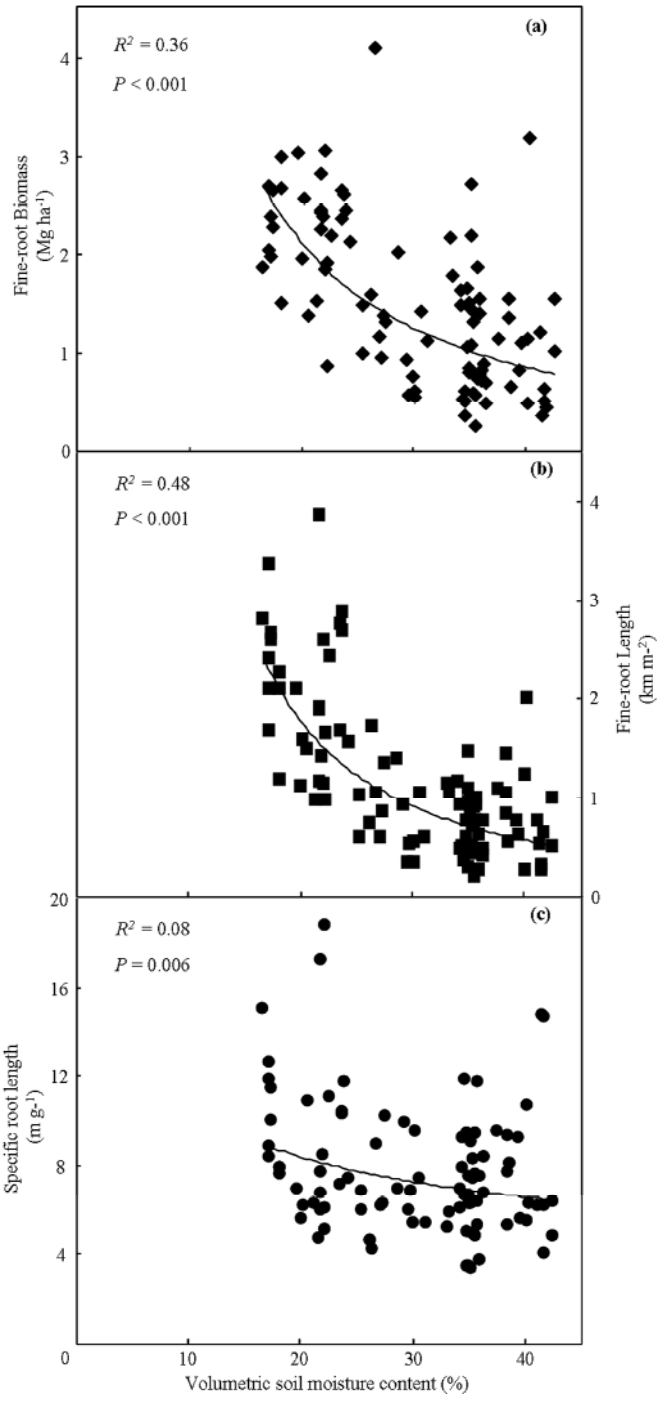

Figure 6.2 The relationship between volumetric soil moisture and fine-root (a) biomass, (b) length and (c) SRL in the surface 30 cm soil layer between April and November 2008

Table 6.1 Mean annual fine-root biomass (live roots dry weight), length and specific root length per diameter class (means ±SE; n = 94)

Diameter classes	Biomass		Length		SRL
(mm)	(g m^{-2})	%	(m m^{-2})	%	(m g^{-1})
0.0 – 0.5	32.3 ±2.1	21.2 ±1.4	920.2 ±71.4	79.0 ±6.1	275
0.5 – 1.0	44.9 ±2.6	29.4 ±1.7	173.8 ±9.7	14.9 ±0.8	39
1.0 – 1.5	39.9 ±3.0	26.2 ±2.0	49.2 ±3.6	4.2 ±0.3	12
1.5 – 2.0	35.2 ±3.0	23.2 ±2.0	21.3 ±1.8	1.8 ±0.2	6

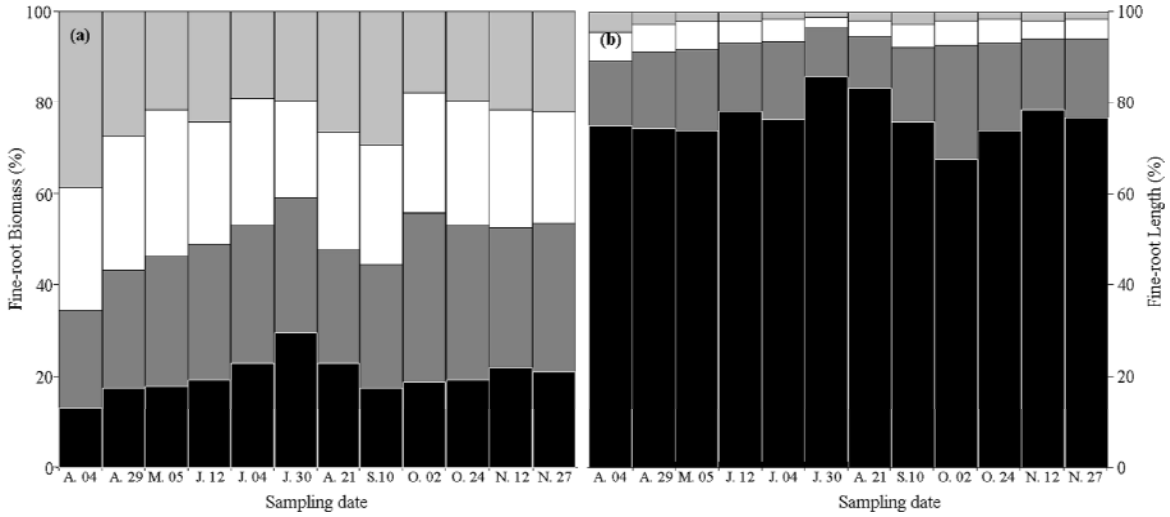

Figure 6.3 Fine-root (a) biomass and (b) length seasonal variation for each diameter class (mm). Black (■) 0.0< d <0.5; dark gray (■) 0.5< d <1.0; white (□) 1.0< d <1.5; gray (■) 1.5< d <2.0

6.2.3 Fine-root production and turnover rate

The annual fine-root production in the 0-30 soil profile was 231 g m^{-2}. Fine-root turnover rate was 0.94 y^{-1} (data not show).

6.3 Discussion

The mean total (live plus dead) fine-root mass in our Turkey-oak stand in the Southern Apennines of Italy (152.4 ± 8 g m-2 and 175.9 ± 7 g m-2, respectively) was consistent with the values reported for the same or similar species. For example, it was 395-398 g m^{-2} for the older stands of *Q. cerris* in central Italy (Claus and George 2005) 298 g m^{-2} for a *Q. alba* stand in Missouri, USA (Joslin and Henderson 1987), and 536-654 g m^{-2} respectively young and old *Q. robur* stand in the Netherlands (Bakker 1998). Moreover, our results concerning live fine roots are similar to those reported in the review by Jackson et al. (1997). The fine-root turnover rate calculated in our Turkey-oak stand was consistent with the values reported in the review by Gill and Jackson (2000).

Few data are available regarding root length. Jackson et al. (1997) estimated the mean standing crop root length in temperate deciduous forests at 3.4 km m^{-2}, almost three times the value measured in our study site (1.16 ± 0.80 km m^{-2}). Concerning SRL, in a meta-analysis, Ostonen et al. (2007) reported a fine-root SRL (diameter < 2mm) ranging between 1.4 to 25 m g^{-1} depending on tree species. Our result (7.68 ± 0.28 m g^{-1}) falls within this range, and is similar to the value reported for *Q. robur* (4.1- 21.3 m g-1; Bakker 1998) and for *Q. cerris* (4.10-6.80 m g^{-1}; Claus and George 2005).

In our *Q. cerris* stand, both fine-root mass and length showed a bimodal seasonal growth pattern. Complex bimodal seasonal patterns have been identified in fine-root biomass in temperate forests (Gaudinski et al. 2001; Tierney and Fahey 2002; Matamala et al. 2003; Trumbore and Gaudinski 2003; Majdi et al. 2005). It has been suggested that this seasonal variation could reflect seasonal variations of water and consequent nutrient availability (Coners and Leuschner 2005; Vanguelova et al. 2005; Mainiero and Kazda 2006) as well as an ontogenic response to local conditions (Chiatante et al. 2005).

In our site, from the beginning of April to the beginning of July, fine-root mass and length were closely related to soil water content patterns. In July, when the mean air temperature exceeded 20 °C and soil water content decreased, fine-root traits were inversely related to soil water content. Teskey and Hinckley (1981) observed that temperature was the dominant factor for oak root growth at low temperatures, but soil water content was the most important factor when temperatures increased above 17 °C. In our study, the summer fine-root peak lasted until the end of August when water in the soil was lowest and air temperature highest, after which fine-root traits decreased. This suggests that root growth/production in the surface 30 cm soil layer was affected by the changes in plant allocation predicted by the functional balance theory (Metcalfe et al. 2008). We found that September and October rainfall events were followed by an increase of soil water content immediately after the appearance of a second fine-root peak. In line with previous studies (Chiatante et al. 2005, 2006), we suggest that this second flush of fine-root production is a recovery mechanism whereby the plant can uptake water and nutrients for winter storage (Cerasoli et al. 2004). This was followed by a decline and consequent arrests of new root production in the autumn coincided with lower temperatures and leaf shedding.

The relationship between the LFRM and DFRM patterns reported herein is consistent with a bimodal pattern in the life cycle of the fine-root component in our Turkey-oak forest stand. In particular, the peaks of live mass were followed by peaks of necromass. Joslin et al. (2006) hypothesised that a tree fine-root system consists of pools of fine roots of different ages. One pool is very dynamic and has a life span < 1 year ("short-lived"), and the other has a life span > 1 year ("long-lived") and consists of older fine roots. In particular, fine-roots produced in spring have shorter life spans that those produced later. It is feasible that the difference we found between the initial and peak value of biomass could be due to the production (flush) of new roots. Moreover, the fact that the peak of fine roots lasts just a few

weeks suggests that most of these new fine roots produced are of the "short-live" type. Furthermore, also the second flush, though less abundant than the first one, could be formed mainly by "short-lived" fine roots because also this second peak lasts only a few weeks. If this is the case, the amount of fine roots present during winter could represent the portion of the root system classifiable as "long-lived" fine roots. As a consequence, the increment between the amount of fine roots measurable at the beginning and at the end of the vegetative season would represent the yearly production of "long-lived" fine roots. In our study, the difference in biomass between April and November was roughly 1%, which indicates that almost all fine roots produced died at the end of the season. Thus, the reduction in root traits observed in our study may be attributed to the shedding of "short-lives" fine roots produced during the season, thereby resulting in a very low net increment in total fine-root mass.

The general increase of fine-root biomass and length we found in summer might derive from a lower amount of rainfall and soil water content coupled with an increase of air temperature that normally occurs in this type of climate. Fine-root production flushes during the drier period could be a strategy to overcome the unfavourable environmental conditions. This hypothesis is reinforced by the inverse relationship found between soil water content and fine-root biomass, length and mean forest fine-root SRL, all of which were higher when the soil was drier. These findings indicate that the soil moisture deficit in the beginning of July led to an increase in root biomass. Our data coincide with reports by Chiatante et al. (2005, 2006) that after the beginning of drought, there is an increase in root mass and length despite a reduction in stomatal conductance. In literature, the mechanisms behind this fine-root growth, as the carbon gain is lowered by the reduction in stomatal conductance with increasing drought stress, suggests that carbon is preferentially channelled into the fine-root production (Dickson and Tomlinson 1996; Thomas and Gausling 2000; in Di Iorio et al. 2011). In our study, fine-root biomass decreased (end of July-beginning of August) only when

drought was prolonged and soil water content reached very low values. As found by Cudlin et al (2007) in a recent meta-analysis, the fact that root growth stops and several roots die (decrease in root length and biomass) might indicate that an overall reduction of the root production becomes more functional when water shortage exceeds a certain limit in content and time. Therefore a relative allocation of growth to below-ground organs is a frequent occurrence during a mild drought, and even the absolute root growth may increase following a mild drought. However, when water stress continues, root growth usually decreases (Joslin et al. 2000).

We found that the peak of mean fine-root SRL in summer coincided with biomass and length peaks. Thus, in summer more root length per unit mass was produced than during the rest of the growing season during which the fine-root length/mass ratio remains constant.

Fine-root SRL represents a mean of all root classes and its value varies proportionally with root class. In our study, the proportion of roots with diameters smaller than 0.5 mm varied in total fine-root biomass and length , respectively from 12% (4 April) to 29% (30 July) and from 74% (4 April) to 85% (30 July). This result shows that roots in this diameter class are major cause of the annual fine-root fluctuation. The increases in mean forest fine-root SRL is a consequence of the increment of the smaller diameter fractions and might imply they are more efficient in exploiting soil water. This hypothesis is in good agreement with the finding of Pregitzer et al. (2002) that the roots in an arbitrary fine-root size-class do not function the same way, and that their carbon cost for construction and maintenance could be different. Our results indicate that the root system is sensitive to soil-water dehydration and responds to an increase in fine-root mass and length which is due mainly to the thinner roots. Our data on the responses of fine roots to soil water content have shed some light on the mechanisms that govern plant-water relationships. These mechanisms are important for

forests growing under natural conditions because they enable plants to survive the typical dry summer in the Mediterranean area, which is likely to become drier and last longer given the increase in temperature expected in this century.

6.4 Acknowledgements

I give special thanks to Dr. Bruno Lassere, Dr. Antonio Montagnoli and Dr. Antonino Di Iorio for their help in field activities. I wish to thank Professor Donato Chiatante for providing many useful comments and suggestions. This work was developed as part of the research project "Trees and Italian forests, sinks of carbon and biodiversity, for the reduction of atmospheric CO_2 and improvement of environmental quality" funded by Italian Ministry of Environment. I also acknowledge SBI (Italian Botanic Society Onlus) for supporting this research.

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Conclusions

In this study considerable variations in the fine-root compartment occur in both Alpine beech forests in relation to forest management and in Apennine Turkey-oak forest in relation to soil moisture deficit.

Concerning to the response of fine roots to forest management practices, conversion from coppice to high forest showed that a 40-year-old coppice stand is characterized by lower fine-root biomass production. Given the importance of fine-root production, this finding suggests an immediate advantage in converting a coppice stand to high forest stand due to the increase of fine-root primary production. On the contrary, this study also showed that harvesting in the converted stands causes a general decrease in the total mass of fine roots with a consequent increase of carbon release. Moreover, the coppice stand showed the highest fine-root biomass coupled with the lowest turnover rate. Therefore, the coppice stand seems to be associated with a higher amount and a longer period of below-ground carbon stock accumulation, at least as regards the fine-root compartment.

These previous results were supported by the finding that fine-root C:N ratio (an indicator of fine-root life-span) was significantly higher in fine roots of the coppice stand than in converted ones. This was because an higher fine-root nitrogen concentration in converted stands (e.g. higher metabolic activity) and an higher fine-root carbon concentration in the coppice stand. While effects on fine-root nitrogen concentration in relation to forest management are well known, nothing is reported about changes in carbon concentration. We suggested that. The histological analysis in the present work showed that the highest carbon concentration was related to differences in fine-root anatomical structure. Fine roots in the Coppice stand showed an higher percentage of phloem instead of xylem, explaining the highest carbon concentration.

An important remark is that the thinnest root component (0-0.5 mm) appears to be more sensitive to forest management than other root diameter classes analyzed (0.5-1; 1.0-2.0 mm). This finding could be useful in evaluations of fine-root response to environmental changes.

Concluding, stand biomass comparison between the three stands has highlighted that forest stands subject to conversion practice have to be accounted as carbon source. In fact the biomass measured in the old-coppice stand was not recovered after 14 years the cutting operations

In regards to the fine-root response to soil moisture deficit, we investigated the characteristics of *Quercus cerris* fine roots during a growing season. Both fine-root biomass and length were influenced by seasonal variations of soil water content and were consistently higher when the soil was drier. We found that peaks constituting the bimodal pattern of fine-root growth were characterised by an increase of the thinner fraction (diameter < 0.5 mm) of the root population. This pattern leads to an increase of the mean fine-root SRL displaying, in summer, the same response of mass and length. While we found a significant relationship between some of the measured fine-root characteristics and soil moisture at our study site, we cannot exclude that other environmental variables such as above-ground growth and soil fertility could contribute to this variation.

Finally, a more comprehensive measurement programme is required to elucidate the effects of other potentially important drivers of fine-root growth patterns and processes (e.g. changes in canopy evapotranspiration) and how these changes would affect the soil carbon stock.