

**Dissertationes Forestales 125**

Biomass production and control of nutrient leaching of  
willows using different planting methods with special  
emphasis on an appraisal of the electrical impedance for  
roots

Yang Cao

School of Forest Sciences  
Faculty of Science and Forestry  
University of Eastern Finland

Academic dissertation

To be presented, with the permission of the Faculty of Science and Forestry of the  
University of Eastern Finland, for public examination in the Auditorium BOR 100 of the  
University of Eastern Finland, Yliopistonkatu 7, Joensuu, on 5<sup>th</sup> August 2011, at 12 o'clock  
noon.

*Title of the dissertation:* Biomass production and control of nutrient leaching of willows using different planting methods with special emphasis on an appraisal of the electrical impedance for roots

*Author:* Yang Cao

Dissertationes Forestales 125

*Thesis supervisors:*

Professor Paavo Pelkonen  
School of Forest Sciences, University of Eastern Finland, Finland  
Professor Tarja Lehto  
School of Forest Sciences, University of Eastern Finland, Finland  
Docent Tapani Repo  
Finnish Forest Research Institute, Joensuu Research Unit, Finland

*Pre-examiners:*

Professor Martin Weih  
Swedish University of Agricultural Sciences, Sweden  
Professor Jan Čermák  
Institute of Forest Ecology, Mendel University of Agriculture and Forestry, Czech Republic

*Opponent:*

Professor John Grace  
School of GeoSciences, University of Edinburgh, UK

ISSN: 1795-7389

ISBN: 978-951-651-339-6 (PDF)

(2011)

*Publishers:*

The Finnish Society of Forest Science  
Finnish Forest Research Institute  
Faculty of Agriculture and Forestry of the University of Helsinki  
School of Forest Sciences of the University of Eastern Finland

*Editorial Office:*

Finnish Society of Forest Science  
P.O. Box 18, FI-01301 Vantaa, Finland  
<http://www.metla.fi/dissertationes>

**Cao, Y.** 2011. Biomass production and control of nutrient leaching of willows using different planting methods with special emphasis on an appraisal of the electrical impedance for roots. *Dissertationes Forestales* 125. 32 p.  
Available at: <http://www.metla.fi/dissertationes/df125.htm>

## ABSTRACT

In many countries there is an increasing interest in willows to be used in biomass production and environmental projects. Vegetative reproduction can be achieved through vertically or horizontally planted cuttings. Conventionally, willow plantations are established by inserting cuttings vertically into the soil. There is, however, a lack of information about the biomass production and its effect on the reduction of nutrient leaching of plantations established through horizontally planted cuttings.

A greenhouse experiment with potted cuttings and a field trial lasting three years were carried out to investigate whether horizontally planted *Salix schwerinii* cuttings have a positive effect on stem yield, root distribution and nutrient leaching in comparison with vertically planted cuttings with different planting densities.

The height of the shoots of horizontally planted cuttings was significantly smaller than that of vertically planted cuttings in the pot experiment, but only during the first two weeks after planting. Thereafter, no significant effect of planting orientation on the stem biomass was observed in the two experiments that were conducted. In both experiments the total stem biomass increased with the planting density. It was also found that the fine root biomass and the specific root length were not affected by the planting orientation or density, while the fine root surface area and the absorbing root surface area (ARSA) were affected only by the planting density. The planting orientation did not affect the nutrient concentrations in the soil leachate, apart from  $\text{SO}_4\text{-S}$  and  $\text{PO}_4\text{-P}$  in the pot experiment. The horizontally planted cuttings were slightly more effective for reducing the  $\text{SO}_4\text{-S}$  leaching and the vertically planted cuttings were slightly more effective for reducing  $\text{PO}_4\text{-P}$  leaching. Lower  $\text{PO}_4\text{-P}$  leaching was observed only in the context of higher planting density.

The ARSA in the pot experiment was assessed by using the earth impedance method. The applicability of this method was further evaluated in a hydroponic study of willow cuttings where root and stem were measured independently. The results showed that electrical resistance had a good correlation with the contact area of the roots with the solution. However, the resistance depended strongly on the contact area of the stem of cuttings with the solution, which caused a bias in the evaluation of root surface area. A similar experimental set-up in hydroponics but combining an approach using of electrical impedance spectroscopy was employed to study the relationship between the electrical parameters and root morphology of willow cuttings. A good fit was obtained between the impedance spectra data and the corresponding proposed lumped models. The model parameters were correlated with the contact area of roots and/or stem in the hydroponic solution.

In conclusion, the horizontal planting method can be used as an alternative method in connection with short-rotation coppice willow used for bioenergy and nutrient leaching. Electrical impedance spectroscopy is a promising new non-destructive method in root research, but further more laboratory and field studies are undoubtedly needed.

**Keywords:** Absorbing root surface, electrical frequency, hydroponic, horizontal orientation, impedance, non-destructive, nutrient leaching, willow.

## ACKNOWLEDGEMENTS

My greatest thanks go to my supervisors, Professor Paavo Pelkonen (School of Forest Sciences, University of Eastern Finland), Professor Tarja Lehto (School of Forest Sciences, University of Eastern Finland), and Docent Tapani Repo (Joensuu unit, Finnish Forest Research Institute), for their constant support, guidance and endless patience. I also thank especially Docent Raimo Silvennoinen (Department of Physics and Mathematics, University of Eastern Finland) and Dr Sirpa Piirainen (Joensuu unit, Finnish Forest Research Institute) for their suggestions and ideas regarding the experimental design, the experimental equipments and the preparation of the manuscripts.

Special thanks are also due to Dr Aki Villa, Mr Erik Kaipainen, and Mr Unto Pieviläinen, the laboratory staff at the Faculty of Science and Forestry, University of Eastern Finland, and the laboratory staff of Finnish Forest Research Institute, Joensuu Research Unit, for their contribution to the field work, to technical issues, and also to running the sample analyses. I also appreciate the assistance provided by the staff of the Botanic Garden of the University of Eastern Finland.

Dr Saija Kaskinen (Language Center, University of Eastern Finland) and Dr John A Stotesbury (English Department, University of Eastern Finland) are also thanked for checking the English of the manuscripts and the summary of my PhD thesis. Similarly, I should like to thank Mr Josef Urban (Mendel University of Agriculture and Forestry, Czech Republic) for his comments on the manuscript.

My thanks also go to all of my Chinese, Finnish and international friends. Because of them, I have thoroughly enjoyed living and studying in Finland. Finally, I would like to extend my deepest gratitude to my parents, and brothers and sisters for their kindness, support, and encouragement. Last but not least, I must also thank the most beautiful lady in my life; my wife, Mei Qu. I thoroughly believe that without her love and support I would not have achieved what I have completed so far.

My work has mainly been funded by the China Scholarship Council (CSC, China). In addition, it was partly funded by the Centre for International Mobility (CIMO, Finland), the University of Eastern Finland, Niemi-säätiö (Finland), and Koneen Säätiö (Finland).

Joensuu, June 2011  
Yang Cao

## LIST OF ORIGINAL ARTICLES

This thesis is based on the following articles, which are listed below, and referred to by Roman numerals. Articles I, III-IV are reproduced with the kind permission from the publishers. Article II is the author version of the submitted manuscript.

- I Cao, Y., Lehto, T., Repo, T., Silvennoinen R. & Pelkonen, P. 2011. Effects of planting orientation and density of willows on biomass production and nutrient leaching. *New Forests* 41:361-377.  
doi: 10.1007/s11056-010-9228-6
- II Cao, Y., Lehto, T., Piirainen, S., Kukkonen, J.V.K. & Pelkonen, P. 2011. Effects of planting orientation and density on the soil solution chemistry and growth of willow cuttings. (Submitted manuscript).
- III Cao, Y., Repo, T., Silvennoinen, R., Lehto, T. & Pelkonen, P. 2010. An appraisal of the electrical resistance method for assessing root surface area. *Journal of Experimental Botany* 61: 2491-2497.  
doi: 10.1093/jxb/erq078
- IV Cao, Y., Repo, T., Silvennoinen R., Lehto, T. & Pelkonen, P. 2011. Analysis of willow root system by electrical impedance spectroscopy. *Journal of Experimental Botany* 62: 351-358.  
doi: 10.1093/jxb/erq276

## The author's contribution

I Yang Cao was responsible for running the experiment and the data analysis, and wrote the manuscript. P. Pelkonen originated the research idea. T. Lehto advised in designing and setting up the experiment. T. Repo and R. Silvennoinen advised in applying the impedance method on root measurement. My co-authors have also commented on the manuscript.

II Yang Cao was responsible for running the experiment and the data analysis, and wrote the manuscript. P. Pelkonen originated the research idea. T. Lehto advised in designing and setting up the experiment. S. Piirainen organized the installation of the lysimeters and analysis of water samples, and advised in the statistical analysis. J.V.K. Kukkonen helped to analyze the DOC of water samples. My co-authors have also commented on the manuscript.

III-IV Yang Cao was responsible for running the experiment and the data analysis, and wrote the manuscript. T. Repo and R. Silvennoinen originated the research idea and also advised on theoretical and technical issues. T. Lehto and P. Pelkonen advised in managing the experiments. My co-authors have also commented on the manuscript.

## Table of Contents

ABSTRACT .....	3
ACKNOWLEDGEMENTS.....	4
LIST OF ORIGINAL ARTICLES.....	5
ABBREVIATIONS .....	7
1 INTRODUCTION.....	9
1.1 Background .....	9
1.2 Cultivation of short rotation willow coppice.....	10
1.3 Willow root system .....	12
1.4 Electrical impedance method on root research.....	13
1.5 Aims of the study .....	15
2 MATERIALS AND METHODS.....	15
2.1 Effects of planting orientation and density of willow cuttings on growth and nutrient leaching (papers I, II) .....	15
2.1.1 Experiment design .....	15
2.1.2 Measurements and data analysis.....	16
2.2 Electrical impedance method for studying willow roots (papers III, IV).....	19
2.2.1 Experiment design .....	19
2.2.2 Measurement and data analysis.....	19
3 RESULTS.....	20
3.1 Effects of planting orientation and density of willow cuttings on growth and nutrient leaching (papers I, II) .....	20
3.2 Electrical impedance method for studying willow roots (papers III, IV).....	22
4 DISCUSSION.....	23
4.1 Effects of planting orientation and density of willow cuttings on growth and nutrient leaching (papers I, II) .....	23
4.2 Electrical impedance method for studying willow roots (papers III, IV).....	25
5 CONCLUSIONS .....	27
REFERENCES .....	28

## ABBREVIATIONS

ARSA	absorbing root surface area
$C_r$	root-solution interfacial capacitance
$C_{sa}$	capacitance of stem above solution level
$C_{sc}$	stem-solution cross-sectional interfacial capacitance
$C_{ss}$	stem-solution longitudinal interfacial capacitance
EIS	electrical impedance spectroscopy
ERSD	estimated relative standard deviation
$R_r$	resistance of root-solution interface
$R_{ra}$	auxiliary resistance of root
$R_{sa}$	resistance of stem above solution level
$R_{sc}$	resistance of stem-solution cross-sectional interface
$R_{sca}$	auxiliary resistance of stem in cross-sectional direction
$R_{ss}$	resistance of stem-solution longitudinal interface
$R_{ssa}$	auxiliary resistance of stem in longitudinal direction
$R_{sup}$	superposition resistance
RSA	root surface area
SRC	short rotation coppice
SRL	specific root length





# 1 INTRODUCTION

## 1.1 Background

In recent times willow has represented a fast-growing and high-yielding woody crop that has been used to resolve an array of environmental problems. However, historically the primary purpose of the cultivation of willow is for the production of baskets, fences, and even medicine. The documented history of the cultivation of willow goes back to at least the second century BC (Keoleian and Volk 2005, Volk et al. 2006). Native Americans maybe firstly used willow cuttings to stabilize stream banks (Shipek 1993). They recognized and utilized the ability of willow species to coppice vigorously and to develop from unrooted cuttings (Keoleian and Volk 2005). European immigrants began cultivating willows in the United States in the 1840s (Volk et al. 2006). In northern Europe, willow plantations were established firstly in the 1800s with the aim of providing primary material for basket-making (Kuzovkina and Quigley 2005).

The original driving force in Sweden for willow research was a predicted shortage of raw material for pulp and paper industry in the late 1960s. The energy crisis in the 1970s stimulated a new motivation to continue research on willows to replace a certain proportion of fossil fuels with renewable woody biomass (Mirck et al. 2005). Currently, Sweden has the largest short rotation coppice (SRC) willow plantations in Europe, around 15 000 ha (Christou et al. 2010, Selkimäki et al. 2010). In the United States the cultivation of willow for bioenergy was reinvigorated in the mid-1980s (Keoleian and Volk 2005). In the early 1980s, studies of SRC willow for bioenergy were initiated in Finland. Experiments with *Salix schwerinii* of Russian origin and *Salix viminalis* of Sweden origin have commonly been carried out in agricultural fields and cut-away peatlands (Tahvanainen and Rytönen 1999). Many exotic willows were susceptible to frost damage and often the biomass production of the indigenous willows, i.e. *Salix phylicifolia*, was greater than that of the exotic ones (Hytönen et al. 1995, Hytönen and Saarsalmi 2009). The greatest interest at present in Finland with respect to energy biomass is not in willow species but in reed canary grass which produces high biomass yields in the Finnish climate (Christou et al. 2010). To select the potential bioenergy crops in China, many experiments have been conducted to compare the fuel characteristics of various exotic crops, i.e. switchgrass and reed canary grass, and the native crops, e.g. *Salix cheilophila* (Xiong et al. 2008).

With increasing understanding of willow biology and production systems, a large theoretical knowledge has been developed and a variety of applications have been demonstrated in SRC willow for bioenergy (Mirck et al. 2005). The rapid expansion of scientific understanding of the inherent eco-physiological properties has promoted the new environmental applications for willow coppice (Volk et al. 2006). Willow coppice has been used to reduce soil erosion and non-point source pollution, to promote stable nutrient cycling, to enhance carbon storage in roots and the soil, to improve ecosystem diversity, and to act as living wind and snow fences (Perttu and Kowalik 1997, Perttu 1999, Kuzovkina and Quigley 2005). For instance, willow cuttings have been planted in the large slop areas, e.g. the Loess Plateau, which is the most severe soil erosion region in China, to prevent both surface erosion and shallow mass movements (Xu et al. 2009). On the basis of Kuzovkina and Volk's (2009) identification, there are 36 essential characteristics for willow, e.g. high growth rate, good rooting ability and high fine root density in the topsoil.

Understanding these inherent characteristics can help us to achieve benefits in biomass production and other environmental performance.

## **1.2 Cultivation of short rotation willow coppice**

To achieve the renewable energy targets set by the Kyoto protocol, an increasing demand for woody biomass has been given high priority, especially in North America, Europe and Australia. According to the UK biomass strategy plans, the area of energy crops would be 350 000 ha by 2020 (Department of the Environment 2007). The long-term goal in the EU is that biomass supply covers 20 % of the region's primary energy (IEA 2001). High-yielding SRC willow, as an alternative source to conventional forestry for biomass production, plays an important role in bridging the huge gap between the future biomass demands and the current supply levels (Mola-Yudego and Pelkonen 2008).

Generally, SRC willow plantations are established by inserting approximately 20 cm long cuttings from one-year-old stems vertically into the soil. During the first year the shoots grow to 2-3 m in height and the root system becomes well developed. The stems are cut off during the winter after the first growing season to enhance multiple branching. The rotation time is typically three years. Because the production may decrease with increasing age, new willow plantations or other crops should replace the stands after about twenty-five years (Elowson 1999, Keoleian and Volk 2005).

For a high-yielding SRC willow plantation, the requirements for the site selection are particularly important, i.e. sufficient water availability, and soil pH between 5.5 and 7.5 (Wickham 2010). Generally, most fertile agricultural lands at low elevation with a slope not exceeding 15% are suitable for willow growing (Wickham 2010). In Sweden, the current area of SRC willow is about 15 000 ha which means about 0.5 % of the total arable land in the country (Selkimäki et al. 2010). The United States has the potential to exploit over 40 million ha of idle or surplus agricultural land for the development of short rotation woody crops (Graham 1994). However, the use of agricultural land for woody biomass production has triggered the controversial between food security and biofuel business. In consequence, the vast available marginal lands have received an increasing attention for crops biofuel crops production (Milbrandt and Overend 2009, Tang et al. 2009). But the abandoned lands may have poor physical properties, poor nutrients, and low pH, which cannot guarantee high production (Wickham 2010).

Conventionally, SRC willow plantations are established by inserting cuttings vertically into the soil but the ability of vegetative reproduction has also been demonstrated by placing cuttings horizontally (Kuzovkina and Quigley 2005). At present, the most commonly used machines are step planters which cut the willow rods (150-250 cm) into 18-20 cm cuttings and insert the cuttings vertically into the soil with a density of 10 000-20 000 cuttings ha<sup>-1</sup>. However, when the commercial willow planter was used for planting in the field, cuttings often fell over within the furrow and were buried in the soil horizontally. In consequence, preliminary experiments have been designed to investigate the influence of planting orientation on the sprouting of cuttings and some companies have been working on the development of new planters (Dieterich and Martin 2008, Lowthe-Thomas et al. 2010). Recently, a lay-flat planter has been under development which lays whole rods horizontally in furrows at 2-8 cm depth (Lowthe-Thomas et al. 2010). The lay-flat planting method has reduced planting costs by up to 48% due to its fast planting rate (0.75 ha h<sup>-1</sup>), and yields have been equivalent to traditional vertically planted willow coppice (Lowthe-Thomas et al.

2010). However, McCracken et al. (2010) have supported the vertical planting of cuttings since horizontal planting with long rods requires more propagating material. In support of environmental construction, horizontal bundles of willow were recommended in early times for use in the site restoration of stream banks and also across sludge fields to dewater the sludge (Gray and Sotir 1996, Vervaeke et al. 2001, Kuzovkina and Quigley 2005, Lovett and Price 2007).

Generally, the stem biomass yield is strongly and positively correlated with planting density. Maximum biomass has been achieved at 20 000 cuttings ha<sup>-1</sup> in Sweden, where the biomass yield did not increase significantly beyond this density (Bergkvist and Ledin 1998, Bullard et al. 2002a and b). Above this planting density the competition within stools, as well as between stools, in a stand caused self-thinning (Wilkinson et al. 2007). A density of more than 25 000 cuttings ha<sup>-1</sup> can result in an increase in stool mortality as a result of competition between the stools, with no significant gains observed (Bergkvist and Ledin 1998).

High yields in SRC willow require intensive management, particularly an adequate supply of nutrients (Adegbidi et al. 2003). To increase our knowledge of nutrient losses as a result of their management, previous studies have been conducted to quantify the nitrate leaching from SRC willow plantations under field conditions and in the context of lysimeters studies. In Denmark, high leaching was only observed during the establishment year in the context of treatments both included and excluded the use of a fertilizer (Mortensen et al. 1998). However, the application of fertilizer did not increase biomass productivity on a coarse sand site, but there was an increase in the nitrogen leaching compared to treatment with no fertilizer. The leaching decreased considerably during the second year, and practically stopped during the third year, and no significant difference was observed, whether there was fertilization at a rate of 75 kg N ha<sup>-1</sup> or none at all (Mortensen et al. 1998). High leaching was also observed during the first and second year after planting in Sweden, but later it was close to zero, despite an average fertilization rate of 100 kg N ha<sup>-1</sup> (Aronsson et al. 2000). Accordingly, it was recommended that fertilization should not be used until the second year (Mortensen et al. 1998, Goodlass et al. 2007). Harvesting and the subsequent regrowth of SRC willow in both Sweden and the UK were not observed to cause any increase in the nitrogen leaching (Aronsson et al. 2000, Goodlass et al. 2007). In addition to the period of establishment high nitrate leaching was also observed when the plantation was finally removed (Goodlass et al. 2007). A lysimeters study conducted in Sweden observed that the irrigation rate had only a slight effect on the nitrate leaching (Aronsson and Bergström 2001). Thus, intensive management of SRC willow should not be thought of as increasing nutrient leaching and thus posing a major threat to the quality of the groundwater (Aronsson et al. 2000).

Since the production of SRC willow removes greater quantities of nutrients from the soil, municipal waste waters and sewage sludge can be used as a nutrient sources in order to increase biomass yields and also to reduce fertilization and irrigation costs (Adegbidi et al. 2003, Perttu 1999). Previous experience in Sweden has indicated that willow vegetation filter approach to the purification of municipal waste waters and sewage sludge may be a practical and economically feasible way to managing and utilizing the residual products (Perttu and Kowalik 1997). However, waste waters contains very high nitrogen concentrations (500-100 mg N l<sup>-1</sup>) (Dimitriou and Aronsson 2004). A threshold for maximum wastewater application needs to be defined that will prevent nutrient leaching into the groundwater (Aronsson and Bergström 2001, Adegbidi and Briggs 2003, Börjesson and Berndes 2006).

SRC willow plantations also have a potential as vegetation filters for mitigating non-point source pollution from agricultural land to watercourses. In high-yielding crop regions of China, large amounts of fertilizer enter the ground water, rivers, and lakes because of low utilization efficiency (only 30-40%) (Zhang et al. 1996). In Finland, 50% of nitrogen and 60% of phosphorus in watercourses come from agricultural areas that cover 7% of the total land area (Granlund et al. 2005). The large-scale movement of non-point source pollution is complex and difficult to control. The natural defense system, consisting of vegetated buffer zones, is the practical strategy for the control of non-point pollution from agriculture. Vegetated buffer zones reduce overland flow and decrease the amount of sediment and nutrients entering streams, thus improving soil properties and stabilizing stream banks (Volk et al. 2006). Buffer zones are very suitable in low-income countries like China as low-cost tools for nutrient leaching reduction (Kuusemets et al. 2000). Compared with grass and tree buffer strips, willow crop is an ideal vegetation type for use in riparian buffers (Kuusemets et al. 2001; Volk et al. 2006). Several characteristics of willow crops make them ideal for use in riparian buffers. The vigorous growth of willow after coppice can effectively remove nutrients from the sites. Rapid and extensive fine root development acts as a nutrient sink and as soil stabilizers soon after willows are planted. Many willow buffers have been established along streams, e.g. in the USA and Sweden (Perttu and Kowalik 1997, Volk et al. 2006). Increasing the amount and distribution of roots in buffers by planting different sizes of cuttings is one approach to enhancing the growth of in the population of microorganisms and increasing the environmental benefits of the riparian system (Volk et al. 2006). The horizontally planted living willows materials have been also explored for their potential use in buffer zones (Kuzovkina and Quigley 2005, Li et al. 2006). In one riparian area of China, the horizontally planted living willows produced more root biomass than those planted vertically (Li et al. 2006). The similar biomass yields of SRC willow differing between the horizontal and vertical planting orientation have also been demonstrated in previous experiments (Lowthe-Thomas et al. 2010, McCracken et al. 2010), but there is a lack of information about the root system of SRC willow between two planting orientations and about the effect of this on nutrient leaching.

### **1.3 Willow root system**

Roots play a critical role for willow as an environmental beneficial bioenergy crop. However, relatively little information exists about root characteristics of SRC willow (Volk et al. 2001). An extensive fibrous root system, high root density, high root tensile strength, deep rooting and long growing period are commonly used to depict the root system of willows (Keoleian and Volk 2005, Kuzovkina and Volk 2009). The active root primordial on the stems can make vegetative reproduction easy (Kuzovkina and Quigley 2005). The root system is widely spread and forms a dense network in the upper layer of the soil in the first year. The root system remains intact during the winter and activate in the early spring (Elowson 1999). A lysimeters study conducted in Sweden indicated that willow root must be able to sustain activity and nitrogen uptake even at low soil temperatures as low as zero Centigrade (Aronsson 2001).

Another study conducted in Sweden has shown that roots typically attain an average depth of 25-30 cm during the first growing season, extending deeper during the second growing season (Rytter and Hansson 1996). Studies have shown that the majority of roots are located in the top 10-20 cm of the soil (Volk et al. 2001, Heinsoo et al. 2009). A large

proportion of the willow root system consists of fine roots (< 2 mm in diameter) (Martin and Stephens 2006), and after four years of growth may amount to more than 90 % of the total (Volk et al. 2001). Roots can reach a depth of 1.3 m and occasionally as deep as 3 m (Crow and Houston 2004).

The shallow distribution of willow root systems may be caused by nitrogen fertilizer application and leaf litter decomposition. The nitrogen concentration (dry mass basis) in willow leaves is around 2.5-3.5 % (Perttu 1998). Leaf litter decomposition and fertilizer increase the nutrient concentration of the soil layer near the surface (Rytter and Hansson 1996). Meanwhile, fertilization has been shown in Estonian to significantly reduce the biomass and annual production of fine roots in the uppermost 10 cm soil layer of SRC willow plantations (Heinsoo et al. 2009). In consequence, fertilizer is not applied to the SRC willow during its establishment year so as to ensure a well-established and deep root system and also to reduce nutrient leaching (Lehmann and Schroth 2003). Fine roots tend to concentrate in the soil where it is well aerated but where it may still have a high capacity for moisture and nutrient retention. Rytter and Hansson (1996) have suggested that the water supplied through surface drip irrigation systems contributes to the high density of willow roots near the soil surface.

The rapid development of an extensive fine root system is an important attribute for SRC willow as a vegetation filter used for reducing nutrient leaching. Widespread root systems were observed during the establishment year of SRC willow plantations in Denmark (Mortensen et al. 1998). When the soil water passes through the well-developed root zone, nutrients appear to be taken up by the effective root system. In the experiment conducted in Sweden it was found that SRC willow plantation fertilized at a rate of 200 kg N ha<sup>-1</sup> caused no leaching of nitrogen into the ground water (Elowson 1999). In addition, around 75-95 % of the nitrogen and phosphorus in the waste water can be taken up by the root system of willow plantations (Börjesson 1999). Increasing the amount and distribution of roots by planting different sizes of cuttings is one feasible approach to improving the treatment efficiency of willow vegetation filter (Volk et al. 2006). Hence, it would seem to be interesting to investigate the effect of planting orientation, both vertical and horizontal, on the characteristics of the root system and also on the potential reduction of nutrient leaching in willow plantations.

#### **1.4 Electrical impedance method on root research**

The need for knowledge about root biomass, growth and decay dynamics may be met only insufficiently as a result of using destructive root sampling methods (Rytter and Rytter 1998). The available root research methods are usually destructive, and cumbersome or may not be suitable for effective study of fine root functionality, e.g. by means of root excavation, sequential coring, the root in-growth bag, minirhizotron (Samson and Sinclair 1994, Majdi 1996), or a scanner set in the soil (Costa et al. 2000). The radar method may detect coarse roots (>19 mm in diameter), but currently the resolution does not permit detection of fine roots (<2 mm in diameter) (Butnor et al. 2003, Hirano et al. 2009).

Because soil and roots contain electrolytes and water, they conduct electric current. When the roots in the soil are subjected to an external electric field, current passes through the system, depending on the electrical properties of the different components in the circuit. This property has been used to characterize a root system both at a single frequency and also at multi-frequencies (Chloupek 1972, Dalton 1995, Aubrecht et al. 2006).

In previous studies the capacitance and/or resistance of a root system has been measured at a single alternating current frequency, and the attributes were assumed to be measurements of absorbing root surface area (ARSA) (Chloupek 1977, Dalton 1995, Preston et al. 2004, Aubrecht et al. 2006). The capacitance measurements were typically carried out at a frequency of 1 kHz and the resistance measurements at 128 Hz. Capacitance has aroused particular interest since the first field studies that were conducted in 1972 (Chloupek 1972, Rajkai 2005). The measurements were made using a capacitance bridge with one electrode set at the base of a plant stem and another in the soil. Practical applications of the capacitance method have been tested with varying success for root investigations involving annual agricultural crops, e.g. red clover and alfalfa (Kendall et al. 1982), tomato (Dalton 1995), maize (van Beem et al. 1998), and sunflower (Rajkai et al. 2005), and even with different genotypes of maize (McBride et al. 2008).

The earth impedance method was introduced to facilitate estimation of the spatial distribution of ARSA in the field (Aubrecht et al. 2006, Čermák et al. 2006). In contrast to the electrode configuration of the capacitance method, the earth impedance method applied four electrodes, two for the driving current and two for measuring the potential. By moving one potential electrode away from the stem, the mean distance of all of the absorbing roots from the tree could be determined by means of the potential characteristics. The estimated ARSA was found to be related to the basal area over a large range of stem diameters (Čermák et al. 2006). Butler et al. (2010) applied the earth impedance method to measure the ARSA of Sitka spruce. They compared the root values with the transpiration leaf area index of the trees in order to understand the relationship between the exchange surface areas between the above- and below-ground parts (Butler et al. 2010). More recently, however, the applicability of the earth impedance method in the context of root measurements has come under debate (Urban et al. 2011).

In the case of electrical impedance spectroscopy (EIS), the characteristic behavior of the system in response to a range of frequencies of the electrical current can be represented by the components of the electrical circuits, i.e. resistors and capacitors. In the past, this method has been used to investigate the properties of plant, animal and human tissues (Tiitta et al. 1999, Altmann et al. 2004, Bayford 2006). In plants, it has been used to reveal the responses of detached, above-ground organs to cold acclimation, freeze-thaw, and heat injury and also to exposure to elevated ozone and carbon dioxide (Zhang et al. 1992, Zhang and Willison 1993, Ryyppö et al. 1998, Repo et al. 2004).

A small number of studies exist concerned with the development of EIS for studying root systems (Ozier-Lafontaine and Bajazet 2005, Repo et al. 2005). Using specific electric analogs to model the impedance spectra of the soil-root-electrodes continuum, the method has been shown to be useful for a non-invasive estimation of root traits, such as the root fresh/dry mass or root length, both for tomato plants in a soil substrate (Ozier-Lafontaine and Bajazet 2005) and also for willow cuttings in hydroponic solution (Repo et al. 2005). Although different electric analogs have been formulated to describe the soil-root-electrodes continuum, the reasonable equivalent circuit will depend on the understanding of the details of electrical current pathways (Dalton 1995).

## 1.5 Aims of the study

The main objective was to investigate the effects of planting orientation and density on the growth of willow cuttings and soil water chemistry in a greenhouse experiment and in a field experiment. The earth impedance method was applied to assess the ARSA by means of a pot experiment. To answer questions concerning the pathway of the current through root system in the impedance method and also to elucidate the relationship between the electrical parameters and morphological attributes, two hydroponic studies were carried out. Their specific objectives were:

1. To investigate the effect of horizontal and vertical planting orientation and planting density on stem growth, root characteristics and reducing nutrient leaching under greenhouse conditions (paper I) and under the three-year field conditions (paper II).
2. To study in hydroponics the impact of the roots and stems of willow cuttings on the electrical current pathways and electrical resistance at a single low frequency (paper III).
3. To explore the relation between electrical parameters, capacitance and resistance, and the contact area of roots and/or stem on a multi-frequency impedance spectrum (paper IV).

## 2 MATERIALS AND METHODS

### 2.1 Effects of planting orientation and density of willow cuttings on growth and nutrient leaching (papers I, II)

#### 2.1.1 Experiment design

Willow cuttings (*Salix schwerinii*) were used in the pot experiment under greenhouse conditions (I) and in the field experiment (II) to investigate the difference between the planting orientations, horizontal and vertical, on the stem yield, root distribution and nutrient leaching with the different planting densities.

The pot experiment (I) was conducted in the winter of 2007-2008 and lasted for sixteen weeks. Dormant cuttings (25 cm in length) were soaked and planted into the plastic pots (base diameter 35 cm, height 35 cm) in the greenhouse of the School of Forest Sciences at the University of Eastern Finland. The soil was taken from the Siikasalmi willow plantation area in eastern Finland. The soil pH was 5.3, and the organic matter content was 5.6 %. The cuttings were planted with horizontal and vertical planting orientations at three planting density levels (one, two and three cuttings per pot). The tops of the vertical cuttings were 2-4 cm above the soil surface, while the horizontal cuttings were placed just below the soil surface. Six planting treatments and one unplanted control treatment were randomly arranged in one block. There were four replications. Deionized water was added twice per

week liter by liter, above the saturation level until the same amount of water leached out of each pot. A commercial fertilizer corresponding to  $45 \text{ kg N ha}^{-1}$  was added after growing for eleven and thirteen weeks. The conditions in the greenhouse were the following: the temperature  $22/15 \text{ }^{\circ}\text{C}$  (day/night), the photoperiod 20 h, and the photon flux density  $320 \mu\text{mol s}^{-1}\text{m}^{-2}$ .

The three-year field experiment (II) was carried out on the former lawn at the Botanic Garden of the University of Eastern Finland ( $62^{\circ}36'\text{N}$ ,  $29^{\circ}43'\text{E}$ , 81 m asl). In June 2008, the experiment area was ploughed and harrowed. The *Salix schwerinii* cuttings (25 cm in length) were planted in the plots ( $2 \times 2 \text{ m}$ ) by hand with horizontal and vertical planting orientations at two planting densities (3 and 9 cuttings per plot corresponding to 7 500 and 22 500 cuttings  $\text{ha}^{-1}$ , respectively) which were randomly arranged in each block. There was also one unplanted plot in each of the four blocks. The tops of the vertical cuttings were 5 cm above the soil surface, while the horizontal cuttings were placed just below the soil surface at a depth of 5 cm. After planting, the experiment area was fenced to prevent animal browsing. However, during the winter of 2008-2009 the shoots were completely grazed by vole. For weed control, each plot was covered with black polythene mulch in May 2009 which still permitted rainfall infiltration. Manual weeding of the paths between plots was carried out each year at the beginning of the growth period. The plantations were not fertilized. They were irrigated during the summer of 2008 by sprinkler equipment to maintain good conditions for the establishment of the plants. In the summer of 2010, the plots were irrigated each Sunday between 4 July and 29 August with the amount 40 mm of water to promote leaching. The soil solution of each plot was collected at two soil profile depths by using zero tension and tension lysimeters within three blocks. One zero tension and one tension lysimeter were installed in each plot in the middle of May 2009. The zero tension lysimeters installed at a depth of 25 cm were made from a polythene plastic funnel, filled with quartz sand, with a collecting area of  $299 \text{ cm}^2$ , and fitted to a sample collection bottle of two liters capacity. The tension lysimeters were installed at a depth of 60 cm below the surface of the soil and connected with a pressure sensitive stopper to a 2 l collecting bottle. The groundwater level was around 160 cm below the soil surface from 19 April to 9 May 2010, but thereafter no groundwater could be detected in the 200 cm deep well. The weather conditions were recorded at the nearest station (network of the Finnish Meteorological Institute) located at a distance from 2 km apart the Botanic Garden. The effective temperature sum (degree days above a threshold of  $+5 \text{ }^{\circ}\text{C}$ ) during the growing period was  $1\,276 \text{ }^{\circ}\text{C d}$  in 2009 and  $1\,513 \text{ }^{\circ}\text{C d}$  in 2010, while the rainfall from April to October was 343 mm in 2009 and 324 mm in 2010.

### 2.1.2 Measurements and data analysis

#### Shoot height and stem yield

In the pot experiment (I), the height of shoots was monitored throughout the experiment. After sixteen weeks of growth, the stems were cut at the root collar and the leaves were separated from the stems. Both were dried to constant mass at  $40 \text{ }^{\circ}\text{C}$  and the nutrients were analyzed using standardized methods. In the field experiment (II), the number of living shoots and shoot-height were measured each month throughout 2009 and 2010. The annual stem production was assessed in the harvest when no leaves were present in the year of 2009 and 2010. The samples were dried at  $105 \text{ }^{\circ}\text{C}$  to constant weight.



## Root system

The earth impedance method was used to estimate the ARSA before harvesting the aboveground biomass in the pot experiment (I). The measurement stage followed the scheme laid down by Aubrecht et al. (2006) and Čermák et al. (2006). First, the root resistivity was assumed to be the same as that of the stem. The resistivity was assessed using four Ag-electrodes (0.5 mm in diameter) placed at equal distances (1.0 cm) from each other along the stem, and the resistance was recorded by means of a ratio meter (128 Hz) (Fluke 1625, USA). Then to measure the resistance of the root-soil system, an electric current was passed through the system via the Ag-electrode set in the stem and through a set of four auxiliary stainless steel electrodes in parallel in the soil (1.5 cm depth) in the fringe area of the pot. The electrical potential in the field between the current electrodes was measured by means of a stainless steel electrode inserted in the soil and an Ag-electrode inserted in the root collar. The electrodes were connected to a ratio meter (Fluke 1625, USA) to record the resistance. The ARSA was calculated according to the equations devised by Aubrecht et al. (2006).

A soil auger (3.5 cm in diameter) was used to collect fine root samples from the upper layer (0-12 cm from soil surface) and the subsoil layer (12-25 cm from soil surface). The live roots were separated from the soil and scanned using an Epson Expression 1640XL scanner and analyzed using the WinRHIZO program (WinRhizo, Régent Instruments Inc, Québec, Canada) to measure the root surface area and root length. The scanned root surface area (RSA) and root length were transformed into the total live fine RSA, and the root length of each pot. The live roots were dried to a constant mass at 40 °C and weighed. The specific root length (SRL) of the live fine roots was calculated as the ratio of the length and the dry weight of the roots by soil layer. The remaining roots were separated from the soil to assess the total root biomass after soaking in water for 12-16 h. The coarse roots (>2 mm in diameter) were separated from the whole root system. The coarse roots and the remaining fine roots were dried to a constant mass at 40 °C and then weighed. The nutrients of the coarse and fine roots were analyzed using standardized methods.

In the field experiment (II), the root mesh net method was used to estimate the fine root production (Lukac and Godbold, 2010). In June 2009, four individual nylon mesh nets (10 cm in width and 30 cm in length, with a 2 mm mesh size) were inserted vertically into the soil with the aid of a steel plate and a hammer, each plot containing a single row. Two root mesh nets were removed in both October 2009 and October 2010, respectively. To remove the nets, soil blocks containing the mesh nets were lifted using a narrow garden spade. Any fine roots ( $\leq 2$  mm in diameter) that had grown through the mesh by at least 2 cm from each side of the nets were used to estimate the annual root production. Two samples from the same soil profile depth were pooled into a single sample. The fine roots were washed out of soil manually and dried at 105 °C to a constant weight. The standing root biomass was measured in 2010 by using an auger core (3.5 cm in diameter) at three soil-profile depths: 0-10, 10-20, and 20-30 cm. The coring locations were around the central willow plant in each plot. Four soil cores were sampled from four directions at distances of 25 cm from the central willow plant. The samples from the same soil profile depth were pooled into a single sample. The fine roots were washed out from the soil manually and dried to a constant weight.

## Nutrient leaching

In the greenhouse experiment (I), the water leachate from the soil for the chemical analyses was collected on the second watering occasion of the week in the ninth, eleventh, and thirteenth weeks following sprouting by placing a clean basin under each pot. The amount of leachate was recorded and stored at  $-18\text{ }^{\circ}\text{C}$  for subsequent nutrient analysis. The water was filtered through  $0.45\text{ }\mu\text{m}$  glass fiber filters (Whatman plc, Kent, UK). The total-N and  $\text{NH}_4\text{-N}$  were detected by using a FOSS Tecator FIAstar 5000.  $\text{NO}_3\text{-N}$ ,  $\text{PO}_4\text{-P}$ , and  $\text{SO}_4\text{-S}$  were detected using the ionic chromatographic equipment (Dionex). Dissolved organic N (DON) was calculated as the difference between the total N and the combined  $\text{NO}_3\text{-N}$  and  $\text{NH}_4\text{-N}$  in the leachate. The amount of nitrogen leached was calculated according to the concentration of the total-N multiplied by the amount of leachate collected.

In field experiment (II), the soil leachates were collected in a polyethylene bottle on the Monday of each week between 15 June and 12 October 2009 and also from 19 April to 11 October 2010. After sampling, the soil solution samples were placed in a cold room ( $4\text{ }^{\circ}\text{C}$ ) and transferred to the laboratory the following day. The pH (PHM 92 Radiometer) and conductivity (CDM 92 Conductivity meter) values were measured from unfiltered samples. The samples were filtered (Schleiche & Schuell GF 52 glass wool filter) and stored in a freezer ( $-18\text{ }^{\circ}\text{C}$ ) for subsequent analyses. The dissolved organic carbon (DOC  $\text{mg l}^{-1}$ ) was measured using TOC-5000A (Total organic Carbon Analyzer, Shimadzu) in 2009 and Multi N/C 2100 (Analytik Jena, Germany) in 2010. The Total nitrogen,  $\text{NH}_4\text{-N}$  and the sum of nitrite and nitrate were measured with the aid of a spectrophotometry (FIA-star 5000 analyzer FOSS TECATOR). If the concentrations proved to be less than the detection limit, a value calculated from half of the detection limits was substituted.

## Data analysis

Repeated measures ANOVA was used to test the effects of planting orientation and density on the height of the shoots and the nutrient concentrations of the leachate for the greenhouse experiment (I). It was also used to test for stand stem production, the average height of the tallest shoot, the number of living stems, and root production between 2009 and 2010 in field experiment (II). The effect of planting orientation and density and their interaction on the biomass, ARSA, coarse root length, and the nutrient concentrations were analyzed using two-way ANOVA. The RSA, SRL and root biomass in the field experiment were compared according to the planting orientation, planting density and soil depths using two-way ANOVA, taking into consideration the depths as a repeated measure. In field experiment (II), significant differences in the soil water chemical concentrations between treatments were tested using a mixed linear model. In the model, the treatment was set to a fixed factor and the plot was a covariate, block and interaction between block and treatment were random factors, and the sampling week was a repeated factor. Concentrations with a logarithm transformation were used in the model. Statistical significance was assessed at a level of 0.05. Statistical analyses were conducted using the SPSS (SPSS, ver.15.0, USA) and PASW software (PASW, ver.18.0, USA).

## 2.2 Electrical impedance method for studying willow roots (papers III, IV)

### 2.2.1 Experiment design

In the experiments conducted in support of papers III and IV, the cuttings of *Salix schwerinii* were raised in the aerated tap water containers under the following greenhouse conditions: air temperature 20 °C, photon flux density 320  $\mu\text{mol s}^{-1}\text{m}^{-2}$ , photoperiod 18/6 h (day /night), and relative humidity 80 %. The cuttings were embedded in the water solution half way by using floating pads. The water was refreshed at intervals of three to four days. Two days before the electrical measurements were taken, the tap water was replaced by a nutrient solution (10 mg N in 1 liter of water with macro- and micronutrients, Riddoch et al., 1991). The conductivity of the hydroponic solution was 54.2  $\mu\text{Scm}^{-1}$ . The specimens were moved within the same containers from the growth chamber to the laboratory so that the electrical impedance measurements could be started the following day. In both studies the measurements were made under the similar experimental set-ups, i.e. root alone ('Root'), stem and root ('Stem and root') and stem alone ('Stem') in contact with the solution. In the experiment conducted in support of paper III, the resistance measurements were made at an alternating current frequency of 128 Hz, while in the experiment for paper IV the complex impedance measurements were made at multi-frequencies (ranging from 60 Hz to 60 kHz).

### 2.2.2 Measurement and data analysis

In experiment III, one light-colored and non-wounded root of each cutting was isolated from the surrounding roots and connected to an electric circuit. The other roots were wrapped in a clean plastic sheet and isolated from the measurement circuitry. A constant electric field (effective voltage of 0.1 V, sine-AC, 128 Hz) (TG215, TTi, Cambs., UK) was applied in order to study the electric resistance of single roots at different immersion depths in the nutrient solution. The depth was adjusted by a micrometer starting from the root apex until the whole intact root was immersed. Voltage and current electrodes (Ag-needles, 0.5 mm in diameter) were inserted in the stem of the cutting and at the bottom of the solution container (110 mm in diameter) (III, Figure 1A). The voltage and current were assessed at each immersion depth (Fluke 8022A Multimeter, John Fluke Inc., Washington, USA). Both the electrical current passing through the root-stem-solution continuum and also the total resistance were calculated. The resistance of the root with a piece of stem included was measured when the stem contacted the solution (III, Figure 1B). Subsequently, then the root was dissected from the cutting, and the resistance of the stem was measured as immersed under immersion at the same depth as in the case of the root (III, Figure 1C).

In experiment IV, the electrical impedance spectra were measured using an impedance gain-phase analyzer (SI1260, Solartron, Farnborough, Hampshire, UK) at 31 frequencies between 60 Hz and 60 kHz. One Ag-electrode (0.5mm in diameter) was inserted into the middle position of the stem above the solution. This position was maintained throughout the measurements. For the sake of an even electric field distribution in the solution, another Ag-electrode was placed at the bottom of the narrow (110 mm in diameter) solution container (IV, Figure 1). The electrodes were connected to the analyzer using coaxial cables. The input voltage level of the sinusoidal signal was 0.1 V (rms). The effect of the system function (which included the solution, the polarization on the surface of the solution electrode, and noise from the surrounding) was eliminated by measuring the IS of the solution without the plant, and then subtracting it from the IS with the plant. The IS was

measured in three experimental set-ups. (A) All roots, together with a piece of stem, were immersed in the solution ('Stem and root') (IV, Figure 1A). (B) One light-colored and non-wounded root from each cutting was immersed in the solution ('Root'). The other roots were sealed away from the circuitry by means of a plastic film (IV, Figure 1B). (C) The roots were dissected and only a part of the stem was immersed in the solution ('Stem') (IV, Figure 1C). The immersion depth of the stem was the same in each measurement set-up.

After the completion of the electrical impedance measurements in experiments III and IV, the roots were dissected and scanned (Epson Expression 1640XL, Epson America, Inc., USA) so that the contact surface area of the roots with the solution could be assessed (WinRhizo, Régent Instruments Inc, Québec, Canada). The base diameter and the immersion depth of the cuttings were recorded.

#### Data analysis

The relationship between the electrical resistance and the root morphology (the root surface area and the number of lateral roots) was determined by using Person correlation analysis and a general linear and nonlinear regression analysis. The resistance of the cuttings with and without the roots was tested by using one-way ANOVA in experiment III. For fitting the measured impedance data in the experiment IV, the equivalent circuit models were developed (IV, Figure 2). The parameters of the equivalent circuits were estimated using a free complex non-linear least squares (CNLS) curve-fitting program (LEVM Version 8.09, J.R. Macdonald, <http://www.jrossmacdonald.com>). Statistical significance was assessed at the level of 0.05. Statistical analyses were performed by using SPSS software (SPSS, ver.15.0, USA).

## 3 RESULTS

### 3.1 Effects of planting orientation and density of willow cuttings on growth and nutrient leaching (papers I, II)

#### Shoot height growth and stem yield

As a result of the extended period of time taken for the shoots of the cuttings planted horizontally to emerge through the surface of the soil, the general height of all of the shoots and the tallest shoot of the horizontally planted cuttings was significantly smaller than that of the vertically planted cuttings in the first two weeks after planting. This difference decreased with time (I, Figure 1). Both in the potted and in the field experiment, the stem biomass yields were not significantly affected by planting orientation. The total biomass yields increased with planting density, while the weight and diameter of individual plants decreased with planting density (II, Table 2).

#### Root system

In both experiment the fine root biomass and the SRL in the potted experiment were not affected by the planting orientation or planting density. The total live RSA and ARSA in each pot were not affected by the planting orientation, but they were significantly affected

by the planting density. The RSA and ARSA increased with planting density. The fine root production at 0-10 cm soil layer was clearly higher during the two consecutive growing seasons in 2009 to 2010 than in the single growing seasons of 2009 (II, Figure 3). In the pot experiment, a small quantity of coarse roots was observed and the cuttings planted horizontally produced more coarse roots than did those planted vertically (I, Figure 1).

### Nutrient leaching

In the pot experiment, the concentrations of total-N,  $\text{NO}_3\text{-N}$ , and  $\text{SO}_4\text{-S}$  in the soil leachate from the pots of the planted treatments were over three times lower than in the unplanted control pots (I, Figure 5). The concentration of  $\text{PO}_4\text{-P}$  was significantly lower in the planted than in the unplanted treatments. The concentration of  $\text{PO}_4\text{-P}$  was affected by planting orientation and planting density. The concentration of  $\text{PO}_4\text{-P}$  in the leachate was higher with the cuttings planted horizontally than with those planted vertically (I, Figure 5). The concentration of  $\text{PO}_4\text{-P}$  in the soil leachate from the pots with the highest planting density was lower than in the case of the two other planting densities. The concentration of  $\text{SO}_4\text{-S}$  in the leachate was also affected by the planting orientation. The concentration of  $\text{SO}_4\text{-S}$  in the soil leachate was lower with the willow cuttings planted horizontally than with those planted vertically (I, Figure 5).

In the field experiment, the number of soil leachate samples collected with zero tension lysimeters from below a depth of 25 cm was rare in 2009 ( $n=42$ ). As a result of irrigation the number of soil leachate samples increased in 2010 ( $n=120$ ). In 2010, the annual mean  $\text{NH}_4\text{-N}$  concentration of soil leachates collected with zero lysimeters at a depth of 25 cm showed no difference between the unplanted and the planted treatments. The average monthly concentration of  $\text{NH}_4\text{-N}$  in April was significantly higher than in irrigation period. The annual mean ( $\text{NO}_2+\text{NO}_3$ )-N concentration of soil leachates collected from below 25 cm was higher in the unplanted treatments than in the planted treatments (II, Figure 4), but no differences were found between the various planted treatments. For the planted treatments, the average monthly concentration of ( $\text{NO}_2+\text{NO}_3$ )-N in April was twice as high as higher as in July and August. The average monthly concentration of DOC from zero lysimeters was slightly higher in the irrigating period than in April, but no differences were found between the months and treatments.

Below the rooting zone (below 60 cm depth), the concentration of  $\text{NH}_4\text{-N}$  in the soil water collected by a tension lysimeter was high in the first water sampling in June 2009 (II, Figure 5). The average annual concentrations of  $\text{NH}_4\text{-N}$  did not differ between 2009 and 2010, nor did they differ between the treatments. The average monthly concentrations of  $\text{NH}_4\text{-N}$  were gradually decreased in 2009 and remained at a low level in 2010.

The concentrations of ( $\text{NO}_2+\text{NO}_3$ )-N in soil water collected by tension lysimeters from a depth of 60 cm were high in the first samples collected after the installation in 2009. The mean annual concentrations of ( $\text{NO}_2+\text{NO}_3$ )-N did not differ between 2009 and 2010, and nor were any differences between treatments observed on annual level. In 2009, the average monthly concentrations in June, July and August were significantly higher than in September and October for all plots. In all plots, the average monthly concentration of ( $\text{NO}_2+\text{NO}_3$ )-N was significantly smaller from April, May and June to July in 2010. Significantly lower concentrations were detected in August, September, and October in comparison to the previous months in 2010 (II, Figure 5).

The concentration of DOC in the soil water collected by tension lysimeter from 60 cm depth was high in the first sampling after installation in 2009. The mean annual

concentrations of DOC did not differ between 2009 and 2010, and no differences between treatments were observed on the annual level. In 2009, the mean monthly concentrations were significantly smaller in all plots from June to July and August, and the concentrations were significantly smaller from July and August to September and October. In the irrigation period of 2010, the mean monthly concentration of DOC in July was significantly higher than in the other months (II, Figure 5). The average annual conductivity and concentration of total-N in the soil water were higher in unplanted than in planted plots in 2010 only. However, there were no differences between the planted plots.

### 3.2 Electrical impedance method for studying willow roots (papers III, IV)

When the root was gradually immersed in the solution, the electrical resistance (at 128 Hz) decreased with increasing immersion depth (III, Figure 2). When the individual root had been more than half-immersed in the nutrient solution, there was a significant correlation between the electrical resistance and root morphology, i.e. the surface area and the number of lateral roots. The electrical resistance had a significant negative correlation with its corresponding root surface area and with the number of lateral roots. When whole individual roots of different plants were immersed in the solution, the resistance and root morphological characteristics correlated significantly. The resistance decreased nonlinearly, along with an increase in the root size (III, Figure 3).

The resistance halved when the root and a piece of the stem were immersed in the solution as compared with immersion of the root alone (III, Figure 4). However, no difference was observed when the stem was in contact with the solution either with or without the root (III, Figure 4). A significant linear relation was found between a cross-sectional area of the stem and the resistance of the stem when immersed with or without the root also immersed in the solution (III, Figure 5).

Similarly, in the EIS the magnitude of the real and imaginary parts of the root in contact with the solution was more than ten times greater (IV, Figure 3B) than that of stem and roots with the solution (IV, Figure 3A), or stem in contact with the solution only (IV, Figure 3C). The fit of the proposed lumped models with the IS data in the three experimental set-ups was good (IV, Table 1).

The EIS parameters were related to the area of contact between the specimens and the solution. In the set-up with stem and roots in the solution, the interfacial capacitances  $C_{ss}$ ,  $C_{sc}$  and  $C_r$ , referring to the longitudinal and cross-sectional interface of the stem and to the interface of the roots with the solution, increased linearly with the increase in the surface area, respectively (IV, Figure 4A, Table 2). The logarithmic superposition resistance  $R_{sup}$  had positive linear relations with the reciprocal of the longitudinal stem surface area and with the stem cross-sectional area in the solution, respectively (IV, Figure 4B, Table 2). The relation between the superposition resistance  $R_{sup}$  and the root surface area in contact with the solution was low (IV, Figure 4B, Table 2). For the root in contact with the solution only, the parameters referring to the root-solution interface, i.e. interfacial capacitance  $C_r$  and resistance  $R_r$ , were dependent on the root surface area. Capacitance  $C_r$  increased linearly (IV, Figure 4A, Table 2), whereas the logarithmic resistance  $R_r$  increased positively with an increase in the reciprocal of the root surface area (IV, Figure. 4B, Table 2). There was no difference between the set-ups with ‘Stem’ and ‘Stem and roots’ in contact with the solution with respect to the relation between the interfacial capacitances  $C_{ss}$  and  $C_{sc}$  and the

superposition resistance  $R_{sup}$  with the longitudinal and cross-sectional area of the stems, respectively (IV, Figure 4, Table 2).

Several common parameters exist in the models for the different set-ups. Hence, it is reasonable to compare those parameters in the different independent measurements with different set-ups. Based on the mean values, most of the parameters for the ‘Stem and root’ and for the ‘Stem’ were close to each other (IV, Table 3). The parameters also bore a similar relation to the contact area of the stem (IV, Figure 4A, Table 3). When the root (‘Root’) was in the solution then the parameter estimates for the stem ( $R_{sa}$ ,  $C_{sa}$ ) differed from the corresponding values in the other two set-ups (paper IV, Table 3). The auxiliary parameter estimates referring to the properties of the root ( $R_{ra}$ ) were lower for the ‘Root’ than for the ‘Stem and root’. The interfacial resistance of the ‘Root’ ( $R_r$ ) was much higher than the superposition resistance ( $R_{sup}$ ) for the set-up ‘Stem and roots’ (IV, Figure. 2A). However, the slope for the interfacial root capacitance ( $C_r$ ) with the root surface area was clearly different for the ‘Root’ than for the ‘Stem and root’ (IV, Figure 4A, Table 2). According to the pooled data of the logarithmic superposition resistance ( $R_{sup}$ ) in the set-up ‘Stem and root’ and the interfacial resistance of the single root ( $R_r$ ) in the set-up ‘Root’ were correlated strongly with the reciprocal of the contact area of the roots in the solution (IV, Figure 4B, Table 2).

## 4 DISCUSSION

### 4.1 Effects of planting orientation and density of willow cuttings on growth and nutrient leaching (papers I, II)

The heights of all of the shoots and the tallest shoots of the horizontally planted cuttings were significantly smaller than those of the vertically planted cuttings but only during the first two weeks after planting. Thereafter, no significant effect of the planting orientation on the stem biomass of the cuttings of the same length could be observed in either of the experiments in this study. The same stem biomass was also obtained between the vertically and horizontally planted cuttings of different lengths in earlier studies. A similar biomass yield produced either by planting 25 cm long cuttings vertically and 90 cm long cuttings horizontally with the same planting density (1 000 cuttings ha<sup>-1</sup>), or between by planting 20 cm long cuttings vertically and 200 cm long cuttings horizontally with a different planting density (Lowthe-Thomas et al. 2010, McCracken et al. 2010). However, the plot experiment reported by McCracken et al. (2010) proved that the 10 cm long willow cuttings planted horizontally, with 25 000 cuttings ha<sup>-1</sup> produced significantly less stem biomass than did 2 m long willow rods with 5 000 cuttings ha<sup>-1</sup>. In addition to the length of the cuttings, the planting depth of the cuttings in the soil also had an influence on the rate of growth.

Apart from considering the stem production as it appeared using two planting orientations, the quantity of the planting material as it appeared using between two planting orientations should also be considered. The same quantity of planting material it was used in two distinct planting orientations produced the same stem production in both experiments. However, the planting material used for planting the long cuttings horizontally proved to be three times more than that used in the planting of short cuttings vertically in the experiments conducted by Lowthe-Thomas et al. (2010) and McCracken et al. (2010).

Significant differences were observed in both present experiments in terms of the stem biomass yields between the different planting densities. This result is consistent with previous studies dealing with the effect of planting density on willow stem biomass yields (Bullard et al. 2002a, Wilkinson et al. 2007). However, there was no difference in the total stem production for either two or three cuttings per pot in the greenhouse experiment, and only twice as much stem biomass was produced in the context of planting densities of 22 500 cuttings ha<sup>-1</sup> and 7 500 cuttings ha<sup>-1</sup> in the present field experiment. The explanation proved by previous studies was that the existing stem biomass production eventually becomes independent of planting density to up to a range of particular planting densities, i.e. 20 000 cuttings ha<sup>-1</sup> (Bergkvist and Ledin 1998, Wilkinson et al. 2007). In contrast to stand production, in both present experiments the average diameter and weight of individual plants were significantly larger at low rather than at high density. The results found by Bullard et al. (2002a and b) also showed that there was a negative nonlinear relation between the weight of the individual plant and planting density.

In both experiments, the fine root biomass, the SRL, and the fine root production were not significantly affected by planting orientation or planting density. However, a UK experiment reported that a greater amount of roots was produced with horizontally planted cuttings at a depth of 5 cm than at 15 cm and also with vertical planting cuttings at a depth of 5 cm (Dieterich and Martin 2008). The effect of planting density on root system was observed in the RSA and ARSA measured using the earth impedance method. Nor was any significant difference in root biomass observed between 10 000 and 111 000 cuttings ha<sup>-1</sup> of SRC willow plantations in the UK. Only the coarse root biomass which makes up only a small portion of the root system was larger in the horizontal treatments and decreased with the increased density in each pot in the present pot experiment. However, previous assumption was that the planting density should prove to change the growth and development of root system just like the effect on aboveground growth and development (Volk et al. 2001). Although many studies have been made in connection with biomass production in SRC willows, few studies have been done of their root biomass distribution and production.

Compared to unplanted pots, the significant effect of willows on reducing nutrient leaching was observed in the pot experiment for the total-N, NO<sub>3</sub>-N, PO<sub>4</sub>-P and SO<sub>4</sub>-S. However, in the field experiment, leaching reduced by willow plantation was observed only for DOC in 2009 and (NO<sub>2</sub>+NO<sub>3</sub>)-N in 2010 from a soil depth below 25 cm, and for total-N in 2010 from a soil depth below 60 cm. The absence of any difference in concentration between planted and unplanted treatments in the field experiment may be due to the small size of the experimental plots, as a result of which the tension lysimeters collected soil water from a larger area rather than just the lysimeter plot (Lehmann and Schroth 2003). Based on the observation of similar root biomass, it appeared that there was no difference in leaching between the planted treatments in two experiments in this study. In the course of irrigation period in 2010, the mean monthly concentration of NH<sub>4</sub>-N remained low and the (NO<sub>2</sub>+NO<sub>3</sub>)-N decreased relative to this situation pertaining before the irrigation period. These results agreed with the findings of a Swedish lysimeter study in which the irrigation rate had only a slight effect on the nitrate leaching (Aronsson and Bergström 2001). However, in the present experiment, the mean monthly concentration of DOC in July was significantly higher than in the other months. Meanwhile, the harvest operation in the winter of 2009 produced no increase in the leaching. This finding concurs with the previous Swedish and UK studies (Aronsson et al. 2000, Goodlass et al. 2007). The explanation may



be that the willow root system was able to sustain activity and nitrogen uptake throughout the winter, despite a soil temperature close to zero (Aronsson 2001).

#### **4.2 Electrical impedance method for studying willow roots (papers III, IV)**

The ARSA was assessed by using the earth impedance method in paper I. However, the results from papers III and IV indicated that the earth impedance method may be not suitable for measuring ARSA properly. In paper III, the electrical resistance was found to correlate with the root morphology when the single root or even the whole root system without the stem was gradually immersed in the solution. This agrees with the assumption of the earth impedance method that the electrical resistance is related to the root area (Aubrecht et al. 2006, Čermák et al. 2006). However, the resistance halved when, in addition to the root, a part of the stem was immersed in the solution. Moreover, the electrical resistance remained approximately the same when the stem, both with and without the root, was in contact with the solution. Similarly, in the IS measurements the magnitude of the real and imaginary parts of the root in contact with the solution was more than ten times higher than that of the stem and roots with the solution or the stem in contact with the solution only. This suggests that in this particular case involving willow cuttings the roots would played a minor role in the total electrical impedance of stem and root in the solution. A similar result has been obtained for Scots pine in laboratory and field studies, where most of the electric charge carriers were found to leave the root system in very proximal parts of the root system (Urban et al. 2011). In addition to the outer surface of the stem, the cut surface of the cuttings used in our experiments probably formed a good contact with the solution through the phloem and xylem of the stem. This seemed to provide a high passage of current (as compared to the roots) between the stem and the solution, resulting in low electrical impedance, the latter being linearly related to the cross-sectional area of the stem (III and IV). A similar linear relation has been observed in maize plants in a nutrient solution (Walker 1965) and also in coniferous and broadleaf woody species under field conditions (Čermák et al. 2006). Both the present study and that made by Urban et al. (2011) experiment suggest that the contact of the stem can cause a strong bias in the evaluation of the ARSA when using the electrical resistance method.

The equivalent circuit model is commonly used to understand the details of the electrical current pathways and electrical properties of different components in a circuit. The models previous proposed took into account the electrode-soil interface, root medium, roots, stem and stem-electrode interface, each of which was represented by a parallel circuit of resistance and capacitance, and also by all of the parallel circuits in series (Dalton 1995, Ozier-Lafontaine and Bajazet 2005). By measuring the components in the circuitry independently, a parallel resistance circuit between the root-solution interface and the stem-solution interface has been proposed in the case of the electrical resistance method (III). This parallel resistance circuit has explained clearly the different roles played by roots and stem in the electrical current pathways. Although Urban et al. (2011) also discovered the strong effect of the stem on the current pathways; they did not explore the intrinsic relationship between the roots and stems.

To clearly express the different roles played by the roots and stem in the electrical models, in paper IV the parallel stem-root circuitry in terms of the resistance and capacitance was proposed in the lumped models for EIS measurement. In the 'Root' set-up, one parallel circuit represents the root-solution interface. In the 'Stem' set-up, one parallel

circuit represents the axial direction of the stem-solution, whereas another parallel circuit represents the radial direction of the stem-solution. These two parallel circuits are in parallel. In the ‘Stem and root’ set-up, three parallel circuits consider the axial and radial directions in the stem-solution and in the root-solution interface. Moreover, the proposed models resulted in a good fit with the data obtained (parameter ERSD values <10 %). Hence, it can be concluded that the proposed lumped models provide a reasonable interpretation of electrical current pathways.

Certain lumped model parameters ( $C_{ss}$ ,  $C_{sc}$ ,  $C_r$  and  $R_{sup}$ ) that were common to the different set-ups correlated highly with the contact area of the roots and stem in the solution. The interfacial root capacitance ( $C_r$ ) correlated linearly with the contact area of the root in the solution in both the ‘Root’ and the ‘Stem and root’ set-up, respectively. Because of the larger root surface area in the ‘Stem and root’ than ‘Root’ set-up, the root-solution interfacial capacitance ( $C_r$ ) was considered likely to be higher for the former than for the latter, while the relation with the root surface area should be the same, respectively. It was found, however, that root-solution interfacial capacitance ( $C_r$ ) was not higher for the former than the latter, and that the slope for these two relations was different. In consequence, there seemed to be some interaction of the root and stem in the set-up ‘Stem and root’. The difference in the slopes for the various interfacial capacitances, i.e.  $C_{ss}$ ,  $C_{sc}$ , and  $C_r$ , with respective areas refers to different electrochemical properties on the surfaces of the stem and root.

The experiment was carried out in a hydroponic solution where two important factors causing variability in the field measurements were fixed, i.e. the soil moisture content and the soil type. In the field measurements, the conductivity of soil will vary depending on the water content and highly mobile ions, such as  $K^+$ , and  $Cl^-$  (Dvorak 1981). Soil properties, i.e. soil moisture, salinity and soil texture, have been observed to affect the capacitance and impedance measurements (Dalton 1995). Hence, it has been recommended to run the measurements of the soil moisture content at a high enough level, i.e. the field capacity (Dalton 1995, van Beem et al. 1998).

The position of the stem electrode should also be considered in the context of the impedance measurements. The position of stem electrode affects the proportion of the stem in the circuit. The stem electrode should be set close to the primary lateral roots and it should remain in the same position throughout the measurements (Dalton 1995, Preston et al. 2004, Rajkai 2005). In the context of beans, for instance, raising the electrode 10 cm upwards from the root collar leads to a linear increase in resistance from below 1 k $\Omega$  to above 150 k $\Omega$ , and to a nonlinear decrease in capacitance from 70 nF to less than 5 nF (Dalton 1995). With regard to the position of the soil electrode, preliminary studies have shown that the distance and depth of the soil electrode would have no effect on the capacitance and resistance (van Beem et al. 1998, Preston et al. 2004, Ozier-Lafontaine and Bajazet 2005).

The cut surface area of the stem formed an open end through the phloem and xylem to the solution in this study. This surface was expected to provide a high passage of current between the stem and the solution. In consequence, it was included in the corresponding circuit models. For seed-originated plants no such open ends exist. In future studies with different species and growing substrates, this part of the circuit needs to be verified and the model revised accordingly.

## 5 CONCLUSIONS

The main aim of this study has been to investigate whether horizontally planted cuttings have a positive effect on stem yield and root distribution and also on reducing nutrient leaching in comparison with vertically planted cuttings with different planting densities. The results of a greenhouse experiment and a field experiment that took three years showed that horizontally planted willow cuttings can result in the same stem yield, root biomass and nutrient leaching as those of conventional, vertically planted willow cuttings of similar size. Meanwhile, it was also found that planting density significantly affected the stem yield but had no effect on the fine root biomass. Hence, horizontal planting can be used as an alternative planting method to achieve biomass production and other environmental performance, for example in buffer zones of riparian areas, stream-banks and wetlands. It would be interesting in the further studies to explore the influence of willow clones, the length and diameter of cuttings, the planting depth of horizontally planted willow cuttings for their ability to stabilize slopes, to control erosion, and to reclaim contaminated sites.

The earth impedance method was applied in the pot experiment to measure the absorptive surface area of a root system. The hydroponic studies showed, however, that a stem in contact with a growth substrate has strong impact on the estimated absorptive root surface area. The impedance measurement at a single-frequency provides a simplified view for an equivalent model of the system only. EIS, as a new approach, has been used to study the electrical parameters and root morphology of willow cuttings. The proposed lumped models were considered the most essential components in the circuitry. A good fit was obtained between the impedance spectra measured and the corresponding lumped models proposed. The model parameters were correlated with the contact area of the roots and/or stem with the solution. Hence, the EIS method provides a useful non-destructive method for studying root systems and their functions. Further studies of the EIS are needed with soil as the growing substrate under different growing conditions.

## REFERENCES

- Adegbidi, H.G. & Briggs, R.D. 2003. Nitrogen mineralization of sewage sludge and composted poultry manure applied to willow in a greenhouse experiment. *Biomass and Bioenergy* 25: 665-673.
- , Briggs, R.D., Volk, T.A., White, E.H. & Abrahamson, L.P. 2003. Effect of organic amendments and slow-release nitrogen fertilizer on willow biomass production and soil chemical characteristics. *Biomass and Bioenergy* 25: 389-398.
- Altmann, M., Pliquet, U., Suess, R. & von Borell, E. 2004. Predication of lamb carcass composition by impedance spectroscopy. *Journal of Animal Science* 82: 816-825.
- Aronsson, P.G. 2001. Dynamics of nitrate leaching and <sup>15</sup>N turnover in intensively fertilized and irrigated basket willow grown in lysimeters. *Biomass and Bioenergy* 21: 143-154.
- & Bergström, L.F. 2001. Nitrate leaching from lysimeter-grown short-rotation willow coppice in relation to N-application, irrigation and soil type. *Biomass and Bioenergy* 21: 155-164.
- , Bergström, L.F. & Elowson, S.N.E. 2000. Long-term influence of intensively cultured short-rotation willow coppice on nitrogen concentrations in groundwater. *Journal of Environmental Management* 58: 135-145.
- Aubrecht, L., Stanek, Z. & Koller, J. 2006. Electrical measurement of the absorption surface of tree roots by the earth impedance method: 1. Theory. *Tree Physiology* 26: 1105-1112.
- Bayford, R.H. 2006. Bioimpedance tomography (electrical impedance tomography). *Annual Review of Biomedical Engineering* 8: 63-91.
- Bergkvist, P. & Ledin, S. 1998. Stem biomass yields at different planting designs and spacings in willow coppice systems 14: 149-156.
- Börjesson, P. 1999. Environmental effects of energy crop cultivation in Sweden: identification and quantification. *Biomass and Bioenergy* 16: 137-154.
- & Berndes G. 2006. The prospects for willow plantations for wastewater treatment in Sweden. *Biomass and Bioenergy* 30:428-438.
- Bullard, M.J., Mustill, S.J., Carver, P. & Nixon, P.M.I. 2002a. Yield improvements through modification of planting density and harvest frequency in short rotation coppice *Salix* spp: Resource capture and use in two morphologically diverse varieties. *Biomass and Bioenergy* 22: 27-39.
- , Mustill S.J., McMillan, S.D., Nixon, P.M.I., Carver, P. & Britt, C.P. 2002b Yield improvements through modification of planting density and harvest frequency in short rotation coppice *Salix* spp: Yield response in two morphologically diverse varieties. *Biomass and Bioenergy* 22: 15-25.
- Butler, A.J., Barbier, N., Čermák, J., Koller, J., Thornily, C., McEvoy, C., Nicoll, B., Perks, M.P., Grace, J. & Meir, P. 2010. Estimates and relationships between aboveground and belowground resource exchange surface areas in a Sitka spruce managed forest. *Tree Physiology* 30: 705-714.
- Butnor, J.R., Doolittle, J.A., Johnsen, K.H., Samuelson, L., Stokes, T. & Kress, L. 2003. Utility of Ground-Penetrating Radar as a Root Biomass Survey Tool in Forest Systems. *Soil Science Society of America Journal* 67: 1607–1615.
- Čermák, J., Ulrich, R., Stanek, Z., Koller, J. & Aubrecht, L. 2006. Electrical measurement of the absorption surface of tree roots by the earth impedance method: 2. Verification

- based on allometric relationships and root severing experiments. *Tree Physiology* 26: 1113-1121.
- Chloupek, O. 1972. The relationship between electric capacitance and some other parameters of plant roots. *Biologia Plantarum* 14:227-230.
- 1977. Evaluation of the size of a plant's root system using its electrical capacitance. *Plant and Soil* 48: 525-532.
- Christou, M., Alexopoulou, E., Panoutsou, C. & Monti, A. 2010. Overview of the markets for energy crops in EU27. *Biofuels, Bioproducts and Biorefining* 4: 605-619.
- Costa, C., Dwyer, L.M. & Hamilton R.I. 2000. A sampling method for measurement of large root systems with scanner-based image analysis. *Agronomy Journal* 92: 21-27.
- Crow, P. & Houston, T.J. 2004. The influence of soil and coppice cycle on the rooting habit of short rotation poplar and willow coppice. *Biomass and Bioenergy* 26: 497-505.
- Dalton, F.N. 1995. In-situ root extent measurements by electrical capacitance methods. *Plant and Soil* 173: 157-165.
- Department of the Environment, Food, and Rural Affairs. 2007. *UK Biomass Strategy*. London. 49 pp.
- Dieterich, B. & Martin, P. 2008. Influence of planting depth and orientation on sprouting of willow cuttings. *Aspects of Applied Biology* 90: 233-238.
- Dimitriou, I. & Aronsson, P. 2004. Nitrogen leaching from short- rotation willow coppice after intensive irrigation with wastewater. *Biomass and Bioenergy* 26: 433-441.
- , Eriksson, J., Adler, A., Aronsson, P. & Verwijst, T. 2006. Fate of heavy metals after application of sewage sludge and wood-ash mixtures to short-rotation willow coppice. *Biomass and Bioenergy* 142: 160-169.
- Dvorak, M., Cernohorska, J. & Janacek, K. 1981. Characteristics of current passage through plant tissue. *Biologia Plantarum* 23: 719-726.
- Elowson, S. 1999. Willow as a vegetation filter for cleaning of polluted drainage water from agricultural land. *Biomass and Bioenergy* 16: 281-290.
- Goodlass, G., Green, M., Hilton, B. & McDonough, S. 2007. Nitrate leaching from short-rotation coppice. *Soil Use and Management* 23: 178-184.
- Graham, R.L. 1994. An analysis of potential land based for energy crops in the conterminous United States. *Biomass and Bioenergy* 6: 175-189.
- Granlund, K., Räike, A., Ekholm, P., Rankinen, K. & Rekolainen, S. 2005. Assessment of water targets for agricultural nutrient loading in Finland. *Journal of Hydrology* 304: 251-260.
- Gray, D.H. & Sotir, R.B. 1996. *Biotechnical and soil bioengineering slope stabilization*. Wiley, New York.
- Heinsoo, K., Merilo, E., Petrovits, M. & Koppel, A. 2009. Fine root biomass and production in a *Salix viminalis* and *Salix dasyclados* plantation. *Estonian Journal of Ecology* 59: 27-37.
- Hirano, Y., Dannoura, M., Aono, K., Igarashi, T., Ishii, M., Yamse, K., Makita, N. & Kanazawa, Y. 2009. Limiting factors in the detection of tree roots using ground-penetrating radar. *Plant and Soil* 319: 15-24.
- Hytönen, J. & Saarsalmi, A. 2009. Long-term biomass production and nutrient uptake of birch, alder and willow plantations on cut-away peatland. *Biomass and Bioenergy* 33: 1197-1211
- , Saarsalmi, A. & Rossi, P. 1995. Biomass production and nutrient uptake of short-rotation plantations. *Silva Fennica* 29: 117-139.

- International Energy Agency (IEA). 2001. Developing a new generation of sustainable energy technologies: long term R&D needs. IEA, Paris, France.
- Kendall, W.A., Pederson, G.A. & Hill, R.R. 1982. Root size estimates of red clover and alfalfa based on electrical capacitance and root diameter measurements. *Grass and Forage Science* 37: 253–256.
- Keoleian, G.A. & Volk, T.A. 2005. Renewable energy from willow biomass crops: life cycle energy, environmental and economic performance. *Critical Reviews in Plant Sciences* 24: 385-406.
- Kuusemets, V., Mander, U., & Ivask, M. 2000. Riparian buffer zones as ecotechnological measures to decrease nutrient losses from agricultural landscapes. Ecosystem service and sustainable watershed management in North China. Beijing, China.
- , Mander, U., Lohmus, K. & Ivask, M. 2001. Nitrogen and phosphorus variation in shallow groundwater and assimilation in plants in complex riparian buffer zones. *Water Science Technology* 44: 615-622.
- Kuzovkina, Y.A. & Quigley, M.F. 2005. Willows beyond wetlands: uses of *Salix* L. species for environments projects. *Water, Air, Soil Pollution* 162: 183-204.
- & Volk, T.A. 2009. The characterization of willow (*Salix* L.) varieties for use in ecological engineering application: Co-ordination of structure, function and autecology. *Ecological Engineering* 35: 1178-1189.
- Lehmann, J. & Schroth, G. 2003. Nutrient leaching. In: Schroth, G., Sinclair, F.L. (ed.). *Tress, crops and soil fertility*. CAB international. p. 151-166.
- Li, X.P., Zhang, L.Q. & Zhang, Z. 2006. Soil bioengineering and ecological restoration of riverbanks at the Airport Town, Shanghai, China. *Ecological Engineering* 26: 304-314.
- Lovett, S. & Price, P. 2007. *Principles for riparian lands management*. Land Water Australia, Canberra.
- Lowthe-Thomas, S.C., Slater, F.M. & Randerson, P.F. 2010. Reducing the establishment costs of short rotation willow coppice (SRC)-a trial of a novel layflat planting system at an upland site in mid-Wales. *Biomass and Bioenergy* 34: 677-686.
- Lukac, M. & Godbold, D.L. 2010. Fine root biomass and turnover in southern taiga estimated by root inclusion nest. *Plant and Soil* 331: 505-13.
- Majdi, H. 1996. Root sampling methods-applications and limitations of the minirhizotron technique. *Plant and Soil* 185: 255-258.
- Martin, P.J. & Stephens, W. 2006. Willow growth in response to nutrient and moisture on a clay cap soil. II: water use. *Bioresource Technology* 97: 449-458.
- McBride, R., Candido, M. & Ferguson, J. 2008. Estimating root mass in maize genotypes using the electrical capacitance method. *Archives of Agronomy and Soil Science* 54: 215-226.
- McCracken, A.R., Moore, J.P., Walsh, L.R.E. & Lynch, M. 2010. Effecting of planting vertical/horizontal willow (*Salix spp.*) cuttings on establishment and yield. *Biomass and Bioenergy* 34: 1764-1769.
- Milbrandt, A. & Overend, R. P. 2009. Assessment of biomass resources from marginal lands in APEC economics. *Energy Work Group*. 52 p.
- Mirck, J., Isebrands, J.G., Verwijst, T. & Ledin, S. 2005. Development of short-rotation willow coppice systems for environmental purposes in Sweden. *Biomass and Bioenergy* 28: 219-228.
- Mola-Yudego, B. & Pelkonen, P. 2008. The effects of policy incentives in the adoption of willow short rotation coppice for bioenergy in Sweden. *Energy Policy* 36: 3062-3068.

- Mortensen, J., Nielsen, K.H. & Jogensen, U. 1998. Nitrate leaching during establishment of willow (*Salix viminalis*) on two soil types and two fertilization levels. *Biomass and Bioenergy* 6: 457-466.
- Ozier-Lafontaine, H. & Bajazet, T. 2005. Analysis of root growth by impedance spectroscopy (EIS). *Plant and Soil* 277: 299-313.
- Perttu, K.L. 1998. Environmental justification for short rotation forestry in Sweden. *Biomass and Bioenergy* 15: 1-6.
- 1999. Environmental and hygienic aspects of willow coppice in Sweden. *Biomass and Bioenergy* 16: 291-297.
- & Kowalik, P.J. 1997. *Salix* vegetation filters for purification of waters and soils. *Biomass and Bioenergy* 12: 9-19.
- Preston, G.M., McBride, R.A., Bryan, J. & Candido, M. 2004. Estimating root mass in young hybrid poplar trees using the electrical capacitance method. *Agroforestry Systems* 60: 305-309.
- Rajkai, K., Vegh, K.R. & Nacs, T. 2005. Electrical capacitance of roots in relation to plant electrodes, measuring frequency and root media. *Acta Agronomica Hungarica* 53: 197-210.
- Repo, T., Oksanen, E. & Vapaavuori, E. 2004. Effects of elevated concentration of ozone and carbon dioxide on the electrical impedance of leaves of silver birch (*Betula pendula*) clones. *Tree Physiology* 24: 833-843.
- , Laukkanen, J. & Silvennoinen, R. 2005. Measurement of tree root growth using electrical impedance spectroscopy. *Silva Fennica* 39: 159-166.
- Riddoch, I., Lehto, T. & Grace, J. 1991. Photosynthesis of tropical tree seedlings in relation to light and nutrient supply. *New Phytologist* 119: 137-147.
- Rytter, R.M. & Hansson, A.C. 1996. Seasonal amount, growth and depth of fine roots in an irrigated and fertilized *Salix viminalis* L. plantation. *Biomass and Bioenergy* 11: 129-137.
- & Rytter, L. 1998. Growth, decay, and turnover rates roots of basket willows. *Canadian Journal of Forest Research* 28: 893-902.
- Ryypö, A., Repo, T. & Vapaavuori, E. 1998. Development of freezing tolerance in roots and shoots of Scots pine seedlings at non-freezing temperatures. *Canadian Journal of Forest Research* 28: 557-567.
- Samson, B.K. & Sinclair, T.R. 1994. Soil core and minirhizotron comparison for determination of root length density. *Plant and Soil* 161: 225-232.
- Selkimäki, M., Mola-Yudego, B., Röser, D., Prinz, R. & Sikanen, L. 2010. Present and future trends in pellet markets, raw materials, and supply logistics in Sweden and Finland. *Renewable and Sustainable Energy Reviews* 14: 3068-3075.
- Shipek, F. 1993. Kumeyaay plant husbandry: fir, water, and erosion management system. In: Blackburn, T.C., Anderson, K. (ed.). *Before the wilderness: environmental management by native Californians*. Menlo Park, CA: Ballena Press. P. 379-388.
- Tahvanainen, L. & Rytönen, V.M. 1999. Biomass production of *Salix viminalis* in southern Finland and the effect of soil properties and climate conditions on its production and survival. *Biomass and Bioenergy* 16: 103-117.
- Tang, Y., Xie, J.S. & Geng, S. 2010. Marginal land-based biomass energy production in China. *Journal of Integrative Plant Biology* 52: 112-121.
- Tiitta, M., Savolainen, T., Olkkonen, H. & Kanko, T. 1999. Woody moisture gradient analysis by electrical impedance spectroscopy. *Holzforschung* 53: 68-76.

- Urban, J., Bequet, R. & Mainiero, R. 2011. Assessing the applicability of the earth impedance method for in situ studies of tree root systems. *Journal of Experimental Botany* Doi: 10.1093/jxb/erq370 (in press).
- Van Beem, J., Smith, M.E. & Zobel, R.W. 1998. Estimating root mass in maize using a portable capacitance meter. *Agronomy Journal* 90: 566-570.
- Vervaeke, P., Luysaert, S., Mertens, J., Vos, De., Speleers, L. & Lust, N. 2001. Dredged sediment as a substrate for biomass production of willow trees established using the SALIMAT technique. *Biomass and Bioenergy* 21: 81-90.
- Volk, T.A., Abrahamson, L.P. & White, E.H. 2001. Root dynamics in willow biomass crops. SUNY College of Environmental Science and Forestry, Syracuse, New York. 30p.
- , Abrahamson, L.P., Nowak, C.A., Smart, L.B., Tharakan, P.J. & White, E.H. 2006. The development of short-rotation willow in the northeastern United States for bioenergy and bioproducts, agroforestry and phytoremediation. *Biomass and Bioenergy* 30: 715-727.
- Walker, J.M. 1965. Electrical A. C. resistance and capacitance of *Zea Mays* L. *Plant and Soil* 23: 270-274.
- Wickham, J., Rice, B., Finnan, J. & McConnon, R. 2010. A review of past and current research on short rotation coppice in Ireland and abroad. COFORD, Dublin. 44p.
- Wilkinson, J.M., Evans, E.J., Bilsborrow, P.E., Wright, C., Hewison, W.O. & Pilbeam, D.J. 2007. Yield of willow cultivars at different planting densities in a commercial rotation coppice in the north of England. *Biomass and Bioenergy* 31: 469-474.
- Xiong, S.J., Zhang, Q.G., Zhang, D.Y. & Olsson, R. 2008. Influence of harvest time on fuel characteristics of five potential energy crops in northern China. *Bioresource Technology* 99: 478-485.
- Xu, Q.J., Zhang, L.M., Wang, W.C. & Zhang, Y. 2009. Biotechnical means of soil stabilization using willow cuttings. *Journal of Soil and Water Conservation*. 64: 105-119.
- Zhang, M.I.N. & Willison, J.H.M. 1993. Electrical impedance analysis in plant tissues: impedance measurement in leaves. *Journal of Experimental Botany* 44: 1369-1375.
- , Stout, D.G. & Willison, J.H.M. 1992. Plant tissue impedance and cold acclimation: a re-analysis. *Journal of Experimental Botany* 43: 263-266.
- Zhang, W.L., Tian, Z.X., Zhang, N. & Li, X.Q. 1996. Nitrate pollution of groundwaters in northern China. *Agriculture Ecosystems and Environment* 59: 223-231.