Inventory of belowground carbon pools and fluxes in a short rotation woody crop

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Analyse van de ondergrondse koolstof–poelen en –fluxen
in een korte–omloop hakhoutplantage

Proefschrift voorgelegd tot het behalen van de graad van Doctor in de Wetenschappen: Biologie aan de Universiteit Antwerpen, te verdedigen door

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UNIVERSITEIT ANTWERPEN
FACULTEIT WETENSCHAPPEN – DEPARTEMENT BIOLOGIE

Antwerpen 2014

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Funding

The research of this thesis has received funding from:
- Erasmus-Mundus External Cooperation, Consortium EADIC – Window Lot 16 financed by the European Union Mobility Programme # 2009-1655/001-001.
- European Research Council under the European Commission's Seventh Framework Programme (FP7/2007-2013) as ERC Advanced Grant agreement # 233366 (POPFULL).
- Flemish Hercules Foundation as Infrastructure contract ZW09-06.
- Flemish Methusalem Programme.

"Perhaps the most scientifically challenging phase of the terrestrial carbon cycle occurs below ground."

(F. Stuart Chapin & R.W. Ruess
In: Carbon cycle: The roots of the matter)

Acknowledgments / Agradecimientos

One of the joys of completion is to look over the journey past and remember all the friends and family who have helped and supported me along this long but fulfilling road.

Foremost, I would like to express my sincere gratitude to my supervisor Prof. Reinhart Ceulemans for the continuous support, guidance and advice throughout of my PhD. His patience and effort in proof reading the drafts, are greatly appreciated. I could not have imagined having a better advisor and mentor for my PhD.

Besides my advisor, I would like to thank Prof. Ivan Janssens and Prof. John King, for their encouragement, insightful comments, and hard questions, always to improve my research; and Prof. Roberto Alvarez, for encouraging me to undertake the research career. The overall PhD experience has been an interesting and rewarding one.

I thank all PLECO members, for the stimulating discussions during lunch and cookie times, for the great disposition to help in the field work, and for all the fun we have had. I gratefully acknowledge the excellent support of Nadine and Nele for all the paperwork, administration and analysis, as well as the generous assistance of all the job students for tedious root picking. I thank specially the POPFULL colleagues with whom I shared most of fun and time in field research.

In the last four years I (and my family) have gathered many friends at PLECO, UA, and Antwerp. It has been a great experience to live in Belgium, the bad weather was easily compensated by the many many friends, the joy of biking everywhere, the diversity of beers, the delicious frietjes and the good chocolate. This together with the countless cultural activities among friends has been fundamental to emotional health needed for my research.

A mi gran compañera de ruta, María Fernanda, por todo lo que hemos crecido aprendiendo a cuidarnos y a cuidar nuestra hermosa Margarita. En estos años también hemos dejado atrás a los nuestros, nos hemos perdido casamientos, cumpleaños y hasta familiares y amigos. Por todo lo aprendido y dejado no tengo más que agradecimiento. Y, por supuesto, un gran agradecimiento a todos los del otro lado del charco, mamá, papá, mis hermanos, los abuelos, la familia y los amigos de siempre, por su apoyo, por sus palabras de aliento y sus visitas! Todo, todo ha sido fundamental.

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Abstract

The substitution of fossil fuels by bioenergy from woody crops is one strategy to reduce CO_2 emissions to the atmosphere. Afforestation with fast-growing woody crops is also relevant for soil organic carbon (SOC) sequestration. Both measures are part of the policies to mitigate climate change. In short rotation woody crop (SRWC) cultures the aboveground biomass is periodically harvested and processed for bio-energy production, where the fixed carbon is released again to the atmosphere. However, the harvest of the biomass implies less carbon (C) input to the soil as compared to a natural forest. The potential of SRWC to store C into the soil and to mitigate the rising atmospheric CO_2 concentration is still not well understood. The primary objective of this contribution is to study the impact of SRWC on SOC of a particular SRWC with fast-growing poplar (*Populus*) trees. The studied SRWC culture has been established on land that was previously used as cropland and as pasture. The large-scale SRWC plantation (18.4 ha) in East-Flanders (Belgium, 51°06'N, 03°51'E) has been managed for four years in two-year rotation cycles. The most important belowground C fluxes were measured during the two rotations of the SRWC. Data of all C fluxes into and out of the soil as well as all C pools belowground were quantified. For the four year study, the main C inputs to the belowground system resulted from leaf fall (~500 g C m^{-2}), from annual weeds (~350 g C m^{-2}), from fine roots (~100 g C m^{-2}) and from harvesting losses (~100 g C m^{-2}). The main C flux coming out from the system was from soil respiration, ranging from 596 to 947 g C m^{-2} y^{-1}, with roots representing about 41-51 % of the total soil respiration. The leaching of dissolved organic carbon represented only a minor proportion (less than 3%) of the C losses. The largest C pool in the soil was situated in the soil organic matter (14000 g C m^{-2}) followed by the belowground woody biomass (240 g C m^{-2}) and fine roots (80 g C m^{-2}). With the repeated SOC measurements (before and after 4 years of SRWC), we found a SOC sequestration of 900 g C m^{-2} (or 9 Mg ha^{-1}), which is similar to the total inputs of C over four years. The balance of SOM C inputs and losses revealed more realistic results than the repeated measurements. To detect significant changes in SOC after an altered land management (from agriculture to SRWC for bio-energy), long-term records are required. However, by assessing the fluxes we can model and simulate the SOC balance and predict future changes. Our results highlight the importance of measuring all carbon fluxes into and out of the soil. This and other relevant data allow us to assess the potential of SRC for bioenergy production and for SOC sequestration.

Samenvatting (Dutch)

Eén van de mogelijke strategieën voor de reductie van atmosferische CO_2-emissies is de vervanging van fossiele brandstoffen door hernieuwbare bio-energie van houtachtige biomassaculturen. De bebossing met snelgroeiende houtachtige gewassen is ook relevant voor de sequestratie van bodem-organische koolstof (SOC). Beide maatregelen maken deel uit van het beleid voor de mitigatie van globale klimaatveranderingen. In korte-omloop hakhoutculturen (KOH) wordt de bovengrondse biomassa periodiek geoogst en aangewend voor de productie van bio-energie, waarbij de gefixeerde koolstof terug vrijkomt in de atmosfeer. De oogst van de biomassa impliceert echter dat er minder koolstof (C) in de bodem wordt gestoken in vergelijking met een natuurlijk bosbestand. Op dit ogenblik is er nog onvoldoende kennis m.b.t. het potentieel van KOH-culturen om C in de bodem te stockeren en de toenemende atmosferische CO_2-concentraties te mitigeren. De belangrijkste doelstelling van deze doctoraatsverhandeling is de studie van de impact van KOH op SOC van een specifieke KOH-cultuur met snelgroeiende populieren (*Populus*). De onderzochte KOH-cultuur werd aangelegd op een terrein dat voorheen als landbouwgrond en als weiland in gebruik was. De grootschalige KOH-aanplanting (18.4 ha) in de provincie Oost-Vlaanderen (België; 51°06'N, 03°51'O) werd in twee-jarige rotatiecycli beheerd gedurende een periode van vier jaren. Alle belangrijke ondergrondse C-fluxen werden gedurende de volledige levensduur van de KOH gemeten. Alle C-fluxen in en uit de bodem, evenals als alle ondergrondse C-poelen werden gekwantificeerd en in detail opgevolgd. De belangrijkste C-inputs ondergronds gedurende de vier jaren van de studie waren afkomstig van de bladval (ca. 500 g C m^{-2}), van de jaarlijkse (on)kruidvegetatie (ca. 350 g C m^{-2}), van de fijne wortels (ca. 100 g C m^{-2}), en van verliezen tijdens de oogst (ca. 98 g C m^{-2}). De grootste C-flux uit het ondergrondse subsysteem kwam van de bodemrespiratie die tussen 596 en 947 g C m^{-2} jr^{-1} lag. De wortels vertegenwoordigden ongeveer 41 to 51% van de totale bodemrespiratie. De uitloging van opgelost organisch koolstof vertegenwoordigde slechts een zeer beperkt aandeel (minder dan 3%) van de koolstofverliezen. De grootste C-poel in de bodem zat in de bodem-organische materie (SOM; 14000 g C m^{-2}), gevolgd door de ondergrondse houtachtige biomassa (240 g C m^{-2}) en de fijne wortels (80 g C m^{-2}). Aan de hand van herhaalde SOC-metingen – voor de aanleg en na vier jaar van KOH-cultuur – werd een SOC-sequestratie van 900 g C m^{-2} (of 9 Mg ha^{-1}) vastgesteld. Deze waarde was vergelijkbaar met de totale C-inputs over vier jaren. De balans van de SOM C-inputs en verliezen toonde meer realistische resultaten dan de herhaalde metingen. Om significante veranderingen in SOC na een verandering in landgebruik vast te stellen (van landbouw naar KOH voor bio-energie) zijn echter lange-termijn observaties vereist. Maar via het kwantificeren van de fluxen kunnen we de SOC-balans wel modelleren en simuleren, en zo toekomstige veranderingen voorspellen. De resultaten van deze doctoraatsverhandeling tonen aan dat het essentieel is om alle koolstoffluxen in en uit de bodem te meten. Deze en andere relevante informatie laat ons toe om het toekomstig potentieel in te schatten van KOH voor de productie van bio-energie en voor de sequestratie van SOC.

Resumen (Spanish)

Una de las posibles estrategias para reducir las emisiones de CO_2 es reemplazar los combustibles fósiles por bioenergía producida a partir de biomasa con leñosas (biocombustibles de biomasa lignocelulósica). La utilización de especies leñosas de altas tasas de crecimiento, como álamos y sauces, han sido propuestas para el secuestro de carbono en la materia orgánica del suelo (SOC). Ambas medidas son parte de las políticas de mitigación del cambio climático. Éste tipo de cultivos forestales se conoce usualmente como SRWC, por su denominación en inglés de *short rotation woody crops*. En los SRWC, la biomasa aérea es cosechada periódicamente y usada para la producción de bioenergía, y así gran parte del carbono fijado es liberado hacia a la atmósfera. La cosecha de esta biomasa implica menos entradas de carbono (C) al suelo en comparación con un bosque natural. Es por esto que existen aún muchos interrogantes sobre el potencial que tendría el cultivo de SRWC para secuestrar C en el suelo y así mitigar el aumento de la concentración de CO_2 en la atmósfera. El objetivo principal de esta tesis fue monitorear el impacto de una plantación de álamos (*Populus sp*.) para SRWC sobre el C del suelo. Las mediciones se realizaron en la plantación SRWC a gran escala (18.4 ha) ubicada en West Flandes (Bélgica, 51° 06'N, 03° 51'E), en tierras que habían sido utilizadas previamente para cultivo y pastoreo. Durante cuatro años, la plantación ha sido manejada en ciclos de rotación de dos años. Durante ese período se midieron los flujos y las reservas de C más importantes del suelo. Todos los flujos de entrada y salida así como de todas las reservas de C en el sistema suelo fueron cuantificados y monitoreados en detalle. Para los cuatro años de estudio, las principales entradas de C fueron a partir de la caída de hojas (aprox. 500 g C m^{-2}), la vegetación espontánea de malezas (aprox. 350 g C m^{-2}), la mortalidad de raíces de álamos (100 g C m^{-2}), y las pérdidas durante la cosecha (aprox. 100 g C m^{-2}). La salida de C más importante del sistema provino de la respiración del suelo, entre 600 y 950 g C m^{-2} $año^{-1}$, representando la respiración de raíces aproximadamente el 41 al 51% de ése total. La lixiviación de carbono orgánico disuelto representó sólo una proporción muy pequeña de las pérdidas de carbono (menos de 3 %). La materia orgánica representó la mayor reserva de C (14.000 g C m^{-2}), seguida por la biomasa subterránea lignificada (240 g C m^{-2}) y las raíces finas (80 g C m^{-2}). A partir de re-muestreos de SOC – antes de la plantación y luego de cuatro años de cultivo con SRWC- se determinó un secuestro de SOC de 900 g C m^{-2} (o 9 Mg ha^{-1}). Éste valor es similar al total entradas de C en los cuatro años. El balance de entradas y salidas de C al suelo mostró resultados más realistas que el re-muestreo. Para detectar cambios significativos en SOC después de un cambio en el uso del suelo (de agricultura a SRWC para la bioenergía) se requieren mediciones a largo plazo. Sin embargo, el balance de flujos permitió estimar cambios de SOC en periodos más cortos y permitirá modelar y simular cambios de SOC a futuro. Nuestros resultados destacan la importancia de medir todos los flujos de entrada y salida de C al suelo. Éste y otros datos relevantes nos permiten evaluar el potencial de SRWC para la producción de bioenergía y el secuestro de SOC.

List of abbreviations and acronyms

B = biomass
BD = soil bulk density
C = carbon
CO_2 = carbon dioxide
Cr = coarse roots
D = dead poplar roots
DM = dry mass; dry matter
DOC = dissolved organic carbon
ESSE = ecosystem scale spatial error
F = F-value
Fr = fine roots
GHG = greenhouse gases
G*r* = root growth
Mr = medium-size roots
N = nitrogen
n = number of samples or number of replicates
NPP = net primary productivity
NRB = not recovered biomass
p = level of significance
P= productivity
PDE = picking duration error
R = respiration
R*gr* = root growth respiration
R*h* = heterotrophic respiration
R*m* = maintenance respiration
R*r* = root respiration
R*s* = soil CO_2 efflux; soil respiration
SD = standard deviation
SE = standard error
SOC = soil organic carbon
SOM = soil organic matter
SRWC = short rotation woody crop
Stu = stump
Tr = total root
TRSE = total relative standard error
UB = uncut biomass
W = weed roots
Wr = weed roots
Ws = soil moisture

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Chapter 1

1. Introduction

1.1. General introduction

In this introductory chapter we describe the importance of full and detailed carbon (C) balance studies in our changing world. What is carbon? Where is carbon? How does it move? History of increasing carbon concentrations in the atmosphere, and its decrease in soils. Within this context the issues of bioenergy, of soil carbon, of their links and their importance are presented and discussed.

1.1.1. Carbon cycle at the global scale: history and future

The element carbon (C) is the 15th most abundant element on the Earth and it is present in all known forms of life. Its abundance, together with the unique diversity of organic compounds and their unusual polymer-forming ability at normal temperatures on Earth, make this element the chemical basis of all known life. About 1500 Pg (1 Pg = 1 Gt =10^{15} g) of organic carbon on Earth is stored in the soil (Batjes 1996), i.e. more than in biota (500 Pg) and in the atmosphere (730 Pg) together. The carbon cycle is the biogeochemical cycle by which carbon is exchanged between the biosphere, the pedosphere, the geosphere, the hydrosphere, and the atmosphere of the Earth (Figure 1.1). Every year 120 Pg of C flows from the atmosphere to the biosphere via the photosynthesis process of plants (Lal 2008). About half of this C (~60 Pg C) is released to the atmosphere as carbon dioxide (CO_2) after being respired by plants. Plants and animals die and produce residues that are transformed, through oxidation by microorganisms, into a complex mix of substances in different stages of microbiological decomposition, and form part of what is called the soil organic carbon (SOC) in the pedosphere (Schnitzer 1991 cited by Lal 2008). The C respired by animals and the microbial decomposition of SOC releases the other half of CO_2 absorbed by plants back to the atmosphere, thus closing the carbon cycle. However, the C cycle is considerably more complex than this short loop. For example, some carbon dioxide is dissolved in the oceans; dead plant or animal matter may become petroleum or coal, which can be combusted with a release of carbon, should bacteria not consume it (Figure 1). The global C balance is the balance of the exchanges (incomes and losses) of C between the C reservoirs, or within one specific loop (e.g., atmosphere ↔ biosphere) of the C cycle. A close look at the C balance of a pool or reservoir provides information about whether the pool or reservoir is functioning as a source or as a sink for CO_2.

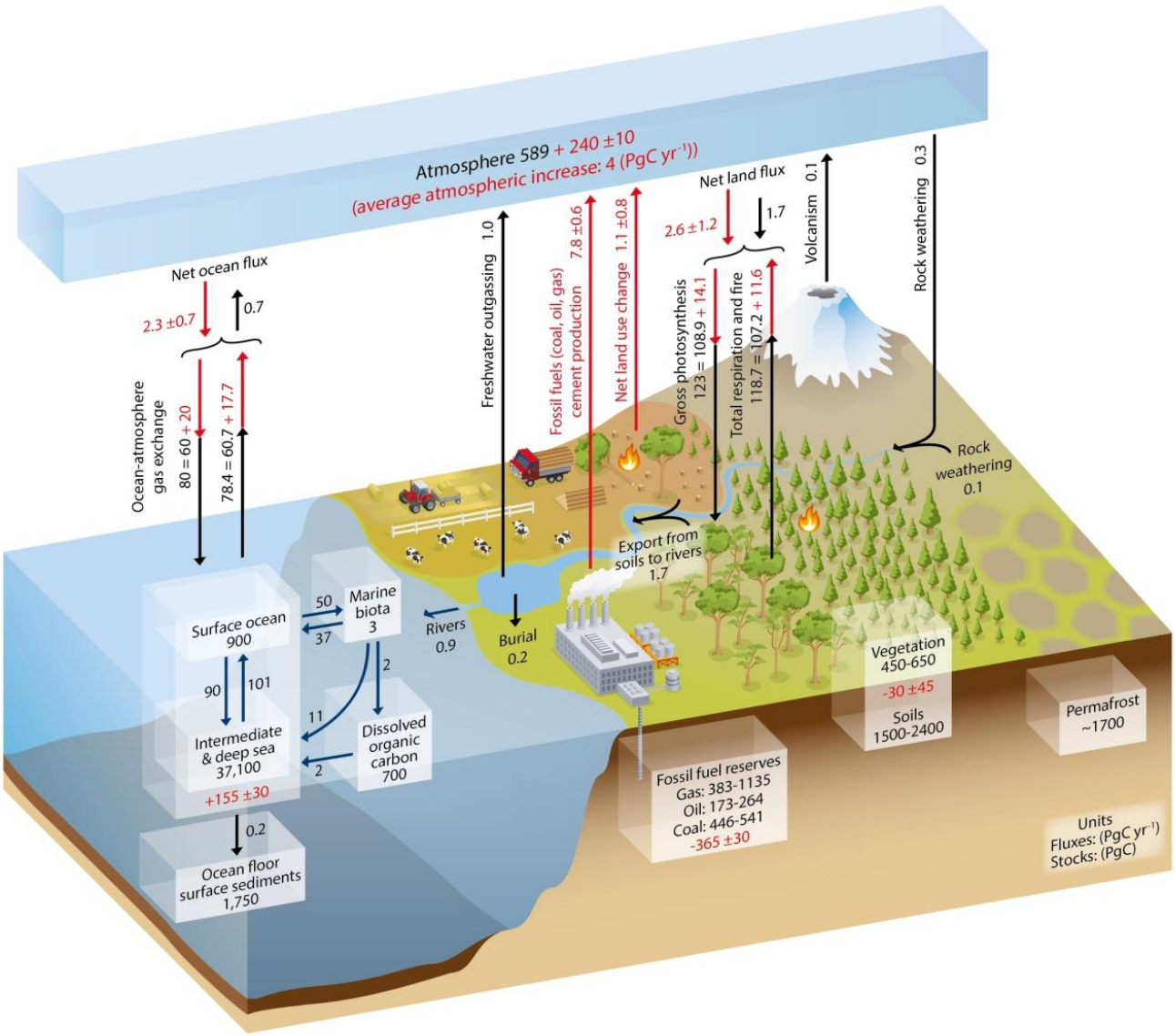


Figure 1.1: Simplified schematic of the global carbon cycle. Numbers represent reservoir mass, also called 'carbon pools' in Pg C (1 Pg C = 10^{15} g C) and annual carbon exchange fluxes (in Pg C yr^{-1}). Black numbers and arrows indicate reservoir mass and exchange fluxes estimated for the time prior to the Industrial Era, about 1750. Red arrows and numbers indicate annual 'anthropogenic' fluxes averaged over the 2000–2009 time period. These fluxes are a perturbation of the carbon cycle during the Industrial Era post 1750. Source: IPCC 2013, WG1 (www.ipcc.org)

Over the last century there has been an unbalance of the global carbon pools and fluxes (Figure 1.2). The concentration of CO_2 has been increasing in the atmosphere (about 30% since the pre-industrial concentration; Follett 2001), while SOC has decreased (Houghton et al. 1983). The increases in the concentration of CO_2, and of other greenhouse gases (GHG), have alarmed scientists because of the contribution of these GHG to the phenomenon of global warming. Agriculture has produced a net carbon flux from the soil to the atmosphere, contributing to the increased greenhouse effect (Le Quéré 2013), and reducing soil fertility and water quality (Lal 2004). Over the last decades this CO_2 flux from the soils has decreased in Europe and in the USA, but has increased in Africa and in Latin America (Houghton et al. 1983). The faster increase of the human population and the cheaper land in the southern continents have created this difference, that is forecasted to

continue for some time in the future (Schulp et al. 2008). Many studies worldwide are trying to estimate the ability of the soil to sequester carbon back from atmosphere (Batjes 2008; Jones et al. 2005; Liang et al. 2005; Schulp et al. 2008), and to reduce the emissions of CO_2. Some authors have further emphasized the importance of this topic by denominating the present time as the 'carbon age' (Lal 2007).

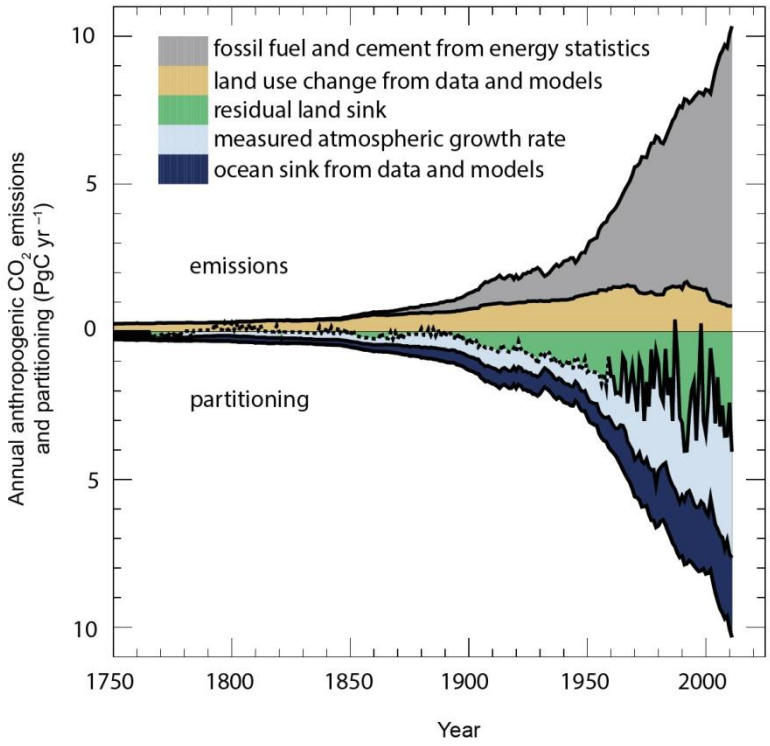


Figure 1.2: Annual anthropogenic CO_2 emissions and their partitioning among the atmosphere, land and ocean (Pg C yr^{-1}) from 1750 to 2011. Source: IPCC 2013, WG1 (www.ipcc.org).

There is a concern about the response of SOC to future climatic conditions, since small losses from this large pool caused important changes in atmospheric carbon dioxide concentration. Smith (2012) stated that it is impossible, and not really necessary, to understand which will be the response of SOC to the future conditions. He suggested that it is better to determine the size and the direction of the change, and the land management practices that can be implemented to protect and enhance SOC pools.

1.1.2. Carbon cycle in the soil of ecosystems

The amount of SOC in a particular soil depends on the C inputs and the balance between stabilization and destabilization processes (for example: protection by clay, aggregates, etc.). The net gain or the net loss of SOC depends on the C that is added to the soil (the C input) and the C that is lost in the decomposition process. The most important SOC input is formed by the residues of aboveground and belowground dead plant materials (Keith et al. 1986). The losses from the soil are mainly from SOC mineralization and from the consumption of residues by animals or from the decomposition by microorganisms (Kononova 1966). Exudates of organic compounds from the roots (rhizodeposition) can also represent a high proportion of the total inputs (Kuzyakov and Domanski 2000). The

vegetation type affects the amount and the spatial distribution of SOC (Berhongaray et al. 2013a; Jobbágy and Jackson 2000). Furthermore, environmental factors and management practices also affect the amount and the allocation pattern of these SOC inputs and outputs that finally determine the SOC content, as well as its spatial and vertical distribution. Carbon inputs are higher in natural ecosystems than in man-made farmland systems (Davidson and Ackerman 1993; Follett et al. 2009). Most of the aboveground biomass of plants in farmland systems is harvested or consumed; so cultivation frequently reduces C input to the soil as compared with natural, unmanaged ecosystems (Houghton and Goodale 2004; Post and Kwon 2000). Higher temperatures in cropped soils (Grant et al. 1995), as well as soil disturbance during the conventional cropping, accelerate the SOC mineralization and increase SOC losses (Reicosky et al. 1997). As a result of less inputs and higher mineralization, cropping causes a depletion of C from the soil. These SOC depletions have been observed mainly in the surface layers of the soil (Davidson and Ackerman 1993; Follett et al. 2009); there is, however, more C at deeper layers than in the top 20 cm of the soil (Jobbágy and Jackson 2000). A few studies have reported losses of deep SOC, although these losses were much lower than in the surface (Berhongaray et al. 2013a; Guo and Gifford 2002; Shrestha et al. 2006). Carbon pools in the soil can be increased in managed ecosystems. If the amounts of C inputs exceed the SOC decomposition, these processes can be reversed and the SOC can be increased. So, there is a potential to return and restore the soil carbon to the previous levels, and even overpass them by sequestering atmospheric carbon in the soil. Agricultural soils have a higher potential for carbon sequestration because they have been losing more carbon than other soils of other ecosystems.

1.1.3. Soil carbon balance in forest ecosystems

Afforestation is highly recommended for carbon sequestration in the soil (Smith et al. 1997). One of the reasons is the higher net primary production (and C inputs) of forests under natural conditions as compared to grasslands and croplands (Aber & Melillo 2001). Although mature forests store more SOC than grasslands (Jobbágy and Jackson 2000; Yang et al. 2007), young afforestations store 20% less SOC than grasslands (Guo et al. 2007). That is because during their first years the C inputs of the recently afforested sites are lower than the SOC losses, but this is reversed after ~40 years when the SOC increases (Davis and Condron 2002; Guo and Gifford 2002). This observation emphasizes the importance of long-term experiments that reach new equilibriums. After a land-use change, a new SOC equilibrium is – on average – reached after 10 years in tropical soils and after 100 years in temperate zones (Smith 2004b). Despite these differences between climatic regions, the IPCC guidelines proposed that 20 years are enough to reach a new equilibrium after a land-use change (IPCC 2006). Thanks to a policy of forest restoration and a stimulation of afforestations, and because forests represent the major land use, the SOC in Europe has increased over the last decennia (Houghton et al. 1983; Janssens et al. 2005; Smith et al. 2006). This has partially offset the human induced GHG emissions. Unfortunately, this C sequestration is not permanent and it seems that forest soils are now reaching an equilibrium (Janssens et al. 2005). Compared to the reduced emissions of other sources of GHG, which can continue indefinitely, carbon sequestration in the soil is

therefore time-limited and finite. This limitation is linked to the sink saturation (Stewart et al. 2007) and because further increases in forest areas are unlikely (Jandl et al. 2007). Moreover, the afforestation has produced indirect land-use changes in other parts of the world. While afforestation and SOC sequestration have been increasing in Europe, large natural areas have been converted to agriculture in South America, in Asia and in Africa with consequent SOC losses (Eglin et al. 2010; Smith and Trines 2006; Vega et al. 2009). There is an increasing demand for land to produce food, fibers and fuels to an increasing population, but there is also an increased demand to restore ecosystems to provide ecosystem services. How to cope with these increasing demands remains a major challenge for the research community and for society in general. While the losses of many ecosystem services are relevant at the local or regional level, the CO_2 and GHG issue remains a global problem.

1.1.4. A particular type of forestry: short rotation woody crops

Short rotation woody crops (SRWC) are defined as high-density plantations of fast growing perennial crops for rotations from 2 to 8 years (Figure 1.3). At the end of each rotation, the trees are harvested at the base of their stump, resulting in the regeneration of new shoots from the stump and the roots. Because of their fast growth and high yield, poplars (*Populus*) and willows (*Salix*) are the most widely used tree species in SRWC cultures. Wood chips from SRWC can be burned, gasified, or co-fired with coal to produce electricity and/or heat, with the advantages that the afforestation brings to the soil as explained above. Therefore, bioenergy crops, such as SRWC, have been considered as management options both to sequester carbon in European croplands, and to (partially) replace the consumption of fossil fuels (Smith 2004a). However, SRWC are more comparable with a crop cultivation than with an afforestation, despite of the woody nature of the poplars or willows. SRWC require lower agrichemical inputs and less fertile land than food crops. Although SRWC cultivation is fully mechanised – from soil preparation, planting and management to harvesting – it is something in between forestry and conventional cropping, since most mechanization is from agricultural machines adapted to SRWC. Due to the relatively recent introduction of SRWC (since the 1970's), some management practices are still under development. For example, weed management is still a major problem, especially in the early years of the culture. All the afore mentioned management applications affect the C cycle, by affecting the SRWC productivity and the C inputs from weeds, harvest losses, etc. As in the case of other agricultural uses (Janssens et al. 2003), the benefit of SRWC for SOC sequestration remains highly uncertain. In summary, SRWC is a relatively new technology with a lot of potential, but with still many questions that are worth to be answered.

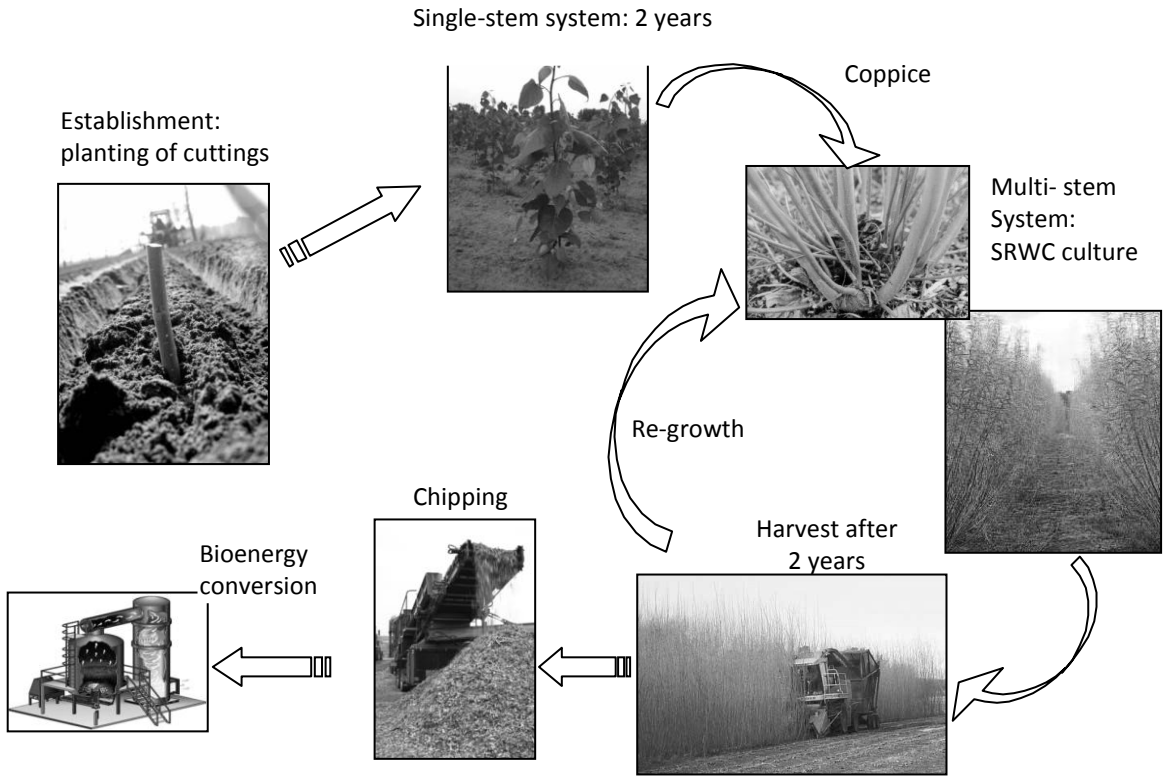


Figure 1.3: Concept of the culture of short rotation woody crop.

1.2 The experimental framework of this thesis: the POPFULL project

The POPFULL project consists of the full analysis (FULL) of an SRWC of poplars (POP) and involves both an experimental approach at a representative field site and a modelling part (POPFULL project; http://uahost.uantwerpen.be/popfull). The overall objectives of the POPFULL project are: (i) to make a full balance of the most important greenhouse gases (CO_2, CH_4, N_2O, H_2O and O_3), (ii) to make a full energy and economic accounting; and (iii) to perform a full life cycle analysis (LCA) of the global warming contribution of SRWC. The overall energy efficiency of the system is being assessed. Eddy covariance techniques are used to monitor net fluxes of all greenhouse gases, in combination with common assessments of biomass pools (incl. the soil) and fluxes. For the energy accounting, colleagues use life cycle analysis (LCA) and energy efficiency assessments over the entire life cycle of the SRWC plantation until the production of electricity and/or heat. A significant process based modeling component integrates the collected knowledge on the GHG and energy balances toward predictions and simulations of the net reduction of fossil GHG emissions (avoided emissions) of the SRWC over different rotation cycles. For the experimental approach a SRWC (18.4 ha) of poplar (*Populus spp*.) and willow (*Salix spp.*) is being monitored during the course of 2+2 years. The harvested materials are transformed into bioenergy using two alternative techniques, i.e. a small-scale gasification and co-combustion in an electricity plant.

1.2.1 Description of the field site

The experimental field site of this thesis is located in Lochristi, Belgium (51°06'N, 03°51'E) and consists of a high-density poplar and willow plantation. Lochristi is located 11 km from Ghent in the province of East-Flanders at an altitude of 6.25 m above sea level with a flat topography. The long-term average annual temperature at the site is 9.5 ºC and the average annual precipitation is 726 mm (Royal Meteorological Institute of Belgium). The region of the field site is pedologically described as a sandy region and has poor natural drainage. The total area of the site is 18.4 ha. The former land-use types were (i) agriculture, consisting of cropland (ryegrass, wheat, potatoes, beets, and most recently monoculture corn with regular nitrogen (N) fertilization at a rate of 200-300 kg ha^{-1} y^{-1} as liquid animal manure and chemical fertilizers), and (ii) extensively grazed pasture (Fig. 1.4; left panel). For more information on the site and planting scheme, see Broeckx et al. (2012a).

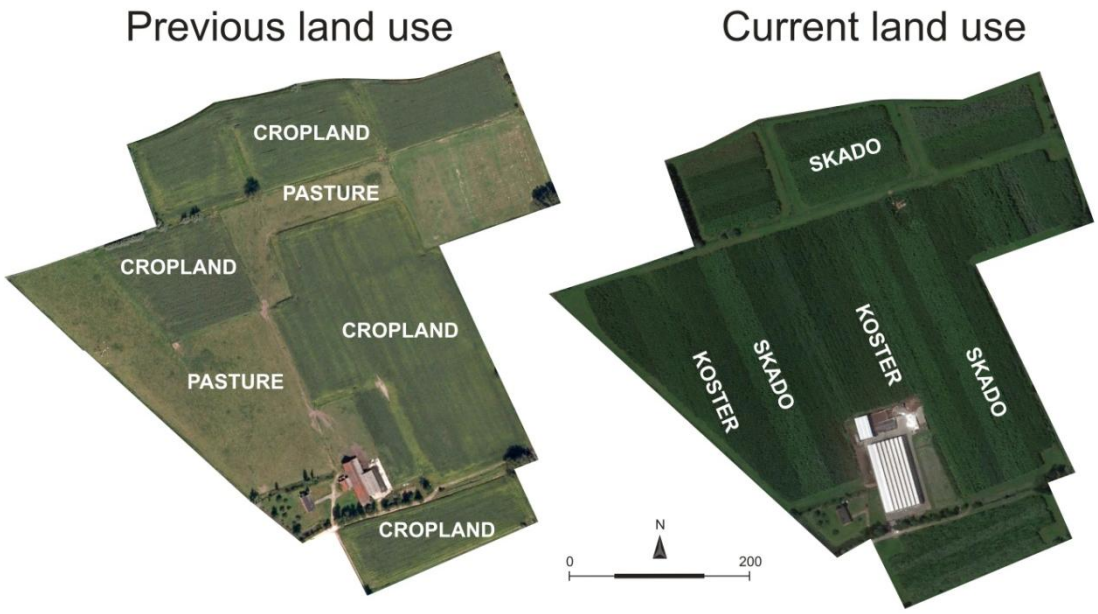


Figure 1.4: Aerial image of the field site before and after the establishment of the SRWC. The map on the left shows the distribution of the previous land-use types, i.e. pasture land and cropland. The map on the right shows the monoclonal blocks indicating the location of the genotypes Skado and Koster. (Source: Google Earth)

A detailed soil analysis was carried out in March 2010, prior to planting. The analysis characterized the soil type as a sandy texture. In the upper soil layer, carbon (C) and nitrogen (N) concentrations were significantly lower in cropland as compared with pasture and decreased exponentially with depth in both former land-use types. Table 1.1 presents a detailed analysis of nutrients and soil variables for both land-use types. For more information on the site and on the soil characteristics, see Broeckx et al. (2012a).

Table 1.1: Soil bulk density, pH, nutrient fractions and particle size distribution of the soil layers on both previous land-use types. Bulk density (BD), C and N were measured at 15 cm increments up to 90 cm depth; other nutrients and texture were measured at 30 cm increments up to 60 cm depth. (Adapted from Broeckx et al. 2012a)

Previous cropland												
Depth	BD	C	N	pH	P	K	Mg	Ca	Na	Clay	Silt	Sand
cm	kg dm^{-3}	%			mg kg^{-1}					%		
0 - 15	1.45	1.48	0.123	5.47	28.4	17.2	12.8	111.4	1.3	11.23	1.62	87.15
15 - 30	1.41	1.42	0.118									
30 - 45	1.49	1.02	0.072	5.87	8.6	12.0	13.7	103.1	1.8	11.48	4.17	84.34
45 - 60	1.51	0.77	0.048									
60 - 75	1.52	0.56	0.037									
75 - 90	1.55	0.38	0.031									
Previous pasture												
0 - 15	1.27	1.95	0.179	5.1	18.6	10.2	14.0	103.1	1.4	11.51	1.93	86.56
15 - 30	1.45	1.12	0.099									
30 - 45	1.50	0.84	0.066	5.6	6.4	5.5	11.1	99.0	1.1	11.09	4.29	84.62
45 - 60	1.53	0.69	0.048									
60 - 75	1.54	0.46	0.032									
75 - 90	1.57	0.34	0.025									

After soil preparation by plowing (40-70 cm depth), tilling and a pre-emergent herbicide treatment, a total of 14.5 ha were planted between 7 and 10 April 2010 with 25 cm long dormant and unrooted cuttings from 12 poplar (*Populus sp*.) and three willow (*Salix sp*.) genotypes in monoclonal blocks in a double-row planting scheme with a commercial leek planter (Broeckx et al. 2012a). The distance between the narrow rows was 75 cm and that between the wide rows was 150 cm (Figure 1.5). The distance between trees within a row was 110 cm, yielding an overall density of 8000 trees per ha. The total length of individual rows ranged from 45 m up to more than 325 m. Manual and chemical weed control was applied during the first and the second years. Neither fertilization nor irrigation was applied during the entire lifetime of the plantation.

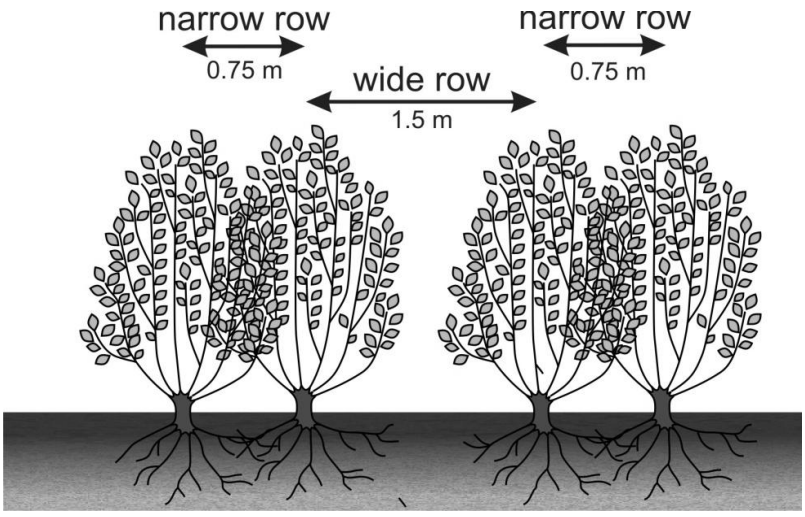


Figure 1.5: Representation of the double-row planting system of the experimental SRWC field site of this thesis.

1.3 Objective of the thesis

The POPFULL project provided the opportunity to study the soil carbon balance in an operational SRWC under the prevailing conditions. The overall objective of the thesis was to quantify the C balance of the soil of a recently established SRWC, and to evaluate the potential of SRWC for soil carbon sequestration. We aimed to provide a better understanding of the belowground carbon cycle of a SRWC, and to quantify all C pools and fluxes in the soil after the land-use change. All management activities (weeding, harvesting, etc.) had been taken into account for the C balance (Figure 1.6).

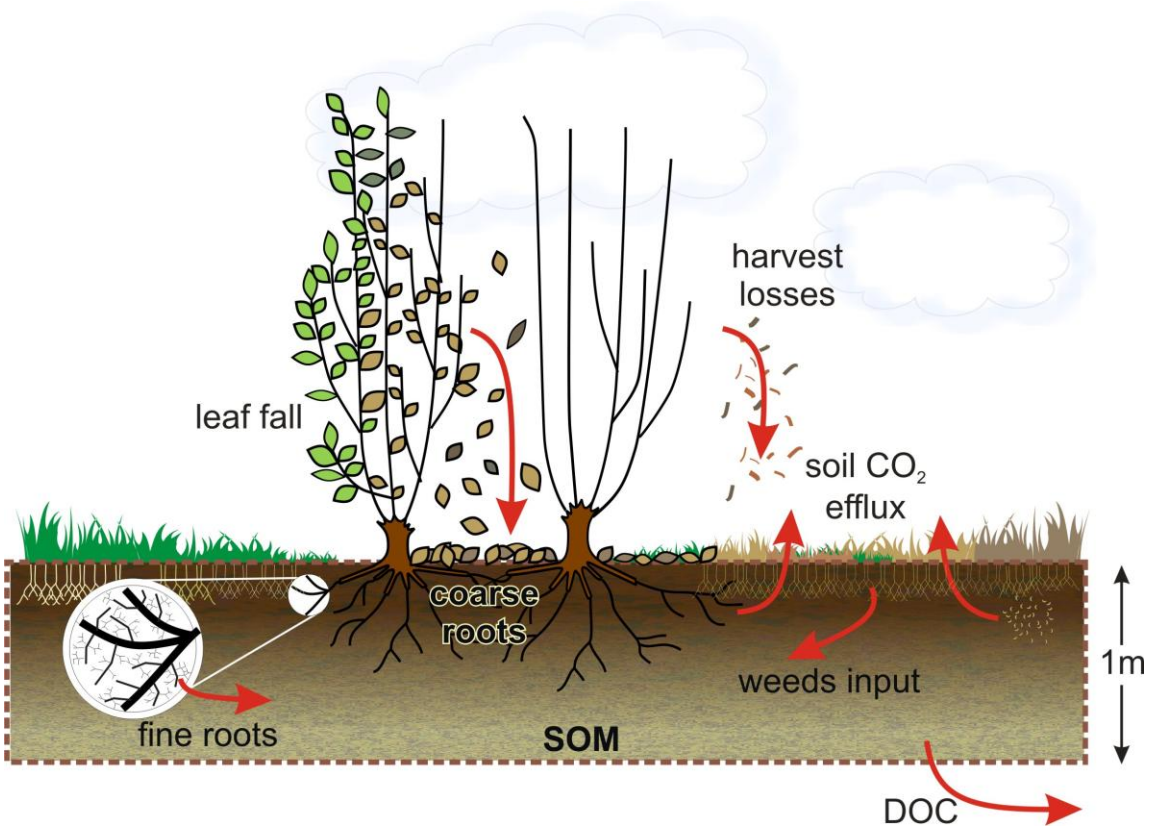


Figure 1.6: Representation of the belowground C pools and C fluxes of a high-density poplar plantation. Red arrows represent C fluxes and the brown dashed line represents the boundaries of the system (soil surface and 1 m depth).

Due to the timing of the rotations of the experimental SRWC and of the sampling of the field data for this thesis, most of the chapters focus on the first two growing seasons, i.e the first two-year rotation of the bioenergy plantation (Figure 1.7). However, in the synthesis (Chapter 7) we include the measurements of the four years (two rotations). Because of the high labor intensity, and in order to limit the variability caused by different species and genotypes, only two poplar genotypes were assessed for the soil carbon balance: i.e. Koster (*P. deltoides* Marsh x *P. nigra* L.) and Skado (*P. trichocarpa* Hook. x *P. maximowiczii* Henry). Both genotypes were chosen because they are genetically and phenotypically contrasting and represented the range of productivity values for the entire plantation (see Broeckx et al. 2012a for more details on the genotypes).

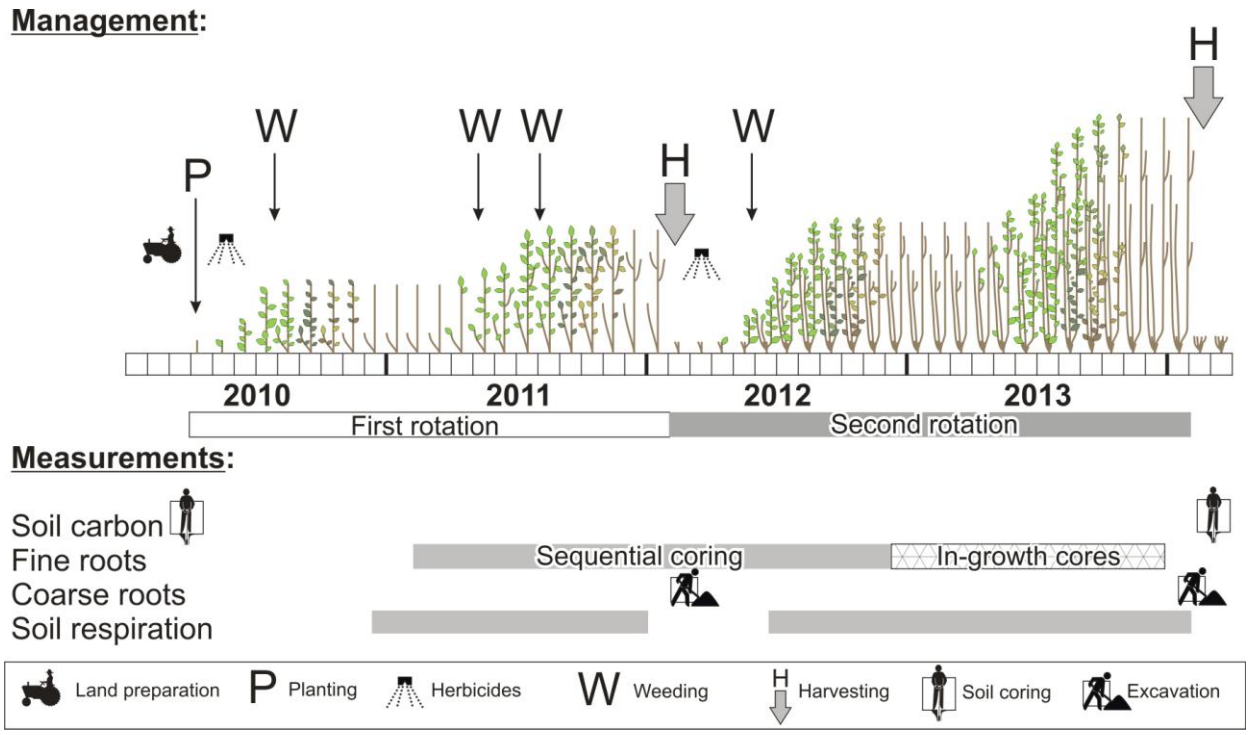


Figure 1.7: Schematic representation of the 2 + 2 rotation cycle of the SRWC plantation, indicating the timing of most relevant management activities and measurements of this thesis.

In Chapter 2 we designed a methodology that reduced the uncertainties in the estimation of one of the most uncertain and dynamic belowground pools, i.e. the fine roots. In Chapter 3, we described the seasonal evolution of fine root biomass from poplars and below-canopy weeds, and we compared several methods for root productivity estimations. We examined the effect of harvesting on the C balance in Chapter 4. In Chapter 5, we quantified the C pools in coarse roots and we linked the coarse root architecture with aboveground parameters. Chapter 6 presents a new method for the partitioning of soil respiration into heterotrophic and autotrophic respiration. The last chapter – Chapter 7 – synthesizes all the information and knowledge after four years of measurements of all C pools and fluxes in a final belowground C balance approach.

Chapter 2

2. A methodology for fine root biomass determination

Based on:
An optimized fine root sampling methodology balancing accuracy and time investment
G. Berhongaray, J.S. King, I.A. Janssens and R. Ceulemans.
Plant and Soil (2013) 366, 351-361.

Abstract
Tree roots are spatially highly heterogeneous and it thus requires large numbers of samples to detect statistically significant changes in root biomass. The objectives of this study were to understand and quantify the sources of error in the assessment of fine root (Fr, <2 mm) biomass during the second year of a high-density *Populus* plantation. Soil cores were collected in winter (n=35) and in summer (n=20), and Fr were picked by hand for varying lengths of time: 1, 2, 5, 20, 40, and 60 min. The root biomass data were used to identify the best combination of the time spent for root picking and the number of samples collected, that minimizes the overall uncertainty (i.e. the combination of the spatial error due to the incomplete sampling and the temporal error due to the incomplete core processing).
On average, 25 min was enough time to pick 90% of the Fr biomass in winter, while in summer only 10 min were needed. In winter fewer samples were needed, but more time for picking was necessary as compared to summer when root biomass was higher. Fine root sampling can be optimized by minimizing the uncertainty of the biomass estimates and simultaneously decreasing root sampling time investment.

Keywords: auger sampling, sampling time, root picking time, spatial error, temporal error

2.1 Introduction

For 250 years various techniques and methods have been developed for studying roots (Evelyn 1662; Noehden 1824), but all methods have their limitations (Jackson et al. 1996; Lauenroth 2000; Nadelhoffer and Raich 1992). Fine roots (Fr) represent only a small fraction of the total root biomass in forest ecosystems (Jackson et al. 1997). But in comparison with their small contribution to the standing root biomass, Fr dynamics play a large role in biomass production and allocation, in plant-soil interactions, and in carbon cycling (Nadelhoffer and Raich 1992; Ostonen et al. 2005; Tufekcioglu et al. 1998). Fine root turnover represents a major carbon cost to the tree (Janssens et al. 2002) and a large carbon input to the soil (Ruess et al. 1996). Within the framework of the changing climate and the increasing demand for ecosystem services provided by forests, the ability to accurately quantify Fr dynamics remains a daunting, but essential challenge that must be overcome (Brunner and Godbold 2007).

Over time a considerable number of methods has been developed to assess Fr biomass and Fr turnover (Böhm 1979; Mancuso 2011; Persson 1980; Publicover and Vogt 1993; Stokes 2000; Waisel et al. 2002). These methods include allometric techniques (e.g. root:shoot or other ratios), the direct excavation of the root system, core sampling, as well as in situ imaging methods (Mancuso 2011; Vogt and Persson 1991). Each of these methods has several sources of error. The analysis of data obtained from root sampling is constrained by the experimental design and by the associated statistical properties of the population of roots sampled. In a comparative study of different techniques for the assessment of biomass of Fr and of medium-sized roots (Mr), soil core sampling provided the same accuracy and was more cost effective than entire tree excavations (Jourdan et al. 2011). However, there is still no “uniform standard approach” for the assessment of Fr biomass, partly because each ecological setting requires a sampling procedure tailored to the specific situation. Therefore, an approach to optimize Fr sampling using soil cores that specifically accounts for the major sources of error would be of great help in forest ecological studies.

Fine root biomass is spatially and temporally highly variable (Metcalfe et al. 2008). In the core sampling method volumetric soil samples are taken manually in the field and washed in the lab to separate roots from the soil (Oliveira et al. 2000). The researcher chooses the number of samples to be taken (normally ranging from 8 to 30), and this decreases the error around the mean (Vogt and Persson 1991). Temporal changes in root biomass can only be detected if the assessments at different points in time are statistically different (Publicover and Vogt 1993). It is thus crucial to minimize the standard deviation of the mean. The power of the assessment thus increases with increasing sample size (Bengough et al. 2000). As root sampling is time consuming, the time and cost associated with increasing sample numbers rapidly increase and often become unrealistic (Metcalfe et al. 2007). For a given time available, the spatial sampling error declines with higher numbers of samples, but comes at the expense of the time that remains available for root picking in the lab (temporal error). The objectives of this study were (i) to understand two sources of

error on the root biomass assessments (spatial and temporal), and (ii) to use experimental data to develop a statistically robust method of minimizing both the spatial and the temporal errors while at the same time decreasing the root sampling time costs. Other minor errors and difficulties are associated with root sampling, as vitality (live/dead), species recognition, loss fractions while picking or through sieves, losses through prolonged storage, soil texture and humidity, etc. But these are not being considered in the present study.

2.2 Materials and Methods

2.2.1 Quantification of root biomass and of duration of root picking

Core sampling was used to assess Fr biomass dynamics during the second year of the plantation. Root biomass was estimated from soil samples collected up to 15 cm depth using an 8 cm diameter x 15 cm deep hand-driven corer (Eijkelkamp, The Netherlands) (Oliveira et al. 2000). 35 samples collected in winter (Feb.—March 2011) and 20 samples collected in summer (July—Aug. 2011), Fr (<2 mm) were picked manually in the laboratory for 1, 2, 5, 20, 40 or 60 min. The time intervals were shorter at the beginning in order to capture the increments of root biomass at early phases of the root picking. Roots from weeds were separated from poplar roots and ignored from here on. At each time roots were washed in a plastic cuvette and weighed to determine the root biomass picked. Fresh biomass collected at each picking duration was later transformed to the proportion picked (see below). After the fresh biomass had been determined, roots were put into paper bags. Roots were dried at 70 ^{o}C to constant mass and expressed in dry matter (DM, g). Root biomass was scaled to g m^{-2}. We carefully quantified the time necessary for each step in the process: (1) the transport to the field site (60 km one way), (2) the collection of the samples in the field, (3) the return transport of the samples to the laboratory, (4) the logistics into the laboratory (incl. handling in and out the freezer, from storage to laboratory, and preparation of the materials for root picking), (5) the root picking at each time, (6) the washing and weighing of the sorted roots. For this purpose a chronometer was used. The time for 10 individual random samples was measured in steps 2, 4, 5 and 6. The transport time in steps 1 and 3 was measured three times.

2.2.2 Picking duration error and ecosystem scale spatial error

The accumulated fresh root biomass at any given duration of picking was expressed as a fraction of the total fresh root biomass at the maximum time (i.e. 60 min of root picking). It was not possible to use a linear model to relate the accumulated proportion of fresh root biomass with the duration of root picking because the residuals did not have the same variance along the distribution, thus failing to support the assumption of homoscedasticity. Therefore Richard's equation was fitted to the transformed data:

$$y = a\,(1 - e^{-bx})^{c} \qquad \text{[Eq. 1]}$$

where y = the proportion of roots picked, x = the duration of root picking, a = the parameter that describes the maximum of the function, b = the parameter that describes the curvature of the function, c = the parameter that describes the lag phase of the function, and e = the base of the natural logarithm (Causton and Venus 1981). The fitted equation was used to estimate the amount of roots picked at all other times. Overlapping of the confidence limits (95%) for each parameter and an ANCOVA of the residuals (with root picking duration as covarying factor) were used to test for differences between the curves fitted to winter and summer samples.

Means and standard deviations were calculated for the proportion of fresh root biomass collected at each duration of picking (1, 2, 5, 20, 40 and 60 min). Using Eq. 2, we then estimated the number of samples that could be processed within a given amount of time invested, i.e. 100, 300, 600, 1200 and 2400 min. The total time invested was divided by the time necessary to process one sample (sample + logistic + duration of root picking + sorting, washing & weighing) to obtain the number of samples that could be processed:

$$n\ (samples) = \frac{Time\ invested\ (min)}{Time\ processing\ (min\ sample^{-1})} \qquad \text{[Eq. 2]}$$

The standard error for each picking duration was obtained by dividing the standard deviation by the square root of the number of samples obtained from Eq. 2. This standard error was then divided by the mean to obtain the relative standard error, defined as the picking duration error (PDE).

From the mean and the standard deviation of the fresh root biomass collected after 60 min of picking, we estimated the ecosystem scale spatial error (ESSE) for different numbers of samples, both for winter and summer samples. The standard error was divided by the mean to obtain the ESSE for all numbers of samples. The different relative standard errors for different numbers of samples were used to assess the spatial variation in the field. More details of the calculation could be found in Berhongaray et al (2013d).

For a different number of samples collected in the field we thus calculated a PDE and an ESSE. Both standard errors were summed to obtain the total relative standard error (TRSE). The PDE and ESSE were plotted against the number of samples, and the minimum TRSE was selected as the optimal number of samples collected for a given time period (e.g. winter, summer).

2.3 Results

Fine root biomass varied significantly among sampling periods. For the subset used for the error analysis (winter n=35, summer n=20), total fresh root biomass at 15 cm depth was 62.4 g m^{-2} (14.0 g DM m^{-2}) in winter *versus* 320 g m^{-2} (75.4 g DM m^{-2}) in summer.

Fresh root biomass increased and PDE decreased with increasing duration of root picking (Figure 2.1). The recovery of roots was faster at the beginning of the picking as there were

still more roots in the sample. The increments of the proportion of roots picked decreased with increasing duration of picking. In general 30% of all Fr were picked after the first minute. Root picking for 60 min instead of 40 min only increased the recovered root biomass by 2%. On average, 25 min of root picking was enough to pick 90% of the root biomass in winter, while 10 min sufficed for the same proportion of roots in summer. The proportion of roots picked after a certain period was proportional to the root biomass in the sample. The time necessary to pick 90% of the Fr biomass decreased with increasing root biomass in the sample.

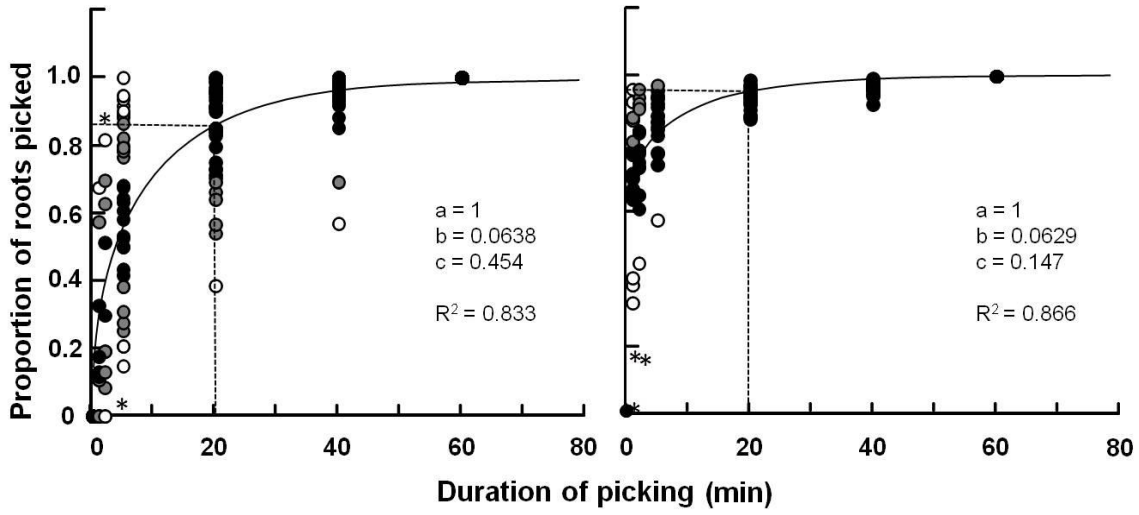


Figure 2.1: Increments in the proportion of fresh Fr biomass picked as a function of the duration of root picking in winter (left) and summer (right panel). Proportions are relative to the maximum root biomass picked after 60 min. Richard's equation ($y = a\ (1- e^{-bx})^c$) was fitted through the data points. Black dots are at <1 SD, grey symbols are at <2 SD, empty symbols are at <3 SD and asterisks are at >3 SD. The dotted line represents the proportion of root picked at a picking duration of 20 min. SD= standard deviation.

Part of the time devoted to process one sample was variable while another part required a constant amount of time (Table 2.1). The time spent per sample in the field and handling in the laboratory was constant. Also the time needed to collect a sample in the field and to transport it to the laboratory was constant. So, these durations were similar for each sample and independent of the duration of root picking. In contrast, the time needed for washing and weighing increased with the duration of root picking, because more roots were retrieved that needed to be washed and weighed. By far most of the time spent for each sample was devoted to manually separating the roots from the soil, i.e. the root picking. An overview of the time cost of a sample collection campaign and the concomitant analysis is shown in Table 2.2. Transport (i.e. the driving time) to the field site was independent of the amount of samples. When only a few samples were taken, the time spent in transport represented a high proportion of the total time invested (including transport). With more than 30 samples the transport represented only 3-5 % of the total time cost. The time spent in root picking, sorting, washing and weighing represented 84-93% of the total time needed to process the samples. Tripling the picking duration (from 20 to 60 min) only doubled the total time needed.

Table 2.1: Time per sample devoted to drive to the field; to collect the samples in the field; to transport samples to the laboratory; to store and to handle the samples in the laboratory; to pick, to sort, to wash and to weigh the roots. The handling in the laboratory includes the transport from the car to the storage room, in and out of the freezers and the oven, and from the storage room to the laboratory. The times to drive to the field, to collect the sample, to transport the samples to the laboratory and to handle in the laboratory were the same for any duration of root picking. All values were rounded to the nearest entire number and are all given in min.

Duration of picking	Sample in the field	Handle in the laboratory	Sort, Wash & Weigh	Total time in lab	Transport to the site	**Total time including transport**
1	4	3	8	16	120	**136**
2	4	3	10	18	120	**138**
5	4	3	11	23	120	**143**
20	4	3	14	41	120	**161**
40	4	3	15	62	120	**182**
60	4	3	16	83	120	**203**

Table 2.2: Time devoted to pick, to sort, to wash and to weigh the roots; to collect the samples in the field; to bring to and to store in the laboratory; and to drive to the field site for different combinations of picking time and number of samples. All values have been derived from Table 2.1. The time to transport (driving time) to the field site was the same for any amount of samples or time picking. All values were rounded to the nearest entire value and are given in min.

Number of samples	Duration of picking per sample	Total time picking	Sample in the field	Handle in the laboratory	Sort, Wash & Weigh	Total time in lab	Transport to site	**Total time including transport**
1	20	20	4	3	14	41	120	**161**
	60	60	4	3	16	83	120	**203**
10	20	200	35	30	140	405	120	**525**
	60	600	35	30	161	826	120	**946**
30	20	600	105	90	420	1215	120	**1335**
	60	1800	105	90	483	2478	120	**2598**
50	20	1000	175	150	700	2025	120	**2145**
	60	3000	175	150	805	4130	120	**4250**

An increase in the duration of root picking was accompanied by a reduction in the uncertainty of the Fr estimation (Figure 2.1). By increasing the duration of root picking by four (from 5 to 20 min) we gained 41% in accuracy. The fitted Richard's equation and the associated PDE significantly differed between the sampling periods ($p = <0.001$; 95% confidence limits of the parameter *c* for winter: 0.340 – 0.566 and summer: 0.089 – 0.204). The ANCOVA further indicated that this difference was not affected by root picking duration ($p = 0.59$). In winter it took 15 min to pick 80% of the roots while in summer it took only 4 min for the same proportion of Fr. Overall, a higher root biomass and a better accuracy were obtained in summer than in winter. Thus, sampling periods greatly affected the time needed to retrieve the root biomass from each sample.

The duration of root picking and the associated PDE played an important role in determining the optimal number of samples to collect (Figure 2.2). For a given total time invested, there was a trade off between the time spent in sampling and in root picking; when we collected more samples, the time available for root picking per sample decreased. Reducing the time of root picking increased the PDE (Figure 2.1). By definition, the PDE

was zero for the maximum time devoted to picking (60 min) and was largest at the minimum time devoted to root picking (1 min). However, as the PDE increased with larger numbers of samples, the ESSE decreased. The minimum ESSE was obtained with the maximum number of samples.

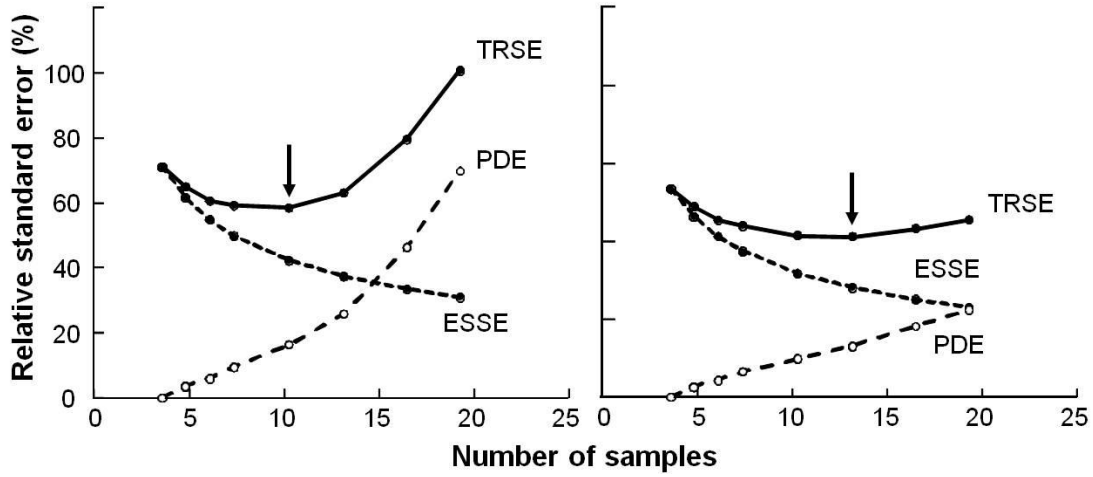


Figure 2.2: Total relative standard errors (TRSE) as a function of the number of samples analyzed. Samples were collected in winter (left) and in summer (right panel) for a total time invested of 300 min. The dotted line with open symbols represents the picking duration error (PDE) for a given time available. The dotted line with solid points represents the ecosystem scale spatial error (ESSE). This is the standard error around the mean given the standard deviation of the different soil cores collected in the field. The solid line represents the sum of both relative standard errors (TRSE = PDE + ESSE). The arrow marks the minimum TRSE.

The magnitude and the importance of the two sources of error were different for sampling periods. The ESSE was similar in both seasons, but in summer the PDE was lower (Figure 2.1) and the minimum TRSE was reached at a higher number of samples than in winter (Figure 2.2). Consequently, the optimal number of samples differed between sampling periods. More samples were necessary to reach the minimum TRSE in summer than in winter.

The optimal number of samples – defined by the minimum TRSE – not only varied with sampling periods (summer *versus* winter), but also with the total time invested (i.e. 100, 300, 600, 1200 and 2400 min). For the same number of samples, the TRSE was reduced by increasing the time invested. An increment of time invested induced an increment in the optimal number of samples. It was, however, less crucial to be very close to the optimum when the total time devoted increased, because TRSE became much less sensitive to changes in the number of samples (Figure 2.3). With more time invested, more samples were needed, but the much smaller sensitivity of the TRSE to changes in the number of samples also allowed a large reduction in the number of samples to be analysed. The optimal number of samples linearly increased with the time invested (Figure 2.4, top panel). When TRSE was increased by 10 % the number of samples could be reduced by 40 % in winter, and by 46 % in summer (Figure 2.4, top panel, dotted lines). The reduction in the number of samples held regardless of the amount of time invested, because the shorter

duration for the collection of the samples was counterbalanced by the longer root picking time.

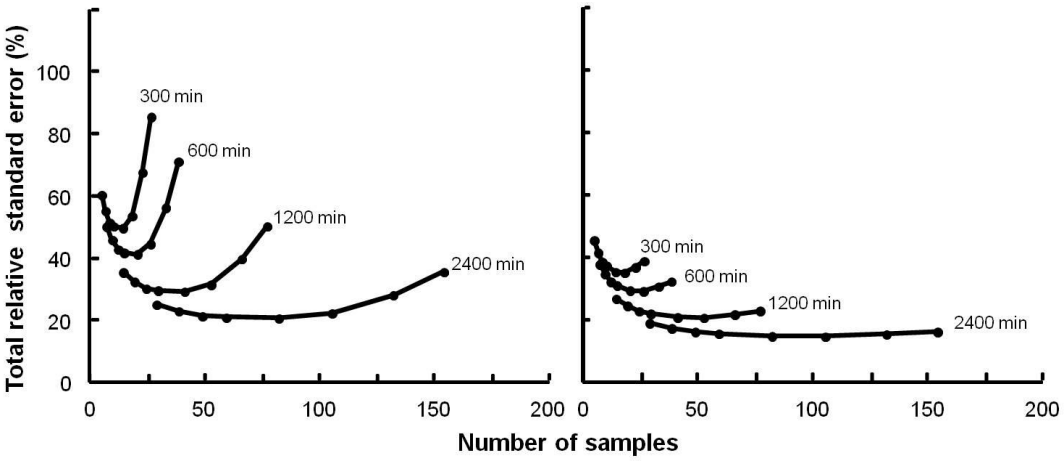


Figure 2.3: Total relative standard errors (TRSE) as a function of the number of samples for different time investments (300, 600, 1200 and 2400 min) for samples collected in winter (left) and in summer (right panel). The lines represent the total (spatial + temporal) relative standard error. Investing more time reduces the TRSE.

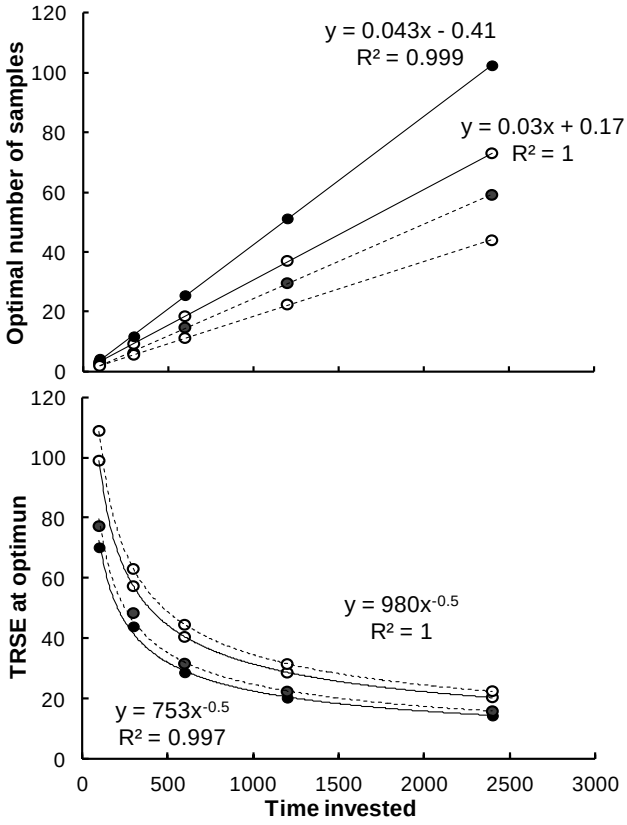


Figure 2.4: Optimal number of samples (top panel) in relation to the total time invested in the root analysis. Filled symbols represent summer samples and open symbols represent winter samples. The solid lines are the optimal number given by the minimum total relative standard error (TRSE). The dotted lines represent the number of samples given by increasing the minimum TRSE by 10%. Total relative standard error (TRSE) at the optimum (lower panel). Filled symbols represent summer samples and open symbols represent winter samples. The solid line represents the TRSE at the optimal number of samples; the dotted lines represent increments of the minimum TRSE by 10%.

The TRSE decreased exponentially with the time invested (Figure 2.4, lower panel). Decreases in the TRSE were around 30-40 % when the time invested was doubled. These decreases were more important when increasing from 300 to 600 min than when going to 1200 min. The TRSE was always lower in the summer samplings than in the winter samplings. The smaller TRSE was associated with more time invested because more time implied more samples, and the number of samples is the denominator in the calculation of

PDE and ESSE. By definition the sum of PDE and ESSE equalled the TRSE. The dotted lines (Figure 2.4, lower panel) show the small increment that represents an increase of the uncertainty by 10 %. The number of samples could be significantly decreased with only a small increase in the uncertainty (Figure 2.4).

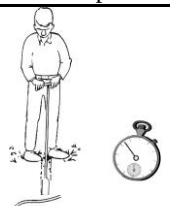
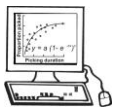
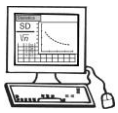
2.5 Discussion

The main objectives of the current study were to understand the sources of error in the estimation of Fr biomass in a young, high-density *Populus* plantation, and to develop a quantitative methodology for optimizing Fr biomass sampling to increase accuracy and decrease time investment costs. Several studies have tried to determine the sample size by only accounting for spatial variation of root biomass distribution (Garten et al. 2007; Liski 1995; Metcalfe et al. 2008). The present study improves this technique by optimizing, for a given time investment, the combination of the picking duration and the spatial errors associated with root sampling. The main message extracted from the study is that sampling effort and time investment processing each core could be minimized in root studies, specially taking into account that after 25 min up to 90% of the roots were already picked. This is an interesting result as most root researchers often pass a lot more time processing cores of similar size. This has been obtained through a statistically robust methodology that is defined by the specific conditions of the experimental design and the ecological conditions of the tree plantation (Table 2.3).

The large time investment, and the resulting financial (i.e. personnel) cost, is the primary limiting factor for field sampling of root biomass. As a consequence, several researchers have tried to decrease the time invested in root sampling and analysis (Benjamin and Nielsen 2004; Levillain et al. 2011; Metcalfe et al. 2007). The time needed for washing and weighing, together with the duration of root picking, represented most of the time spent per sample. The time to collect the sample in the field and to transport it to the laboratory was constant for any duration of root picking and only represented a small proportion of the total time, especially in comparison with root excavations (Rodrigues de Sousa and Gehring 2010). The time to drive to the field site is only applicable for the specific situation of this study, but it gives an idea of the proportion of time that was needed for a campaign of root sampling in the field. All this information could be useful to estimate the time cost (in amount of work hours, Table 2.2), to optimally design a field campaign for root sampling. The required amounts of time and the associated cost of the research are very relevant for realistic project proposals.

Most of the time spent was invested in separating the Fr from the soil (Table 2.1). Generally, the amount of root biomass retrieved from a soil sample increases with the duration of the root picking time, while the error decreases (Metcalfe et al. 2007). In our case, the proportion of roots retrieved did increase with the time invested in root picking, but the relationship differed greatly between the sampling periods. This has implications for optimizing the root sampling design. The reason for the easier root picking in summer was probably the higher connectivity or clumping of a larger root biomass.

Table 2.3: Description of the step-by-step procedure to reproduce the proposed methodological approach. SD = standard deviation, PDE = picking duration error, ESSE = ecosystem scale spatial error, TRSE = total relative standard error.

Step	Procedure
	1. Collect samples in the field and quantify the time needed to collect, to transport and to handle each sample.
	2. Pick the roots for different durations of time (i.e. 1, 2, 5, 20... min). Sum the time needed to collect and to handle (step 1) with the time for washing, sorting and weighing (step 2) for each picking duration. Use this information (time) in step 4 (Eq. 2, time processing).
	3. Convert the biomass picked at each interval as a proportion of the root biomass picked at the maximum duration. Fit Eq. 1 to the increments in the proportion of roots picked with time, and obtain the mean and SD for each picking duration.
Eq. 2	4. Set an available time (time invested) for the root sampling and calculate the number of samples possible for each different root picking duration (step 2) using the Eq. 2.
	5. *Estimate the picking duration error (PDE):* For each picking duration, divide the SD (step 2) by the square root of the number of samples (step 4). Plot PDE versus the number of samples prescribed by root picking duration and the available time (Figure 2.2, dotted line).
	6. *Estimate the ecosystem scale spatial error (ESSE):* Take SD of root biomass picked at the maximum picking duration (i.e. 60 min) and divide by the square root of the number of samples (step 4) and then by the mean of the biomass picked at the maximum picking duration to get the ESSE. Plot ESSE versus the number of samples (Figure 2.2 dashed line)
	7. Sum ESSE and PDE to obtain the total relative standard error (TRSE). Plot TRSE versus the number of samples (Figure 2.2, solid line). Get the optimum sample number with the minimum uncertainty. If the uncertainty is above your expectation, return to step 4 and increase the time available. If the uncertainty is lower than your expectation reduce the time invested in step 4.

Differences in the proportion of root biomass picked and the PDE with the duration of picking, defined the optimal number of samples for each season. The optimal number of samples was defined by the error of the estimation of the correct root mass and the time needed to separate the roots from soil. For a given distribution the precision of a statistical estimator increases with an increasing number of replicate samples (Underwood 1997). In the present study more samples decreased the TRSE, but this also meant that there was less time available to process the roots. Most of the time spent with the samples was devoted to manually separating/picking roots from the soil (Table 2.1), in line with recent observations of Rodrigues de Sousa and Gehring (2010). On the other hand the optimal number of samples increased linearly when we had more time available to determining root biomass (Figure 2.4, above panel). If we had chosen 600 min instead of 300 min to process the samples, the optimal number of samples would have more than doubled.

The second source of error examined in the current work was the ESSE, i.e. the random error associated with the spatial variation in root biomass distribution. This error has

received particular attention from many authors (Metcalfe et al. 2008; Publicover and Vogt 1993). The present analysis demonstrates that if we increased TRSE by an acceptably small amount (by increasing ESSE and reducing PDE, Figure 2.2), the number of samples collected could be decreased significantly (Figure 2.4). An increase of 10% of the TRSE allowed us to decrease the number of samples by more than 40 % in both seasons. The decreases in numbers of samples held regardless of the amount of time invested, because the time reduced to take samples was employed in longer pickings. Although this reduction in the number of samples collected does not necessarily mean a reduction in the total time invested in studying roots, it means a reduction in the amount of time spent in the field, in the number of samples to carry/transport, in the storage capacity needed in the laboratory, and in the amount of data management. All of these time durations translate directly into decreased costs, potentially freeing up resources that could be devoted to other aspects of the research.

Ideally, the root sampling methodology should be determined by the objectives of the study, by the experimental design, and by biological characteristics of the root systems being studied. Fine root biomass varies seasonally, normally peaking in summer (Lukac et al. 2003; Santantonio and Santantonio 1987). Therefore, some authors have suggested to decrease sampling intensity during periods of expected high root biomass (Vogt et al. 1998). Our study clearly shows that more samples were needed in summer when root biomass was high compared to winter (Figure 2.2). The ESSE for both seasons was virtually the same, and therefore the difference resulted mainly from the PDE. Summer samples had more root biomass and inter-connections between roots, resulting in a shorter duration for root picking and in more time available for sampling. These results suggest that it is necessary to vary the sampling intensity, not only in the number of samples, but also in the duration of picking (i.e. separating roots from soil).

Root sampling should also follow the spatial variation in root distribution. The current study focused on the top layer of the soil only, and on specific times during the growing season. Root biomass tends to decrease with depth (Jackson et al. 1996) while the ESSE increases (Trumbore et al. 2006). Fine root depth profiles differ between tree species (De Baets et al. 2007), between clones (Al Afas et al. 2008), and even for the same clone with differences in management (Mulia and Dupraz 2006). Furthermore root biomass and composition (diameter, species, etc.) change during the year, and differently for top layers and deeper soil layers (Burke and Raynal 1994; Janssens et al. 2002; Santantonio and Santantonio 1987). These factors have to be considered in order to calculate the number of samples throughout the year (Vogt et al. 1986). By minimizing the combined spatial and temporal errors, our methodology maximizes the efficiency of root sampling allowing a more effective allocation of resources to account for the myriad of factors that must be considered in the design of accurate, cost-effective studies of Fr dynamics.

2.5 Conclusion

In conclusion, most of the roots were retrieved in the first minutes of the picking. But, more time to pick roots per sample was needed during the winter, where lower root biomass was present, than during the summer sampling periods. In the sampling made in winter, the minimum total relative standard error (TRSE) occurred at a smaller number of samples than in the summer sampling. In winter, the smallest error was achieved by taking fewer samples, but picking them a bit longer. In summer, with a larger biomass, taking more samples and picking them faster provided the smallest error. Our understanding of the sources of error allowed us to optimize the time invested in root sampling, processing and analysis.

Chapter 3

3. Dynamics of fine roots

Based on:
Fine root biomass and root turnover of two fast-growing poplar genotypes in a short-rotation coppice culture
G. Berhongaray, I.A. Janssens, J.S. King and R. Ceulemans
Plant and Soil (2013)373, 269-283

Abstract
The quantification of root dynamics remains a major challenge in ecological research because root sampling is laborious and prone to error due to the unavoidable disturbance of the delicate soil-root interface. The objective of the present study was to quantify the distribution of the biomass and turnover of roots of poplars (*Populus*) and the associated understory vegetation during the second growing season of a high-density short rotation coppice culture. Roots were manually picked from soil samples collected with a soil core from narrow (75 cm apart) and wide rows (150 cm apart) of the double-row planting system from two genetically contrasting poplar genotypes. Several methods of estimating root production and turnover were compared. Poplar fine root biomass was higher in the narrow rows than in the wide rows. In spite of genetic differences in above-ground biomass, annual fine root productivity was similar for both genotypes (ca. 44 g DM m^{-2} y^{-1}). Weed root biomass was equally distributed over the ground surface, and root productivity was more than two times higher compared to poplar fine roots (ca. 109 g DM m^{-2} y^{-1}). Early in SRWC plantation development, weeds result in a significant root competition with the poplar crop, but may confer certain ecosystem services as carbon input to the soil and retention of available soil N until the trees fully occupy the site.

Keywords: fine root biomass, root production, *Populus*, weeds, soil cores

3.1. Introduction

Strategies to store carbon (C) in the soil have the promise to recapture soil organic C lost due to disturbance associated with intensive agriculture, helping to mitigate the rapidly rising atmospheric CO_2 concentration. Fine roots (Fr) are very important for water and nutrient uptake, but they also represent an important component of the ecosystem C cycle (Jackson et al. 1997). Fine root productivity often exceeds above-ground productivity in forest ecosystems, due to high rates of turnover (Janssens et al. 2002). Consequently, the process of Fr production and turnover represents a large C input to the soil. How this process responds to changes in environmental conditions and to management directly impacts ecosystem C sequestration in a changing climate.

Species of the genus *Populus* show high variation in aboveground growth, phenology and biomass productivity (Laureysens et al. 2003; Laureysens et al. 2005; Singh 1998). Strong genetic control of allometric biomass partitioning to roots has also been reported (Al Afas et al. 2008; King et al. 1999), as has the seasonal evolution of root biomass among genotypes (Al Afas et al. 2008). High-density short-rotation plantations of poplar and/or willow (*Salix*) for the production of bioenergy often use a double-row planting design (Deraedt and Ceulemans 1998; Dillen et al. 2010), that could affect biomass production and distribution. In plantations with double-row planting systems (e.g. alternating narrow and wide rows), one might expect a higher root biomass in the narrow rows because of closer proximity to the trees. In addition, machine traffic occurs in the wide rows, possibly inducing soil compaction (Ampoorter et al. 2012). Roots may preferentially explore the planting row where soil compaction is lower (Bengough et al. 2006; Laclau et al. 2004), i.e. in the narrow rows solely based on the shorter distance to the tree.

Roots from competing herbaceous plants often remain unquantified in studies of C-cycling in tree-based ecosystems (Bakker et al. 2009). However, short-rotation woody crop (SRWC) cultures with poplar or willow are more comparable to crop cultivation than with forestry, despite of the use of woody plants. In agricultural systems, weeds consist of a spontaneous herbaceous vegetation that competes with the crop. Aboveground, weeds compete for light (Curt et al. 2005) and belowground they compete for water and nutrients (Kabba et al. 2007). Nitrogen availability for the poplars in a SRWC has been shown to be reduced by the fine roots of weeds that occupy part of the soil (Welham 2007). In mature temperate forests, the contribution of the herbaceous understory vegetation to the total Fr biomass is minimal (Bauhus and Messier 1999; Meinen et al. 2009). However, herbaceous competition can be significant in recently established tree plantations, such as SRWC, even when the herbaceous competition is controlled (Curt et al. 2005; Dickmann and Stuart 1983). Some studies have assessed the effects of weed competition on the establishment and productivity of poplar plantations (Kabba et al. 2007; Welham et al. 2007; Pinno and Belanger 2009; Otto et al. 2010), but very few have quantified their ecological impact (e.g. on carbon dynamics). Notwithstanding the negative effects, a large amount of herbaceous root biomass in the soil may reduce soil erosion (De Baets et al. 2007), increase nutrient retention (thus, avoiding losses from leaching and denitrification) (Hobbie 1992), and

increase carbon inputs to the soil (Alvarez et al. 2011) before trees have completely occupied the site. The presence of weeds generally has a negative impact on tree growth, but may confer other positive ecological attributes.

The objectives of the present study were to describe the distribution of the biomass of Fr of different size classes, and to quantify Fr production and turnover in a high-density SRWC poplar plantation and associated understory. We hypothesized that (i) soil carbon inputs from the roots of annual weeds may be equal to or exceed those from Fr of the poplar trees, and (ii) tree Fr biomass is higher in the narrow rows as compared to the wider rows of a double-row planting system. We expected weed root biomass to be less in the narrow rows because of the proximity to the trees. Both hypotheses were proposed with the goal of gaining a better understanding of the C-cycling dynamics of a *Populus* bioenergy SRWC in the early years after establishment.

3.2. Materials and Methods

3.2.1. Experimental site

All data were collected at the large-scale POPFULL project (Chapter 1; Broeckx et al., 2012a). Temperature and precipitation evolution during the studied year (2011) are presented in Figure 3.1. Despite the weed control measures described in chapter 1, there was high abundance of common agricultural weeds within the SRWC plantation (360 g aboveground DM m^{-2} in May 2011), including thistles (*Carduus spp., Circium spp.*), *Urtica spp., Capsella bursa-pastoris* L.*, Convolvulus spp., Matricaria chamomilla* L.*, Taraxacum officinale* Weber and various species of *Gramineae*. As nutrients and water were not limiting at the site, no fertilization or irrigation were applied during the study.

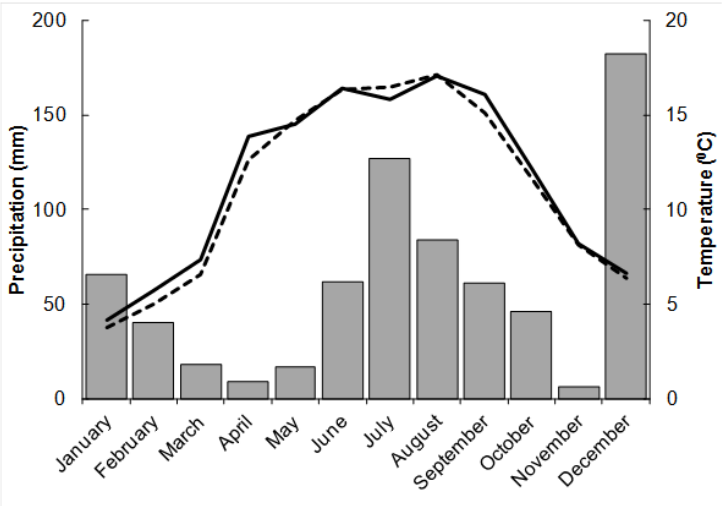


Figure 3.1: Seasonal evolution (2011) of a number of meteorological parameters monitored on a mast at the field site. Air temperature (solid line), soil temperature (dashed line) and precipitation (grey bars) are shown during the entire year.

3.2.2. Estimation of root biomass

All data for the present study were obtained from soil samples collected during the second year (2011) of the SRWC plantation. Fine root biomass dynamics of two phenotypically and genetically contrasting poplar genotypes, i.e. Skado (*P. trichocarpa* Hook. *x P. maximowiczii*

Henri.) and Koster (*P. deltoides* Marsh. × *P. nigra* L.) (Broeckx et al. 2012a) were quantified. Between Feb. and Dec. 2011 the two selected genotypes grew in stem diameter (measured at a height of 22 cm) from 28.8 mm to 46.4 mm (Skado) and from 20.7 mm to 37.4 mm (Koster). Over the same period stem height increased from 276.2 mm to 567.3 mm (Skado) and from 204.7 mm to 340.4 mm (Koster). Fine root biomass was estimated from soil samples collected down to a depth of 15 cm using an 8 cm diameter x 15 cm deep hand-driven corer (*cfr*. Oliveira et al. 2000), collected every two weeks from Feb. to Nov., 2011. An extra sampling was performed in Jan. 2012, for genotype Skado. Sample locations were randomized separately for narrow and wide rows: 10 samples per row and per genotype, at each sampling date. The distance from the sample to the nearest tree was measured with a tape measure (to the nearest cm). Samples were transported to the laboratory and stored in a freezer until processed. Samples were thawed and roots were manually picked for 5 min, washed, dried with tissue paper and weighed. From an earlier methodological study (Chapter 2; Berhongaray et al. 2013d), the 5 min picking duration was found to be the optimum trade-off between duration of root picking and number of samples that could be realistically processed. Roots were sorted into poplar and weed roots, and the total fresh root weight was determined after the 5 min picking duration. Shortly after the first picking, the samples were picked for another 15 min (20 min in total), sorted and put in paper bags for dry mass determination. Poplar roots were sorted from weed roots based on morphological characteristics. Poplar roots showed a brown colour and a dense ramification pattern, while weed roots (W) had a lighter colour and less ramification. Live poplar roots were classified in four diameter classes: <1 mm (L1; very fine roots), 1-2 mm (L2; fine roots), 2-5 mm (L3; medium-size roots) and >5 mm (L4; defined here as coarse roots). In the current study, we arbitrarily defined Fr biomass as roots with a maximum diameter of 2 mm (i.e. diameter classes L1 and L2).

Dead poplar roots (D), which were observed only in the L1 diameter class, were sorted from live roots based on the dark colour and the lack of cohesion of the periderm (Janssens et al. 1999). It was impossible to discriminate live from dead roots for the annual weeds. Sorted roots were dried at 65 °C to constant mass. Subsamples of dried roots were ground, and analysed for C mass fraction with an NC-2100 element analyzer (Carlo Erba Instruments, Italy) using a complete dry combustion technology. Every second sampling date samples were not transported to the laboratory, but immediately processed in the field where total fresh root weight was estimated. From these samples processed in the field, roots were manually picked for 5 min, washed, dried with tissue paper, sorted in poplar roots and weed roots, and their total fresh weight determined. Fresh root weight of one sample core picked for 5 min was converted into total root mass (from 20 min picking duration) using Richard's equation (Berhongaray et al. 2013d) and expressed in g DM m^{-2}. Root mass was converted to C mass using the average root C mass fraction, and expressed in g C m^{-2}. Therefore, we calculated the total Fr biomass on an approximately two-weekly basis, separately for narrow and wide rows. To quantify potential soil compaction, we took eight soil samples with a corer in Feb., 2012, and estimated soil bulk density for both wide and narrow inter-row spacing.

3.2.3. Estimation of fine root productivity and root turnover

There is no universally accepted method for estimating Fr biomass, productivity and turnover. Several methods have been proposed to estimate Fr productivity (see Vogt et al. 1998 for a comprehensive review). A number of studies combined multiple methods to characterize plant root dynamics in various terrestrial ecosystems (Burke and Raynal 1994; Levillain et al. 2011; Steele et al. 1997). Although the primary intention of the present study was not to compare different methodologies of estimating Fr production, we used four methods based on core sampling to provide a range of estimates for the poplar trees (Fr; diameter classes L1 and L2). The four methods were:

1. The "max-min" method was the simplest method used. This method estimates Fr productivity by subtracting the annual minimum root biomass from the annual maximum biomass (Burke and Raynal 1994).
2. The "sequential core" technique (Milchunas 2009) was applied using three variants of this technique. Two of these variants estimate Fr productivity by summing the increases in Fr biomass between sampling dates and by only using data of Fr biomass (Publicover and Vogt 1993). For the "sequential core" productivity estimates, we used all the positive increments between sampling dates in a more liberal estimation, while for the "significant differences in sequential core" productivity estimates we used only the statistically significant increments (ANOVA / LSD means) in a more conservative approach (Milchunas 2009). For the third variant, i.e. the "sequential core of all-roots", we used total (biomass + necromass) Fr mass data. This last variant was applied to compare poplar Fr with data from weed roots where no sorting in biomass and necromass was done.
3. The "decision matrix" method (Fairley and Alexander 1985) calculates productivity, mortality and disappearance of Fr between consecutive sampling dates using data of Fr biomass and necromass.
4. The "compartment flow" method (Santantonio and Grace 1987) uses a pool and flux approach. The method defines two pools, i.e. biomass and necromass. Productivity, mortality and decomposition are the flows. As root decomposition was not measured in the current study, an annual dead root decomposition rate of 50% was estimated for the (poplar) necromass based on studies from the region (Kalhe et al. 2007; Silver and Miya 2001). This is a rough assumption, as Fr productivity may equal decomposition at times of no change in the pool of live biomass and necromass.

Medium (L3) and coarse (L4) roots are highly variable in the soil, and therefore it is not recommended to estimate their biomass by core sampling (Levillain et al. 2011). Since no distinction between live and dead root mass could be made for weed roots, total root productivity of weeds was calculated in two ways: (i) by subtracting the annual minimum weed root mass from the annual maximum (comparable with the max-min method referred to above); and (ii) by summing all the positive differences in total weed root mass between sampling dates (comparable with the sequential core of all-roots method referred

to above). The approaches used for weed roots were also applied for poplar root mass, and are presented as variants of "all-roots", including biomass and necromass without distinction (L1+L2+D). In all methods, we used the average of root mass over both wide and narrow rows (n=20 per sampling date) weighted by the proportion of the ground area occupied. Additionally, poplar Fr productivity was calculated for wide and narrow rows separately, and subsequently averaged taking into account the proportional area occupied by the wide and the narrow rows. In the last case, the root productivity was calculated for each row using a smaller number of samples (n=10) and then averaged. All the root productivity and mortality estimates calculated for each sampling date were summed and expressed in g DM m^{-2} $year^{-1}$. The cumulative root productivity over the year was converted to C using the measured Fr C mass fraction.

Fine root turnover rate is defined as the rate at which the roots are being renewed every year (Vogt and Bloomfield 1991). Several approaches have already been proposed to estimate root turnover rate in mature (and/or "steady state") ecosystems (Gill and Jackson 2000). However, it remains an issue how to estimate Fr turnover in a dynamic, growing ecosystem. We calculated Fr turnover rate for each diameter class (L1 and L2) using two equations:

$$\frac{root\ productivity}{mean\ root\ biomass} = root\ turnover\ rate_{\ (mean)} \qquad \text{[Eq. 1]}$$

$$\frac{root\ productivity}{mean\ root\ biomass} = root\ turnover\ rate_{\ (mean)} \qquad \text{[Eq. 2]}$$

Both equations are used in the literature (Brunner et al. 2013), but *a priori*, Eq. 1 may over-estimate root turnover rate while Eq. 2 may under-estimate root turnover rate.

3.2.4. Statistical analysis

Analysis of variance (ANOVA) was used to test for differences in Fr biomass between genotypes (Skado *vs*. Koster) and between rows (wide *vs.* narrow), as well as to test for differences in C concentration between the six root classes/categories (W, D, L1, L2, L3, L4). Genotype, row (wide *vs.* narrow) and root class were considered as the main factors in the ANOVA. A two-way ANOVA tested differences in root biomass between genotypes and between rows using sampling date as a co-variate. Another two-way ANOVA was run to compare differences in C concentration between genotypes and between root classes. Differences were considered significant at $P \leq 0.05$. Differences in root productivity between rows, between genotypes and between plant communities (poplar *vs.* weeds) were examined by a simple comparison of the estimated values, since no replicate estimates could be made to assess their uncertainties.

3.3. Results

At the end of the growing season, total (above + below-ground) standing biomass was 1130 g DM m^{-2} and 1700 g DM m^{-2} for Koster and Skado, respectively (Broeckx et al 2012a). Net primary production (NPP; above- + below-ground) was estimated at 800 g DM m^{-2} y^{-1} and 1400 g DM m^{-2} y^{-1} for Koster and Skado, respectively (Verlinden et al. 2013c).

3.3.1. Poplar fine root biomass

Total root biomass varied during the course of the year (Figure 3.2). Total root biomass, averaged over (narrow and wide) rows and months, was 19 ±8 g DM m^{-2} for both genotypes in winter (Feb.-Mar.) *vs.* 69 ±7 g DM m^{-2} (genotype Skado) and 140 ±30 g DM m^{-2} (genotype Koster) at the end of the growing season (Oct.-Nov.). Fine root biomass (<2 mm) in Nov. accounted for 38-47 g DM m^{-2}, nearly 60% of total root biomass sampled (Figure 3.3). The two genotypes differed significantly in both total and Fr biomass. Peaks in total root biomass (Figure 3.2) were due to the occasional presence of coarse roots (>5 mm) in the samples (Figure 3.3). Nevertheless, there was a consistent increase of Fr biomass over the course of the year.

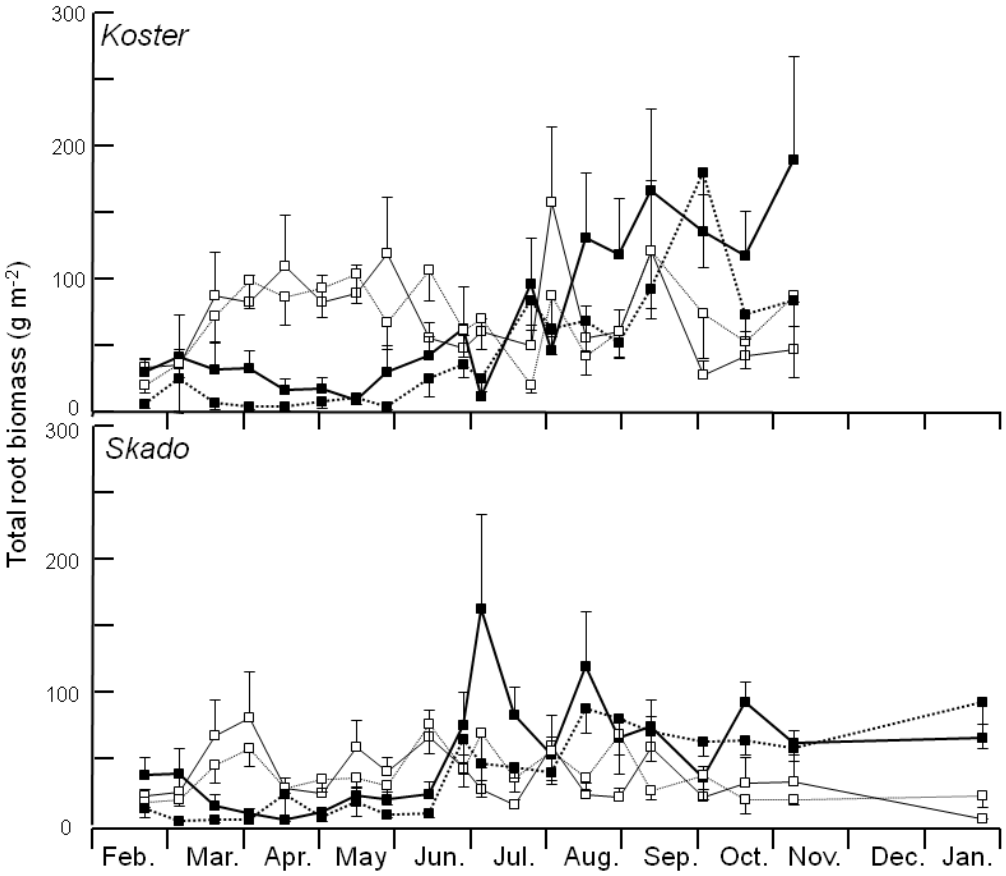


Figure 3.2: Seasonal evolution (2011) of the total root mass from poplars (filled symbols) and weeds (open symbols) in narrow (solid line) and wide rows (dotted line) for genotypes Koster (top panel) and Skado (lower panel). Each point represents the mean of ca. 10 samples. Bars above the mean represent the standard error for samples in the narrow rows, and bars below the mean data point for samples in the wide rows. An extra root sampling in Jan. 2012 was included for genotype Skado.

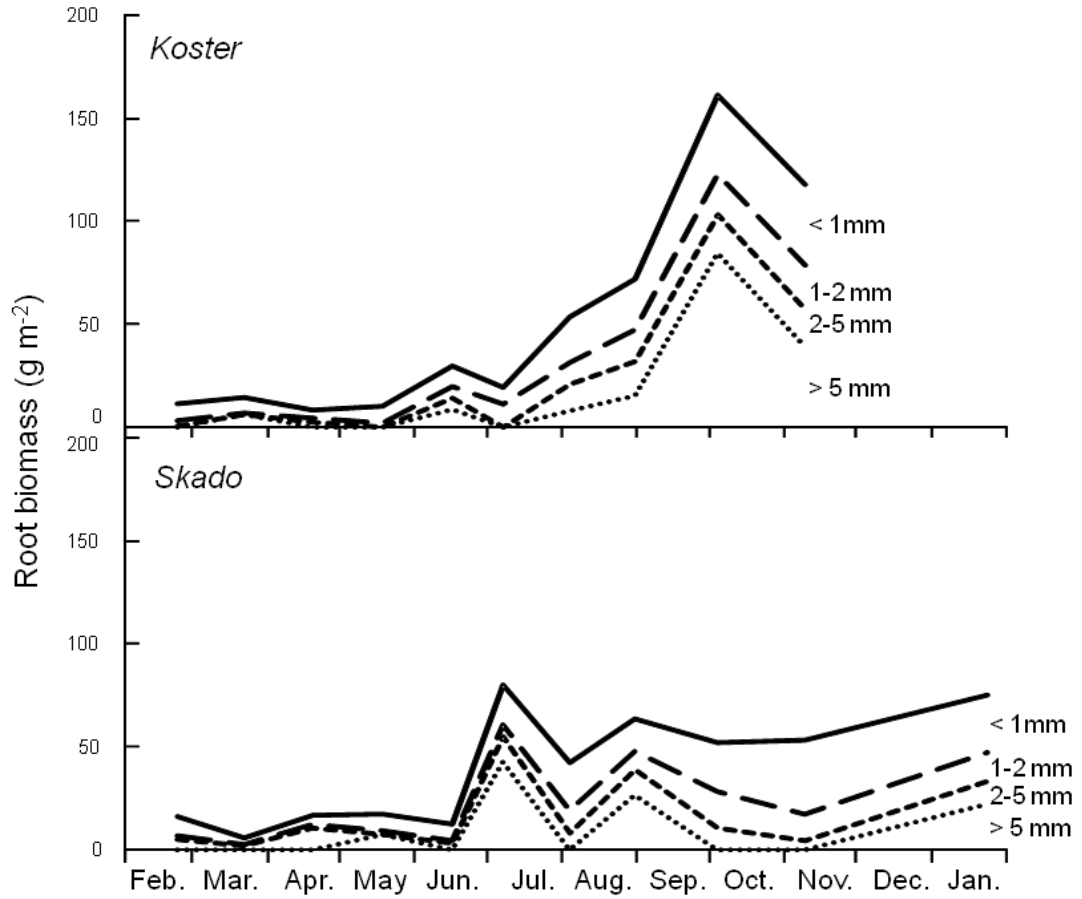


Figure 3.3: Seasonal evolution (2011) of the root mass for different root diameter classes of poplar roots for genotypes Koster (top panel) and Skado (lower panel). Each line represents the mean evolution of 20 values. An extra root sampling in Jan. 2012 was included for genotype Skado.

Fine roots represented 2.2% of the total standing biomass in Skado *vs.* 4.1% in Koster, thus representing a higher proportion for the genotype with the lower standing biomass. On average, Fr biomass (<2 mm, L1+L2), represented 60% of the total root mass; live medium-size and coarse roots (L3+L4) represented 33% (Figure 3.3), while dead roots accounted for only a minor proportion (6%) of the total root mass (data not shown). The C concentration was lowest (36 % of C) in the finest root category (<1 mm), without significant differences between necromass and biomass. No significant differences in root C concentration were found between genotypes (Table 3.2).

Table 3.1: Statistical results of the two-ways analysis of variance on the effect of the factors genotypes and rows on poplar total root mass, Fr mass and weed root mass. Sampling date was used as a co-variate. Genotypes: Skado and Koster; row: narrow and wide; root classes: W=weed roots, D=dead roots (necromass), L1=<1 mm, L2= 1-2 mm, L3= 2-5 mm, L4=>5 mm. Fr= fine roots; p= level of significance; F= F-value.

Factor	Total root biomass		Fr biomass		Weed root biomass	
	F	p	F	p	F	p
Genotype	5.1	0.024	5.0	0.026	44.6	<0.0001
Row	12.0	0.001	2.6	0.107	0.0	0.986
Genotype x Row	0.6	0.424	1.5	0.219	0.0	0.894
(Sampling date)	101.4	<0.0001	198.2	<0.0001	0.5	0.503

Table 3.2: Statistical results of the two-ways analysis of variance on the effect of the factors genotypes and root class on carbon concentration, as well as results of the Tukey t-test. Sampling date was used as a co-variate. Genotypes: Skado and Koster; row: narrow and wide; root class: W=weed roots, D=dead roots (necromass), L1=<1 mm, L2= 1-2 mm, L3= 2-5 mm, L4=>5 mm; p= level of significance. Significant differences in C% between root classes are followed by different letters

	carbon (%)	
	F	P
Genotype	0.002	0.966
Root class	61.8	<0.0001
Genotype x root class	2.7	0.021
(Sampling date)	0.0004	0.985

Root class	*n*	carbon (%)	
W	97	28.2	a
D	45	35.5	b
L1	92	36.6	b
L4	12	40.3	c
L3	35	40.7	c
L2	54	41.7	c

For both genotypes total root biomass was significantly higher in the narrow rows than in the wide rows (Table 3.1). Even when the sampling date was used as a covariate, total root biomass was higher in the narrow rows than in the wide rows for both genotypes. However, Fr biomass was significantly higher in the narrow rows compared to the wide rows, only in genotype Skado. The distance from the nearest tree was not a significant term in the regression models (data not shown). Average bulk density in the upper 15 cm soil layer was significantly lower ($p < 0.05$) in the narrow than in the wide rows: 1.48 (±0.04) g cm^{-3} *vs.* 1.56 (±0.05) g cm^{-3}, respectively.

3.3.2. Weed root biomass

In the first months of sampling (Feb.-June), weed root biomass was five times larger than that of poplar roots (Figure 3.2). In summer, the ratio of weed to poplar root mass was reversed when poplars became dominant. Weed root biomass was two times higher under Koster than under Skado throughout the entire growing season. Despite the higher poplar root biomass in the narrow rows, weed roots were widely distributed over the entire field, with no significant differences between rows (Table 3.1). Weed roots accounted for ca. 50-100 g DM m^{-2} and remained constant throughout the growing season (Figure 3.2). On average, C concentration of weed roots was lower than that of poplar roots (Table 3.2).

3.3.3. Fine root productivity and turnover rate

Estimates of Fr productivity and turnover differed according to the method of calculation (Table 3.2). Averaging over genotype, poplar Fr productivity (L1+L2) was lowest using the 'significant differences in sequential core' method (21.6 g DM m^{-2} y^{-1}), followed by the 'max-min' method (41.2 g DM m^{-2} y^{-1}), the 'sequential core' method (46.8 g DM m^{-2} y^{-1}), the 'sequential core of all-roots' (47.8 g DM m^{-2} y^{-1}), the 'decision matrix' method (51.4 g DM m^{-2} y^{-1}) and the 'compartment flow' method (53.2 g DM m^{-2} y^{-1}). Since it was based on Fr productivity estimates, the same ranking was obtained for Fr turnover rates. This ranking of methodological estimates was quite consistent across root diameter classes and genotypes. Averaged across methods, Fr productivity of both genotypes was nearly identical, i.e. 43.7 g DM m^{-2} y^{-1} *vs.* 43.6 g DM m^{-2} y^{-1} for genotypes Koster and Skado,

respectively. In both genotypes, 68% of the annual productivity of Fr was accounted for by the finest root class (<1 mm; L1).

Root turnover rate can be estimated from observations of the median root lifespan or from the ratio of the Fr productivity to biomass. Our estimated turnover rate for roots of <2 mm was 1.8 to 3.4 y^{-1} using the mean Fr biomass, and 0.3 - 1.4 y^{-1} using the maximum Fr biomass. Consequently the Fr turnover rate was between 2.3 and 3.9 times higher using the mean of the Fr biomass than using the annual maximum Fr biomass. Overall, Fr <2 mm diameter lived approximately 3 to 9 months depending on the estimated Fr turnover rate. Using the 'sequential core of all-roots', 'decision matrix' or 'compartment flow' methods, the calculated turnover was slightly higher (2-6 % higher) in very Fr (<1 mm; L1) than in Fr (1-2 mm; L2), while it was (9 to 75%) lower when using the 'max-min' and the two other 'sequential core' methods.

Similar to poplar, values of weed root productivity differed according to the method used for the calculation (Table 3.4). Averaging weed root mass across poplar genotypes, weed root productivity was lower using the 'max-min' method (70.5 g DM m^{-2} y^{-1}) than with the 'sequential core' method (127.1 g DM m^{-2} y^{-1}). This ranking was consistent with the ranking found for the poplar root estimates. When all methods were averaged, weed root productivity was 50% higher under genotype Koster (i.e. 120.7 g DM m^{-2} y^{-1}) than under genotype Skado (77.0 g DM m^{-2} y^{-1}). Weed roots had lower C concentrations than poplar roots (Table 3.2), but their production exceeded at least two times the poplar Fr productivity (Table 3.4). Considering that on an annual basis the Fr production is an input to the soil (turnover rate >1 year, Table 3), the total root C input to the soil was on average 17.0 g C m^{-2} y^{-1} for the poplar trees, and 44.8 g C m^{-2} y^{-1} for the weeds.

Table 3.3: Fine root productivity and turnover rate of two root diameter classes from two poplar genotypes during their second year of growth estimated using different methodological approaches: "significant differences in sequential core" (sequential core (sign.)), "max-min", "sequential core" (sequential core (live roots)), "sequential core of all-roots" (sequential core (all-roots)), "decision matrix" and "compartment flow". The results of the different methods were ranked from left to right in ascending order of productivity estimation. The dashed line divides the methods that occasionally provide unrealistic results (to the left) from the methods with more realistic estimations (to the right). DM= dry mass.

		Weeds		Poplar	
		Max-min (all-roots)	Sequential core (all-roots)	Max-min (all-roots)	Sequential core (all-roots)
Production	(g DM m^{-2} y^{-1})				
Koster		109.5	156.0	46.0	48.7
Skado		72.1	98.3	37.7	46.8
Turnover	(y^{-1})				
Koster		1.5	2.1	2.2	2.3
Skado		1.8	2.4	2.0	2.5

Table 3.4: Root productivity and root turnover rate of two poplar genotypes and of weeds estimated using two different approaches: "max-min" (max-min (all-roots)) and "sequential core of all-roots" (sequential core (all-roots)). Root production was calculated using the total fine root mass (live + dead). DM= dry mass.

			Sequential core (sign.)	Max-min	Sequential core (live roots)	Sequential core (all-roots)	Decision matrix	Compartment flow
Production	(g DM m^{-2} y^{-1})							
Koster	<1 mm (L1)		23.5	27.6	29.0	31.9	33.2	33.8
	1-2 mm (L2)		0.0	16.2	16.8	16.8	16.8	16.9
	Total		23.5	43.7	45.8	48.7	50.0	50.7
Skado	<1 mm (L1)		11.4	25.5	30.9	30.0	37.5	39.7
	1-2 mm (L2)		8.3	13.1	16.8	16.8	15.4	16.0
	Total		19.7	38.6	47.8	46.8	52.9	55.7
Turnover	(y^{-1})							
Koster	<1 mm (L1)	mean	1.8	2.1	2.2	2.4	2.5	2.6
		max	0.8	0.9	1.0	1.0	1.1	1.1
	1-2 mm (L2)	mean	0.0	2.3	2.4	1.3	2.4	2.4
		max	0.5	0.8	1.0	0.6	0.9	1.0
Skado	<1 mm (L1)	mean	1.0	2.2	2.6	2.3	3.2	3.4
		max	0.4	0.9	1.1	1.0	1.3	1.4
	1-2 mm (L2)	mean	1.7	2.7	3.4	1.3	3.1	3.2
		max	0.3	1.0	0.6	0.6	1.2	0.6

Poplar Fr production differed between narrow and wide rows (Table 3.5). When averaging all methods, Fr productivity was 25% higher in the narrow rows. But when the max-min method was used, wide rows were 10% more productive than the narrow rows. Averaging over wide and narrow rows, Fr productivity was 46% higher than the estimates obtained with the two respective methods, "max-min" and "sequential core-all-roots" (Table 3.3).

Table 3.5: Fine root productivity in narrow and wide rows for two poplar genotypes during their second year of growth estimated using different methodological approaches: "max-min", "sequential core" (sequential core (live roots)), "decision matrix" and "compartment flow". Root productivity from root diameter classes <1 mm (L1) and 1-2 mm (L2) were summed. Values were ranked from left to right in ascending order of productivity estimation. The dashed line divides the methods that occasionally provide unrealistic results (to the left) from the methods with more realistic estimations (to the right).

		Max-min	Sequential core (live roots)	Decision matrix	Compartment flow
Production	(g DM m^{-2} y^{-1})				
Koster	Narrow rows (L1+L2)	54.4	77.3	90.3	91.0
	Wide rows (L1+L2)	63.9	72.1	73.8	76.4
	Average	60.1	73.1	78.5	80.4
Skado	Narrow rows (L1+L2)	51.3	93.4	89.0	89.0
	Wide rows (L1+L2)	52.2	46.0	84.7	71.4
	Average	51.4	61.2	85.3	76.5

3.4. Discussion

3.4.1. Poplar root biomass

We observed a constant increase in Fr and in total root biomass during the year, with significant differences between genotypes. The active Fr growth started in June-July, possibly in response to an increase in precipitation (Fig.1 and Figure 3.2). The increasing Fr production continued until Oct., which is longer than the production of the aboveground biomass. This could indicate a shift in carbon allocation from aboveground biomass to belowground biomass towards the end of the growing season (Scarascia-Mugnozza 1991; Dickmann & Pregitzer 1992). Root growth generally continues longer than shoot growth, even after leaf abscission (Lyr & Hoffmann 1967; Cannell & Willett 1976). That root growth is favored over shoot growth after the growing season as has been previously reported for mature forests (Burke & Raynal 1994) and young poplar plantations (Heilman et al. 1994).

The productivity and the proportion of total biomass allocated to Fr were consistent with other studies across a broad range of species and ages. Fine roots represented 2.2% of total (above- + belowground) biomass in Skado and 4.1% in Koster. Curiel Yuste et al. (2005) found that Fr accounted for 1.6% of total biomass in mature pines, and 2.1% for a 70-year-old oak stand in Belgium. In a nine-years old poplar SRWC plantation in Belgium, genotypic differences in Fr biomass (in the upper 15 cm of the soil) ranged between 25 and 44 g DM m^{-2} (Al Afas et al. 2008). In a two-year-old poplar plantation in the USA, Fr biomass (<1 mm) ranged from 25 to 65 g DM m^{-2} with higher values for nitrogen rich soils (Pregitzer et al. 2000). On the other hand, in a nutrient gradient experiment carried out in a deciduous forest, it was found that low soil nutrient levels resulted in a high biomass allocation to Fr to increase nutrient uptake (Tateno et al. 2004). The high Fr biomass in our plantation could be explained by the fertile soil and adequate water supply driving high tree productivity (Broeckx et al. 2012a). Trees were still in the early, exponential phase of stand development and growing with no apparent limitation of nutrients or water.

In a double-row plantation, samples taken in narrow rows and in wide rows have different mean root mass and different standard deviation. Therefore, the samples have to be considered as belonging to different statistical populations, and each data set has to be processed separately. We hypothesized that tree Fr biomass would be higher in the narrow rows as compared to the wider rows, and that the proximity of the trees would explain these differences. In general, total root biomass was significantly higher in the narrow rows, but not for Fr biomass (diameter < 2 mm). Soil properties around an individual tree are normally affected by the distance to the tree stem (Zinke 1962). Based on our random sampling, we did not find an effect of the distance from the nearest tree on Fr biomass (data not shown). A methodological experiment carried out on six-year-old *Eucalyptus* trees found an effect of tree size on Fr biomass in samples taken with augers, but the authors did not find an effect of the distance to the nearest tree (Levillain et al. 2011). However, we observed that total root biomass was lower in the wide rows, especially at the beginning of the growing season. It appears that roots preferentially explored the planting

row where soil compaction was lower (Laclau et al. 2004), due to less traffic from tractors and other machinery. Later in the growing season these differences in root biomass between rows were lower. This could have been due to avoidance of competition in the narrow rows where the trees were closer and root abundance was already high.

Fine roots have commonly been defined as roots with a diameter less than 2 mm (category L1 + L2) (Persson 1980; Vogt et al. 1981; Janssens et al. 2002). This is a simplification that implies that all roots within this Fr category have a similar or a comparable function. However, in many cases it has been shown that a high proportion of the "fine root" class is occupied by roots finer than 1 mm diameter (Bauhus and Messier 1999; King et al. 2002; Pinno et al. 2010) and only these very Fr (<1 mm; category L1) are highly dynamic during the growing season (Santantonio and Santantonio 1987). Our results confirmed that there was more root mass in the very Fr class (<1 mm, L1; Figure 3.3) and that these very Fr were more productive than those of the larger diameter classes (Table 3.3).

3.4.2. Weed root biomass

Some of the samples collected in the current study contained only weed roots and no tree roots at all, in particular where trees were further apart from one another. However, weed roots were spatially homogeneously distributed over the field site over the entire growing season. Higher weed root biomass under Koster might be explained by the fact that there was more light transmitted to the herbaceous canopy under this smaller (aboveground) genotype as compared to Skado. Genotype Koster also had lower maximum leaf area index and later leaf phenology than genotype Skado (unpublished data, and see Broeckx et al. 2012b). In ecosystem studies on roots, it is necessary to separate live roots of different plant species/genotypes because they may have asynchronous phenology, which could lead to errors when estimating root productivity based on sequential differences in root biomass.

In crops or in SRWC plantations, associated annual plants are traditionally considered pests and not a valuable product, perhaps explaining why weed root production is so rarely reported. Weeds are usually considered a negative factor in poplar and SRWC plantations (Pinno and Belanger 2009). However, annual plants do have an important function within the agro-ecosystem. For example, the high density of weed roots in the topsoil could drastically reduce soil erosion (De Baets et al. 2007) in periods when poplar roots are less abundant. Moreover, weed root mass growing during the dormant period of the poplars can help to decrease the nutrient leaching during winter (McLenaghen et al. 1996; Wyland et al. 1996). Here we quantified root biomass of the entire weed community (multiple species), but we did not characterize interspecific differences in root biomass, root spatial distributions, or competition strategies that may be important components of weed communities (Kabba et al. 2007). Annual weeds may thus have an impact on the establishment of the poplar trees (Kabba et al. 2007) and on their productivity (Otto et al. 2010; Pinno and Belanger 2009; Welham et al. 2007), but they also play a relevant ecological role.

3.4.3. Fine root production and turnover rate

The developmental stage of trees influences Fr productivity and root turnover. In mature forests, Fr productivity has been reported to range between 50-520 g DM m^{-2} y^{-1} (Pinno et al. 2010; Steele et al. 1997), and in young tree plantations between 60-420 g DM m^{-2} y^{-1} (Block 2004; Lukac et al. 2003). When young and mature plantations were compared in the same study (Block 2004), Fr productivity was lower in the younger plantation. In our plantation, we estimated a Fr productivity of approximately 53 g DM m^{-2} y^{-1}. Fine roots represented 3.9% and 6.3% of net primary productivity (NPP) for Skado and Koster, respectively, which is much less than the 10% reported for a mature broadleaf deciduous forest (Curiel-Yuste et al. 2005). Despite genetic and above-ground NPP differences between both genotypes, they did not differ in Fr productivity. This may be relevant for plant ecological research and for genetic selection (Dickmann et al. 2001). For example, differences in the belowground versus aboveground allocation are relevant for the adaptation/selection of specific genotypes to different soil types, or for early rooting, etc. (Crow and Houston 2004).

Our estimates of Fr turnover are in the same order of magnitude as those reported for two-year-old hybrid poplars derived from ratios of Fr productivity to mean annual Fr biomass, that is, between 1.9 y^{-1} and 2.7 y^{-1} (Block 2004). Using maximum Fr biomass, turnover rate for Fr in an SRWC plantation in Italy ranged from 1.1 – 1.4 y^{-1} (Lukac et al. 2003). These rates, obtained through different methods, confirm that multiple root cohorts can be produced during one growing season. However, they also suggested that the value of the Fr turnover rate depends on the methodology applied. The turnover rates reported in these studies imply fine *Populus* root longevities of 4 to 11 months, consistent with the broader literature (Pregitzer et al. 2000; Block 2004).

By quantifying both tree and weed root production, data from the current study support the hypothesis that soil carbon inputs due to weed roots may be equal to or exceed those due to poplar Fr. This occurred despite the fact that we used operational levels of weed control to facilitate plantation establishment. This finding is important because it confirms the importance of accounting for root production of associated annual plants when calculating ecosystem C balances of SRWC or other tree crop plantations, especially during the early phases of the plantation. In agro-ecosystems, aboveground C input from weeds has been reported to range between 150 and 2500 kg ha^{-1} (Alvarez et al. 2011; Poudel et al. 2002). This weed biomass also needs to be included in agro-ecosystem carbon balances (Alvarez et al. 2011).

3.4.4. Method comparison

The aim of the present study was not to compare different methodologies for estimating Fr productivity, but to better understand plant root dynamics and quantify Fr turnover rates in a fast-growing SRWC by using a combination of several methods (e.g. those proposed by Burke and Raynal 1994; Levillain et al. 2011; Steele et al. 1997). Our Fr biomass

productivity estimates obtained via four different methods were within the range of the values reported for other poplar plantations (Block 2004). The lowest estimates were obtained with the most restrictive method; in one specific case this method even yielded a production rate of zero. We therefore recommend caution when using only statistically significant differences for the calculation of productivity using the sequential coring technique. Methods with obviously meaningless values should not be used: for instance when negative or zero productivity values are obtained in a system with a clear increase in root biomass (Milchunas 2009). Among the methods used here, the higher estimates were obtained with the compartment flow method, an approach that has been highly recommended (Publicover and Vogt 1993). In general, the calculation of Fr productivity does not include carbon remobilization from senescent roots to live roots, nor the growth of Fr into a larger size classes, or losses due to herbivory (Hunter 2008). However, these processes have been considered insignificant compared to the large error of estimations attributable to the method of calculation itself (Publicover and Vogt 1993).

The sum of root biomass and necromass resulted in higher productivity estimates than using the live root biomass only. In the literature, Fr productivity is often calculated with the max-min and the sequential core methods, using only live root biomass (Burke and Raynal 1994; Publicover and Vogt 1993; Trumbore et al. 2006). Apparently, not sorting the roots into live and dead roots produced better productivity estimates than using live root biomass only. For example, in a hypothetical situation where there were no differences in live root biomass measured between sampling dates, zero root production would be estimated. But, in the same situation with a consistent increase in necromass, total root mass (biomass + necromass) would result in an estimation of root production. These results illustrate the usefulness of sorting Fr into live and dead categories when the max-min or the sequential core methods are applied.

Fine root productivity estimates were higher in the narrow rows than in the wide rows (Table 3.5) and, on average, they were higher than when the calculation was not done for each row independently (cfr. the results presented in Table 3.1). This higher estimation of the narrow and wide rows together was a mathematical artifact of the calculation procedure. When the root productivity was estimated for each row, the number of samples was halved and consequently the deviation of the data for each row increased. In the methods that do not focus on the significant differences there is a higher probability to report biomass differences between sampling dates if the deviation is larger at each sampling date. Therefore, a higher root productivity was estimated when the number of samples was reduced.

On top of the different calculation approaches, also methodological artifacts could affect the results: (i) differences in the sorting into the various root classes between the persons involved in the sample processing; (ii) small mineral particles attached to the roots even after washing; (iii) live roots could be mistakenly sorted as dead roots as a result of freezing damages; (iv) the sampling interval could be so long that root productivity is underestimated (Publicover and Vogt 1993). Other sources of error can be caused by the

tools used; for example, core augering is not well-suited to estimating coarse root biomass (>10 mm) (Levillain et al. 2011; Rodrigues de Sousa and Gehring 2010).

The present study focused on the top layer (15 cm) of the soil only and at specific times of the growing season. Root biomass tends to decrease with depth, with most Fr occurring in the upper 15 cm of the soil (Jackson et al. 1996; Janssens et al. 2002). In addition, Fr (their biomass, diameter, plant species, etc.) changes over the year, and differently for surface and deep soil horizons (Burke and Raynal 1994; Janssens et al. 2002; Santantonio and Santantonio 1987). Therefore, it is recommended that root sampling design takes into account root distributions and phenology, and be done at frequent enough intervals to capture temporal dynamics. Although the present study focused on a short-time period after plantation establishment, it is the most critical period of land use change from agriculture into SRWC. A characterization of the effects in early as well as in later stages of plantation development is needed to fully parameterize ecosystem models needed to scale effects of bioenergy cropping on C cycling across the landscape and in response to changes in resource availability and climate.

3.5. Conclusions

We found that annual soil carbon inputs from root production and turnover of annual weeds far exceeded those from the poplar trees during the early stages of land conversion from agriculture to SRWC bioenergy cropping. Further, tree Fr biomass was higher in the narrow rows as compared to the wider rows when a double-row planting system was used, but weed root biomass was uniformly distributed. Genotypic differences between *Populus* clones were expressed in terms of standing Fr biomass, but not in annual root productivity, which could have ecological and management implications. More research is needed to fully examine the potential of the genus *Populus* under SRWC for bioenergy to offset rising atmospheric CO_2, but care must be taken to characterize all parts of the system, including weeds.

Chapter 4

4. Effect of harvesting on soil carbon inputs

Based on:
Comparative analysis of harvesting machines on an operational high-density short rotation woody crop (SRWC) culture: one-process versus two-process harvest operation.
G. Berhongaray, O. El Kasmioui and R. Ceulemans.
Biomass and Bioenergy (2013) 58, 333-342.

Abstract:
Short rotation woody crops (SRWC) are being studied and cultivated because of their potential for bioenergy production. The harvest operation represents the highest input cost for these short rotation woody crops. We evaluated three different harvesting machines representing two harvesting systems at one operational large-scale SRWC plantation. On average, 8 ton ha^{-1} of biomass was harvested. The cut-and-chip harvesters were faster than the whole stem harvester; and the self-propelled harvester was faster than the tractor-pulled. Harvesting costs differed among the harvesting machines used and ranged from 388 € ha^{-1} to 541 € ha^{-1}. The realized stem cutting heights were 15.46 cm and 16.00 cm for the tractor-pulled stem harvester and the self-propelled cut-and-chip harvester respectively, although a cutting height of 10 cm was requested in advance. From the potential harvestable biomass, only 77.4% was harvested by the self-propelled cut-and-chip harvester, while 94.5% was harvested by the tractor-pulled stem harvester. An increase of the machinery use efficiency (i.e. harvest losses, cost) is necessary to reduce costs and increase the competitiveness of biomass with other energy sources.

Key words: biomass harvesting; harvesting efficiency; harvesting looses; cost; New Holland SRC harvester; Ny vraa; Stemster;

4.1. Introduction

Within the framework of the production of bioenergy from fast-growing trees, various aspects have already been studied and documented over the past decennia: importance of species and genotypes to be used (Halford and Karp 2011; Willebrand et al. 1993); impact of coppicing in short rotation cultures (Dallemand et al. 2008; Dillen et al. 2010); length of (coppice) rotation cycle (Al Afas et al. 2008; Herve and Ceulemans 1996); interaction between soil type and genotype (Broeckx et al. 2012b). Theoretical studies and practical field experiments have led to the introduction of bioenergy plantations in several regions of the world. To bring the concept of the culture of bioenergy from the experimental to the commercial scale, efforts have been made toward a further mechanization of the culture: mechanical planting, weed management (Welham et al. 2007), nutrient and herbicide applications, irrigation (Ibrahim et al. 1998; Linder and Rook 1984) and harvesting (Felker et al. 1999; Hannum 2009). For most of the management operations existing agricultural techniques have been modified and applied. In a short rotation biomass culture agricultural management approaches are being applied to woody crops. Since the main difference between agricultural crops and woody biomass crops is in the harvest of the crop, progress on the mechanization of the harvesting process has been slow thus far (Dallemand et al. 2008; Jossart 1994).

Although different harvesting machines have already been developed, mainly two different harvesting approaches have been developed for short rotation woody crops (SRWC), i.e. the harvest-and-chip system (Spinelli et al. 2009) and the harvest-and-storage system (Schweier and Becker 2012) (Figure 4.1). The harvest-and-chip system can be performed with a self-propelled cut-and-chip front harvester or with a tractor-pulled cut-and-chip side harvester. In most cases the self-propelled cut-and-chip front harvester is a converted corn harvester with a specific coppice header for SRWCs. In both cases chips are produced from wet stems, collected in an attached trailer or an additional tractor-trailer combination, and stored as wet chips. The storage of wet chips implicates a risk of dry matter looses, and further drying might be necessary. In the harvest-and-storage system, wet stems are cut, transported to a storage location to dry, and chipped afterwards to obtain dry chips. The storage of cut stems, also called 'rods', avoids the problems with wet chips. The expected productivity of the self-propelled cut-and-chip front harvester is 35.6 Mg of fresh biomass per scheduled machine hour, and 19 Mg for the harvest-and-storage system, but with similar operational costs (Schweier and Becker 2012; Spinelli et al. 2009). The lower the moisture content of the obtained chips, the higher the calorific values for energy conversion. An overview of additional advantages and disadvantages of each system can be found in earlier studies (Schweier and Becker 2012; Spinelli et al. 2009).

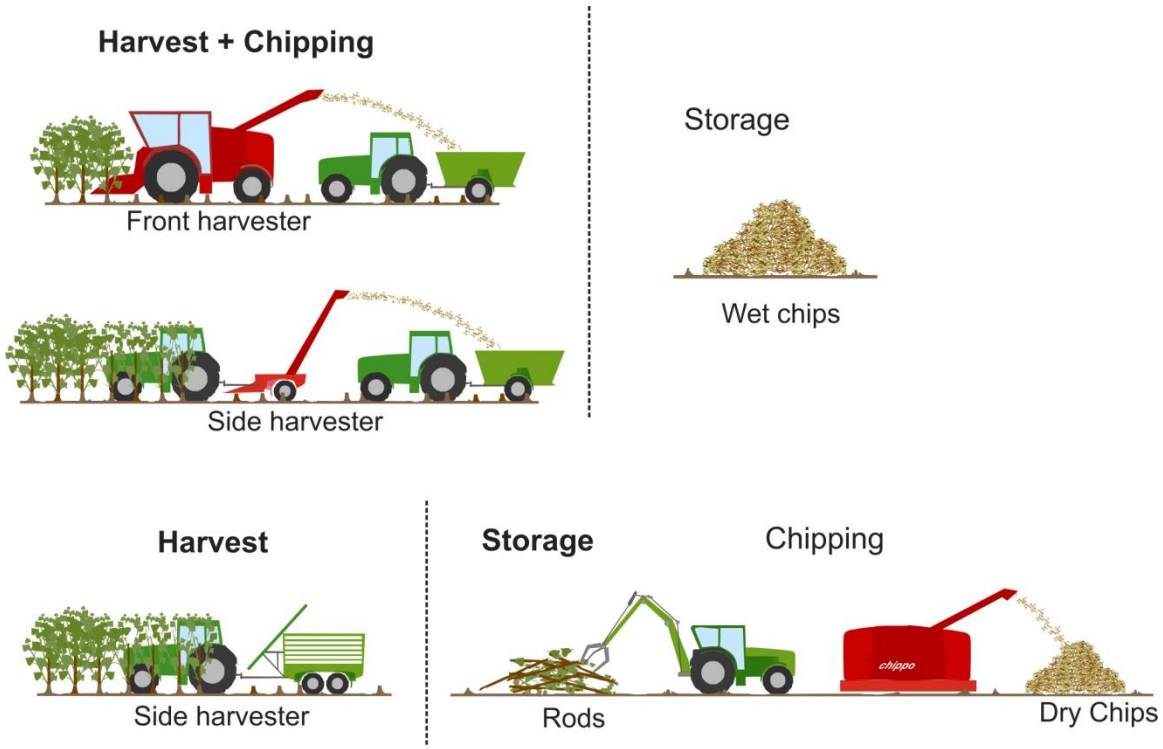


Figure 4.1: Representation of the harvest-and-chip and the harvest-and-storage systems. The harvest-and-chip system can be performed with a self-propelled cut-and-chip front harvesting machine or with a tractor-pulled cut-and chip side harvesting machine. In both cases the final product are wet chips. The harvest-and-storage system is operated using a tractor-pulled whole stem harvester. In this harvest system the final product could be dry chips at sizes and moisture as demanded.

Machinery costs represent the highest input costs for biomass production (Silveira 2005 cited in Hannum 2009). Consequently, harvesting costs make up a large share of the total costs of biomass produced from SRWCs and might amount up to 45% of the total cultivation costs (El Kasmioui and Ceulemans 2012). This is due to the fact that harvesting is mostly subcontracted by the farmer, as a harvesting machine is excessively expensive to be owned and used by a single farmer. Typical harvest rates (excluding transportation costs) charged by Belgian and Danish subcontractors range from 400 € ha^{-1} for a tractor-pulled stem harvester, over 600 € ha^{-1} for a tractor-pulled cut-and-chip harvester to 950 € ha^{-1} for a self-propelled cut-and-chip harvester (El Kasmioui and Ceulemans 2012).

The present study extends previous analysis by: (i) evaluating three different harvesting machines representing two harvesting systems at the same plantation; (ii) assessing the efficiency and performance of these harvesters on a field plantation at an operational scale; and (iii) discussing the economic potential, advantages and disadvantages of the different harvesters and harvesting systems.

We have been operating and intensively monitoring an operational bioenergy plantation with fast-growing poplar and willow trees in Flanders, Belgium (see http://uahost.uantwerpen.be/popfull) since three years. The plantation was harvested after the first two-year rotation cycle. In this paper we compare and report on the performance of the three harvesting machines that were used to harvest this large-scale SRWC plantation.

4.2. Materials and Methods

4.2.1. Description of the site

The field site is located in Lochristi, Belgium (51°06'N, 03°51'E) and consists of a high-density poplar and willow plantation (POPFULL project; Chapter 1). Lochristi is located 11 km from Ghent in the province of East-Flanders. After initial soil sampling and site preparation, 12 poplar (*Populus sp*.) and 3 willow (*Salix sp*.) genotypes were planted in monoclonal blocks in a double-row planting scheme on 7-10 Apr. 2010 with a commercial leek planter (Broeckx et al. 2012b). The distance between the narrow rows was 75 cm and that of the wide rows was 150 cm. The distance between trees within a row was 110 cm, yielding an overall density of 8000 trees per ha. The total length of individual rows ranged from 45 m up to more than 325 m. An area of 14.5 ha was planted on a total of 18.4 of former agricultural (pasture and crop) land. Manual and chemical weed control was applied during the first and the second year. Neither fertilization nor irrigation was applied during the entire lifetime of the plantation thus far. A detailed description of the site, the plantation lay-out, the soil conditions and the planted materials have been published previously (Broeckx et al. 2012b).

4.2.2. Harvest operation and harvesting equipment

On 2-3 Feb. 2012 – i.e. after a first rotation cycle of two years – the entire plantation was harvested. For this harvest three different harvesting machines were used: (1) a self-propelled cut-and-chip harvester of New Holland (available in Belgium), (2) a tractor-pulled cut-and-chip harvester of Ny Vraa (transported from Denmark), and (3) a tractor-pulled whole stem harvester of Nordic Biomass (transported from Denmark) (Figure 4.1). The first harvester is a front-operated single-pass cut-and-chip harvester of New Holland, consisting of a forage harvester (type: FR9090) and a coppice header (type: 130 FB). This harvester is mostly accompanied by an additional tractor-trailer combination to collect the biomass chips, as it was in our case. The second harvester is a side-operating and tractor-pulled single pass cut-and-chip harvester, consisting of a tractor (type: JD 6920) equipped with a harvesting implement of Ny Vraa (type: JF Z200) and – if desired – with an attached trailer to collect (and automatically unload) the chips. In our case, this harvester was accompanied by an additional, separate tractor-trailer combination to collect the chips, instead of an attached trailer (Figure 4.2). The third harvester is a side-operated tractor-pulled stem harvester of Nordic Biomass that consists of a tractor (type: JD 8520T) and an (inseparable) harvest-trailer combination (type: Stemster MKIII). This harvester does not need an accompanying tractor with trailer (Figure 4.3). The three different harvesting systems are schematically represented in Figure 4.1; their technical characteristics and financial information sheets are summarized in Table 4.1. The technical characteristics (weight, biomass storage, required power, etc.) as well as the sales prices of the tractor-pulled cut-and-chip harvester and the stem harvester were taken from the technical documentation available on the official website of the manufacturing companies, Ny Vraa and Nordic Biomass, respectively (Bioenergy 2011; Biomass 2010) completed with

information acquired from personal communications with the managers of both companies (Table 4.1). The characteristics of the self-propelled cut-and-chip harvester were obtained from personal communication with Xavier Desmyter, who owns and operates the described harvester, and from a study by De Dobbelaere (2011).

Table 4.1: Technical and financial specifications of the three harvesting machines that were compared in this study. Specifications are based on the information provided by the manufacturers unless otherwise indicated. Source: for Stemster http://www.nordicbiomass.dk; for Ny Vraa http://www.nyvraa.dk; for New Holland De Dobbelaere (2011) and http://www.newholland.com.

Harvester/coppice head (type) Tractor/basis machine (type)	Stemster MKIII JD 6920	130 FB FR9090	JF Z200-HYDRO/E JD 8520T
Manufacturer harvester (company, country)	Nordic Biomass, Denmark	New Holland, Belgium	Ny Vraa, Denmark
Manufacturer tractor (company, country)	John Deere, USA	New Holland, Belgium	John Deere, USA
Principle of operation	Whole-stem harvester	Cut-and-chip	Cut-and-chip
Weight harvester (Mg)	7	13.1	1.5
Weight tractor (Mg)	6	n/a	6
Maximum harvestable diameter (cm)	15-20	10-15	4-6
Biomass storage capacity (Mg)	4.5	n/a	n/a
Cost of purchase (€)	175,000 (tractor) 215,000 (harvester)	350,000 (forage harvester) 85,000 – 90,000 (coppice head)	125,000 (tractor) 46,000 (harvester)
Horsepower (HP)	150	768	255

Figure 4.2: View of the tractor-pulled cut-and-chip harvester operating at the short rotation woody crop operating on willows.

Figure 4.3: View of the tractor-pulled whole stem harvester (on the left) and the self-propelled cut-and-chip harvester with the trailer-tractor combination (on the right) operating at the same short rotation woody crop poplar plantation.

The three harvesting machines harvested different parts of the plantation. The self-propelled cut-and-chip harvester harvested approx. 7 ha, while the tractor-pulled cut-and-chip harvester and stem harvester harvested 1 ha and 6.5 ha, respectively. Professionally skilled and experienced drivers operated the harvesting machines during the harvest. Before harvesting we had requested a cutting height of 7-10 cm above soil level to all 'operators'. A schematic representation of which parts of the plantation were harvested by each harvesting machine is shown in Figure 4.4.

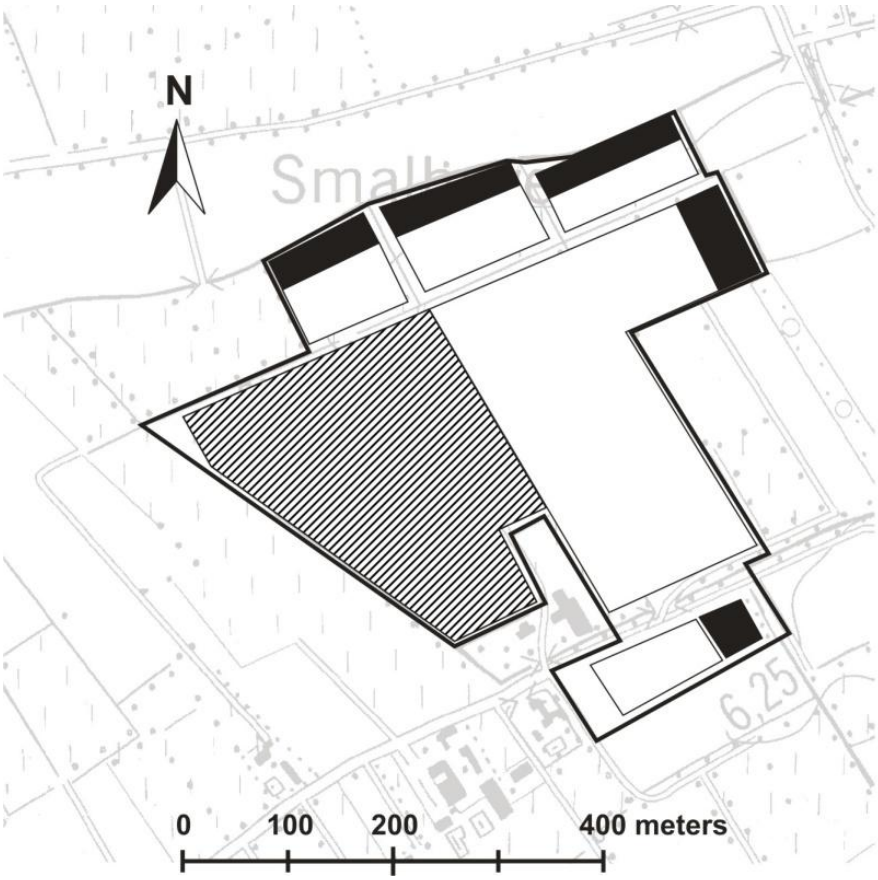


Figure 4.4: Lay-out of the short rotation woody crop plantation and harvested areas per harvesting machine. Black areas = willows area, harvested by the tractor-pulled cut-and-chip side harvester; hatched area = poplars area harvested by the tractor-pulled whole stem harvester; white area = poplars area harvested by the self-propelled cut-and-chip front harvester.

4.2.3. Data collection during the harvesting operating

The harvesting rate of each harvester was calculated by dividing the recorded total duration of the harvest of each harvesting machine by the actually harvested surface area by the machine. The tractor-pulled stem harvester harvested shorter rows and had to turn more than the self-propelled cut-and-chip harvester, giving the last mentioned harvesting machine a competitive advantage in terms of harvesting rate. The stem harvester, however, was not able to harvest the long rows, as it was only able to collect rods from rows up to 200 m of length, before the storage capacity was reached. The plantation existed of several rows up to 300 m. The tractor-pulled stem harvester is only able to harvest such long rows if it is accompanied by a shuttle wagon which collects the harvested stems when the attached trailer is full before finishing the row. The tractor-pulled cut-and-chip harvester only harvested part of the willows at the plantation, as it was not able to harvest (poplar) trees with a diameter larger than 4-6 cm (see plantation lay-out, Figure 4.4).

4.2.4. Cost analysis

To calculate the hourly costs of using the machinery for the harvest we used the guidelines of the American Agricultural Economics Association (AAEA) (AAEA 2000). These costs were divided into operating and ownership costs. The operating costs include maintenance, fuel, lubrication, and labor costs. The ownership costs include the depreciation costs, the opportunity costs associated with the financial capital invested in the assets and other costs such as property taxes, housing and insurance.

The fuel consumption by the different harvesters and by the tractor-trailer combination was recorded during the harvest (Table 4.2). We calculated the fuel costs, using a diesel price of 0.95 € l^{-1}, which was the official fuel price for agricultural use in Sep. 2012 in Belgium (Economie 2012). For the remuneration of the machine operators we used the average Belgian hourly labor cost of 35 € h^{-1} (Eurostat 2011). Due to the transport of the harvesting machine to the field site and the time required to lubricate and service the machines, the actual hours of labor generally exceed the field machine time (AAEA 2000; Edwards 2009). Therefore, we multiplied the hourly labor cost by 1.1 to calculate the labor costs required for the different harvest operations, as previously suggested by Edwards (2009) and as applied by Smeets et al. (2009) and El Kasmioui and Ceulemans (2012).

Table 4.2: Overview of the costs and characteristics of the equipment (harvesting machine, tractor, trailer) used for the harvest of the short rotation woody crop plantation of this study. HP: horse power; n/a: not applicable

Equipment	Purchase price (k€)	Annual use (h y^{-1})	Life-time (y)	Maintenance costs (€ h^{-1})	Lubricant use (€ h^{-1})	Salvage value (k€)	Fuel use (l h^{-1})	Operating rate (h ha^{-1})	Operating and ownership costs exc. labor (€ h^{-1})	Harvest costs inc. labor (€ ha^{-1})	Combined tractor
Tractor – 150 HP	125	800	12	8.4	0.242	31.3	n/a	n/a	25.8		n/a
Tractor – 255 HP	175	800	12	11.8	0.397	43.8	n/a	n/a	36.2		n/a
Ny Vraa	46	500	8	15.9	n/a	9.7	30	1.7	83.6	387.7	150 HP
Nordic Biomass	215	500	8	74.1	n/a	45.3	24	2	195.7	540.9	255 HP
New Holland	437.5	500	8	52.5	1.233	92.2	33	1.3	212.5	464.1	n/a
Trailer – 40 m^3	44	800	10	15.6	n/a	7.8	20	n/a	41.7		150 HP

The salvage values, required to compute the depreciation and opportunity costs, were calculated as a percentage of the purchase price based on the calculation methodology suggested by Bowers (1994), mentioned by the AAEA (2000) (Table 4.2). We assumed an (economic) lifetime of 8 years for the harvesters, of 10 years for the trailer and of 12 years for the tractor. Given the limited land area of SRWCs in Belgium (and its neighboring countries) we assumed a moderate annual use of 500 h y^{-1}, which corresponds to an annual harvestable area between 250 and 380 ha, depending on the operation rate. We assumed a higher annual utilization for the tractor and the trailer, however, as this equipment can be used for other agricultural purposes than the harvest of SRWCs.

The depreciation and opportunity costs were calculated using the capital recovery formula, which annualizes these two components together. This method amortizes the original costs of the asset (i.e. purchase price) less the present value of the salvage value over its lifetime to calculate the annual capital service cost (CSC) (AAEA 2000)

$$CSC = \frac{PP - \frac{SV}{(1+r)^n}}{\frac{1 - \frac{1}{(1+r)^n}}{r}} \qquad \text{[Eq.1]}$$

where *PP* is the purchase price of the machines (€), *SV* is the salvage value (€), *r* is the discount rate, and *n* is the lifetime of the equipment in years. The discount rate used in the calculations equaled 4 % y^{-1}. Data on housing costs, property taxes and insurance vary widely from country to country and from farm to farm. We therefore calculated these costs as a percentage of the purchase price as suggested by the AAEA (2000). The AAEA suggested adding an annual cost of 2% of the purchase price to the CSC to calculate the annual ownership costs.

4.2.5. Data collection after the harvest

Harvest losses were estimated from samples collected at the field site after the harvest, i.e. early Mar. 2012. These losses were only estimated in the area of the field site planted with poplar for reasons of comparison. In order to control the variability caused by different species and genotypes, looses were only measured in two poplar genotypes: i.e. Koster and Skado. Those genotypes were chosen because they are genetically and phenotypically contrasting and represented the range of productivity for the entire plantation (see Broeckx et al. 2012 for more details of the genotypes). Woody stem biomass that was supposed to have been harvested, but remained on the field was considered as harvest losses. Two types of harvest losses were considered: (i) uncut biomass (UB) due to a different realized cutting height than the requested cutting height of 7-10 cm; and (ii) cut, but not recovered biomass (NRB) (Monti et al. 2009).

To estimate the UB, 20 stumps were selected randomly on the areas harvested by the two harvesting machines, and the height of the remaining stump from the soil surface was measured with a simple ruler (accuracy 1 mm). We considered a height of 10 cm above the soil surface as the upper threshold. The biomass present between the 10 cm threshold and the realized cutting height was estimated using the stump height and the bulk density of the stump biomass. For the bulk density estimation 20 stumps of different diameters (from 20 mm to 60 mm) were manually cut by a handsaw in the field. The height and the diameter of the cut portion of the stump was measured with a digital caliper (accuracy 0.01 mm), and weighted with a precision balance (accuracy 0.01 g) after oven drying at 70°C.

The stump diameter and weigh, for the bulk density estimation, were measured including the bark. Stump bulk density was estimated from the dry mass (DM) and the volume of the cylinder estimated from stump height and diameter. A linear allometric equation was established linking bulk density to stump diameter. Using data of a diameter inventory of the entire plantation reported previously (Verlinden et al. 2012) and the allometric equation, an estimation of the average biomass per centimeter of stump height was made for the harvested field area. The estimated UB above the highest threshold (10 cm) was considered as biomass loss. Although the biomass cut below the lower threshold (7 cm) is a gain in the biomass yield, it was not considered as harvested biomass. Harvesting below the 7 cm was avoided because of the potentially negative impact on the resprouting (Ledin and Alriksson 1992).

To estimate the NRB, harvested woody debris and woody biomass material were collected from the soil surface on four areas of 1 m^2 within the land area harvested by each harvesting machine on the two genotypes (Skado and Koster). The collected biomass material and debris were brought to the laboratory and dried in a drying oven at 60-70^{0}C until constant weight. The NRB losses were expressed in g DM m^{-2}. Differences between harvesting machines were tested for the UB and the NRB with a one-way analysis of variance (ANOVA) and a Tukey post-hoc test ($p=0.05$).

For the self-propelled cut-and-chip harvester we also performed a more refined analysis. The NRB was classified in stem and branches at one hand, and in woody chips on the other hand. The cut stem and branch biomass laying on the soil was considered as collection loss, i.e. the woody stem was cut, but the harvesting machine failed to collect the woody biomass to transport it into the chipping system of the machine. Biomass chips remaining on the soil after harvest were considered as a transfer loss from the harvester to the additional tractor-trailer combination (Figure 4.1). For the tractor-pulled stem harvester only cut stems and branches were measured in the field.

The harvesting efficiency (Eff) of the harvesting machine was calculated as follows:

$$\text{Eff}\ (\%) = (\text{Potential harvestable biomass} - \text{NRB} - \text{UB})/\text{Potential harvestable biomass} \quad [\text{Eq.2}]$$

where potential harvestable biomass is the standing biomass above 7 cm at harvest. This potential harvestable biomass yield was calculated using the allometric equations previously developed and reported (Broeckx et al. 2012a). For these equations, 120 two-year-old trees were harvested by a handsaw in Dec. 2011, before the mechanical harvest. The stumps were cut at 7 cm stem height, as this value was considered the lowest harvestable threshold by the harvesting machine. Potential harvestable biomass, NRB and UB were all expressed in g DM m^{-2}. Although we acknowledge that some water may remain in the biomass when it is dried at 70 °C, all the DM was obtained with the same methodology.

4.2.6. Data collection at the onset of the next rotation

After the harvest on 2-3 Feb. 2012, the stumps started resprouting and produced new shoots from the end of Mar. 2012 onward. Stump mortality was assessed in July 2012 – i.e. five months after the harvest – to evaluate the possible impact of the (two) harvesting machines on the resprouting success (i.e. coppice ability) of the poplars. The number of missing stumps in at least one complete single row per monoclonal block (i.e. between 70 and 330 stumps per row) were counted. A total of 34 rows and 4927 stumps were surveyed (aprox. 2500 per harvesting machine). Stump mortality rates were calculated as the percentage (%) of dead stumps in relation to the number of stumps that were alive before the harvest. These latter ones were available from the detailed counting of Summer 2011. We assumed that missing or dead stumps – since the counting in 2011 – were due to the harvesting operations. An overall mortality rate was calculated by combining all genotypes. A T-test was applied to evaluate whether the differences in the percentage of dead stumps were statistically different between the harvesting machines.

4.3. Results and Discussion

4.3.1. Harvest yield

After two years of growth approximately 230 Mg of (fresh) woody chips were harvested from the 14.5 ha planted with trees. The potential harvestable biomass calculated with the allometric relationship equation ranged from 468 g DM m^{-2} to 1167 g DM m^{-2}.The mean dry mass yield was 8 Mg ha^{-1} for the two-year rotation, which was lower than the average values reported for SRWCs under European conditions (Don et al. 2012). However productivity values of the first rotation period are generally lower than for subsequent rotations due to the early establishment from unrooted cuttings and the initial root development (Deraedt and Ceulemans 1998). The moisture content on a wet basis of the freshly harvested biomass was 50 %. The chemical composition of the harvested SRWC chips from our plantation were reported earlier (Njakou Djomo et al. 2012).

4.3.2. Harvesting cost and machine productivities

In this analysis we calculated the ownership and operation costs for the different harvesters, including labor costs, to estimate the (hourly) cost to own and operate the studied harvesters. Table 4.2 provides an overview of the calculated ownership and operation costs for the three harvesters and the accompanying tractor-trailer combination based on data collected from the harvest of our plantation. Table 4.2 also includes the productivity in tons per hour for each harvester. One should, however, take into account that this study was conducted on the first rotation of a very low-yield plantation (with a dry mass yield of approximately 4 Mg ha^{-1} y^{-1}). Therefore caution is required if the results are extrapolated to other sites or conditions. This caution also applies for the harvesting costs per oven-dried ton (odt) harvested biomass reported in the next paragraph.

The ownership and operation costs of the tractor-pulled cut-and-chip harvester of Ny Vraa – without considering the tractor-trailer combination to collect the chips – amounted to 83.6 € h^{-1}, excluding labor costs. This equaled a total harvesting cost, including the tractor-trailer combination and labor costs, of 387.7 € ha^{-1} or 48.5 € odt^{-1}, considering a yearly biomass increment of 4 odt ha^{-1} $year^{-1}$ and a rotation of two years. For the self-propelled cut-and-chip harvester of New Holland the ownership and operation costs equaled 212.5 € h^{-1}, whereas the harvesting costs amounted to 464.1 € ha^{-1} or 58.0 € odt^{-1}. For both the tractor-pulled cut-and-chip harvester and the self-propelled cut-and-chip harvester, the large differences between the hourly operation costs and the overall harvesting costs were due to the fact that these harvesting systems required an additional tractor-trailer combination (and driver) to collect the chips. Equipping these harvesters with an attached (and specially designed) trailer, however, would most probably decrease the total harvesting costs considerably. Unfortunately, a cost assessment of these scenarios was not possible, as these harvesters were not equipped with an attached trailer during the harvest at our operational plantation. So we were unable to record data regarding fuel consumptions and operation rates for these scenarios. The ownership and operation costs of the tractor-pulled stem harvester of Nordic Biomass amounted to 195.7 € h^{-1}, whereas the harvesting costs were 540.9 € ha^{-1} or 67.6 € odt^{-1}. Although the tractor-pulled stem harvester did not require an additional tractor-trailer combination (and driver) as the stems were collected in the machine's storage space, the total harvesting costs of this harvester were higher than the other two harvesters. This is mainly due to the high operation rate of the tractor-pulled stem harvester (Table 4.2). It is, however, important to mention that the stem harvester and the chip harvesters produce completely different products. Therefore, the harvesting costs of the stem harvester could not be straightforwardly compared with the other harvesters. The rods produced by the stem harvester still need to be chipped to deliver the same final product (i.e. woody biomass chips), which incurs additional costs. According to recent literature (El Kasmioui and Ceulemans 2012; Schweier and Becker 2012), post-harvest chipping costs vary between 15 and 20 € odt^{-1}, making the harvest and storage system even more expensive if woody biomass chips are to be delivered. At the POPFULL plantation approximately 95.4 Mg of fresh biomass (50 % moisture content on wet basis) was chipped at a total costs of 1.035 €, corresponding to a cost of 21.68 € odt^{-1}. In spite of its financial drawbacks, this harvesting system has the advantage to let the biomass air-dry on the field (no need for extra storage space) until it reaches the required moisture content before chipping the material. This increases the quality of the biomass delivered and as a consequence the price of the biomass chips. At our plantation, however, the rods were chipped on site right after harvesting.

4.3.3. Efficiency of the harvesting machines

The harvest loss analysis was done without including the tractor-pulled cut-and-chip harvester, because this harvester was not able to harvest the larger (poplar) trees. In Dec. 2011 the mean stem diameter (measured at a height of 22 cm) was 40.8 mm (± 0.16, n=4928) for poplars and 24.3 mm (± 0.42, n=289) for willows. Although a cutting height of

7-10 cm had been requested at the start of the harvest, the realized stem cutting height was 15.46 cm and 16.00 cm for the tractor-pulled stem harvester and the self-propelled cut-and-chip harvester, respectively (Table 4.4). As a result, an average of 5.5 cm and 6.0 cm of woody stem – per individual harvested stem – was lost as it remained on the field. None of the harvesting machines cut below the lower threshold (7 cm). No statistically significant differences were found between the two harvesting machines, but the tractor-pulled stem harvester had a more variable cut height than the self-propelled cut-and-chip harvester (Figure 4.5). Based on the established allometric relations, the UB averaged 37.2 g DM m^{-2}. This value was much lower than the UB reported for switchgrass, which accounted for 400 g DM m^{-2} (Monti et al. 2009). On average 6.5 g DM m^{-2} (i.e. 65 kg ha^{-1}) of biomass was lost for every centimeter of stem height that we harvested above the threshold height in our two-year-old trees. The attainable cutting height should be minimal to harvest as much material as possible. The lower the cutting height, however, the more contamination with soil particles among the wood chips might occur.

Table 4.3: Potential harvestable biomass and not recovered biomass (NRB) of two harvesting machines. Observations on two genotypes (Skado and Koster) and two former land-use types (cropland and pasture) after the harvesting campaign at the short rotation woody crop plantation field site. C=cropland, P=pasture.

Harvesting machine	Genotype	Former land-use type	Potential harvestable biomass (g m^{-2})	*n*	NRB (g m^{-2})	(%)
Self-propelled cut-and-chip harvester	Skado	C	1167	4	322.8	27.7%
	Skado	P	982	4	105.3	10.7%
Tractor-pulled whole stem harvester	Skado	P	982	4	35.3	3.6%
	Koster	C	468	4	14.3	3.0%
	Koster	P	657	4	2.1	0.3%

Table 4.4: Comparative results of the performance of two harvesting machines based on the observations after the harvesting campaign at the short rotation woody crop plantation field site. Data only refer to poplar.

Harvesting machine	Tractor-pulled whole stem harvester	Self-propelled cut-and-chip harvester	Approach, Source
Mortality after harvest (%)	0.68	0.54	Observations 5 months after harvest
Not recovered biomass (g DM m^{-2})	35.3	105.3	Left-overs quantified at field site on the same clone (Skado)
Harvesting height (cm)	15.46	16.00	Measured at field site
Efficiency (%)	93.4	68.7	Potentially harvestable biomass, uncut biomass and not recovered biomass

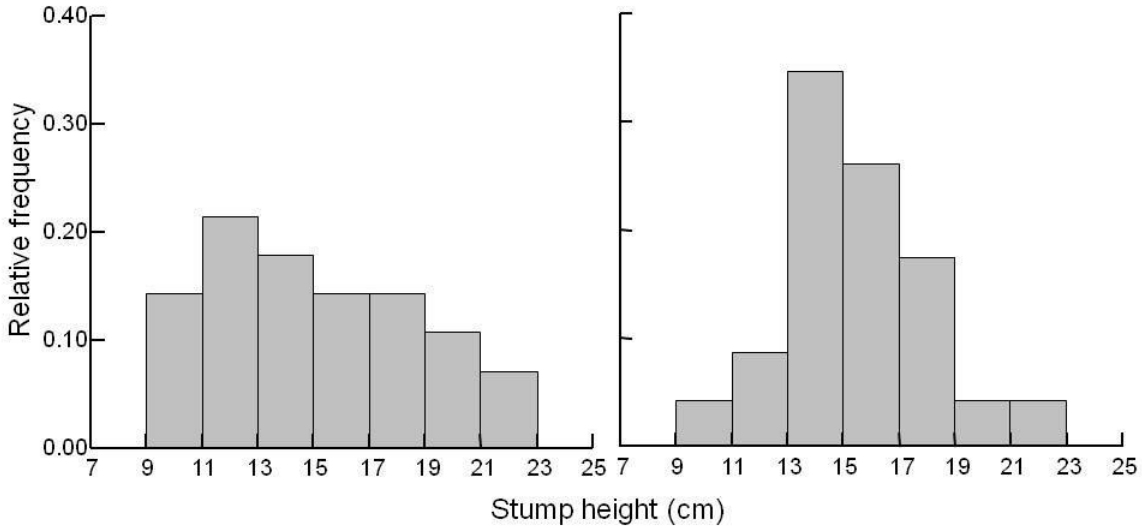


Figure 4.5: Relative frequency of the stump height above the soil (cutting height) for the tractor-pulled whole stem harvester (left panel) and the self-propelled cut-and-chip harvester (right panel).

On average, losses by NRB accounted for 17.2 g DM m^{-2} for the tractor-pulled stem harvester versus 214.0 g DM m^{-2} for the self-propelled cut-and-chip harvester (Table 4.3). In the self-propelled cut-and-chip harvester, NRB losses consisted of 97.2 g DM m^{-2} front losses of cut biomass that the machine failed to chip, and 116.8 g DM m^{-2} of biomass chips lost during the transfer from the harvester to the tractor-trailer combination. In analogy with grain crops, front losses are linked to the design of the cutting table and the mode of operation of the harvester (Klinner and Biggar 1972). The high front losses found in the self-propelled harvesting machine could be due to the relatively low harvesting or operating rate of the harvesting machine during the operation (Table 4.2). There might also be chip losses during the chipping of the rods harvested by the stem harvester. But this chipping process can be operated on a concrete floor and the lost chips recovered afterwards.

Considering all the losses, only 77.4% of the potentially harvestable biomass was harvested on average by the self-propelled cut-and-chip harvester, while the tractor-pulled stem harvester collected 94.5% of the potentially harvestable biomass (Table 4). In terms of losses, the UB accounted for ca. 3.6% of the biomass for both harvesting machines. Under the same conditions (genotype: Skado and land: pasture), the NRB differed between both harvesting machines; it accounted for 3.6% and 10.7% for the tractor-pulled stem harvester and the self-propelled cut-and-chip harvesting machine, respectively. There was not a clear relation between potential harvestable biomass and NRB (Table 4.3). As far as we know, losses after harvest of SRWC poplars and willows have never been carefully quantified or assessed. A harvest efficiency of 64% of the potentially harvestable biomass has been reported for switchgrass (Monti et al. 2009). As machinery costs – and harvest machinery in particular – represent the highest input costs for biomass production (Silveira (2005) cited in Hannum 2009) the harvest efficiency should be increased to reduce overall costs and increase the competition of biomass with other energy sources.

The overall mortality rate, expressed as the percentage (%) of dead stumps, after harvesting was very low (i.e. less than 1%) as shown by the successful resprouts (Table 4). A T-test showed that differences between both harvesting machines were not significant ($P < 0.05$). High reductions in the number of stems produced due to mechanical damage have been reported for willow plantations, but damaged plants compensated by producing larger stems (Souch et al. 2004). In our study, mechanical damage was not a major problem for the resprouting success.

A number of additional pro's and con's could be considered when selecting the appropriate harvesting system or machine for the harvest of a SRWC (Table 4.5). The side harvesting machine requires a pre-designed plantation scheme (Figure 4.6), as it needs an empty row or a previously cut row where the tractor can drive. In contrast, a front harvesting machine can start the harvest operation in any row of the plantation. The stem harvester was not able to harvest the long rows before the storage capacity was reached; for rows with a length of more than 200 m a cut-and-chip harvester was needed. According to the manufacturer, this machine is also able to harvest longer rows if accompanied by a shuttle wagon. Although we did not quantify the differential impact of the harvesters on the soil, a recent comparative study showed that various forest harvesters had a different impact on soil compaction and changed soil density accordingly (Ampoorter et al. 2012; Souch et al. 2004). Lighter machines with wide tire dimensions are recommended to decrease soil contact pressure. Most of the advantages and disadvantages of the operated machines are summarized in Table 4.5.

Table 4.5: General comparison of the three studied harvesting systems

	Self-propelled cut-and-chip harvester	Tractor-pulled cut-and-chip harvester	Tractor-pulled whole stem harvester
Collection of biomass	Additional tractor-trailer combination required	Additional tractor-trailer combination required - Trailer attached to the same tractor in option	Trailer attached to the same tractor
Compaction of the soil	High (if not frozen)	Low (if on tracks)	Moderate (if on tracks)
Maximum diameter (cm)	15	4-6	15-20
Final product	Biomass chips (10-45 mm)	Biomass chips (5-30 mm)	Whole stems/rods (additional chipping required)
Availability in Belgium	Available	Not available	Not available
Storage capacity	Dependent on the trailer	Dependent on the trailer	Max. 5 Mg
Access to the field	Able to harvest any plantation design	Pre-designed plantation scheme required	Pre-designed plantation scheme required

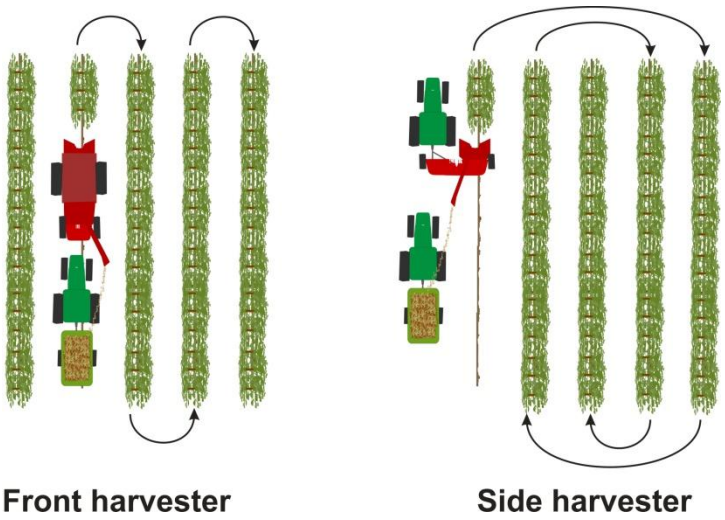


Figure 4.6: Representation of the turnings for a front harvesting machine and for a side harvesting machine. The front harvesting machine can start to harvest in any row of the plantation and turn to any row. The side harvest machine needs an empty row or a harvested row where the tractor pulling the machine can drive. This results in longer turnings.

Given a number of limitations of our study, caution is required if the results are extrapolated to other sites or conditions. Firstly, this study was conducted on the first rotation of a very low-yield plantation. Secondly, we did not specifically design the study for the harvest test. However, very few studies have been conducted on a comparison of different commercial harvesters at a plantation of this size (14.5 ha).

4.4. Conclusion

In conclusion, this study confirmed that harvesting machines have their specific advantages and disadvantages. The harvesting machines that we evaluated differed in their operational cost (e.g. one-step operation vs. two-steps operation), their harvest capacity (i.e. stem diameter, row length), their harvest efficiency (i.e. losses) and the final product (chips or rods). In the selection of the appropriate harvesting machine, speed performance should be the second priority; the first priorities should be the success of the resprout, the efficiency of the harvesting process and the quality of the final product. To minimize the impact on the soil light-weighted machines are to be preferred.

Chapter 5

5. Assessment of coarse roots and fine root distribution

Based on:
Below- *versus* aboveground biomass in two *Populus* genotypes in a short rotation coppice plantation: architecture, genotypic differences and root profiles
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(Manuscript in preparation)

Abstract
Few studies have examined the belowground components of poplars (*Populus* spp.) in high-density, short-rotation biomass plantations. We were particularly interested in the root architecture in relation to biomass allocation patterns of two genotypes. The root system of 20 selected trees from genotypes Skado and Koster were excavated for coarse (Cr) and medium-sized (Mr) roots determination. The soil coring technique was used to determine fine root (Fr; Ø <2 mm) mass at different soil depths. Allometric equations were fitted between Cr and Mr and stem diameter. The highest Fr biomass was detected in the upper 15 cm of the soil and no genotypic differences were detected at any soil depth. After harvesting we found a reduction of the weed root biomass, which was explained by the higher canopy closure. The Cr biomass was higher in Skado (135.6 g DM m^{-2}) than in Koster (113.4 g DM m^{-2}). The root:shoot ratio decreased exponentially with stem diameter. A similar below-ground architecture was found for genotypes that significantly differed in aboveground crown architecture. Maximum root depth was correlated with water table depth. Both genotypes showed a relatively shallow, but extensive root system. We found no mirroring of above- and belowground structures.

Keywords: coarse roots; fine roots; root:shoot; allometry; topology; biomass allocation

5.1. Introduction

Fast-growing trees as poplars (*Populus* spp.) are intensively studied, in particular because of the potential use of their biomass for renewable energy production. In a short rotation woody crop (SRWC) culture poplars are harvested every two to five years and the produced woody biomass is converted into bio-energy. Several ecological, physiological and genetic aspects of SRWC have been examined to further improve biomass yield (Dickmann et al. 2001; King et al. 1999; Laureysens et al. 2005). Within this framework there is a particular interest in selecting individuals that prioritize allocation of biomass to harvestable and economically valuable organs (i.e. stems, branches), which implies a reduced allocation of biomass to roots. Although the belowground parts are crucial for woody biomass production, there are disproportionally few studies on these tree organs.

Any root system consists of roots of different sizes and with different functions. The larger or coarse roots (Ø >5 mm) contribute to tree stability (Stokes 2000); they provide a network for the transport of water, nutrients and metabolic compounds (Coutts 1987), and they act as a storage organ during dormant periods (Schulze et al. 2005a). Coarse roots are frequently subdivided in different medium-sized root classes (Ø = 2-5 mm) with similar functions. However, the most intimate contact with the soil – essential for the uptake of water and nutrients– is realized by the many, very active fine roots (Ø < 2 mm) (Schulze et al. 2005b). The same amount of carbon allocated belowground may produce different root systems, with a varying relative contribution of the different root diameter classes (Janssens et al. 2002). The distribution of roots over the soil profile confers different ecological properties to the plant and to the soil (Jackson et al. 1996; Jobbágy and Jackson 2000). Large trees with deep root systems experience less effect of drought than smaller trees as the deep roots can provide water from deeper soil layers (Duursma et al. 2011). But the impact of water deficit on root growth is not straightforward. Some studies observed an increase in carbon allocation to roots with a decrease in water availability (Guo et al. 2010; Yin et al. 2005; Zhang et al. 2004), whereas others found no effect (Souch and Stephens 1998) or even a decrease in the allocation to roots (Dickmann et al. 1996). In comparison to the effect of water deficit, the effects of excess soil water on belowground biomass are well understood. In soils with a permanently high water table trees develop shallow root systems (Rewald et al. 2011). Roots and their distribution with soil depth contribute to water (Nosetto et al. 2005) and nutrient (Jobbágy and Jackson 2003) cycling in the soil profile as well as to carbon sequestration in the deep soil (Jobbágy and Jackson 2000). Root distribution highly determines the erodability of the top soil (De Baets et al. 2007), and represents an important component of the ecosystem carbon and nutrient storage and cycles (Jackson et al. 1997; Jayawickreme et al. 2011). Moreover, the vegetation can be more important for the control of water and nutrient cycles than abiotic factors (Hobbie 1992).

Genotypic differences in aboveground tree growth and/or in the plasticity of crown morphology in relation to changes in light availability and competition are relatively well understood (Benomar et al. 2012; Nelson et al. 1981; Wu and Hinckley 2001). The

belowground root distribution and architecture might be related to the aboveground differences in growth or crown morphology. Despite the considerable number of theoretical and modelling studies on belowground architecture (e.g. Berntson 1997; Crawford and Young 1990; Fitter et al. 1991), very few empirical or experimental studies addressed the relation between belowground root architecture and aboveground crown structure. This relationship between below-ground and aboveground tree parts has been mostly studied in terms of biomass ratios and generally expressed in the so called root:shoot ratio. This root:shoot ratio is genotype dependent in *Populus* (King et al. 1999) and in other tree genera. However, the explanation for those genotypic differences in root:shoot ratio remains unclear. Functional equilibrium, metabolic control and the hormonal signal of the root-shoot communication have been suggested as explanations, but none of these mechanisms explains the variation in poplar (Friend et al. 1994). Coordinated studies of the above- and belowground processes are important to understand the whole-tree physiology (Neuman 1993).

We were particularly interested in aspects of root architecture that are related to different carbon allocation patterns of contrasting *Populus* genotypes. Within this context our hypotheses were: (i) root:shoot ratio is independent to tree size (larger trees have a bigger root system), between individuals of the same populations or across different *Populus* genotypes; (ii) allocation to fine roots is dependent to the tree size (genotypes with larger trees produce more fine roots); (iii) the soil water table is a strong determinant of the root:shoot ratio by limiting the rooting depth; and (iv) above- and belowground tree architecture are correlated (e.g. more branches aboveground correspond to more below-ground branching). The and the answers to these four hypotheses are discussed in the context of a higher soil resource use efficiency and of the avoidance of competition for below-ground resources.

5.2. Materials and Methods

5.2.1. Experimental site

The experimental site was the POPFULL field described in Chapter 1. Just to refresh the memory, the distance between tree rows was alternating 75 cm (narrow inter-rows) and 150 cm (wide inter-rows). The spacing between trees within a row was 110 cm, yielding an overall theoretical tree density of 8000 trees per ha. One year after of the planting, an overall average mortality of 18.2 % was observed on the plantation (Broeckx et al. 2012a). Re-planting with one-year old unrooted plantlets reduced the mortality to a plantation average of 15 %.

Water table depth was monitored on a monthly basis in seven water tubes spread across the field site using a 2 m tape-measure. As nutrients and water were not limiting at the site (Broeckx et al. 2012a), no fertilization or irrigation were applied during the study. A more detailed description of the plantation lay-out, management and plant materials used, can be found in Chapter 1, in Broeckx et al. (2012a) and in Berhongaray et al. (2013c).

5.2.2. Data collection in the field

All data for the present study were obtained from samples collected during the second year of the first rotation (2011) and the first year of the second rotation (2012) of the plantation. Due to the high labor intensity with belowground analyses, this study was restricted to two genotypes with a contrasting aboveground habitus, i.e. Koster (*P. deltoides* Marsh x *P. nigra* L.) and Skado (*P. trichocarpa* Hook. x *P. maximowiczii* Henry). Both genotypes were selected as being the most representative for the plantation based on their parentage, origin and area coverage in the plantation (Broeckx et al. 2012a). In this contribution, "plot" is defined as a combination of previous land-use type and genotype (with different density and mortality; Table 5.1).

Table 5.1: Aboveground (stems + branches) and belowground (stump + medium-sized + coarse roots) woody biomass from two two-year old poplar genotypes (Skado and Koster) grown on two former land-use types (cropland and pasture). The above- and belowground biomass components were estimated using allometric relations and diameter inventories. Leaf and fine root biomass were not included. Mean (±SE); DM= dry mass; n = number of samples; P= former pasture; C= former cropland.

Genotype	Land-use type	Density (trees m^{-2})	Mortality (%)	Aboveground (g DM $tree^{-1}$)	Belowground (g DM $tree^{-1}$)	Root:shoot Ratio
Skado	C	0.767	6.3%	1562	717	0.46
Skado	P	0.723	24.8%	1735	782	0.45
Koster	C	0.687	18.0%	765	534	0.70
Koster	P	0.687	17.6%	1175	670	0.57

Belowground woody biomass from tree excavation: medium-sized roots (Mr) coarse roots (Cr) and stump (Stu)

In Feb.-Mar. 2012, the root system of 20 selected trees (10 per genotype) was completely excavated. For each of the two genotypes five trees of different stem diameter (Ø ranging from ~20 mm to ~60 mm at 22 cm height above the soil) were selected from each of both former land-use types. Immediately after the harvest in Feb. 2012, the remaining stumps (Stu) and roots of the selected trees were excavated from the Voronoï polygon confined by an area of 1.1 m x 1.125 m (planting distance within the rows x sum of half inter-row distances). All roots within this 1.238 m^2 area were collected, assuming that roots from adjacent trees within the sampled area compensated for roots of the excavated tree growing outside the sampled area. Excavation depth was limited to 60 cm, as very few roots were observed under 60 cm (see Results section). Roots that penetrated below 60 cm during the excavation were not recovered by complete excavation, but were rather pulled out. Medium-sized roots (Mr, Ø = 2-5 mm) and coarse roots (Cr, Ø > 5 mm) were collected separately in the 0-15 cm and 15-60 cm soil layers from both the narrow and the wide inter-rows. Total dry mass of the Mr and Cr as well as of the remaining 15 cm high Stu was determined in the laboratory after oven drying at 70°C until a constant weight was reached. Dried root mass was ground for subsequent carbon (C) and nitrogen (N) analyses. An average of the C mass fraction of all samples per organ and per root class was used to

calculate the belowground woody C pool. As for the aboveground components, below-ground biomass values at the tree level (i.e. Mr and Cr) were scaled up to the plantation level by using the specific planting density and mortality of each plot.

Fine roots (Fr) from soil core sampling

The soil coring technique was used to determine fine root (Fr; Ø<2 mm) mass of both genotypes (Berhongaray et al. 2013c). A soil core sampling at different depths was performed in Aug. 2011 and Aug. 2012. In Aug. 2011 sampling was performed in six different soil layers (0-15 cm, 15-30 cm, 30-45 cm, 45-60 cm, 60-75 cm and 75-90 cm, whereas in Aug. 2012 four different soil layers (0-15 cm, 15-30 cm, 30-45 cm and 45-60 cm) were sampled. An 8 cm diameter x 15 cm deep hand-driven corer (Eijkelkamp Agrisearch equipment, The Netherlands) (cfr. Oliveira *et al*. 2000) was used. The number of samples differed at each depth depending on the expected intrinsic variability of the Fr mass. Based on our previously described approach and methodology (Berhongaray et al. 2013d), the number of samples varied from 20 in the upper soil layers to 10 in the deeper layers. In order to compare the effect of the previous land-use type on the seasonal dynamics of Fr, soil samples from the top 15 cm were collected from genotype Skado in Spring (May 2011) and Summer (Aug. 2011) of the first rotation as well as in Winter (Feb. 2012), Spring (May 2012) and Summer (Aug. 2012) of the second rotation. Immediately after collection in the field, all samples were transported to the laboratory and stored in a freezer until processed.

Fine roots were picked from each sample by hand while: (i) separating weed roots (W) from poplar roots, and (ii) sorting poplar roots in dead and living roots. The sorting of dead and living Fr was based on the darker color and the poorer cohesion between the cortex and the periderm of the dead roots (Janssens et al. 1999). After washing, fine roots were oven dried at 70°C for 1-4 days to determine the dry root mass. Fine root mass of one core sample picked for *x* min (i.e. 5 to 20 min) was converted into total Fr mass in the sample (i.e. after 60 min picking duration) using Richard's equation (Berhongaray et al. 2013d) and expressed in g DM m^{-2}. Subsamples of dried Fr were ground for further C and N-analyses. More details on Fr collection and data processing can be found in Berhongaray et al. (2013c; 2013d).

Aboveground biomass

The aboveground woody mass data were calculated from previously published data for the two genotypes (Verlinden et al. 2013a). A detailed inventory of the stem diameter distribution and mortality was carried out for each genotype in Dec. 2011, by measuring stem diameter at 22 cm above soil level of one entire row per monoclonal block and by counting the number of missing trees. Based on the stem diameter distribution of the plantation reported in Verlinden et al. (2013a) ten trees of each genotype were selected for destructive harvest, covering the widest possible stem diameter range. In Dec. 2011 and after the inventory, stem diameter (D) at 7 cm and at 22 cm was measured on the selected

trees with a digital caliper (model CD-15DC, Mitutoyo Corporation, Japan, 0.01 mm precision), before the tree was harvested at 7 cm above soil level. After determination of dry mass (DM) of each stem, allometric relationships were established between stem diameter and aboveground dry mass, fitted as $DM=a \cdot D^b$ for both genotypes (for genotype specific regression coefficients, see Broeckx et al. 2012a). As the coppicing with harvesting machines was performed at 15 cm (Berhongaray et al. 2013a) instead of 7 cm, the 8 cm biomass remaining in the Stu was subtracted from the aboveground biomass using the volume ($8\ cm \cdot \pi \cdot (D_{7cm}/2)^2$; D_{7cm}= stem diameter at 7 cm) and the bulk wood density (524±9 mg DM cm^{-3}) as previously reported in Berhongaray et al. (2013b).

Chemical analysis of biomass samples

Root samples were analyzed for their C and N mass fractions by dry combustion using a NC-2100 element analyzer (Carlo Erba Instruments, Italy). Root mass was converted to C mass using the average root C mass fraction, and expressed in g C m^{-2}.

5.2.3. Data analysis

Root topological analysis

All Mr and Cr removed from the soil during the excavation were cleaned and photographed with a digital camera, providing a clear picture of the root system of the trees under the natural conditions. A white board with a metric scale was used to remove background noise and to compare the root systems at the same scale after analysis. The maximum depth of the root system was visually determined from the photographs. The pictures were digitally skeletonized using Illustrator® software (Adobe Systems Software Ireland Ltd.). The pictures enabled the precise imaging and the reconstruction of the root systems as well as the reconstruction of the root skeletons for topological analysis.

Root architecture generally refers to the spatial configuration of the root system. It includes root topology (root branching pattern; see Berntson 1997; Fitter et al. 1991) and root biomass distribution (at different depths and for different root diameter classes; see Bauhus and Messier 1999; Guo et al. 2004). Root topology refers more to the inter-connections (pattern) of individual root segments (root branches). In this study root architecture refers to the root topology or the branching pattern of the roots. From the skeletonized images, the branching structure of the root system was analyzed using the architectural analysis proposed by Fitter et al. (1991). This method is useful for the description of functional properties of root systems, and in particular for describing excavated root systems for which it is not always possible to identify the developmental branching orders. In line with this methodology we separated the links that terminated in a meristem (exterior link) from the ones that terminated in a node (interior link). Since no branching angles could be determined on roots extracted from the soil, the architectural analysis was done comparing topological indices. The geometry of the structure was

represented mathematically and resolved into several components and indices (Figure 5.1):

1) magnitude (μ): the number of exterior links downstream one link. It is a term that refers either to the entire system or to any particular link within the root system;

2) altitude (a): the number of links in the longest unique path from the base line to an exterior link;

3) total exterior path length (p_e): the sum of the number of links in all possible unique paths from the base link to all exterior links;

4) topological index: the ratio log (altitude) / log (magnitude) calculated for each root system;

5) altitude-slope: the slope of the regression of log (a) on log (μ);

6) path length-slope: the slope of the regression of log (p_e) on log (μ).

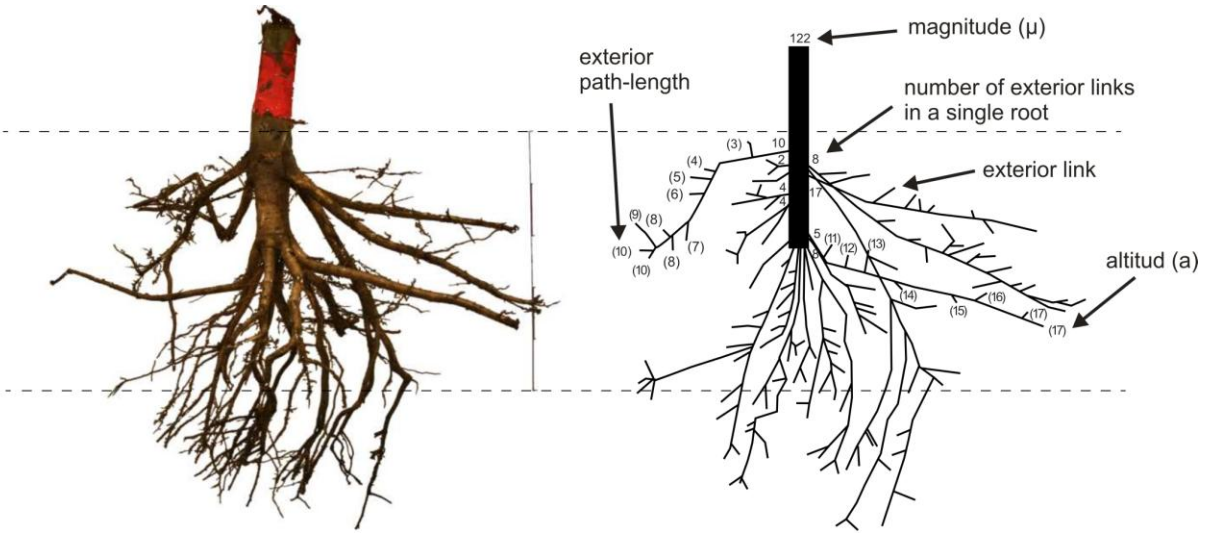

Figure 5.1: Root system from a poplar tree, photographed (on the left) and skeletonized (on the right). The exterior links and the exterior path-length (numbers in brackets) were identified from the skeletonized roots. The exterior links were shortened to avoid overlapping and to better identify the roots. The magnitude (μ; sum of all the exterior links) and the altitude (a; maximum exterior path-length) were calculated for each root system.

The extreme values of these components and indices are reflected in the herringbone (branches confined in the main axis) or in the dichotomous (random branching) topology of the root system. Higher parameters and slopes indicate a herringbone pattern of branching while lower values indicate a dichotomous branching pattern. More details and further explanations can be found in Fitter (2002). The root topology was analyzed by comparing the topological parameters a and p_e as well as the altitude-slope and the pathlength-slope for both genotypes.

Allometric relationships

Allometric equations were used to scale-up belowground woody biomass components based on stem diameter at 22 cm. Stu, Cr and Mr biomasses were plotted against stem diameter at 22 cm, and allometric power and exponential equations were fitted. The most reliable equations with higher R^2 were selected. Using the data from the stem diameter

inventory and the allometric equations, we estimated the average belowground woody biomass and Stu biomass per tree.

Root:shoot ratio

Most commonly, the root:shoot ratio is defined as the root biomass divided by the shoot biomass. The distinction between 'root' and 'shoot' biomass is generally made at the ground surface level, with the term 'root' referring to all biomass below the ground surface, and 'shoot' being all biomass above the ground surface. In the present study, the root:shoot ratio was calculated using only woody biomass (Cr, Mr, Stu, stem and branches), and excluding Fr and leaves. As the studied trees were planted in a SRC plantation, we considered harvesting height as the upper limit for the belowground biomass, instead of the ground surface. The belowground biomass was defined as all what remained in the field after the mechanical harvesting, and the aboveground as the biomass that is frequently harvested.

Water table depth

Mean annual water table depth was calculated from the measurements and interpolated to get an estimate of the (mean annual) water table across the entire field of the SRWC plantation. Ordinary kriging was performed using Spatial Analyst tool in ArcGIS 10.1, as it is the most suitable interpolation method for groundwater table (Sun et al. 2009). Exponential and spherical semivariogram models were selected for the kriging interpolation. From the kriging interpolation maps, the mean water table depth was estimated for each plot.

Statistical analysis

A two-way analysis of variance (ANOVA) was performed using land-use type and genotype as fixed factors, also including their interactions. In the case of a significant genotype effect, pairwise comparisons were performed using a Tukey post-hoc test ($P \leq 0.05$).

5.3. Results and Discussion

5.3.1. Fine roots

Biomass of the Fr in the first year of the second rotation (2012) was for all depths not significantly different from the second year of the first rotation (2011), except for genotype Koster where Fr biomass in the upper soil layer was increased in 2012 as compared to 2011 (Figure 5.2). In Skado, Fr biomass was higher in the former cropland than in the former pasture (Table 5.2). A higher Fr biomass in the former cropland could be expected because of the higher nitrogen concentrations in the cropland soil (Broeckx et al. 2012a; Pregitzer et al. 2000). The higher weed presence and the intensive weed management in the former pasture land caused a higher mortality by mechanical and chemical treatments

(Broeckx et al. 2012a). No genotypic differences in Fr biomass were detected at any soil depth. The highest Fr biomass was detected in the upper 15 cm. On average, Fr biomass in the upper 15 cm accounted for 63.6 ± 16.4 g DM m^{-2}, which is slightly higher than values reported for SRWC poplar on nutrient poorer soils in the same region (Al Afas et al. 2008). The Fr biomass present in the upper 15 cm of the soil represented 44.3% and 50.1% of the total Fr in the 0-60 cm profile of Skado and Koster, respectively.

Table 5.2: Fine root biomass (<2 mm) of poplars grown on two previous land-use types (cropland and pasture). Significant differences (at p≤0.05) in the same sampling date are marked with an asterisk (*). 2011= second year of the first rotation, 2012= first year of the second rotation. Mean (±SE); DM= dry mass; n= number of samples.

Year/season		*n*	Cropland (g DM m^{-2})	Pasture (g DM m^{-2})	sign.
2011	Spring	20	21.0 (±6.5)	7.3 (±3.2)	
	Summer	20	74.3 (±10.5)	43.4 (±7.0)	*
2012	Winter	25	74.8 (±7.8)	52.6 (±5.5)	*
	Spring	25	63.9 (±6.2)	60.8 (±13.5)	
	Summer	11	68.7 (±10.5)	39.9 (±4.5)	*

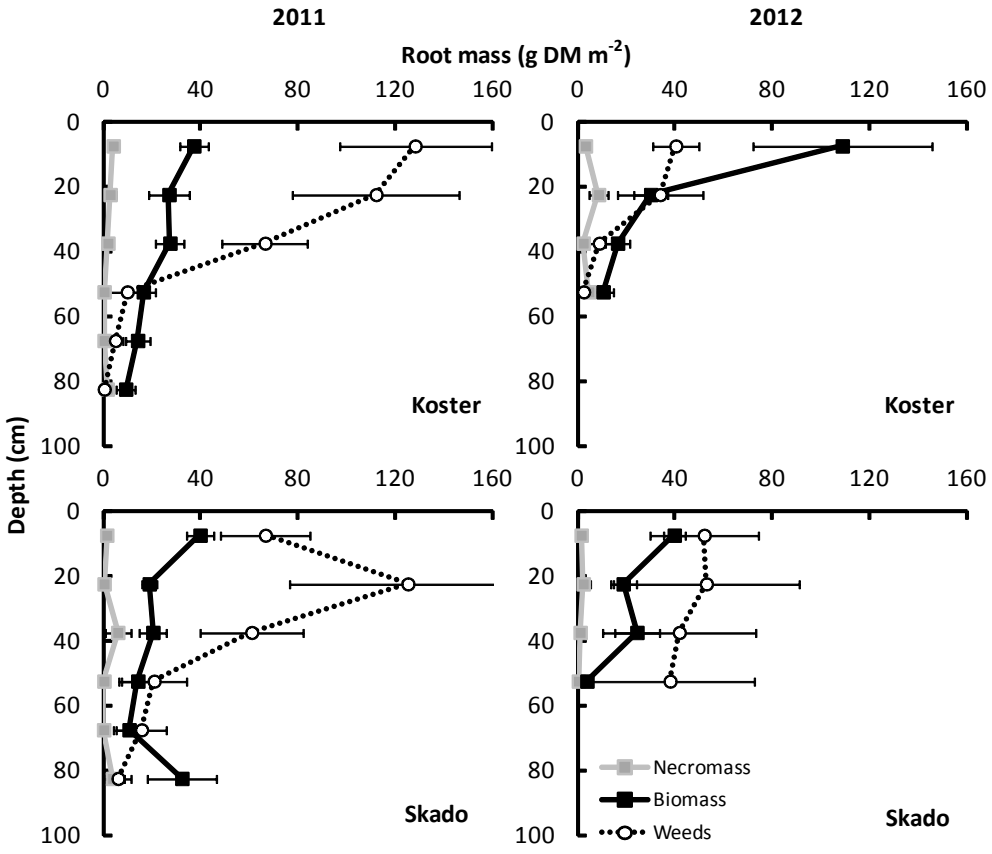

Figure 5.2: Vertical distribution of fine root mass (∅ <2 mm) of poplars and weeds under two poplar genotypes and for two consecutive years (2011 and 2012). Genotype Koster: top panels; genotype Skado: bottom panels. 2011: second year of first two-year rotation; 2012: first year of second two-year rotation. Error bars indicate standard error of the mean; DM= dry mass.

In the second year of the first rotation (2011), WR biomass, mostly from grasses, was significantly higher than Fr of poplar in the upper 45 cm of the root profile. This difference was not detected any more in the first year of the second rotation (2012), which could be explained by the higher canopy closure of the poplars and the lower weed presence after the coppice (unpublished observations and personal communication of Stefan

Vanbeveren). Overall, in 2011 the WR showed a strong vertical distribution with a significant concentration in the upper 30 cm, while in 2012 the WR were more evenly distributed over the soil profile than the Fr. In native ecosystems tree roots show deeper rooting profiles than grass species (Jackson et al. 1996).

Root category	*n*	C%
WR	179	30.4 (±0.38) a
D	103	35.9 (±0.61) b
FR	334	36.6 (±0.31) b
MR	28	42.0 (±0.40) c
CR	50	42.3 (±0.30) c
Stu	20	43.5 (±0.51) c

Table 5.3: Carbon (C) concentration (in %) of different belowground components. WR= weed roots, D= dead fine roots (∅ <2 mm), Fr= fine roots (∅ <2 mm), Mr= medium-sized roots (∅ 2-5 mm), Cr= coarse roots (∅ >5 mm), Stu= stumps. Different letters indicate significant differences in carbon concentration between different components (Tukey, $p<0.05$). Mean (±SE); n=number of samples.

5.3.2. Medium-sized and coarse roots

For trees of the same stem diameter class, no significant differences in Cr biomass were detected, neither between genotypes nor between previous land-use types. Consequently one single allometric equation was established to scale-up Cr biomass of the two genotypes across both previous land-use types using the stem diameter frequency distribution (Figure 5.3). It was, however, not possible to establish an allometric equation for Mr (Figure 5.3). The up-scaled standing Cr biomass in Dec. 2011 significantly differed between genotypes (Table 5.1). The Cr biomass was higher in Skado (135.6 g DM m^{-2}) than in Koster (113.4 g DM m^{-2}). These Cr biomass values were lower than the values of 390-2980 g DM m^{-2} reported for older and less dense plantations (Puri et al. 1994; Toenshoff et al. 2013; Tufekcioglu et al. 1998). The low Cr biomass values could probably be attributed to the limited rooting depth, i.e. almost no Cr roots were found below 60 cm. As poplar is an opportunistic rooter, it does not produce roots at deep soil layers when there is sufficient water available or a high water table (Hallgren 1989). The latter was the case at the site of this study (average water table depth 85 cm). Since we used only one unique allometric equation to scale-up Cr, the genotypic differences in Cr were due to differences in the stem diameter frequency distribution (Figure 5.3), in the final planting density and/or in the mortality rate (Table 5.1). On average, Skado had significantly larger stem diameters than Koster (Verlinden et al. 2013a).

5.3.3. Root system

On the previous pasture the top soil (0-15 cm depth) was dominated by Fr (Figure 5.4). A lot of horizontal, lateral roots just below the soil surface were observed during the tree excavation. On average, 41% of the total root biomass was composed by fine roots in Koster versus 28% in Skado. We hypothesized that larger trees produce more fine roots. Although genotype Skado showed larger tree dimensions (higher biomass and stem diameter) with larger Cr (Table 5.1, Figure 5.3), there were no differences in Fr biomass with genotype Koster. This observation could be interpreted as a rejection of our second hypothesis, but it is based on a comparison of two different genotypes. If we consider only genotype Skado, and we compare the Fr at two different previous land-use types, we

observed a higher total (above- and belowground) biomass (Table 5.1) and a higher Fr mass (Table 5.2) on the previous cropland; this then confirmed our second hypothesis. The comparisons of Fr biomass between the two former land-use types could only be done for the first 0-15 cm.

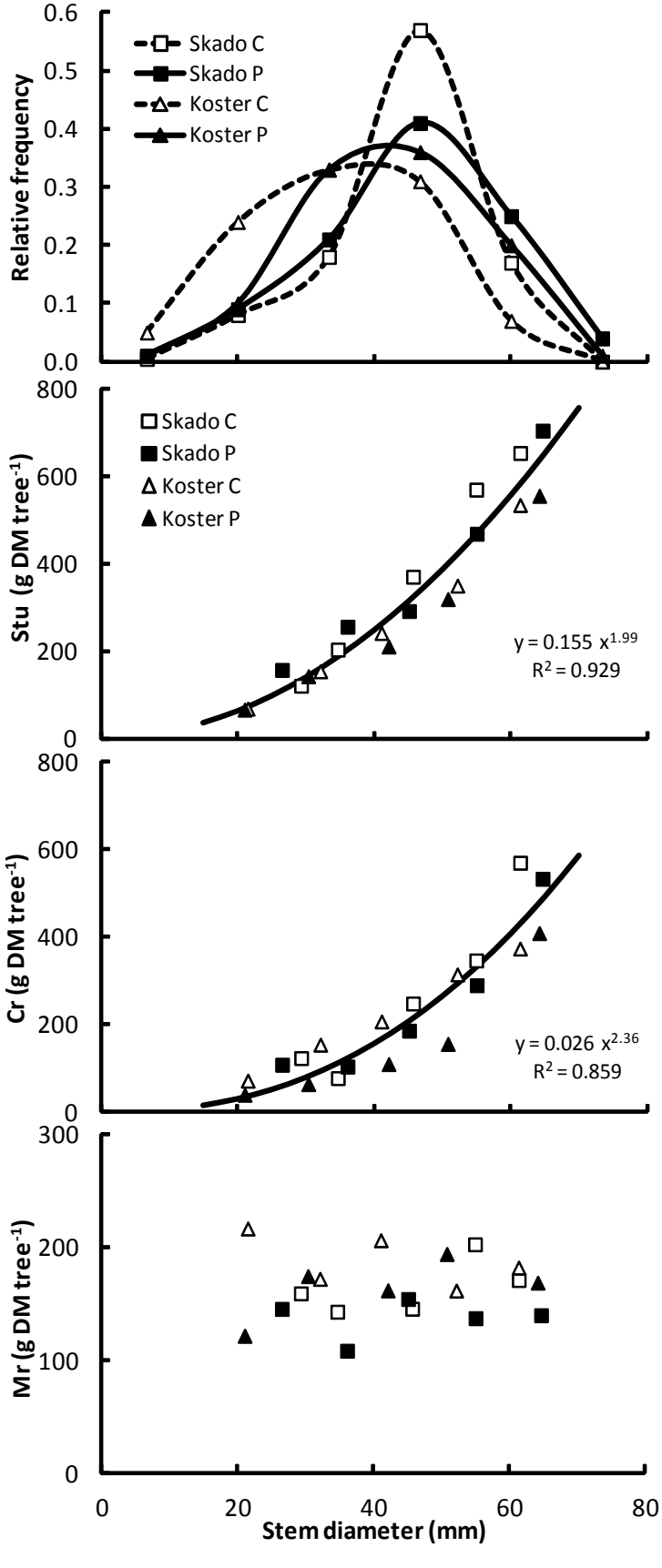

Figure 5.3: Stump, coarse root (Ø >5 mm) and medium-sized root (Ø 2-5 mm) biomass in the area occupied by a single tree (Voronoï polygon) in relation to its stem diameter (at 22 cm). An exponential equation was fitted to the allometric relationship between root biomass and stem diameter. Medium-sized root biomass was homogenously distributed over the stem diameter range, and no equation was fitted. DM= dry mass; C= previous cropland; P= previous pasture land.

Deeper in the soil (15-60 cm depth) the presence of Fr decreased, while the amount of Mr and Cr increased. This pattern differed from other observations on trees that reported a decreasing proportion of Mr and Cr with depth, and consequently, an increasing proportion of Fr at deeper soil layers (Lyr and Hoffmann 1967). We think that this might be explained by the young age (only second growing year) of the trees in our study. As the trees were still in their exponential growth phase, they were investing more in Fr than in Cr to occupy the fertile soil of the site. Moreover, Cr are always initially Fr before they grow in diameter and become Cr. The C concentration of the roots increased with root diameter; C concentration was lowest (36 % of C) in the Fr without significant differences between necromass and biomass. There were no significant differences in root C concentration between both genotypes.

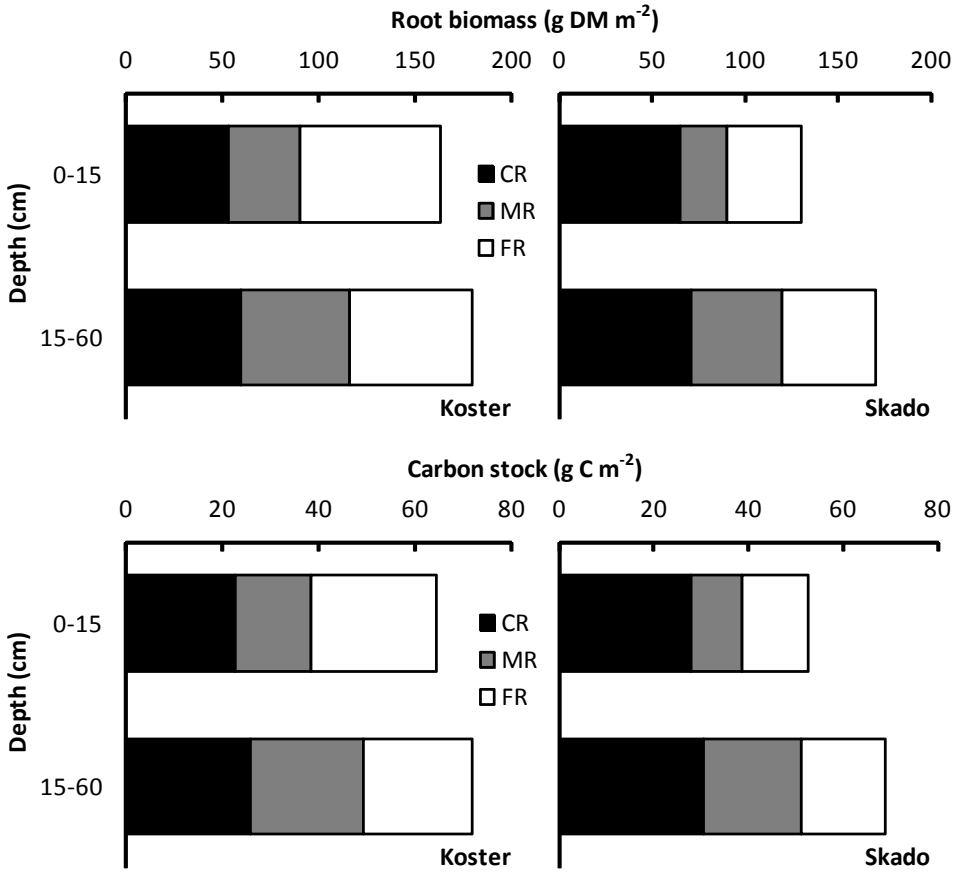

Figure 5.4: Vertical distribution of root biomass by diameter class (fine Ø <2 mm, medium-sized Ø 2-5 mm, and coarse roots Ø >5 mm) in Koster and Skado. Stump biomass was excluded. DM= dry mass.

5.3.4. Root:shoot ratio and root:shoot architecture

At the end of the second growing season of the first rotation (Dec. 2011) total (= above- and belowground) standing woody biomass was higher in Skado than in Koster (Table 5.1). Cr and Mr represented 17% of the total standing woody biomass in Skado *vs.* 23% in Koster. The Stu represented 14% of the total standing woody biomass in Skado *vs.* 16% in Koster, thus representing a higher belowground proportion for the genotype with the lower standing biomass. The root:shoot ratio exponentially decreased with stem diameter in the same way for both clones (Figure 5.5). As for Cr biomass the genotypic differences in root:shoot ratios were attributed to differences in mean stem diameter. For young Scots

pines an increment of the root:shoot ratio with stem diameter increment was reported, different from our findings (Xiao and Ceulemans 2004). This could be explained by the fact that these evergreen trees were growing on poor forest soils. Moreover, we found that the root:shoot ratio increased with increasing aboveground biomass. This observation differed from a literature review (Mokany et al. 2006), where they found a decrease in the root:shoot ratio with increases in the aboveground biomass. A possible explanation is that Mokany et al. (2006) compared different ecosystems at geographically different locations, so that not only increases in aboveground biomass but also variations in climatic and soil characteristics were involved. In our experiment, trees of different sizes exposed to the same soil and climatic conditions were exposed. We found that biomass allocation (to above- versus belowground) was not under strong genetic control, in contrast to some other studies that compared poplar genotypes (King et al. 1999; Yin et al. 2005). However, we compared only two genotypes under non-limiting growth conditions.

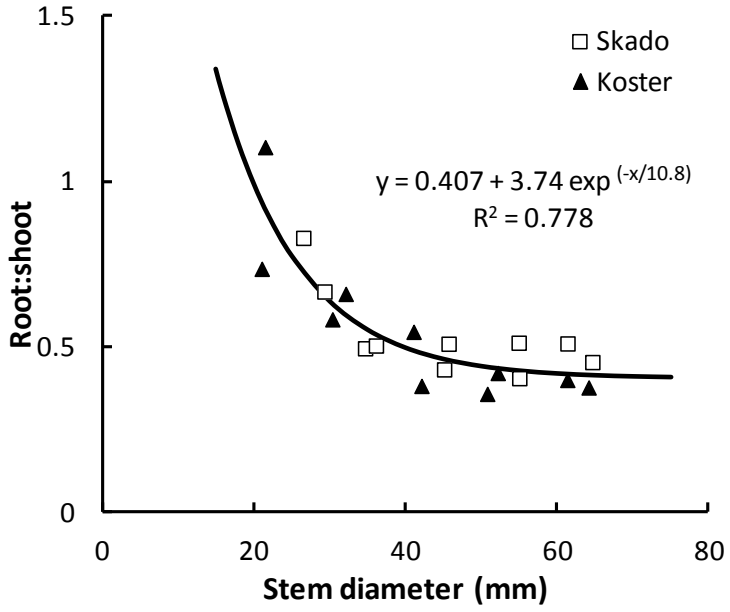

Figure 5.5: The ratio of below/aboveground biomass for the genotypes Koster and Skado in relation to tree stem diameter. Belowground biomass includes stump, coarse and medium-sized roots; aboveground biomass is composed by stem and branches.

Our observations on the root:shoot ratio may also have differed from other studies based on the different definitions that are used. The distinction between below- and aboveground biomass is based on the arbitrary position of the ground surface. In some ecosystems, a considerable proportion of the roots occur above the ground surface and likewise, part of the stem biomass sometimes occurs below the soil surface (Mokany et al. 2006). There might be some disagreement on considering the 15 cm of Stu aboveground as a belowground component, but the Stu only represented 5-6% of the total tree biomass. The root:shoot ratio does not represent the total carbon allocation to the tree compartments, since it does not incorporate the considerable loss of carbon resulting from respiration and senescence (turnover). So, the root:shoot ratio only represents the net effects of carbon allocation. Although root:shoot ratios may only be rough indicators of physiological processes affecting carbon allocation, they are of high value in providing estimates of belowground plant biomass from aboveground biomass. For example, multiplying the biomass of the tree organs by the turnover and decomposition rates implies that C

allocation in trees strongly influences forest carbon cycling. Consequently a proper understanding of carbon allocation is an important issue in the context of best management practices for biomass production and carbon sequestration in the soil.

The fundamental structure of the root systems did not significantly differ between both genotypes (Figure 5.6). The logarithmic plot of a to μ showed for both genotypes a strong and linear relationship over the entire range of μ. Figure 5.7 schematically depicts the Cr system of the two genotypes under the two previous land-use types. Although Koster and Skado had a very different aboveground (crown) structure (Broeckx et al. 2012b), their topological index of the coarse roots did not significantly differ (Figure 5.7). The mean topological index of 0.62 (Figure 5.6) was lower than the values reported for other plant species (Bouma et al. 2001), and it showed a pattern close to a dichotomous or random branching. The topological index was independent of stem diameter or tree size, which confirmed previous studies that showed no effect of primary root diameter (Bouma et al. 2001) nor of plant size (Glimskar 2000) on branching patterns.

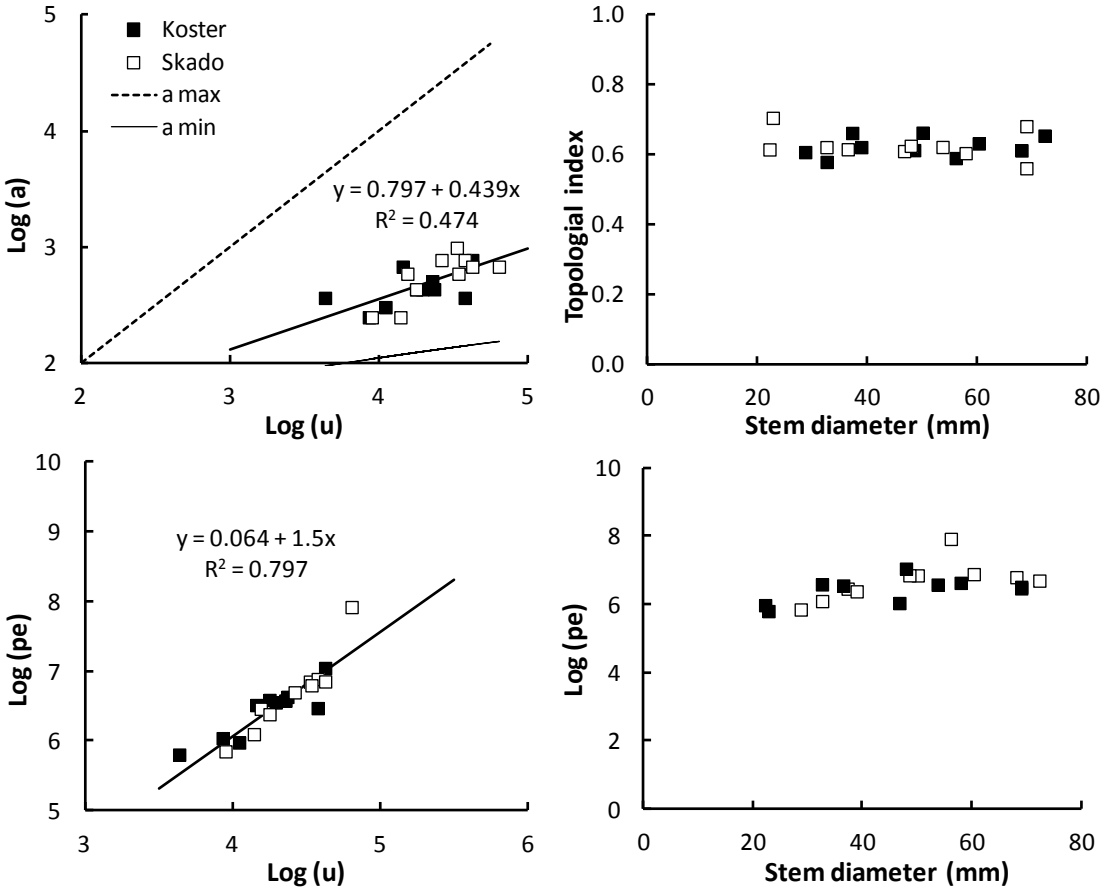

Figure 5.6: Relationship between topological components and indices of root systems. Altitude (a) *vs.* magnitude (μ) of tree root systems (top left); total exterior pathlength (pe) *vs.* magnitude (bottom left); topological index (altitude/magnitude) *vs.* stem diameter (top right) and total exterior pathlength (pe) *vs.* stem diameter (bottom right).

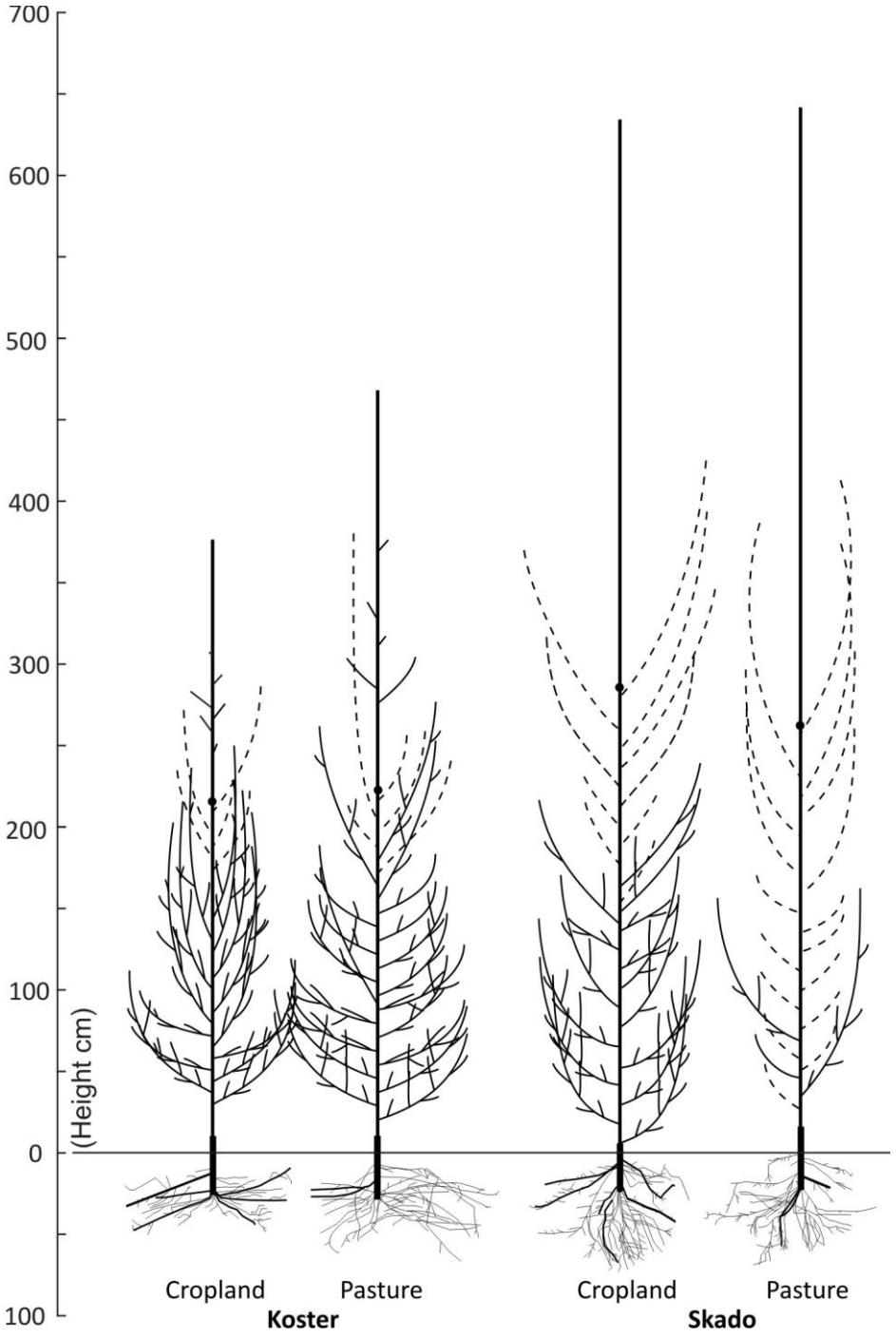

Figure 5.7: Schematic representation of the above- and belowground architecture of the two poplar genotypes on both former land-use types. Reconstruction from the most representative (average tree) selected trees at the end of the second year of the first rotation. The dark black dot on the stem above 2 m indicates the end of the first year height growth. Dotted branches = proleptic branches, developed from lateral meristems that have been formed during the previous growing season; Full-line branches = sylleptic branches, that develop from current-year lateral meristems (Broeckx et al. 2012b).

These results rejected our hypothesis of similarity between above and belowground tree architecture. A possible explanation for the similar belowground architecture of genotypes that significantly differ in aboveground architecture could be the non-limiting growth conditions. This plantation was almost not limited in nutrient or water (Broeckx et al. 2012a), except for a short dry period in June 2011 (Broeckx et al. 2013). Trees did, therefore, not need complicated or strong structures to capture water or nutrients (Fitter and Stickland 1991). But, as light is a limiting factor in the very dense SRWC plantation, the trees competed for light through their aboveground canopy architecture (and leaf display; Broeckx et al. 2012b). In short, the limiting factor determines the architecture of the organ.

5.3.5. Rooting depth

We observed a shallow root system in both genotypes, and the water table was a strong determinant of the rooting system depth (Fig. 8), in line with the natural riparian habitat of poplars. Typically, poplar trees have relatively shallow but widespread root systems (Dobson and Moffat 1999). It is uncommon for roots to penetrate to a depth below 2 m, with 80–90% of the roots of most plant species generally found within the top 60 cm of the soil profile (Jackson et al. 1996). This expected result of water table limitations on the rooting depth did not confirm our third hypothesis. Although the water table depth limited the rooting depth and the tree size, the root:shoot ratio was only correlated with the tree size and not with the genotype nor the water table depth (data not shown).

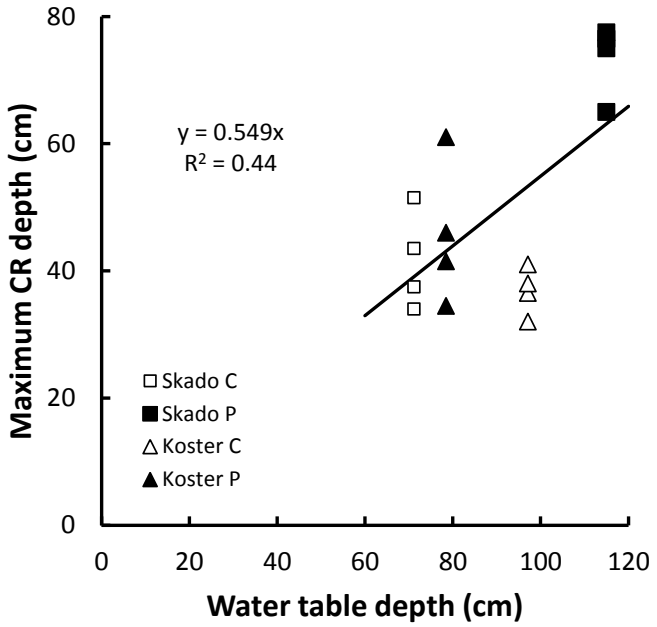

Figure 5.8: Maximum coarse root (Cr) depth in relation to the mean annual water table depth for the two poplar genotypes (Koster and Skado) on the two previous land-use types. C: cropland; P: pasture. The linear regression is significant at p=0.05.

5.3.6. Methodology used

In this study we used the technique of core sampling for the determination of Fr biomass, and tree excavation for the biomass estimations of Mr and Cr. The core sampling methodology is recommended for the samping of uniform roots, such as for Fr biomass. With increasing root diameters the (spatial) variability of the lateral root distribution also increases; so the sampling of an increasing amount of soil volume enables a better sampling of this belowground heterogeneity.

5.4 Conclusions

We found that larger trees had a bigger root system, but not necessarily that larger trees produced more fine roots. The soil water table was a strong determinant of the rooting depth. The poplar genotypes only rooted in the upper 30 cm, and they showed relatively shallow, but widespread root systems. Using a more accurate representation of the branching of tree roots we have shown that genotypic differences in aboveground architecture were not reflected belowground.

Chapter 6

6. Partitioning soil respiration

Based on:
Partitioning soil respiration using high-resolution measurements of soil respiration and root biomass
G. Berhongaray, M.S. Verlinden, I.A. Janssens and R. Ceulemans
(Manuscript to be submitted)

Abstract
Several studies have suggested that overall increases in soil respiration (R_s) observed during the last decennias are more closely related to increases in plant (root) respiration (R_r) than to increases in soil organic carbon (SOC)-derived mineralization (R_h). Understanding the mechanisms that determine changes in R_s is important in global change research, since an increase in R_h might eventually induce an important positive feedback to climate change. Using high-resolution measurements of soil respiration and root biomass, we partitioned R_s in its three major components, R_h, root maintenance (R_m) and root growth (R_{gr}) respiration. We found that R_h accounted from 41 to 51% of the total annual R_s, and it represented a high portion of R_s in winter and a minor proportion in summer. These results allowed us to close the soil carbon balance of a short rotation coppice culture of fast-growing poplars.

Keywords: heterotrophic respiration; autotrophic respiration; root growth; maintenance respiration; *Populus*; SRWC

6.1. Introduction

Soils can be either a source or a sink for atmospheric CO_2. Soils in Europe (Ciais et al. 2010) and worldwide (Houghton et al. 1983; Schimel et al. 2001) have been losing carbon for many years, and efforts are being made to halt this process and sequester carbon (C) back into the soil to mitigate climate change (Batjes 2008; Jones et al. 2005; Liang et al. 2005; Schulp et al. 2008). However, the evaluation of the impact of changes in management and in land use on soil organic carbon (SOC) requires very long-term records (Guo and Gifford 2002), and this is not suitable for decisions on the short term. The C mass balance approach, as the balance of soil C inputs and losses, can help us to predict changes in SOC based on short-term records (Giardina and Ryan 2002), but we still lack a lot of knowledge on the C that is released from the soil.

Soil respiration (R_s) is defined as the flux of carbon dioxide (CO_2) from the soil surface to the atmosphere. It is the second largest terrestrial carbon CO_2 flux and it has significantly increased over the last two decades (Bond-Lamberty and Thomson 2010). Changes in the rates of R_s could potentially change the C balance of terrestrial ecosystems and act as a feedback mechanism to climate change (Trumbore et al. 2006). R_s is composed of CO_2 coming basically from two different sources: (i) microbial decomposition of SOC and (ii) root respiration. It is still unclear whether the increased C loss observed during recent decades (Bond-Lamberty and Thomson 2010) comes from an increased activity of plant roots (autotrophic respiration, R_r) or is caused by an increased SOC decomposition (heterotrophic respiration, R_h). Moreover, R_r is the result of three major respiration processes, i.e. respiration for maintenance of root biomass (R_m), root growth (R_{gr}) and ion uptake. Research aimed at partitioning carbon releases between R_r versus R_h is absolutely needed to better predict the net response of soil carbon stores to climate change.

Regardless of its importance for quantifying the soil C balance, the partitioning of R_s still remains a scientific challenge (Baggs 2006). Although several methods have been developed, there is no universally accepted method for the partitioning of R_s (Subke et al. 2006). The root exclusion technique, either by trenching or by the installation of collars, significantly disturbs the soil. A good use of the isotopic technique is costly and with many methodological issues. *In vitro* lab techniques include measurements of small fragments of roots, or soil incubations (Fukuzawa et al. 2011). These *in vitro* methods provide very precise results. They enable researchers to examine the relationships between R_s rate and environmental factors, and they also provide relationships and parameters for the development and improvement of models. However, usually the representativeness of these parameters for *in situ* conditions is poor. Although the parameters obtained *in vitro* may not be entirely correct, we can use them to better understand the relationships between the parameters *in situ*.

A non-invasive method for R_s partitioning is the regression technique, in which the relationship between R_s and root biomass is extrapolated to the intercept, yielding R_h (Rodeghiero and Cescatti 2006; Xu et al. 2001). This is an indirect way for partitioning R_s

that can provide an approximate quantification of the contribution of roots to total soil respiration (Baggs 2006). However, this method does not take into account the seasonal evolution of belowground processes, such as root growth and seasonal changes in root biomass. In a recent study Ogle et al. (2014) emphasized the importance of process based models to explain the partitioning of ecological processes. Linear models generally do not incorporate an underlying process model, and they thus lack predictive ability. It is thus nearly impossible to use the results of linear models to predict how the contributions of ecological components will change over time, across space, or in response to disturbances.

Although the methods mentioned above do not provide an answer to the partitioning issue, they have revealed interesting relationships. For example, it is well known that R_s increases almost exponentially with increases in soil temperature (T_s). The exponential effect of T_s has also been found independently for R_h and R_r (Moyano et al. 2008). The sensitivity of R_r to T_s is mainly determined by R_m which is highly temperature dependent, while R_{gr} is unaffected by temperature (Kuzyakov and Gavrichkova 2010). Several studies reported a different sensitivity of R_h and R_r to temperature, but more recent results suggest that there is no difference in the temperature sensitivity between both sources of CO_2 (Subke et al. 2006). The effect of soil moisture (W_s) on soil respiration is well understood for water limited systems, such as the Mediterranean forests (Talmon et al. 2011). However, the moisture limitation to R_s is difficult to address in non-water limited systems. The effects of W_s and T_s on R_s can be additive or integrative (Talmon et al. 2011; Xu et al. 2001). Furthermore, R_s is closely linked to plant growth and plant structure (Barba et al. 2013; Sampson et al. 2007). Root respiration (R_{gr} and R_m) largely depends on the amount of carbon allocated belowground (Kuzyakov and Gavrichkova 2010; Vargas et al. 2011). Thus, R_r and root proliferation are likely to be sensitive to seasonal changes in photosynthetic activity. The similarity in the seasonality of soil respiration and of aboveground plant variables has already been demonstrated (Curiel-Yuste et al. 2004). Other studies (Kuzyakov and Gavrichkova 2010; Moyano et al. 2008; Vargas et al. 2011; Wingate et al. 2010) reported similar connections, but as far as we know no study was able to relate the separate components of R_s, R_h and R_r, to the seasonality of the ecosystem.

Many biogeochemical models have been applied for European ecosystems to report changes in belowground C after land-use changes as a reporting requirement for the Kyoto protocol. These models use different pools and fluxes related to the belowground compartment, such as R_s, R_h, R_r and C inputs. Using models with a fixed rate for R_s partitioning should, however, be avoided for correct ecosystem based C budgets. The long-term goal of our study is to evaluate the effect on SOC of land-use change to short-rotation woody crops as an alternative to fossil fuels, which helps to mitigate the rapidly rising atmospheric CO_2 concentrations. In this contribution we used chamber techniques to measure R_s, and we took advantage of *in situ* spatial differences in fine root biomass and production, and in R_s (Berhongaray et al. 2013c; Verlinden et al. 2013b) to fit non-linear models. The objective of this contribution is to offer support for an easy and straightforward method for partitioning of R_s into its autotrophic and heterotrophic components.

6.2. Materials and Methods

6.2.1. Experimental site

The data for the present contribution come from the experimental field site of the large-scale POPFULL project (Chapter 1).

6.2.2. Measurement of soil CO_2 efflux

Soil respiration (R_s) was measured continuously throughout the year using an automated soil CO_2 flux system (LI-8100, LI-COR Biosciences, Lincoln, NE, USA). Sixteen long-term chambers operating as closed systems were connected to an infrared gas analyzer through a multiplexer (LI-8150, LI-COR Biosciences, Lincoln, NE, USA). Half of the 16 chambers were installed in the narrow inter-rows, the other half in the wide inter-rows. Soil CO_2 efflux was gap-filled for the periods without measurements by an Artificial Neural Network analysis (using MATLAB; 7.12.0, 2011Mathworks, Natick, Massachusetts, USA) based on soil temperature, which was continuously monitored throughout the year. Values of CO_2 efflux where integrated over time to obtain the cumulated CO_2 efflux. Throughout the growing season, weeds were manually removed from the soil chambers to prevent CO_2 uptake. After the measurements the chambers were removed, and only poplar roots were observed in the collars. More details of the R_s measurements and gap filling can be found in Verlinden et al. (2013b).

6.2.3. Environmental variables

Meteorological and soil variables were recorded half hourly at the site in one location *ca.* 30 m away from the location of the soil CO_2 measurements. Soil water content (W_s, $m^3\ m^{-3}$) was measured using a probe (TDR model CS616; Campbell Scientific) placed at 20 cm depth. Soil temperature (T_s) at 0-10 cm depth was measured using a thermocouple (model TCAV-L, Campbell Scientific, Logan, UT, USA) every 10 s and the 30 min averages were stored on a data logger. Mean W_s and T_s were calculated for the period between sampling dates (see below for more details on root sampling dates).

6.2.4. Fine root biomass

The data of the evolution of fine root biomass come from Berhongaray et al. (2013c). From 22 Feb. 2011 to 24 Jan. 2012 the upper 15 cm soil layer was sampled approximately every two weeks (a total of 21 sampling campaigns) using an 80 mm diameter 150 mm deep hand-driven corer (Eijkelkamp Agrisearch equipment, The Netherlands). At every sampling campaign 20 samples were collected half in the narrow and half in the wide inter-row spacings, randomly spread over the planting area. Fine roots (Fr; Ø < 2 mm) were picked from the sample by hand while (i) separating out weed roots from poplar roots, (ii) sorting poplar roots in two diameter classes (0-1 and 1-2 mm), and (iii) sorting poplar roots in dead and living roots. Further details on the sorting, on the washing and on the dry root

biomass determination can be found in Berhongaray et al. (2013c). There was a significant difference in Fr biomass between wide and narrow inter-row spacings when compared in a t-test (Berhongaray et al. 2013c).

6.2.5. Coarse root biomass

Coarse root woody biomass increment during the study period was estimated from allometric equations, diameter inventories and transition functions. After the R_s measurements, in February 2012, 20 trees of different stem diameters (from 20 mm to 60 mm at 22 cm height) were selected and their complete root system was excavated separately for the narrow and the wide inter-rows. The excavations were done over an area of 1.1 m × 0.375 m (planting distance in the rows × half the narrow inter-row distance) for the narrow inter-rows, and over an area of 1.1 m × 0.75 m (planting distance in the rows × half the wide inter-row distance) for the wide inter-rows. All roots within these sampling areas were collected, assuming that roots from adjacent trees compensated for roots of the selected tree growing outside the sampled area. The excavation depth was limited to 0.6 m, as very few roots were observed deeper than 0.6 m. All coarse roots (Ø > 2 mm) were sampled, and the total dry biomass of these coarse roots (Cr) was determined after oven drying at 70 ºC in the laboratory until constant weight. The allometric equations were constructed based on the excavations of tree root systems and the measured stem diameter as previously described by Verlinden et al. (2013c) and in Chapter 5.

From the stem diameter inventory described by Verlinden et al. (2013c) the average Cr biomass was estimated for both the beginning and the end of the growing season. The growth pattern of Cr between both growing seasons was simulated using a sigmoid function. Since it was not possible to monitor the evolution of Cr biomass through the growing season and since most growth processes follow a sigmoid curve, we used the evolution of leaf area index (LAI) to estimate the Cr growth (Figure 6.1). The LAI has been shown to be a reliable descriptor of aboveground growth (Broeckx et al. 2013), and we assumed that the above- and the belowground growth occurred at the same time and showed the same pattern. The LAI data were transformed to cumulative LAI values and then different sigmoid functions were fitted to the increment of cumulative LAI with time (Fig. 1). The best fit was obtained with the cumulative symmetric double sigmoid function:

$$LAI = \frac{a}{2c}\left[2d\,ln\left(exp\left(\frac{2x+c}{2d}\right)+exp\left(\frac{b}{d}\right)\right)-2d\,ln\left(exp\left(\frac{x}{d}\right)+exp\left(\frac{2b+c}{2d}\right)\right)+c\right] \quad \text{[Eq. 1]}$$

where *x* is the day of the year, *a* represents the transition magnitude or height, *b* represents the midpoint of the transition *c* controls the width of the transition, and *d* controls the shape of the transition.

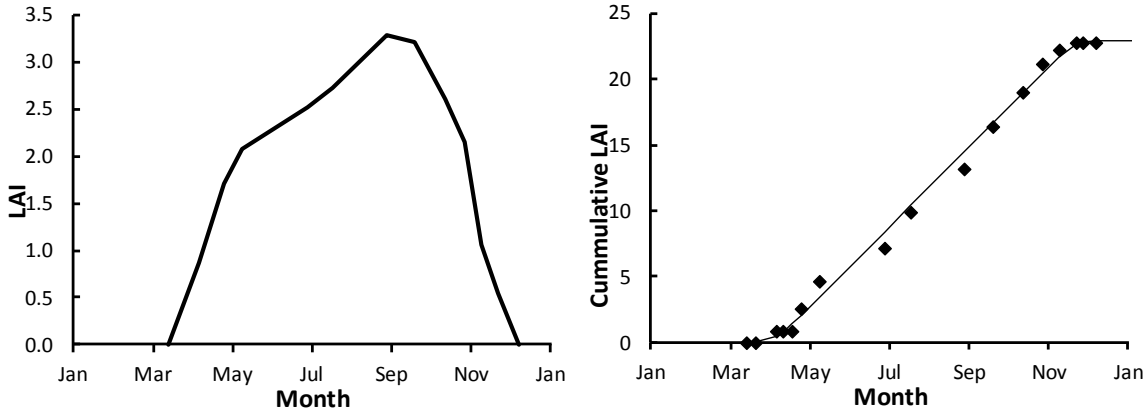

Figure 6.1: Seasonal evolution of leaf area index (LAI, left panel) and the cumulated values of LAI (right panel) of the poplar plantation during the second growing season (of the first rotation) i.e. 2011. The line in the right panel represents the sigmoid fit to the data: Eq. 1, a= 22.95, b=154.1, c= 231.4, d= 5.053. (after Broeckx et al 20112b)

Cr growth was then estimated as a function of cumulative LAI, using Eq. 1. For the Cr growth over time, parameter *a* was replaced by the Cr biomass at the end of the growing season, and the initial Cr was added to the estimations.

6.2.6. Partitioning of soil respiration

The study period included 21 sampling campaigns from 1 January 2011 until 24 January 2012, that corresponded with the 21 sampling campaigns of Fr. For our double-row poplar plantation, we have previously reported spatial differences in root biomass and production (Berhongaray et al. 2013c) and also in soil respiration (Verlinden et al. 2013b) between wide inter-rows and narrow inter-rows. For the periods between the 21 root sampling campaigns we calculated the mean F_r biomass and productivity, as well the mean C_r biomass; we cumulated the R_s and we averaged the hourly data T_s and W_s, in order to obtain one value per variable per period.

Based on the spatial and the temporal variation in root biomass and soil respiration we partitioned the R_s into heterotrophic (R_h) and root derived respiration (R_r):

$$R_s = R_h + R_r \qquad \text{[Eq. 2]}$$

The rate of CO_2 produced by the roots and by the rhizosphere (R_r) can be further separated into two major components of respiration: the maintenance and the growth component. A more complex method identified three components of respiration, namely growth, maintenance and ion uptake (Lambers et al. 2002). However, the ion uptake and root growth are closely related since both refer to plant growth (Scheurwater et al. 1998). In our case, ion uptake was integrated in the R_{gr} compartment:

$$R_r = R_m + R_{gr} \qquad \text{[Eq. 3]}$$

R_m is the CO_2 production for the maintenance of root biomass; this rate is assumed to be linearly related to the root biomass to be maintained. R_{gr} is the cost associated to form new root structures, and is assumed to be proportional to the growth rate of the roots. Only poplar fine root biomass was used for the partitioning, since weeds were manually removed from the soil chambers (see above in the description of the *measurements of soil CO_2 efflux*). The root growth rate (Gr) was determined as the slope of the total root biomass increment (*Br*) between two sampling dates of the 21 Fr sampling campaigns versus the time (*t*) between those sampling dates:

$$Gr = \frac{\Delta Br}{\Delta t} \quad \text{[Eq. 4]}$$

R_s was then calculated for each sampling period by cumulating over time since the previous sampling date and decomposed in three major components:

$$R_s = R_h + R_m + R_{gr} \quad \text{[Eq. 5]}$$

We used non-linear models to estimate R_s based on the spatio-temporal variation in root biomass and root growth, and the temporal variation in soil temperature (T_s) and in soil water content (W_s). To build the non-linear models we used information and relationships previously published in the literature. We therefore used the same parameter (β_2) to describe the sensitivity of R_h and R_m to T_s. As a result of the relationships described above in the introduction, we used the following two equations:

$$R_h = \beta_1 * e^{Ts * \beta_2} \quad \text{[Eq. 6]}$$

$$R_m = \beta_3 * B_r * e^{Ts * \beta_2} \quad \text{[Eq. 7]}$$

The respiration derived from growth is linearly related to the growth rate (Scheurwater et al. 1998) as follows:

$$R_{gr} = \beta_4 * G_r \quad \text{[Eq. 8]}$$

We designed non-linear models that either considered or did not consider the W_s effect, and both the multiplicative as well as the additive effect of W_s to the exponential response of T_s (Curiel-Yuste et al. 2003). In line with the literature, and considering equations [6], [7] and [8] above, we designed the following three non-linear models for partitioning of the R_s:

$$R_s = \beta_1 * e^{Ts * \beta_2} + \beta_3 * B_r * e^{Ts * \beta_2} + \beta_4 * G_r \quad \text{[Eq. 9]}$$

$$R_s = \beta_1 * W_s * e^{Ts * \beta_2} + \beta_3 * B_r * e^{Ts * \beta_2} + \beta_4 * G_r \quad \text{[Eq. 10]}$$

$$R_s = \beta_1 * e^{Ts * \beta_2} + \beta_3 * B_r * e^{Ts * \beta_2} + \beta_4 * G_r + \beta_5 * W_s \quad \text{[Eq. 11]}$$

The R_h component was calculated by setting B_r and G_r to zero; the R_r component was determined as the difference between R_s and R_h.

6.2.7. Statistical analysis

The optimization of the non-linear models was done following the downhill simplex method of Nelder and Mead (1965). The significance of the variables and of the parameters was determined. Slopes and intercepts of predicted vs. observed data were compared by the t-test (Fila et al. 2003). Root mean square error (RMSE) (Kobayashi and Salam 2000) was calculated for each estimation methodology and we looked at the trade-off between the goodness of fit of the model and the complexity of the model using the corrected Akaike information criterion (AICc) (Burnham and Anderson 2002):

$$AICc = -2\,ln(L) + \frac{2k\,(k+1)}{n-k-1} \qquad \text{[Eq. 12]}$$

where k is the number of parameters in the model, L is the maximized value of the likelihood function for the estimated model, and n is the number of samples.

6.3. Results

The T_s rapidly increased from about 5 ºC in March 2011 to 15 ºC in May (Figure 6.2). This fast increment coincided with a high G_r in the same period. While T_s remained constant from May to September, a severe drought period started in May, which decreased W_s and stopped G_r. This (temporary) stop in G_r was more evident in the narrow inter-rows than in the wide inter-rows. W_s started to be restored in June, followed by G_r. Throughout the whole growing season, B_r was higher in the narrow rows than in the wide inter-rows. In general, the pulses of G_r were also higher in the narrow rows. Together with the temporal differences in T_s and W_s these spatial differences in B_r and G_r allowed us to fit the non-linear models.

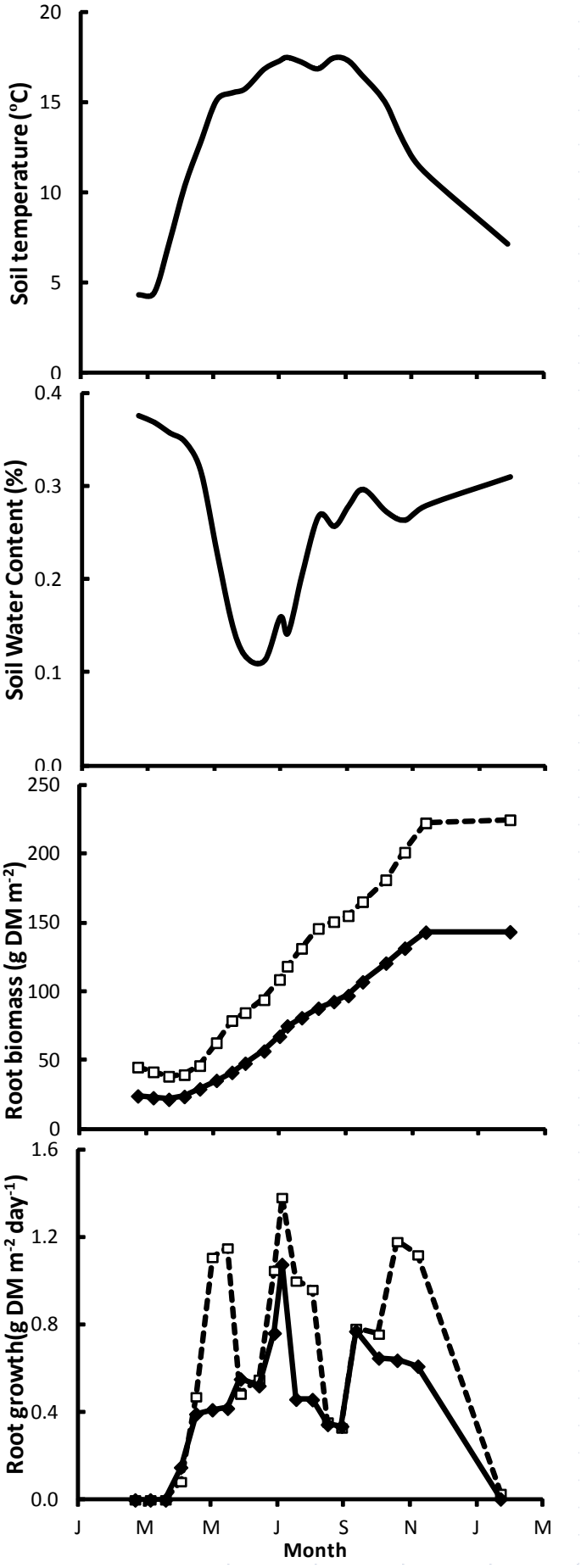

Figure 6.2: Seasonal evolution of soil temperature, soil water content, root biomass and root growth during the second year of the first rotation (2011). White squares represent the root biomass and root growth in the narrow inter-rows, and the black squares are those in the wide inter-rows.

All the models performed quite well with similar RMSE and AICc (Table 1). The AICc differences were smaller than two units, which make them very similar. Parameters β_1 and β_2 from model 3 presented very high p-values, reducing the significance of those parameters.

Table 6.1: Different simplified root respiration model of SWRC and the values of the estimated parameters. Adjusted parameters for heterotrophic, root maintenance and root growth respiration estimations, and the p-values were given. The root mean square error (RMSE) and the corrected AIC (AICc) of each model were also given.

Model	Syntax	Parameters					RMSE	AICc
		β_1 (p-value)	β_2 (p-value)	β_3 (p-value)	β_4 (p-value)	β_5 (p-value)		
Model 1	[Eq. 9]	0.225 (0.013)	0.118 (<0.001)	0.00163 (0.016)	0.835 (0.016)		0.580	31.5
Model 2	[Eq. 10]	0.510 (0.023)	0.144 (<0.001)	0.000975 (0.104)	1.43 (<0.001)		0.610	33.2
Model 3	[Eq. 11]	0.0439 (0.455)	0.182 (0.003)	0.000573 (0.338)	1.14 (0.003)	1.79 (0.032)	0.535	29.3

The three models had a tendency to underestimate R_s at high rates (Figure 6.3). The calculated fluxes showed a different annual course for each source. Regardless of the model used, R_h and the R_m varied throughout the year with minimum values in winter and peak values in summer (Figure 6.3). The measured cumulated R_s for the duration of the intensive study period was 790 g C m^{-2}, and the estimated was 810, 780 and 830 g C m^{-2} for models 1, 2 and 3 respectively. All models also estimated a peak in R_h earlier than in R_m. The calculated R_h showed a steady decrease in the relative contribution to R_s until November (Figure 6.3). In the winter (2011-2012) the relative contribution of R_h increased, but to a lower proportion as compared to the previous year, where the root mass was much lower (Figure 6.2). Mean values of R_h and R_r revealed significant exponential relations with mean T_s (Figure6.3). The second model (with W_s interactions) simulated a strong decrease in R_h during the drought period in spring 2011.

On an annual basis, R_h accounted from 41 to 51% of the total annual R_s. It varied from 82%, 86% and 95% in the first winter, and to 44%, 29% and 38% in summer for models 1, 2 and 3, respectively. The cost for root growth was estimated to be 0.87, 1.5 and 0.95 g C g^{-1} DM^{-1} for model 1, 2 and 3 respectively.

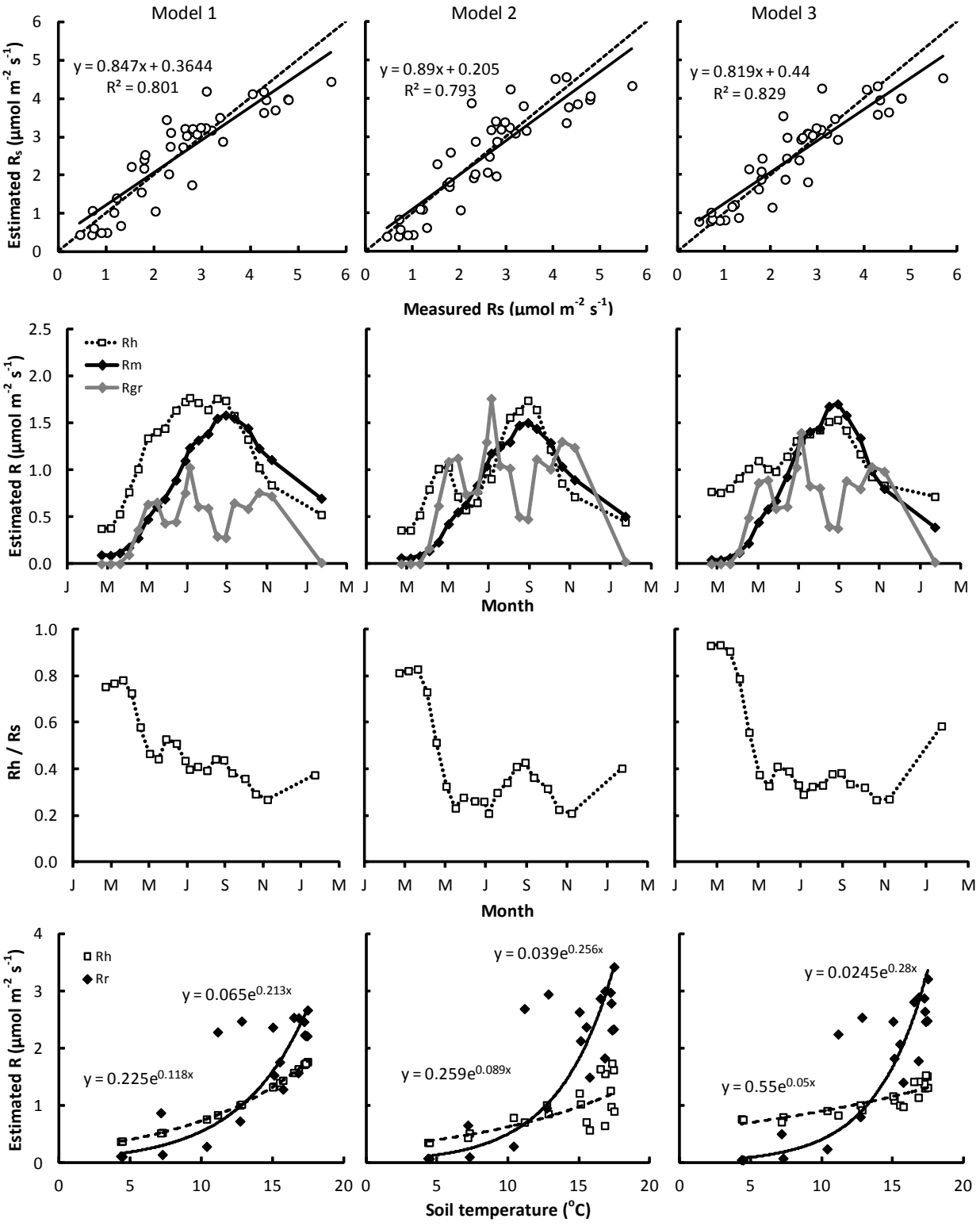

Figure 6.3: Performance and partitioning of soil respiration (R_s) components with the three models. Measured vs. predicted R_s (top row panels). Prediction of the three components: heterotrophic respiration (R_h), root maintenance respiration (R_m) and root growth respiration (R_{gr}) (second row panels). Proportion of R_h to total R_s (third row panels). Exponential curve fitting between soil temperature and root derived respiration (R_r) and R_h. (lower= bottom panels)

6.4. Discussion

The partitioning of the different R_s components using sequential sampling dates allowed us to estimate the seasonal trends in the root contribution to R_s (Figure 6.3, second row panel). The parameterisation of these non-linear models with *in situ* data has potentially a very interesting application in biogeochemical models.

Based on the spatial and temporal variation in B_r and R_s we partitioned the R_s into R_h and R_r. We assumed that R_h was equal in the narrow and in the wide inter-rows, an assumption that we consider reasonable. The soil was ploughed prior to planting and the soil was well mixed in the year before the measurements, so very small spatial differences in the soil are expected after the ploughing. Moreover, compared with the R_r, the spatial differences in R_h influencing R_s can be considered to be small. The higher R_s in the narrow rows in our plantation was reported previously by Verlinden et al. (2013b). In the present contribution, we related the higher R_s to higher B_r and G_r in the narrow inter-rows, and consequently derived the higher R_r. The tree proximity played an important role in the spatial differences in R_s. This result complements the results of Tang and Baldocchi (2005) where R_s was higher in the proximity of trees. The same authors also used a non-destructive method to partition R_s in an Oak dominated savannah.

The use of the approach presented here is more labor intense than the root regression method, as it requires sequential root data. However, many studies have field observations of sequential root data available and can potentially be used with the methodology proposed here. For example, Tomotsune et al. (2013) presented spatial and temporal variability in monthly measurements of B_r and R_s; however, instead of applying one general model, they applied a single linear model for each sampling date. This resulted in some cases in unrealistic predictions (e.g. negative R_r, because R_h was higher than R_s). The use of non-linear models requires a proper understanding of the underlying assumptions. Non-linear models provide better mechanistic insights and predictive ability, much more than can be obtained with ‘‘standard’’ linear models approaches (Ogle et al. 2014).

Depending on the model used, R_h varied from 82 to 95% in the first winter to 29-44% in summer. The contribution of R_h has been estimated to be between 10 and 90% of R_s (Hanson et al. 2000), with an average of 60 %. Our models predicted the proportion of R_h within the range of previous studies, but they also predicted that the contribution to R_s is not constant as it is occasionally assumed. The peak in R_h was earlier than the peak of R_m. Similar observations were found using the root exclusion method in a mature forest in France, where the peak of autotrophic respiration was later than that of the heterotrophic component (Moyano et al. 2008).

All the models showed a higher sensitivity of R_r to T_s than of R_h to T_s (Figure 6.3; bottom panel). It is quite common to see in the literature the use of a single, fixed coefficient (generally represented by the Q_{10}) for the exponential function between R_s and T_s. However, it has been demonstrated that this Q_{10} varies among ecosystems and across

temperature ranges (Curiel-Yuste et al. 2004). This variation was related, in a mixed temperate forest experiment, to the different temperature sensitivities of the various components of R_s (Boone et al. 1998). In this last mentioned experiment, R_r, which produced a large portion of total R_s, was more temperature-sensitive than the R_h. Model 1 only uses T_s as the environmental variable, which make it easily applicable. Soil temperature data are easily obtained as most automatic soil chambers for soil CO_2 efflux measurements have an integrated soil temperature sensor. However, if soil temperature is not available it can be estimated from air temperature measurements.

Models 2 and 3 directly include W_s for the R_h estimations, but not the model 1. The effect of soil moisture or W_s on soil respiration is well understood for water limited systems, such as the Mediterranean forests (Talmon et al. 2011). However, the moisture limitation to R_s is difficult to address in non-water limited systems such as our plantation. We therefore designed non-linear models that either considered and did not consider the W_s effect. Moreover, the effect of W_s and T_s on R_s can be additive or integrative (Talmon et al. 2011; Xu et al. 2001), and that is the difference between models 2 and 3.

In the model syntax there is no apparent inclusion of the effect of W_s on R_r, but in reality it was indirectly included in the root growth compartment. W_s affected root growth (Broeckx et al. 2013), and indirectly affected the R_r (see Barba et al. 2013 for a detailed discussion of this indirect effect). This was evidenced in the G_r and R_{gr} patterns. The obvious decrease in G_r in the narrow inter-rows, as compared to the wide inter-rows, could be explained by a faster water depletion in the narrow inter-rows, where trees were closer to each other. Most probably the trees first consumed the water available in the narrow inter-rows, and then they explored the wide inter-rows. This could differently affect the course of W_s in the narrow vs. the wide inter-rows, but unfortunately we cannot confirm this hypothesis since the W_s data were not partitioned per row. At a scale of meters, Tang and Baldocchi (2005) did not find significant and systematic differences in W_s along a transect of tree distances.

Considering the average C fraction of 0.40 (Berhongaray et al. 2013c), the root growth respiration coefficient ranged from 2.1 to 3.6 g C respired per g root C produced. This is by far higher than the root growth respiration coefficients of 0.28 (Ågren et al. 1980) used for roots of a Scots pine forest in Belgium (Janssens et al. 2002). This calculated C use efficiency for root growth proposed by Ågren et al. (1980) was re-examined and suggested to be far higher than reasonable (Hogberg et al. 2002). However, other estimations of growth respiration coefficient for a herbaceous vegetation, for a mature forest and for a young Eucalyptus cuttings are also in that range, from 0.21 to 0.29, and much smaller than our prediction (Bouma et al. 1996; Mata et al. 1996; Thongo M'Bou et al. 2010). Global estimates of forest belowground C use efficiency (which includes roots and mycorrhizae) of 0.50 have been reported that did not differ between forest types, but this might depend on the applied methodology, and the coefficients could vary from 0.35 to 0.55 (Chen et al. 2011). In conclusion, caution has to be taken to use our estimated coefficient for root growth respiration since they are much higher than previously reported values.

High plant productivity is often associated with a high photosynthetic capacity per unit of leaf area. Recently, better insights in respiratory processes have been available. This aspect warrants at least an equal attention, as under optimal nitrogen supply, up to 50% in young vegetative plants (van der Werf 1996) and 42% in mature forest (Vicca et al. 2012) of the daily assimilated carbon is consumed in autotrophic respiration. A fast growth can only occur if resources are acquired at a minimum carbon cost (i.e. with minimum respiration), but information on the costs of roots remains scarce (Vicca et al. 2012). At the regional level, the ecosystem carbon balance of European forests is determined by total respiration (Valentini et al. 2000), which is basically dominated by soil respiration (Janssens et al. 2001). So, a proper understanding of plant respiration (including root respiration) is crucial for the selection of fast growing trees for higher yields, and for a better understanding of the carbon balance in the context of bioenergy production.

6.3. Conclusion

We made use of high-resolution measurements of root biomass and R_s to partition R_s in its components, using three different non-linear models. This partitioning is essential for the evaluation of the potential of SRWC for two reasons. First, it is crucial a more accurate quantification for the ecosystem C budget. And secondly, it is fundamental for the understanding of plant respiration and the costs of growth within the context of the selection of fast growing trees for higher yields.

Chapter 7

7. Synthesis: toward a belowground carbon balance

7.1. Introduction

The overall framework, the theoretical background and the objectives of the thesis were presented in Chapter 1. In this synthesis chapter we combine results of the five main 'results' chapters of the thesis, i.e. Chapters 2-6, with some new data. Chapters 2 to 6 present research findings from the first rotation of the SRWC poplar plantation. In the second rotation (2012-2014), the same measurements were repeated and included in this synthesis chapter to present a complete analysis of the four years of the SRWC experiment (two two-year rotations). Each of the previous chapters contains its own literature study, materials and methods, results and discussion. This synthesis was prepared as a separate stand-alone chapter and we present a summary of the methodology and the results; for specific details we refer to the corresponding chapter. In this synthesis the research findings of our large-scale project are discussed in view of the relevant literature. We also incorporate a general discussion about the potential of the SRWC approach, and we end with concluding remarks and perspectives.

7.2. Materials and Methods

7.2.1. Experimental site

The experimental site for this thesis was described in detail in Chapter 1. Briefly, in April 2010, 18.4 ha of agricultural land (including former cropland and pasture) were converted to SRWC. Twelve poplar (*Populus sp.*) and three willow (*Salix sp.*) genotypes were planted in monoclonal blocks in a double-row planting scheme. The large-scale SRWC plantation in East-Flanders (Belgium) was managed in two-year rotation cycles, for two rotations (four years in total; 2010-2014). Because of the high labor intensity, and in order to control the variability caused by the different species and genotypes, only two poplar genotypes were intensively measured for the belowground carbon inventory: i.e. Koster (*P. deltoides* Marsh x *P. nigra* L.) and Skado (*P. trichocarpa* Hook. x *P. maximowiczii* Henry).

7.2.2 Carbon pools

Soil organic matter

The C content in the soil organic matter (SOM), known as the soil organic C (SOC), was assessed before the plantation establishment (March 2010) and after the second rotation (March 2014). A random sampling was performed at 110 locations in March 2010, of which 60 locations matched with the current distribution of the two studied genotypes Skado and Koster. These 60 locations were revisited and the soil was re-sampled in March 2014, of which half were sampled in each former land-use type, and within each land-use type half in each row spacing. In March 2010 the soil was sampled up to a depth of 90 cm, while the repeated sampling in March 2014 was only up to 60 cm depth. In both campaigns, an aggregate sample was taken every 15 cm by core sampling (Eijkelkamp Agrisearch equipment, The Netherlands). Bulk density (BD) samples were taken independently in each campaign. C mass fractions were determined in three replicates per sample (see below under section *7.2.4 Chemical analysis of soil and biomass samples*). From the C mass fractions and BD, the carbon pool per 15 cm depth interval was calculated and cumulated over 90 cm for the 2010 samples and over 60 cm for the 2014 samples. SOC data were transformed to equivalent soil mass to account for differences in BD between the treatments (i.e. previous land-use type and row spacing). The estimations of SOC at equivalent soil mass were performed for masses of 200, 400, 650 and 900 kg m^{-2} using spline functions as described previously in Berhongaray et al. (2013a). The soil mass was used as the independent variable and SOC as the dependent variable. Interpolations were made by adding or by removing a portion of the soil to reach the desired soil mass assuming that transitions between soil layers were smooth and continuous.

Stumps, coarse and medium-sized roots

Woody root biomass was determined by excavation of the root system immediately after the two harvests. In February 2012, five trees of different stem diameters (from 20 mm to 60 mm at 22 cm height above the soil) were selected within both genotypes (Koster and Skado) for each of both former land-use types. In February 2014, only four trees per genotype and per land-use type were excavated. In both excavation campaigns, the remaining stumps (Stu) and roots were excavated over an area of 1.1 m x 1.125 m (planting distance in the rows x sum of half inter-row distances). All roots within this area were collected, assuming that roots from adjacent trees compensated for roots of the selected tree growing outside the sampled area. The excavation depth was limited to 60 cm, as very few roots were observed under 60 cm (Chapter 5). Coarse roots (Cr; Ø > 5 mm) and medium-sized roots (Mr; Ø = 2-5 mm) were sampled; total dry biomass of these roots (Cr) and of the remaining 15 cm high stump was determined after oven drying at 70°C. Since no significant effect was found for genotype or former land-use type, all data were pooled. Belowground woody biomass and stump biomass were plotted against stem diameter for the first rotation and against basal area for the second rotation. An allometric power regression was fitted. An estimation of the average belowground woody biomass and

stump biomass pool was made from the diameter inventory of each sampling year, i.e. winter 2012 and winter 2014 as was already explained in Chapter 5. Dried root wood was grated for CN-analysis. An average of the C mass fractions was used for calculating the belowground woody C pool.

Fine roots

The fine root (Fr, Ø < 2 mm) biomass pool was annually estimated using the soil core methodology. Intact soil samples were taken using an 8 cm diameter x 15 cm deep hand-driven corer (Eijkelkamp Agrisearch equipment, The Netherlands) at the end of each growing season, i.e.: winter 2011 (Dec. 2010 – Feb. 2011), winter 2012 (Dec. 2011 – Feb. 2012), winter 2013 (Dec. 2012), winter 2014 (Dec. 2013 – Jan. 2014). Winter samples were taken only in the first 15 cm. Samples from different depths were collected in two summers, i.e.: Aug. 2011 and Aug. 2012. In Aug. 2011 sampling was performed in six different soil layers (0-15 cm, 15-30 cm, 30-45 cm, 45-60 cm, 60-75 cm and 75-90 cm, whereas in Aug. 2012 four different soil layers (0-15 cm, 15-30 cm, 30-45 cm, 45-60 cm) were sampled. During each sampling campaign, samples were transported to the laboratory and stored in a freezer until processed. All roots were picked from the sample by hand while (i) separating out weed roots from poplar roots, (ii) sorting poplar roots in dead and living roots, and (iii) sorting poplar roots in diameter classes. The roots were sorted by visual inspection as described in Chapter 3, as well as in Berhongaray et al. (2013c). Following washing, fine poplar roots were oven dried at 70°C for 1-4 days to determine the standing root biomass per soil surface area and expressed in g DM m^{-2}. More details on root collection and on data processing can be found in Chapters 2 and 3, as well as in Berhongaray et al. (2013c; 2013d).

7.2.3 Carbon fluxes

7.2.3.1 Belowground inputs

Fine roots

Sequential soil coring was used to determine Fr mass and Fr production for the second growing season of the first rotation (i.e. 2011) and the first growing season of the second rotation (i.e. 2012). From Feb. 2011 to Nov. 2012 the upper 15 cm of soil layer was sampled every 2-3 weeks (except for the winter when we decreased the sampling intensity) using an 8 cm diameter x 15 cm deep hand-driven corer (Eijkelkamp Agrisearch equipment, The Netherlands). During 2011, 20 samples were collected at every sampling campaign for each genotype. During 2012, the number of samples was different at each sampling date, following the expected intrinsic variability of the Fr biomass based on the experience of the previous year (i.e. 2011). Based on our previously described approach and methodology (Berhongaray et al. 2013d; Chapter 2) the number of samples in 2012 varied from 12 in winter to 20 in summer. At each sampling campaign in 2011 and in 2012, half of the samples were collected in the narrow and half in the wide rows, randomly

distributed over the planted area within the former pasture land-use type. The samples were transported to the laboratory and stored in a freezer until processed. Once in the laboratory, fine roots were picked from the sample by hand while (i) separating out weed roots from poplar roots, and (ii) sorting poplar roots in dead and living roots. The sorting of dead and living roots was based on the darker colour and the poorer cohesion between the cortex and the periderm of the dead roots (Janssens et al. 1999). Following washing, fine poplar roots were oven dried at 70°C for 1-4 days to determine the dry root biomass per soil surface area. Fr weight of one sample core picked for *x* min (i.e. 5 to 20 min) was converted into total Fr mass in the sample (i.e. after 60 min picking duration) using Richard's equation (Berhongaray et al. 2013d) and expressed in g DM m^{-2}. Subsamples of dried roots were ground for C and N-analysis. More details on root collection and data processing can be found in Berhongaray et al. (2013c; 2013d; Chapters 2 and 3).

For 2011 and 2012, root production (*P*) was calculated using the "desicion matrix" approach (Fairley and Alexander 1985). All differences in biomass and necromass were taken into account during the calculation, assuming that the living and dead pools were continuously changing. This approach was better than using the significant differences between root mass of consecutive sampling dates, especially in the case of high frequency resolution sampling (Brunner et al. 2013), such as in our sampling campaign. For the annual production calculation, all productivity values from sampling periods were summed up from the beginning to the end of the year. More details on root productivity calculations and on the comparison of different methods can be found in Berhongaray et al. (2013c; Chapter 3).

In the second growing season of the second rotation (2013), Fr production was estimated with the in-growth core technique. In Dec. 2012, ten 2.2-mm mesh-bags (10 cm diameter × 0.40 m depth) were installed for each genotype, 20 in total. Each mesh bag was refilled with root-free original soil obtained from the root biomass assessment (see above under section 7.2.2.3). Root-free soils were stored in plastic bags and care was taken to refill the holes with exactly the same stratification. The in-growth cores were harvested after one year in Dec. 2013. The in-growth cores were divided in two samples from 0-15 cm depth and from 15-30 cm depth, and the separated samples were stored in plastic bags until processed. Consequently, only the first 30 cm of the in-growth cores was used to make it comparable to the 15 cm increment soil coring approach, and the bottom 10 cm of the in-growth cores (from 30 to 40 cm) were discarded. The samples were processed in the same way as the samples from the soil coring approach. The P was estimated from the quantity of total root mass produced (biomass and necromass) in the considered period of time and expressed in g DM m^{-2} y^{-1}.

The turnover time is widely used to estimate root derived C inputs to the soil. An assumption of this root turnover method is that annual fine root production equals fine root mortality on an annual basis. However, the approach of the turnover rate is only valid in steady-state systems, as e.g. mature forests, but not in growing systems such as our SRWC poplar plantation. In mature forests, the same amount of roots produced, die at the

end of the growing season and represents the C inputs. In a growing system, part of the productivity is used to form the growing standing biomass. We used the following methodology to estimate C inputs from roots (I_{root}) that consider the increments in root biomass:

$$I_{root} = (P - \Delta Br) * C\% \quad \text{[Eq. 1]}$$

where *P* is the root productivity in g DM m^{-2} y^{-1}; ΔBr is the difference between root biomass at the end and at the beginning of the growing season in g DM m^{-2} y^{-1}; and C% is the fraction of carbon (g C g DM^{-1}). In our study this methodology only applies to the fine roots. Since no mortality was evidenced in medium-size and coarse roots, the productivity of these last mentioned root classes equals the ΔB and the C input is zero.

From the in-growth technique we got evidence for the same vertical distribution of Fr and root P, *i.e.* the proportion of P at one specific soil depth was similar to the proportion of Fr at the same depth. For years 2011 and 2012 the C inputs from Fr were extrapolated up to 60 cm depth using the measured P from the first 15 cm and the vertical distribution of Fr in each year.

7.2.3.2 Aboveground inputs

Leaf fall

Leaf litter was collected during the period of leaf fall from early Sep. to Dec. in two plots of 5 x 6 trees for each genotype within each former land-use type (n=8). In each plot three perforated litter traps (litter baskets) of 57 cm × 39 cm were placed on the ground along a diagonal transect between the rows covering the wide and the narrow inter-row spacings. Every two weeks the litter traps of each plot were emptied and leaf dry biomass was determined after oven drying at 70°C. Collected leaf biomass was cumulated over time to obtain the yearly leaf C input (I_{leaves}).

Weeds

Aboveground biomass from weeds was measured after they reached the maximum standing biomass (after flowering) only in two growing seasons, *i.e.:* Aug. 2011 and Aug. 2013. In 2011, six randomly distributed plots of 1 m^2 were harvested under each genotype and from the previous pasture land area. In 2013, four plots of 1 m^2 were harvested under each genotype and previous land-use type combination, *i.e.* 16 plots in total. In each plot, the weeds were cut at ground level and placed in paper bags. The collected weed biomass was dried and the DM expressed in g DM m^{-2}. Annually the weeds died and the total biomass was considered as an input to the soil. We estimated the aboveground annual C input from the weeds (I_{weed}) assuming a C mass fraction of 50% (Larcher 2003).

Harvest losses

The two harvests took place after the first rotation on 2-3 Feb. 2012, and at the end of the second rotation on 18-20 Feb. 2014. Harvest losses were estimated from samples collected at the field site after both harvests, i.e. early March 2012 and mid March 2014. Two different harvest techniques were used and compared during each harvest, two mechanical harvesters in Feb. 2012, and a mechanical vs. a manual harvesting in Feb. 2014. To estimate the harvest losses, harvested woody debris and woody biomass material were collected from the soil surface on four areas of 1 m^2 within the land area harvested by each harvesting technique for the two genotypes. The collected biomass material and debris were transported to the laboratory and dried in a drying oven at 60-70^{0}C until constant weight. The harvest losses were expressed in g DM m^{-2}, and later expressed as C inputs ($I_{harvest}$) using the C mass fraction. More details can be found in Chapter 4 (Berhongaray et al. 2013b).

7.2.3.3 Carbon outputs

Soil CO_2 efflux

Soil CO_2 efflux was continuously monitored using an automated soil CO_2 flux system (LI-8100, LI-COR Biosciences, Lincoln, NE, USA). Sixteen long-term chambers operating as closed systems were connected to an infrared gas analyzer through a multiplexer (LI-8150, LI-COR Biosciences, Lincoln, NE, USA). The 16 chambers were spatially distributed over the plantation (Figure 7.1). Soil CO_2 efflux was extrapolated for the periods without measurements by a Neural Network analysis based on soil temperature, which was also continuously monitored throughout the year. Values of CO_2 efflux where integrated over time to obtain the cumulated CO_2 efflux. More details can be found in Verlinden et al. (2013b) and in Chapter 6.

Figure 7.1: The LI-8100 soil chambers in operation at the field site (photo M.S. Verlinden).

Partitioning of soil respiration

To calculate the SOC balance (see below under section 7.2.5. Carbon balance) we quantified the contribution of roots and SOM decomposition to the CO_2 emission from the soil. The

soil CO_2 efflux (R_s) is the results of CO_2 release coming from two main sources: (i) microbial decomposition of SOM (heterotrophic respiration, R_h) and (ii) root derived respiration (autotrophic respiration). We partitioned R_s based on the spatial and the temporal variation in root biomass, in soil temperature, in soil water content and in soil respiration, following the methodology described in Chapter 6, as follows:

$$R_s = R_h + R_m + R_{gr} \quad \text{[Eq. 2]}$$

where R_m is the CO_2 from the maintenance of root biomass, this rate is assumed to be linearly related to the root biomass to be maintained; R_{gr} is the cost of the formation of new root structures, and is assumed to be proportional to the growth rate of the roots; R_h is consequently assumed to be the C output from the SOM pool. The results were annualized and expressed in g C m^{-2} y^{-1}.

Dissolved organic carbon

For the analysis of dissolved organic carbon (DOC) in the soil, 10 groundwater samples were taken monthly from Aug. 2011 until July 2013 from six PVC water tubes (length x diameter: 2 m x 5 cm) distributed randomly under the two genotypes. Water samples were collected using a 2 m plastic tube connected to a glass bottle by applying a vacuum of 60 kPa. After collection, the samples were stored at 4°C and sent to an external laboratory (SGS, Antwerp, Belgium) within the next 24 hours. DOC concentrations were determined with a Shimadzu TOC VPH analyzer (Shimadzu corp., Japan, 2001) with IR detection after thermal oxidation.

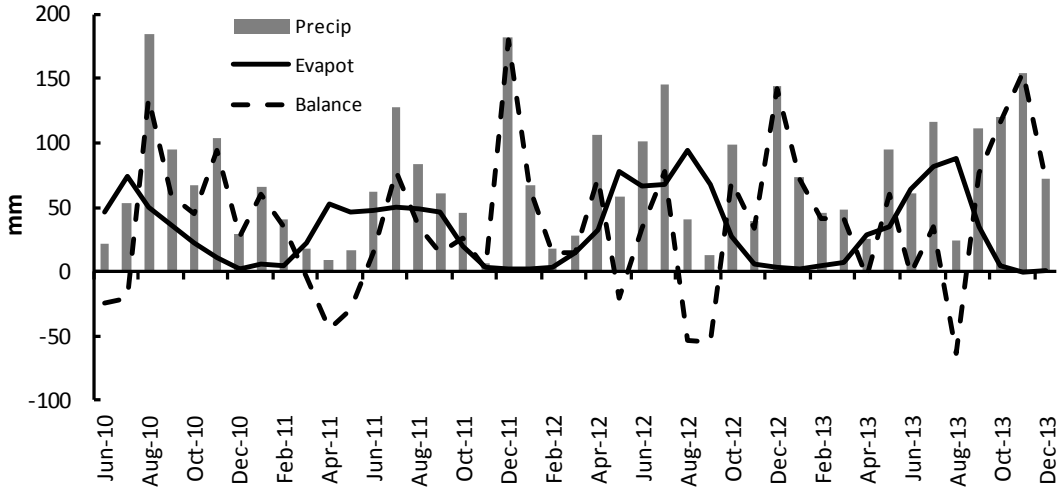

Figure 7.2: Course of the monthly precipitation, evapotranspiration and water balance during the four years of the two rotations.

Leaching from the belowground system (see below section 7.2.5 for a description of the system) was estimated using DOC concentrations and the soil water balance. The soil water balance was calculated as the difference between the monthly cumulative precipitation minus the monthly evapotranspiration, considering positive values as water excess and leaching (Figure 7.2). Precipitation was monitored from June 2010 onwards using a tipping

bucket rain gauge (model 3665R, Spectrum Technologies Inc., Planfield, USA) installed next to the eddy covariance mast. A LI-7000 fast response gas analyzer (LiCor, Lincoln, USA) was used to continuously measure latent heat from air samples at the eddy covariance mast from June 2010 onwards. Latent heat flux was converted into evapotranspiration using air temperature and latent heat of vaporization. The annual leaching of DOC was calculated by summing the monthly products of DOC concentrations and water excess. For months without DOC data, the average DOC concentration was used. The annual DOC leaching was also calculated using annual averages of DOC concentration, and annual precipitation and evapotranspiration.

7.2.4 Chemical analysis of soil and biomass samples

Soil samples as well as dried biomass from wood, leaves and roots were grated and analyzed by dry combustion with an NC element analyzer (NC-2100 element analyzer, Carlo Erba Instruments, Italy). Soil and plant mass were converted to C mass using the average C mass fraction, and expressed in g C m^{-2}. For the upscaling we used averages per land-use type and per genotype. The means from different row spacings were calculated separately and then the scaled-up averages were calculated taking into account the proportion of the land area each row spacing occupied.

7.2.5 Carbon balance

Inventory of belowground C pools and fluxes

The boundaries of the belowground system that we considered for our carbon balance were the top of the soil surface and a soil depth of 60 cm. In line with the conservation mass balance theory, the outputs from the belowground system should be equal to the inputs minus any change in storage over a defined time period. Therefore, C released from the soil (R_S) or lost in the leaching (DOC) should be equal to the inputs from aboveground poplar leaf litter, from harvest losses, from annual weed biomass plus the total belowground C allocation (TBCA) to roots from poplar trees minus any change in the belowground C pools (SOM, Stu, Cr, Mr, Fr) per unit of time (Figure 7.3).

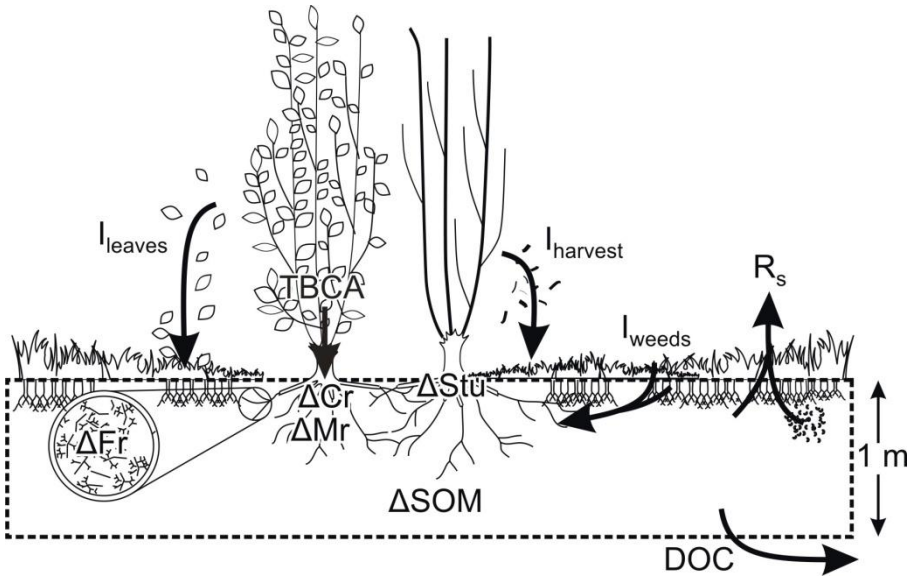

Figure 7.3: Schematic representation of the belowground carbon balance approach showing the fluxes that have been quantified. The dashed lines indicate the boundaries of the belowground compartment. Acronyms and abbreviations have been defined in the text.

SOM carbon balance

The SOM C balance, also referred to as SOC balance, is the balance between the C inputs and losses from the SOM pool only (Figure 7.4). Unlike the belowground C balance (Figure 7.3), the SOC balance considers respiration (R_h) from the SOM decomposition only; C inputs from the roots are included and TBCA is not required.

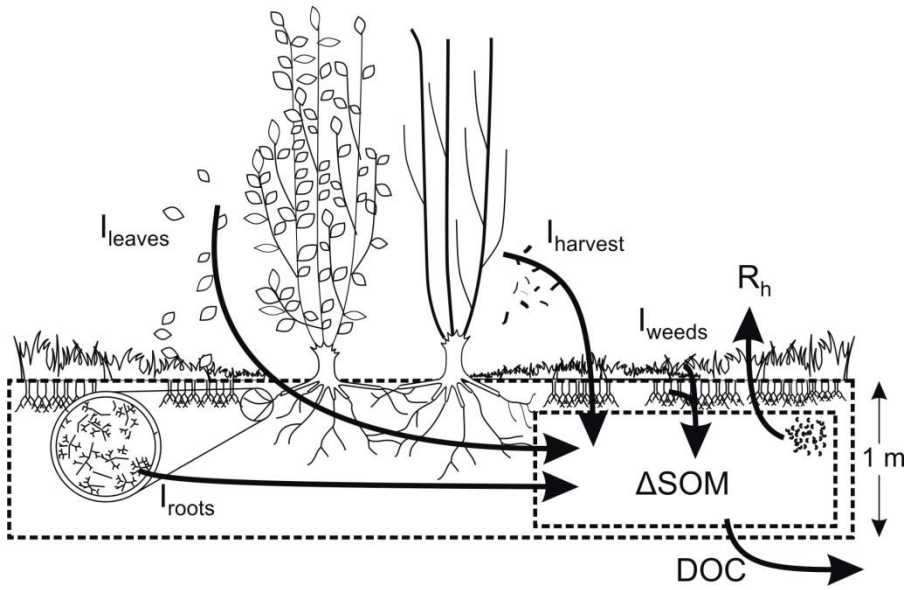

Figure 7.3: Representation of the soil organic matter (SOM) C balance approach. The dashed lines around Δ-SOM indicate the boundaries that are being considered for the SOM C balance. All acronyms and abbreviations have been defined in the text.

7.2.6 Statistical analyses

Data were analyzed with different lineal models. Mostly a two-way analysis of variance (ANOVA) was performed using land-use type and genotype as fixed factors, also including

their interactions. More complicated models considered climate, plant and soil variables. These were tested as covariates ($p \leq 0.05$) and included in the model when significant. In the case of a significant genotype effect, pairwise comparisons were performed using a Tukey post-hoc test ($p \leq 0.05$). Regression and correlation analyses were performed to search for relationships between variables, which significance was tested by an F test ($p \leq 0.05$).

7.2.7 Uncertainty analysis

The primary obstacles for applying the C balance approach were: (i) the quantification of the annual fluxes of the inputs, the outputs and the changes in the C pools with a reasonable precision, and (ii) the accumulation of errors in the calculation of the C balance as a sum of many components, each with their own error. These errors were due to the intrinsic variability of the measured variable and to errors in the measurements. Furthermore in our SRWC plantation spatial variability was generated by the double row planting system, by the different genotypes and by the previous land-use types.

A sensitivity analysis was carried out to assess the effect of the estimations of the variables and the assumptions on the obtained results. The elasticity method (i.e., the ratio of the change in the results to the change in the input data) was used to perform the sensitivity analysis. The ratio of change of the data was calculated using the range provided by either the different former land-use types, by the different genotypes or due to the intrinsic variability of the estimations. When no differences between genotypes were found in a certain variable, for example Fr, we used the standard error as the range of change in the input data. In case we found differences between the previous land-use types, we used the range provided by the mean of each land-use type. Similarly as for the land-use types all other comparisons were made, as in the case of genotypes, of harvesting method, etc.

7.3. Results

7.3.1 Carbon pools

As for nearly all terrestrial biomes, the largest C pool in the soil was situated in the SOM. The SOC pool in the first 60 cm of the soil before the planting rounded 10.3 kg C m^{-2} (103 Mg C ha^{-1}) versus 14.0 kg C m^{-2} (140 Mg C ha^{-1}) after four years of SRWC (Table 7.1). Changes in BD were also observed, especially in the wide rows. Before planting, the vertical distribution of C differed between both land-use types. In the first layer (0-15 cm) the C% was higher in previous pasture, while in the second layer (15-30 cm) the C was higher in previous cropland. This vertical distribution was disrupted during the ploughing just before the planting of the SRWC. Furthermore, in 2014 the C% was higher in the second layer of the previous pasture as compared to the previous cropland indicating that the soil was ploughed upside down. The soil layer that was the top layer in 2010, has been found in 2014 at a depth of approx. 30 cm. After the conversion to SRWC the C% presented a clear spatial distribution, with higher values in the narrow rows than in the wide rows.

Table 7.1. Soil bulk density (kg dm^{-3}), carbon concentrations (%) and carbon content (kg m^{-2}) in the SOM at different depths before the planting (2010) and after four years of SRWC (2014). No differences were detected between genotypes (Skado and Koster) and data were pooled. The means are presented for both previous land-use types, and for both narrow and wide rows. Values from narrow and wide rows were averaged taking into account the proportional area they occupied per m^{-2}. BD= bulk density; C= carbon.

	2010						2014									
	Cropland			Pasture			Cropland					Pasture				
							Narrow		Wide		Average	Narrow		Wide		Average
Depth	BD	C		BD	C		BD	C	BD	C	C	BD	C	BD	C	C
cm	kg dm^{-3}	%	kg m^{-2}	kg dm^{-3}	%	kg m^{-2}	kg dm^{-3}	%	kg dm^{-3}	%	kg m^{-2}	kg dm^{-3}	%	kg dm^{-3}	%	kg m^{-2}
0 - 15	1.48	1.54	3.40	1.29	1.91	3.69	1.47	1.50	1.49	1.47	3.30	1.47	1.43	1.49	1.46	3.24
15 - 30	1.44	1.41	3.04	1.43	1.29	2.77	1.12	1.38	1.53	1.40	2.91	1.12	1.71	1.53	1.76	3.65
30 - 45	1.48	1.07	2.36	1.45	1.10	2.39	1.79	1.46	1.72	1.27	3.48	1.79	1.43	1.72	1.48	3.82
45 - 60	1.48	0.80	1.78	1.49	0.98	2.19	1.73	1.08	1.78	1.01	2.74	1.73	1.63	1.78	1.22	3.59
60 - 75	1.59	0.57	1.36	1.51	0.57	1.30										
75 - 90	1.56	0.35	0.81	1.58	0.36	0.85										

When SOC changes were analyzed at the same soil mass (Table 7.2), both former land-use types lost C in the first (or top) layer (carbon loss of 0-200 kg m^{-2}~ 0-15 cm). In the former cropland C losses were also found in the second layer (200-400 kg m^{-2}~ 15-30 cm). However, after losses in the first layers, we found an accumulation of C in the deeper layers for both land-use types. An overall sequestration of C was found in the entire soil profile (0-900 kg m^{-2}~ 0-60 cm) with repeated soil samplings. At equivalent soil masses the SOC pool in the 0-900 kg m^{-2} (0-60 cm) layer before the planting rounded 11080 g C m^{-2} and increased to 11980 g C m^{-2} after four years of SRWC (Table 7.2). The higher SOC sequestration was evidenced in the previous cropland.

Table 7.2. Carbon in SOM at equivalent soil mass before planting (2010) and after four years of short rotation woody crop (2014). No differences were detected between genotypes (Skado and Koster) and data was pooled and the mean are presented. The difference between 2010 and 2014 (Δ) is also given.

	2010		2014		Δ	
Soil mass	Cropland	Pasture	Cropland	Pasture	Cropland	Pasture
kg m^{-2}	kg C m^{-2}					
0-200	3.08	3.79	3.02	2.94	-0.07	-0.85
200-400	2.88	2.58	2.78	2.82	-0.10	0.24
400-650	2.78	2.75	3.66	3.26	0.88	0.52
650-900	1.99	2.33	2.75	2.72	0.76	0.40
0 - 900	10.72	11.44	12.21	11.75	1.48	0.31

The total accumulation of C in Cr, Mr and Stu after four years of SRWC was smaller than the changes in SOC (Table 7.3). The annual change in C stored in the Cr averaged 18.4 g C m^{-2} y^{-1}. This annual change in C was much larger in genotype Skado on the previous cropland, with 22.5 g C m^{-2} y^{-1}, than in the other treatments, which averaged 17.0 g C m^{-2} y^{-1} (data not shown). The higher Cr for "Skado cropland" per unit of land area (i.e. m^{-2}) compared to "Skado pasture" could be explained by the lower tree mortality that resulted in a higher plant density per area (see Chapter 5). The belowground woody biomass (Cr + Stu) increased by 30% after the first rotation. By the fourth year, the plantation had sequestered a total of 240 g C m^{-2} in belowground woody biomass. The Mr

biomass remained constant between both sampling campaigns. The Mr biomass represented about 22% of the total root biomass. Fine root biomass at a depth of 0-15 cm increased from winter 2010 until winter 2012. There was no significant increase of Fr biomass, even a small reduction, in winter 2013. This small reduction was observed during the growing season of 2012, i.e. the year after the harvest (winter 2012). The data of Fr biomass in winter 2014 indicate that there was a large increment in Fr in the last growing season (i.e. 2013). In general, Fr biomass was lower in previous pasture than in previous cropland. After the four years of SRWC (i.e. in winter 2014) Fr biomass in previous cropland was almost twice the biomass in previous pasture land (data not shown). No differences were found in Fr between both genotypes. Among the plant C pools belowground the highest amount of C was stored – after four years of SRWC – in the woody biomass (Cr and Stu), followed by the Fr and Mr.

Table 7.3. Carbon pools belowground: fine roots (Fr), medium-size roots (Mr), coarse roots (Cr), stumps (Stu) and soil organic matter (SOM), before planting (winter 2010) and at the end of each growing season (winters 2011, 2012, 2013 and 2014). No differences were detected in Fr for genotypes (Skado and Koster) under both previous land-use types (cropland and pasture). Fr data was pooled and the mean and SE are presented. For the other pools, significant differences were detected; the mean and the range given by the mean values of the combination of genotype*land-use type are presented.

		Fr (0-1 mm)		Fr (1-2 mm)		Mr (2-5 mm)		Cr (>5 mm)		Stu		SOM	
	Depth	*mean*	*SE*	*mean*	*SE*	*mean*	*range*	*mean*	*range*	*mean*	*range*	*mean*	*range*
								g C m^{-2}					
Winter 2010	*0-15 cm*	0.00	-	0.00	-	0.00	-	0.00	-	0.00	-	3472.5	(3260 - 3700)
	0-60 cm	0.00	-	0.00	-	0.00	-	0.00	-	0.00	-	10325	(9570 - 11600)
Winter 2011	*0-15 cm*	4.5	(±1.48)	1.2	(±0.29)	-	-	-	-	-		-	-
	0-60 cm	-	-	-	-	-	-	-	-	-	-	-	-
Winter 2012	*0-15 cm*	14.2	(±0.77)	7.1	(±1.28)	18.2	(26 - 65)	40.1	(27 - 51)	-	-	-	-
	0-60 cm	33.9	-	21.0	-	41.2	(74 - 118)	51.9	(33 - 67)	129.3	(93 -156)	-	-
Winter 2013	*0-15 cm*	10.4	(±0.92)	6.1	(±0.81)	-	-	-	-	-	-	-	-
	0-60 cm	24.8	-	12.0	-	-	-	-	-	-	-	-	-
Winter 2014	*0-15 cm*	22.6	(±1.96)	13.2	(±4.03)	19.6	(32 - 68)	34.8	(32 - 43)	-	-	3241.65	(3170-3330)
	0-60 cm	54.1	-	26.0	-	41.4	(86 - 120)	73.6	(66 - 90)	167.6	(152 - 205)	14045.6	(11000-15260)

7.3.2 Carbon inputs

The annual leaf fall represented the largest C input to the soil. The total amount of leaf fall increased with the age of the trees, from 2010 to 2013. This C input was exceeded only by the aboveground inputs from weeds in the former pasture land in 2011 and by the Fr in the year 2012, just after the harvest. After the first harvest we evidenced a very high Fr mortality that resulted in a large C input into the soil. During the early stages of land conversion from agriculture to the SRWC, annual soil C inputs from weed roots far exceeded those from the poplar trees (Table 7.4). This was more evident in the former pasture land than in the previous cropland. The contribution of weed inputs decreased as trees grew older and bigger, while the harvest losses increased. However, the C inputs to the soil after both harvests strongly depended on the operated harvesting machine. The losses during the harvesting reached up to 10.7% of the potential harvestable aboveground biomass with the self-propelled cut-and-chip harvester (see Chapter 4). These C inputs due to the harvest losses were as high as the Fr C inputs.

Table 7.4: Inputs and outputs (release) of carbon(C) from the belowground soil system for both previous land-use types. Data from differen genotypes (Skado and Koster) was pooled and the means are presented. nd= no data. The acronyms and abbreviations have been explained in the text. Harv = losses after harvest. All values are in g C m^{-2} yr^{-1}. Total cumulative values over four years and the annual SOC balance are presented in bold.

	Aboveground inputs			Belowground inputs		Output			SOC
	Leaves	Weeds	Harv	Weeds	Fr	Rs	Rh	DOC	**Balance**
Pasture									
2010	43	*nd*	-	*nd*	*nd*	*nd*	*nd*	6.9	***nd***
2011	151	231	23.9	67.5	18.5	674	326	7.3	**158.8**
2012	156	*nd*	-	63.4	130.8	563	233	10.0	**106.9**
2013	191	7.4	43.4	8.8	40.5	612	253	13.5	**24.8**
Total	**541**	**238**	**67.4**	**139.7**	**189.8**		**812**	**37.8**	**326.6**
Cropland									
2010	34	*nd*	-	*nd*	*nd*	*nd*	nd	6.9	***nd***
2011	91	*nd*	75.2	46.0	70.3	440	211	7.3	**64.1**
2012	154	*nd*	-	15.4	175.4	547	226	10.0	**108.8**
2013	182	8.2	53.2	0.8	20.2	565	234	13.5	**16.5**
Total	**461**	**8.2**	**128.4**	**62.2**	**266.0**		**671**	**37.8**	**254.1**

7.3.3. Carbon losses

Over the three years of the measurements, soil surface CO_2 efflux (R_s or "soil respiration") averaged across treatments was 567 g C m^{-2} $year^{-1}$. For all treatments, R_s was higher in summer than in winter. R_s continuously increased from 2011 to 2013 in the former cropland, while in the previous pasture it remained quite stable. Overall R_s was much higher in the previous pasture and under the genotype Skado. Narrow rows had higher R_s rates than the wide rows (Chapter 6). This was related to the higher root biomass in the narrow rows (Chapter 3). The variation in the monthly R_s was correlated both with soil temperature at 10 cm and with root biomass increment. This allowed us to describe the relationship for soil respiration partitioning in root related (autotrophic; R_r) and heterotrophic respiration (R_h).

We observed a cumulative increase of DOC over the three years that it was studied (2011, 2012 and 2013). The leaching of DOC calculated on a monthly basis increased exponentially from 7.9, to 9.3, 12.8 and 14.5 g C m^{-2} in 2010, 2011, 2012 and 2013 respectively (data not shown). The DOC leaching calculated on an annual basis was a bit lower, because the water balance was lower than the one calculated monthly. But it also increased exponentially as presented in Table 7.4. There was no difference in DOC concentration between the former land-use types; since the water balance was made-up for the entire canopy of both former land-use types, we assumed the same DOC leaching.

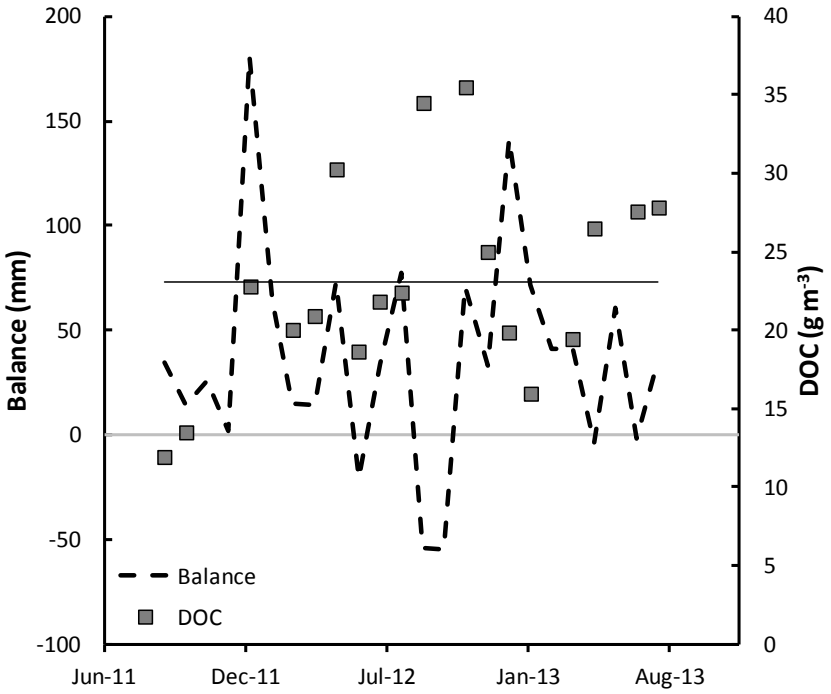

Figure 7.4: Water balance and DOC concentration in the soil during the soil water sampling campaigns from Aug. 2011 to July 2013. The dashed line represents the monthly water balance of the measured precipitation minus evapotranspiration. The squares represent the monthly average of DOC, and the black line shows the average DOC = 23 g C m^{-3}. DOC= dissolved organic carbon (modified after M. Camino Serrano).

7.3.4. Carbon balance

The main C inputs to the soil resulted from the leaf litter fall, annual weeds and harvesting losses (Table 7.4). The total C inputs ranged from a potential minimum of 730 g C m^{-2} to a potential maximum of 1530 g C m^{-2} depending on the genotype, the previous land-use type and the used harvesting machine. The main C flux released from the soil came from soil respiration; the leaching of DOC represented only a very minor proportion (less than 3 %). The total C released from the soil ranged from 634 g C m^{-2} to 984 g C m^{-2} for the four years. Overall, the SOC balance showed a small C increase after four years. If we add the C stored in the woody biomass pools, the belowground system resulted in a net gain of C after four years of SRWC in both former land-use types.

7.4. Discussion

7.4.1 Belowground pools and fluxes, and SOM C balance

Our results indicated an increase of 900 g C m^{-2} (or 9 Mg ha^{-1}) in the SOM pool, which seems to be a large value when compared with the total inputs which are in the same range (Table 7.4). The belowground woody biomass (Stu, Cr, Mr) represented the second C pool of the SRWC system. This long-term belowground biomass also contributed to enhance the C sequestration along the four-year sequence (Pacaldo et al. 2014). The value observed for the C sequestration (240 g C m^{-2}) was much higher than the 90 g C m^{-2} reported for an SRWC plantation in Canada (Arevalo et al. 2011). This might be due to the higher planting density in our field.

Although not all fluxes were continuously measured, especially in the former cropland, we were able to identify and quantify the main fluxes. Our SOM C balance showed small C increases in the system. This positive balance depended on the genotypes, on the weed control, and on the harvesting machines (Figure 7.5). The magnitude of the reported positive balance is in the same order as the annual R_h. So, this positive balance has to be interpreted with care since we lack R_s data in the first (establishment) year. If we assume that R_s in the first year was similar to that in the other three years, the balance may be close to zero.

In the selection of the appropriate management, the choice of the suitable genotype, the process of weeding and the efficiency of the harvesting process are all important for the SOC sequestration. Some C fluxes as weed inputs, harvesting losses and DOC are hardly considered in soil C balances. These C balance-related processes are usually considered negligible and difficult to quantify or to measure. We here demonstrated that they cannot be neglected and that they can be as important as other C fluxes. The quantification of the soil C balance of SRWCs for bioenergy is necessary to evaluate the C sequestration potential of this bioenergy system.

Effect of the previous land-use type

We found that the C balance was less positive in the previous cropland than in the former pasture land. This was explained by the higher C inputs in the former pasture. These higher inputs in the former pasture came in particular from leaf fall and from weeds. However, the C inputs were measured with less intensity (fewer locations and occasions) in cropland, and several sources of C inputs were missing. This might have slightly changed the C balance in favor of the previous cropland. Moreover, the C losses via the R_h were lower in the cropland. This confirmed the observed higher accumulation of SOC with the repeated soil sampling approach.

Changes of the total SOC pool as a result of land-use change from cropland and pasture to SRWC in Central Europe have been reported recently (Walter et al. 2014) and ranged from -1.3 to 1.4 Mg C ha^{-1} yr^{-1} (converted from cropland) and from -0.6 Mg C ha^{-1} yr^{-1} to +0.1 Mg C ha^{-1} yr^{-1} (converted from pasture). Overall there was no SOC change in the study of Walter et al. (2014) which is in line with results of a 20-year chronosequence for SRWC plantations in the USA (Pacaldo et al. 2013). These findings suggest that the C inputs from short-term components (as Fr, leaves, weeds) did not result in a SOC accumulation over time. In contrast a chronosequence of SRWCs in Canada showed that soils initially lost C while after two years soil C levels increased and reached the initial values in the seventh year (Arevalo et al. 2011).

As the number of studies on belowground and SOC C balance of SRWC is still very limited, we also compared our observations with a number of studies on afforestation. For example, the previous land-use type significantly affects the C sequestration potential of afforested sites (Jandl et al. 2007). Chronosequence studies on afforestation also showed

that initially soils loose C, but later they show net gains of C (Guo and Gifford 2002). Pasture soils already have high C stocks and high root densities in the upper part of the mineral soil to start with; so the afforestation has a small impact on SOM. In contrast, croplands are more depleted in SOM (Berhongaray et al. 2013a), and have a higher potential to sequester SOC. It is, however, preferably to avoid the conversion of agricultural land because of the competition with food production. Marginal lands can/should be the main target for future SRWC cultivation. But the potential for C sequestration of these areas has been poorly analysed thusfar. The rate of soil C sequestration is slower than changes in the aboveground C, and it takes decades until net gains occur in former arable soils. Forest floors accumulate C rather quickly, but most of it in a labile form and for a limited time.

Effect of harvesting

Our study showed that harvesting represents a high C input to the soil. Overall, the inputs were as high as the Fr inputs. The C inputs from the harvest losses were higher in the former cropland, which can possibly be explained by the higher aboveground biomass productions (and yields) in the cropland. This demonstrates that harvesting operation has an effect on the C balance of the system. Litter fall is temporarily reduced in frequently harvested tree plantations (Jandl et al. 2007); this reduces forest floor accumulation and contributes to lower soil C stocks. The input of harvest losses into the soil may compensate for the less litter fall inputs. However, we found an increased belowground input from Fr mortality after harvest. Apart from the changed C inputs, the harvest might have secondary effects. For example, harvesting changes the microclimate. Decomposition of forest floor C is temporarily stimulated after harvest, because the soil becomes warmer and possibly wetter due to the reduced evapotranspiration (Piene and Vancleve 1978). Moreover, the harvested field is more exposed to wind and to erosion. Field studies in timber plantations showed that SOC decreased with increasing harvest intensity (Nave et al. 2010).

Presence of weeds

We found very high annual C inputs from weeds, especially in the first rotation. In crops or in SRWC plantations, associated annual plants are traditionally considered pests and not a valuable product (Pinno and Belanger 2009). This explains perhaps why weed production is rarely reported. However, annual plants do have an important function within any agro-ecosystem. Planting of annual 'cover crops' in periods of non-growth has been proposed as one of the most promising strategies to offset the removal of C inputs in bioenergy crops (Blanco-Canqui 2013). These 'cover crops' provide additional biomass C inputs, but represent an extra cost for the farmer. The mixtures of annual weed species, mimicking the native vegetation, grow spontaneously and are the most adaptable species for a specific environment. They do not have the risk of establishment failure of the 'cover crops', and do not have any cost. In addition to the C inputs, the high density of weed roots in the topsoil could drastically reduce soil erosion (De Baets et al. 2007) in periods when poplar roots are less abundant. Moreover, weed root mass growing during the dormant period of the poplars can help to decrease the nutrient leaching during winter (McLenaghen et al. 1996).

Species that occupy different ecological niches can complement each other so that the biomass production of a mixed stand is higher than that of a pure stand (Fae et al. 2009). Annual weeds may thus have an impact on the establishment of the poplars (Kabba et al. 2007) and on their productivity (Otto et al. 2010), but they also play a relevant ecological role.

Soil CO_2 efflux

R_s constituted the largest flux to return belowground C to the atmosphere, and it represented the combined R_r and R_h. This R_s has been estimated to represent 55% of the total ecosystem respiration in our SRWC (Verlinden et al. 2013), with roots representing about 41-51 % of the total R_s (Chapter 6). The current study revealed a large R_s during the four years of SRWC, ranging from 596 to 947 g C m^{-2} y^{-1}. These values are within the range of R_s values of 740-970 g C m^{-2} y^{-1} obtained in different willow SRWC plantations in the USA under a similar planting scheme and comparable climatic conditions as our plantation. Other measurements of R_s on poplar SRWC plantations were recorded over shorter time periods and might be not comparable (Arevalo et al. 2011).

In the former cropland, there was an increasing R_s throughout the years. This might contradict results from other SRWCs where R_s remained rather constant over the years after agricultural lands were converted to SRWC (Arevalo et al. 2011). However, this increase was not observed in the previous pasture land. The higher R_s in the pasture compared with the cropland might be attributed to the higher initial SOC in previous pasture and the higher root biomass and growth of genotype Skado.

DOC

The annual DOC leaching increased exponentially though the years, and we found that this was driven by the water balance. With regard to our DOC measurements very similar annual estimates (7 to 13 g C m^{-2} y^{-1}) have been reported for forests in Belgium and Germany (Borken et al. 2011; Gielen et al. 2011). Moreover, for forests (Gielen et al. 2011) as well as for agroecosystems (Brye et al. 2001) it has already been shown that the inter-annual variability of DOC fluxes is primarily driven by the water balance, in line with our observations.

7.4.2 Uncertainties

In general, soil characteristics are highly spatially variable over short distances. A high degree of uncertainty is created by the low capture of the spatial heterogeneity in the R_s estimations. The measurements of R_s were concentrated on a rather small area of the plantation due to various logistic reasons, as the restricted length of the instrument cables and the necessity of mains power supply (Verlinden et al., 2013). The other variables were measured over a larger area of the plantation and might have a lower spatial uncertainty. For the SOC determination we best captured the spatial heterogeneity. Uncertainties were

also created by the upscaling models, by the calculation methods, etc. For example, the uncertainties associated with our estimations of the DOC leaching highly depended on the water balance estimation. Uncertainties in the estimations of Fr productivity were associated with the method used (Chapter 3), as well as with the R_s partitioning (Chapter 6).

Aboveground inputs from weeds were also subject to a high uncertainty. This high uncertainty was created by the high spatial heterogeneity and the rather low sampling intensity and frequency. Due to time constraints and logistical management issues, aboveground weed biomass was measured with few replicates, only in two out of the four years, and in one year only in the previous pasture land area.

The uncertainties in the SOC balance calculation might be so large that one can question the overall direction of the change in the balance. Figure 7.5 shows the sensitivity analysis on the SOM C mass balance approach. It represents the change in the SOC balance by the change in one variable only. The graph shows that the balance is very sensitive to the inputs from leaf litter and from weeds, and to the release of CO_2 from R_h. Moving from one genotype to another might change the balance almost two folds.

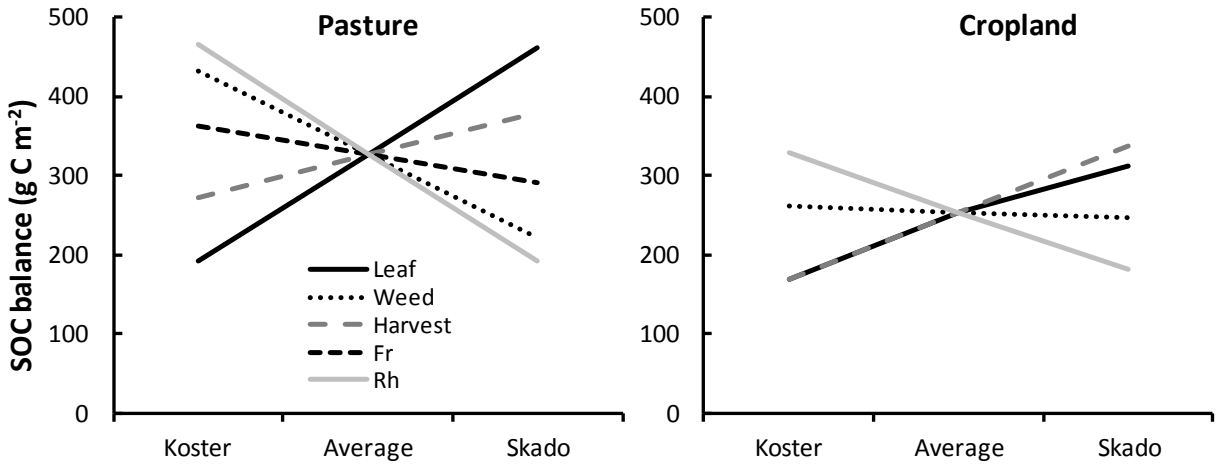

Figure 7.5: Schematic representation of the results of a rough sensitivity analysis of the SOC balance for the two previous land-use types. The acronyms and abbreviations have been explained in the text.

The large uncertainty in the accumulation of errors in the calculation of the C balance is obvious when we calculate the balance with the minimum possible C inputs and maximum possible C losses and *vice versa*. The range was from -162 to 820 g C m^{-2} in the former pasture, while from -52 to 172 g C m^{-2} for the former cropland. This large ranges is as it is a sum of many components, each with their own error.

The proportion of the flux to the pool has a major impact on the uncertainty. For instance, with the repeated SOC measurements, we found a C sequestration of 900 g C m^{-2} (or 9 Mg ha^{-1}), which is similar to the total inputs of C over four years. In theory, this might be possible in a system that does not loose C, which is not possible in nature.

It is important to gain a better understanding as to whether soils are a net source or a net sink of C, and, if possible to make some estimate of the imbalance between the inputs and outputs of C to the soil. While calculations of the SOC have been made, little is known about the error bounds surrounding such estimates. In our study, this was tackled using a large number of replicates and a high sampling frequency.

7.4.3. Potential of SRWC

Across their full life cycle, biofuels can be carbon neutral (no net effect on atmospheric CO_2 and other GHG), carbon negative (a net reduction in GHG), or carbon sources (a net increase in GHG). This depends on how much CO_2 and other greenhouse gases – expressed as CO_2 equivalents – are removed from or released into the atmosphere during crop growth as well as on how much fossil CO_2 is released during management and transport (Njakou Djomo and Ceulemans 2012; Njakou Djomo et al. 2011). Bioenergy production is expected to increase exponentially and biomass-for-energy will probably be harvested at large scales in the near future. The implications of the removal of this biomass on SOC pools and fluxes deserve attention. It has been recognized that SRWC cultivation on marginal lands can be a better alternative than bioenergy sources from agricultural crops (Blanco-Canqui 2013; Njakou Djomo and Ceulemans 2012). However, at the moment life cycle analysis techniques are not accurate enough to predict those implications on the SOC balance, primarily because of the complexity and the scarcity of reliable data. Our results might help toward the develop of a carbon neutral source of energy. Our preliminary results showed a small C increase of the belowground system of a SRWC. However, results from the entire life of an SRWC (around 20 years) should be considered to substantiate the C storage potential of this type of bioenergy crop.

Concerning the impact on the hydrological cycle, we found low levels of DOC in the water table. Evapotranspiration rates for poplar SRWC seems to be a bit higher than for arable crops (Ceulemans et al. 1996; Fischer et al. 2013). But this slightly higher water consumption is largely compensated by the proved better groundwater quality achieved with the low-disturbance crop management of SRWC as compared to arable crops (Brye et al. 2001). A similar comparison with regard to plant diversity indicates an increase of diversity if SRWC is planted in areas that are dominated by agriculture. In our plantation the inter-rows were occupied by a large diversity of annual plant species that provide services to the ecosystem. Moreover, animal diversity in terms of invertebrates, rodents and birds is considerably higher in SRWC as compared to arable crops (Stauffer et al. 2014; Personal communication from local hunters and from Lien Deleye). The diverse impact of SRWC on soil and ecosystem characteristics illustrates the multiple functions of SRWC. The

SOC increase is not the only benefit, but also many other environmental services of growing SRWC instead of other energy crops.

7.5 Conclusion

To detect significant changes in SOC after a changed land management (from agriculture to SRWC for bioenergy), long-term records are required. But by assessing the fluxes we can model and simulate the SOC balance and predict future changes. Different approaches and methods have been combined in this thesis for the SOC balance. C inputs due to weed roots may equal or exceed those due to poplar fine roots, especially during the early phases of the plantation. Harvesting influenced the dynamics of above and belowground C inputs, as well as the soil environment. Leaching of DOC represented a negligible component of the C balance. Large amounts of C were stored in the belowground woody biomass, which represents a long-term C pool. Our results are relevant for the first four years after establishment, most crucial, but maybe not the most representative. However, our results highlight the importance to measure all C fluxes into and out of the soil. This and other relevant data allow us to assess the potential of SRC for bio-energy production and for potential SOC sequestration.

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