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Development of leaf area and above-ground biomass of different *Populus* genotypes in a bio-energy plantation

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Ontwikkeling van bladoppervlakte en bovengrondse biomassa bij diverse *Populus* klonen in een bio-energieplantage

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

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Promotor

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Summary

To meet the growing energy demand – caused by the increasing world population and industrialization – while reducing anthropogenic CO_2 emissions in the context of global warming, various alternative renewable energy sources are being explored. Solar, wind and water are perhaps the best known. Nevertheless bio-energy, either extracted from biomass waste streams or actively cultivated biomass, is one of the most promising and readily available renewable energy sources in the European Union in the near future. Fast-growing trees have a considerable potential of storing carbon, and offer opportunities as renewable (bio-)energy resource when planted in short-rotation coppice (SRC) cultures. The process of energy generation is assumed to be CO_2 neutral as the carbon (C) released during the conversion of cultivated biomass into energy was primarily absorbed during plant growth. The generated bioenergy thereby replaces energy from burning of fossil fuels and potentially avoids CO_2 emissions, and hence mitigates climate change. Within this context, an in depth study of the sequestration and energetic efficiency of an SRC culture with poplar was the main objective of the European research project POPFULL, of which this thesis is part.

Productivity (or biomass yield) is the main indicator to evaluate the efficiency for bio-energy in terms of carbon assimilation, energy generation and economic profitability of an SRC culture. This thesis aims to obtain more fundamental insights in and a better understanding of structural and functional (process-based) production determining factors of different poplar genotypes. The poplar genotypes (*Populus* spp.) were planted in April 2010 in high-density (8,000 trees per ha) in the operational SRC plantation (18.4 ha) of the POPFULL project in Lochristi, Belgium. Production related characteristics under strong genetic control are a key factor in breeding and selection of genotypes when aiming at yield maximization in SRC bio-energy plantations. Growth and wood production primarily depend on photosynthetic efficiency and on the expansion of the photosynthetic area. Therefore, genotypic variation in total leaf area, in leaf area duration, in the distribution of leaves within the tree and in the photosynthetic capacity – all affecting the efficiency of light interception and carbon fixation – was examined in detail during the first rotation (2010-2012) of the SRC culture. Realized growth was studied in relation to naturally occurring seasonal and year-to-year variation in environmental conditions, such as water availability and incoming radiation.

Leaf area index (LAI) was a reliable estimator of harvestable woody biomass production during the first rotation of the SRC bio-energy plantation of this thesis. High LAI values were reached either by a high number of small leaves (e.g. genotype Hees) or by fewer large leaves (e.g. genotype Skado). The branch volume rather than the

number of branches contributed to high LAI values. However, a high proportion of the total leaf area was observed on sylleptic branches in several genotypes, contributing to rapid canopy development during the establishment phase. The lower photosynthetic capacity per unit of leaf area was compensated for by the large leaf area in some genotypes, which hampered the detection of a relationship between carbon assimilation rate and growth. Genotypic ranking was similar for leaf area duration (LAD) and maximum LAI (LAI_{max}), suggesting a combined strategy of high LAI_{max} and long growing seasons in highly productive genotypes.

The time between bud burst and bud set closely corresponded to the ecosystems carbon uptake period, defined as the period with a net ecosystem exchange smaller than zero, during the second growing season (2011). Year-to-year variation in the time of bud phenology revealed that cumulated air temperature was the main driver of bud burst, but that the variation was also affected by cumulated short-wave radiation. As expected from previous observations, photoperiod was decisive for timing of bud set, influenced also by (minimum and night) air temperature.

Genotypic and seasonal variation in photosynthesis and water-use efficiency (WUE) were examined using combined gas exchange and stable isotope discrimination measurements. Stomatal control upon photosynthetic assimilation rate was observed as the primary response to low soil water availability and to high vapour pressure deficit. A parallel increase in WUE was found, estimated as intrinsic water-use efficiency (WUE_i) through carbon isotope discrimination ($\Delta^{13}C$). As expected from the theory, $\Delta^{13}C$ scaled negatively with WUE_i, but the relationship varied among genotypes and throughout the growing season. We found no straightforward relationship between growth determinants and water-use efficiency – estimated as $\Delta^{13}C$ and WUE_i – and our results suggested that a considerable effect of the temporal scale of integration might explain this.

Large genotypic variation in production related characteristics was confirmed under the prevailing conditions, suggesting the potential of further selection for high-yielding genotypes for high-density and short-rotation managed plantations. Continued breeding of new genotypes is important and necessary to maximize yields in a given environment and management, which is crucial for the deployment of future SRC bio-energy plantations.

Samenvatting

Om de groeiende vraag naar energie – als gevolg van de groeiende wereldbevolking en de industrialisatie – te kunnen beantwoorden en om de antropogene CO_2-emissies te reduceren in het kader van de opwarming van de aarde, worden verschillende alternatieve hernieuwbare energiebronnen onderzocht. Zon, wind en water zijn wellicht de bekendste. Maar bio-energie – zowel gewonnen biomassa uit afvalstromen als actief geteelde biomassa – heeft in de Europese Unie een groot potentieel voor de nabije toekomst. Snelgroeiende bomen hebben een aanzienlijk potentieel voor koolstofopslag en zijn geschikt als hernieuwbare energiebron wanneer ze gecultiveerd worden in korte-omloop-hakhout (KOH) aanplantingen. De energieopwekking op basis van gecultiveerde biomassa wordt als CO_2-neutraal aanzien vermits de koolstof die vrijkomt bij de omzetting van de biomassa in bio-energie in de eerste plaats werd opgenomen tijdens de groei van de plant. Bij de bio-energieproductie wordt energie uit de verbranding van fossiele brandstoffen vervangen, dit kan CO_2-emissies vermijden en de daarmee gepaarde klimaatverandering afremmen. In deze context wordt een diepgaande studie uitgevoerd naar de koolstofvastlegging en de energetische efficiëntie van een KOH-cultuur met populier in het Europese onderzoeksproject POPFULL, waarvan dit proefschrift deel uitmaakt.

Productiviteit (biomassa-opbrengst) van een KOH cultuur is de belangrijkste indicator om de efficiëntie voor bio-energie in termen van koolstofassimilatie, energie-opwekking en economische rendabiliteit te evalueren. Dit proefschrift tracht meer fundamentele inzichten te verkrijgen in en een beter begrip te bekomen van zowel structurele als functionele productie-bepalende determinanten van verschillende populierengenotypen. Deze populieren (*Populus* spp.) werden in april 2010 aangeplant met een hoge plantdichtheid (8,000 planten per ha) in de operationele KOH-plantage (18.4 ha) van het POPFULL project in Lochristi, provincie Oost-Vlaanderen. Productie-gerelateerde kenmerken onder sterke genetische controle zijn een belangrijke factor in de selectie van genotypes voor de maximalisatie van de oogst in KOH-plantages voor bio-energie. Groei en houtproductie zijn hoofdzakelijk afhankelijk van de fotosynthetische efficiëntie en de expansie van de fotosynthetische oppervlakte. Daarom werd de genotypische variatie in totale bladoppervlakte, in bladoppervlakte-duur, in de verdeling van de bladeren in de kruin en in de fotosynthetische capaciteit – welke allemaal een invloed hebben op de efficiëntie van lichtinterceptie en koolstof-vastlegging – in detail onderzocht gedurende de eerste rotatie van de KOH-plantage. De gerealizeerde groei werd bestudeerd in relatie tot natuurlijke seizoensgebonden en jaarlijkse variaties in omgevingsomstandigheden, bijvoorbeeld de waterbeschikbaarheid en de inkomende straling.

Bladoppervlakte-index (LAI) was een betrouwbare indicator van de oogstbare houtige biomassaproductie tijdens de eerste rotatie van de KOH-plantage in deze studie. Hoge LAI-waarden werden ofwel bereikt door een groot aantal kleine bladeren (bv. genotype Hees) of door een kleiner aantal grote bladeren (bv. genotype Skado). Het takvolume had een grotere invloed op LAI dan het aantal takken. Echter, een groot deel van het totale bladoppervlak bevond zich op sylleptische takken in verschillende genotypes, welke een aanzienlijke bijdrage leverden aan de snelle ontwikkeling van het bladerdek tijdens de vestigingsfase. De lagere intrinsieke fotosynthesecapaciteit per eenheid bladoppervlak werd gecompenseerd door de grote totale bladoppervlakte in sommige genotypes, wat de detectie van een relatie tussen koolstofassimilatie en groei belemmerde. De resultaten duidden op een gecombineerde strategie van hoge maximale LAI (LAI_{max}) en lange groeiseizoenen in hoog productieve genotypen.

De tijd tussen de knopopening en knopzetting kwam nauw overeen met de periode waarin een netto ecosysteem-koolstofopname werd waargenomen in het tweede groeiseizoen (2011). Jaarlijkse variatie in fenologische observaties wees erop dat de gecumuleerde luchttemperatuur de belangrijkste determinant van de knopopening was, maar dat deze ook beïnvloed werd door de gecumuleerde kortgolvige straling. Zoals verwacht uit eerdere studies was de fotoperiode bepalend voor de timing van knopzetting, maar een additioneel effect van (minimum en nacht-) luchttemperatuur werd waargenomen.

Genotypische en seizoenale variatie in fotosynthese en in *water-use* efficiëntie (WUE) werd onderzocht met behulp van gecombineerde gasuitwisselings- en stabiele-isotoop-discriminatie-metingen. Stomatale controle van de fotosynthetische assimilatie werd waargenomen als de belangrijkste respons op de lage beschikbaarheid van bodemwater en het hoge dampdruk-deficit. Een parallelle toename van WUE werd gevonden, gemeten als intrinsieke *water-use* efficiëntie (WUE_i) en C-isotoop-discriminatie ($\Delta^{13}C$). Zoals verwacht op basis van de theorie was $\Delta^{13}C$ negatief gecorreleerd met WUE_i, maar de relatie varieerde tussen genotypen en gedurende het groeiseizoen. We vonden geen duidelijke relatie tussen de groeideterminanten en WUE – geschat als $\Delta^{13}C$ en WUE_i – en onze resultaten suggereren dat een aanzienlijk effect van de temporele schaal van integratie dit zou kunnen verklaren.

Grote genotypische variatie in productie-gerelateerde kenmerken werd bevestigd onder de heersende omstandigheden, wat het potentieel van verdere selectie voor hoog-productieve genotypen suggereert voor KOH-plantages in diverse regio's. De continue zoektocht naar nieuwe genotypen is belangrijk en noodzakelijk om de opbrengsten in een bepaalde omgeving en beheer te maximalizeren, hetgeen cruciaal is voor de toepassing van toekomstige KOH plantages voor bio-energie.

List of publications

Broeckx LS, Verlinden MS, Berhongaray G, Zona D, Fichot R, Ceulemans R (2013) The effect of a dry spring on seasonal carbon allocation and vegetation dynamics in a poplar bioenergy plantation. *Global Change Biology Bioenergy*, doi: 10.1111/gcbb.12087.

Broeckx* LS, Verlinden* MS, Ceulemans R (2012) Establishment and two-year growth of a bio-energy plantation with fast-growing *Populus* trees in Flanders (Belgium): effects of genotype and former land use. *Biomass and Bioenergy,* 42: 151-163.
* Both authors equally contributed to this manuscript

Broeckx LS, Verlinden MS, Vangronsveld J, Ceulemans R (2012) Importance of crown architecture for leaf area index of different *Populus* genotypes in a high-density plantation. *Tree Physiology*, 32: 1214-1226.

Njakou Djomo S, El Kasmioui O, De Groote T, Broeckx LS, Verlinden MS, Berhongaray G, Fichot R, Zona D, Dillen SY, King JS, Janssens IA, Ceulemans R (2013) Energy and climate benefits of bioelectricity from low-input short rotation woody crops on agricultural land over a two-year rotation. *Applied Energy*, 111: 1-9.

Verlinden MS, Broeckx LS, Van den Bulcke J, Van Acker J, Ceulemans R (2013) Comparative study of biomass determinants of 12 poplar (*Populus*) genotypes in a high-density short-rotation culture. *Forest Ecology and Management*, 307: 101-111.

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Verlinden MS, Broeckx LS, Zona D, Berhongaray G, De Groote T, Camino Serrano M, Janssens IA, Ceulemans R (2013) Net ecosystem production and carbon balance of an SRC poplar plantation during its first rotation. *Biomass and Bioenergy*, 56: 412-422.

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Synthesis

Development of leaf area and above-ground biomass of different *Populus* genotypes in a bio-energy plantation

Broeckx L.S.

This overarching synthesis acts as an integrated synthesis of the five main chapters of the thesis. The synthesis presents the overall framework as well as the specific large-scale research project in which the thesis fits. The theoretical background based on the literature is followed by a summary and integrated discussion of the primary research findings, and completed with concluding remarks and perspectives. As each chapter is a published or (to be) submitted scientific article of an international peer-review journal, it contains its own literature study, materials and methods, results and discussion. This synthesis is to be read as a stand-alone compilation of all the chapters of this Ph.D. thesis.

1. General introduction

1.1. Biomass for energy

As the world population and economic development continue to grow, so will the global energy demand. The International Energy Outlook (IEO) recently projected an increase of the global energy consumption by more than 50% between 2010 and 2040 (IEO 2013). Currently, the global energy supply is about 80% fossil fuel-based (Fig. 1). A higher consumption than supply of oil and natural gas in many industrialized countries increased their energy dependency, with 54% of the EU's gross inland energy use being imported in 2010 (Eurostat 2013a). Meanwhile, increasing evidence supports the large contribution of anthropogenic greenhouse gas (GHG) emissions to global warming since the industrial revolution (IPCC 2011). Mainly the use of fossil fuels induced an increase in atmospheric CO_2 concentration of 100 ppm (an increase of 36%) over the last 250 years, with a much higher increase rate during the last decades (IPCC 2007 & 2011). Carbon dioxide emissions due to human-induced land-use change, in particular deforestation, considerably contributed to the CO_2 growth rate as well. Due to its long residence time in the atmosphere and the high emission rate, CO_2 is the most important GHG. The observed increase in atmospheric GHG concentrations is associated with a changing climate as evidenced from rising global average surface temperature and sea level (IPCC 2007). Projections of global warming indicate a temperature increase by 2100 ranging between 1.8 and 4.0 °C depending on the scenarios that are being used (IPCC 2007).

Environmental concerns, increasing fossil fuel prices and the demand for energy security and diversity have been the key drivers for governmental policies to invest in renewable energy sources. In 2010, 13% of the global primary energy demand was supplied by renewable energy (IEA 2012; Fig. 1). In Belgium renewables contributed 4.1% to the gross final energy consumption in 2011 (Eurostat 2013b). The European Commission (EC) addressed these energy issues in binding 20-20-20 targets, aiming at (i) a 20% reduction of GHG emissions in comparison to the 1990 levels, (ii) a share of 20% renewable energy in the gross final energy consumption, and (iii) a 20% reduction of primary energy use through improved energy efficiency by 2020 (Directive 2009/28/EC). The energy targets were implemented in national action plans for each of the member states, with a target of a 13% share of renewable energy sources for Belgium (Eurostat 2013c).

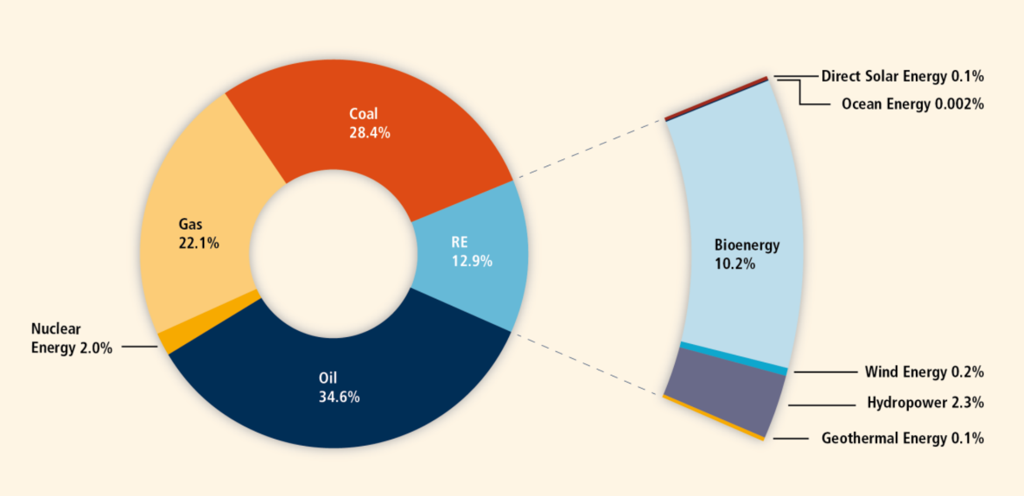

Fig. 1 Shares of energy sources in the total global primary energy supply in 2008 (IPCC, 2011)

Besides hydropower, solar, wind and geothermal energy, biomass – generally referred to as bio-energy – made up the largest share (80%) of the global renewable energy supply in 2008 (IPCC 2011; Fig. 1). Bio-energy is a versatile energy source that can substitute fossil fuels in all its forms, in contrast to other renewables. For example, liquid biofuels remain the only renewable energy source that can directly substitute oil in the transport sector (COM 2005/628), a key economic sector which is responsible for almost 30% of the EU's energy consumption (Eurostat 2013d). Bio-energy can be extracted from residues of forestry, agriculture and processing industries, as well as from municipal and other organic waste streams. Biomass can also be actively grown in cultures of annual and perennial energy crops, combining their carbon sequestration potential with bio-energy production. Cultivation of energy crops for bio-energy is assumed to be CO_2 neutral since the carbon released during the conversion was primarily absorbed during plant growth. Via substitution of fossil fuels, bio-energy could potentially avoid CO_2 and other GHG emissions, hence mitigate climate change.

The need for an increasing growth of renewable energy generation and the amendment towards the use of non-food feedstock suggest that the study of and investment in biomass production remain relevant to meet the EU targets (COM 2013/175).

The use of biomass for energy in the EU is still below its potential (COM 2006/848). Bio-energy is expected to play a central role in the development of sustainable energy sources in the EU, up to half of the 20% target (COM 2010/11). Significant progress has been made in the renewable electricity sector in the EU, with a steep increase of biomass electricity. In Flanders, green power production was for 42% dependent on biomass in 2011, wood being the most important feedstock (Vito, 2011). The highest growth potential is in biomass-fired combined heat and power stations and efficient household installations. In this context, fast-growing woody energy crops deserve an in depth study of their sequestration and energetic efficiency, which is the overall objective of the European research project POPFULL (see section 1.3.1.).

1.2. Short-rotation coppice (SRC)

1.2.1. Definition

According to the Flemish forest decree a short-rotation coppice (SRC) culture is defined as ‘a cultivation of fast-growing woody crops where the above-ground biomass is periodically harvested in total, maximum 8 years after the planting or after the previous harvest’ (Staatsblad 19/05/2006, Vlaams Bosdecreet Artikel 4 §14bis1). Generally, the rotation cycle varies between two and five years (with an expected lifetime of 20-25 years), generating biomass for energy, paper or fiber. The concept of SRC for bio-energy production originates from the 1960’s (Hansen, 1991) but coppicing, i.e. cutting the trees at the stem base to stimulate regrowth from the stump and/or from the roots to increase biomass production, is an ancient technique in forestry. At first the 1973 oil crisis and later the EU set-aside policy (1980s) in response to the agricultural overproduction, renewed the interest in SRC cultivation (Dillen et al. 2011a). Currently, the CO_2 emission mitigation and energy production potential of SRC are being explored in response to environmental and energy security concerns.

In SRC cultures for bio-energy, fast-growing trees – most commonly *Populus* and *Salix* spp. – are planted in high densities, ranging from 6,000 to 20,000 ha^{-1} (Sage 1999, Bergante et al. 2010). Short-rotation coppice cultures can be established from one-year old long shoots, from rooted or from unrooted (dormant) hardwood cuttings, the latter being mostly applied (Fig. 2). Both single- and twin-row planting schemes have been described and applied, each with their benefits and disadvantages. The main advantage of the Swedish twin-row design is the fast canopy closure; hence optimal light capture is reached shortly after planting. In this planting scheme, trees are planted in rows with alternating distances of 0.75 m and 1.50 m, the wheels of agricultural machines straddling the narrow row. The single-row design, often used in Italy, facilitates weed management; but lower planting densities induce longer rotation cycles (Spinelli et al. 2009, Dillen et al. 2011a).

Weed management is crucial during the establishment year(s) and each year after coppicing, given the light-demanding nature of poplars, a pioneer species (see further under 1.2.3). Mechanical and/or chemical weed control reduces competition for light, water and nutrients and hence improves growth and survival (Ledin and Willebrand 1996, Otto et al. 2010). In case of a careful follow-up, mortality of the hardwood cuttings during the establishment year is generally very low for commercial genotypes (Dickmann and Stuart 1983, Ceulemans and Deraedt 1999, Trnka et al. 2008), but was shown to be genotype dependent (Broeckx et al. 2012a, Laureysens et al. 2003). A first coppice often takes place after one or two growing seasons to stimulate multi-stem regrowth from the established root system (Sims et al. 2001, Al Afas et al. 2008a). An increased growth performance has been found as compared to non-coppiced systems with poplar (Hervé and Ceulemans 1996, Tschaplinski and Blake 1989, Dickmann et al. 2001). In addition, multi-stem coppice systems are easily harvestable with agricultural harvesting machines. In poplar there is generally a lower number of heavier stems as compared to willow coppice systems due to a stronger apical dominance in poplar (Ceulemans et al. 1996).

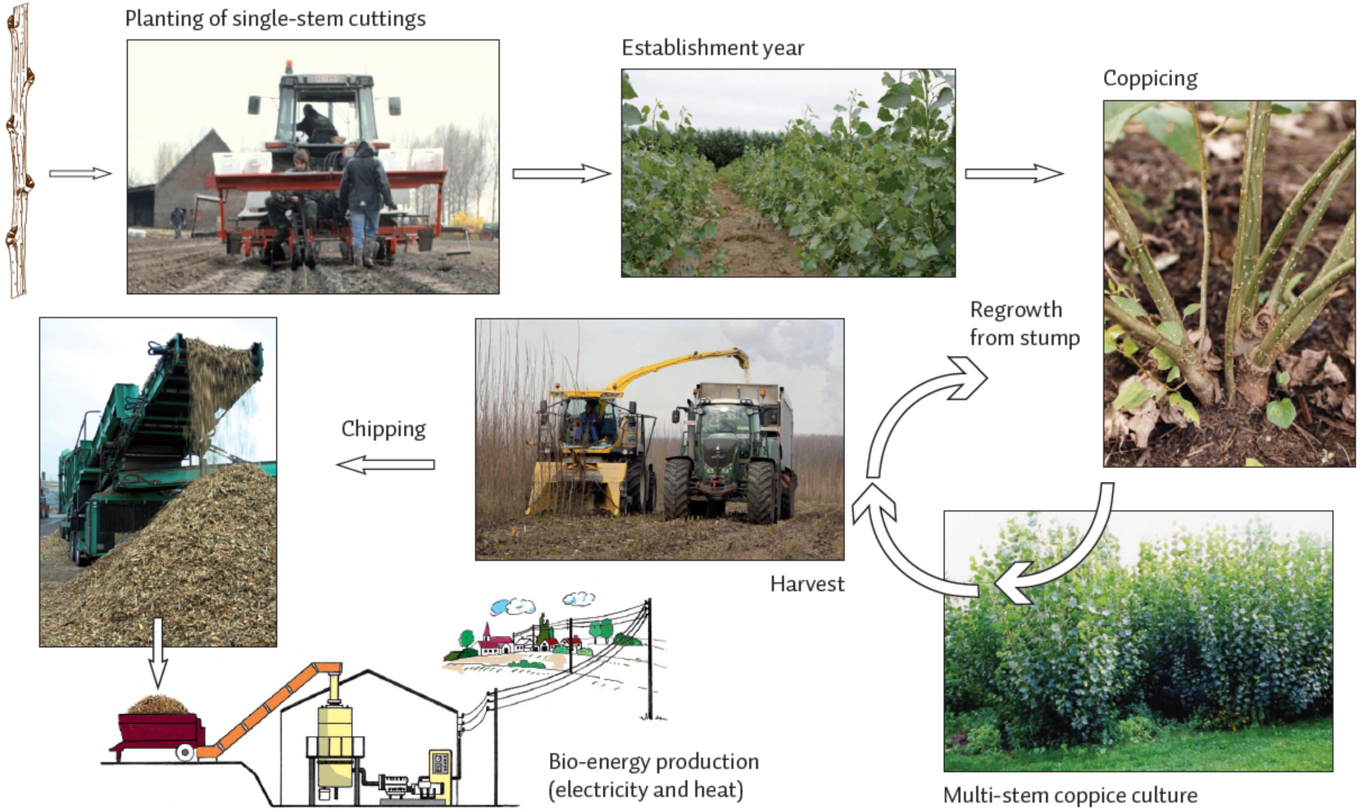

Fig. 2 Short-rotation coppice management of woody energy crops, the principle.

The harvest takes preferentially place in winter, when frozen soils prevent soil compaction due to the heavy harvesting machines. In addition, harvesting after the trees have shed their leaves minimizes the extraction of nutrients out of the ecosystem, limiting the need for fertilization. Two harvesting strategies are common, either combining coppicing and chipping in situ, or harvesting whole stems and chipping afterwards (Berhongaray et al. 2013).

Dried wood chips can be used for co-combustion or gasification, to produce green power and/or heat. With calorific values around 19 GJ ton^{-1} dry mass (Verlinden et al. 2013a), electricity generation varies between 1450 and 1950 kWh per ton depending on the conversion efficiency (ranging between 27.7% for combustion and 37.2% for gasification; Njakou Djomo et al. 2011). Depending on site conditions – i.e. climatic conditions, nutrient and soil water availability – yields of woody dry mass (DM) vary between 5 and 16 Mg ha^{-1} y^{-1} (Njakou Djomo et al. 2011, Ceulemans and Deraedt 1999). Taking an average yield of 10 Mg ha^{-1} y^{-1}, the yearly electricity use of 3 to 5 average Belgian households could be covered with 1 ha of poplar SRC.

1.2.2. Possibilities and constraints

The large-scale implementation of SRC for bio-energy requires changes in the landscape of which the environmental impacts mostly depend on the reference system. Positive effects on soil properties, including reduced soil erosion, soil organic carbon sequestration and soil texture, water retention and fertility due to reduced tillage, have been reported for SRC established on cropland (Rowe et al. 2009). Several studies also suggest a phytoremediation potential of poplar and willow in SRC for bio-energy purposes when established on contaminated sites (Meers et al. 2007, Van Slycken et al. 2013, Laureysens et al. 2004 & 2005). In terms of biodiversity, higher species richness of plants, invertebrates and birds and a comparable diversity and density of soil micro-arthropods were observed in willow SRC plantations in comparison to arable controls (Rowe et al. 2009, Volk et al. 2004). Additionally, planting of woody SRC counteracts negative impacts of fragmentation as the SRC crops act as ecological corridors through which species can migrate between natural areas (Sage 1998). In comparison with agricultural crops, poplar and willow SRC are characterized by a higher water-use as a result of high transpiration rates, large leaf area and considerable rooting depth (Hall et al. 1998, Allen et al. 1999, Tschaplinski et al. 1994, Fischer et al. 2013). The limitation of productivity by water availability reduces the potential of SRC without irrigation in (semi-)arid regions.

The very few studies on both the GHG and energy balances confirmed the energetic and ecological viability of SRC cultures with poplar and willow; e.g. significantly higher energy yields and lower GHG emissions of SRC were found in comparison to coal (cfr. review by Njakou Djomo et al. 2011). However, there was a large variability in the results due to methodological issues and a lack of coherence in the system boundaries. Standardization is requested for reasons of comparability, in particular since both GHG and energy balances are highly site- and region-specific. On the other hand the financial feasibility is a major constraint in the development of SRC plantations for bio-energy production. A recent review concluded that SRC cultures are not economically viable in most regions without government support (El Kasmioui and Ceulemans 2012). Results were highly variable due to differences in production costs and yields in addition to the variability in the calculation techniques. Also in Flanders, poplar SRC is not profitable due to the low biomass prices and the high lease costs for land (El Kasmioui and Ceulemans 2013), in combination with the absence of stable subsidies and/or (establishment) grants.

The implementation of SRC cultures for energy has been shown successful and profitable in Scandinavian countries; e.g. 90% of the electricity consumption and heat in the Swedish city Enköping depends on willow plantations (Wright 2006). Such large-scale development of SRC plantations in combination with a centralized conversion plant is not feasible in Belgium, given the land scarcity. The lack of juridical transparency adds to the distrust of Belgian farmers to invest in SRC bio-energy plantations. Since 2006 SRC has been removed from the forest decree, allowing SRC on agricultural land. However, the absence of SRC for timber reclamation in the land tenure law complicates the practice of SRC as an agricultural crop.

1.2.3. The genus *Populus*

Although naturally primarily occurring in the northern hemisphere, the species of the genus *Populus* (Salicaceae) have a widespread geographical distribution across a broad range of ecological habitats. Poplars are dioecious species, with separate male and female trees. The ca. 30 species are classified in six sections, morphologically and ecologically distinguished from each other (Eckenwalder, 1996). Large genetic differentiation along climatic gradients has resulted from (i) migration, typically seed dispersal through wind and rivers; (ii) natural selection adapting to the local environment; and (iii) mutation. In addition, both natural and anthropogenic hybridization further generated genetic variability.

Ecologically, poplars are fast-growing pioneer species, as reflected in their light demanding, indeterminate growth (Eckenwalder, 1996). Despite their natural occurrence on river banks, they occupy a broad range of environmental conditions. Because of their fast growth, their easy vegetative propagation and growth under controlled conditions, poplars are cultivated worldwide for wood products, mostly paper, veneer and lumber (Bradshaw et al. 2000, Dillen et al. 2011a). The disadvantages for cultivation are the high susceptibility of poplars to diseases and their high water-use associated with high growth rates, although water-use efficiency is highly variable among species and genotypes (see below). Hence, the large genetic diversity has been exploited in breeding and selection programs for high-yielding and disease-tolerant genotypes. Hybrids used in cultivation are mainly derived from species in the Aigeiros, Tacamahaca and Populus sections. The advantage of hybridization is the combination of specific growth characteristics and heterosis, defined as the superiority of the offspring as compared to the parental average characteristics (Stettler et al. 1996). The cultivation of poplar for bio-energy purposes remains scarce (Dillen et al. 2011a). Therefore, selection and breeding programs have so far mainly focused on straight, single-stem growth, not including coppice ability as a selection criterion. Besides wood production, poplars are also widely used as windbreaks in agricultural areas, soil protection and phytoremediation, reforestation, etc. (Zsuffa et al. 1996).

Due to their large genetic variation and ease of hybridization, poplars have been the subject of intensive study of quantitative genetics. The large seed abundance to offer adequate-size F1 families and the rather short generation interval – with an early expression of morphological and physiological characteristics – further contribute to its adoption as a model tree for physiological and molecular genetics studies (Taylor 2002). It was the first tree genus with a fully sequenced genome (Tuskan et al. 2006). The current progresses in genetics and biotechnology further improve the efficiency and precision of breeding and genotype selection (Dillen et al. 2011a).

1.3. POPFULL-project

1.3.1. Objectives

This thesis fits within the framework of a broader European research project named POPFULL, referring to the full accounting of the energetic, economic and GHG balance of an SRC with poplar. Overall, the project aims at studying the sequestration and energy production potential of an operational SRC, substituting fossil fuels and saving CO_2 emissions (Fig. 3). More specifically, the following objectives are addressed in POPFULL:

- Assessment of the full GHG balance of the main greenhouse gases (CO_2, CH_4, N_2O, O_3 and H_2O) of a poplar SRC;
- Energy efficiency accounting and Life Cycle Assessment (LCA) of poplar as an energy crop;
- Analysis of the economic profitability of an SRC bio-energy plantation in Flanders.

An experimental field site, operationally managed, was established where all field data are collected – both at the tree and the ecosystem scale – for a period of 2 + 2 years (2010-2014). Net fluxes of all GHG are monitored using the eddy covariance technique (Zona et al. 2013a & b). To meet the project's goals, quantification of the full carbon balance is included by combining a stock-change and a flux-based approach (Verlinden et al. 2013b). The inventorized stocks of harvestable and non-harvestable biomass combined with CO_2 exchange measurements – at the soil (Verlinden et al. 2012c), the leaf (see chapters 4 and 5) and the ecosystem (Zona et al. 2013b, Verlinden et al. 2013b) levels – determine the yield and sequestration potential. Field data are used to analyze the potential of poplar SRC to mitigate GHG emissions by means of a modeling approach, using a process based simulation model (ORCHIDEE). The energy input/output ratio is quantified taking into account all management practices from the plantation establishment up to the biomass-to-energy conversion through a full LCA (Njakou Djomo et al. 2013).

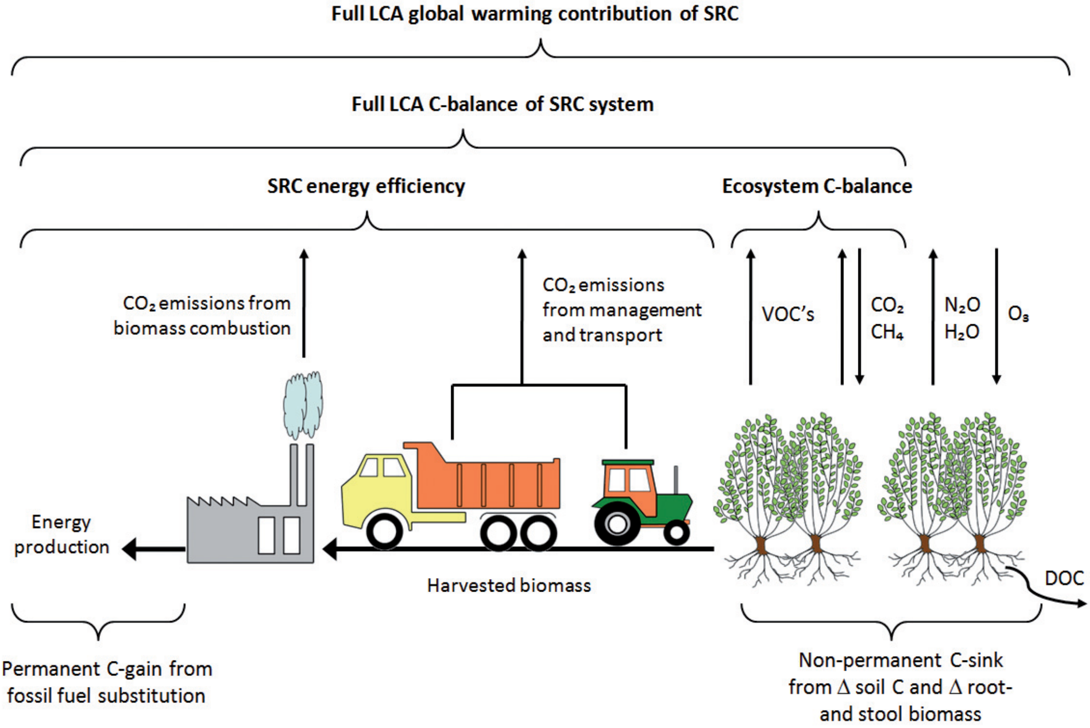

Fig. 3 Graphical description of the main objectives addressed in the large-scale POPFULL project. LCA = Life Cycle Assessment, SRC = Short-Rotation Coppice, VOC's = Volatile Organic Compounds, DOC = Dissolved Organic Carbon, C = Carbon.

1.3.2. Site description

The experimental site of POPFULL and of this thesis is located in Lochristi, Belgium (51°06’44” N, 3°51’02” E), about 11 km from the city of Ghent (Province East of Flanders). The site has an altitude of 6.25 m above sea level with a flat topography. The long-term average annual temperature and precipitation at the site are 9.5 °C and 726 mm respectively. The site has a total area of 18.4 ha of former agricultural land consisting of extensively grazed pasture as well as cropland (ryegrass, wheat, potatoes, beets, and most recently monoculture corn with regular nitrogen (N) fertilization at a rate of 200-300 kg ha^{-1} y^{-1} as liquid animal manure and chemical fertilizers).

Based on a detailed soil survey prior to planting (as detailed in chapter 1), the soil type was characterized as a sandy texture with a clay-enriched deeper soil layer, corresponding to the pedological description of the region as a sandy region with poor natural drainage. The texture of the mineral soil did not statistically differ among the soil layers up to 60 cm depth. In the upper 0-15 cm soil layer C and N mass fractions were significantly lower in previous cropland (1.48 ± 0.32 % and 0.12 ± 0.03 %, respectively) as compared to previous pasture (1.95 ± 0.36 % and 0.18 ± 0.03 %, respectively). Soil bulk density in this upper layer was significantly higher in previous cropland (1.45 ± 0.07 g cm^{-3}) than in pasture (1.27 ± 0.10 g cm^{-3}). C and N mass fractions further decreased exponentially with depth. The availability of nutrients K, P, Mg, Na and Ca did not differ between former land use types; averages are reported in chapter 1.

A total of 14.5 ha, excluding the headlands, were planted between 7-10 April 2010 with twelve poplar (*Populus*) and three willow (*Salix*) genotypes, all commercially available. After soil preparation by ploughing (40-70 cm depth), tilling and pre-emergent herbicide treatment, 25 cm-long dormant and unrooted cuttings were planted. The cuttings were soaked in water 24 h prior to planting. The planting was performed with an agricultural leek planting machine. The cuttings were planted in a double-row planting scheme with alternating distances of 0.75 m and 1.50 m between the rows and 1.10 m between trees within the rows, corresponding to a tree density of 8,000 ha^{-1}. The plantation was designed in large monoclonal blocks of eight double rows wide (Fig. 4) that cover the two types of former land use (cropland and pasture).

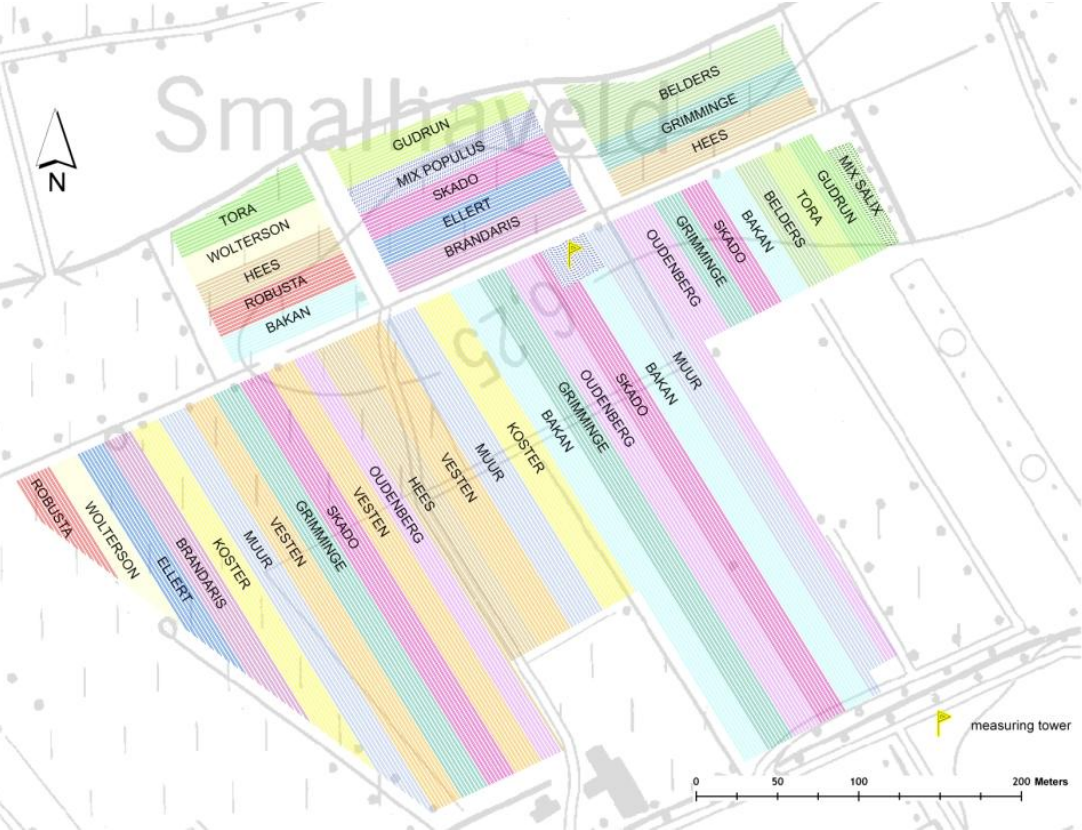

Fig. 4 Map of the plantation lay-out showing monoclonal blocks and indicating the location of the flux measurement (eddy covariance) tower (cfr. yellow flag). Genotypes Tora, Gudrun and Belders represent willow species and hybrids; all other genotypes represent poplar species and hybrids (see Table 1).

As expected, cutting mortality was very low in terms of sprouting after the planting (Dickmann and Stuart 1983, Ceulemans and Deraedt 1999, Broeckx et al 2012a), with significant genotypic variation in both timing and survival rate (Broeckx et al. 2012a). During the first months after planting intensive weed control – mechanical, chemical and manual – was applied to decrease competition for light and nutrients. Considerable mortality (18.2%) was the result of competition with weeds and damage during weed control. Hence, weed management was found a crucial factor in the establishment success of the bio-energy plantation (Buhler et al. 1998, Broeckx et al. 2012a). A 2 + 2 rotation scheme was applied (Fig. 5), with the first harvest in February 2012 yielding an average of 4 Mg DM ha^{-1} y^{-1} (with 2 and 6 Mg DM ha^{-1} y^{-1} during the first and second growing seasons, respectively). No significant increase in mortality was found after coppicing the entire plantation (Berhongaray et al. 2013), confirming the high resproutability of the genus. A standing biomass of 10 Mg ha^{-1} was estimated, based on an intensive diameter inventory (see also section 2.1.2.) at the end of first growing season after the harvest.

1.3.3. Plant materials

The planted genotypes represented different species and hybrids of *Populus deltoides* Bartr. (ex Marsh.), *P. maximowiczii* Henry, *P. nigra* L., and *P. trichocarpa* Torr & Gray (ex Hook) and *Salix viminalis* L., *S. dasyclados* Wimm., *S. alba* L. and *S. schwerinii* E.Wolf. This thesis focuses on (all) poplar genotypes only; information on the parentage and the origin of the twelve poplar genotypes is given in Table 1. Although they have been selected for growth under local environmental conditions, the different genotypes contain parental material of different origin (climate and habitat related), reflecting a wide variety of growth strategies. All genotypes are commercially available, directly employable in commercial SRC plantations. However, the genotypes have not been selected specifically for coppicing. Instead, selection criteria of wood quality mainly focused on the use as plywood, when grown in pruned, more widely spaced single-stem stands. Hence, their growth performance in high-density bio-energy plantations is thusfar unknown.

Table 1 Overview of the twelve poplar (*Populus*) genotypes planted in the operational SRC plantation of this thesis. Parentage, botanical section, place of origin, gender, and the year of the cross and of the commercialization are presented.

Genotype	Parentage	Section	Place of origin	Gender	Year of cross/ commercialization
Bakan[1]	T × M	Tacamahaca	(Washington US x Oregon US) x Japan	♂	1975/2005
Skado[1]	T × M	Tacamahaca	(Washington US x Oregon US) x Japan	♀	1975/2005
Muur[1]	D × N	Aigeiros	(Iowa US x Illinois US) x (Italy x Belgium)	♂	1978/1999
Oudenberg[1]	D × N	Aigeiros	(Iowa US x Illinois US) x (Italy x Belgium)	♀	1978/1999
Vesten[1]	D × N	Aigeiros	(Iowa US x Illinois US) x (Italy x Belgium)	♀	1978/1999
Ellert[2]	D × N	Aigeiros	Michigan US x France	♂	1969/1989
Hees[2]	D × N	Aigeiros	Michigan US x France	♀	1969/1989
Koster[2]	D × N	Aigeiros	Michigan US x The Netherlands	♂	1966/1988
Robusta[3]	D × N	Aigeiros	Eastern US x Europe	♂	1885-1890/?
Grimminge[1]	D × (T × D)	Aigeiros x (Tacamahaca x Aigeiros)	(Michigan US x Connecticut US) x (Washington US x (Iowa US x Missouri US))	♂	1976/1999
Brandaris[2]	N	Aigeiros	The Netherlands x Italy	♂	1964/1976
Wolterson[2]	N	Aigeiros	The Netherlands	♀	1960/1976

[1] genotypes produced by the Institute for Nature and Forest Research (INBO, Geraardsbergen, Belgium)

[2] genotypes produced by the Research Institute for Forestry and Landscape Planning "De Dorschkamp" (Wageningen, The Netherlands)

[3] genotype originating from an open-pollinated *P. deltoides* tree, first commercialized by the nursery Simon-Louis Frères (Metz, France)

1.4. Objectives of the thesis

The POPFULL-project provided the opportunity to study the realized growth of selected, commercially available poplar genotypes in an operational SRC regime under the prevailing conditions. When searching for the deployment of superior genotypes it is essential to screen a large variety of genotypes for their growth performance. The overall objective of the thesis was to improve our knowledge of the determinants of productivity of a high-density poplar SRC plantation for the production of bio-energy. Production related characteristics under strong genetic control are key factors in breeding and selection for genotypes when aiming at yield maximization in SRC bio-energy plantations. Therefore, the identification of functional and structural parameters, and of their interactions contributing to genotypic differences in productivity, supports selection decisions for tree improvement. To reach the overall objective the following research topics were addressed more in particular:

- Genotypic variation in maximum leaf area index (LAI_{max}) and identification of the underlying mechanisms determining LAI (tree architecture, individual leaf area (LA), number of leaves, ...);
- Genotypic variation in leaf area development, either assessed as leaf area duration (LAD) or through bud phenology, including its relationship with ecosystem carbon uptake;
- Genotypic and seasonal variation in photosynthetic and water-use efficiency (WUE), both at the leaf level and the ecosystem scale;
- Identification and interpretation of the relationships of the above mentioned parameters and processes with growth and biomass production.

Due to the timing of the SRC plantation and of the sampling of the field data for this thesis, the thesis mainly focuses on the first two growing seasons, i.e. the first two-year rotation of the bio-energy plantation (Fig. 5).

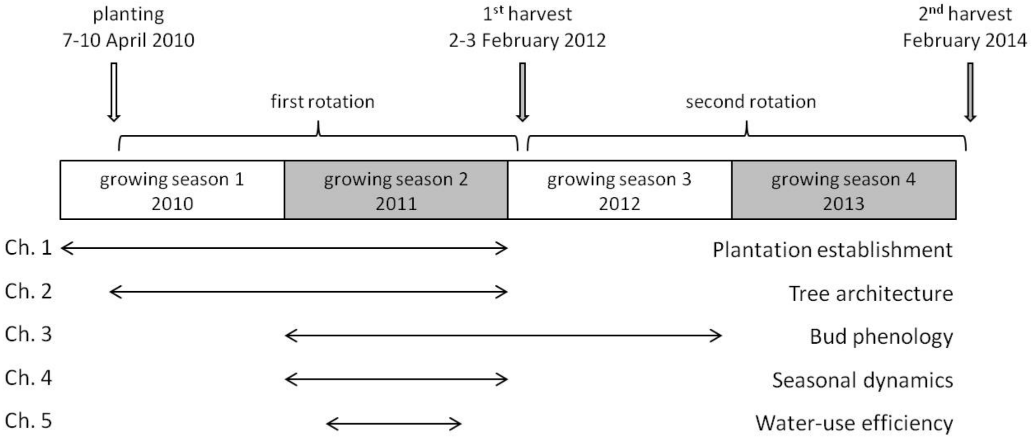

Fig. 5 Schematic representation of the 2 + 2 rotation cycle of the SRC plantation, indicating the time frames covered in the different chapters of the thesis.

1.5. Theoretical background based on the literature and identification of research questions

The success of an SRC plantation depends on the yield, i.e. the above-ground harvestable woody biomass production. Growth and wood production primarily depend on photosynthetic efficiency and on the expansion of the photosynthetic area, the latter being determined by the leaf area and tree architecture (Monteith 1977, Hallé et al. 1978). The photosynthetic area determines the plant's capacity to intercept solar radiation, which is linearly related to biomass production (Cannell et al. 1988). Light-use efficiency, i.e. the ratio of biomass accumulation to intercepted radiation, depends on leaf area index (LAI), leaf area duration (LAD), tree architecture and leaf photosynthetic capacity (Isebrands et al. 1983). In addition, genotypes can differ in leaf, phenological and tree architectural traits as well as in their relationship to growth when grown under different environmental conditions (Dillen et al. 2007 & 2009). Hence, the interactions between genotype and environment determine the realized growth of a poplar SRC culture. Based on the existing literature Fig. 6 shows an integrated scheme of factors underlying biomass production, either directly or indirectly. The interaction between genotype and environment determines the expression of both structural and process-based parameters that affect the efficiency of carbon uptake during photosynthesis. Genotype specific carbon uptake rate and allocation of photosynthates to the different tree compartments determine the amount of harvestable woody biomass, depending on resource availability (light, water and nutrients). So, the overall research question of the thesis was formulated as: which are the functional and structural components that determine yield in a high-density short-rotation plantation? In this thesis measurements of production related parameters were collected at the leaf, the tree and/or the ecosystem scales, for morphologically and genetically differing genotypes. Since no manipulative experiments were included in the POPFULL project, genotypic variation in relation to the prevailing environmental conditions was used to analyze the critical – functional and structural – determinants of productivity.

Large genotypic variability in poplar has been shown for total leaf area, for LAD, for the distribution of leaves within the tree and for photosynthetic capacity, as well as for biomass production (Scarascia-Mugnozza et al. 1989, Gielen et al. 2002, Monclus et al. 2006, Dowell et al. 2009). Total leaf area is generally assessed through the leaf area index (LAI), defined as the total one-sided leaf area per unit ground surface area (Watson 1947, Chen and Black 1991). LAI represents the total photosynthetically active surface area and has been reported to correlate with biomass production in poplar SRC cultures (Larson and Isebrands 1972, Taylor et al. 2001a, Pellis et al. 2004a, Broeckx et al 2012a). LAI is one of the most important early selection criteria among production related characteristics (Ceulemans et al. 1993, Al Afas et al. 2005, Verlinden et al. 2013a). Non-destructive indirect measurements of LAI can therefore be used to estimate the standing biomass of an SRC plantation. However, the high variability among LAI assessment methods indicates that direct measurements are necessary to validate the indirect assessments (Jonckheere et al. 2004). A good correlation with biomass production has been reported for both total (Larson and Isebrands 1972, Pellis et al. 2004a) and individual leaf area (Ridge et al. 1986, Bunn et al. 2004, Marron et al. 2007) in poplar SRC.

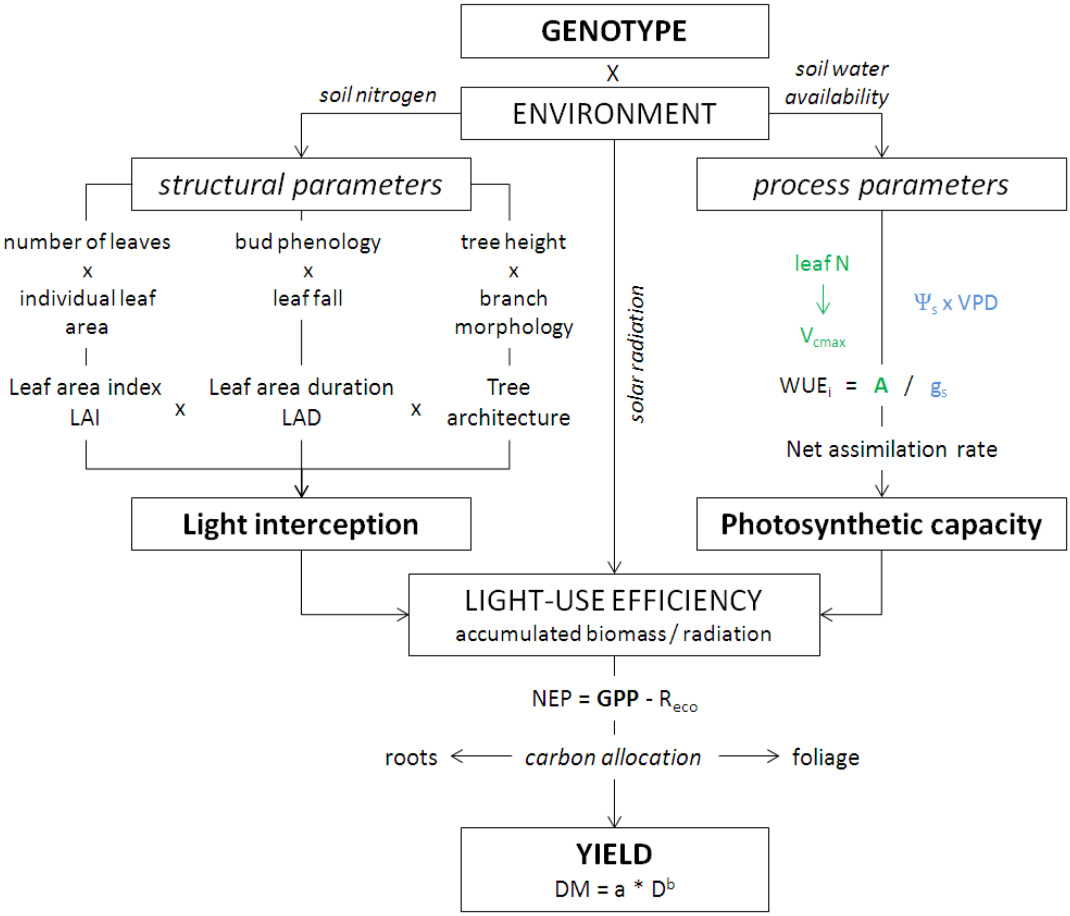

Fig. 6 Integrated scheme of factors underlying biomass production and yield, including genotype x environment interactions affecting structural and process-based growth parameters (adapted from Ceulemans 1990). Parameters in the same color indicate the main drivers; LAI = leaf area index, LAD = leaf area duration, N = nitrogen, A = net assimilation rate, g_s = stomatal conductance, V_{cmax} = maximum carboxylation rate, Ψ_s = soil water potential, VPD = vapour pressure deficit, WUE_i= intrinsic water-use efficiency, NEP = net ecosystem productivity, GPP = gross primary production, R_{eco} = ecosystem respiration, DM = above-ground woody dry mass, D = stem diameter, a,b = regression coefficients.

Particularly in trees, the distribution, orientation and display of the total leaf area in the canopy is as important as the total amount of leaf area. Tree architecture determines the orientation and the distribution of the leaves; together with the canopy density tree architecture significantly influences radiation interception (Hallé et al. 1978). Therefore, tree architecture, characterized by branch morphology, is intimately related to stand productivity (Ceulemans et al. 1990). With regard to branch morphology and branch structure, two types of branches can be distinguished in poplar and in some other tree genera: (i) proleptic branches developed from lateral meristems that have been formed during the previous growing season, and (ii) sylleptic branches that develop from current year lateral meristems (Hallé et al. 1978, Remphrey and Powell 1985). Syllepsis is highly genotype dependent (Ceulemans et al. 1990, Dunlap et al. 1995, Gielen et al. 2002, Zeleznik 2007) and shows high heritability (Wu and Stettler 1996, Rae et al. 2004, Marron et al. 2006, Dillen et al. 2009) indicating its potential for breeding and selection purposes. Based on previous observations of high leaf area development on sylleptic branches (Scarascia-Mugnozza et al. 1989, Ceulemans et al. 1990, Zeleznik 2007) and their high proportion of carbon allocation to the stem (Scarascia-Mugnozza et al. 1999), a correlation between syllepsis on the one hand, and biomass production and LAI on the other hand can be expected. However, the relationship between syllepsis and LAI has not been examined in high-density plantations with poplar.

Beside the spatial display and the amount of the light intercepting leaf area, also the temporal evolution and the total duration of the leaf area are important determinants of growth and productivity. Several definitions of the duration of the growing season can be defined and have been used in literature; (i) first, the growing season length could be defined as the period of leaf expansion or LAD (Dowell et al. 2009), (ii) a second definition delimits the growing season between the time of bud burst and bud set (bud phenology; Rousi and Pusenius 2005) and (iii), thirdly, the period with a net ecosystem exchange (NEE) smaller than zero defines the net carbon uptake period (CUP; Falge et al. 2002). In this latter case a negative NEE indicates a net photosynthetic carbon uptake by the canopy. LAD was calculated as the sum of the LAI measured on two consecutive sampling dates divided by two and multiplied by the number of days between the samplings. The LAD throughout the growing season was then calculated by summing the sequential LAD results, integrating the absolute LAI and the growing season length (Ceulemans and Deraedt 1999, Dowell et al. 2009, Broeckx et al. 2012a). The starting point of the phenological growing season was assessed through the timing of bud burst, more precisely the day of the year when buds were sprouting, with a tip of the small leaves emerging out of the bud scales, which couldn't be observed individually (UPOV, 1981). Completion of bud set was defined as the day of the year when an apical bud was present but not fully closed, bud scales were predominantly green and no more rolled-up leaves were present (Rohde et al. 2011a). The underlying mechanisms of the annual cycle of growth dormancy are still unclear. Experimental data of different species, ecotypes and cultivars are required to identify the underlying ecophysiology of the annual growth cycle and to include variation in phenological responses in climate change modeling (Hänninen and Tanino 2011).

Therefore the importance of the light intercepting leaf area for the overall productivity of poplar SRC was examined in this thesis. Both absolute leaf area and leaf area development were studied in terms of their genotypic variation, and as decisive determinants of biomass production. The following research questions concerning the structural drivers of productivity were addressed:

1. How large is the variation in maximum LAI among the 12 poplar genotypes; and what are the main underlying factors contributing to the genotypic differences in LAI?
2. What were the critical factors in the establishment success and for rapid canopy closure of the poplar SRC?
3. Is the LAI (development) related to biomass production (productivity) in high-density plantations?

Plant productivity is controlled by water and nutrient availability, a.o. through carbon allocation between roots and leaves (Loustau et al. 2001). Fertilization and irrigation experiments clearly showed that net primary productivity (NPP) is to different extents limitated in agricultural crops (Garabet et al. 1998, Cosentino et al. 2007) and in forests (Linder 1987, Campoe et al. 2013). The available nitrogen influences NPP through the investment in photosynthetic enzymes (Loustau et al.

2001). The relationship between leaf N concentration and the photosynthetic capacity, expressed in terms of the maximum rate of carboxylation (V_{cmax}), has been demonstrated for various plant and crop species. However, few data are available on the variability over the growing season and there is increasing evidence that net ecosystem productivity (NEP) is incorrectly simulated when seasonal fluctuations in photosynthetic parameters are ignored (Grassi et al. 2005).

The assimilation of CO_2 during photosynthesis coincides with a flux of water vapour out of the leaf, i.e. transpiration. Both processes are controlled by the stomatal opening, i.e. stomatal conductance. At the leaf level, the ratio of the net assimilation rate to stomatal conductance to water vapour determines the intrinsic water-use efficiency (WUE_i; µmol CO_2 mol^{-1} H_2O) (Fichot et al. 2009), measured at light saturation and ambient CO_2 concentration in this thesis ($WUE_{i\text{-}sat}$):

$$WUE_{i-sat} = \frac{A_{sat}}{g_{s-sat}}$$

Whole-plant WUE, i.e. the biomass accumulation per unit water loss (Larcher 2003), is gaining the interest of breeders given the assumed correlation between productivity and water availability (Heilman and Stettler 1990, King et al. 2013). As the whole-plant WUE is difficult to measure, WUE_i is used as a proxy since it is considered the main source of variation in whole-plant WUE (Cernusak et al. 2008). Discrimination against the heavier (^{13}C) isotope of carbon occurs during photosynthesis, mostly by diffusion of CO_2 from the atmosphere to the intercellular spaces (4‰) and during the carboxylation by the Rubisco enzyme (27-30‰) (Warren 2006). The isotopic discrimination ($\Delta^{13}C$) can be calculated as the difference between the $^{13}C/^{12}C$ ratios of the plant material and the air ($\delta^{13}C$):

$$\Delta^{13}C = \frac{\left(\delta^{13}C_{air} - \delta^{13}C_{plant}\right)}{\left(1 + \left(\frac{\delta^{13}C_{plant}}{1000}\right)\right)} \quad [‰]$$

Given the dependence on CO_2 diffusion through stomata of both $\Delta^{13}C$ and the $A_{sat}/g_{s\text{-}sat}$ ratio, ^{13}C discrimination is negatively related to WUE_i (Farquhar and Richards 1984).

High growth rates of poplar are generally linked to high water consumption (Allen et al. 1999, Zsuffa et al. 1996, Meiresonne et al. 1999), and poplars are also rather sensitive to drought (Liang et al. 2006, Lindroth et al. 1994, Monclus et al. 2009). A large genotypic variability was reported in water-use efficiency (estimated as ^{13}C discrimination; Marron et al. 2005, Dillen et al. 2008, Fichot et al. 2011, Rasheed et al. 2013, Toillon et al. 2013) and in the degree of tolerance to drought (Monclus et al. 2006, Bonhomme et al. 2008). Although high productivity is the primary target of most breeding programs, it might not be related to high water-use efficiency (Marron et al. 2005; Monclus et al. 2005 & 2006; Bonhomme et al. 2008; Dillen et al. 2011b; Rasheed et al. 2011). The identification of genotypes combining high productivity and high WUE would be very useful for poplar cultivation under future climatic conditions, but the complexity of both traits has led to conflicting results on the WUE-growth relationship.

The effect of resource availability, i.e. soil nitrogen and water, was observed and monitored but not manipulated at the experimental field site of the thesis. Photosynthetic and water-use efficiency were studied in terms of their seasonal and genotypic variation, which allows the identification of the underlying driving factors of variability. Specific research questions on process-based parameters were:

4. Did the former land use type affect biomass production of the SRC plantation, e.g. through the different soil N concentration in both previous land use types?
5. What were the main drivers of genotypic and seasonal fluctuations in photosynthetis and water-use efficiency, both at the leaf and ecosystem scale?
6. Was $\Delta^{13}C$ a good estimator of WUE in different poplar genotypes; and was WUE related to growth determinants?

2. Presentation and discussion of the main results

2.1. Structural growth determinants

2.1.1. Leaf area index

Direct LAI measurements were performed either through leaf fall (outlined in detail in chapter 1) or through whole-tree leaf harvest (further described in chapter 2) while indirect LAI readings were collected with an LAI-2000/2200 Plant Canopy Analyzer (LiCor, Lincoln, NE, USA) based on the light extinction through the canopy. Measurements of the maximum LAI reached during the growing season (LAI_{max}) were significantly correlated between both methods, validating the frequent indirect measurements throughout the growing season in this thesis (Fig. 7; see chapters 1 and 2 for a more detailed description and discussion of the LAI validation procedure). The observed underestimation of LAI with the indirect measurements in the higher range of LAI values could probably be explained by the effect of clumping; in higher trees the indirect measurements were less capable of detecting the increasing number of leaf layers in the canopy. Plantation weighted averages of 1.2 and 1.1 in GS1, and of 2.6 and 3.1 in GS2 were found for LAI_{max} from indirect and direct measurements, respectively (as reported and analysed in chapter 1).

Significant genotypic variation in LAI_{max} was observed and reflected the parental origin with *P. nigra* genotypes being in the lower end of the range, as compared to the hybrids, in the first and second growing seasons (Table 2). Genotypes and parentages differed in the strategy to achieve high LAI. In terms of LA, a high number of small leaves (genotype Hees) and a low number of large leaves (genotype Skado) both generated among the highest LAI_{max} (Broeckx et al. 2012b, Verlinden et al. 2013a). But an intermediate strategy of leaf production and LA has already been observed to reach the highest LAI in a poplar SRC culture (Taylor et al. 2001b). In contrast to previous studies (Rae et al. 2004, Marron et al. 2006, Zeleznik et al. 2007), neither the number of branches nor the branched proportion of the stem (relative canopy depth), affected LAI_{max} (as shown and discussed in chapter 2).

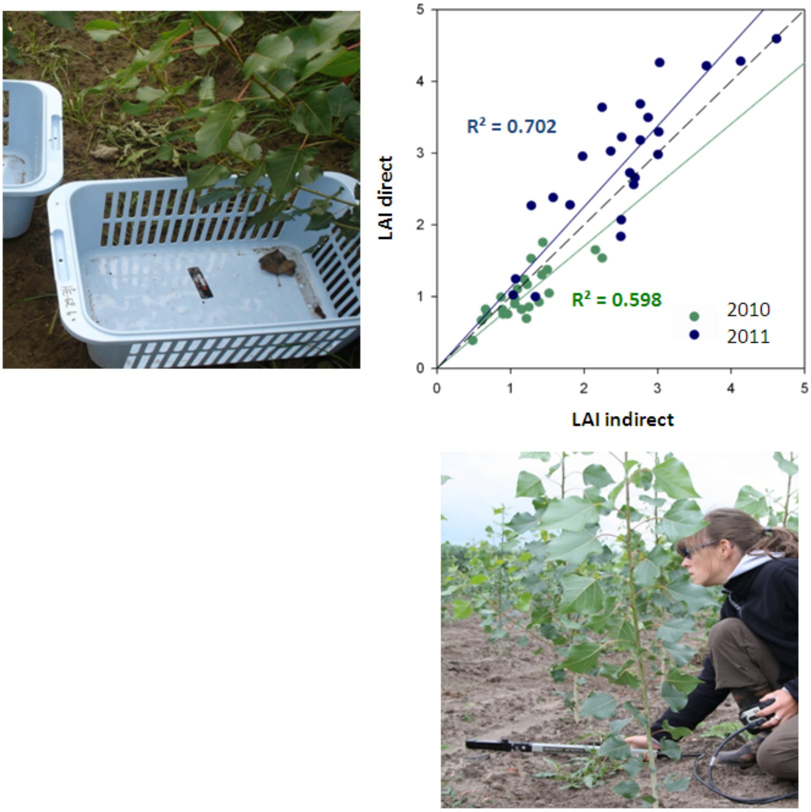

Fig. 7 Correlation between indirect (LAI-2000/2200; LiCor, Lincoln, NE, USA) and direct (leaf fall) measurements of maximum leaf area index (LAI_{max}, m^2 m^{-2}) in the first (2010; green) and second (2011; blue) growing seasons. The 1:1 line (dashed), regression lines and correlation coefficients (R^2) are presented. The predetermined ratio between fresh leaf area and leaf dry weight, i.e. the specific leaf area (SLA), was used to calculate direct LAI_{max} from leaf fall per unit ground surface area.

These observations are based on a subset of all genotypes of the plantation as we studied branching patterns of only four genotypes differing in parental origin and tree architecture. These four genotypes did not include the genotype with the highest number of branches, i.e. genotype Hees that was also the most productive genotype during the first rotation (Verlinden et al. 2013a). Branch dimensions – branch diameter and branch length – were significantly and positively correlated with LAI_{max}. Stem diameter at 22 cm above soil level (D) – being significantly correlated to tree height (H) – and canopy depth – defined as tree height minus the insertion height of the lowest branch – were the best predictors of LAI_{max} in both the first and the second growing seasons (cfr. research question 1; further discussed in chapter 2). Phenotypic plasticity of branch characteristics in response to environmental conditions (Wu and Stettler 1998) and spacing (Nelson et al. 1981, Benomar et al. 2012) was previously described in poplar. This implied that the reported results reflect the suitability of the studied genotypes when planted at high planting density under similar growth conditions. The results also emphasize the importance of studying tree architecture in combination with other growth determinants (Nelson et al. 1981). Fastigiate genotypes Skado and Wolterson showed amongst the highest and the lowest total leaf area, respectively.

The number of sylleptic branches did neither affect stem volume index ($VI = D^2*H$) nor LAI_{max} in the studied genotypes (see chapter 2 for more details). This was in contrast to previous findings on poplar (Rae et al. 2004, Marron et al. 2006, Dillen et al. 2009), emphasizing effects of genotype, environment and their interactions (Wu and Stettler 1998, Zeleznik 2007). For three of the four studied genotypes there was a high contribution of sylleptic branches to the total leaf area of the tree in the given environment (Fig. 8). Sylleptic branches were the main contributors to increased leaf area in an optimal as compared to a suboptimal environment in *P. trichocarpa x P. deltoides* hybrids (Wu and Stettler 1998). The high contribution of proleptics to the total leaf area in the high-yielding genotype Skado confirmed earlier observations for *P. trichocarpa* hybrids (Stettler et al. 1988, Dunlap et al. 1995). The main stem leaves potentially contributed to the high yields of genotype Skado, based on reported leaf properties as high specific leaf area, optimal leaf exposition and long leaf life-span of main stem leaves (Scarascia-Mugnozza et al. 1999, Casella and Ceulemans 2002, Niinemets et al. 2004, Peltoniemi et al. 2012). Combined with the higher translocation efficiency of photosynthates from sylleptics (Scarascia-Mugnozza et al. 1999), the observed results emphasized the importance of leaf distribution among different branch categories.

Table 2 Production related characteristics and woody biomass production of different *Populus* genotypes in the first and second growing seasons, indicating genotypic variation and ranking (values in bold and italics indicate the highest and lowest genotypic averages respectively). Values between brackets indicate standard deviations.

	Hybrid	Genotype	Stem diameter at 22 cm [mm]	Tree height [cm]	Volume index [dm^3]	Biomass [Mg ha^{-1}]	LAI$_{max}$ indirect [m m^{-2}]	LAD [m^2 day m^{-2}]
Growing season 1 (2010)	T x M	Bakan	24.39 (7.28)	**263 (55)**	1.88 (1.16)	3.21 (1.81)	0.97 (0.37)	99 (40)
		Skado	23.84 (7.31)	246 (45)	1.68 (0.99)	3.58 (1.78)	1.47 (0.13)	151 (24)
	D x N	Muur	23.93 (6.17)	243 (36)	1.67 (0.89)	2.54 (1.30)	1.02 (0.15)	96 (16)
		Oudenberg	23.83 (5.80)	252 (39)	1.72 (0.89)	2.56 (1.22)	0.98 (0.13)	95 (8)
		Vesten	25.99 (6.34)	248 (38)	**1.92 (0.97)**	2.60 (1.31)	1.32 (0.18)	118 (16)
		Ellert	21.09 (6.58)	*199 (33)*	1.26 (0.90)	2.03 (1.37)	1.30 (0.12)	113 (15)
		Hees	**26.21 (6.23)**	206 (22)	1.90 (0.98)	**3.89 (1.93)**	**1.87 (0.44)**	**183 (54)**
		Koster	21.42 (7.34)	210 (31)	1.29 (0.92)	1.79 (1.24)	1.10 (0.18)	104 (19)
		Robusta	22.79 (6.94)	203 (37)	1.32 (0.81)	2.85 (1.56)	1.69 (0.59)	141 (62)
	D x (T x D)	Grimminge	24.82 (6.47)	255 (44)	1.82 (1.05)	2.80 (1.51)	1.08 (0.55)	114 (49)
	N	Brandaris	*19.14 (6.29)*	214 (34)	*1.01 (0.68)*	1.34 (0.86)	*0.58 (0.12)*	53 (9)
		Wolterson	19.90 (6.70)	237 (47)	1.24 (0.82)	*1.27 (0.78)*	0.74 (0.27)	73 (29)
Growing season 2 (2011)	T x M	Bakan	40.33 (10.68)	497 (99)	9.42 (5.30)	10.41 (5.28)	1.91 (0.56)	298 (99)
		Skado	44.91 (11.43)	**555 (92)**	**12.89 (7.17)**	12.58 (5.63)	3.34 (0.79)	562 (153)
	D x N	Muur	40.16 (9.46)	434 (73)	8.27 (4.45)	8.65 (4.24)	2.56 (0.62)	360 (68)
		Oudenberg	39.53 (8.33)	460 (60)	8.24 (3.95)	8.03 (3.47)	2.25 (0.33)	290 (32)
		Vesten	46.69 (10.67)	476 (68)	11.66 (5.51)	11.52 (5.34)	2.90 (0.29)	397 (49)
		Ellert	37.76 (10.89)	373 (85)	7.46 (5.13)	7.89 (4.83)	2.85 (0.38)	406 (42)
		Hees	**46.94 (11.56)**	407 (48)	11.88 (6.36)	**14.43 (7.23)**	**4.37 (0.92)**	**650 (117)**
		Koster	38.00 (12.92)	344 (74)	6.78 (5.06)	6.76 (4.60)	2.63 (0.39)	371 (74)
		Robusta	33.11 (10.51)	321 (64)	4.52 (3.41)	6.72 (4.24)	1.82 (0.64)	231 (28)
	D x (T x D)	Grimminge	41.25 (9.61)	447 (79)	8.67 (4.72)	9.52 ((4.70)	2.77 (0.71)	379 (96)
	N	Brandaris	*27.58 (7.97)*	*290 (54)*	*2.75 (1.71)*	*3.04 (1.73)*	*1.05 (0.14)*	*147 (21)*
		Wolterson	34.01 (9.40)	368 (75)	5.34 (3.36)	4.29 (2.31)	1.57 (0.55)	183 (57)

D = *Populus deltoides*, M = *Populus maximowiczii*, N = *Populus nigra*, T = *Populus trichocarpa*

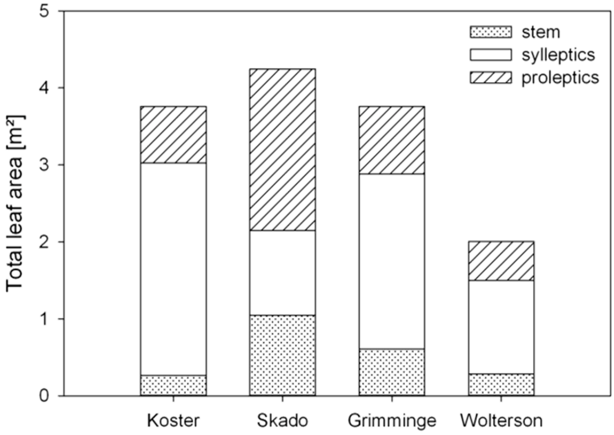

Fig. 8 Total leaf area (per tree) on the main stem (dotted), on sylleptic (white) and on proleptic (hatched) branches. (adapted from chapter 2).

A fast development of leaf area and a large amount of leaf area have been observed on sylleptic branches, which indicates the contribution of syllepsis to early canopy closure (Scarascia-Mugnozza et al. 1989, Ceulemans et al. 1992). The observed correlation between sylleptic branch dimensions and LAI_{max} during the establishment year, confirmed the ability of syllepsis to quickly fill the available space with leaves (Remphrey and Powell 1985; cfr. research question 2). A decreasing importance of branchiness in time to achieve high LAI was further confirmed by a decreased genotypic variation in branchiness index in the second growing season in comparison to the establishment year (Fig. 9; see further details in chapter 2), while genotypic variation in both LAI_{max} and stem diameter increased with time (Table 2; see details in chapter 1).

Genotypic ranking was similar for LAD and LAI_{max} (Table 2) suggesting a combined strategy of high LAI_{max} and long growing seasons; both parameters were also significantly intercorrelated (Verlinden et al. 2013a). These findings indicate that lower LAI values were not compensated for by the length of the growing season in the studied genotypes.

2.1.2. Leaf area development

Leaf area development showed the typical annual cycle of deciduous trees in temperate regions, representing a hyperbolic curve between spring bud burst and autumn leaf fall (Fig. 10). The increasing LAI until the end of the growing season reflected the indeterminate growth habitus of the poplar genus (Howe et al. 2000). The timing of LAI_{max} closely corresponded to the timing of bud set, determining the end of height growth and of leaf production (Rousi and Pusenius 2005). In addition, stem diameter increment was delimited by bud set. High gross primary production (GPP) and hence carbon uptake until the end of September were confirmed by the high net photosynthesis rates and stomatal conductances at the leaf level (see section 2.2 below). A net carbon uptake of [96 ± 15] g C m^{-2} was observed during the 2011 growing season (Zona et al. 2013b, Verlinden et al. 2013b). A comparable duration of the growing season length was found for two definitions of length of the growing season (i.e. the time between bud burst and bud set, vs. the CUP; Fig. 10), although they address processes at different scales.

The gradual processes of spring bud burst and autumn bud set were quantified by means of weekly visual scoring (as explained in more detail in chapter 3). The timing was expressed in growing degree days (GDD, °C) to assess heat forcing requirement, i.e. the accumulation of air temperature above a threshold value. Therefore, the calculation of GDD involved the summation of the daily average temperature minus a threshold temperature starting at 1 January (Pellis et al. 2004b, Rousi and Pusenius 2005). Different threshold temperatures (0 °C, 2.5 °C, 5 °C and 7.5°C) were compared because of reported clinal differences in the base temperature for dormancy release (Myking and Heide 1995). As expected from previous observations (Pellis et al. 2004b, Rohde et al. 2011a & b), large genotypic variation in bud phenology was observed at the tree level (Fig. 11). This variation was smaller in bud set as compared to bud burst, confirming previous findings on poplar (Farmer 1993, Pellis et al. 2004b) and emphasizing the strong environmental control of the end of season phenology (Aitken and Adams 1996).

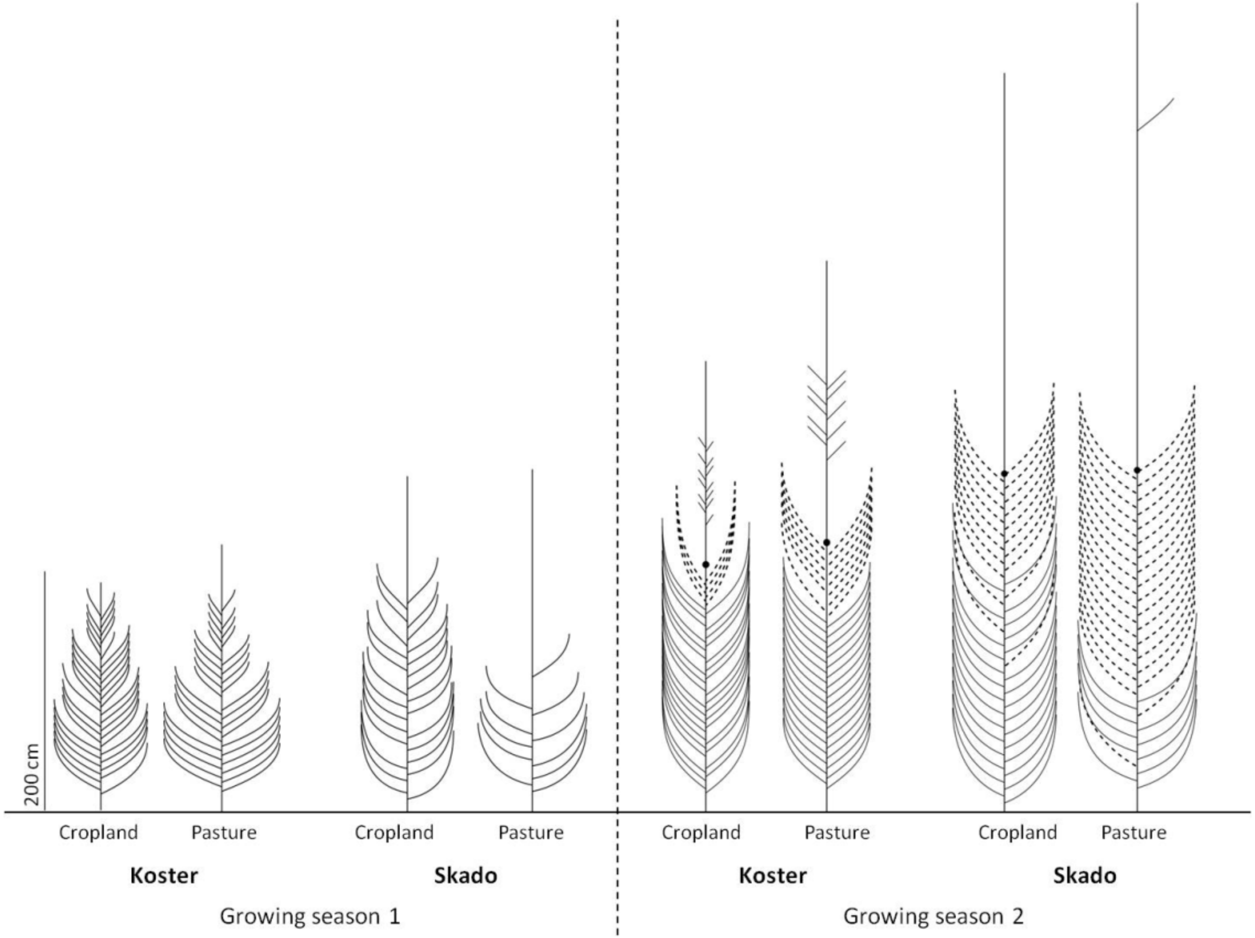

Fig. 9 Schematic representations of the two-dimensional tree architecture for genotypes Koster (*P. deltoides* x *P. nigra*) and Skado (*P. trichocarpa* x *P. maximowiczii*) during the first (2010) and second (2011) growing seasons (adapted from chapter 2).

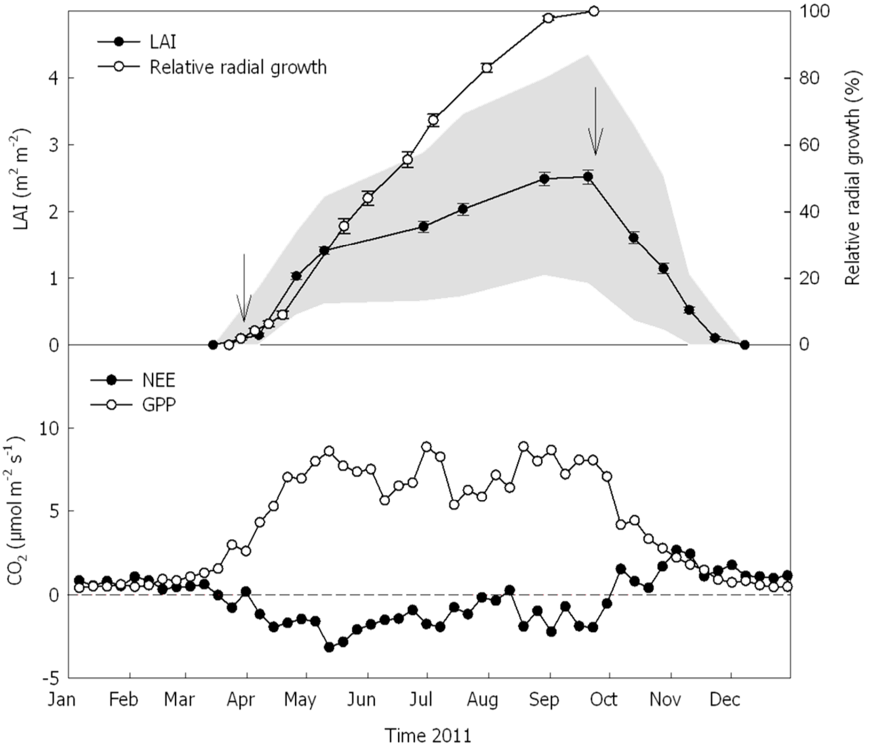

Fig. 10 Time course during the second growing season of the first rotation (2011) of CO_2 fluxes and tree growth: (top panel) relative radial stem growth and leaf area index (LAI) development. Arrows indicate plantation average timing of bud burst (left) and bud set (right), error bars indicate standard errors; (bottom panel) net ecosystem exchange (NEE) and gross primary production (GPP) measured using the eddy covariance technique (adapted from chapter 4).

Since the timing of bud burst and bud set is crucial for tree growth (maximizing the use of optimal growth conditions) and survival (avoiding frost damage), it is under strong genetic selection. Genotypes with early bud burst reached full bud set late, generating the longest growing season, and vice versa. Different environmental factors were identified as the main drivers of the timing of bud burst and bud set. While an accumulation of atmospheric temperature mainly determined the completion of bud burst, the timing of bud set was mainly controlled by the day of the year and hence the photoperiod. The latter confirms the adaptation of bud set to the latitude of origin (Pauley and Perry 1954), which was reflected in significant differences among parentage groups (Fig. 11). However, bud burst was also affected by the cumulated short-wave radiation. The (minimum and night-) temperature also influenced the timing of bud set. The threshold temperature decisive for bud burst – and hence for plant growth – was between 5 °C and 7.5°C for the studied genotypes.

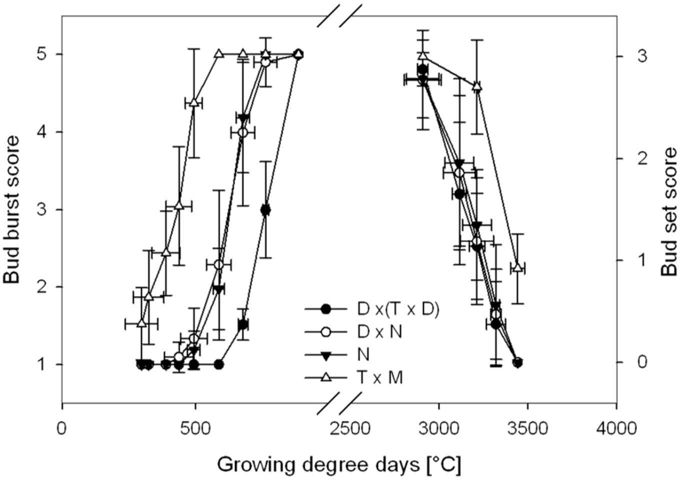

Fig. 11 Timing of spring bud burst (left) and bud set (right) per parentage group as a function of growing degree days, during the second growing season (2011). Error bars indicate standard deviations. Completion of full bud burst and bud set were chosen at scores 4 and 1 respectively (scores are described in detail in chapter 3). D = *Populus deltoides*, M = *P. maximowiczii*, N = *P. nigra*, T = *P. trichocarpa*

Early leaf fall was observed in the first growing season in genotype Robusta, as a consequence of a severe rust infection (*Melampsora* spp.). Yield reductions have been reported as a result of foliar rust infection causing early leaf shedding (Dunlap and Stettler 1996). Reduced biomass production was indeed observed with increasing rust infection in our experimental plantation (Verlinden et al. 2013a). Resistance to diseases is included as a selection criterion in most breeding programs, given the high variation available in the genus *Populus*. However, cultivars with qualitative rust resistance remained free of rust no longer than five years after commercialization due to the rapid evolution of new pathogen strains (Dowkiw and Bastien 2004). Therefore, the high sensitivity of genotype Robusta was expected, being among the oldest genotypes commercially available; it emphasized the importance of continued breeding and selection of new resistant (or tolerant) genotypes.

2.1.3. Relationship between leaf area and wood production

At the end of each growing season, the above-ground standing biomass was estimated through an intensive diameter inventory (explained in more detail in chapter 1). Both LAI_{max} and LAD were good indicators of above-ground woody biomass production, either expressed as VI (Broeckx et al. 2012a) or dry mass (DM) (Verlinden et al. 2013a; cfr. research question 3). Dry mass has been estimated from diameter measurements using allometric relationships between stem diameter and above-ground woody DM:

$$DM = a * D^b$$

where a and b are regression coefficients specific for each genotype (all values are reported in chapter 4). Both LAI_{max} and LAD were lowest for the two *P. nigra* species, which were among the lowest productive genotypes. Superior growth performance of hybrids, generally used in commercial plantations, has been previously observed (Scarascia-Mugnozza et al. 1997). However, *P. nigra* and *P. trichocarpa* were among the most productive genotypes at the end of the fourth growing season in an SRC in Belgium (Al Afas et al. 2008, Dillen et al. 2011b). These results highlight the importance of monitoring growth performance over several rotations as the production and mortality rates are significantly affected by the number of harvests or coppice cycles. Overall, the highest productivity (VI) was observed for genotypes with fewer, heavier branches (Broeckx et al. 2012b), as was expected from the positive correlation between branch dimensions and LAI_{max}. The branchiness index, defined as the ratio of branch volume index to total VI of the tree, was significantly – although only at the $\alpha = 0.10$ significance level – related to VI in the first growing season only. This observation emphasized the decreasing importance of branchiness for biomass production with time after the first growing season. Although the correlation between growing season length and biomass production was not significant, the early bud flush of Bakan and Skado was the most important trait distinguishing the T x M genotypes in a hierarchical cluster analysis (Verlinden et al. 2013a). Combined with the late bud set, growing season length contributed to high wood production. The large genotypic variability in production related traits described above, i.e. leaf area, phenological traits and tree architecture indicate a high potential for further breeding and selection of new high-yielding genotypes for SRC bio-energy plantations.

No effect of former land use type was found on leaf area development, although higher soil N concentrations in former pasture land were reflected in higher leaf N concentrations in trees grown on former pasture land (as described in chapter 1). In contrast, a higher growth performance in terms of VI was observed in trees planted on former cropland during the establishment year. This difference was most likely related to the spatial variability in weed management, given the higher weed pressure in former pasture. Higher seed abundance in former pasture was expected as compared to former cropland where chemical weed control was applied annually. Similarly, relative canopy depth was affected by former land use only during the establishment year. The land use effect was outperformed by the genotypic variation during the second growing season, perhaps due to the non-limiting nutrient conditions in both former land use types (see chapters 1 and 2 for more detailed information). The observed land use effect on the number of branches (Fig. 9) could be attributed to differences in soil N concentration, since the concentration of specific plant hormones has been reported to affect syllepsis (Cline and Dong-Il, 2002) and to regulate apical dominance (Zeleznik, 2007). However, as branch number was not correlated to LAI_{max} and biomass production, no significant effect of former land use type on growth performance was found in this field site, in response to research question 4.

2.2. Process-based growth determinants

2.2.1. Photosynthesis and water-use efficiency

Whereas leaf area was found to be an important growth determinant, a reduction of photosynthesis per unit leaf area was previously found for some genotypes to compensate for a large leaf area (Tharakan et al. 2005, Marron et al. 2007). Therefore, leaf gas exchange measurements were performed (with a portable

LiCor-6400 instrument; LiCor, Lincoln, NE, US) throughout the second growing season (2011), assessing the assimilation rates of six out of the twelve planted genotypes differing in total and individual leaf area as well as in biomass production (see chapters 4 and 5 for a complete description). Seasonal and genotypic variation in photosynthesis – measured as the net assimilation rate at saturating light and a CO_2 concentration of 400 ppm (A_{sat}) – was largely affected by the stomatal response to environmental conditions through changing stomatal conductance to water vapour ($g_{s\text{-}sat}$). The intrinsic photosynthetic capacity – quantified as the maximum carboxylation rate (V_{cmax}) and the maximum rate of electron transport (J_{max}) (Farquhar et al. 1980) – was estimated from the response of A_{sat} to varying CO_2 concentrations at the site of carboxylation (C_c) (see chapter 5 for more details). The observed relationship between V_{cmax} and leaf nitrogen content (N_{area}) indicated the dependence of photosynthetic capacity on the investment of N in the photosynthetic apparatus (Wilson et al. 2000, Grassi et al. 2005, Xu and Baldocchi 2003). Although V_{cmax} and A_{sat} depend on different drivers, a significant correlation was found between A_{sat} and V_{cmax}.

As for leaf area, a high genotypic variability in photosynthetic traits was found among the six selected genotypes (Figs. 12 & 13). However, the highest photosynthetic performance was observed for the lowest yielding genotype Wolterson; this genotype showed a low total and individual leaf area. However, high-yielding genotypes were previously found to combine high maximum photosynthesis with large leaves (Ceulemans and Impens 1980, Isebrands et al. 1988). Besides differences in leaf area, the correlation between photosynthetic assimilation rate and yield might be obscured by: (i) the saturating conditions of the gas exchange measurements in absence of diurnal and seasonal fluctuations; (ii) an upscaling effect not taking into account genotypic differences in day and dark respiration at the whole tree level; and (iii) a difference in the time scale of integration between the yield estimates (over an entire growing season) and the (instantaneous) photosynthesis measurements.

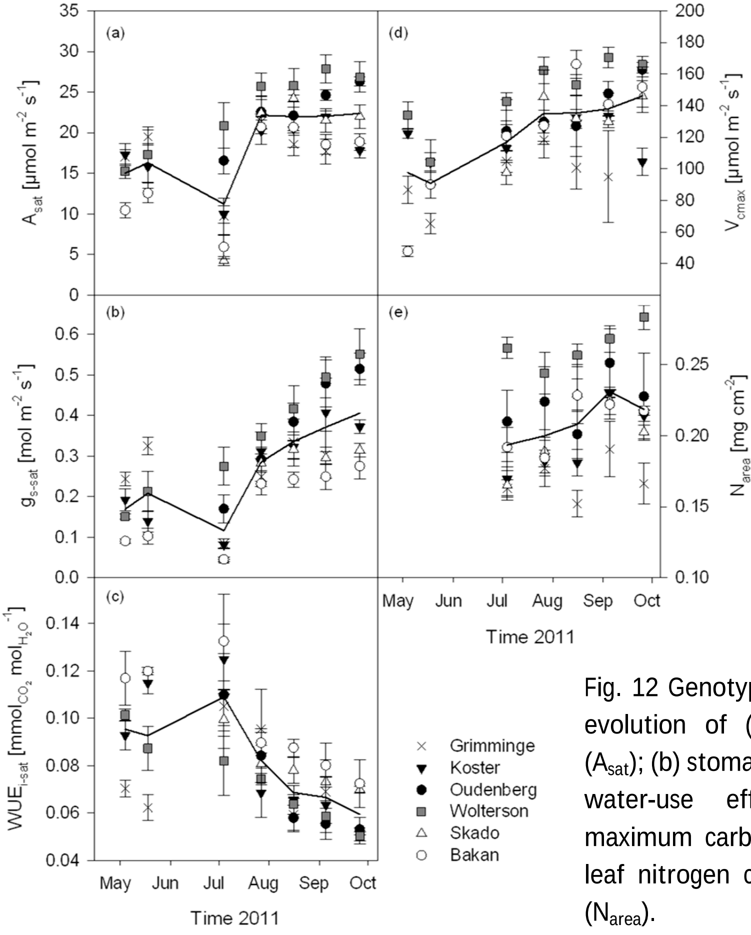

Fig. 12 Genotypic variation and seasonal evolution of (a) net assimilation rate (A_{sat}); (b) stomatal conductance ($g_{s\text{-}sat}$); (c) water-use efficiency (WUE_i); (d) maximum carboxylation rate (V_{cmax}); (e) leaf nitrogen content on an area basis (N_{area}).

Substantial genotypic variation was observed in WUE_i, confirming previous results (Rasheed et al. 2013, Monclus et al. 2005 & 2006, Dillen et al. 2011b). This genotypic variation could be caused by variation in A_{sat} or in $g_{s\text{-}sat}$, or a combination of both, since both variables can vary independently (Gilbert et al. 2011; cfr. research question 5). This is reflected in the non-linear relationship between A_{sat} and $g_{s\text{-}sat}$, with A_{sat} reaching a genotype dependent saturation with increasing $g_{s\text{-}sat}$ (Fig. 13). While variation along the curve is mainly driven by changes in soil water potential (Ψ_s), variation in elevation of the curve is related to photosynthetic capacity. The strong correlation between WUE_i and $g_{s\text{-}sat}$ suggested stomatal control as the main driver of the variation in WUE_i. The latter was confirmed by a lack of correlation between WUE_i and V_{cmax} and by the counterintuitive negative relationship between WUE_i and A_{sat}, due to overruling variation in $g_{s\text{-}sat}$ (as reported in chapter 5). The same $WUE_{i\text{-}sat}$ might be observed for genotypes with low photosynthetic capacity operating at low stomatal conductance as for genotypes with high intrinsic photosynthetic capacity and high stomatal conductance (Gilbert et al. 2011). Hence, selecting for high WUE_i is not directly related to high carbon assimilation and productivity, emphasizing the importance of examining the underlying drivers of WUE_i.

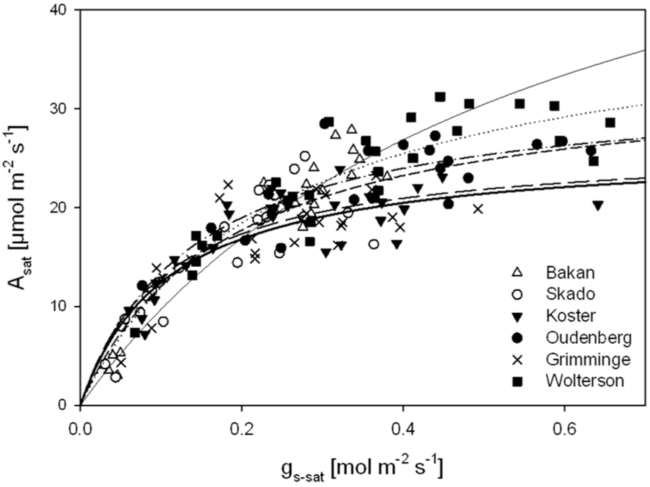

Fig. 13 Hyperbolic response of net assimilation rate (A_{sat}) to stomatal conductance ($g_{s\text{-}sat}$) for six selected genotypes; short dashed = Bakan, long dashed = Skado, bold = Koster, dash-dot = Oudenberg, dotted = Grimminge, solid = Wolterson (from chapter 5).

2.2.2. Stable isotope discrimination

As expected from the theory, bulk leaf ^{13}C discrimination ($\Delta^{13}C_{bl}$) scaled significantly and negatively with $WUE_{i\text{-}sat}$ for the plantation of this thesis, confirming previous results for wheat (Farquhar and Richards 1984) and for (poplar) trees (Lauteri et al. 1997, Ripullone et al. 2004, Monclus et al. 2006, Fichot et al. 2011). However, Ripullone et al. (2004) were unable to detect a significant negative relationship between $\Delta^{13}C$ and WUE_i in poplar. In this thesis, the observed relationship exhibited some scatter, being largely dependent on genotype and timing throughout the growing season (Fig. 14; cfr. research question 6). The variability throughout the growing season can be mainly ascribed to the difference in the time scale of integration that is considered; $\Delta^{13}C$ integrates over the entire leaf lifespan while instantaneous WUE is highly responsive to varying environmental conditions.

Since $\Delta^{13}C$ can be readily measured, it has been widely used as an indirect estimate of WUE_i. However, variation in $\Delta^{13}C$ doesn't distinguish between variation in photosynthetic capacity and in stomatal conductance, both driving the variation in WUE_i alone or in combination (see also section 2.2.1). Due to a faster evaporation of the lighter ^{16}O isotope, the oxygen isotope discrimination during transpiration reflects the genetic variation in g_s. This suggests that a combined use of $\Delta^{13}C$ and $\Delta^{18}O$ allows distinguishing among the sources of variation in WUE_i (Barbour 2007). The analysis of bulk leaf ^{18}O isotopic composition ($\delta^{18}O_{bl}$) revealed a significant relationship with $g_{s\text{-}sat}$ (Fig. 14) – supporting $g_{s\text{-}sat}$ as the main driver of variation in WUE_i – but failed to detect any relationship with $\Delta^{13}C_{bl}$. This could be ascribed to the use of organic matter $\delta^{18}O_{bl}$ in absence of source water $\delta^{18}O$ measurements, hence not reflecting xylem water $\delta^{18}O$ from the transpiration stream (see chapter 5 for more details).

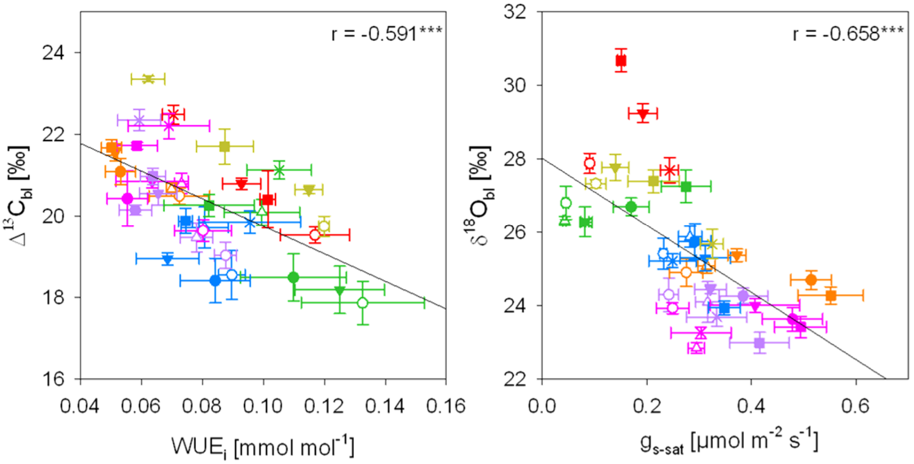

Fig. 14 Relationship between intrinsic water-use efficiency (WUE_i) and bulk leaf ^{13}C discrimination ($\Delta^{13}C_{bl}$; left panel), and relationship between stomatal conductance to water vapour ($g_{s\text{-}sat}$) and the bulk leaf ^{18}O isotopic composition ($\delta^{18}O_{bl}$; right panel). r values indicate Pearson correlation coefficients, ***= $P < 0.001$ (adapted from chapter 5). Colors indicate different measurement campaigns during the growing season, red = 4-6 May, yellow = 18-20 May, green = 4-8 July, blue = 27-29 July, purple = 16-19 August, pink = 5-9 September, orange = 26-30 September 2011.

The relationship between WUE and growth performance is of interest for plantation management, especially for large-scale plantations used for biomass production. The absence of a trade-off between growth and $\Delta^{13}C$ in poplar suggested the potential of identifying high-yielding genotypes with high WUE (Monclus et al. 2006, Chamaillard et al. 2011, Toillon et al. 2013). However, contrasting results illustrate the importance of a correct interpretation of the ecophysiological relationship between WUE and growth. Both traits depend on different physiological drivers and they differ in the time scale of integration that is considered (Fig. 15). Hence, depending on the environmental conditions, an independent variation could inhibit the detection of a WUE-growth relationship. Relative growth rate (RGR) – measured as stem diameter increment – and $\Delta^{13}C$ integrated a similar time period of several weeks, explaining the negative correlation in absence of a correlation between RGR and WUE_i (Fig. 15). When averaged over the growing season, no significant correlation was found between RGR and $\Delta^{13}C$. Similarly, only seasonal average $\Delta^{13}C$ was negatively correlated to final tree biomass at the end of the growing season, confirming the importance of the time scale of integration when the relationship between WUE and growth is addressed (cfr. research question 6). The absence of a correlation with A_{sat} and SLA suggested that RGR was mainly dependent on the allocation of biomass to the photosynthetic compartment, i.e. the leaf mass ratio (LMR) (Shipley 2006). Hence, the lack of a positive correlation between WUE_i and RGR is in line with the observation that $g_{s\text{-}sat}$ drives most of the variation in WUE_i (Fig. 15). Although no correlation was found between LA and RGR, LA was significantly correlated to the end-of-season tree dry mass (eos TDM), i.e. the woody biomass estimated from the end of season stem diameter using allometric relationships (see section 2.1.3.).

2.2.3. Effect of seasonal changes in water availability

An atypical dry spring to summer period during the second growing season (2011) provided the opportunity to examine the interacting effect of low water availability and seasonality on growth physiology and ecosystem dynamics, although no irrigated control plots were available. The seasonal evolution of all meteorological parameters is presented in chapters 4 and 5.

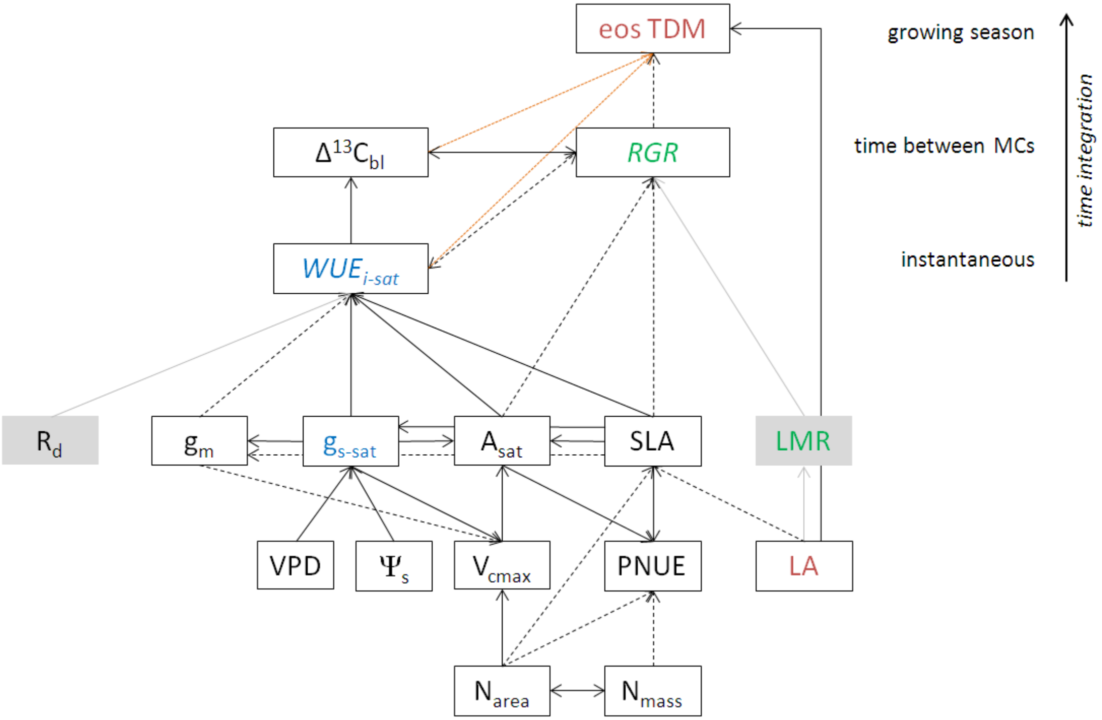

Fig. 15 Schematic representation of the (lack of) relationship between WUE and growth and their physiological drivers. Parameters in the same color indicate the main drivers. Solid arrows indicate significant correlations; dashed arrows indicate non-significant correlations; gray arrows indicate correlations not examined in this thesis but assumed (based on literature); and orange arrows indicate significant correlations when averaged over the entire growing season (see chapter 5 for more details). eos TDM = end of season tree dry mass, $\Delta^{13}C_{bl}$ = bulk leaf carbon isotope discrimination, RGR = relative growth rate, MC = measurement campaign, $WUE_{i\text{-}sat}$ = intrinsic water-use efficiency, R_d = day respiration, g_m = mesophyll conductance, $g_{s\text{-}sat}$ = stomatal conductance, A_{sat} = net assimilation rate, SLA = specific leaf area, LMR = leaf mass ratio, VPD = vapour pressure deficit, Ψ_S = soil water potential, V_{cmax} = maximum carboxylation rate, PNUE = photonsynthetic nitrogen use efficiency, LA = individual leaf area, N_{area} = leaf nitrogen concentration on an area basis, N_{mass} = leaf nitrogen concentration on a mass basis.

During the drought period the minimum values of Ψ_S reached in the plantation were -1.8 MPa at 20 cm depth in the beginning of June and -1.2 MPa at 40 cm depth in mid-July 2011. These values suggested that the trees were potentially suffering from drought, which affects several plant- and ecosystem-scale processes. Both ecosystem scale (from eddy covariance measurements) and leaf level (from leaf gas exchange) assessments indicated a stomatal control upon photosynthesis as the primary response to low soil water availability and to high vapour pressure deficit (VPD), addressing research question 5.

A decrease in leaf productivity, as reflected in a reduced leaf area development (Fig. 10), was observed when soil water availability was rapidly decreasing in the near surface soil layer (May-June 2011). A simultaneous decrease in GPP and less responsive ecosystem respiration (R_{eco}) resulted in a parallel decrease in net ecosystem productivity (NEP = GPP – R_{eco}). Increasing sensible heat (H) with decreasing Ψ_S at 20 cm depth indicated an increase in leaf temperature due to the reduced transpiration. These results imply stomatal closure, confirmed by the rapid saturation of evapotranspiration (ET) with increasing VPD at low water availability in the upper soil layer. High air temperature and the low GPP – even at low VPD during this period of low Ψ_S at 20 cm depth – suggested that water availability was indeed the limiting factor. Water-use efficiency at the plantation scale was defined as the ratio of GPP to ET (see chapter 4 for a more in-depth discussion). An increased ecosystem WUE was observed with decreasing soil water potential at 20 cm depth, due to a faster decrease in ET in comparison to GPP. The latter emphasized that stomatal control was the main cause of the reduced ecosystem productivity in June.

At the leaf level, a reduced net assimilation rate (A_{sat}) and reduced stomatal conductance ($g_{s\text{-}sat}$) were observed when soil water availability was low at both 20 and 40 cm soil depth, when a peak in ambient VPD and T_{air} were observed (early July). However, this response to low soil water potential was highly genotype dependent (Fig. 12). This could be ascribed to local differences of soil water depletion due to genotypic differences in total leaf area; bigger trees encountering rapid and more severe water shortage due to high transpiratory water loss.. No effect on WUE_i was observed of reduced soil water availability at 20 cm soil depth, in the absence of an increased VPD and T_{air}. A constant N_{area} with varying SLA and $\Delta^{13}C$ suggested that N_{area} was not responsive to water stress, although there was a weakly negative relationship with WUE_i. Divergent results have been reported in the literature (Reich et al. 1989, Poorter and Evans 1998, Grassi et al. 2005, Han 2011). A rather constant N_{area} during the drought period explained the absence of a reduction of V_{cmax} in response to low water availability; this indicated no change in N allocation to the photosynthetic apparatus (Rubisco and chlorophyll). These observations confirmed that the reduction in A_{sat} was due to stomatal control rather than to a biochemical limitation.

2.3. Conclusions

Among the studied structural production determinants, LAI was clearly the main determinant of harvestable woody biomass production in the SRC bio-energy plantation of this thesis. The strategy to reach high LAI values, i.e. the number of leaves, LA and the number of branches, was of secondary importance. This result enables that a non-destructive LAI assessment can be used as an early selection criterion for productivity as well as a reliable estimator of standing biomass in high-density SRC plantations. In terms of process-based parameters, the lower photosynthetic capacity per unit of leaf area was highly compensated for by the large leaf area in some genotypes. This hampered the detection of a relationship between carbon assimilation rate and growth. Carbon isotope discrimination ($\Delta^{13}C$) scaled negatively with intrinsic water-use efficiency (WUE_i), both estimating the efficiency of water consumption, but the relationship varied among genotypes and throughout the growing season. We found no straightforward relationship between growth determinants and water-use efficiency – estimated as $\Delta^{13}C$ and WUE_i – and our results suggested that a considerable effect of the temporal scale of integration might explain this.

Given the site specificity, generalization of the results is not straightforward. The plantation was established on a fertile agricultural soil, providing non-limiting growth conditions in terms of soil nutrients. It is uncertain whether the same genotypic ranking in biomass production would be conserved under other climatic, soil and/or management conditions. The latter highlights one of the limitations of this thesis; the effects of a naturally occurring low water availability was studied without an intended manipulation of water supply. As a consequence, no irrigated control plots were available.

Large genotypic variation in both functional and structural production determinants was confirmed under the prevailing conditions, suggesting the potential of further selection for high-yielding genotypes for high-density and short-rotation managed plantations. Continued breeding of new genotypes is important and necessary to maximize yields in a given environment and for a specific management option. However, this does not imply that only one selected high-yielding genotype should be planted in large-scale SRC plantations (so called monocultures). Finding a compromise should be advised between: (i) an optimal spread of risk and a sustainable SRC management by planting a mix of different genotypes and; (ii) the use of only one (or very few related) genotype(s) which is preferred to harmonize and ease harvesting, hence optimizing industrial applications.

This thesis has for the first time analyzed the main productivity determinants of SRC poplar at the scale of an operational bio-energy plantation. This type of study is necessary to further examine the potential of biomass from SRC as a greenhouse gas neutral and energy efficient bio-energy source. An unavoidable limitation of this thesis is the studied time-frame, i.e. only the first rotation was monitored, including the establishment year. This first rotation might not be representative in terms of growth performance as reflected by the increasing genotypic variation in LAI. In addition, focusing on the first rotation implied the study of a non-coppiced – mainly single stem – stand. This emphasizes the necessity of more research in the future on the longer-term and over different rotation cycles, of the POPFULL and other SRC plantations. Including the assessment of genotypic variation in coppice ability is also essential for the successful employment of future SRC plantations for bio-energy. And last, but not least, the genotypic diversity in below-ground productivity in relation to the above-ground harvestable biomass production also deserves further research.

Chapter 1

Establishment and two-year growth of a bio-energy plantation with fast-growing *Populus* trees in Flanders (Belgium): effects of genotype and former land use

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Abstract

In April 2010, a large-scale short-rotation coppice (SRC) plantation was established with mainly poplar (*Populus* spp.) on a former agricultural site (cropland and pasture) in Flanders. The twelve selected genotypes planted were assessed on establishment and production characteristics during the first two years of growth and were found highly productive, with a volume index ranging between 1.00 (± 0.68) and 1.93 (± 0.97) dm^3 in growing season 1 (GS1) and between 2.75 (± 1.70) and 11.91 (± 6.33) dm^3 in growing season 2 (GS2). Despite high survival rates of the cuttings after planting, competitive weeds and management operations increased tree mortality during the growing season from 3.4 % up to 18.2 % averaged over the entire plantation. Weed control therefore turned out to be the key factor in the establishment success. Only a minor influence of former land use was observed during GS1, which is explained by the non-limiting nutrient conditions on both former cropland and pasture, and which disappeared during GS2. These productive soils also explained the high growth rates, with an average tree height of 247 cm and 445 cm and stem diameter (at 22 cm height) of 25.21 mm and 40.73 mm after GS1 and GS2 respectively. Genotypic and parentage variations were found to be less pronounced during GS1, and increased during GS2 as expected. The maximum leaf area index and total leaf area duration were shown to be good indicators of production and growth performance. The results of this paper confirm the high potential of SRC with poplar on agricultural land for bio-energy purposes.

Keywords

Short-Rotation Coppice, weed control, stem cuttings, leaf area index, volume index, Huber value

1. Introduction

To meet its 20/20/20 objective, the 27 EU member states seek to reduce their CO_2 emissions by 20 % and to raise the share of renewable energy by 20 % by 2020 (Communication from the Commission 2008). Bio-energy from biomass is one of the most interesting renewable energy sources within the EU (Communication from the Commission 2007). Biomass for bio-energy can be extracted from different residue streams from agriculture, forestry and processing industries, but can also be actively grown in cultures of annual or perennial crops. For the latter, fast-growing trees are one of the most promising alternatives for the production of biomass when planted in a short-rotation coppice (SRC) regime. Poplar (*Populus* spp.) and willow (*Salix* spp.) are the most commonly used species for SRC in Europe (Herve and Ceulemans 1996; Galinski et al. 1991; Verwijst 2001; Aylott et al. 2008). Both species are generally propagated and grown from hardwood cuttings. Poplar is particularly suitable for SRC cultures in temperate regions because of its high growth rate and biomass yield, its easy vegetative propagation from cuttings and coppice ability, and its genetic diversity enabling growth under a wide range of environmental conditions (Bradshaw et al. 2000; Dillen et al. 2011a). On average poplar trees in a coppice culture in temperate European conditions have dry mass yields of 10-15 Mg ha^{-1} y^{-1} (Laureysens et al. 2004; Labrecque and Teodorescu 2005). Compared with other woody energy crops, *Populus* spp. has the advantages of a comprehensive scientific base on the ecophysiology and productivity of the genus (Stettler et al. 1996; Dillen et al. 2011a). SRC cultures with poplar could therefore play a promising role in achieving part of the European renewable energy objectives.

Different land use areas can be used for SRC, ranging from agricultural land, set-aside land and previously forested land to marginal land and rights-of-way of roads and highways. An SRC culture has the ability to protect the soil against erosion and nitrate leaching (Isebrands et al. 2001). Some studies reported the possibility of phytoremediation of contaminated soils by SRC cultures with poplar (Laureysens et al. 2005; Meers et al. 2007). Moreover, SRC plantations generally have a higher biodiversity compared to traditional agriculture (Volk et al. 2004; Rowe et al. 2009).

Much information has been gathered about the cultivation, production and technological aspects of SRC cultures, leading to successful small-scale applications of SRC for bio-energy (Meiresonne 2006). Nevertheless, the success of an SRC culture largely depends on the establishment and the first-year performance of the trees (Kauter et al. 2003; Otto et al. 2010). In general, the survival rate of dormant hardwood cuttings during the establishment year is high for commercial poplar clones. However, there is also large genotypic variation in initial rooting as affected by soil type and climatic conditions, especially soil moisture (Ceulemans and Deraedt 1999; Zalesny et al. 2005; Bergante et al. 2010). Cuttings are particularly prone to drought during the first weeks after planting (Dickmann and Stuart 1983). Since poplars are light-demanding pioneer species, weed control in an SRC culture is especially critical during the period of establishment (Buhler et al. 1998; Dillen et al. 2011a). It is, however, unknown how a genotype may interact with former land use to affect the establishment success.

In the present study different selected poplar genotypes were assessed on establishment success and production characteristics during the first two years of an SRC culture, i.e. growing season 1 (GS1) and growing season 2 (GS2). The plantation was established on former agricultural land with two different land use types, cropland and pasture. We hypothesized that (1) genotypic variation in production characteristics will occur, quantified as stem diameter and as leaf area development, and (2) differences in production characteristics will occur on different former land use types resulting from differences in soil characteristics. The use of different genotypes in a plantation is strongly recommended to reduce the risk of large yield losses due to diseases or infestations. It is important to study the potential of these genotypes on different land areas for successful future SRC plantations. The available genotypic variation in growth characteristics can be exploited in future breeding and selection of poplar genotypes. To test these hypotheses, we established a large scale experimental SRC culture with different poplar genotypes.

2. Materials and Methods

2.1. Site description

The experimental site is located in Lochristi, Belgium (51°06’44” N, 3°51’02” E), about 11 km from the city of Ghent (Province East of Flanders) at an altitude of 6.25 m above sea level with flat topography. The long-term average annual and growing season temperature at the site is 9.5 °C and 13.72 °C, respectively. Average annual and growing season precipitation is 726 mm and 433 mm, respectively (Cermak et al. 1998; Royal Meteorological Institute of Belgium 2011 & 2012a). The region of the field site is pedologically described as a sandy region and has poor natural drainage (Ameryckx et al. 1989). The total area of the site is 18.4 ha. The former land use was (i) agriculture, consisting of cropland (ryegrass, wheat, potatoes, beets, and most recently monoculture corn with regular nitrogen (N) fertilization at a rate of 200-300 kg ha^{-1} y^{-1} as liquid animal manure and chemical fertilizers), and (ii) extensively grazed pasture.

Prior to planting, a detailed soil survey was carried out in March 2010 by analysis of soil samples taken at 110 locations, spatially distributed over the two former land use types. Bulk density and aggregate soil samples were taken at 15 cm intervals, up to 90 cm depth, by core sampling (Eijkelkamp Agrisearch equipment, Netherlands). Carbon (C) and nitrogen (N) mass fractions were determined in the laboratory by dry combustion (CN element analyzer, Carlo Erba Instruments, Italy). Soil texture, pH and nutrient concentrations were assessed on an aggregate sample of the upper 30 cm and 30-60 cm layers of the soil by the Pedological Service of Belgium (Heverlee). Based on the soil analyses, the soil type was characterized as a sandy texture with a clay-enriched deeper soil layer. The particle size distribution of mineral soil did not statistically differ among the soil layers up to 60 cm depth (Table 1).

In the upper 90 cm of the soil the average total C and N contents were not significantly different between pasture (C content of 106.0 ± 30.4 Mg ha^{-1}, N content of 9.4 ± 1.4 Mg ha^{-1}) and cropland (C content of 111.7 ± 32.9 Mg ha^{-1}, N content of 9.1 ± 2.1 Mg ha^{-1}). In the upper 0-15 cm soil layer C and N mass fractions were, however, significantly (P = 0.000) lower in cropland (1.48 ± 0.32 % and 0.12 ± 0.03 %, respectively) as compared to pasture (1.95 ± 0.36 % and 0.18 ± 0.03 %, respectively). Soil bulk density in this upper layer was significantly higher in cropland (1.45 ± 0.07 g cm^{-3}) than in pasture (1.27 ± 0.10 g cm^{-3}). C and N mass fractions further decreased exponentially with depth (Fig. 1). The availability of nutrients K, P, Mg, Na and Ca did not differ between former land use types; averages are reported in Table 1.

Table 1 Soil pH, nutrient mass fractions and particle size distribution (PSD) of the soil layers at 0-30 cm and 30-60 cm depth. Weighted averages for both land use types pasture and cropland over the total land area are presented. PSD indicates that the soil has a texture of loamy sand.

			0-30 cm	30-60 cm
	pH - KCl		5.34	5.78
Nutrient mass fraction [mg kg^{-1}]	P		246.9	77.5
	K		145.4	95.1
	Mg		132.6	127.1
	Ca		1082.5	1015.5
	Na		13.3	15.2
PSD [%]	clay	< 2 µm	11.34	11.33
	silt	2-10 µm	0.56	1.45
		10-20 µm	0.37	0.96
		20-50 µm	0.81	1.80
	sand	> 50 µm	86.93	84.45

KCl = Potassium chloride, P = Phosphor, K = Potassium, Mg = Magnesium, Ca = Calcium, Na = Sodium

2.2. Plant material and plantation establishment

A total of 14.5 ha were planted between 7-10 April 2010 with twelve selected poplar (*Populus*) genotypes, all commercially available. Three selected willow (*Salix*) genotypes were also planted at the same field site. The genotypes represented different species and hybrids of *Populus deltoides*, *P. maximowiczii*, *P. nigra*, and *P. trichocarpa* (Table 2; Buitenveld 2007; Michiels et al. 2010a; Michiels et al. 2010b) and *Salix viminalis*, *S. dasyclados, S. alba* and *S. schwerinii.* In this study, only results from the poplar genotypes are presented. The twelve poplar genotypes represent four different parentages.

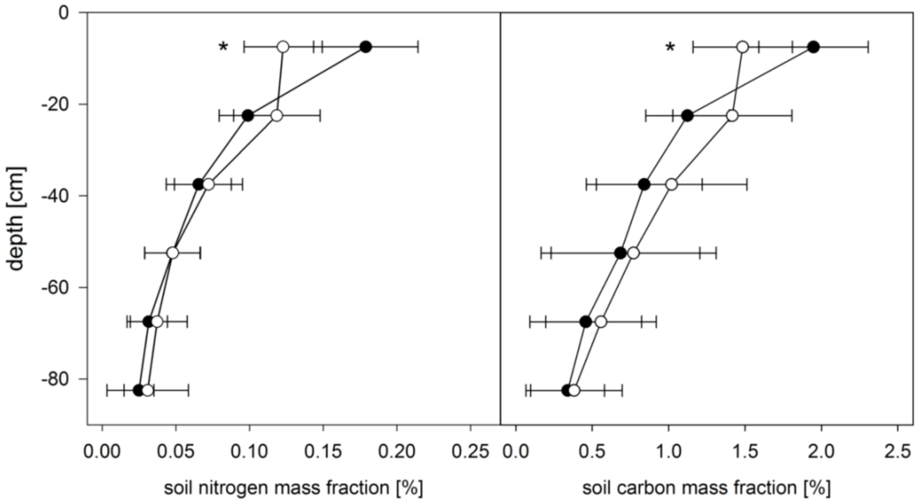

Fig. 1 Profiles of carbon (right) and nitrogen (left) mass fractions of the soil before the plantation establishment in cropland (white circles) and pasture land (black circles). Error bars indicate standard deviation of the mean. * indicates a significant difference between both land use types at the $\alpha = 0.05$ level.

After soil preparation by ploughing (40-70 cm depth), tilling and pre-emergent herbicide treatment, 25 cm long dormant and unrooted cuttings were planted. The cuttings were soaked in water 24 h prior to planting. The planting was performed with an agricultural leek planting machine. The cuttings were planted in a double-row planting scheme with alternating distances of 0.75 m and 1.50 m between the rows and 1.10 m between trees within the rows, corresponding to a tree density of 8,000 ha^{-1}. The plantation was designed in large monoclonal blocks of eight double rows wide (Fig. 2) that cover the two types of former land use (cropland and pasture). The minimum of two and maximum of four replicated blocks of each genotype with row lengths varying from 90 m to 340 m, were based on the available number of cuttings and the spatial configuration of the site.

Table 2 Overview of the twelve poplar (*Populus*) genotypes of this study planted in a short-rotation coppice culture. Parentage, place of origin/provenance, botanical section, year of the cross and of the commercialization, gender and selection criterion of the genotypes are shown.

Genotype	*Parentage*	*Place of origin*	*Section*	*Year of cross/ commercialization*	*Gender*	*Selection criterion*
Bakan[1]	T × M	(Washington US x Oregon US) x Japan	Tacamahaca	1975/2005	♂	plywood
Skado[1]	T × M	(Washington US x Oregon US) x Japan	Tacamahaca	1975/2005	♀	plywood
Muur[1]	D × N	(Iowa US x Illinois US) x (Italy x Belgium)	Aigeiros	1978/1999	♂	plywood
Oudenberg[1]	D × N	(Iowa US x Illinois US) x (Italy x Belgium)	Aigeiros	1978/1999	♀	plywood
Vesten[1]	D × N	(Iowa US x Illinois US) x (Italy x Belgium)	Aigeiros	1978/1999	♀	plywood
Ellert[2]	D × N	Michigan US x France	Aigeiros	1969/1989	♂	plywood
Hees[2]	D × N	Michigan US x France	Aigeiros	1969/1989	♀	plywood
Koster[2]	D × N	Michigan US x The Netherlands	Aigeiros	1966/1988	♂	plywood
Robusta[3]	D × N	Eastern US x Europe	Aigeiros	1885-1890/?	♂	plywood
Grimminge[1]	D × (T × D)	(Michigan US x Connecticut US) x (Washington US x (Iowa US x Missouri US))	Aigeiros x (Tacamahaca x Aigeiros)	1976/1999	♂	plywood
Brandaris[2]	N	The Netherlands x Italy	Aigeiros	1964/1976	♂	plywood
Wolterson[2]	N	The Netherlands	Aigeiros	1960/1976	♀	plywood

D = *Populus deltoides*, M = *Populus maximowiczii*, N = *Populus nigra*, T = *Populus trichocarpa*

[1] produced by the Institute for Nature and Forest Research (INBO, Geraardsbergen, Belgium)

[2] produced by Vermeerderingstuinen Nederland (Zeewolde, The Netherlands)

[3] genotype originating from an open-pollinated *P. deltoides* tree, first commercialized by the nursery Simon-Louis Frères (Metz, France)

During the first months after planting intensive weed control – mechanical, chemical and manual – was applied to decrease competition for light and nutrients (Table 3). With the exception of glyphosate, none of the herbicides used specify poplar as an approved crop species for the use of these chemicals. Herbicides that have proven effective in the establishment of poplar plantations in experimental trials were chosen based on the weed species present in the field (personal communication F. Goossens, ILVO), but these chemicals can not be used legally in commercial plantations. Plantation management was extensive, without fertilization or irrigation. In the winter after GS1 the largest gaps in the plantation due to cutting mortality, were re-planted with one-year old unrooted cuttings, hereafter referred to as interplanting.

2.3. Measurements

Measurements of survival, growth performance and various production characteristics were performed on all twelve poplar genotypes. All measurements were taken during the first two years after planting between May 2010 and December 2011.

2.3.1. Leaf characteristics

Leaf area index (LAI) was assessed in different replicated measurement plots (consisting of 6 × 5 trees) for each genotype within each former land use type, i.e. two plots in GS1 and four plots in GS2 × 12 genotypes × 2 land use types. LAI was assessed using direct and indirect methods. The (cross calibrated) LAI-2000 and LAI-2200 Plant Canopy Analyzer (LiCor, Lincoln, NE, USA) was used to measure LAI indirectly in GS1 and GS2 respectively, by comparison of above and below canopy readings with a 45° view cap. These indirect LAI measurements were taken monthly throughout the growing season from July to November in GS1 and from April to November in GS2 to monitor leaf area development and to determine maximum LAI (LAI_{max}). In each plot, two diagonal transects were made between the rows, and along each transect measurements were taken with the sensor parallel to the row and perpendicular to the row.

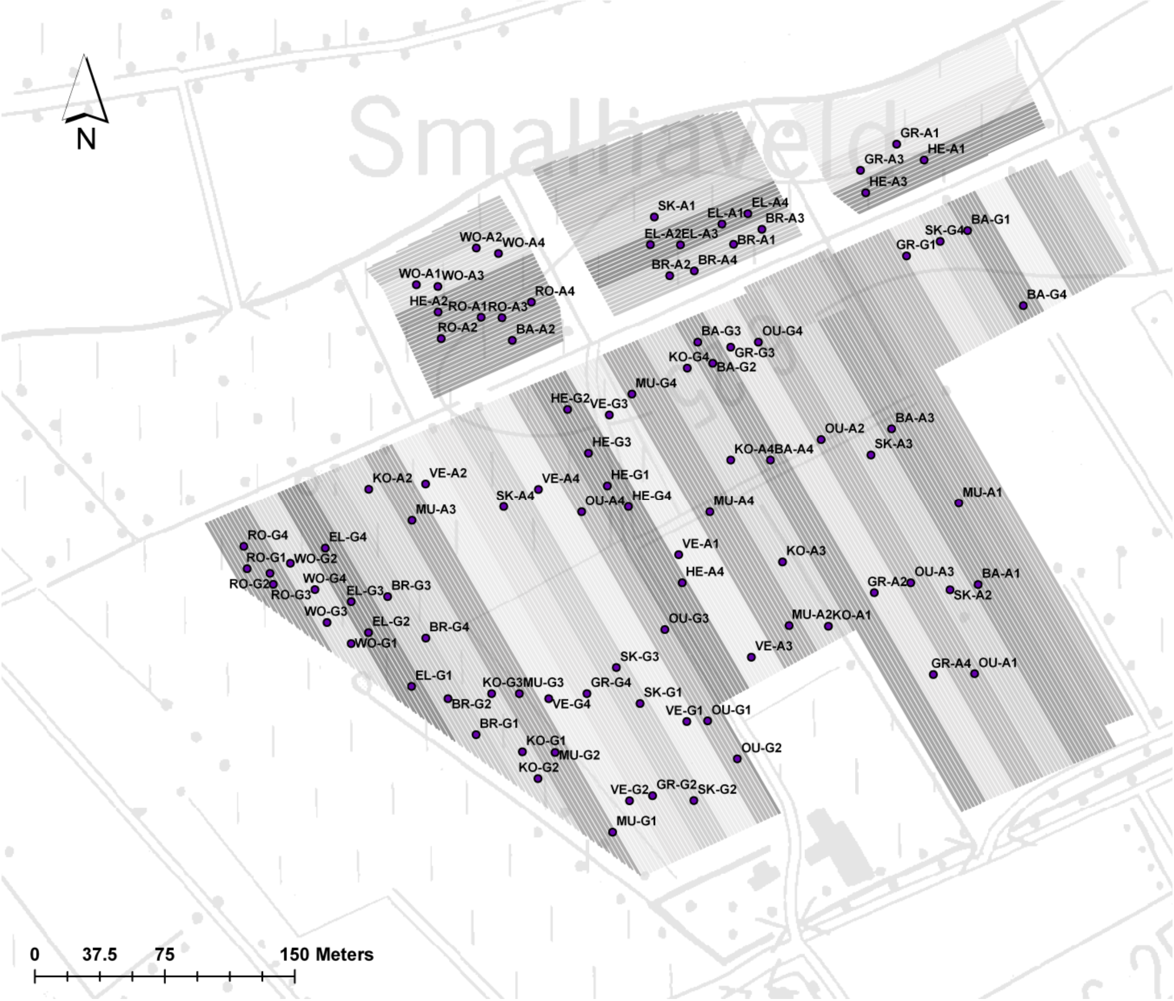

Fig. 2 Map of the plantation lay-out showing the monoclonal blocks and the measurement plots on both former land use types. A = former cropland, G = former pasture, poplar genotypes BA = Bakan, BR = Brandaris, EL= Ellert, GR = Grimminge, HE = Hees, KO = Koster, MU = Muur, OU = Oudenberg, RO = Robusta, SK = Skado, VE = Vesten and WO = Wolterson.

Table 3 Description and timing of weed control during the establishment year (2010) and the second growing season (2011).

	Category	Description	Target species	Frequency (Date)	Application area	Damage on the trees
Manual	Dutch hoe	Manual removal of weeds by pulling the hoe through the soil and cutting the weeds just under the surface	All weeds that are higher than the sprouting trees	± 450 man-hours (June-August 2010)	Areas with high weed growth (i.e. weeds shading the sprouting trees)	Accidental removal of the sprout with the hoe
Chemical*	Roundup[1] (glyphosate)	3.5 l ha^{-1} sprayed with a tractor mounted sprayer before planting	General herbicide	Single treatment (26 March 2010)	Areas with former pasture land (5.8 ha)	No assignable effect
	AZ 500[2] (isoxaben) + Kerb 400 SC[2] (propyzamide)	0.3 l ha^{-1} AZ + 1 l ha^{-1} Kerb sprayed with a tractor mounted sprayer before sprouting of the trees	Pre-emergent (broadleaf weeds) + soil herbicide (wide range of weeds)	Single treatment (18 April 2010)	Planted area (14.5 ha)	No assignable effect
	Tomahawk[3] (fluroxypyr) + Actirob[4]	1 l ha^{-1} Tok + 0.5 l ha^{-1} Acti sprayed with a tractor mounted sprayer with protective caps	Systemic herbicide (broadleaf weeds) + efficiency enhancing additive	Single treatment (24-28 June 2010)	Problem areas in terms of weed growth (6 ha)	Local tree damage by contact with the herbicide + accidental damage by tractor wheels
	Matrigon[2] (clopyralid) + Actirob[4]	1 l ha^{-1} Mat + 0.5 l ha^{-1} Acti sprayed with a tractor mounted sprayer with protective caps	Systemic herbicide (thistles) + efficiency enhancing additive	Single treatment (30 June – 7 July 2010)	Problem areas in terms of weed (in particular thistles) growth (6 ha)	Local tree damage by contact with the herbicide + accidental damage by tractor wheels
	Aramo[5] (tepraloxydim) + Actirob[4]	2.5 l ha^{-1} Ar + 0.5 l ha^{-1} Acti sprayed with a tractor mounted whole field sprayer	Systemic herbicide (monocotyls) + efficiency enhancing additive	Single treatment (13 July 2010)	Problem areas in terms of weed (grasses) growth (7 ha)	Local tree damage by tractor wheels and spraying arms
Mechanical	Tractor-pulled hoe	Weed control by agitating the soil surface in the 0.75 m and 1.50 m wide rows between the trees	Non-selective	Dual treatment (± 19 May 2010 and ± 3 June 2010)	Planted area (14.5 ha)	Local tree damage by tractor wheels and hoe
	Mill	Milling of the soil surface with a small tractor in the 1.50 m wide rows between the trees	Non-selective	Single treatment (± 25 August 2010)	Planted area (14.5 ha)	Local tree damage by tractor wheels and mill
	String trimmer	Trimming the weeds in the 0.75 m and 1.50 m wide rows	Priority to thistles and weeds that are higher than 30 cm	± 450 man-hours (April-September 2011)	Areas with high weed growth	Accidental removal of the tree with the trimmer
	Mulcher	Mulching in the 1.50 m wide rows	Non-selective	Dual treatment (± 28 May 2011 and ± 8 June 2011)	Planted area (14.5 ha)	Limited damage
	Heavy duty grass mower	Mowing in the 0.75 m wide rows	Non-selective	Single treatment (1-2 August 2011)	Planted area (14.5 ha)	Limited damage

*chemical herbicides are represented by brand name, followed by the active chemical between brackets, numbers refer to the producers/distributors

[1] Monsanto®; [2] Dow Agrosciences™; [3] Agronica®; [4] Bayer CropScience®; [5] Certis®

Leaf area duration (LAD) [m^2 day m^{-2}] was calculated as the integrated area below the seasonal LAI curve for each genotype as a function of day number of the year (Ceulemans and Deraedt 1999; Dowell et al. 2009). Direct LAI assessment consisted of leaf litter collection at the end of GS1 and GS2 during leaf fall, from September to December. Three 0.57 m × 0.39 m litter traps were placed on the ground along a diagonal transect between the rows in 48 plots. Litter traps were emptied every two weeks and LAI_{max} was determined using the weight method (Jonckheere et al. 2004). LAI_{max} was calculated from the cumulated dry weight of the leaf litter collection using a predetermined ratio between fresh leaf area and leaf dry weight, also called specific leaf area (SLA) [m^2 kg^{-1}]. Fresh leaf area was measured with a Li-3000 Leaf Area Meter (LiCor, Lincoln, NE, USA). C and N mass fractions of the leaves were determined by dry combustion (CN element analyzer, Carlo Erba Instruments, Italy) of a mixed subsample of three randomly selected mature leaves of different leaf area and on different tree heights per plot on 8 October 2010 in GS1 and on 13 September 2011 in GS2 (when LAI_{max} was reached). A weighted average LAI_{max} of the plantation was calculated based on the relative area covered by each genotype within the plantation.

2.3.2. Stem characteristics

Stem diameter (D), tree height (H) and mortality were measured in one entire row (between 80 and 310 trees) within each monoclonal block. D and H were assessed as the main growth characteristics after GS1, in February 2011, and repeated after GS2. Stem diameters were measured with a digital caliper (Mitutoyo, CD-15DC, UK, 0.01 mm precision) at 22 cm above soil level. This height level – recommended and used for poplar by Ceulemans et al. (1993) and Pontailler et al. (1997) – is above the conical stem base and is low enough to avoid measurement errors because the stems were relatively small. A subset of a minimum of ten stem diameters for each LAI plot was collected. If more than one stem had sprouted from one cutting, the number of stems – called shoots – was counted and diameters were measured for each shoot. The total stem basal area (BA) at 22 cm height was calculated as the sum of the basal areas of all shoots within one tree. The Huber value (HV) was calculated as the ratio between the stem basal area and the total leaf area within each plot (Huber 1928). Tree height was calculated using the predetermined relationship between D and H for each genotype, where H was measured with a telescopic rule (Nedo mEssfix-S, NL, 1 mm precision). Stem volume index (VI) was calculated for each genotype as the sum of $D^2 \cdot H$ of all shoots within one tree (Pontailler et al. 1997; Causton 1985) and was considered as the main growth indicator of GS1 growth. Mortality was estimated by counting the number of missing trees in one or two entire rows (80 to 310 trees) within each monoclonal block. This was done one month after planting (May of GS1), after weed control (August of GS1) and once more in July of GS2 (after interplanting). Since genotypes Ellert, Hees and Koster had not yet fully sprouted at the time of the first mortality assessment, mortality of these genotypes has been excluded from calculation of the average plantation mortality. Mortality assessments were used to calculate effective planting density in each monoclonal block. A weighted average over the plantation was calculated for all variables, based on the relative planted area of each genotype within the plantation. The coefficient of variation was used as an indication of the variability of each production characteristic within each genotype or parentage. Production characteristics D, H, BA, VI and HV were correlated (Pearson correlation) with possible explanatory variables LAI_{max}, LAD, mortality and the number of shoots within each tree.

2.3.3. Statistical analysis

A nested analysis of variance (nested ANOVA) was used to analyze the influence of genotype, former land use and plot on the production characteristics described above, with (i) genotype/parentage and land use as fixed factors, and (ii) plot nested within genotype/parentage and land use as a random factor. Data were tested for normality by means of a Kolmogorov-Smirnov test. Normal distribution was not significant in all groups and no transformation was found to normalize the data. Hence, normality was considered acceptable since no non-parametric alternative for a nested ANOVA was found (Logan 2010). When no significant land use effect or interaction between land use and genotype/parentage were found, these factors were removed from the model. The Tukey's students range test

(HSD) was used as a post-hoc test in case of a significant genotype/parentage effect. LAI_{max} measurements were averaged within each plot and plots were used as replicates within each genotype × land use combination. Because of the low number of plots, a non-parametric Kruskal-Wallis test was used, followed by Mann-Whitney U to test each pair. A P-value smaller than 0.05 was considered significant. The software package SPSS (SPSS Inc., Chicago, IL, USA) was used for all analyses.

3. Results

3.1. Establishment success

Six weeks after planting initial mortality was 3.4 % on average in the plantation. This number does not include genotypes Ellert, Hees and Koster which had not yet fully sprouted at the time of the mortality assessment. Mortality increased to 18.2 % when weed control treatments were finished (Fig. 3). Interplanting after GS1 reduced mortality to a weighted average of 15 % in the entire plantation, as assessed during GS2. A large genotypic variation in mortality was observed in the plantation, ranging between 10 % and 21 % after GS2. After two growing seasons genotypes Brandaris and Vesten had the lowest and highest survival rates, respectively.

3.2. Production characteristics

Despite differences in soil characteristics in the upper 15 cm of the soil, only small differences were found in production related characteristics between former land use types in GS1 (Table 4). These land use differences almost disappeared in GS2, except for the number of shoots per tree. Weighted averages of 1.17 and 1.06 in GS1, and 2.63 and 3.07 in GS2 were found for LAI_{max} from indirect and direct measurements, respectively. Both methods were significantly correlated ($R^2 = 0.47$ in GS1 and $R^2 = 0.61$ in GS2 with $P < 0.01$). Most of the genotypes in the current study reached LAI_{max} in the beginning of October in GS1, except for genotypes Oudenberg and Robusta (early September). In GS2, LAI_{max} was reached between the end of August and mid-September. Significant genotypic differences in LAI_{max} were found in both growing seasons (Table 4), with genotype Brandaris having the lowest (0.58 ± 0.12 in GS1 and 1.05 ± 0.14 in GS2) and genotype Hees (1.87 ± 0.44 in GS1 and 4.37 ± 0.92 in GS2) having the highest LAI_{max}, measured indirectly (Fig. 4). A significant parentage effect was found for indirect measurements in GS1 and for both direct and indirect measurements in GS2. Species *P. nigra* had a significantly lower LAI_{max} compared to the hybrids in both GS1 and GS2.

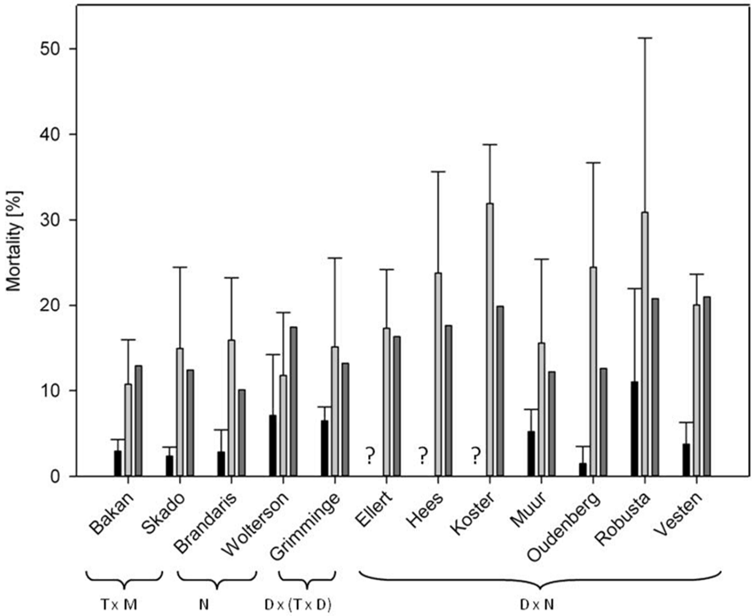

Fig. 3 Mortality for the twelve poplar genotypes after planting (black bars), after weed control (light grey bars) during the establishment year (2010) and after interplanting (dark grey bars) at the end of the second growing season (2011). Error bars indicate standard deviation of the mean; (?) indicates cuttings had not fully sprouted at the time of mortality assessment. D = *Populus deltoides*, M = *Populus maximowiczii*, N = *Populus nigra*, T = *Populus trichocarpa.*

Table 4 Results of the analysis of variance (ANOVA) of genotype, parentage, land use and plot effects on production characteristics during the first and second growing seasons.

	n	Genotype	Land use	Plot (genotype)	Parentage	Land use	Plot (parentage)
Growing season 1							
Stem diameter at 22 cm	4907	(*)	0	***	*	0	***
Basal area	4907	*	0	***	*	0	***
Number of shoots	4907	***	**	***	***	0	***
Stem height	4907	***	*	***	0	0	***
Volume index	4907	0	*	***	0	0	***
LAI_{max} indirect[1]	48	**	0	-	**	0	-
LAI_{max} direct[1]	48	*	0	-	0	0	-
Huber value indirect[1]	48	*	0	-	(*)	0	-
Huber value direct[1]	48	0	0	-	0	0	-
Growing season 2							
Stem diameter at 22 cm	4907	***	0	***	**	0	***
Basal area	4907	***	0	***	*	0	***
Number of shoots	4907	***	*	***	***	0	***
Stem height	4907	***	0	***	***	0	***
Volume index	4907	***	0	***	**	0	***
LAI_{max} indirect[1]	96	***	0	-	***	0	-
LAI_{max} direct[1]	48	**	0	-	**	0	-
Huber value indirect[1]	96	***	0	-	0	0	-
Huber value direct[1]	48	0	0	-	0	0	-

LAI_{max} = maximum leaf area index, measured (in)directly; significance: 0 = $p > 0.10$; (*) = $0.05 < P \leq 0.10$; * = $0.01 < P \leq 0.05$; ** = $0.001 < P \leq 0.01$; *** = $P \leq 0.001$; n = sample size; [1] Non-parametric Kruskal-Wallis test

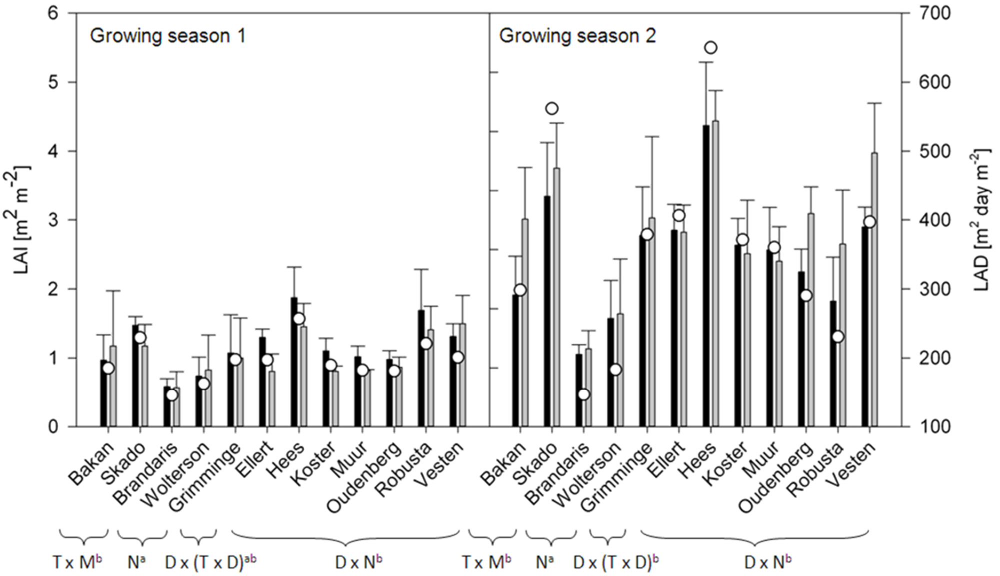

Fig. 4 Maximum leaf area index (LAI$_{max}$), measured in an indirect (black bars) and direct (grey bars) way, and leaf area duration (LAD) (circles) for twelve poplar genotypes – grouped by parentage – during GS1 and GS2. ^{a,b}Homogeneous subsets for indirect LAI measurements, groups which do not share a letter are significantly ($P < 0.05$) different; Error bars indicate standard deviation of the mean; D = *Populus deltoides*, M = *Populus maximowiczii*, N = *Populus nigra*, T = *Populus trichocarpa*.

The higher N mass fraction in the upper 15 cm soil layer in former pasture compared to cropland was reflected in a significantly higher leaf N mass fraction ($P = 0.009$ in GS1 and $P = 0.000$ in GS2) in trees planted on former pasture land (Fig. 5).

Stem diameter was 25.21 mm (weighted average) across genotypes at the end of GS1. Height measurements showed a weighted average of 247.4 cm (GS1) and were linearly correlated with D. VI was not significantly different among genotypes, although some genotypic variation was observed for stem BA and H. In line with LAI$_{max}$, a significantly lower D was found in *P. nigra* (genotypes Brandaris and Wolterson) in comparison to the hybrids. A low significant parentage effect was found for D and stem BA. The number of shoots that sprouted from one cutting was significantly different among genotypes and parentages. A strong plot effect within genotypes and parentages was found for all stem characteristics

(Table 4), indicating a spatial variability in growth performance that was not correlated to former land use. High spatial variability was also reflected in the high variability observed for most production characteristics (Fig. 6), suggesting within genotype and parentage variability.

During GS2, a weighted average of 40.73 mm and 444.6 cm were found for D and H respectively. Strong significant ($P < 0.05$) genotype and parentage effects were found for all production related characteristics (Table 4).

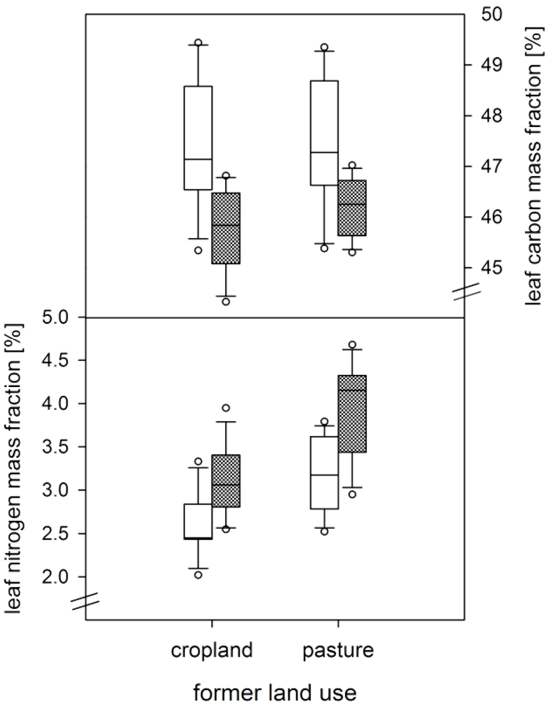

Fig. 5 Boxplots of carbon (top) and nitrogen (bottom) mass fractions in the leaves of the different poplar genotypes (n=12), planted on two former land use types. Plantation averages are shown. White and dotted boxes represent GS1-data and GS2-data respectively. GS = growing season.

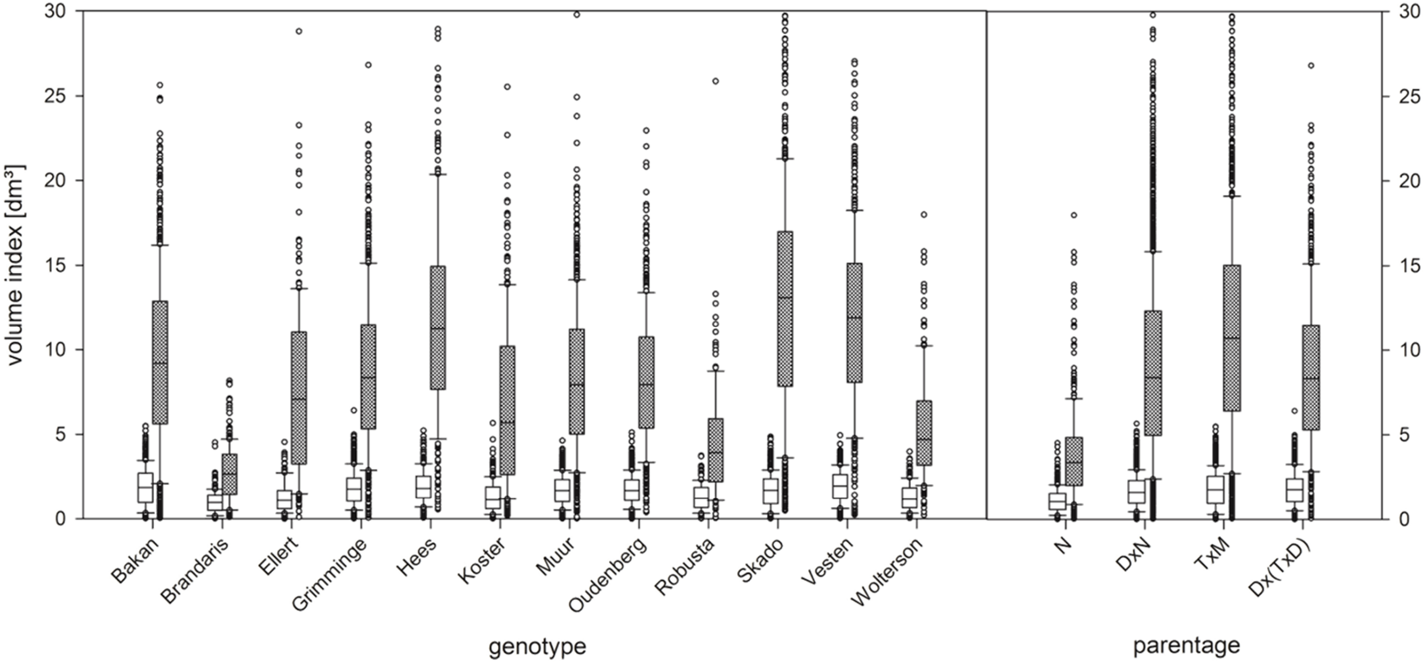

Fig. 6 Volume Index [dm³] of trees after GS1 (white boxes) and GS2 (dotted boxes) arranged by poplar genotype (left panel) and parentage (right panel). D = *Populus deltoides*, M = *Populus maximowiczii*, N = *Populus nigra*, T = *Populus trichocarpa*; GS = growing season.

Table 5 Correlation between production characteristics and explanatory variables (Pearson correlation)

	n	Volume index	Diameter	Basal Area	Height
Growing season 1					
LAI_{max} indirect	48	++	+++	+++	0
LAI_{max} direct	48	++	+++	+++	++
LAD	48	++	+++	+++	0
No. of shoots	4907	0	- - -	+++	- - -
Mortality[1]	34	0	0	0	-
Growing season 2					
LAI_{max} indirect	96	+++	+++	+++	+++
LAI_{max} direct	48	+++	+++	+++	+++
LAD	96	+++	+++	+++	+++
No. of shoots	4907	0	- - -	+++	- - -
Mortality[1]	34	0	0	0	0

[1] mortality data were collected and correlated with growth characteristics on a row level instead of a plot level, as for the other production parameters; LAI_{max} = maximum leaf area index; LAD = leaf area duration; n = sample size; significance: 0 = $P > 0.05$; + = $0.01 < P \le 0.05$; ++ = $0.001 < P \le 0.01$; +++ = $P \le 0.001$ for positive correlations, equally with – for negative correlations.

LAI_{max}, both from direct and indirect measurements, and LAD were strongly and linearly correlated to VI, D and BA for both growing seasons (Table 5; Fig. 7). This suggests that LAI_{max} and LAD were good indicators of production. A significant correlation with tree height was observed in GS2 as well, whereas in GS1 this was only observed for the direct LAI_{max} measurements. Lower D and H values were found with a higher number of shoots within each tree (Table 5). There was no significant correlation between mortality and VI, indicating that differences in growth performance were not caused by differences in effective planting density.

4. Discussion

An evaluation of factors affecting the first-year growth and survival is reported, since the establishment year is crucial for the success of any bio-energy plantation. Analysis of genotypic variation in production related characteristics is important to identify genotypes with high potential for SRC cultures, in relation to soil characteristics and former land use.

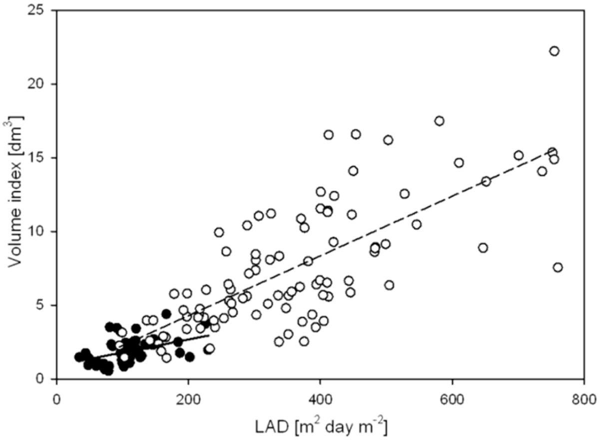

Fig. 7 Linear correlation between leaf area duration (LAD) and volume index for twelve poplar genotypes during GS1 (black circles) and GS2 (white circles). Linear regression is significant for GS1 (solid line, $R^2 = 0.20$) and for GS2 (dotted line, $R^2 = 0.64$) at the $\alpha = 0.01$ level. GS = growing season.

The lower C mass fraction in the upper soil layer in former cropland (Fig. 1) was caused by the annual harvesting of the crop and the removal of crop residues. A higher bulk density of the soil in cropland can be expected because of soil compaction and surface sealing by annual ploughing and regular access by agricultural machines. High uptake of N-fertilizer by the crops could explain the lower N mass fraction in the upper layer of the cropland compared to pasture, where N-rich manure remains in the field and where surplus manure coming from intensive cattle and pig farming is spread. Overall, the N mass fraction in the field site was high, but comparable with other agricultural areas in Flanders because of high N deposition (30-40 kg ha^{-1} y^{-1}) and N-fertilizer application (17-250 kg ha^{-1} y^{-1}) in this area (Zona et al. 2011). Since N and C mass fractions in the upper soil layer (0-15 cm) were significantly higher in former pasture land, a higher growth and production could be expected on this soil type. Indeed, higher leaf N mass fractions were found in trees planted on former pasture land. However, there was only a limited effect of former land use on the measured production characteristics in GS1 and this effect disappeared during GS2. Despite significant differences, soil nutrients were clearly not limiting in either of the former land use types. Spatial variability can likely be explained by differences in weed management, since weed growth was spatially variable in the plantation. High spatial variability in mortality supports this hypothesis, since locally several adjacent trees died from mechanical and chemical treatments (Table 3). In addition, large spatial variability in the water table in the plantation could contribute to spatial variability in growth performance or indirectly to weed growth (personal communication M. Camino Serrano).

Our results confirm the high potential for the establishment and productivity of SRC with poplar on former agricultural land in terms of survival rate and growth during the first two years of the plantation. It should be noticed that these results were obtained under optimal site conditions in terms of soil quality. Survival of the planted dormant cuttings was very high, more than 96 %, despite a very dry period in April 2010 (GS1) after planting (15.7 mm compared to a long-term monthly average of 51.3 mm in Ukkel, Belgium (Royal Meteorological Institute of Belgium 2012b). A large genotypic variation in survival rate was observed, likely driven by genotypic variation in response to the environmental conditions (Bergante et al. 2010). The delayed sprouting of genotypes Ellert, Hees and Koster compared to the other genotypes could be partly explained by a lower rooting capacity of genotypes with the *P. deltoides* parentage (female *P. deltoides* parent from the same origin; Table 2). Rooting capacity of dormant cuttings of *P. deltoides* is generally low (Randall and Krinard 1977) and genotypically highly variable (Dickmann and Stuart 1983; Zalesny et al. 2005). Weed competition and weed control were the most important factors in the establishment of the plantation (cfr. Buhler et al. 1998). Poplars are known to be susceptible to competition for available water and light, mainly during the establishment year (Dickmann and Stuart 1983). In addition, herbicide damage and losses due to mechanical weed control significantly reduced the survival rate and growth in the plantation (Table 3). Hence, genotypic variability in survival rate possibly also reflects genotypic variation in resistance to weeds or to weed control.

Even during GS2 canopy closure was not fully reached. In a comparable multi-clonal SRC plantation, stable LAI was not reached during the first year of the second rotation at a tree density of 10,000 ha^{-1} (Al Afas et al. 2005). The strong decrease in LAI of genotype Robusta after week 35 of GS1 can be explained by a rust infection (*Melampsora larici-populina*), an important determinant of early leaf fall (Dunlap and Stettler 1996). No significant signs of early leaf fall or growth reduction caused by rust infection were observed for the other genotypes. During

GS2, a shift in the growing season occurred; LAI_{max} was reached one month earlier as compared to GS1 due to the early growth start in 2011 from the established rooting system.

Significant genotypic variability in LAI was observed among the different poplar genotypes during GS1 and GS2, confirming previous observations (Ceulemans et al. 1993; Taylor et al. 2001; Al Afas et al. 2005). From the observations of this study, genotypes with large individual leaves (Skado, Bakan and Grimminge) tended to have high LAI. Indeed, maximal individual leaf area has been reported as a main determinant of total leaf area (Marron et al. 2005; Dillen et al. 2009). However, the highest LAI was observed in genotype Hees, which can probably be explained by the fast production of many small leaves. Taylor et al. (2001) reported an intermediate strategy of leaf production and individual leaf area to achieve the highest LAI. More research on the rate of leaf production and individual leaf area is required to clarify both strategies. Total leaf area determines the amount of carbon uptake and has been previously suggested as a good indicator of biomass yield of a SRC with poplar (Larson and Isebrands 1972; Taylor et al. 2001b; Pellis et al. 2004). In this study, LAI_{max} was indeed found to be a good determinant of above-ground biomass production, assessed through VI.

In terms of D and H the first-year growth performance of the poplar genotypes in this SRC was comparable with previous reports (Ceulemans et al. 1992; Barigah et al. 1994; Filat et al. 2010) or higher (Tharakan et al. 1998; Al Afas et al. 2008) under similar conditions. During GS1, no significant genotypic variation was found for D or VI, although different studies reported clonal variation in biomass yield (Ceulemans et al. 1992; Laureysens et al. 2004; Paris et al. 2011). Most likely, the large spatial variability in the first-year of growth and establishment, due to the heterogeneity in weed pressure and management practices, obscured the genotypic variability in our analysis. In addition, a high variability within genotypes during the establishment year is likely due to a large heterogeneity in cutting quality, mainly depending on cutting diameter (Dickmann and Stuart 1983). Indeed, large within-genotype variability was found for most production characteristics, as expressed by large variation between different measurement plots. We expect these within-genotype variations (and competition with weeds) to decrease in the following years.

GS2 data confirm an increasing genotypic variability in production characteristics, also outperforming the limited land use effects that were observed during GS1. But there was still a high spatial variability, expressed as differences among plots, probably due to growth delays of trees from lower quality cuttings and to the continuing spatially heterogeneous weed control. The parentage effect observed during GS1 was more strongly expressed in GS2. Pure *P. nigra* genotypes showed the lowest LAI and lowest diameter, indicating a significantly lower growth performance compared to the hybrid genotypes. In commercial plantations – both traditional and SRC cultures – interspecific hybrids are frequently used because of their heterosis, a phenomenon defined as the superiority of the offspring compared to the parental trees (Stettler et al. 1996). Nevertheless Al Afas et al. (2008) mentioned pure *P. nigra* and *P. trichocarpa* genotypes amongst the most productive genotypes in a SRC culture at the end of the fourth growing season after coppicing. These results might indicate that the hybrids may not sustain the higher productivity over several rotations.

5. Conclusion

Only minor genotypic variation was found in production related characteristics during GS1, but some significant parentage effects were observed. Genotypic and parentage variation in growth performance were strongly expressed during GS2. Although significant differences in soil C and N mass fractions in the 0-15 cm soil layer were demonstrated between former cropland and pasture, this was reflected only to a small extent in different growth performance of the trees planted on both former land use types. This former land use effect was clearly outperformed by the genotypic variation during the second growing season. High spatial variability in production characteristics among the different genotypes was likely influenced by weed management, which turned out to be the key factor in the establishment success of an SRC plantation.

Acknowledgements

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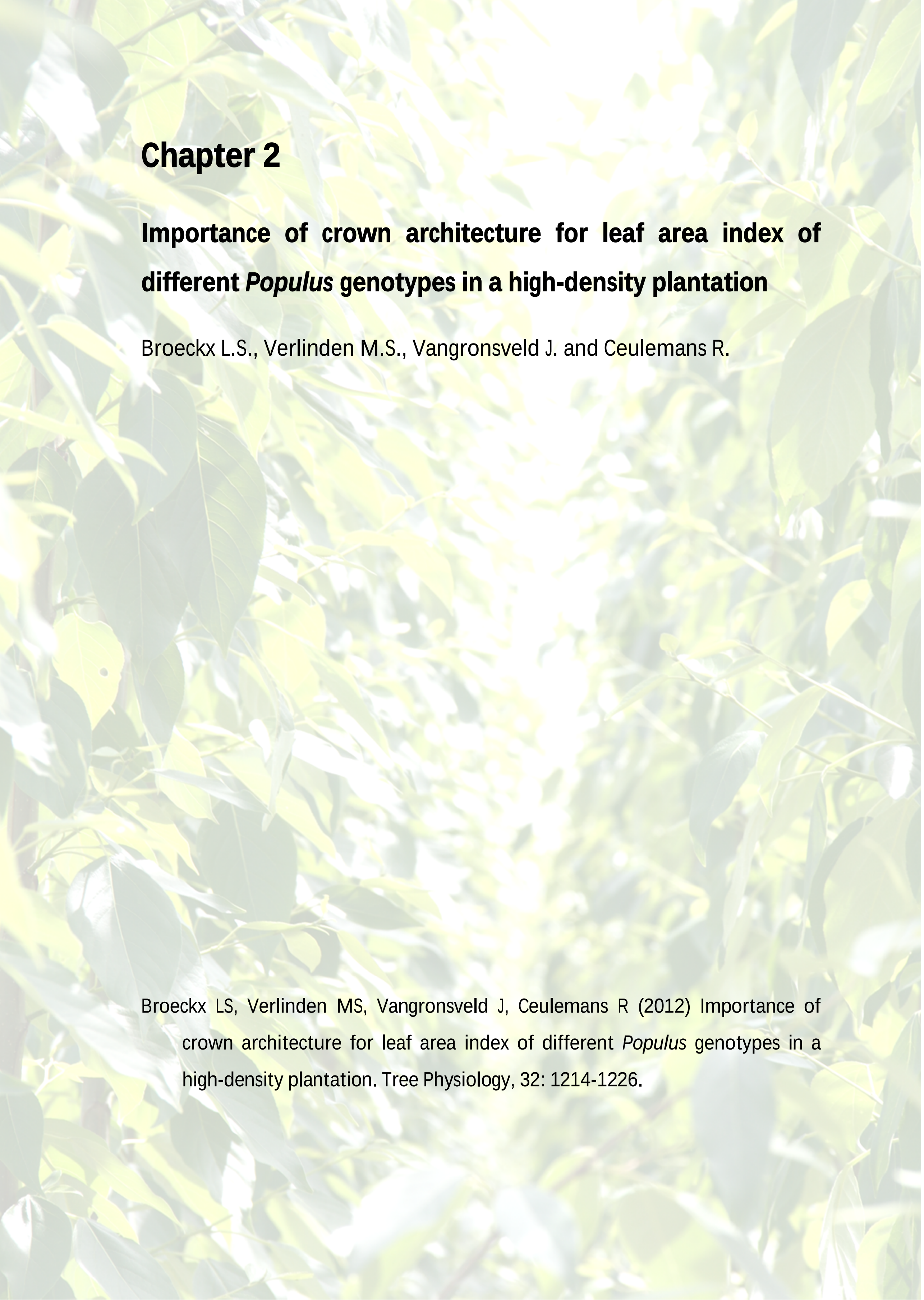

Chapter 2

Importance of crown architecture for leaf area index of different *Populus* genotypes in a high-density plantation

Broeckx L.S., Verlinden M.S., Vangronsveld J. and Ceulemans R.

Broeckx LS, Verlinden MS, Vangronsveld J, Ceulemans R (2012) Importance of crown architecture for leaf area index of different *Populus* genotypes in a high-density plantation. Tree Physiology, 32: 1214-1226.

Abstract

Crown architecture is an important determinant of biomass production and yield of any bio-energy plantation since it determines leaf area display and hence light interception. Four *Populus* genotypes – of different species and hybrids and with contrasting productivity and leaf area – were examined in terms of their branch characteristics in relation to crown architecture during the first and second growing seasons after plantation establishment. The trees were planted at high-density (8,000 ha^{-1}) on two different former land use types, cropland and pasture. We documented significant differences in branch architecture among the genotypes and for the first year among the former land use types. Land use effects only affected factors not related to canopy closure and wood production, and disappeared after the first growing season. This suggested that both former land use types were equally suited for the establishment success of a poplar bio-energy plantation. Tree height and branch dimensions – branch diameter and branch length – were the most important determinants of wood production and maximum leaf area index. Despite the secondary importance of the number of sylleptic branches, these branches contributed significantly to the total leaf area in three out of the four studied genotypes. This indicated that enhanced syllepsis accelerates leaf area development and hence carbon assimilation, especially in the early stages of a high-density plantation with poplar.

Keywords

Branching pattern, syllepsis, genotypic variation, volume index

1. Introduction

An increasing energy demand in combination with global warming has renewed the interest in using plants as a potential carbon-neutral energy source. Fast-growing hardwood trees as poplar (*Populus)*, grown under a short-rotation coppice regime, can be suited as a bio-energy source when their yield is sufficiently high (Hansen 1991, Aylott et al. 2008, Rowe et al. 2009). Canopy structure is an important determinant of the productivity – and thus of the yield – of these plantations since it comprises leaf orientation and distribution, thereby affecting canopy density, light interception and carbon assimilation (Hallé et al. 1978, Duursma and Mäkelä 2007, Kim et al. 2011). Identifying and quantifying the underlying factors contributing to canopy structure are therefore important for a better understanding and optimization of stand productivity.

An important part of the canopy structure is determined by the woody crown architecture, i.e. the branching pattern (Gielen et al. 2002). Trees of the temperate regions produce proleptic branches that develop from lateral meristems that have gone through a period of rest (winter dormancy) (Remphrey and Powell 1985). Some temperate tree genera such as *Populus, Alnus, Larix, Tsuga* and *Prunus*, as well as many tropical trees, also produce sylleptic branches that develop from meristems that were formed in the same year as the branches (Hallé et al. 1978). The additional leaf area on these sylleptic branches contributes to increasing canopy density, which is especially important in the first years of plantation development when canopy closure is not yet reached (Ceulemans et al. 1992, Scarascia-Mugnozza et al. 1989). A larger and often faster development of leaf area has been observed on sylleptic branches as compared to the main axis (Ceulemans et al. 1990, Scarascia-Mugnozza et al. 1989, Zeleznik 2007). In addition, sylleptic branches contribute more to the overall tree growth because of a higher proportion of carbon allocation to the main stem in comparison to proleptic branches (Scarascia-Mugnozza et al. 1999). A large genotypic variability in crown architecture has already been reported within the genus *Populus* (Ceulemans et al. 1990, Dunlap et al. 1995, Gielen et al. 2002, Zeleznik 2007) as well as a strong genetic control and heritability of the syllepsis phenomenon (Wu and Stettler 1996, Rae et al. 2004, Marron et al. 2006, Dillen et al. 2009). This variability and heritability indicate a high potential for further breeding and selection of new high-yielding genotypes. Although syllepsis is an important component of crown architecture, the relationship between the occurrence of sylleptic branches and leaf area index (LAI) has to our knowledge not been examined in high-density plantations with *Populus*. LAI is defined as one half of the total leaf area per unit ground surface area (Chen and Black 1991), representing the photosynthetically active surface area of the canopy.

In this study, variations in crown architecture were examined in relation to LAI among four different *Populus* genotypes grown in a high-density bio-energy plantation. All measurements were performed during the first two growing seasons after plantation establishment. The general objective was to generate a better insight in the most important crown structure determinants affecting light interception. This information could be used for the selection of particular genotypes for bio-energy plantations and for interspecific hybridization in search of an architectural ideotype (Wu and Stettler 1996 & 1998). We hypothesized that genotypes differ in branch characteristics and that contrasting branch characteristics result in alternative growth strategies in terms of crown architecture including the quantity and distribution of foliage. Additionally, the aim of the present study was to evaluate to what extent the LAI is determined by crown architecture and related branch characteristics. A second hypothesis was that sylleptic and proleptic branches differ in their contribution to the total leaf area of the tree and hence to carbon assimilation. A secondary objective was to study the effect of former land use on the crown architectural and LAI characteristics, since the plantation was established on two different land use types.

2. Materials and Methods

2.1. Site description

In April 2010 an experimental bio-energy plantation was established in Lochristi, East-Flanders, Belgium (51°06’44” N, 3°51’02” E; 6.25 m above sea level). The total area of the site is 18.4 ha of former agricultural land consisting of cropland, recently a monoculture of maize, and extensively grazed pasture. The long-term average annual temperature at the site is 9.5 °C and the average annual precipitation is 726 mm, equally distributed over the year. A detailed soil analysis prior to planting characterized the soil type as a sandy texture, with clay-enriched deeper soil layers. In the upper soil layer, carbon (C) and nitrogen (N) concentrations were significantly lower in cropland as compared to pasture and decreased exponentially with depth in both former land use types. For more information on the site and on the soil characteristics, see Broeckx et al. (2012).

2.2. Plant materials and plantation lay-out

The planted area represents 14.5 ha and consists of twelve poplar (*Populus*) and three willow (*Salix*) genotypes set up in large replicated monoclonal blocks. At least one block (minimum 950 and maximum 4900 trees) of each genotype was planted on each of the former land use types, cropland and pasture. In this study four poplar genotypes – i.e. Grimminge, Skado, Koster and Wolterson representing four different species and hybrid parentages – were examined (Table 1). After soil preparation, 25 cm long dormant and unrooted hardwood cuttings were planted on 7-10 April 2010 at a density of 8,000 ha^{-1}. The cuttings were planted in a double-row design with alternating distances of 0.75 m and 1.5 m between the rows and 1.1 m between the individuals within each row. Neither irrigation nor fertilization were applied. More details on the plantation establishment and on the lay-out can be found in Broeckx et al. (2012). For this study, measurements and observations were performed on 16 measurement plots located within the monoclonal blocks (2 plots x 2 land uses x 4 genotypes) and consisting of 6 x 5 trees each (48 m^2).

Table 1 Overview of the four *Populus* genotypes studied in the short-rotation coppice plantation of the experiment. Parentage, place of origin, botanical section, year of the cross and of the commercialization, gender and available breeders information on crown structure are shown.

Genotype	Parentage	Place of origin	Section	Year of cross/ commercialization	Gender	Crown structure
Grimminge[1]	D × (T × D)	(Michigan US x Connecticut US) x (Washington US x (Iowa US x Missouri US))	Aigeiros x (Tacamahaca x Aigeiros)	1976/1999	♂	not available
Skado[1]	T × M	(Washington US x Oregon US) x Japan	Tacamahaca	1975/2005	♀	not available
Koster[2]	D × N	Michigan US x The Netherlands	Aigeiros	1966/1988	♂	closed, broad pyramidal crown thin branches
Wolterson[2]	N	The Netherlands	Aigeiros	1960/1976	♀	relatively broad crown heavy upright lateral branches in older trees

D = *Populus deltoides*, M = *P. maximowiczii*, N = *P. nigra*, T = *P. trichocarpa*; US = United States of America; [1] produced by the Research Institute for Nature and Forest (INBO, Geraardsbergen, Belgium); [2] produced by Vermeerderingstuinen Nederland (Zeewolde, The Netherlands)

2.3. Measurements of crown architecture

2.3.1. Branching pattern

Tree and branch characteristics were measured in detail on six randomly selected trees per plot in February 2011, at the end of the first growing season (GS1) (Fig. 1). The same measurements were repeated in the same plots on four trees per plot in January 2012, at the end of the second growing season (GS2). We selected the same trees as in GS1 if possible, but new trees were randomly selected if the former trees were damaged. Thus, all assessments of tree structure and crown architecture were made on the dormant and leafless winter habitus of the trees. Stem diameter (D) at 22 cm above ground level and tree height (H) were measured. The number of buds on the main stem and the number of branches of each branch category – up to the second-order branches (cfr. Burk et al. 1983) – were counted. The relative number of branches was calculated by correcting the number of branches of each tree for H. The different branch categories include sylleptic branches of GS1 (SYL1), proleptic branches (PROL) and sylleptic branches of GS2 (SYL2) (see Fig. 1). Branch characteristics were assessed for maximum 10 branches in GS1 and for maximum five branches per branch category in GS2. The branch diameter (D_b) was determined at 1 cm above the insertion to the stem. The branch length was measured (i) along the branch (BLx), and (ii) as a straight line between the branch insertion and the branch tip (BLy) (Fig. 1). The branch angles of origin (α) and of termination (β) were determined, according to Burk et al. (1983). Insertion heights of the lowest (I_l) and the highest branch (I_h) were measured for each branch category. A telescopic pole (Nedo mEssfix, NL, 1 mm accuracy), a digital caliper (Mitutoyo, CD-15DC, UK, 0.01 mm accuracy), a tape measure and an in-house made inclinometer (Kockelbergh and Assissi 2000) were used to perform all measurements of tree height, of tree and branch diameters, of branch length and of branch angles, respectively.

The internodal distance of the stem was calculated as H divided by the sum of the number of buds and the total number of branches. The canopy depth was defined as $H\text{-}I_l$ while relative canopy depth (%) was expressed as that part of the stem that carried branches in relation to H. Branch curvature was determined as α/β. Volume indices of branch and stem biomass (VI_{branch} and VI_{stem}, respectively) were estimated from $D_b^2 \cdot BLx$ and $D^2 \cdot H$ respectively (Causton 1982, Ceulemans et al. 1992, Pontailler et al. 1997). These indices were used as a proxy for above-ground woody biomass. Total volume index was defined as the sum of VI_{branch} and VI_{stem}. The ratio of the estimated branch biomass to the estimated total above-ground biomass was chosen as the branchiness index (Ceulemans et al. 1990).

Genotypic phenograms (Gielen et al. 2002) were constructed as schematic representations of the two-dimensional crown structure of the four genotypes in Inkscape 0.48 (Open Source vector graphics editor). Mean values of H and of the branch characteristics were used to construct a genotypic phenogram for every genotype on both former land use types. For GS1 mean values were calculated of I_l and I_h for all sylleptic branches, and mean values of BLx, BLy, α and β for four groups of sylleptic branches in each genotype x land use combination. These four groups consisted of the highest branches on the tree, medium high branches, medium low branches and the lowest branches on the tree. The number of branches in each group varied according to the number of measured branches. For GS2 mean values of I_l, I_h, BLx, BLy, α and β were calculated for each branch category in each genotype x land use combination.

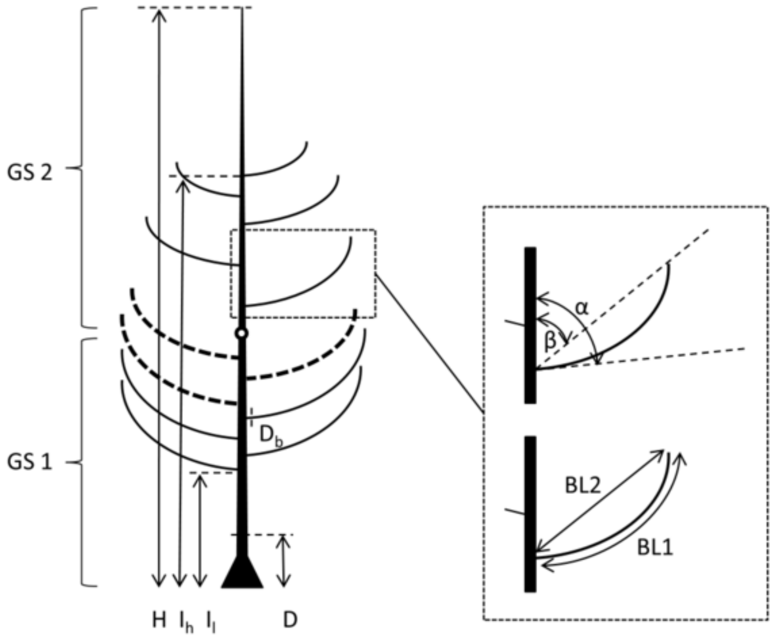

Fig. 1 Schematic representation of the measurements of tree and branch characteristics: (H) tree height, (I_h) insertion height of the highest branch, (I_l) insertion height of the lowest branch, (D) stem diameter at 22 cm above soil level, (D_b) branch diameter at 1 cm above the insertion to the stem, (α) branch angle of origin, (β) branch angle of termination, (BLx) branch length along the branch and (BLy) branch length as a straight line between the branch insertion and branch tip; (GS1) first growing season, (GS2) second growing season. Proleptic branches are represented by dotted lines, sylleptic branches by thin solid lines. The small open circle on the main stem indicates the end of the first growing season (GS1).

2.3.2. Leaf area index

Leaf Area Index (LAI) was measured indirectly with cross calibrated LAI-2000 and LAI-2200 Plant Canopy Analyzers (LiCor, Lincoln, NE, USA) in GS1 and GS2 respectively, by comparison of above- and below-canopy readings (LiCor Inc. 2011). In each measurement plot the readings were made along two diagonal transects in two directions – parallel and perpendicular to the rows – and with a 45° view cap. Monthly measurements, from July to November 2010 in GS1 and from April to November 2011 in GS2, were used to determine maximum leaf area index (LAI_{max}).

To validate the indirect LAI measurements of the Plant Canopy Analyzers a whole-tree leaf harvest of every third leaf was performed early September 2011 (GS2) on three randomly selected trees of each genotype in previous cropland (3 trees x 1 land use x 4 genotypes). The leaves of each branch category (sylleptics and proleptics), and of the main stem were collected separately. The number of leaves was counted and multiplied by three and the individual leaf area was measured with a LI-3000 leaf area meter (LiCor, Lincoln, NE, USA). D and H were measured for each tree as described above. The LAI from the destructive leaf harvest was calculated for each tree.

2.3.3. Statistical analysis

The mixed procedure (Littell et al. 1996, West et al. 2006) was used to analyze the effects of genotype and former land use type on crown architecture. The restricted maximum likelihood (REML) approach is used in this procedure. A mixed model with genotype, former land use type and the genotype x land use interaction as fixed factors, and plot as a random factor – nested within genotype and former land use type – was applied. In the case of a significant genotype effect, pairwise comparisons of the means were performed using the Bonferroni adjustment. An ANOVA was used to test for differences in the slopes of the D_b to BLx regression curves between the different branch categories and for differences in total leaf area between genotypes. The difference in α and β between sylleptic and proleptic branches (overall) and the difference in D_b between SYL1 and PROL (per genotype) were analyzed with a T-test. Data were tested for normality by means of a Kolmogorov-Smirnov test. A significance level of $P < 0.05$ was applied. Spearman correlation coefficients were calculated between branch characteristics related to crown architecture and LAI_{max} with plot as a replicate. Pearson correlation coefficients were calculated between branch characteristics and VI_{stem}. All statistical analyses were performed in SPSS (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Branching pattern

Mean values of H, number of branches, insertion height of branches, branch length, branch curvature and internodal distance on the main stem are schematically represented in the genotypic phenograms (Fig. 2). Trees on the former cropland produced significantly more SYL1 branches compared to trees on the former pasture (Fig. 2; Table 2), resulting in a difference in branchiness index between the two former land use types in GS1 (Fig. 3). A higher number of PROL was produced in GS2 in trees grown on former pasture compared to trees grown on previous cropland (Fig. 2; Table 2). The difference in relative canopy depth between the two former land use types decreased from 18% in GS1 to less than 1% in GS2. Therefore, former land use type had an effect on canopy depth – as part of the crown architecture – only in GS1 and this effect disappeared after the establishment year (Table 2). No significant plot effects were found in any of the crown characteristics indicating low between measurement plot variability.

In both growing seasons, significant genotypic differences were found for most branch characteristics related to crown architecture (Table 2). The four genotypes differed in the number of branches of each branch category, indicating genotypic variations in the overall branching pattern. Genotypes Grimminge and Skado had fewer branches than Koster and Wolterson. However, branch volume index was higher in Skado in GS2 (Fig. 4), due to higher D_b and BLx (Fig. 5). The greatest number of SYL1 was found for Koster while the fewest SYL1 were found for

Wolterson (Fig. 2). However, this latter genotype produced significantly more PROL than the other genotypes with a smaller D_b and BLx as compared to PROL of the other genotypes (Fig. 5). In addition, PROL were inserted lower on the main stem than SYL1 in Wolterson which was not observed in any of the other genotypes (Fig. 2).

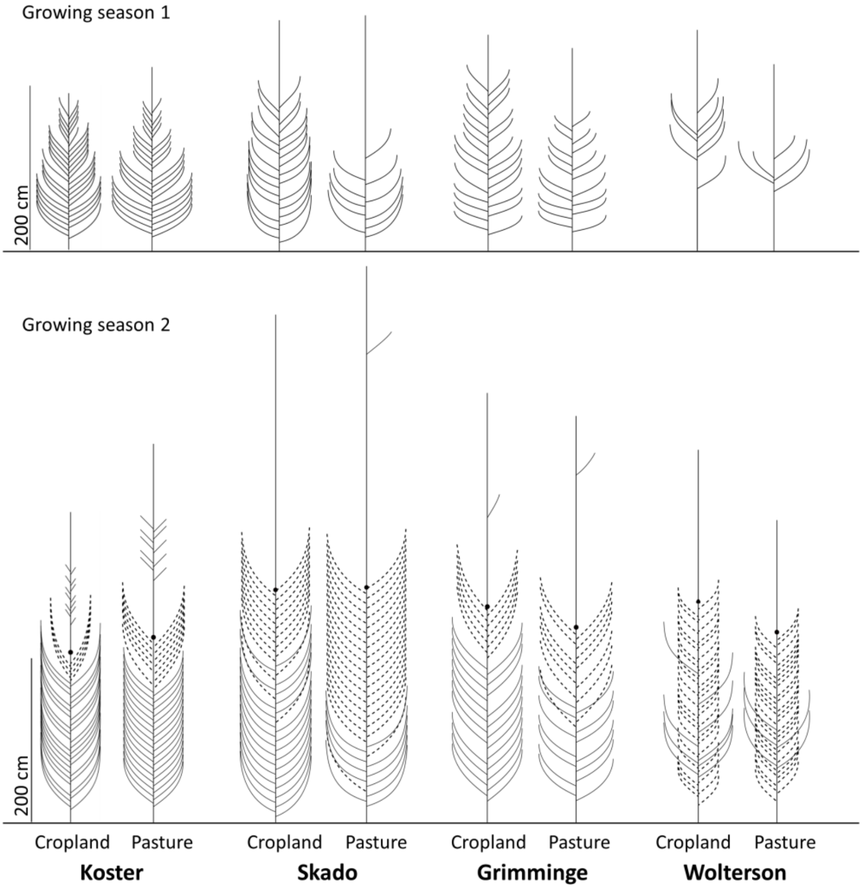

Fig. 2 Genotypic phenograms, constructed as a schematic representation of the two-dimensional crown structure, for the four studied poplar genotypes on both former land use types. Proleptic branches are represented by dotted lines; sylleptic branches by thin solid lines. The small black dot on the main stem indicates the end of the first growing season.

Canopy depth in Wolterson was thus only influenced by PROL, since SYL2 were absent in this genotype. A significantly greater number of SYL2 was formed by genotype Koster in comparison to the other genotypes (Fig. 2). The D_b was linearly correlated with BLx, with SYL2 showing a different relationship compared to SYL1 and PROL (Fig. 5). In genotype Wolterson a significant difference was found in D_b between SYL1 and PROL ($P < 0.001$), which was not found in the other genotypes. The α of SYL1 was significantly greater in Skado, so these branches were more horizontally positioned at the insertion point compared to the other genotypes. Overall, a significant difference was found between sylleptic and proleptic branches for α ($P < 0.001$) and β ($P = 0.002$), proleptic branches being more vertically oriented.

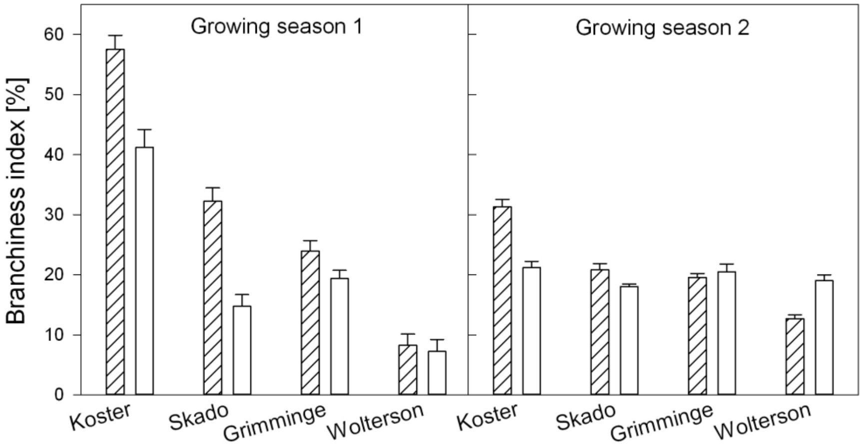

Fig. 3 Branchiness index (± SE), i.e. the ratio of the branch volume index to the total volume index, for the four studied poplar genotypes on both former land use types: cropland (hatched bars) and pasture (white bars).

Significant genotypic differences were found in relative canopy depth in both GS1 and GS2 (Table 2). Genotype Koster developed a significantly larger branched portion of the stem compared to the other genotypes. In GS1 this could be attributed to the I_h and in GS2 this could be attributed to the presence of SYL2. The deepest crowns were observed in Skado and the smallest crown depths in Wolterson, mainly due to the difference in tree height.

Table 2 Output of the mixed model (REML) used to analyze differences in various tree and branch characteristics between four genotypes and two former land use types. Genotype, former land use and the genotype x land use interaction were used as fixed factors and plot was used as a random factor.

	Branch and tree characteristics	Land use	Genotype	Land use x Genotype
Growing season 1				
Tree	Relative canopy depth	***	***	-
	Insertion height lowest branch	-	**	-
	Insertion height highest branch	**	-	-
	Internodal distance	-	***	-
	Branchiness index	*	***	-
	Volume index branches	-	(*)	-
	Volume index stem	-	-	-
Sylleptic branches	Relative number	*	***	-
	Length	-	-	-
	Diameter	-	*	-
	Branch curvature	-	**	-
Growing season 2				
Tree	Relative canopy depth	-	*	-
	Internodal distance	-	***	-
	Relative number of branches	-	***	*
	Branchiness index	-	**	*
	Volume index branches	-	**	-
	Volume index stem	-	*	-
Sylleptic branches GS1	Relative number	**	***	-
	Length	-	(*)	-
	Diameter	-	*	-
	Insertion height lowest branch	-	**	-
	Insertion height highest branch	**	-	-
	Relative number of 2nd order branches	-	*	-
	Branch curvature	-	*	-
Proleptic branches	Relative number	*	***	-
	Length	-	***	-
	Diameter	-	***	-
	Insertion height lowest branch	***	***	***
	Insertion height highest branch	-	-	-
	Branch curvature	(*)	-	-
Sylleptic branches GS2	Relative number	-	***	(*)
	Length	-	(*)	-
	Diameter	-	(*)	-
	Insertion height lowest branch	*	***	-
	Insertion height highest branch	(*)	*	-
	Branch curvature	**	***	***

(*) = $0.05 < P \leq 0.10$; * = $0.01 < P \leq 0.05$; ** = $0.001 < P \leq 0.01$; *** = $P \leq 0.001$

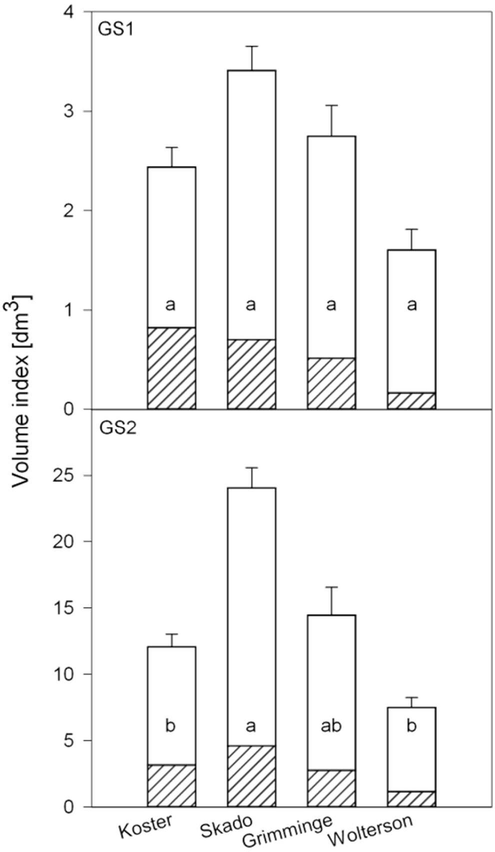

Fig. 4 Volume indices of the four studied genotypes in the first (GS1) and second growing seasons (GS2). Hatched bars represent branch volume index and white bars represent stem volume index. Error bars indicate standard error of the total volume index. Letters a and b indicate homogeneous subsets following post-hoc tests; genotypes that do not share the same letter are significantly different in total volume index at the $\alpha = 0.05$ level.

A representation of VI_{branch} and VI_{stem} in both growing seasons is found in Fig. 4. Total volume index was not significantly different among genotypes in GS1. Genotypic differences in total volume index occurred in GS2, with Skado having the largest (21.7 ± 1.4 dm^3, mean ± SE) and Wolterson having the least (6.9 ± 0.7 dm^3) wood volume. Branchiness index was close to but not quite significantly related to VI_{stem}, positively in GS1 and negatively in GS2 (Table 3). The branchiness index decreased from an average (across all four genotypes) of 26% (± 4) in GS1 to 13% (± 1) in GS2 (Fig. 3), indicating that the proportion of branch biomass decreased with time due to the increasing importance of the main stem biomass. At the end of GS1 Wolterson had the least (8% ± 2) and Koster the greatest (50% ± 3) branchiness index, but these genotypic differences disappeared at the end of GS2.

Average branch diameter, branch length, α of SYL1 and to a lesser extent branch curvature of SYL1 were the most important branch characteristics in determining VI_{stem} in both growing seasons, as attested by correlation coefficients (Table 3). A significant quadratic relationship was found between absolute canopy depth and VI_{stem} over both growing seasons (Fig. 6). I_l and I_h of SYL1 were negatively and positively correlated with VI_{stem}, respectively, in GS1 (Table 3). So the lower the lowest branch and the higher the highest branch, the greater VI_{stem} was reached in the establishment year. These correlations were weaker or disappeared during GS2, and the VI_{stem} was instead strongly and positively related to the I_h of PROL and to I_h and I_l of SYL2.

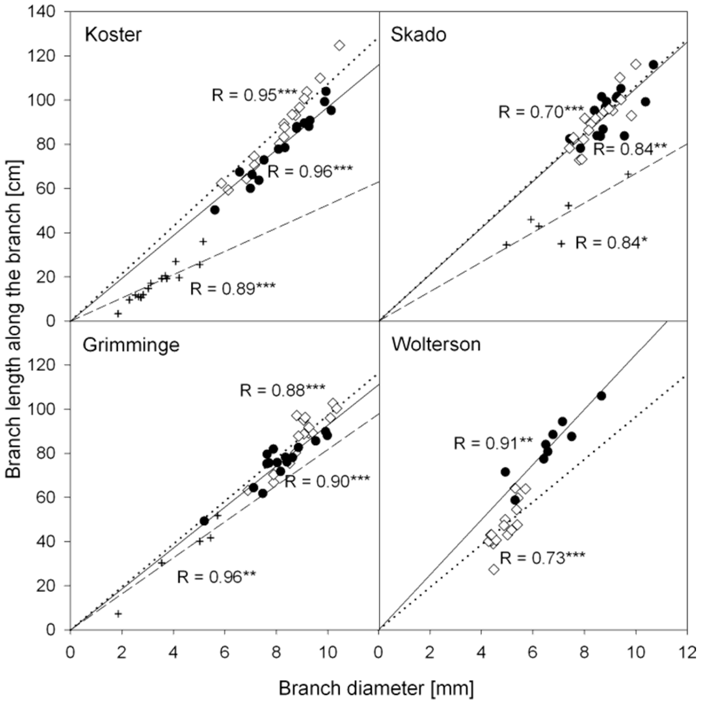

Fig. 5 Relationships between branch diameter and branch length along the branch for the four studied poplar genotypes. Diamonds (◊) represent proleptic branches (dotted regression lines), black circles (•) represent sylleptic branches of the first growing season (solid regression lines) and crosses (+) represent sylleptic branches of the second growing season (dashed regression lines). Each point represents the mean value per tree. Pearson correlation coefficients (R) are represented. * = $0.01 < P \leq 0.05$; ** = $0.001 < P \leq 0.01$; *** = $P \leq 0.001$

Table 3 Pearson correlation coefficients between branch characteristics and stem volume index (VI_{stem}) in the first (n = 96) and second (n = 64) growing seasons, as well as Spearman correlation coefficients between branch characteristics and maximum leaf area index (LAI_{max}) in the first and second growing seasons (n = 16).

	VI_{stem}	VI_{stem}	LAI_{max}	LAI_{max}
Growing season	1	2	1	2
Tree height	0.82***	0.93***	0.65**	0.89***
Stem diameter at 22 cm	0.94***	0.94***	0.83***	0.86***
Branchiness index	$0.18^{(*)}$	-0.19	0.29	-0.05
Internodal distance on the stem	0.56***	0.72***	0.65**	0.72**
Relative number of SYL1	0.09	-0.02	0.23	0.17
Relative number of PROL	/	-0.30*	/	-0.19
Relative number of SYL2	/	-0.14	/	0.39
Insertion height lowest SYL1	-0.28**	$-0.23^{(*)}$	-0.51*	-0.41
Insertion height highest SYL1	0.43***	0.08	0.17	-0.18
Insertion height lowest PROL	/	0.06	/	0.16
Insertion height highest PROL	/	0.78***	/	0.59*
Insertion height lowest SYL2	/	0.86***	/	0.83**
Insertion height highest SYL2	/	0.90***	/	0.87***
Branch diameter SYL1	0.79***	0.52***	0.87***	$0.49^{(*)}$
Branch diameter PROL	/	0.58***	/	0.70**
Branch diameter SYL2	/	0.84***	/	0.66*
Branch length along the branch SYL1	0.69***	0.45***	0.61*	0.44
Branch length along the branch PROL	/	0.56***	/	0.76***
Branch length along the branch SYL2	/	0.89***	/	$0.59^{(*)}$
Angle of origin SYL1	0.57***	0.73***	0.80***	0.66**
Angle of origin PROL	/	0.58***	/	$0.49^{(*)}$
Angle of origin SYL2	/	0.05	/	0.66*
Branch curvature SYL1	0.42***	0.27*	0.39	0.41
Branch curvature PROL	/	0.06	/	0.51*
Branch curvature SYL2	/	0.24	/	0.52

$^{(*)}$ = $0.05 < P \leq 0.10$; * = $0.01 < P \leq 0.05$; ** = $0.001 < P \leq 0.01$; *** = $P \leq 0.001$; no superscript = non-significant; / = not applicable; SYL1 = sylleptic branches of the first growing season, PROL = proleptic branches, SYL2 = sylleptic branches of the second growing season.

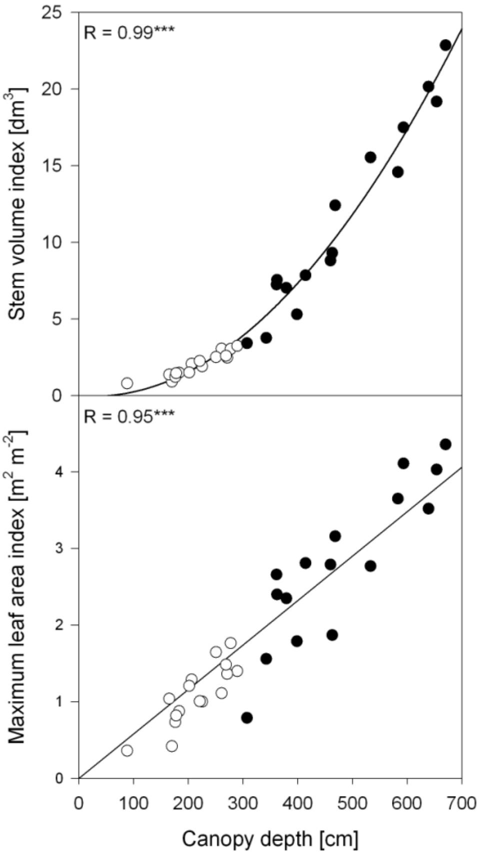

Fig. 6 Canopy depth in relation to stem volume index (top panel) and to maximum leaf area index (bottom panel) during the first (open symbols) and second (closed symbols) growing seasons. Each point represents the mean value per plot. Correlation coefficients (R) are presented.

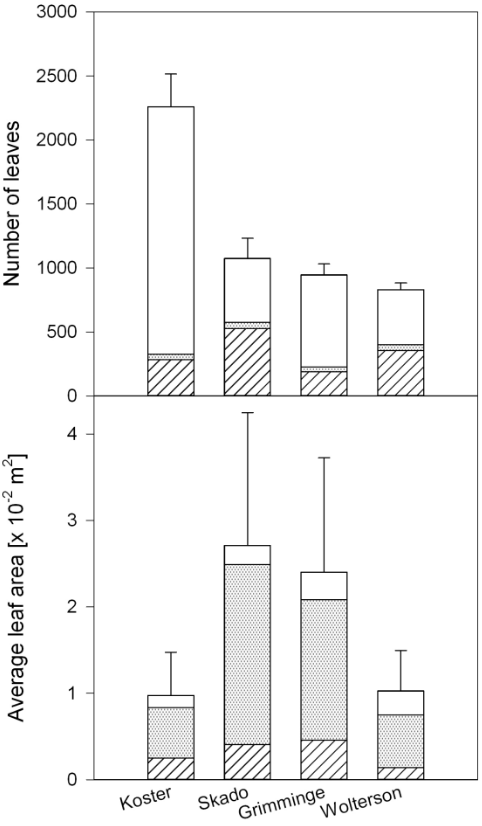

Fig. 7 Number of leaves (top panel) and average leaf area of individual leaves (bottom panel) per genotype (n = 3) during the second growing season. Hatched bars represent leaves on proleptic branches, white bars represent leaves on sylleptic branches and dotted bars represent leaves on the main stem. Error bars indicate standard error of the whole tree average number of leaves and individual leaf area.

3.2. Leaf area index

The H and D were significantly and positively correlated with LAI_{max} in both growing seasons (Table 3). The internodal distance was also found to be a good determinant of LAI_{max}, longer internodes resulting in a larger LAI_{max}. Branch dimensions of SYL1 showed significant and positive correlations with LAI_{max} in GS1, but these correlations disappeared in GS2; branch dimensions of PROL and SYL2 were found to determine LAI_{max} in GS2. Absolute canopy depth was significantly and positively correlated with LAI_{max} (Fig. 6) while relative canopy depth showed no correlation with LAI_{max} ($P = 0.3$ and $P = 0.7$ in GS1 and GS2 respectively). Since I_l was significantly correlated with LAI_{max} only in GS1, we conclude that crown depth – derived from H and I_l – was important for LAI_{max} during the establishment year, but that H was the main determinant of LAI_{max} in the subsequent growing season. The α was positively correlated with LAI_{max} in all branch categories, indicating that LAI_{max} increased with a more horizontal orientation of the branches.

The LAI from the destructive leaf harvest was strongly correlated with the indirect LAI measurements in the corresponding cropland plots ($R = 0.98$, $P = 0.02$). Many small leaves were produced on the proleptic and sylleptic branches as compared to few large leaves on the main stem (Fig. 7). Average leaf area of individual leaves was the least on sylleptic branches in all genotypes except for Wolterson. The greatest number of leaves was developed on sylleptic branches in genotypes Koster and Grimminge, while genotypes Skado and Wolterson had an approximately equal number of leaves on proleptic and sylleptic branches. Total leaf area per tree was greater in Skado compared to the other genotypes but not significantly ($P = 0.12$) different (Fig. 8). Proleptic and sylleptic branches contributed differently to the total leaf area of the tree: more than 60% of the leaf area was provided by the sylleptic branches in genotypes Koster, Grimminge and Wolterson, whereas almost 50% of the leaf area in Skado was developed on proleptic branches.

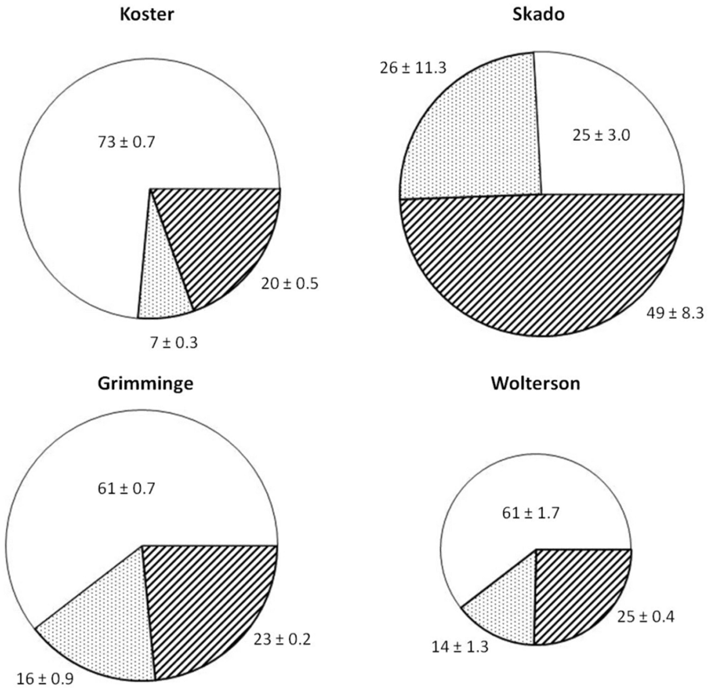

Fig. 8 Leaf area distribution between the main stem and the branches per genotype (n = 3). The values represent the percentage (%) of the total leaf area per tree. The size of the pie chart is relative to the total leaf area per tree for each genotype. White = leaf area on sylleptic branches; dotted = leaf area on the main stem; hatched = leaf area on proleptic branches.

4. Discussion

As hypothesized, large genotypic variability in crown architecture was found. This confirms earlier observations (Ceulemans et al. 1990, Dunlap et al. 1995, Gielen et al. 2002, Zeleznik 2007) but now for locally selected genotypes examined in a high-density plantation. Genotype Koster developed the greatest number of SYL1 creating a cone shaped crown. Since this genotype was also greatest in SYL2, Koster showed the most intensive syllepsis. SYL1 dimensions – especially branch length – were closely correlated to LAI_{max} only in GS1, indicating the importance of syllepsis for rapid canopy closure and efficient light interception especially during the establishment year (Ceulemans et al. 1992, Scarascia-Mugnozza et al. 1989). Indeed, syllepsis was previously described as an opportunistic strategy to fill the available space with leaves (Remphrey and Powell 1985). Since branch length and branch diameter were closely correlated, branch diameter measurements may be used as simple and reliable predictors of LAI (Burk et al. 1983). In contrast to previous observations (Rae et al. 2004, Marron et al. 2006, Zeleznik 2007, Dillen et al. 2009), the number of sylleptic branches was not related to LAI_{max} or VI_{stem}. Differences in genotypes and in planting densities may explain these different observations, since poplars are known for their plasticity in crown architecture – in particular syllepsis – in response to spacing (Hinckley et al. 1992, Nelson et al. 1981) and environmental conditions (Wu and Stettler 1998). Although Benomar et al. (2012) reported no effect of between tree competition on the level of syllepsis, superior growth was found in the poplar genotype with the highest number of sylleptics. The greatest number of PROL was observed in genotypes Skado and Wolterson, the genotypes with the largest and smallest VI_{stem} respectively. This suggests that the number of PROL was also not a determinant of the total volume index of the tree. From these results we conclude that it’s not the number of branches, but branch dimensions – branch diameter and branch length – that determine stem biomass. Genotypes with fewer, heavier branches (e.g., Grimminge) were more productive than genotypes with many light branches (e.g., Wolterson). Low branch dimensions in Wolterson resulted in a narrow cylindrical crown structure and hence low LAI_{max}. Considering LAI as an important determinant of woody biomass (Larson and Isebrands 1972, Heilman et al. 1996, Pellis et al. 2004), this narrow crown structure is not to be preferred in high-density plantations. In contrast to Ceulemans (1990), narrow crowns of genotype Wolterson resulted in the least wood volume in this study.

In terms of branchiness index, genotypes only differed during the establishment year. In addition, a non-significant trend was observed from a high branch proportion contributing to VI_{stem} in GS1 to a low branch proportion contributing to VI_{stem} in GS2. Hence total wood volume in GS2 – mainly provided by the stem – was largely determined by H and D. In line with these observations, Ceulemans et al. (1992) reported a weak correlation between stem volume and the number of sylleptics during the first year, which had disappeared by the fourth year. High carbon allocation from sylleptics to the root system, as reported by Scarascia-Mugnozza et al. (1999), could have a positive feedback on total plant growth with time and should be addressed to understand the potential role of sylleptics in productivity. The branchiness indices after the second growing season found in this study were lower than those reported by Heilman and Stettler (1985) for *P. trichocarpa* x *P. deltoides* hybrids. In a four-year-old plantation with a tree density of 7,000 ha^{-1} this study by Heilman and Stettler (1985) noted significant genotypic differences. Development of wider crowns was indeed observed before in wider spaced poplar plantations (Nelson et al. 1981).

The strong relationships observed between sylleptic branch dimensions and LAI_{max} may have important applications in terms of management of a high-density plantation and of future breeding and selection programs of poplar. Rapid canopy closure and efficient light interception limit the understory growth of annual herbs during the first years of growth. This is advisable since weed control is one of the most crucial factors in the establishment success of a bio-energy plantation (Dickmann and Stuart 1983, Ledin and Willebrand 1996, Bulher et al. 1998, Broeckx et al. 2012). In addition, the branch angle determines leaf area display and light interception (Medhurst and Beadle 2001). Genotype Skado showed a more horizontal orientation of SYL1 and PROL at the insertion point in combination with the greatest wood production among the studied genotypes. This was confirmed by the positive correlation between α of SYL1 and PROL and both LAI_{max} and VI_{stem}. Overall, the more vertical orientation of proleptic branches could be attributed to competition for light as an effect of planting density (Nelson et al. 1981). During the establishment year the developing SYL1 did not compete for light with neighboring trees at the current planting density. Indeed, genotype Koster – having wide crowns and hence the highest canopy density due to interacting branches of neighboring trees – showed the most vertically oriented proleptic branches. Breeding and selection programs of poplar might use the described branch characteristics as possible criteria for the selection of genotypes with potentially high and fast LAI development (Larson and Isebrands 1972, Heilman et al. 1996, Pellis et al. 2004). Such a focus has been shown to be very effective in increasing yields in many agronomic species (Ford et al. 2008).

Since absolute canopy depth was strongly related to LAI_{max} while relative canopy depth was not, tree height seemed to be more decisive to LAI_{max} than the proportion of the main stem that is carrying branches. However, the leaves developed on the main axis contributed the least to the total leaf area of the tree. When hybrid poplars grow older, this proportion decreases. Indeed, Stettler et al. (1988) reported that the current terminal contributed between 9 and 41% of the total leaf area at the end of the third growing season, while this proportion fell to only 1% in a five-year-old hybrid poplar (Isebrands and Nelson 1982). However, these main stem leaves – mainly in the upper canopy – can be of significant physiological importance due to their high specific leaf area (Casella and Ceulemans 2002), optimal leaf exposition and leaf properties (Niinemets et al. 2004, Peltoniemi et al. 2012) and seasonal longevity (i.e. last abscised) (Scarascia-Mugnozza et al. 1999). Their direct attachment to the stem promotes allocation of photosynthates to the main stem and the top position is favorable for light interception (Isebrands and Nelson 1982). We also observed a different contribution of proleptic and sylleptic branches to the total leaf area, as hypothesized. The sylleptic branches carried a significant amount of leaf area in all genotypes and were thus important for growth and productivity. A high contribution of proleptics to total leaf area as in genotype Skado was previously observed in *P. trichocarpa* genotypes and hybrids (Stettler et al. 1988, Dunlap et al. 1994). Leaf distribution among different branch categories can be an important production determinant because sylleptic branches were found to have a higher translocation efficiency of photosynthates (Scarascia-Mugnozza et al. 1999). Genotype Wolterson had the lowest total leaf area among the studied genotypes in combination with the lowest number of SYL1. And yet there was no correlation between the number of SYL1 and LAI_{max}. This confirmed the previous conclusion that tree height and branch dimensions were more decisive for LAI_{max} than the number of branches.

The observed effects of former land use type on canopy depth, on the number of branches and on related characteristics could be attributed to the difference in soil quality, in particular the higher N concentration in the upper soil layer in former pasture compared to former cropland. Cline and Dong-Il (2002) reported increasing and inhibiting effects of auxine respectively cytokinine on syllepsis in hybrid poplar clones. The auxine/cytokinine ratio affects apical dominance and lateral bud outgrowth in poplar (Zeleznik 2007). As we observed significantly different N concentrations in the leaves of trees planted on the two different former land use types (Broeckx et al. 2012), the N concentration could have altered the concentration of the two afore mentioned plant hormones. Indeed, land use differences in the number of SYL1 and PROL were found in this study, indicating a possible influence of N. Additional hypotheses for the land use differences are spatial variability in cutting quality, in water table and in spatially heterogeneous weed management (cfr. Broeckx et al. 2012a). Relative canopy depth and branchiness index were affected by former land use type only in the first growing season, indicating that the land use effect decreased with time. No land use differences were found in characteristics affecting LAI_{max}, thus former land use type was not controlling canopy closure and hence establishment success in this study.

5. Conclusion

We documented large variations in branch characteristics related to crown architecture among four poplar genotypes grown in a high-density bio-energy plantation. Former land use affected branch and crown characteristics, but the effect decreased in time and it did not affect factors related to canopy closure and wood production. Although the number of branches was of secondary importance, branch dimensions can significantly contribute to stem volume and LAI_{max} during the early stages of a high-density plantation. In three out of four studied genotypes, sylleptic branches did contribute most to the total leaf area of the tree. This indicated the importance of the syllepsis phenomenon to the establishment success and wood production of a bio-energy plantation with poplar during the first growing season. Overall, total wood volume was mainly supplied by the stem – and hence tree height – whereas branches contributed only a minor part after the second growing season. The four genotypes studied reflect different branching strategies in crown and leaf area development for optimal light interception and wood production. Production related branch characteristics could be included in future breeding and selection programmes for improved yield of high-density plantations with poplar.

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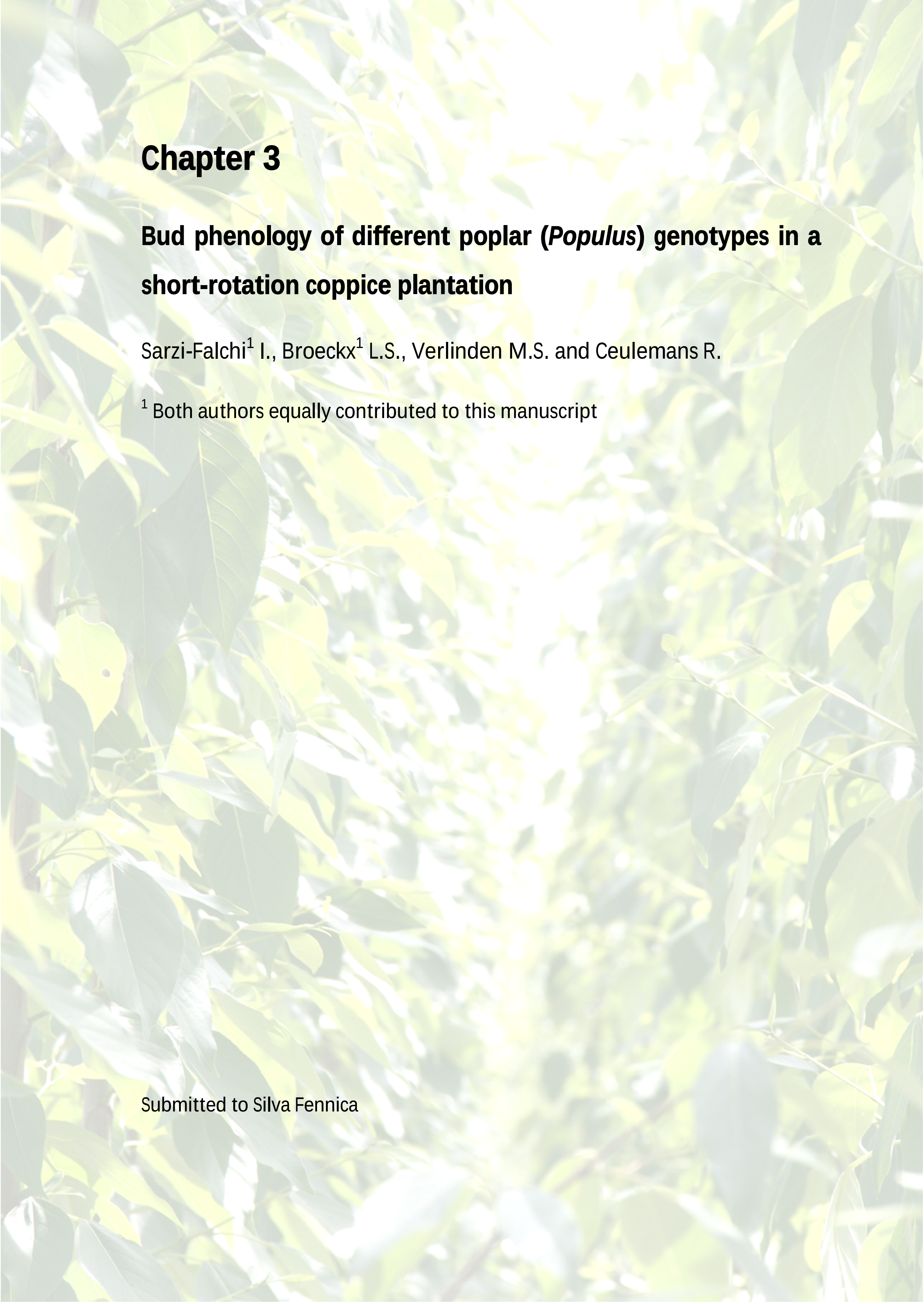

Chapter 3

Bud phenology of different poplar (*Populus*) genotypes in a short-rotation coppice plantation

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Abstract

Phenological bud traits (bud burst, bud set) determine the length of the carbon uptake period and hence also biomass production of trees. These traits were studied in a short-rotation coppice plantation with 12 different poplar (*Populus*) genotypes, representing four parentage groups. The timing of both bud burst and bud set, as well as the length of the growing season, were examined in two consecutive years, i.e. before and after the first coppice of the plantation. Significant differences among parentage groups, as well as among genotypes, were observed in the day of the year (DOY) and growing degree-days (GDD0) required to reach full bud burst and full bud set, and hence in the length of the growing season, indicating high genetic diversity. Bud burst ranged from day of the year (DOY) 81 (genotype Skado) to DOY 111 (genotype Grimminge); bud set ranged from DOY 251 (genotype Robusta) to DOY 275 (genotype Skado). The more narrow time span in bud phenology between the first and the last genotype for bud set (24 days) as compared to bud burst (30 days) suggested that genotypic variability in bud set was smaller than in bud burst. Length of the growing season ranged between 150 days (genotype Grimminge) and 194 days (genotype Skado). Earlier starters ended the growing season later, and thus had a longer growing season. Our results indicated that the time of bud burst was not only influenced by air temperature, but also by incoming solar radiation. The time of bud set was primarily influenced by photoperiod, but also by air temperature. Cumulated temperatures were a stronger determinant for bud development as compared to growing degree-days, suggesting the importance of low temperatures.

Keywords

bud burst, bud set, length of the growing season, growing-degree days, photoperiod

1. Introduction

Temperate and boreal trees alternate periods of active growth with dormancy in response to the annual cycle of the seasons (Myking and Heide 1995, Häkkinen et al. 1998, Howe et al. 2003). These repeated biological processes are defined as phenological events (Schwartz 2003); they synchronize the growing season to favourable environmental growth conditions (Rohde et al. 2011a). The timing of bud burst and bud set are assumed to be adapted to the local climate, and thus regulated by environmental conditions (Schwartz 2003). These adaptations are crucial for tree growth and survival (Pauley and Perry 1954, Partanen et al. 1998, Linkosalo et al. 2006, Junttila and Hänninen 2012) and are therefore subject to strong genetic selection (Viherä-Aarnio et al. 2006). For example, early bud burst and late bud set increase the risk of damage by a late spring or by an early autumn frost (Bailey and Harrington 2006, Dillen et al. 2009). On the other hand, late bud burst and early bud set do not allow the maximum use of the growth resources of a site (Linkosalo et al. 2006), and thus negatively affect forest production. So, the phenological response to the annual cycle of the seasons is of relevance to forest economics. The interactions between bud phenology and the environment are furthermore crucial for developing a good understanding of ecosystem sensitivity to global warming, potentially affecting the carbon uptake period and global carbon storage (Jach et al. 2001, Menzel 2002, Hänninen and Tanino 2011).

Air temperature has previously been reported to be the main driver of bud burst in temperate and boreal trees (Pauley and Perry 1954, Tanja et al. 2003, Rousi and Heinonen 2007), but some studies suggest light as an additional factor (Häkkinen et al. 1998, Girault et al. 2008). Completion of bud burst is affected by both low and high temperatures: (i) chilling is required to release dormancy (Partanen et al. 1998, Bailey and Harrington 2006, Junttila and Hänninen 2012), and (ii) the subsequent accumulation of air temperature above a threshold value, called heat forcing, determines the timing of bud burst (Myking and Heide 1995, Linkosalo et al. 2006, Howe et al. 2003). Heat forcing is often measured in growing degree-days (GDD, °C) (McMaster and Wilhelm 1997, Rousi and Heinonen 2007), with a threshold value above which physiological activity is enabled (Idso et al. 1978).

Bud set, i.e. growth cessation and dormancy induction, is strongly controlled by the shortening photoperiod in late summer and autumn (Rohde et al. 2011b). The interaction between the timing of bud set and photoperiod is highly correlated with the latitude of the genotypes' provenance (Pauley and Perry 1954); high-latitude genotypes show early bud set as an adaptive trait to the local conditions. There is increasing evidence that temperature affects the sensitivity of poplar to day length (Howe et al. 2000, Mölmann et al. 2005, Kalcsits et al. 2009, Rohde et al. 2011b,). The degree of the effect of temperature on day length is crucial to determine the impact of future climate conditions. In contrast to the generally accepted extension of the growing season under future higher temperatures, several studies reported that warm temperatures advanced bud set in some woody plants (Tanino et al. 2010). In addition, delayed bud burst was previously projected under climate warming in some species, including *Populus trichocarpa* (Murray et al. 1989). Experimental data of different species, ecotypes and cultivars are required to identify the underlying ecophysiology of the annual growth cycle and to include variation in phenological responses in climate change modeling (Hänninen and Tanino 2011).

A large phenotypic variation and strong genetic control in the timing of bud burst (Frewen et al. 2000, Pellis et al. 2004a) and bud set (Rohde et al. 2011a & b) have been reported for the genus *Populus* (poplar). This genotypic variation is exploited in the cultivation of poplar for wood production under different environmental conditions. Poplar is also favoured as a model tree in phenological studies, because of its high genetic variability. To reach renewable energy targets poplar is being cultivated in many countries (Taylor 2002, Laureysens et al. 2004, Labrecque and Teodorescu 2005, Dillen et al. 2009) as short-rotation coppice (SRC) for bio-energy (Aylott et al. 2008). Poplar is suitable for bio-energy production because of its high growth rate and high biomass production, its easy vegetative reproduction, its high genetic variability and good adaptation to different environmental conditions (Ceulemans et al. 1992). In the light of future breeding and selection programs, observations of bud burst and bud set – defining the carbon uptake period – are a crucial factor in the study of poplar growth, development and hence wood production. Although the effect of the coppice management, resulting in the regrowth of multiple stems from a stump (Tschaplinski and Blake 1995, Pellis et al. 2004a), could be expected to affect bud phenology, studies including the effect of coppice on phenological traits of *Populus* are scarce (Pellis et al. 2004b).

The main objective of this study was to examine genotypic differences in the bud phenology of 12 selected poplar genotypes that differ in their parentage, place of origin, growth and production. The effects of air temperature and solar radiation on spring bud burst, autumn bud set and hence the length of the growing season, were studied over two consecutive years. The poplar genotypes, grown under an SRC regime, were studied in the second and the third year after plantation establishment, i.e. before and after the first coppice. We hypothesized (i) significant variation among the genotypes, from different parentage groups, in the timing of bud burst and bud set, and hence in growing season length; (ii) air temperature as the main factor influencing bud burst; (iii) photoperiod as the main driver for bud set and (iv) year-to-year variation due to a combined effect of variation in weather conditions and management (non-coppiced versus coppiced).

2. Materials and methods

2.1. Field site and experimental plantation

Twelve poplar (*Populus* spp.*)* genotypes, selected for fast growth and high wood production, were examined at an existing SRC plantation in Lochristi (51°07'N; 03°51'E; 6.5 m above sea level), in the East-Flanders province of Belgium. The 12 poplar genotypes included pure species as well as hybrids, and belonged to one of the four parentage groups of the species *Populus trichocarpa* Torr & Gray (ex Hook) (T), *P. maximowiczii* Henry (M), *P. deltoides* Bartr. (ex Marsh.) (D) and *P. nigra* L. (N) (Table 1). The 12 genotypes represented seven sibling groups (Table 1). Siblings are genotypes sharing one or two parent trees (although unknown for sib2). The long-term average annual temperature at the site is 9.5 ^{o}C and the average annual precipitation is 726 mm (Royal Meteorological Institute of Belgium; http://www.meteo.be).

Table 1 Description of the twelve poplar (*Populus*) genotypes of this study. Parentage group, sibling group, place of origin/provenance, latidute/longitude of provenance, year of the cross and gender are presented (adapted from Broeckx et al. 2012a).

Genotype	Parentage group	Parental code	Sibling group	Place of origin	Latitude/Longitude	Year of cross	Gender
Bakan[1]	T × M	S.724-116 × S.122-3	Sib1	(Washington USA x Oregon USA) x Japan	(48°24'N, 122°5'W × 45°30'N, 122°40'W) × 43°03'N, 141°20'E	2005	♂
Skado[1]	T × M	S.724-116 × S.122-3	Sib1	(Washington USA x Oregon USA) x Japan	(48°24'N, 122°5'W × 45°30'N, 122°40'W) × 43°03'N, 141°20'E	2005	♀
Ellert[2]	D × N		Sib 2	Michigan USA x France	ca 42°N, 84°W × ca 48°N, 6°E	1969	♂
Hees[2]	D × N		Sib 2	Michigan USA x France	ca 42°N, 84°W × ca 48°N, 6°E	1969	♀
Koster[2]	D × N		Sib 2	Michigan USA x The Netherlands	ca 42°N, 84°W × 51°18'N, 3°43'E	1966	♂
Muur[1]	D × N	S.513-60 × S.132-4	Sib 3	(Iowa USA × Illinois USA) x (Italy x Belgium)	(ca 42°N, 93°W × ca 40°N, 88°W) × (45°8'N, 8°27'E × 50°46'N, 3°52'W)	1978	♂
Oudenberg[1]	D × N	S.513-60 × S.157-3	Sib 3	(Iowa USA × Illinois USA) x (Italy x Belgium)	(ca 42°N, 93°W × ca 40°N, 88°W) × (45°8'N, 8°27'E × 50°46'N, 3°52'W)	1978	♀
Robusta[3]	D × N		Sib 4	Eastern USA x Europe	unknown	1885-1890	♂
Vesten[1]	D × N	S.513-60 × S.157-4	Sib 3	(Iowa USA × Illinois USA) x (Italy x Belgium)	(ca 42°N, 93°W × ca 40°N, 88°W) × (45°8'N, 8°27'E × 50°46'N, 3°52'W)	1978	♀
Grimminge[1]	D × (T × D)	(S.333-44 × S.336-16) × S.910-1	Sib 5	(Michigan USA x Connecticut USA) x (Washington USA x (Iowa USA x Missouri USA))	(ca 42°N, 84°W × ca 41°N, 72°W) × (48°24'N, 122°5'W × (ca 42°N, 93°W × ca 38°N, 91°W))	1976	♂
Brandaris[2]	N × N		Sib 6	Brummen, The Netherlands x Italy	52°05'N, 6°09'E × 45°8'N, 8°27'E	1964	♂
Wolterson[2]	N		Sib 7	Doesburg, The Netherlands	51°59'N, 6°06'E	1960	♀

T= *Populus trichocarpa* Hook; M= *Populus maximowiczii* Henry; D= *Populus deltoides* March.; N= *Populus nigra* L.

[1] produced by the Institute for Nature and Forest Research (INBO, Geraardsbergen, Belgium); [2] produced by Vermeerderingstuinen Nederland (Zeewolde, The Netherlands); [3] genotype originating from an open-pollinated *P. deltoides* tree, first commercialized by the nursery Simon-Louis Frères (Metz, France); ca: circa

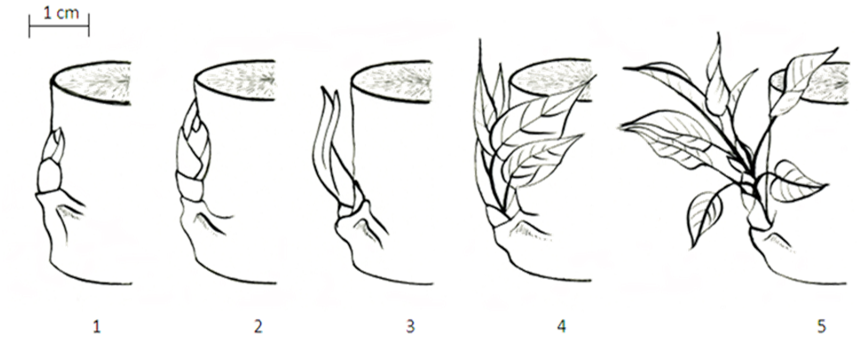
The plantation is established on land previously used for arable agricultural land or as extensively grazed pasture. Based on a detailed soil survey prior to planting, the soil was characterized as a sandy texture with a clay-enriched deeper soil layer (Broeckx et al. 2012a).

In April 2010 more than 110,000 unrooted hardwood cuttings (25 cm long) were planted in large monoclonal blocks, designed with a minimum of six and a maximum of ten double rows, on a total of 14.5 ha. The minimum number of replicated blocks per genotype was two and the maximum was four, with row lengths varying between 90 m and 340 m. Within the double rows, plants were on average 1.1 m apart, with alternating inter-row distances of 0.75 m and 1.5 m, corresponding to a tree density of 8,000 ha^{-1}. Intensive weed control (chemical and mechanical) was applied during the establishment rotation (first two years), but no fertilizer or irrigation has been applied since the planting. A more detailed description of the plantation and of the management regime has been previously reported (Broeckx et al. 2012a).

At the end of the second growing season – on 2-3 February 2012 – the entire plantation was coppiced at an average height of 15 cm, with commercial SRC harvesting machines (Berhongaray et al. 2013). After the harvest, regrowth of multiple shoots per stump occurred, in contrast to the single stem growth after planting.

2.2. Phenological assessments

Bud burst and bud set of each genotype were visually and intensively scored during the spring and autumn, before (year 2011) and after (year 2012) the coppice. The bud burst scoring system was based on those specifically developed for poplar by Turok et al. (1996) and Pellis et al. (2004b). The discrete scores of one to five are shown and described in Fig. 1. During the spring of 2011 (late February – mid-May) the gradual process of bud burst and leaf unfolding was quantified each week by assigning scores to eight plots of four trees of each genotype. In the spring of 2012 (early April – mid-May), one of the five scores was assigned to each monoclonal block on a weekly basis. Assessments of the early onset of bud burst in 2012 were made from daily webcam pictures collected automatically at the plantation, combined with detailed genotype-specific pictures from early spring. Phenological observations in spring 2011 were made on individual buds on the upper part of the main current-year axis, while in 2012 buds on the coppiced tree stumps were scored.



1 Dormant bud completely encapsulated by the bud scales; completely dry outlook and brown color

2 Buds sprouting, with a tip of the small leaves emerging out of the scales; leaves cannot be observed individually

3 Buds completely opened with leaves still clustered together; scales still present; leaves individually visible, but folded on it

4 Leaves diverging with their blades still rolled up; scales may be present or absent; folded leaves, starting to open; leaf venation visible

5 Leaves completely unfolded (but smaller in size than mature ones); lengthening of the axis of the shoot evident; scales absent. Development of leaves from the elongation of the lateral branch

Fig. 1 Description of the five discrete classes of the scoring system for bud burst in poplar. The specific drawing shown here is for genotype Robusta (*Populus deltoides* x *P. nigra*).

The autumn phenology, i.e. the end of tree height growth and of leaf production was assessed using a bud set scoring system based on that of Rohde et al. (2011b). Seven discrete scores were used as shown and described in Fig. 2. Analogous to bud burst, scores were assigned each week to eight plots of four trees of each genotype in the autumn of 2011 (late August – early October). In the autumn of 2012 (late August – mid-October) two plots of four trees were assigned to one of the seven scores on a weekly basis. For the bud set scoring we observed the apical bud in both years, i.e. on the main axis in 2011 and averaged over the resprouting shoots in 2012.

3	Apical shoot fully growing, > 2 rolled-up young leaves
2.5	Last leaves still rolled-up, last leaves at equal height, internodes become shorter
2	Shoot/internode growth reduced, second last leaf fully stretched, last leaf bright green
1.5	Transition from shoot to bud structure, color of second last leaf is comparable to older leaves, last leaf partially rolled
1	Apical bud not fully closed, bud scales predominantly green, no more rolled-up leaves
0.5	Apical bud fully closed, color between green and red, stipules of the two last leaves still green
0	Apical bud red-brown

Fig. 2 Description of the seven discrete classes of the scoring system for bud set in poplar.

A complete set of meteorological data has been continuously recorded from a station at the field site, from the beginning of June 2010 to the present day (Zona et al. 2013a). The growing degree-days (GDD, ^{o}C) were calculated by applying the equation GDD = $\sum(T_{av}-T_b)$, where T_{av} represents the daily average air temperature and T_b the base or threshold temperature (Baker et al. 1984). The starting date of the degree-day summation was 1 January (in line with Pellis et al. 2004b, Rousi and Pusenius 2005) and the T_b of 0 ^{o}C (GDD0), 2.5 °C (GDD2.5), 5 °C (GDD5) and 7.5 °C (GDD7.5) were chosen because of reported clinal differences in the base temperature for dormancy release (Myking and Heide 1995), given the wide geographic distribution of *Populus* species. Daily average minimum (T_{min}) and maximum (T_{max}) air temperatures were cumulated starting on 1 January for both 2011 and 2012, as well as the total incoming short wave radiation (SWR_{cum}; W m^{-2}). A SWR threshold of 20 W m^{-2} was used to differentiate between day and night temperatures (SWR < 20 W m^{-2} = night and SWR ≥ 20 W m^{-2} = day).

Cumulated daily average day (T_{day}) and night (T_{night}) temperatures were calculated as from 1 January 2011 and 2012.

2.3. Data analysis

Due to the absence of statistical replications for bud burst and the limited amount of replicates for bud set in 2012, statistical analyses were restricted to the 2011 data set, using plot averages as replicates. The average scores for each replicate were plotted as a function of time and fitted to a sigmoidal curve (Matlab R2012a, Mathworks, Natick, MA, USA). Based on these fitted equations the day of the year (DOY), SWR_{cum}, cumulated temperatures (T_{day}, T_{night}, T_{min}, T_{max}) and GDD (using different threshold temperatures) when each replicate reached full bud burst (= score 4) and full bud set (= score 1) were calculated. The length of the growing season was determined as the time between bud burst score 4 and bud set score 1 for the above mentioned variables. Due to the considerable uncertainty on the latitude of origin for several genotypes, the experimental design could not provide sufficient statistical power to detect significant differences in bud phenology among ecotypes. Latitude of origin was therefore not included in further analysis. We performed an analysis of variance (ANOVA) to examine the effects of parentage group, sibling group and genotype on the timing of bud burst and of bud set, and on the length of the growing season. Genotypic averages were considered significantly different when the adjusted *p* value of the ANOVA test was < 0.05. The Tukey's studentized range test (HSD) was used as a post-hoc test in case of significant parentage group or genotype effects. All statistical analyses were performed using the SPSS (SPSS Inc., Chicago, IL, USA) software.

Both years of observation were compared in terms of the timing of bud phenology by plotting spring and autumn bud development for both 2011 and 2012 as a function of time: (i) the evolution of bud burst and bud set with GDD0 was plotted for each parentage group for both 2011 and 2012; (ii) a plantation average bud development was plotted as a function of DOY, of SWR_{cum} and of GDD using different threshold temperatures; (iii) plantation average bud development was examined in terms of T_{max} versus T_{min} on the one hand and T_{day} versus T_{night} on the other hand, for both 2011 and 2012 (i.e. before and after coppice).

3. Results

Table 2 shows the DOY, SWR_{cum} as well as the GDD0 with T_b = 0 °C, on which the four parentage groups, the seven sibling groups and the 12 individual genotypes reached full bud burst (score 4) and full bud set (score 1). The length of the growing season is also shown for the same variables. Genotypic ranking did not change between different thresholds of GDD; only minor differences in significance levels of variation among genotypes and siblings were observed. Therefore, only the results of the GDD0 analysis were presented in Table 2. In 2011, significant differences in the timing of bud burst and bud set were observed among parentage groups and among genotypes for the DOY, for SWR_{cum} and for the GDD0, as well as in the growing season length (Table 2). The T x M parentage group reached full bud burst significantly earlier (26 March) – expressed in DOY (85), in SWR_{cum} ($0.2*10^6$ W m^{-2}) and in GDD0 (472 °C) – as compared to the other parentage groups. In addition, the T x M hybrids reached full bud set – i.e. had their buds completely closed – significantly later than the other parentage groups. Consequently, the T x M genotypes showed the longest growing season (195 days, $1.9*10^6$ W m^{-2} and 2944 GDD0) (Table 2 and Fig. 3). The D x (T x D) backcross group completed bud burst latest in the year, i.e. on 21 April (DOY 111, $0.5*10^6$ W m^{-2} and 804 GDD0) and it was the earliest to end growth on 18 September – for DOY (261), SWR_{cum} ($1.8*10^6$ W m^{-2}) and for GDD0 (3201) – resulting in the shortest growing season (150 days, $1.3*10^6$ W m^{-2} and 2398 GDD0). Hence, a wide range of 27 days, $0.2*10^6$ W m^{-2} and 333 GDD0, and a considerable difference of 13 days, $0.09*10^6$ W m^{-2} and 215 GDD0 were observed in the timing of bud burst and bud set, respectively, among parentage group averages. This corresponded to a difference of 40 days, $0.3*10^6$ W m^{-2} and 548 GDD0 in the length of the growing season.

The parentage group D x N, and the pure N species, showed a similar pattern of bud phenology (Table 2 and Fig. 3) in 2011. Both the D x N and N groups reached full bud burst on 11 April (DOY 101 and $0.4*10^6$ W m^{-2}). Bud set was completed on 19 September (D x N) and 20 September (N) (DOY 262 and 263, respectively corresponding to $1.8*10^6$ W m^{-2}). This corresponded to a similar growing season length for both groups (160 and 162 days, respectively). Similarly small differences were observed in the SWR_{cum} and GDD0 necessary for bud burst ($0.0004*10^6$ W m^{-2} and 1 GDD0 difference respectively) and bud set ($0.008*10^6$ W m^{-2} and 18 GDD0 difference respectively) with non-significant $0.008*10^6$ W m^{-2} and 20 GDD0 differences in the length of the growing season between parentage groups D x N and N.

In analogy with the parentage group ranking, Skado (on 22 March or DOY 81, 438 GDD0 and $0.22*10^6$ W m^{-2}) and Bakan (on 29 March or DOY 88, 506 GDD0 and $0.28*10^6$ W m^{-2}) were the first two genotypes to reach full bud burst in spring 2011. The latest genotype was Grimminge on 21 April or DOY 111, 804 GDD0 and $0.48*10^6$ W m^{-2}. Although the full-sibs Bakan and Skado are very closely related (Table 1), their time for bud burst was significantly different in number of days (7 days difference), as well as in GDD0 (68 GDD0 difference) and in SWR_{cum} ($0.06*10^6$ W m^{-2}). Both genotypes, Bakan and Skado, required significantly less heat forcing and incoming radiation for bud burst than the other genotypes, as indicated by the significant difference in GDD0 and SWR_{cum} with the other genotypes. Some less related genotypes within the parentage group D x N, reached full bud burst within a smaller time span, with a range of only 11 and 10 days (129 and 117 GDD0) between the earliest (Robusta and Muur) and the latest (Koster) genotypes, respectively (Table 2). Within the D x N parentage group, sibling groups 2 and 3 (Table 2) showed smaller coefficients of variation in DOY, GDD0 and SWR_{cum} in comparison to the overall D x N parentage group.

Table 2 Time of bud burst (phenology score 4), and of bud set (phenology score 1) as well as the length of the growing season of twelve poplar (*Populus* spp.) genotypes belonging to four parentage groups and 7 sibling groups, during the second year after planting (before coppice, 2011). Bud burst, bud set and length of the growing season are expressed as day of the year (DOY), SWR_{cum} (W m^{-2}) and as growing degree-day (GDD0, ^{o}C). Numbers between brackets represent coefficients of variation.

		Bud burst			Bud set			Growing season length		
		DOY	SWR_{cum}	GDD0	DOY	SWR_{cum}	GDD0	DOY	SWR_{cum}	GDD0
Genotype	Bakan	88 (0.8)^{b}	283541 (2.0)^{b}	506 (2.1)^{b}	274 (1.3)^{ef}	1917938 (1.3)^{f}	3408 (1.7)^{e}	186 (2.0)^{f}	1634397 (1.6)^{d}	2901 (2.1)^{e}
	Brandaris	99 (0.9)^{d}	362887 (2.3)^{d}	642 (1.5)^{d}	258 (2.2)^{ab}	1814059 (1.8)^{ab}	3151 (2.7)^{ab}	159 (3.5)^{cd}	1451172 (2.3)^{b}	2509 (3.3)^{abc}
	Ellert	101 (1.3)^{de}	382377 (3.6)^{e}	665 (2.5)^{de}	259 (1.7)^{bc}	1822029 (1.5)^{bc}	3177 (2.0)^{bc}	159 (3.6)^{bcd}	1439652 (2.8)^{b}	2511 (3.1)^{abc}
	Grimminge	111 (0.6)^{i}	483352 (2.1)^{h}	805 (2.1)^{h}	261 (1.9)^{bcd}	1832436 (1.6)^{bcd}	3201 (2.2)^{bcd}	150 (3.3)^{a}	1349084 (2.5)^{a}	2396 (3.1)^{a}
	Hees	102 (0.6)^{ef}	389968 (1.7)^{e}	675 (1.0)^{e}	267 (0.2)^{d}	1868963 (0.3)^{de}	3287 (0.3)^{d}	165 (0.6)^{de}	1478995 (0.6)^{bc}	2612 (0.5)^{cd}
	Koster	107 (1.6)^{h}	432368 (4.0)^{g}	731 (3.2)^{g}	258 (2.5)^{ab}	1814840 (2.0)^{ab}	3152 (3.2)^{ab}	151 (5.1)^{ab}	1382473 (3.6)^{a}	2421 (4.8)^{a}
	Muur	97 (1.4)^{c}	338678 (4.0)^{c}	614 (3.0)^{c}	266 (0.6)^{cd}	1858218 (0.6)^{cde}	3263 (0.8)^{cd}	169 (1.6)^{e}	1519540 (1.4)^{c}	2650 (1.5)^{d}
	Oudenberg	102 (2.1)^{ef}	393335 (4.7)^{ef}	679 (3.3)^{ef}	266 (1.0)^{cd}	1864261 (0.9)^{de}	3274 (1.2)^{cd}	164 (2.5)^{de}	1470926 (2.0)^{bc}	2595 (2.1)^{cd}
	Robusta	96 (0.7)^{c}	329464 (2.6)^{c}	602 (2.2)^{c}	251 (2.7)^{a}	1775907 (2.4)^{a}	3051 (3.8)^{a}	156 (4.8)^{abc}	1446443 (3.5)^{b}	2448 (5.1)^{ab}
	Skado	81 (1.6)^{a}	222413 (5.3)^{a}	438 (2.6)^{a}	275 (0.9)^{f}	1924177 (0.9)^{f}	3425 (1.3)^{e}	194 (1.6)^{g}	1701763 (1.4)^{e}	2987 (1.7)^{e}
	Vesten	104 (0.6)^{g}	410393 (1.7)^{f}	700 (1.4)^{f}	265 (0.5)^{cd}	1853378 (0.3)^{bcde}	3253 (0.5)^{bcd}	160 (1.0)^{cd}	1442985 (0.7)^{b}	2553 (0.9)^{bcd}
	Wolterson	103 (0.8)^{fg}	400939 (1.2)^{ef}	687 (1.0)^{ef}	268 (0.5)^{de}	1875111 (0.4)^{e}	3301 (0.6)^{d}	165 (1.0)^{de}	1474173 (0.6)^{bc}	2614 (0.7)^{cd}
Parentage group	DxN	101 (3.9)^{b}	382369 (9.6)^{b}	667 (6.8)^{b}	262 (2.5)^{a}	1836799 (2.2)^{a}	3208 (3.2)^{a}	161 (4.6)^{b}	1454430 (3.5)^{b}	2541 (4.2)^{b}
	Dx(TxD)	111 (0.6)^{c}	483352 (2.1)^{c}	805 (2.1)^{c}	261 (1.9)^{a}	1832436 (1.6)^{a}	3201 (2.2)^{a}	150 (3.3)^{a}	1349084 (2.5)^{a}	2396 (3.1)^{a}
	N	101 (2.2)^{b}	381913 (5.4)^{b}	665 (3.7)^{b}	263 (2.5)^{a}	1844585 (2.1)^{a}	3226 (3.0)^{a}	162 (3.1)^{b}	1462672 (1.8)^{b}	2561 (3.1)^{b}
	TxM	85 (4.5)^{a}	252977 (13.0)^{a}	472 (7.8)^{a}	275 (1.1)^{b}	1921057 (1.1)^{b}	3416 (1.5)^{b}	190 (2.8)^{c}	1668080 (2.5)^{c}	2944 (2.4)^{c}
Sibling group	sib1	85 (4.5)^{a}	252977 (13.0)^{a}	472 (7.8)^{a}	275 (1.1)^{e}	1921057 (1.1)^{e}	3416 (1.5)^{e}	190 (2.8)^{d}	1668080 (2.5)^{c}	2944 (2.4)^{d}
	sib2	103 (2.9)^{d}	401571 (6.4)^{d}	690 (4.9)^{d}	261 (2.3)^{bc}	1835277 (1.9)^{bc}	3205 (2.8)^{bc}	158 (5.0)^{bc}	1433706 (3.8)^{b}	2515 (4.4)^{bc}
	sib3	101 (3.6)^{cd}	380802 (8.9)^{cd}	664 (6.2)^{cd}	265 (0.7)^{cd}	1858619 (0.7)^{cd}	3263 (0.9)^{cd}	164 (2.8)^{c}	1477817 (2.6)^{b}	2599 (2.1)^{c}
	sib4	96 (0.7)^{b}	329464 (2.6)^{b}	602 (2.2)^{b}	251 (2.7)^{a}	1775907 (2.4)^{a}	3051 (3.8)^{a}	156 (4.8)^{ab}	1446443 (3.5)^{b}	2448 (5.1)^{ab}
	sib5	111 (0.6)^{e}	483352 (2.1)^{e}	805 (2.1)^{e}	261 (1.9)^{bc}	1832436 (1.6)^{bc}	3201 (2.2)^{bc}	150 (3.3)^{a}	1349084 (2.5)^{a}	2396 (3.1)^{a}
	sib6	99 (0.9)^{bc}	362887 (2.3)^{bc}	642 (1.5)^{bc}	258 (2.2)^{b}	1814059 (1.8)^{b}	3151 (2.7)^{b}	159 (3.5)^{bc}	1451172 (2.3)^{b}	2509 (3.3)^{bc}
	sib7	103 (0.8)^{d}	400939 (1.2)^{d}	687 (1.0)^{d}	268 (0.5)^{d}	1875111 (0.4)^{d}	3301 (0.6)^{d}	165 (1.0)^{c}	1474173 (0.6)^{b}	2614 (0.7)^{c}
	OVERALL	99 (8.0)	369143 (18.2)	645 (14.7)	264 (2.9)	1851776 (2.6)	3245 (3.7)	165 (8.1)	1482633 (6.7)	2600 (7.2)

T= *Populus trichocarpa* Hook*;* M= *P. maximowiczii* Henry*;* D= *P. deltoides* March.*;* N= *P. nigra* L. a - i homogeneous subsets, groups which do not share the same letter within each column are significantly ($P < 0.05$) different, separately for parentage groups and for genotypes.

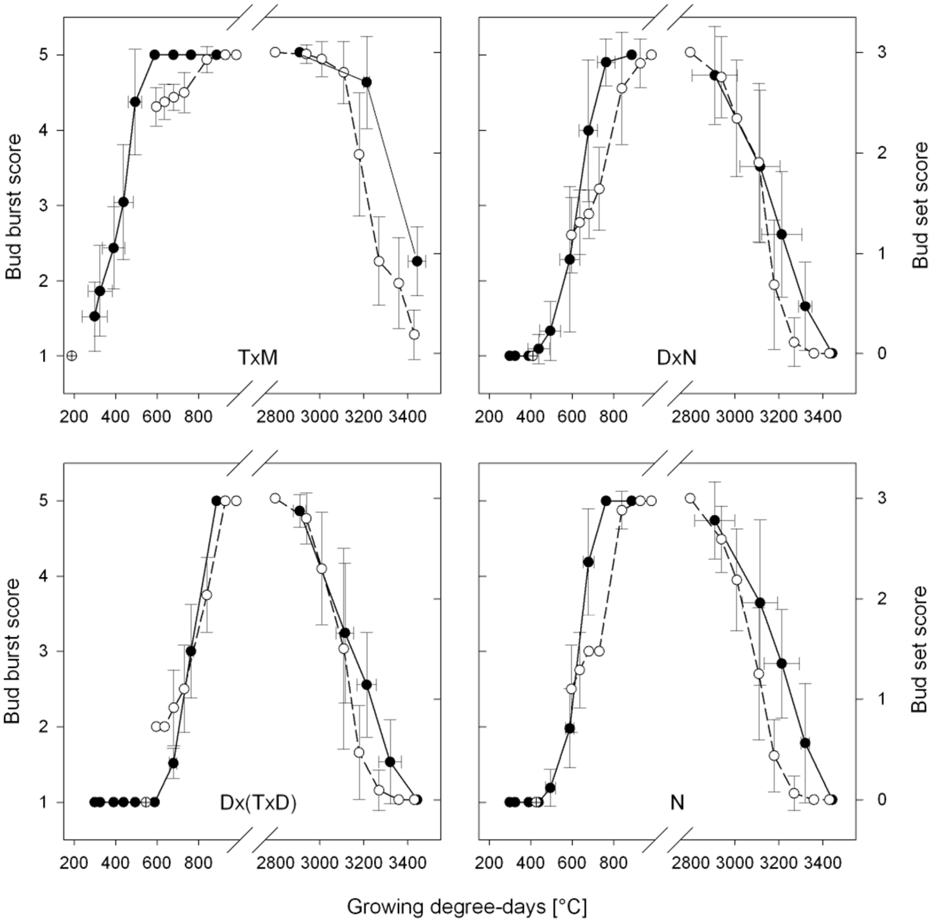


Fig. 3 Time course of spring phenology (bud burst; left axis) and autumn phenology (bud set; right axis), expressed in terms of five and seven discrete scores, respectively, reached at a specific growing degree-day (^{o}C), for the four parentage groups of the 12 poplar genotypes, before (2011 – black circles) and after (2012 – white circles) coppice. The open white circles with a black cross indicate the beginning of bud burst in 2012, estimated from webcam pictures taken in the plantation. The thin horizontal and vertical lines represent the standard deviations of the means. T= *Populus trichocarpa;* M= *P. maximowiczii;* D= *P. deltoides;* N= *P. nigra*.

Brandaris, Ellert, Hees, Oudenberg, Wolterson and Vesten all reached full bud burst within a short time period, i.e. between 9 April (Brandaris; DOY 99) and 14 April (Vesten; DOY 104) and between 642 GDD0 ($0.36*10^{6}$ W m^{-2}) and 700 GDD0

($0.41*10^6$ W m^{-2}), although these genotypes do not all belong to the same parentage group (Table 1). A significant difference in timing of bud burst was found between Brandaris and Wolterson, which belong to the same parentage group N. Brandaris reached full bud burst four days earlier as compared to Wolterson. Genotype Robusta was the first to finish the growing season (8 September or DOY 251), but due to an early bud burst (DOY 96) the length of its growing season (156 days) did not differ significantly from the length of the growing season of most other genotypes.

As for bud burst, some genotypes representing different parentage groups reached full bud set within a short time span. For example, no significant difference in the timing of bud set was found among genotypes Ellert, Vesten and Grimminge, ranging from 16 September to 22 September (Table 2). There was no variation in the bud set timing between Hees (24 September), Muur (22 September) and Oudenberg (23 September), all three belonging to the D x N parentage group (Table 2). Also for bud set, the variation within sibling groups 2 and 3 was smaller as compared to the overall D x N parentage group for DOY, GDD0 and SWR_{cum} (Table 2). A significant difference in the timing of bud set was found between Brandaris and Wolterson, both N genotypes: Brandaris reached full bud set ten days earlier than Wolterson (DOY 258 versus DOY 268, respectively). Combined with the earlier bud burst of Brandaris as compared to Wolterson, no significant difference was found in the growing season length between both genotypes. Genotypes Bakan and Skado were the latest to finish the growing season; both genotypes had all their buds closed on 1 and 2 October (DOY 274 and 275), respectively. The length of the growing season of Bakan (186 days) and Skado (194 days) was the longest compared with the other genotypes that ranged from 151 days (Koster) to 169 days (Muur).

We observed a smaller time span in bud phenology between the first and the last genotype for bud set (23 days) than for bud burst (30 days). Similarly, a smaller range of SWR_{cum} between the first and last genotype was observed for bud set ($1.5*10^6$ W m^{-2}) in comparison to bud burst ($2.8*10^6$ W m^{-2}). However, there was no substantial variation in GDD0 and cumulated temperature between the first and the last genotypes in timing of bud set as compared to bud burst. Overall, coefficients of variation were smaller for bud set as compared to bud burst for all parameters examined (Table 1).

Parentage group ranking in timing of bud burst and bud set was similar in 2011 and 2012, i.e. before and after the coppice (Fig. 3). The bud burst process started at the same time in both years, both for DOY and GDD0 (Figs. 3 & 4). In 2012 we observed a decreased bud development rate in April, indicated by a deviation from the sigmoidal course of bud burst (Fig. 4). This effect was most pronounced for the T x M parentage group (Fig. 3). Compared to April 2011, April 2012 was characterized by lower temperatures and lower incoming solar radiation (Fig. 4). Hence, full bud burst was delayed in the year after coppice in terms of DOY (Fig. 4). Although the bud set process started at the same time before and after the coppice, a higher rate of bud closure in 2012 was indicated by a faster decrease of the bud set curves in terms of GDD0 (Figs. 3 & 5). Considering the parentage groups, fewer growing degree-days were required to reach full bud set in 2012 compared to 2011 (Fig. 3). Lower temperatures and incoming solar radiation were recorded in the second half of September in 2012 as compared to 2011. However, a very similar DOY and SWR_{cum} time course of bud set was observed between both years of observation (Fig. 4).

Fig. 5 compares the effect of using different base temperatures for the timing of full bud burst and full bud set. A higher number of GDD, both above 0 °C and 2.5 °C, was needed to reach full bud burst in 2012 as compared to 2011. A closer correspondence between both years was found with a base temperature of 5 °C. When a threshold of 7.5 °C was used, less GDD were required to reach full bud burst in 2012 in comparison to 2011. As for bud set, no difference in correspondence between both years was found using base temperatures of 0 °C or 2.5 °C. An increasingly lower number of GDD to reach full bud set was found in 2012 when increasing the threshold to 5 °C and 7.5 °C.

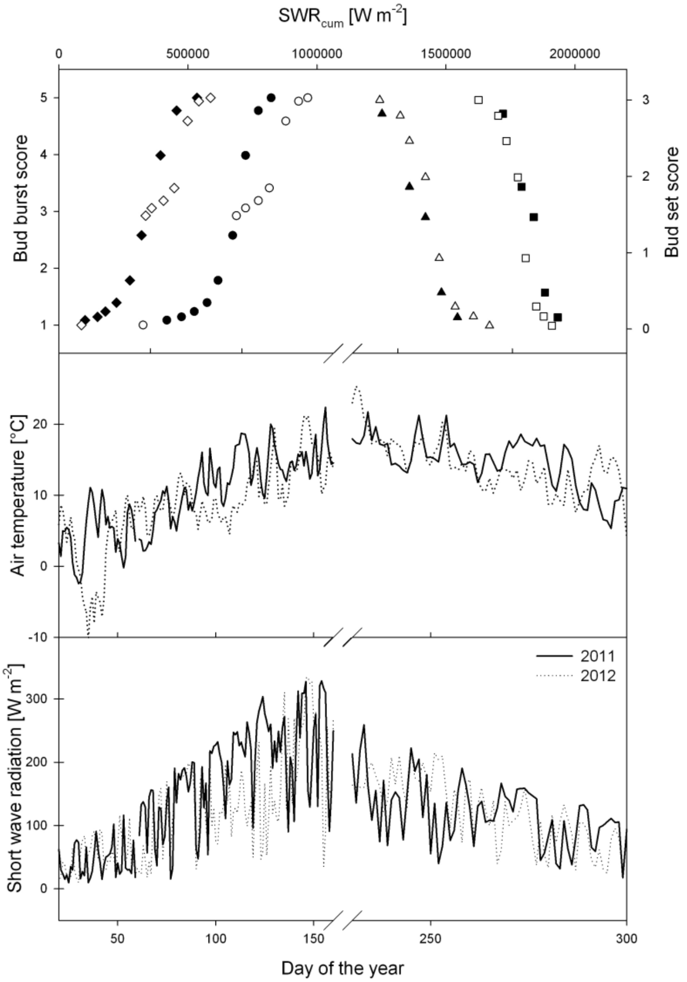


Fig. 4 Top panel: Time course of spring phenology (bud burst) in terms of day of the year (circles) and cumulated short-wave radiation (SWR$_{cum}$; diamonds) and autumn phenology (bud set), in terms of day of the year (triangles) and SWR$_{cum}$ (squares) in two consecutive years of observation, 2011 (black symbols) and 2012 (white symbols). An overall average score for the entire plantation of all poplar genotypes is shown. Middle panel: Daily average air temperature (^{o}C) measured from the meteorological mast at the field site before (2011) and after coppice (2012). Bottom panel: Solar radiation measured from the mast at the field site before (2011) and after coppice (2012).

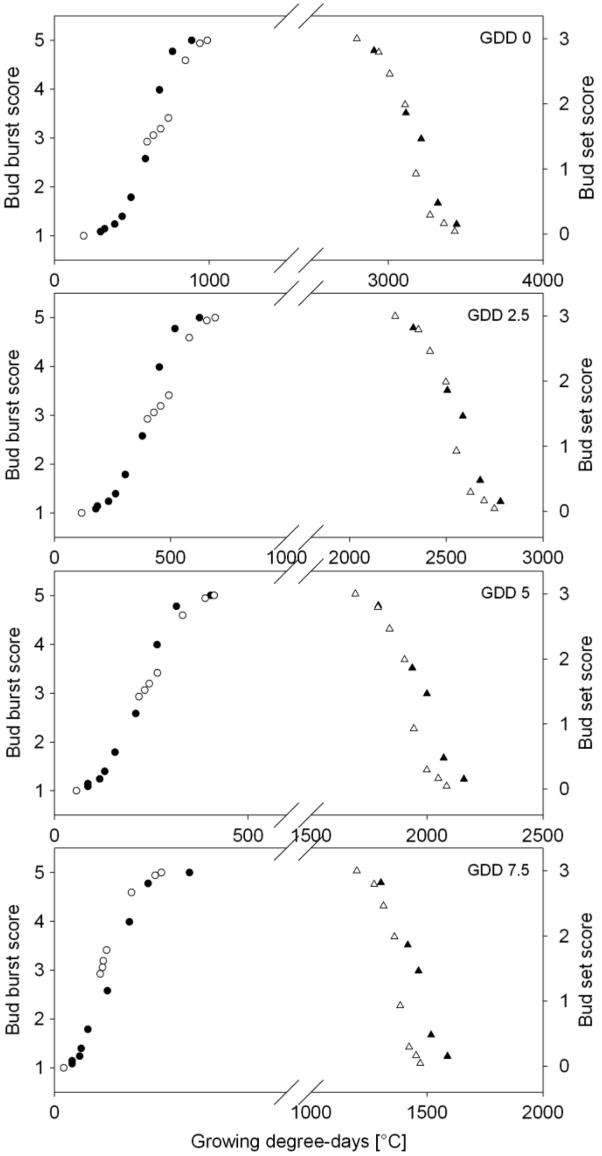


Fig. 5 Time of bud burst (circles) and bud set (triangles) of 12 poplar (*Populus* spp.) genotypes in two consecutive years of observation, 2011 (black symbols) and 2012 (white symbols), using different threshold temperatures of growing degree days, 0 °C (GDD0), 2.5 °C (GDD2.5), 5 °C (GDD5) and 7.5 °C (GDD7.5).

Development of spring phenology highly corresponded between both years when expressed in function of cumulated temperature (Fig. 6). Similar results were found for T_{max} versus T_{min} and for T_{day} versus T_{night}. In contrast, a higher correspondence in the time course of autumn phenology was observed in terms of T_{min} and T_{night} as compared to T_{max} and T_{day} (Fig. 6).

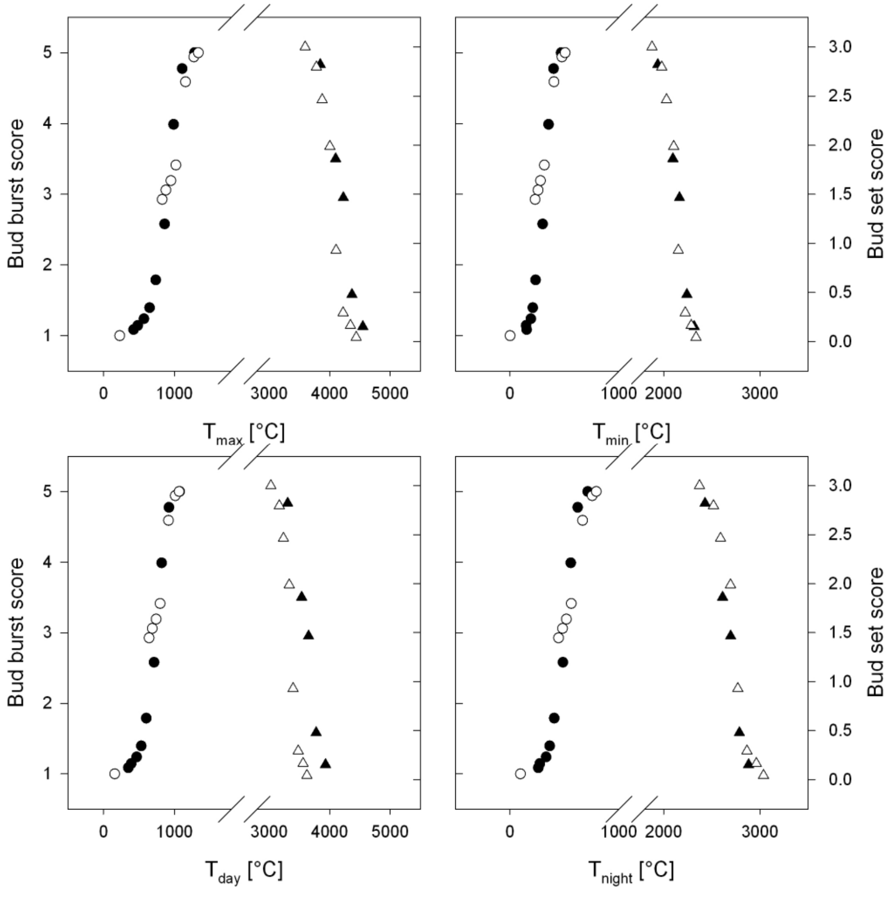


Fig. 6 Time of bud burst (circles) and bud set (triangles) as a function of cumulated maximum (T_{max}) and minimum (T_{min}) temperatures (top graphs) and of cumulated day (T_{day}) and night (T_{night}) temperatures (bottom graphs). Correspondence between two consecutive years of observation were examined, 2011 (black symbols) and 2012 (white symbols).

4. Discussion

4.1. Genotypic differences

We observed significant differences among parentage groups, sibling groups and among genotypes in different bud phenological traits (bud burst, bud set and length of the growing season) in line with earlier studies on poplar and willow (Ceulemans et al. 1992, Weih 2009, Pellis et al. 2004b). These observations also reaffirmed the genetic control of phenological traits in poplar (Pauley and Perry 1954, Howe et al. 2003). Smaller variation within sibling groups for both bud burst and bud set indicated at least partial heritability of phenological traits (Marron et al. 2010). Since for bud set the time span between the earliest and the latest genotype (24 days) was smaller as compared to bud burst (30 days), we infer that genotypic variability in bud set was smaller than in bud burst. This was also evidenced by the higher overall variation in bud burst as compared to bud set. This was in line with previous findings on poplar (Farmer 1993, Pellis et al. 2004b), but in contrast with other forest trees (Howe et al. 2003) which showed higher genetic variation for bud set compared to bud burst. This finding suggested that the environmental control was more important for autumn phenology in comparison to spring phenology (Aitken and Adams 1996).

Overall, the timing of bud burst in 2011 fell within the time range of bud burst observed by Pellis et al. (2004b) in a poplar SRC plantation in non-coppice years (DOY 85-111), despite differences in parentage groups. In this study, D x N genotypes reached full bud burst earlier when compared to the findings of Pellis et al. (2004b), who analysed the same hybrid combinations, although different genotypes. An earlier bud burst was observed for genotype Wolterson in our study as compared to that of Pellis et al. (2004b). These differences might be explained not only by the year-to-year variations in weather conditions between both studies, but also by the differences in tree age and environmental conditions.

Earlier starters ended the growing season later, and thus had a longer growing season length. This characteristic enables the plants to use the favourable spring and autumn conditions more efficiently, producing a positive effect on productivity (Weih 2009). The opposite was also observed, i.e. later starters ended the growing season earlier, and thus had a shorter growing season length, as observed in other studies (Yu et al. 2001). This might be a strategy to avoid the risk of frost damage in early spring and late autumn (Weih 2009).

4.2. Main factors influencing bud burst and bud set

Previous findings suggest that air temperature is the main factor controlling bud burst (Pauley and Perry 1954, Pellis et al 2004b, Rohde et al. 2011a), either through the chilling requirement or through the heat forcing (Pauley and Perry 1954, Menzel 2002, Heide 2003, Gould et al. 2012). The higher correspondence between both years for cumulated temperature in comparison to GDD, suggested the importance of low (chilling) temperatures to release dormancy (Junttila and Hänninen 2012). Comparing different base temperatures, the physiological threshold for bud burst, i.e. plant growth, was found between 5 °C and 7.5°C when averaged over all genotypes. As suggested earlier (Häkkinen et al. 1998), incoming radiation probably played an important role as well; the incoming solar radiation in the early spring was lower in 2012 compared to 2011. The higher correspondence among both years in the time of bud burst when corrected for incoming SWR as compared to DOY emphasized this assumption. Although for poplar, it has been reported that neither light nor its periodicity directly influence the break of dormancy (Pauley and Perry 1954).

The closeness in DOY and incoming SWR between both years of observation confirms our hypothesis that photoperiod is the main driver for bud set (Pauley and Perry 1954, Howe et al. 2003, Pellis et al. 2004b, Luquez et al. 2008, Weih 2009, Rodhe et al. 2012). But our results indicated that air temperature also plays a role, confirming the previous report by Rohde et al. (2011a). In our study the 2012 bud set date (average of all genotypes) coincided with the 2011 bud set both in terms GDD0 and GDD2.5. However, slightly less heat forcing, i.e. growing degree-days, was needed for the process of bud set in 2012 compared to 2011, as explained by the lower temperatures during bud set in 2012 as compared to 2011. These results indicated at least a partial contribution of air temperature to bud set, with a lower physiological threshold as compared to bud burst.

Cumulated temperature was a better predictor of both the time of bud burst and bud set, in comparison to GDD above a certain threshold temperature. Maximum versus minimum temperatures similarly affected the time of bud burst, as well as day versus night temperatures. In contrast, minimum temperatures tended to be more decisive for the time of bud set in comparison to maximum temperature although the difference was small. Our results suggested a stronger dependence of the time of bud set on night temperature as compared to day temperature. A more important effect of night temperature on terminal dormancy, interacting with day length, was previously reported in *Betula* spp. (Junttila 1980, Downs and Bevington 1981).

4.3. Year-to-year variation

No studies have specifically examined the effect of coppicing on phenological traits. One might assume that coppicing has an influence on phenology due to the changed root/shoot ratio or to an altered phytohormone regulation and carbon metabolism (Tschaplinski and Blake 1995). Year-to-year variation in this study was the result of an interacting effect of varying weather and growth conditions, and of management issues (i.e. non-coppiced versus coppiced). The variation could be largely explained by correcting for temperature and light conditions, suggesting a high contribution of weather conditions to the year-to-year differences in the observations. However, due to the interaction between year and management, an effect of coppice could not be excluded.

In our study the onset of the bud burst process before and after coppice was very similar, apart from the delay in April of the growing season after coppice. Pellis et al. (2004b) observed that full bud burst was delayed after coppice compared to the timing of bud burst in the following growing seasons, suggesting an effect of coppicing on spring phenology. Concerning bud set, the timing was very similar before and after coppice. The DOY of the start and end of the bud set process were really close. No evidence was found that coppicing affects bud cessation (Pellis et al. 2004b).

5. Conclusion

We concluded that: (i) there is significant variation in the bud phenology among the studied poplar parentage groups, sibling groups and genotypes, confirming genetic control and potential for future selection of locally adapted genotypes; (ii) bud burst was driven mainly by air temperature, showing an influence of incoming solar radiation also; (iii) bud set is driven mainly by photoperiod but also to a lesser extent by (minimum and night) air temperature, and (iv) year-to-year variation in the time of bud burst and bud set was related to variation in weather conditions but the experimental design did not allow to exclude an effect of coppicing on bud phenological traits of the studied poplar genotypes.

Acknowledgements

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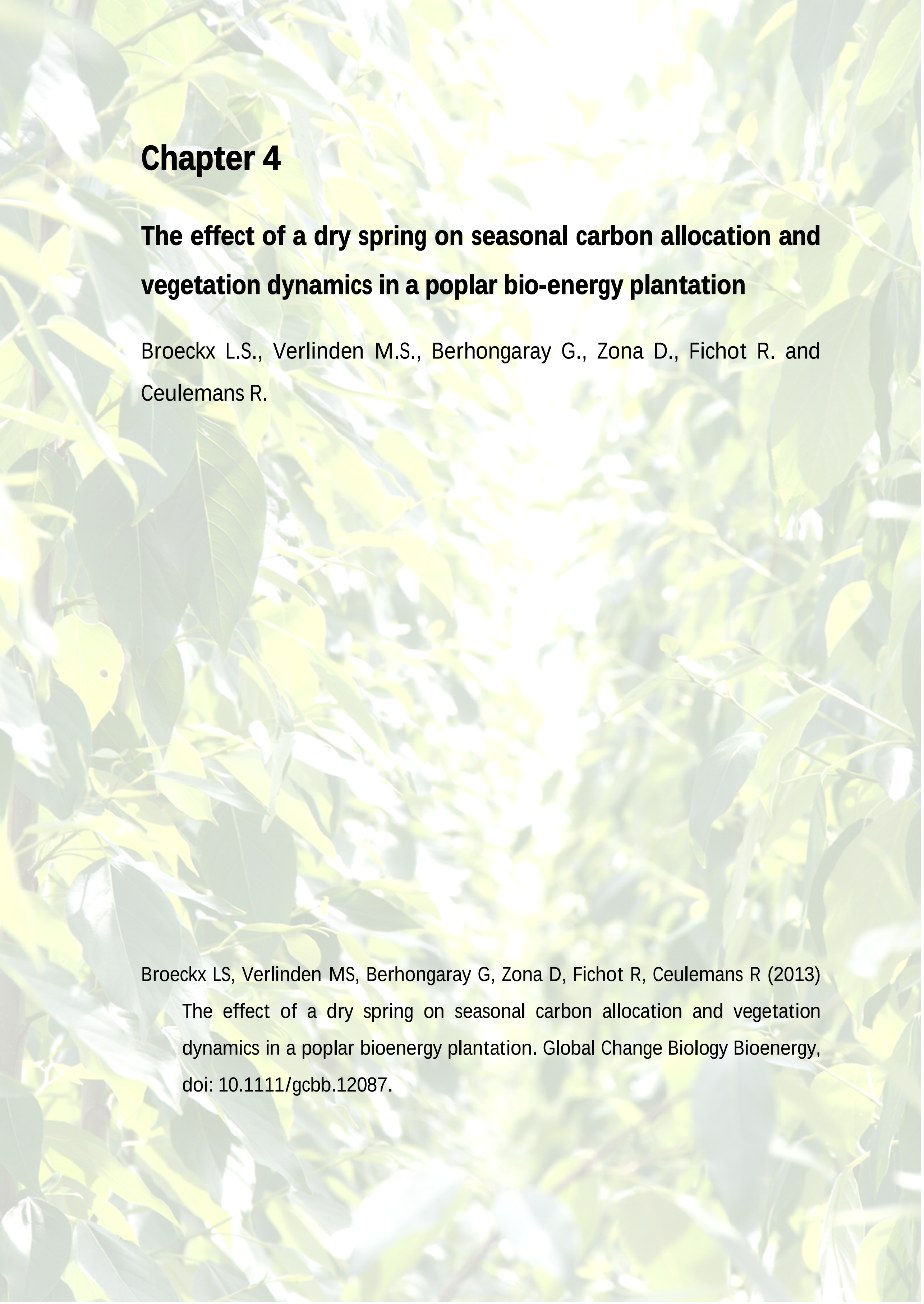

Chapter 4

The effect of a dry spring on seasonal carbon allocation and vegetation dynamics in a poplar bio-energy plantation

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Abstract

In this study the seasonal variation in carbon, water and energy fluxes as well as in net primary productivity (NPP) of different tree components is presented for a two-year-old poplar (*Populus* spp.) plantation. A thorough ecophysiological study was performed at ecosystem scale, at tree and at leaf level, in this high-density bio-energy plantation. Seasonal variation in NPP and fluxes was analyzed in relation to meteorological parameters at the field site. The growing season length in terms of carbon uptake was controlled by leaf area development until the maximum leaf area index (LAI_{max}) was reached. Afterwards, a shift to below-ground carbon allocation was observed. A dry period in spring caused a reduced leaf area production as well as a decrease in net ecosystem exchange (NEE) and gross primary production (GPP) due to stomatal closure. Water-use efficiency (WUE) and fine root growth increased in response to limiting soil water availability in the root zone. When soil water availability was not limiting, GPP was controlled by a decrease in solar radiation and air temperature. The results of this study indicate that the productivity of recently established bio-energy plantations with fast-growing trees is very sensitive to drought. The interaction between soil water availability and factors controlling ecosystem GPP is crucial in assessing the CO_2 mitigation potential under future climate conditions.

Keywords

Populus spp., intra-annual variability, net ecosystem exchange, net primary productivity, growing season length, soil water availability, water-use efficiency, short-rotation coppice

1. Introduction

Forest ecosystems play a crucial role in the global carbon cycle (Kauppi et al. 1992, Birdsey et al. 1993, Grace 2004), in particular by mitigating the increasing anthropogenic CO_2-emissions (Kauppi et al. 1993, Bonan 2008). In response to an increasing global energy demand this mitigation potential is applied by increasing the use of biomass from forestry for bio-energy (Burger 2009, Aguilar et al. 2011). In this context, Europe has committed in the development of short-rotation coppice cultures (SRC) of fast-growing tree species, as poplar (*Populus* spp.) and willow (*Salix* spp.) (Kauter et al. 2003, Aylott et al. 2008, AEBIOM 2011). It is therefore important to assess the efficiency of these bio-energy cultures – an intermediate between forestry and agronomy – to sequester carbon, and hence to mitigate climate change.

Productivity of poplar and willow SRC is largely dependent on the environment in terms of soil characteristics and climatic conditions (Hofmann-Schielle et al. 1999, Crow and Houston 2004, Dillen et al. 2007, Hillier et al. 2009). Environmental stresses as drought, heat or pests can strongly affect the woody biomass production and hence the carbon mitigation potential of bio-energy plantations. Both the intensity and the frequency of extreme climatic events are predicted to increase under future climate conditions (Easterling et al. 2000, Meehl and Tebaldi 2004, Beniston et al. 2007), with a projected dryer summer climate in central-western Europe (Schär et al. 2004, Seneviratne et al. 2006). Fast growing trees, such as poplars and willows, are especially sensitive to and might be possibly threatened by these climatic extremes (Migliavacca et al. 2009). Their leaf and stem growth decrease in response to water deficit (Lindroth et al. 1994, Monclus et al. 2009).

A better understanding of the interaction among environmental conditions, ecosystem responses and ecophysiological characteristics controlling tree growth is required to optimize the wood production of high-density SRC under projected climatic conditions (Calfapietra et al. 2010). Increased drought due to climate change is predicted to reduce energy production and carbon mitigation for perennial energy crops, such as *Miscanthus x giganteus* and *Phalaris arundinacea* (Hastings et al. 2009, Zhou et al. 2011). On the other hand, drought tolerance improved under elevated atmospheric CO_2 concentrations in a number of lignocellulosic bio-energy crops (Oliver et al. 2009). Hence, the study of carbon allocation patterns to different tree components throughout the growing season in relation to changing environmental conditions remains relevant (Kozlowski 1992, Barbaroux et al. 2003, Campioli et al. 2011).

With the long-term goal of assessing whether SRC are efficient in mitigating climate change, an experimental SRC with poplar (POPFULL: http://webh01.ua.ac.be/popfull/) is being intensively studied in Flanders (Belgium). The objective of the present study is to analyze the seasonal variations in net ecosystem exchange (NEE), photosynthesis and carbon allocation between organs in relation to environmental parameters in this type of high-density plantations. An atypical dry spring to summer period during the year of investigation (2011) provided the opportunity to examine the effect of drought on ecosystem dynamics. Given the increasing risk of summer droughts, ecosystem water-use is gaining interest in climate change research. Divergent results have been reported in studies examining the controlling factors of ecosystem water-use efficiency (WUE) (Krishnan et al. 2006, Reichstein et al. 2007a, Yang et al. 2010). In this context, environmental control of the plantation WUE was studied throughout the second growing season after planting. Because poplar is known for its vulnerability to drought (Rood et al. 2000, Marron et al. 2003, Larchevêque et al. 2011), we hypothesized that plantation productivity would decrease during the dry period (Souch and Stephens 1998). An increased WUE (Monclus et al. 2006, Chamaillard et al. 2011) and root-shoot ratio were expected in response to dry conditions (Yin et al. 2005, Guo et al. 2010). Therefore, an in depth data collection and analysis were performed using an integration of different hierarchical scales: leaf level, tree level and ecosystem scale.

2. Materials and Methods

2.1. Study site

The study was carried out within an operationally managed bio-energy plantation of poplars, established in April 2010 in Lochristi, East-Flanders, Belgium (51°06'44" N, 3°51'02" E; 6.25 m above sea level). The total area of the site is 18.4 ha of former agricultural land consisting of cropland (65% of the surface area, recently grown with a monoculture of maize) and extensively grazed pasture (35% of the surface area). The long-term average annual temperature at the site is 9.5 °C and the average annual precipitation is 726 mm, equally distributed over the year. A detailed soil analysis prior to planting characterized the soil type as a sandy texture, with clay-enriched deeper soil layers. In the upper soil layer, carbon (C) and nitrogen (N) concentrations were significantly lower in former cropland as compared to former pasture, and decreased exponentially with depth in both former land use types. For more information on the site and the soil characteristics, see Broeckx et al. (2012a).

Large replicated monoclonal blocks were established on a total of 14.5 ha, with twelve poplar genotypes representing different species and interspecific hybrids of *Populus deltoides* Bartr. (ex Marsh.), *P. maximowiczii* Henry, *P. nigra* L., and *P. trichocarpa* Torr & Gray (ex Hook) (Table 1). After soil preparation, 25 cm long dormant and unrooted hardwood cuttings were planted on 7-10 April 2010 at a density of 8,000 ha^{-1}. The cuttings were planted in a double-row design with alternating distances of 0.75 m and 1.50 m between the rows and 1.1 m between the individuals within each row. Neither irrigation nor fertilization were applied. More details on the plantation establishment and on the lay-out can be found in Broeckx et al. (2012a). All measurements reported in this paper were performed between January and December 2011, i.e. during the second growth year of the plantation. Depending on the study scale and on the measurement technique, the measurements were performed on a different number of genotypes, ranging from two genotypes for below-ground measurements to twelve genotypes for leaf area index and ecosystem scale measurements (Table 1). Although the number of genotypes varied, the choice of genotypes was nested hierarchically and always represented phenotypically and genetically contrasting genotypes.

Table 1 Overview of the twelve poplar genotypes planted in a short-rotation coppice culture. Parentage, genotype and measurements performed on each genotype have been indicated. Regression coefficients a and b as well as the coefficient of determination R^2 of the genotype specific allometric relationship are also shown ($DM = a * D^b$, where DM and D represent the above-ground woody biomass and the stem diameter at 22 cm, respectively).

Parentage	Genotype	a	b	R^2	Measurement
T × M	Bakan[1]	0.162	2.397	0.998	EC – LAI – bud phenology – stem diameter – Ψ_S – leaf gas exchange
T × M	Skado[1]	0.688	2.030	0.970	EC – LAI – bud phenology – stem diameter – Ψ_S – leaf gas exchange – R_{soil} – fine roots
D × N	Muur[1]	0.124	2.422	0.994	EC – LAI – bud phenology
D × N	Oudenberg[1]	0.180	2.333	0.999	EC – LAI – bud phenology – stem diameter – Ψ_S – leaf gas exchange
D × N	Vesten[1]	0.072	2.574	0.988	EC – LAI – bud phenology
D × N	Ellert[2]	0.135	2.385	0.996	LAI – bud phenology
D × N	Hees[2]	0.218	2.279	0.996	EC – LAI – bud phenology
D × N	Koster[2]	0.152	2.344	0.999	EC – LAI – bud phenology – leaf gas exchange – fine roots
D × N	Robusta[2]	0.200	2.282	0.997	LAI – bud phenology
D × (T × D)	Grimminge[1]	0.127	2.454	0.998	EC – LAI – bud phenology – stem diameter – Ψ_S – leaf gas exchange – R_{soil}
N	Brandaris[2]	0.150	2.348	0.991	LAI – bud phenology
N	Wolterson[2]	0.139	2.380	0.996	LAI – bud phenology – leaf gas exchange

D = *Populus deltoides*, M = *P. maximowiczii*, N = *P. nigra*, T = *P. trichocarpa*; EC = genotypes highly represented in the footprint of the eddy covariance tower; LAI = leaf area index; Ψ_S = soil water potential; R_{soil} = soil respiration; [1] produced by INBO (Geraardsbergen, Belgium); [2] produced by Vermeerderingstuinen Nederland (Zeewolde, The Netherlands).

2.2. Environmental parameters

Meteorological parameters were recorded half-hourly at the study site. Air temperature (T_{air}) and relative humidity were recorded on an extendable eddy covariance (EC) mast at 5.4 m above the ground surface using Vaisala probes (model HMP45C, Vaisala, Helsinki, Finland). The incoming short-wave radiation (SWR, 0.3 to 3 µm) was collected using a pyranometer (model CNR1, Kipp & Zonen, Delft, The Netherlands). Precipitation (P) was recorded using a tipping bucket rain gauge (model 3665R, Spectrum Technologies Inc., Plainfield, IL, USA), while the water table was recorded with a pressure transducer (model PDCR1830, Campbell Scientific, Logan, UT, USA) installed in a pipe inserted into the ground to a depth of 1.85 m. More information on the logging and the gap filling of the environmental factors can be found in Zona et al. (2013a & b).

Soil water content (SWC, m^3 m^{-3}) was measured half-hourly using moisture probes (TDR model CS616, Campbell Scientific, Logan, UT, USA) placed at depths of 20 cm and 40 cm at one location close to the EC mast. As a complement, soil water potential (Ψ_S) was measured half-hourly from June to November 2011 using calibrated equitensiometer probes (type EQ-2, Delta-T Devices Ltd, Cambridge, UK) installed at depths of 20 cm and 40 cm at four locations around the EC mast in four different genotypes (Table 1). We opted for characterizing soil water availability along the growing season through the time-course of Ψ_S values, averaged among the four locations. Therefore Ψ_S values (only available from June to November 2011) were extrapolated to the whole year based on the relationship observed between SWC and Ψ_S measurements at each measuring depth. A feed forward Neural Network (Matlab R2012a, Mathworks, Natick, MA, USA) was used to interpolate missing values. The correlation between predicted and measured values ranged between 0.83 and 0.98 for the different soil depths.

2.3. Eddy covariance measurements

Ecosystem scale CO_2, water vapour and energy fluxes were continuously (at 10 Hz) measured with standard EC techniques. The system included a sonic anemometer

(Model CSAT3, Campbell Scientific, Logan, UT, USA) for the measurement of the three-dimensional wind components, wind speed, wind direction, and the energy fluxes. The sonic anemometer and the inlets of the sampling lines were situated at 5.8 m above the surface during the first period (1 January 2011 to 31 August 2011); afterwards they were raised to 6.6 m (after 31 August 2011). The location of the mast was chosen according to the prevalent wind direction, to maximize the footprint of the EC mast, as described in Zona et al. (2013a). A LI-7000 fast-response gas analyzer (LiCor, Lincoln, NE, USA) was used to measure CO_2 and latent heat (LE) fluxes. Latent heat flux was converted into evapotranspiration (ET) using T_{air} and latent heat of vaporization (λ; Rebmann et al. 2012):

$$ET = \frac{LE}{\lambda}$$

Fluxes of CO_2, LE and sensible heat (H) were calculated using the EdiRe software (R. Clement, University of Edinburgh, UK) and averaged over 30 min. The Bowen ratio was defined as the ratio between H and LE (Bowen 1926, Teuling et al. 2010). The ratio of ET over P was called the evaporative index (Williams et al. 2012). Half-hourly net ecosystem exchange (NEE) data were partitioned into gross primary production (GPP) and ecosystem respiration (R_{eco}). Accordingly, the net ecosystem productivity (NEP) was defined as (Gower et al. 2001, Krishnan et al. 2006):

$$NEP = GPP - R_{eco}$$

A filter was used to set negative GPP values to zero. The ecosystem water-use efficiency (WUE) was defined as the GPP/ET ratio (Krishnan et al. 2006, Granier et al. 2007, Reichstein et al. 2007a). For more details on the EC system and post-processing of the data, see Zona et al. (2013b).

2.4. Soil CO_2-efflux

The CO_2-efflux from the soil, i.e. soil respiration (R_{soil}), was measured on an hourly basis by an automated soil CO_2-flux system (LI-8100, LiCor, Lincoln, NE, USA). Sixteen chambers operating as closed systems were connected to an infra-red gas analyzer through a multiplexer (LI-8150, LiCor, Lincoln, NE, USA). The chambers were located in the neighbourhood of the EC mast – within the footprint – and spatially distributed to cover both former land use types in two genotypes (Table 1, Verlinden et al. 2013c). The system was installed at the end of March 2011 and continuously logged soil CO_2-efflux for each chamber successively every hour until the end of the year. Soil CO_2-efflux was extrapolated for January to March 2011 by a feed forward Neural Network analysis (Matlab R2012a, Mathworks, Natick, MA, USA) based on the close relationship with soil temperature. The correlation between predicted and measured values ranged between 0.84 and 0.93 for the different measurement chambers. Soil temperature was monitored all year at 0-10 cm depth using a thermocouple (TCAV-L, Campbell Scientific, Logan, UT, USA) at one location close to the EC mast.

2.5. Leaf gas exchange and photosynthetic parameters

Leaf gas exchange measurements were performed with a portable open-path gas exchange measurement system (LI-6400, LiCor, Lincoln, NE, USA) equipped with a leaf chamber fluorometer (LI-6400-40, LiCor, Lincoln, NE, USA). Measurements were completed in monthly measurement campaigns from May to September 2011 on four replicate trees of six genotypes located on former cropland. All measurements were done in the upper canopy on the most recently matured leaf of the current-year shoot. Leaves were first acclimated for 10 min in the chamber at the atmospheric CO_2 concentration (400 ppm) and under a saturating photosynthetic photon flux density (PPFD) of 1500 µmol s^{-1} m^{-2}. Afterwards light-saturated assimilation rate at atmospheric CO_2 concentration (A_{sat}) and stomatal conductance ($g_{s\text{-}sat}$) were recorded, before establishing the response of the net assimilation rate to varying intercellular CO_2 concentrations (A-C_i curve). Each curve consisted of 10 steps of external CO_2 concentrations set in succession to 400, 300, 250, 150, 100, 50, 500, 750, 1000 and 1250 ppm. Leaves were allowed to equilibrate at least 3 min at each step before data were logged. All measurements were done at a constant block temperature (25°C) and at a controlled vapour pressure deficit (VPD) of ca. 1 kPa (1.07 kPa ± 0.03, mean ± SE). Day respiration (R_{day}) was estimated from the A-C_i curves by fitting the biochemical photosynthesis model of Farquhar (Farquhar et al. 1980).

2.6. Radial stem growth

Stem diameter at 22 cm above soil level was regularly measured for four genotypes between March and September 2011 with a digital caliper (Mitutoyo, CD-15DC, Japan, 0.01 mm precision). Measurements were performed on five trees per genotype, chosen to represent the diameter distribution of each genotype using the quantils of the total approach (Cermák 1991, Cermák & Kučera 1994). Since the effect of former land use on stem diameter diminished during the second growing season (Broeckx et al. 2012a) only stem growth of trees located on former cropland were monitored. Data are presented as the relative radial growth for 2011, i.e. stem diameter expressed relative to the end-of-season stem diameter.

2.7. Leaf area index (LAI) and bud phenology

Canopy development was monitored by measuring LAI on a monthly basis from April to November 2011. Measurements were performed in four replicated measurement plots per former land use type for each genotype. An LAI-2200 Plant Canopy Analyzer (LiCor, Lincoln, NE, USA) with a 45° view cap was used, by comparison of above- and below-canopy readings. In each plot two diagonal transects were defined between the rows; measurements were then taken along each transect with the sensor parallel to the row and perpendicular to the row (cfr. Broeckx et al. 2012b). A weighted average LAI was calculated according to the ground surface area occupied by each genotype in the plantation at each monthly measurement.

For the assessment of the timing of bud break and bud set a phenological score was assigned weekly to each of the twelve genotypes. A sigmoidal curve was then fitted through the evolution of weekly scores (Matlab R2012a, Mathworks, Natick, MA, USA) and used to calculate the day of the year at which the score – corresponding to the following descriptions – was reached: the timing of spring bud flush was defined according to the score describing the apical bud as “bud sprouting, with a tip of the small leaves emerging out of the bud scales, which couldn't be observed individually” (based on UPOV, 1981); the end of leaf production, associated with autumnal bud set and the end of stem height growth, was defined as the time when “an apical bud was present but not fully closed, bud scales were predominantly green and no more rolled-up leaves were present” (Rohde et al. 2010). These phenological observations were used to define the length of the growing season as the time between bud flush and bud set, corresponding to the time between leaf appearance and maximum LAI (LAI_{max}). In comparison, a second definition of the length of the growing season is used further in the discussion, i.e. the period with a net CO_2 uptake from the atmosphere as indicated by NEE<0 for a period of more than one week (carbon uptake period, CUP).

2.8. Fine root production

Root biomass was quantified every two weeks from February to November 2011. At each campaign 20 soil samples per genotype of the upper 15 cm soil layer were collected using a hand-driven corer (Eijkelkamp Agrisearch equipment, Netherlands; Oliveira et al. 2000). In the laboratory roots were then hand-picked, washed, sorted in poplar and weed roots, and finally oven-dried at 70°C to estimate root biomass. In addition, poplar roots were also sorted in living and dead roots every two sampling dates, i.e. once a month. Living roots were further classified into four diameter classes: <1 mm (class L1), 1-2 mm (class L2), 2-5 mm (class L3) and >5 mm (class L4). Dead roots were only observed in the <1 mm class (L1), and were separated from living roots based on the dark colour and the lack of cohesion of the periderm (according to Janssens et al. 1999). Below-ground biomass production was constrained to poplar fine root biomass production. Fine roots were defined as roots with diameter <2 mm (classes L1 + L2). More details on root collection and data processing can be found in Berhongaray et al. (2012).

2.9. Net primary productivity (NPP)

Biomass measurements of each tree component were used to calculate NPP in terms of g C m^{-2} day^{-1}. For each tree component, monthly average NPP/GPP ratios were calculated. Standing biomass was estimated using allometric relationships established between above-ground woody dry mass (DM) and stem diameter (D):

$$DM = a * D^b$$

where a and b are regression coefficients specific to each genotype (cfr. Laureysens et al. 2004). Ten trees per genotype, representing the population diameter range, were harvested at the end of the second growing season, to establish genotype-specific allometric relationships (Table 1). Specific leaf area (SLA, the ratio between fresh leaf area and leaf dry mass), measured in September 2011 from six leaves of different size and tree height per genotype, was used to estimate leaf biomass from LAI measurements (Jonckheere et al. 2004). Biomass increment of stems and leaves was calculated as the difference between two consecutive biomass estimations and the time between the data collection. Fine root productivity between sampling dates was estimated using the decision matrix of Fairley and Alexander (1985). This method calculates production, mortality and decomposition of fine roots between consecutive sampling dates using data of fine root biomass and necromass. Carbon (C) concentrations of leaves (from leaf gas exchange measurements), stems (from harvested trees) and fine roots (averaged over growing season) and nitrogen (N) concentrations of leaves were determined from ground plant material by dry combustion using a CN element analyzer (Carlo Erba Instruments, Italy). Carbon concentrations were considered constant during the growing season, carbon concentration of dry mass being a conservative parameter (Vande Walle et al. 2005).

2.10. Data integration and analysis

Weekly averages were calculated for all continuously monitored parameters (NEE, GPP, R_{eco}, R_{soil}, $\Psi_{S,}$ T_{air}, P, SWR, VPD and heat fluxes). In addition, midday fluxes of NEE, GPP, R_{eco} and VPD (constrained between 10h and 16h local time for the entire year) were used since photosynthetic carbon uptake was restricted to daytime and since leaf and tree level measurements were performed within this time range. All continuous and discontinuous measurements were plotted over time to analyse seasonal trends and to identify possible patterns of interaction. Short-term regressions between continuous and derived parameters (GPP, SWR VPD and ET) were established for periods with a different water status: both a period with limiting water availability at 20 cm depth (15 May to 20 June 2011) and a period with low water availability at 40 cm depth (15 June to 20 July 2011) were compared to a period with ample soil water availability (August 2011). All plots and regressions were made in SigmaPlot Version 12.0 (Systat Software, San Jose, CA).

3. Results

3.1. Seasonal variability

Based on bud phenology measurements, the growing season started on 31 March 2011 (± 11 days) – averaged over the different genotypes in the plantation – corresponding with leaf expansion and the onset of stem diameter increment (Fig. 1a). At the onset of the growing season high NPP/GPP ratios for leaf and stem biomass were found, with maxima of 24% in March and 21% in April, respectively. In terms of ecosystem CUP the growing season started on 31 March 2011, in accordance with the first definition. A short period of net carbon uptake (by definition excluded from the CUP) occurred earlier, starting on 13 March and net carbon uptake temporarily decreased to zero around 31 March. A rather symmetrical seasonal NEP pattern was observed, slightly shifted towards the left, i.e. towards the beginning of the growing season (Fig. 2a).

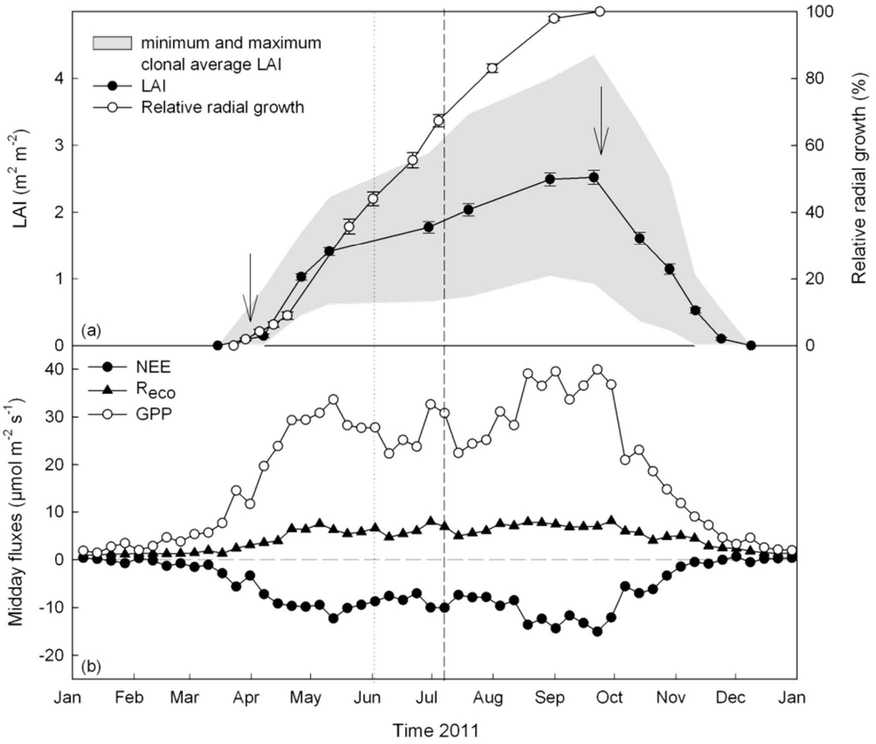

Fig. 1 Time course in 2011 of CO_2 fluxes and tree growth. (a) Relative radial stem growth and leaf area index (LAI) development; arrows indicate plantation average timing of bud burst (left) and bud set (right); error bars indicate SEs. (b) Midday CO_2 fluxes between 10h and 16h. Vertical dotted and dashed lines represent the minimum soil water potential at 20 cm depth (before June rainfall events) and restoration of soil water potential (before the July heavy rainfall event) respectively.

Stem diameter increment, reflecting stem biomass production, stopped in September 2011 (Fig. 1a). No more leaves were produced from 23 September (± 7 days) onward – averaged over the plantation – once LAI had reached a maximum. The average plantation LAI_{max} was rather low (LAI_{max} = 2.63; Fig. 1a). An increase in fine root production was concomitantly observed in September (Fig. 3). Overall, the above- to below-ground productivity ratio decreased during the growing season (Fig. 3). The ecosystem stopped being a carbon sink on 1 October (Fig. 2a). Over the whole year, the poplar SRC was a moderate carbon sink of 95.7 g C m^{-2}

(± 15.3) (Zona et al. 2013b). Leaf, stem and fine root dry biomass production corresponded to 2 Mg ha^{-1} y^{-1}, 5.8 Mg ha^{-1} y^{-1} and 0.5 Mg ha^{-1} y^{-1} respectively. It is worth noting that the photosynthetically active season, as defined by CUP, ended shortly after bud set (Fig. 1a).

On a yearly basis, R_{soil} contributed 54% to R_{eco} (Fig. 2b). A decreasing trend in the contribution of R_{soil} to R_{eco} was, however, observed towards the end of the year. A significant and positive linear correlation was observed between SWR and T_{air} which in turn positively affected VPD. Consequently, a closely related seasonal time-course was observed between SWR and VPD (Fig. 4a). Vapour pressure deficit and GPP showed a reversed seasonal pattern during the growing season, reflecting a reduced carbon uptake at high VPD values. Overall, the ecosystem WUE increased during the growing season (Fig. 5a).

3.2. Dry spring conditions

Spring 2011 was particularly dry at our site. The monthly precipitation at the site in March, April and May represented only 26%, 16% and 25% of the long-term average monthly precipitation respectively, as measured by the Royal Meteorological Institute (Ukkel, 50°47’55’’ N, 4°21’29’’ E). The monthly precipitation amounts measured in Ukkel, were statistically characterized as exceptional (i.e. occurring once every 30 years) for March 2011, normal for April 2011 and very atypical (i.e. occurring once every ten years) for May 2011 (Royal Meteorological Institute). Low precipitation was mirrored by a progressive decrease in water table of almost 1 m, i.e. from 45 cm below the soil surface at the beginning of March to a minimum of 142 cm below the soil surface at the beginning of July 2011 (Fig. 4b). However, the effects on soil water potential (Ψ_S) were visible only from May onwards continuing until July (Fig. 4c). Values of Ψ_S reached a minimum of -1.8 MPa at 20 cm depth in the beginning of June and a minimum of -1.2 MPa at 40 cm depth in mid-July (Fig. 4c). These values were close to and even exceeded the wilting point (i.e. at Ψ_S of -1.6 MPa; Granier et al. 2007, Migliavacca et al. 2009).

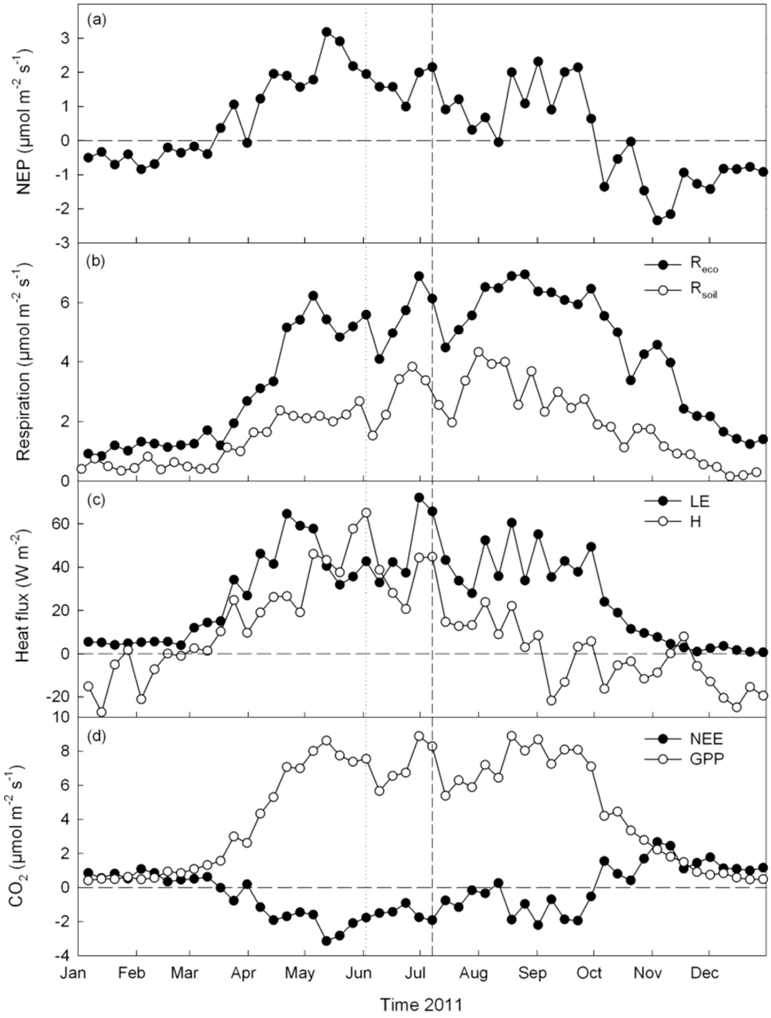

Fig. 2 Time course in 2011 of CO_2 and energy fluxes. (a) Net ecosystem productivity (NEP = GPP-R_{eco}). (b) Ecosystem (R_{eco}; eddy covariance (EC) measurements) and soil respiration (R_{soil}; chamber measurements). (c) Latent (LE) and sensible (H) heat fluxes. (d) Net ecosystem exchange (NEE) and gross primary production (GPP); negative NEE values indicate carbon uptake. Vertical dotted and dashed lines represent the minimum soil water potential at 20 cm depth (before June rainfall events) and restoration of soil water potential at 20 cm depth (before the July heavy rainfall event) respectively.

Given this naturally occurring drought, we acknowledge that we studied the interacting effect of soil water availability and seasonality, in absence of an irrigated control plot. A decrease in leaf NPP and consequently a decreased leaf growth rate were observed in May and June 2011 (Figs. 1b & 3), when soil water availability was low in the near surface soil layer. The reduced leaf area development was accompanied by a decrease in midday GPP and NEE (Fig. 1b). NEE and GPP indeed showed a similar seasonal time-course as the evolution of LAI during the growing season. This seasonal trend was less pronounced in midday R_{eco} (Fig. 1b). At the leaf level, net assimilation rate (A_{sat}) was reduced in response to low water availability in the near surface soil layer while day respiration (R_{day}) was not affected (Fig. 6a). A temporary return towards carbon neutrality occurred in August when the ecosystem was recovering from drought (Fig. 2a,d). Simultaneously with the decreasing leaf production, fine root productivity reached a temporary maximum in June (Fig. 4), indicating a stimulation of fine root production in response to limiting water availability in the near surface soil layer. In contrast, stem biomass growth was only slightly affected by the dry conditions (Fig. 1a), as supported by the seasonal pattern in stem NPP (Fig. 3).

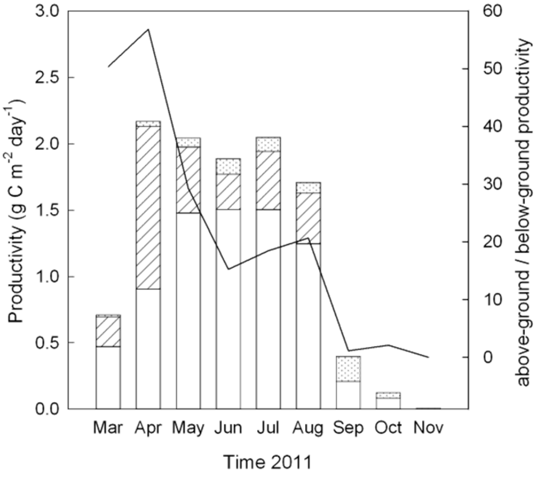

Fig. 3 Time course in 2011 of biomass allocation between different tree components: dotted bars represent fine root biomass production, hatched bars represent leaf biomass production and white bars represent stem biomass production. The black line represents the ratio between the above-ground (stem + leaf) and below-ground (fine roots) productivity.

A decrease in GPP was observed towards the beginning of June (Fig. 2d), in response to decreasing Ψ_S at 20 cm depth (Fig. 4c). GPP was not responsive to VPD when water availability was limiting at 20 cm depth (Fig. 7a). A modest increase in the Bowen ratio with decreasing Ψ_S at 20 cm depth was observed (Fig. 5a). This was caused by an increasing H and decreasing LE (Fig. 2c). A rapid saturation of ET with VPD occurred at low water availability in the upper 20 cm soil layer (Fig. 7b). A local maximum in WUE was reached shortly before the minimum Ψ_S at 20 cm depth (Fig. 5a). At the leaf level, a simultaneous reduction in $g_{s\text{-}sat}$ and A_{sat} was observed towards the beginning of July (Fig. 6a,b). A similar, but less pronounced, spring reduction was found in leaf nitrogen (N) concentration (Fig. 6b). An increase in GPP was observed in response to the June rainfall event (Figs. 2d & 4b) that caused an increase in Ψ_S at 20 cm depth (Fig. 4c), although the water table did not change at this time (Fig. 4b). Simultaneously, Ψ_S at 40 cm depth was decreasing as a delayed effect of the spring dry period by depletion of deeper soil water (Fig. 4c). A peak in ET and evaporative index corresponded to the decreasing soil water availability at 40 cm depth (Fig. 5b). A second decrease in GPP was observed during the intense rainfall event in the beginning of July (Figs. 2d & 4b). This sudden rainfall caused an increase in water table and a recovery of Ψ_S back to 0 MPa (Fig. 4c). A strong decrease in SWR and T_{air} were observed in line with reduced GPP (Fig. 4a,d). A trend of increasing GPP with T_{air} and SWR was observed when soil water availability was low at 40 cm depth (Fig. 7c,d).

4. Discussion

4.1. Growing season patterns

High NPP/GPP ratios for leaf and stem diameter in the beginning of the growing season are in line with previous findings for beech (Campioli et al. 2011). This reflects the growth strategy of deciduous trees in temperate regions, maximally exploiting optimal spring growth conditions (Campioli et al. 2011).

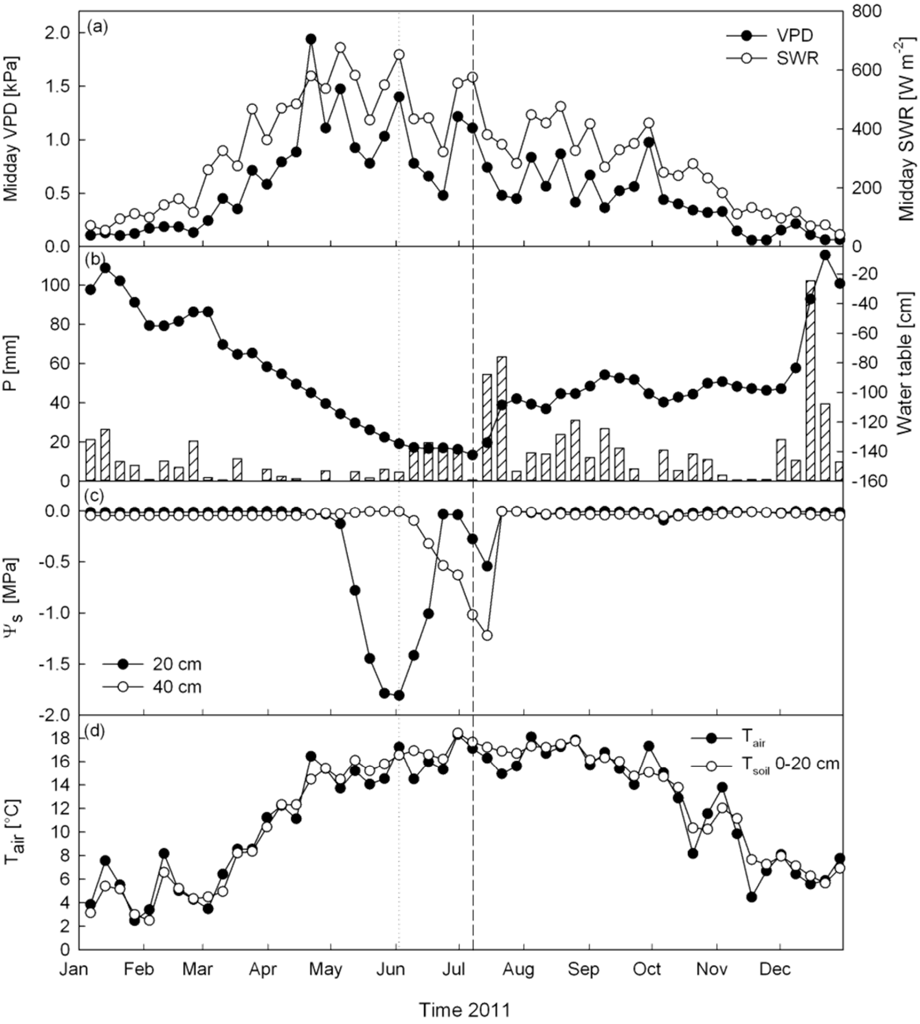

Fig. 4 Time course in 2011 of meteorological parameters. (a) Vapour pressure deficit (VPD) and short-wave radiation (SWR). (b) Precipitation (P; hatched bars) and water table (black dots) in cm below the surface. (c) Soil water potential (Ψ_S) at 20 and 40 cm soil depths. (d) Air (T_{air}) and soil temperature (T_{soil}) in the upper 20 cm soil layer. Vertical dotted and dashed lines represent the minimum soil water potential at 20 cm depth (before June rainfall events) and restoration of soil water potential at 20 cm depth (before the July heavy rainfall event) respectively.

An early net carbon uptake event of less than one week from 13 March onward was caused by increasing GPP. This early ecosystem carbon uptake reflected the photosynthetic carbon uptake by the understory vegetation at one hand, and the genotypic variation in timing of bud flush on the other hand. The ecosystem carbon uptake started when environmental conditions favored photosynthesis and strongly increased when the earliest genotypes started to flush leaves. A temporary decrease to zero in net carbon uptake around 31 March was explained by reduced SWR and hence reduced GPP, in addition to increasing respiration at the very beginning of the growing season, offsetting GPP. High photosynthetic rates in the beginning of the growing season resulted in high NEP, when SWR was high and R_{soil} was still low because of the low soil temperature (Verlinden et al. 2013c). This observation was previously found for temperate deciduous forests (Falge et al. 2002).

The timing of LAI_{max} near the end of the growing season reflects the indeterminate growth habitus of poplar (Howe et al. 2000). A similar seasonal LAI development was observed in the perennial energy crop switchgrass (Albaugh et al. 2012). A typical temperate annual cycle was observed with a fast increase of NEP in spring associated with photosynthetic C uptake (Krishnan et al. 2006). A very close relationship was observed when comparing both definitions of the duration of the growing season (i.e. the time between bud burst and bud set versus the CUP), although they address processes at different scales. The increasing fine root production in September could indicate a shift in carbon allocation from above-ground biomass to below-ground biomass towards the end of the growing season (Scarascia-Mugnozza 1991, Dickmann and Pregitzer 1992), as evidenced by a decreasing above- to below-ground productivity ratio. Root growth generally continues longer than shoot growth even after leaf abscission (Lyr and Hoffmann 1967, Cannell and Willett 1976). The fact that root growth is favored over shoot growth at the end of the growing season has been previously reported not only for mature forests (Burke and Raynal 1994) but also for young poplar plantations (Heilman et al. 1994).

The low LAI_{max} suggested a rather open stand where a fraction of the net radiation reached the soil surface (cfr. Granier et al. 2000). The coinciding seasonal course of SWR and soil heat flux (data not shown) suggested that at least part of the solar energy was indeed absorbed by the soil in our plantation. Total ecosystem ET in open stands is generally not proportional to tree LAI and hence to tree transpiration (Granier et al. 2000), which was mirrored in the different seasonal time-courses of ET and LAI. Canopy openness had therefore promoted an increase in transpiration of the understory weed vegetation with incoming radiation (cfr. Granier et al. 2000), especially in the beginning of the growing season when LAI was low and incoming SWR was rather high.

The observed contribution of R_{soil} to R_{eco} (54%) was in the lower end of the range reported for European forest ecosystems (Janssens et al. 2001), although this plantation was recently established and accompanied by severe soil disturbance due to ploughing. Since roots, litter and soil microbial activity are the main contributors to soil respiration, the young age of the plantation and the still developing root system likely explained this low proportional contribution. A positive correlation between R_{soil} and root biomass was observed in the plantation (Verlinden et al. 2013c), with low root biomass values compared to other studies (Vande Walle et al. 2007, Abou Jaoudé et al. 2011). Several factors may explain the decreasing trend of this relative contribution, including (i) the limited leaf fall in the soil chambers causing a higher contribution of leaf decomposition in the plantation as compared to the chambers; (ii) a higher ecosystem respiration at the end of the year due to an increase in wood volume and an increase in perennial weed biomass as compared to the beginning of the season; and (iii) a low soil respiration at the end of the year due to a high local water table and a low soil temperature possibly underestimating the contribution of R_{soil} to R_{eco}. The first factor could be considered an artifact of the limited chamber area of the R_{soil} measurements. The effect of water table was previously described (Verlinden et al. 2013c), with water saturated soils causing surface sealing and anaerobic conditions, and hence reduced diffusion of gases and of microbial activity. Earlier studies also reported an exponential response of soil respiration to soil temperature and a decrease in soil respiration at low and high soil water contents (Vincent et al. 2006, Lee et al. 2010), affecting seasonality of R_{soil}.

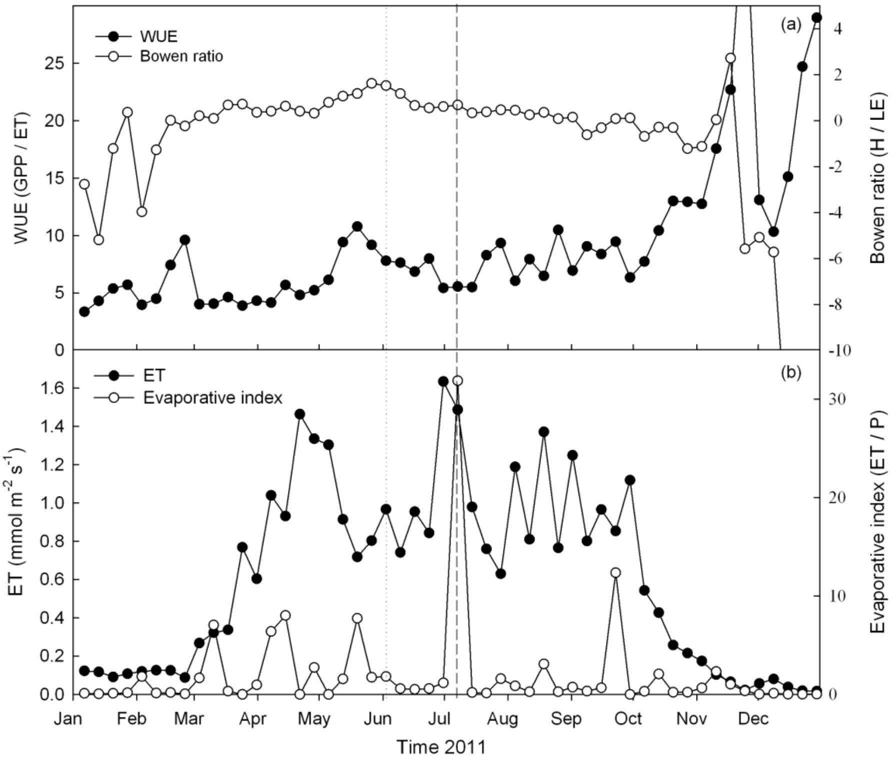

Fig. 5 Time course in 2011 of (a) water-use efficiency (WUE) and Bowen ratio [ratio between sensible (H) and latent (LE) heat flux] and of (b) evapotranspiration (ET) and evaporative index [ratio between ET and precipitation (P)]. Vertical dotted and dashed lines represent the minimum soil water potential at 20 cm depth (before June rainfall events) and restoration of soil water potential (before the July heavy rainfall event) respectively.

4.2. Impact of the dry spring

The minimum values of Ψ_S reached in the plantation suggested that the trees were potentially suffering from drought, affecting several plant- and ecosystem-scale processes. Over the growing season (April-September), cumulative ET (284 mm) was more than compensated by cumulative rainfall (360 mm). However, seasonal variations were observed, with an average evaporative index of 1.62 during the first half of the growing season (April – June) indicating drought, as compared to 0.52 during the second half of the growing season (July – September). Seasonal variation in the evaporative index has already been related to lower ET values in drier periods in a switchgrass bio-energy plantation (Wagle et al. 2012).

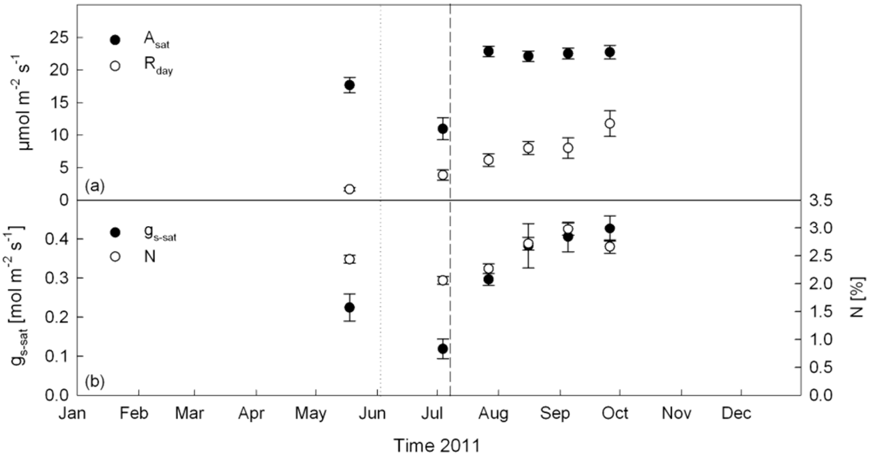

Fig. 6 Time course in 2011 of photosynthetic parameters at the leaf level: (a) net assimilation rate (A_{sat}), at saturating light conditions [photosynthetic photon flux density (PPFD = 1500 µmol m^{-2} s^{-1}) and atmospheric CO_2 concentration (400 ppm)], and day respiration (R_{day}) and (b) stomatal conductance (g_{s-sat}) and leaf nitrogen concentration (N). Vertical dotted and dashed lines represent the minimum soil water potential at 20 cm depth (before June rainfall events) and restoration of soil water potential (before July heavy rainfall event) respectively.

4.3. Effects on net primary productivity

The observed decrease in leaf NPP and hence in leaf growth rate in response to low water availability was reported in previous studies: a decreased LAI was found in container-grown hybrid poplar under drought conditions (Souch and Stephens 1998), in willow SRC stands in response to soil water deficits (Lindroth et al. 1994) as well as in other perennial energy crops such as several *Miscanthus* species, reed canary grass and sugarcane (Smit and Singels 2006, Zub and Brancourt-Hulmel 2010, Zhou et al. 2011). A decreased maximum individual leaf area in response to drought has also been reported in hybrid poplar (Monclus et al. 2006 & 2009), confirming that leaf production and expansion are very sensitive to drought in fast-growing species such as poplar and willow. The lower correspondence in seasonal time-course of R_{eco} compared to GPP with the evolution of LAI was in line with previous observations of a stronger response of GPP to increasing drought in European forests (Granier et al. 2007). Similar observations of a larger effect of soil water availability on gross ecosystem productivity than on R_{eco} were already reported for a hybrid poplar plantation in the southern boreal forest region of Canada (Cai et al. 2011).

Observations of increased carbon allocation to the roots in response to water stress were previously reported for poplar seedlings (Zhang et al. 2004, Yin et al. 2005, Guo et al. 2010), as well as for other tree species (Tomlison and Anderson 1998, Leuschner et al. 2001). However, during the first year of their experiment on water-use in hybrid poplar, Souch and Stephens (1998) found no effect of drought on biomass partitioning between roots, stems or branches. A reduced allocation to fine roots in the upper soil layers in response to low soil moisture has been reported before in poplar (Dickmann et al. 1995) and in balsam fir (Olesinski et al. 2011). Older trees have fewer possibilities and needs for expansion of their root systems, but in newly planted trees root growth tends to explore the soil volume and to increase the ability to compete with neighboring plants. Consequently, root growth uses reserves during periods of low supplies of photosynthates (Persson 2002). In poplar, active root growth in response to a declining water table might be an adaptive trait related to its natural riparian habitat (Rood et al. 2003).

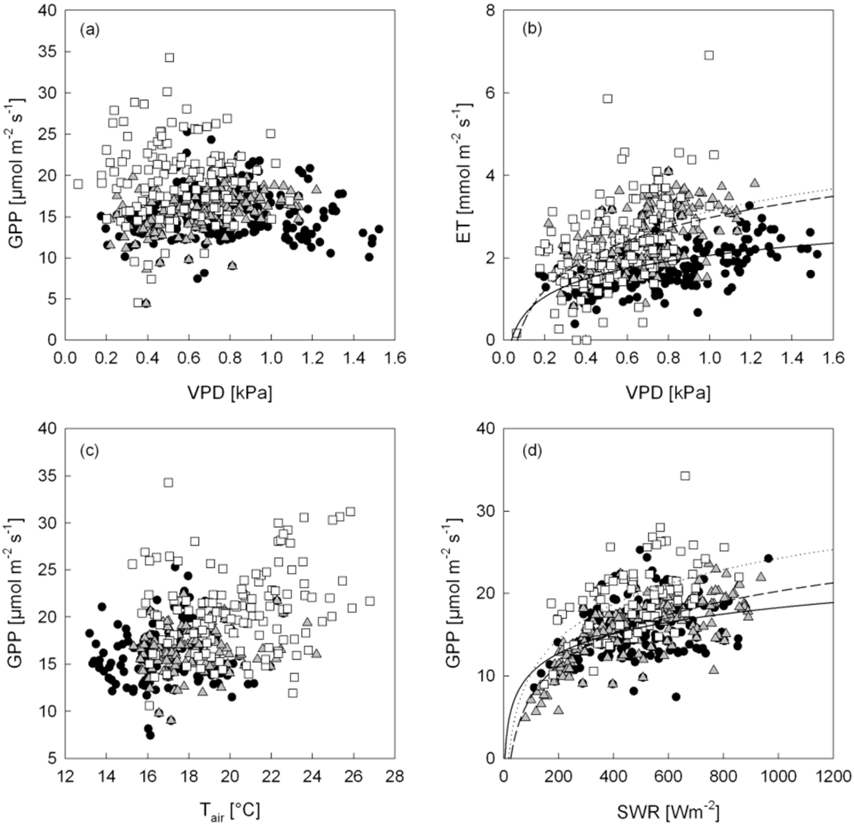

Fig. 7 Short-term regressions for three periods with different soil water status during the 2011 growing season (white squares = ample soil water; grey triangles = dry at 40 cm depth; black circles = dry at 20 cm depth). (a) Response of midday GPP to midday VPD (10 ≥ T_{air} ≥ 20 °C; 300 ≥ SWR ≥ 700 Wm^{-2}). (b) Response of midday ET to VPD (10 ≥ T_{air} ≥ 20 °C; 300 ≥ SWR ≥ 700 Wm^{-2}). (c) Response of midday GPP to midday T_{air} (0.5 ≥ VPD ≥ 1 kPa; 300 ≥ SWR ≥ 700 Wm^{-2}). (d) Response of midday GPP to midday SWR (0.5 ≥ VPD ≥ 1 kPa; 10 ≥ T_{air} ≥ 20 °C). GPP, gross primary production; T_{air}, air temperature; VPD, vapour pressure deficit; SWR, short-wave radiation; ET, evapotranspiration.

4.4. Effects on ecosystem and leaf level gas exchange

Increasing H when water availability was limiting at 20 cm depth suggested that the higher energy load from incoming radiation increased leaf temperature and hence sensible heat, due to reduced transpiration. Reduced photosynthetic rates, induced by a decrease in $g_{s\text{-}sat}$ with increased VPD were previously observed in *P. tremuloides* stands (Hogg et al. 2000). The similar reduction in stomatal conductance and A_{sat} towards the beginning of July implied a strong stomatal control upon photosynthesis (Regier et al. 2009, Larchevêque et al. 2011). The high T_{air} during this period and the limited GPP even at low VPD emphasized that water availability was the limiting factor. These results were supported by a rapidly saturating ET with increasing VPD when soil water availability was low in the upper 20 cm. Decreasing photosynthetic rates when soil water potential was below the wilting point in the upper 20 cm soil layer (-1.8 to -1.6 MPa) suggested water and nutrient uptake occurred in this near surface soil layer where a significant part of fine roots occurred (cfr. Al Afas et al. 2008; unpublished results). Sufficient water availability at 40 cm depth (0 to -0.3 MPa) indicated that deeper roots could not support the necessary water uptake for the whole system at this time, in contrast to previous studies (Lyr and Hoffmann 1967, Rood et al. 2003). The increasing ecosystem WUE with decreasing soil water availability and decreasing g_s was in line with earlier studies (Baldocchi 1997, Williams 1998, Krishnan et al. 2006).

Reduced leaf N concentration due to water stress was previously reported for poplar, in combination with a reduced net assimilation rate and g_s (Yin et al. 2009). Reduced leaf N concentration may have been one cause of reduced NPP considering the positive correlation between N content and fresh stem biomass as previously reported by Monclus et al. (2009). However, the minimum N concentration in the leaves of more than 2% suggested that N was not the limiting factor of A_{sat}. The reduced leaf N concentration during the spring dry period could have been caused by a lower N uptake due to either (i) a reduced transpiration caused by stomatal closure; (ii) a reduced soil N availability (Larsen et al. 2011); and/or (iii) a reduced uptake capacity of the roots (Kreuzwieser and Gessler 2010) in response to low water availability. The hypothesis of a limitation in N availability was supported by the progressive decrease in N_2O emission and the occurrence of N_2O uptake at the end of the growing season 2011 (Zona et al. 2013a). A similar limitation in N uptake during drought showed from an increased effect of N input on the yield of *Miscanthus* spp. under water stress (Zub and Brancourt-Hulmel, 2010).

The constant Bowen ratio during the June rainfall event reflected high transpiration rates and less sensible heat, indicating a reduced stomatal control despite low A_{sat} and $g_{s\text{-}sat}$. This was supported by a sharp increase in ET, associated with a peak in the evaporative index. A short-term increase in $g_{s\text{-}sat}$ and hence tree transpiration in response to rainfall as also observed by Granier et al. (2007) could explain this high carbon uptake – that could not be observed due to the discontinuous leaf gas exchange measurements – in combination with evaporation of water intercepted by the canopy. The maximum GPP reached by the end of June was associated with an increase in SWR and T_{air}, contributing to high photosynthetic rates.

Decreased GPP during the July rainfall event, with latent heat still exceeding sensible heat, with decreased but not limiting soil water availability and reduced VPD, suggested that stomatal control of GPP was less likely. This was also supported by the further increasing A_{sat} and $g_{s\text{-}sat}$ values. Decreasing SWR and T_{air} in line with reduced GPP suggested temperature as the limiting factor of photosynthetic carbon uptake at that time. These results were confirmed by the stronger control of GPP by air temperature when water was non-limiting in the root zone. Both stomatal control due to water limitation in the superficial root zone and increased VPD, reduced the effect of temperature on GPP. This observation was supported by a faster saturation of GPP with increasing SWR when water was more limiting. A stronger control of solar radiation on the carbon assimilation rate as compared to VPD was previously observed in a boreal aspen stand (Hogg et al. 2000), with sufficient water availability. Similarly, Reichstein et al. (2007b) reported a significant correlation between GPP and water availability in more water-limited (Southern European) sites, while in rarely water-limited (Northern European) sites a significant correlation between GPP and T_{air} was found.

5. Conclusion

In this study we analyzed the seasonal dynamics in CO_2, water and energy fluxes during the second growth year of a poplar bio-energy plantation. The results showed that the plantation was already a modest carbon sink at this time, with the carbon uptake period closely corresponding to leaf area development up to LAI_{max}. The unusually dry period that occurred in spring allowed us to evaluate the impact of low water availability on ecosystem productivity. As we hypothesized, a reduced leaf area development was found in response to low water availability in the near surface soil layer. High SWR and VPD induced strong stomatal control on carbon uptake when soil water availability was limiting in the upper soil layer. An increase in ecosystem WUE was observed in association with stomatal closure. Decreasing SWR and T_{air} were the limiting factors of photosynthetic carbon uptake when water was not limiting in the root zone. These results indicate that an increasing frequency and intensity of summer heat and drought, could affect the productivity of recently established bio-energy plantations with fast-growing trees, when the developing root system is not able to provide the necessary water from deeper soil layers.

Acknowledgements

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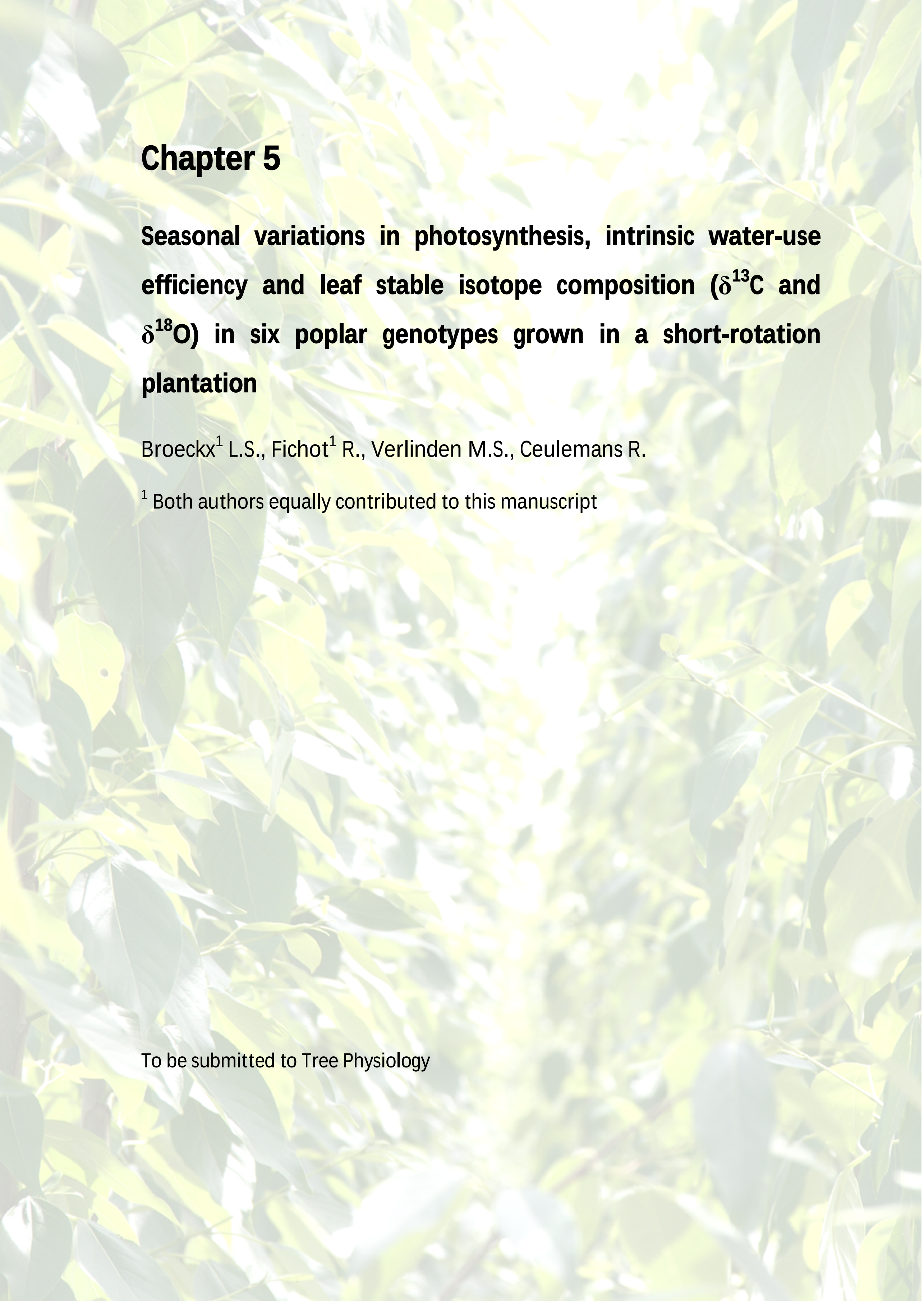

Chapter 5

Seasonal variations in photosynthesis, intrinsic water-use efficiency and leaf stable isotope composition ($\delta^{13}C$ and $\delta^{18}O$) in six poplar genotypes grown in a short-rotation plantation

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[1] Both authors equally contributed to this manuscript

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Abstract

Photosynthetic carbon assimilation and the associated transpiratory water loss play an important role in the efficiency of bio-energy-devoted cultures with fast-growing trees. We investigated and characterized (i) seasonal variations in photosynthetic parameters and water-use efficiency (both estimated as intrinsic water-use efficiency, WUE_i, and leaf stable isotope discrimination, $\Delta^{13}C$) for six poplar genotypes in a short-rotation plantation and (ii) how genotypes and timing throughout the growing season affected relationships among the studied traits. Photosynthetic assimilation showed substantial genotype by time interaction in the degree of stomatal limitation in response to low soil water availability. Stomatal rather and biochemical limitation was emphasized by constant intrinsic photosynthetic capacity (V_{cmax}) with time, highly related to leaf nitrogen on an area basis (N_{area}) for most genotypes. An increased WUE_i in response to low soil water availability was observed, depending on the genotype, with stomatal conductance as the main driver of variation in WUE_i. As expected from the theory, $\Delta^{13}C$ scaled negatively with WUE_i but the relationship was variable among genotypes and throughout the growing season. Poor correlations between $\Delta^{13}C$ and growth parameters were confirmed, that could be largely ascribed to time scale considerations.

Keywords

short-rotation coppice, water deficit, stomatal conductance, maximum rate of carboxylation, leaf nitrogen, photosynthetic nitrogen-use efficiency

1. Introduction

Fast-growing tree species, such as poplar and willow, implemented in short-rotation bio-energy cultures represent a promising renewable energy source (AEBIOM 2012). The success of this renewable bio-energy partly depends on the production or yield rates that can be achieved. The large genetic variability found within the *Populus* genus (Dunlap and Stettler 1998, Al Afas et al. 2005) offers the possibility to select highly productive genotypes. Photosynthetic parameters and stomatal conductance play a crucial role in CO_2 assimilation and water-use of trees. Seasonal variations in photosynthetic parameters largely affect modeling of ecosystem carbon uptake and energy fluxes, determining the efficiency of these bio-energy cultures. In most studies, seasonal patterns of leaf photosynthesis are examined as the assimilation rate at saturating light and ambient CO_2 concentration (A_{sat}). Although saturated assimilation is generally related to intrinsic photosynthetic capacity (V_{cmax}), the relationship could be hampered by stomatal limitations. Therefore, V_{cmax} is usually considered in modeling ecosystem carbon fluxes, however little is known about seasonal variation in V_{cmax} (Wilson et al. 2000, Xu and Baldocchi 2003). Because of the effort needed to estimate V_{cmax}, much attention is being given to the relationship between V_{cmax} and the easily measurable leaf nitrogen concentration (N) as a potential estimator of seasonal variation in photosynthetic capacity. The V_{cmax}-N is however not universal and although variability with species (Ripullone et al. 2004) and nitrogen availability (Reich et al. 1989) has been well-described, information on seasonal patterns in the relationship is scarce (Wilson et al. 2000, Grassi et al. 2005).

The high productivity of poplar has been associated with high water-use (water consumption) (Allen et al. 1999, Zsuffa et al. 1996, Meiresonne et al. 1999) and with high sensitivity to drought (Liang et al. 2006, Lindroth et al. 1994, Monclus et al. 2009). The increasing probability of seasonal droughts (Easterling et al. 2000, Seneviratne et al. 2010) and the prospects of freshwater scarcity (Berndes 2002) stress the importance of accounting for traits such water-use efficiency (WUE) and drought tolerance as selection criteria for biomass production under future climate conditions (King et al. 2013). At the whole plant level, WUE is defined as plant dry matter production per unit water loss during transpiration. Substantial species and genotypic variation has been reported (Cernusak et al. 2007, Linderson et al. 2007, Rasheed et al. 2013). At the leaf level, intrinsic water-use efficiency is defined as the instantaneous ratio between net CO_2 assimilation rate (A) and stomatal conductance to water vapour (g_s), i.e. the intrinsic WUE (WUE_i). Carbon isotope discrimination ($\Delta^{13}C$) is often used as an indirect estimate of WUE_i. Discrimination against the heavier (^{13}C) isotope of carbon occurs during photosynthesis, mostly during diffusion of CO_2 from the atmosphere to the intercellular spaces (4‰) and during the carboxylation by the Rubisco enzyme (27-30‰) (Warren 2006). Given the dependence on CO_2 diffusion through stomata of both $\Delta^{13}C$ and the A/g_s ratio, ^{13}C discrimination is expected to scale negatively with WUE_i (Farquhar and Richards 1984). In line with the theory, a negative relationship between $\Delta^{13}C$ and WUE_i has been reported in herbaceous and (tree) species (Farquhar et al. 1989, Ponton et al. 2002), including poplar (Ripullone et al. 2004, Monclus et al. 2006, Fichot et al. 2011, Rasheed et al. 2013).

The detection of a negative linear relationship among $\Delta^{13}C$ and WUE_i can be obscured by differences in the time scale of integration, i.e. $\Delta^{13}C$ integrating the entire leaf life span and WUE_i being highly responsive to the changing environmental conditions. For instance, stomatal response often provides the primary limitation of carbon uptake during drought. An increased WUE in response to stomatal closure was reported in previous studies (Lauteri et al. 1997, Limousin et al. 2010, Chaves et al. 2002). Considering the relationship between photosynthetic capacity and leaf N, seasonal changes in water and nutrient availability largely influence factors driving the variation in WUE_i. Therefore, selecting genotypes for high WUE_i does not necessarily result in a selection towards higher productivity, as this depends very much on the source of variation that mainly drives the variation in WUE_i (Gilbert et al. 2011). The oxygen isotope discrimination during transpiration has been shown to reflect the genetic variation in g_s. This suggests that a combined use of both $\Delta^{13}C$ and $\Delta^{18}O$ might be the way to distinguish between the sources of variation in WUE_i (Barbour 2007).

The study of WUE in relation to growth performance is of interest for plantation management, especially for large-scale plantations managed for the purpose of biomass for energy. The absence of a trade-off between growth and $\Delta^{13}C$ in poplar suggested the potential of identifying high yielding genotypes with high WUE (Monclus et al. 2006, Chamaillard et al. 2011, Toillon et al. 2013). However, both positive (Zhang et al. 2004) and negative (Rasheed et al. 2013) correlations between growth and $\Delta^{13}C$ have been observed as well. These contrasting results – that depend on the species, site and growth determinants considered – illustrate the importance of a correct interpretation of the ecophysiological relationship between WUE (estimated as $\Delta^{13}C$) and growth, since both traits depend on different physiological drivers. Depending on the seasonal variation in environmental conditions, independent variation of both WUE and growth could hamper the detection of any WUE-growth relationship.

The objectives of this study were (i) to investigate and characterize seasonal variations in photosynthetic parameters, WUE_i and leaf stable isotope composition (^{13}C and ^{18}O), and; (ii) to examine how genotypes and timing throughout the growing season affect the relationships between the studied traits. The following research questions were addressed: (i) is $\Delta^{13}C$ a good estimator of WUE_i for different poplar genotypes and throughout the growing season; (ii) was the variation in WUE_i among genotypes and with environmental conditions driven by either A or g_s (estimated as $\delta^{18}O_{bl}$ as well); and (iii) did WUE_i correlate to growth parameters, and if so, was this relationship affected by genotype and/or the time scale of integration considered? The occurrence of low soil water availability during the growing season was studied in relation to seasonal variation of the above mentioned parameters. Simultaneous measurements of leaf N concentration allowed analyzing the seasonality of photosynthetic capacity.

2. Materials and Methods

2.1. Experimental site and plant material

The experimental site was located in Lochristi, East-Flanders, Belgium (51°06'44" N, 3°51'02" E; 6.25 m above sea level). The poplar bioenergy plantation was established in April 2010 on 18.4 ha of former agricultural land. The long-term average annual temperature at the site is 9.5 °C and the average annual precipitation is 726 mm, equally distributed over the year. A detailed soil analysis prior to planting characterized the soil type as a sandy texture, with clay-enriched deeper soil layers. After soil preparation, 25 cm long dormant and unrooted hardwood cuttings were planted at a density of 8,000 ha^{-1} in a double-row design, with alternating distances of 0.75 m and 1.50 m between the rows and 1.1 m between the individuals within each row. A total area of 14.5 ha was planted with 12 selected poplar genotypes representing different species and hybrids of *Populus deltoides*, *P. maximowiczii*, *P. nigra*, and *P. trichocarpa*, arranged in large monoclonal blocks. Neither irrigation nor fertilization was applied. Additional information on the site, the soil characteristics and the plantation lay-out can be found in Broeckx et al. (2012a). For this study, six out of the twelve poplar genotypes were retained, covering the different parentages present in the plantation: Koster and Oudenberg (*Populus deltoides* Bartr. (ex Marsh.) x *P. nigra* L.), Bakan and Skado (*P. trichocarpa* Torr & Gray (ex Hook) x *P. maximowiczii* Henry), Grimminge (*P. deltoides* x (*P. trichocarpa* x *P. deltoides*)) and Wolterson (*P. nigra*) (Broeckx et al. 2012a). All measurements reported in this paper were performed between May and September 2011, i.e. during the second growing season of the plantation.

2.2. Meteorological parameters

Meteorological parameters were recorded half-hourly using a meteorological mast installed at the experimental site. Air temperature and relative humidity data, recorded using Vaisala probes (model HMP45C, Vaisala, Helsinki, Finland), were used to calculate vapour pressure deficit (VPD). The amount of precipitation was measured with a tipping bucket rain gauge (model 3665R, Spectrum Technologies Inc., Plainfield, IL, USA). Moisture probes (TDR model CS616, Campbell Scientific, Logan, UT, USA) placed at depths of 20 cm and 40 cm close to the mast were used to measure soil water content (SWC, m^3 m^{-3}). As a complement, soil water potential (Ψ_S) was measured from June to November 2011 using calibrated equitensiometer probes (type EQ-2, Delta-T Devices Ltd, Cambridge, UK) installed at depths of 20 cm and 40 cm at four locations around the mast. We chose to characterize soil water availability along the growing season through the time-course of Ψ_S values, averaged among the four locations. Therefore Ψ_S values were extrapolated for the missing period (May-early June) based on the relationship observed between SWC and Ψ_S measurements at each measuring depth. A feed-forward Neural Network (Matlab R2012a, Mathworks, Natick, MA, USA) was used to interpolate missing values. The correlation between predicted and measured values ranged between 0.83 and 0.98 for the different soil depths (cfr. Broeckx et al. 2013).

2.3. Leaf gas exchange, chlorophyll content and photosynthetic nitrogen-use efficiency

Leaf gas exchange measurements were performed repeatedly on the same trees during the 2011 growing season in seven measurement campaigns (MC): 4-6 May (MC1), 18-20 May (MC2), 4-8 July (MC3), 27-29 July (MC4), 16-19 August (MC5), 5-9 September (MC6), 26-30 September 2011 (MC7). For the six genotypes, measurements were done on four replicate trees located close to the mast with a LI-6400 open path photosynthesis system (LiCor, Lincoln, NE, USA) equipped with a leaf chamber fluorometer (LI-6400-40, LiCor, Lincoln, NE, USA). Measurements were taken in the upper canopy, on the first fully mature sunlit leaf of the current-year main axis. To minimize differences in leaf age across MCs, we sampled leaves of the same leaf rank. Leaves were first acclimated for 10 min in the chamber at a CO_2 concentration of 400 ppm and under a photosynthetic photon flux density

(PPFD) of 1500 µmol m^{-2} s^{-1}; preliminary test experiments showed that this PPFD was enough to ensure saturating light conditions for all genotypes. Afterwards, light-saturated assimilation rate at atmospheric CO_2 concentration (A_{sat}, µmol m^{-2} s^{-1}) and stomatal conductance ($g_{s\text{-}sat}$, mol m^{-2} s^{-1}) were recorded before establishing the response of the net assimilation rate (A) to varying intercellular CO_2 concentrations (C_i), i.e. the A-C_i curve. Each curve consisted of 10 steps of external CO_2 concentrations set in succession to 400, 300, 250, 150, 100, 50, 500, 750, 1000 and 1250 ppm (Monclus et al. 2006). Leaves were allowed to equilibrate at least 3 min at each step before data were logged. Net assimilation rates were corrected for the effect of CO_2 diffusion, according to the instrument manual (LI-6400XT Version 6), using a diffusion correction term of 0.46 µmol s^{-1}. Before logging at each step of the A-C_i curves, steady state (F_s) and maximum fluorescence (F_m') were measured during a light-saturating pulse and the efficiency of the photosystem II (Φ_{PSII}) was determined as:

$$\Phi_{PSII} = \frac{(F_m' - F_s)}{F_m'}$$

Then, the CO_2 concentration in the chamber was set back to 400 ppm. Once the net assimilation rate had stabilized, the response to varying light intensities was recorded (A-light curve). Leaf photosynthesis was measured at eight PPFD intensities in the following order: 1500, 1000, 800, 600, 400, 200, 100, 0 µmol m^{-2} s^{-1}. A minimum of 2 min of leaf equilibration was set at each step before data were logged. Dark respiration was defined as the absolute CO_2 exchange rate measured during the last step of the A-light curve. All measurements were done at a constant block temperature (25°C) and at a controlled vapour pressure deficit (VPD) close to 1 kPa (1.2 ± 0.04, mean ± SE). Intrinsic water-use efficiency under saturating conditions ($WUE_{i\text{-}sat}$, $mmol_{CO2}$ mol_{H2O}^{-1}) was calculated as the ratio between the values of A_{sat} and $g_{s\text{-}sat}$ obtained from the A-C_i and A-light curves under reference conditions (PPFD of 1500 µmol m^{-2} s^{-1} and CO_2 concentration of 400 ppm).

Once gas exchange measurements were completed, a minimum of six chlorophyll readings was taken on the same leaf with a portable chlorophyll content meter (CCM-200, Opti-Sciences Inc., Hudson, NH, USA). Total chlorophyll content (Chl) was estimated from the CCM values according to the equations reported in Richardson et al. (2002). The leaf sampled was then harvested and the individual leaf area (LA) was measured using a LI-3000 leaf area meter (LiCor, Lincoln, NE, USA). A subsample was punched out of the leaf lamina to determine specific leaf area (SLA, cm^2 g^{-1}) after drying at 70 °C; SLA was only available from MC3 onwards. The dried leaf material was then ground and used for the assessment of the leaf nitrogen (N) content per unit mass (N_{mass}, mg g^{-1}) with an elemental analyzer (Carlo Erba, NA 1500-NC, Milan, Italy). Values of N_{mass} were converted to N content per unit area (N_{area}, mg cm^{-2}) using SLA values. Photosynthetic nitrogen use efficiency (PNUE, µmol mg^{-1} s^{-1}) was finally calculated as the ratio of A_{sat} (µmol m^{-2} s^{-1}) to N_{area}.

2.4. Estimation of mesophyll conductance and photosynthetic parameters

The mesophyll diffusion conductance to CO_2 from the sub-stomatal cavities to the chloroplast (g_m) was estimated by combining gas exchange and chlorophyll fluorescence measurements (Pons et al. 2009). The rate of photosynthetic electron transport (J_{ETR}) was calculated as:

$$J_{ETR} = \alpha * 0.5 * PPFD * \Phi_{PSII}$$

where α is the leaf absorptance and 0.5 is the fraction of photons absorbed by photosystem II. Absorptance was derived from CCM reading according to Bauerle et al. (2004) after conversion of the CCM readings to SPAD values (Richardson et al. 2002). Mesophyll conductance was then estimated following the equation of Harley et al. (1992):

$$g_m = \frac{A}{C_i - \frac{42.7[J_{ETR} + 8(A + R_d)]}{J_{ETR} - 4(A + R_d)}}$$

where 42.7 is the CO_2 compensation point in absence of dark respiration, as taken from Bernacchi et al. (2001), and R_d is the mitochondrial respiration in the light, taken as half of the dark respiration obtained from the A-light curves (Piel et al. 2002, Niinemets et al. 2005). The values of g_m were then used to convert A-C_i curves to A-C_c curves, with C_c being the CO_2 concentration in the chloroplast stroma calculated as (Limousin et al. 2010, Misson et al. 2010):

$$C_c = C_i - \frac{A}{g_m}$$

The maximum carboxylation rate (V_{cmax}) and the maximum rate of electron transport (J_{max}) were estimated by fitting the A-C_c curves to the biochemical photosynthesis model of Farquhar et al. (1980) using the routine developed by Sharkey et al. (2007). The Michaelis constant of Rubisco for carbon dioxide (K_c), the inhibition constant of Rubisco for oxygen (K_o) and the photocompensation point ($\Gamma*$) used for fitting were taken from Sharkey et al. (2007).

2.5. Carbon and oxygen stable isotope analyses

Isotopic analyses were performed at the Stable Isotope Laboratory of the James Hutton Institute (Invergowrie, Dundee, UK). Bulk leaf carbon isotope composition ($\delta^{13}C_{bl}$) was determined on the leaves used for gas exchange measurements. Subsamples of ground leaf material were enclosed and weighed in tin capsules and combusted in a continuous flow isotope ratio mass spectrometer (IRMS) (Delta V, Thermo Fisher Scientific, Bremen, Germany). The CO_2 produced by combustion was purified and its $^{13}CO_2/^{12}CO_2$ ratio was analyzed by the IRMS. The $\delta^{13}C_{bl}$ (‰) was expressed relative to the Pee Dee Belemnite (PDB) standard (Craig 1957). The accuracy of measurements was assessed by repeated measures of laboratory standards and was ± 0.08 ‰ (standard deviation). Carbon isotope discrimination between the atmosphere and the bulk leaf organic matter ($\Delta^{13}C_{bl}$, ‰) was then calculated as in Farquhar et al. (1989):

$$\Delta^{13}C_{bl} = \frac{(\delta^{13}C_{air} - \delta^{13}C_{bl})}{\left(1 + \left(\frac{\delta^{13}C_{bl}}{1000}\right)\right)} (‰)$$

with $\delta^{13}C_{air}$ assumed to equal -8‰.

The same leaf powder used for $\delta^{13}C$ analyses was used to measure the ^{18}O composition of bulk leaf matter ($\delta^{18}O_{bl}$). Leaf material was enclosed and weighed in silver capsules. Analyses were conducted with a continuous flow IRMS (Delta Plus XP, Thermo Fisher Scientific, Bremen, Germany) interfaced with a high temperature elemental analyzer. Bulk leaf oxygen isotope composition was expressed relative to the sea mean ocean water (VSMOW) standard and the analytical precision for repeated measurements was ± 0.09 ‰ (standard deviation).

2.6. Tree and leaf growth estimates

The stem diameter of the trees used for leaf gas exchange was measured repeatedly at each MC at 22 cm above the soil with a digital caliper (Mitutoyo, CD-15DC, UK, 0.01 mm precision). Tree dry mass (TDM) – defined as the above-ground woody dry mass per tree – was then estimated at each date from diameter measurements using genotype-specific allometric relationships established at the experimental site (see Broeckx et al. 2013). Biomass estimates between two MC were used to compute the relative growth rate (RGR, g g^{-1} day^{-1}) as:

$$RGR = \frac{ln(TDM_{MCx+1}) - ln(TDM_{MCx})}{t_{MCx+1} - t_{MCx}}$$

As a complement, the number of new leaves produced between two MCs was counted in order to calculate the leaf increment rate of the main axis (dTLN/dt; day^{-1}).

2.7. Statistical analyses

The mixed procedure for repeated measurements was used to analyze the effects of genotype and seasonality on photosynthetic, isotopic and growth parameters. Measurements were performed seven times during the growing season (repeated variable 'MC') on the same four replicate trees of each genotype (subject variable 'Genotype x Tree'). A mixed model with genotype, MC and the interaction – indicating the genotype specific behavior in time – was used. The unstructured repeated covariance type was used in this procedure. When significant genotype or MC effects were found, pair-wise comparisons of the means were performed using the Bonferroni adjustment. Pearson correlation coefficients were calculated between photosynthetic, isotopic and growth parameters using genotypic averages per MC. Analysis of covariance (ANCOVA) was used to test whether the $\Delta^{13}C$-WUE_i and V_{cmax}-N_{area} relationships varied among MCs or genotypes, with $\Delta^{13}C$ or V_{cmax} as the dependent variable, WUE_i or N_{area} as covariates and MC or genotype as categorical variable. All statistical analyses were performed in SPSS 20.0 (IBM Corp., SPSS Statistics for Windows, Armonk, NY).

3. Results

The time course for precipitation, air temperature, daytime maximum VPD and soil water availability at the site during the 2011 growing season (May – September) is presented in Fig. 1. A first drop in soil water potential (Ψ_s) was observed at the end of May in the upper soil layer (20 cm depth), with Ψ_s peaking at *ca.* -1.8 MPa close to MC2, while there was no apparent response in the 40 cm depth layer; the precipitation and the lower VPD that occurred afterwards led to a progressive recovery. A second drop in Ψ_s around -1.5 MPa was observed in mid-July (close to MC3) for both soil layers, concomitantly with high air temperatures and high VPD. The high amount of precipitation after mid-July combined with a progressive decrease in VPD resulted in the recovery of Ψ_s up close to zero for the rest of the growing season (MC4 to MC7).

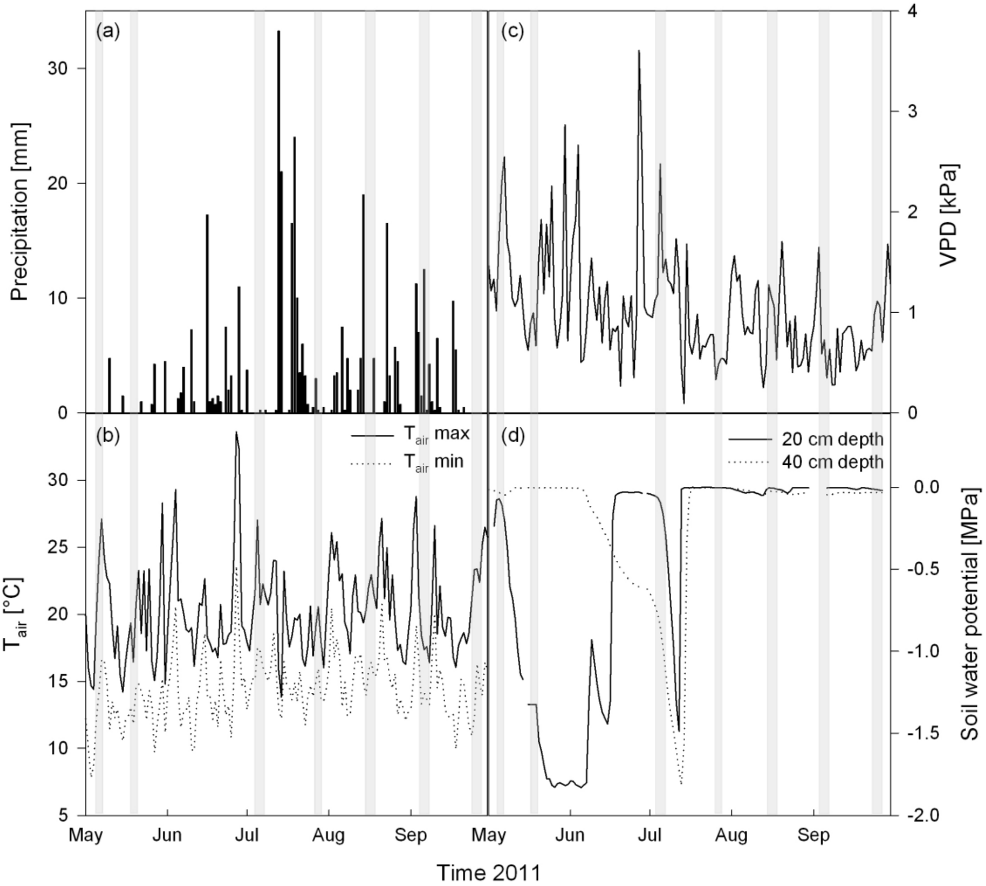

Fig. 1 Seasonal time course of the main meteorological parameters during the period of this study (May – September 2011): (a) precipitation; (b) daily minimum (T_{min}) and maximum (T_{max}) air temperature; (c) daytime maximum vapour pressure deficit (VPD) and (d) soil water potential at 20 cm (solid line) and 40 cm (dotted line) soil depths. Gray bars indicate the timing of gas exchange measurement campaigns (MCs).

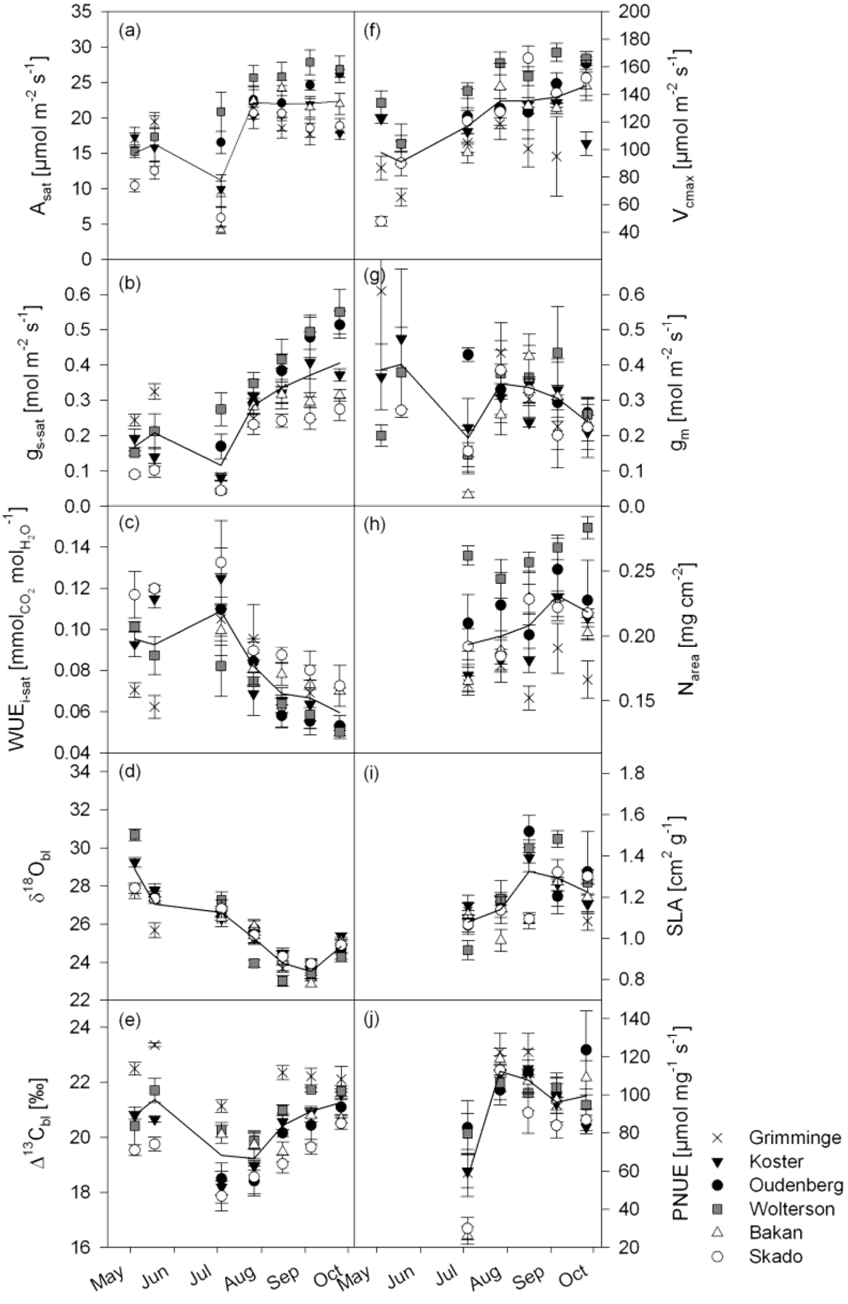

Fig. 2 Seasonal evolution of (a) net assimilation rate (A_{sat}); (b) stomatal conductance ($g_{s\text{-}sat}$); (c) intrinsic water-use efficiency (WUE_i); (d) bulk leaf oxygen isotope concentration ($\delta^{18}O$); (e) bulk leaf carbon isotope discrimination ($\Delta^{13}C_{bl}$); (f) maximum carboxylation rate (V_{cmax}); (g) mesophyll conductance (g_m); (h) area-based leaf nitrogen content (N_{area}); (i) specific leaf area (SLA) and; (j) photosynthetic nitrogen-use efficiency (PNUE). Data points represent genotypic means (±SE) for six poplar genotypes of different parentages: T×M (Bakan, Skado), D×N (Koster, Oudenberg), D×(T×D) (Grimminge), and N (Wolterson). The black line represents the average seasonal pattern across genotypic averages.

Overall, most of the leaf traits differed significantly among genotypes and fluctuated along the MCs (Fig. 2, Table 1). Considering the average pattern across the six genotypes, A_{sat}, g_{sat} and $\Delta^{13}C_{bl}$ exhibited a similar time course with a pronounced decrease in mid-July (MC3) – when soil water potential was low at both 20 and 40 cm depth – and a progressive increase towards the end of the growing season (Fig. 2). Values of $WUE_{i\text{-}sat}$ followed an opposite trend (Fig. 2). The time course observed for $V_{cmax,}$ J_{max} and g_m was slightly different; V_{cmax} and J_{max} increased progressively during the growing season and g_m decreased, especially in July (MC3; Fig. 2). The overall means of V_{cmax} and J_{max} were 125.2 µmol m^{-2} s^{-1} and 172.4 µmol m^{-2} s^{-1} respectively (Fig. 2), both parameters being strongly and linearly correlated ($r = 0.85$; $P < 0.001$). Although a significant MC effect was found (Table 1), the seasonal trend in N_{area} was less clear, potentially due to missing data points in the beginning of the growing season. On the other hand, PNUE was lower in July at low soil water availability (MC3; Fig. 2), but – as for N_{area} – response to water availability was unclear due to missing data points. Looking further in detail, the six genotypes did not respond in the same way or with the same amplitude with time, as indicated by the significant genotype × MC interactions observed for most traits (Table 1; see also Fig. 2). Differences among genotypes were particularly reinforced during the dry period around MC3 (Fig. 2). The genotypes Wolterson and Oudenberg were clearly less responsive than the other genotypes in terms of A_{sat}, $g_{s\text{-}sat}$, $WUE_{i\text{-}sat}$ and PNUE (Fig. 2). Overall, the genotype Wolterson exhibited the highest values of A_{sat}, $g_{s\text{-}sat}$, V_{cmax} and N_{area} throughout most of the growing season (Fig. 2), while $WUE_{i\text{-}sat}$ was in the lower end of the genotypic range (Fig. 2). The ranking of the other genotypes changed substantially during the growing season, although the genotypes Bakan and Skado remained consistently in the lower end of the range for A_{sat} and $g_{s\text{-}sat}$ (Fig. 2). The genotype Skado exhibited the lowest $\Delta^{13}C_{bl}$ and the highest $WUE_{i\text{-}sat}$ throughout the entire season (Fig. 2); at the opposite, the highest $\Delta^{13}C_{bl}$ values were observed for the genotype Grimminge, which also exhibited the lowest V_{cmax} and J_{max} values with an early decrease from August onwards (MC5-7; Fig. 2). In contrast to other traits, the genotypic ranking for N_{area} was only slightly changed with time (no significant genotype × MC interaction, Table 1; Fig. 2), Wolterson and Grimminge generally exhibiting the highest and lowest values, respectively (Fig. 2).

Table 1 Output of the mixed model analysis showing the effects of genotype and time in the season (MC) on photosynthetic, isotopic and growth parameters. The different parameters have been identified and described in the text.

Parameters	df	genotype	df	MC	df	genotype*MC
A_{sat}	5	***	6	***	25	***
$g_{s\text{-}sat}$	5	***	6	***	25	***
$WUE_{i\text{-}sat}$	5	**	6	***	25	*
V_{cmax}	5	**	6	***	25	***
J_{max}	5	**	6	***	25	***
g_m	5		6	***	24	***
$\Delta^{13}C_{bl}$	5	***	6	***	26	*
$\delta^{18}O_{bl}$	5	**	6	***	26	***
PNUE	5	**	4	***	19	
N_{area}	5	***	4	**	20	
Chl_{tot}	5	***	3	***	15	**
SLA	5		4	***	20	**
dTLN/dt	5	***	3	***	15	***
TDM	5	***	4	***	20	***
RGR	5		3	***	15	***

* = $0.01 < P \leq 0.05$; ** = $0.001 < P \leq 0.01$; *** = $P \leq 0.001$; MC = measurement campaign

Values of A_{sat} and $g_{s\text{-}sat}$ were significantly but non-linearly related ($A_{sat} = g_{s\text{-}sat}/(0.0055+0.0288^{*}\ g_{s\text{-}sat})$; $R^2 = 0.776$, $P < 0.001$) with A_{sat} reaching saturation at high $g_{s\text{-}sat}$ (Fig. 3). Although the curvature was conserved, the elevation of the curve differed among genotypes (Fig. 3), Wolterson displaying the highest values of A_{sat} at high $g_{s\text{-}sat}$ while Skado and Koster displayed the lowest values. When pooling all data, both A_{sat} and $g_{s\text{-}sat}$ were strongly and negatively related to $WUE_{i\text{-}sat}$ (Fig. 4). To examine the observed variability related to genotypic variation relative to seasonal variation, relationships were further dissected either between genotypes or between MCs. When pooling data across dates, A_{sat} and $g_{s\text{-}sat}$ were still significantly related to WUE_i within each genotype with the exception of Grimminge for A_{sat} (Table 2). When pooling data across genotypes, the relationship between $g_{s\text{-}sat}$ and $WUE_{i\text{-}sat}$ was always significant irrespective of the MC considered, although weaker at MC3 (Table 2); in contrast, the A_{sat}-$WUE_{i\text{-}sat}$

relationship was only significant in the beginning of the growing season (MC1 and 2; Table 2).

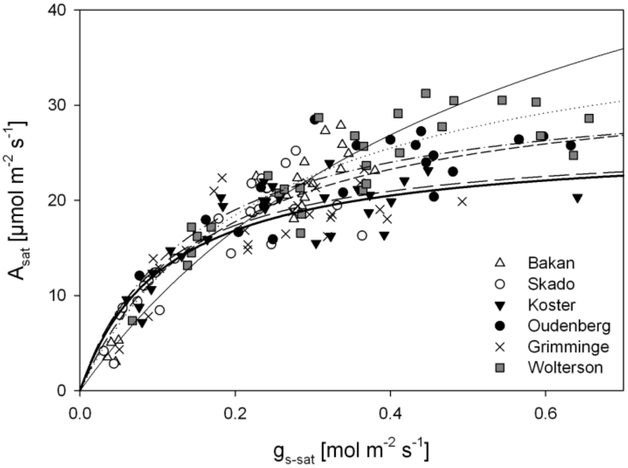

Fig. 3 Hyperbolic response of PPFD saturated net assimilation rate (A_{sat}) to stomatal conductance ($g_{s\text{-}sat}$) for different genotypes; short dashed = Bakan ($R^2 = 0.92$), long dashed = Skado ($R^2 = 0.82$), bold = Koster ($R^2 = 0.71$), dash-dot = Oudenberg ($R^2 = 0.67$), dotted = Grimminge ($R^2 = 0.66$), solid = Wolterson ($R^2 = 0.80$), with $P < 0.001$ for all regressions.

As expected from theory, $\Delta^{13}C_{bl}$ scaled significantly and negatively with $WUE_{i\text{-}sat}$ although the relationship exhibited some scatter (Fig. 4). When pooling across dates, the relationship was significant for the four genotypes Skado, Koster, Oudenberg and Grimminge only (Table 2); the relationship was also variable in time and not significant for MC4 and 6 (Table 2). A significant and negative non-linear relationship was observed between $\delta^{18}O_{bl}$ and $g_{s\text{-}sat}$ for pooled data (Fig. 5; $g_{s\text{-}sat} = 0.002 + 27.283*\exp(-0.183*\delta^{18}O_{bl})$, $R^2 = 0.342$, $P \leq 0.001$). This relationship was found significant for all genotypes except Grimminge (Table 2), while it was highly variable throughout the growing season (Table 2). No significant correlation was recorded between $\delta^{18}O_{bl}$ and $\Delta^{13}C_{bl}$ for pooled data (Fig. 5) and the relationship was only punctually significant when considering data per genotype or per MC (Table 2).

Correlations among other physiological variables are given in Table 3. A_{sat} and V_{cmax} were significantly and positively correlated. No correlation was found between V_{cmax} and g_m. V_{cmax} scaled positively with leaf N content, especially when expressed on an area basis (Table 3), and the relationship did not change significantly during the growing season (data not shown). V_{cmax} and N_{area} scaled positively with Chl. N_{area} was significantly and negatively correlated to $WUE_{i\text{-}sat}$ but no relationship could be observed with $\Delta^{13}C_{bl}$. A significant and negative relationship was found between $WUE_{i\text{-}sat}$ and PNUE, although genotype specific correlations were not significant (data not shown). No correlation was observed between N_{area} and SLA while N_{mass} was significantly and positively correlated to SLA.The maximum rate of carboxylation was not correlated to SLA, but negatively to $\Delta^{13}C_{bl}$.

Table 2 Pearson r coefficients between $WUE_{i\text{-}sat}$ and $\delta^{18}O_{bl}$ on the one hand and A_{sat}, $g_{s\text{-}sat}$ and $\Delta^{13}C_{bl}$ on the other hand, per genotype and per measurement campaign (MC) throughout the growing season.

Pearson r		n	A_{sat}	$g_{s\text{-}sat}$	$\Delta^{13}C_{bl}$
$WUE_{i\text{-}sat}$	Bakan	20	**-0.456***	**-0.672****	-0.362
	Skado	28	**-0.489***	**-0.791*****	**-0.484***
	Koster	28	**-0.580****	**-0.893*****	**-0.478***
	Oudenberg	20	**-0.594****	**-0.869*****	**-0.719*****
	Grimminge	24	-0.233	**-0.812*****	**-0.627****
	Wolterson	28	**-0.638*****	**-0.898*****	-0.328$^{(*)}$
	MC1	16	**-0.553***	**-0.859*****	**-0.618***
	MC2	16	**-0.562***	**-0.942*****	**-0.937*****
	MC3	24	-0.169	**-0.513***	**-0.653****
	MC4	24	0.145	**-0.766*****	-0.232
	MC5	24	0.050	**-0.738*****	**-0.562****
	MC6	24	-0.259	**-0.876*****	-0.228
	MC7	20	-0.203	**-0.757*****	**-0.488***
$\delta^{18}O_{bl}$	Bakan	20		**-0.533***	-0.037
	Skado	28		**-0.765*****	-0.075
	Koster	28		**-0.587****	0.048
	Oudenberg	20		**-0.722*****	**-0.565****
	Grimminge	24		-0.386$^{(*)}$	-0.028
	Wolterson	28		**-0.725*****	-0.233
	MC1	16		-0.166	-0.299
	MC2	16		**-0.645***	**-0.656****
	MC3	24		**0.576****	0.204
	MC4	24		**-0.434***	-0.194
	MC5	24		-0.346$^{(*)}$	-0.113
	MC6	24		0.051	**-0.458***
	MC7	20		**-0.458***	-0.277

$^{(*)}$ = 0.05 < P ≤ 0.10; * = 0.01 < P ≤ 0.05; ** = 0.001 < P ≤ 0.01; *** = P ≤ 0.001

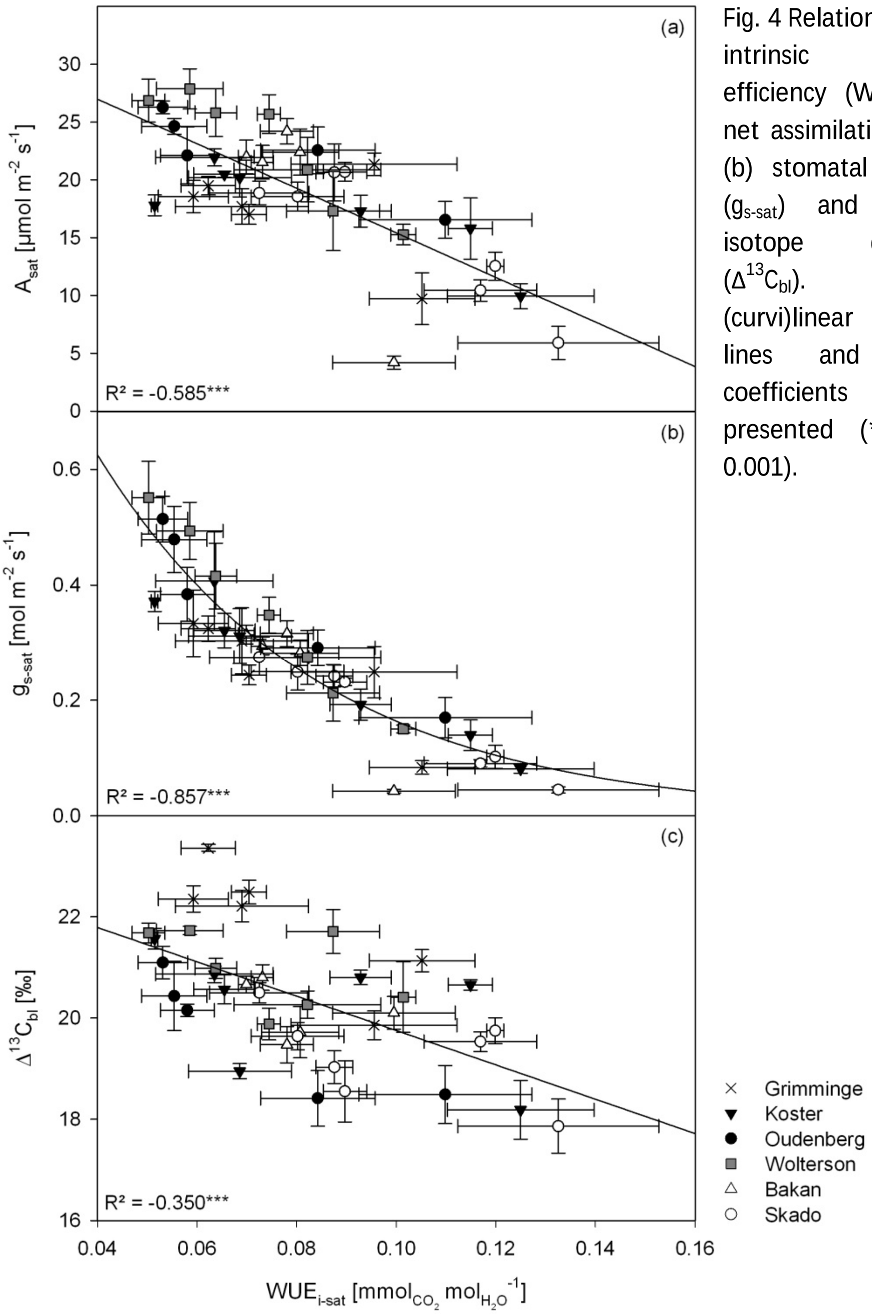

Fig. 4 Relationship between intrinsic water-use efficiency (WUE_i) and (a) net assimilation rate (A_{sat}); (b) stomatal conductance ($g_{s\text{-}sat}$) and (c) carbon isotope discrimination ($\Delta^{13}C_{bl}$). Significant (curvi)linear regression lines and correlation coefficients (R²) are presented (*** = P ≤ 0.001).

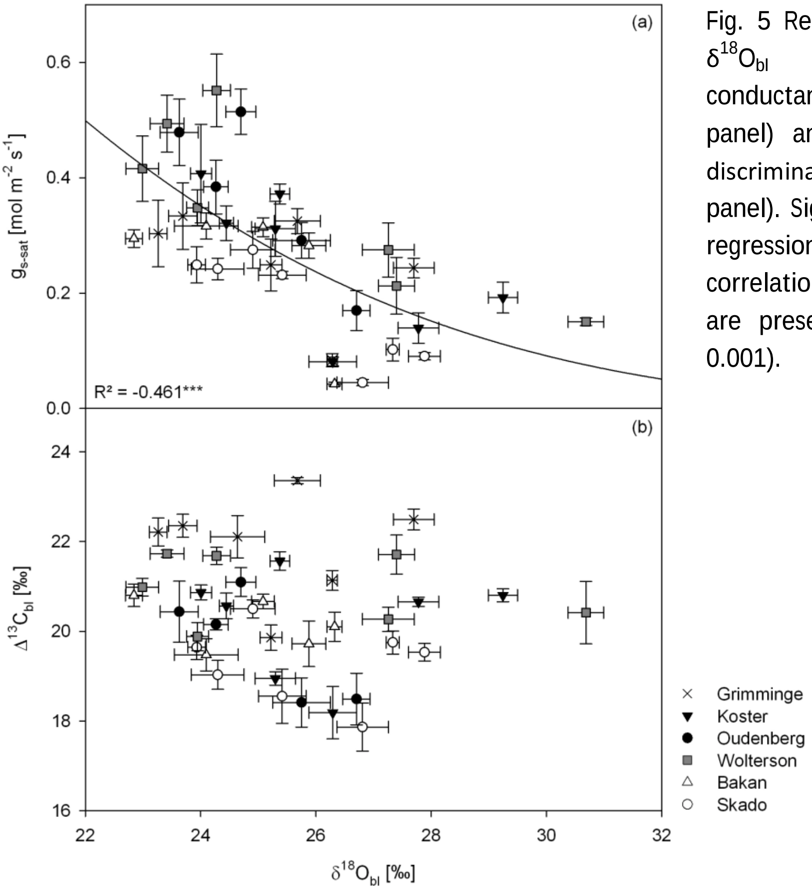

Fig. 5 Relationship between $\delta^{18}O_{bl}$ and stomatal conductance ($g_{s\text{-}sat}$, top panel) and carbon isotope discrimination ($\Delta^{13}C$; bottom panel). Significant curvilinear regression line and correlation coefficient (R²) are presented (*** = $P \leq 0.001$).

Genotypes differed significantly for TDM, RGR and dTLN/dt but responded differently with time as indicated by the significant genotype × MC interactions recorded (Table 1, Fig. 6). Tree dry mass increased progressively until the end of September, genotypes Bakan and Koster starting to slow down earlier than others (Fig. 6a). The highest and lowest TDM values were consistently found for the genotypes Skado and Wolterson, respectively (Fig. 6a); the genotypic ranking for RGR and dTNL/dt was highly variable throughout the growing season. Relative growth rate was neither significantly related to LA nor to SLA, but RGR was positively related to dTNL/dt ($r = 0.283$, $P = 0.012$). LA scaled positively to TDM ($r = 0.533$, $P < 0.001$) and Chl ($r = 0.609$, $P < 0.001$).

Table 3 Pearson correlation coefficients (r) between photosynthetic and isotopic parameters and leaf characteristics based on individual tree measurements throughout the growing season (n ≥ 116).

	A_{sat}	$g_{s\text{-}sat}$	g_m	V_{cmax}	Chl	N_{mass}	N_{area}	PNUE	SLA
WUE_i	**-0.517*****	**-0.818*****	0.049	-0.156$^{(*)}$	-0.069	**-0.287*****	-0.134	**-0.460*****	**-0.457*****
$\Delta^{13}C$	**0.188***	**0.369*****	0.049	**-0.223****	0.082	**0.250****	-0.104	**0.347*****	**0.438*****
A_{sat}	1	**0.803*****	**0.490*****	**0.552*****	**0.268****	**0.456*****	**0.538*****	**0.782*****	**0.253****
$g_{s\text{-}sat}$		1	**0.179***	**0.405*****	0.135	**0.425****	**0.415*****	**0.632*****	**0.438*****
g_m			1	0.019	-0.015	**0.251****	**0.191***	**0.569*****	0.143
V_{cmax}				1	**0.535*****	**0.376*****	**0.677*****	0.167$^{(*)}$	0.005
Chl					1	**0.455*****	**0.454*****	0.046	0.121
N_{mass}						1	**0.786*****	0.165$^{(*)}$	**0.482*****
N_{area}							1	-0.081	0.144
PNUE								1	**0.431*****

$^{(*)}$ = 0.05 < P ≤ 0.10; * = 0.01 < P ≤ 0.05; ** = 0.001 < P ≤ 0.01; *** = P ≤ 0.001

Relationships between traits related to growth and carbon uptake on the one hand, and WUE estimates ($WUE_{i\text{-}sat}$ and $\Delta^{13}C_{bl}$) on the other hand were highly dependent upon the traits and the time scale considered (Table 4); the relationships were analyzed for the time integration considered by the different parameters on the one hand (simultaneous), and for growing season integration on the other hand (seasonal average). For simultaneous measurements, this indicates an integration of the leaf life span for $\Delta^{13}C_{bl}$, LA and SLA, while both dTNL/dt and RGR integrate the period between MC_x and MC_{x-1}. Seasonal average values of LA, SLA, dTNL/dt, $WUE_{i\text{-}sat}$ and $\Delta^{13}C_{bl}$ per tree integrate the whole growing season, averaging all MCs. For RGR and TDM, the integration over the growing season is represented by the biomass increment between the first and last MC and the final TDM at the end of the growing season, respectively. Specific leaf area was significantly correlated with both $WUE_{i\text{-}sat}$ and $\Delta^{13}C_{bl}$; a higher SLA resulted in a lower WUE (Table 4). LA was significantly related to $WUE_{i\text{-}sat}$ and $\Delta^{13}C_{bl}$ only when considering the seasonal average; larger leaves were associated with a higher WUE (Table 4). No relationship was observed between dTNL/dt and WUE estimates (Table 4). Final TDM was negatively related to $\Delta^{13}C_{bl}$ when averaged over the entire growing season, but there was no correlation when measured over a shorter time period during the growing season (Table 4). Inversely, the RGR was significantly and negatively correlated to $\Delta^{13}C_{bl}$ when calculated over the same time period, while there was no correlation when seasonal averages were considered. $WUE_{i\text{-}sat}$ was not correlated to RGR, irrespective of the time scale considered (Table 4).

4. Discussion

In this study, all measurements were performed on mature leaves of similar leaf age, under normalized conditions of light intensity, leaf temperature and VPD. The observed seasonal variations could be therefore largely ascribed to variations in resource availability. However, despite variations along the growing season as well as among genotypes, leaf gas exchange rates recorded were overall consistent with previously published data for several poplar species and hybrids (Ripullone et al. 2004, Monclus et al. 2006, Gornall and Guy 2007, Fichot et al. 2010 & 2011).

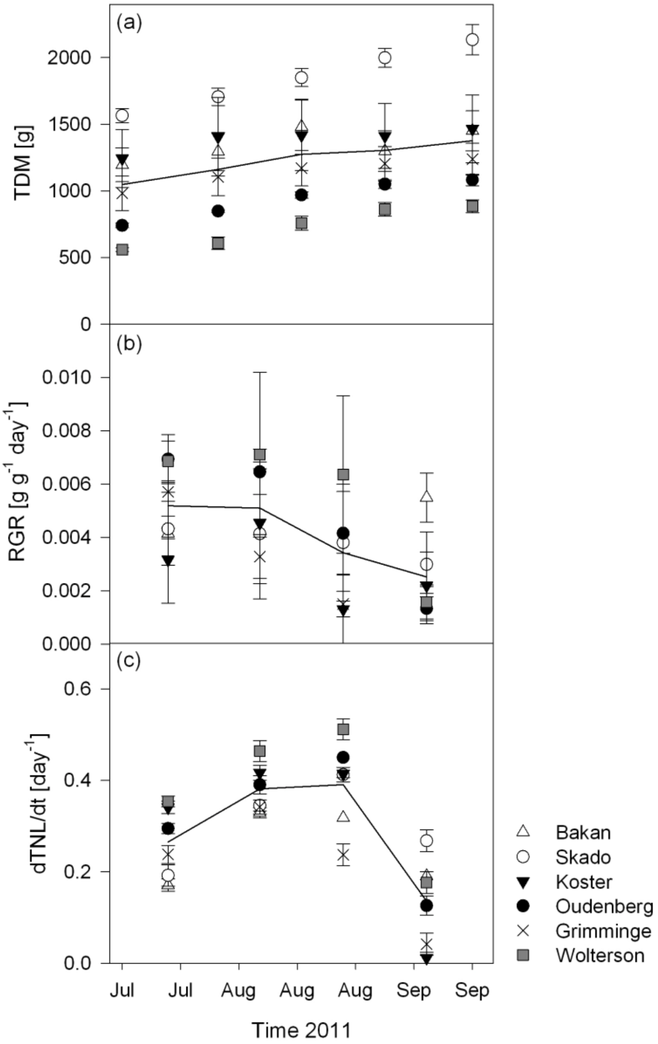

Fig. 6 Seasonal evolution of growth related traits for different genotypes: (a) tree dry mass (TDM); (b) relative growth rate of the stem (RGR) and; (c) the number of new leaves produced (dTNL/dt).

Table 4 Pearson correlation coefficients using different time scales of integration (simultaneous measurements in each MC or averaged over the growing season). The different parameters and acronyms are defined and explained in the text.

	n	n	$\Delta^{13}C_{bl}$		$WUE_{i\text{-}sat}$	
	simulta-neous	seasonal average	Simulta-neous	seasonal average	Simulta-neous	seasonal average
d(TLN)/dt	90	24	-0.175	-0.121	0.056	-0.006
TDM	120	24	-0.183	**-0.429***	-0.003	**0.559****
RGR	84	24	**-0.446***	-0.070	0.061	-0.123
LA	115	24	0.119	**-0.412***	-0.158	**0.511***
SLA	115	24	**0.438*****	0.398$^{(*)}$	**-0.457*****	-0.370$^{(*)}$

$^{(*)}$ = $0.05 < P \le 0.10$; * = $0.01 < P \le 0.05$; ** = $0.001 < P \le 0.01$; *** = $P \le 0.001$

A high photosynthetic carbon uptake was sustained until late in the season. Indeed, although measurements lasted until the very end of September, A_{sat} and V_{cmax} were still close to maximal values. The high photosynthetic carbon uptake rates were associated with a high N investment in leaves until late in the growing season, as could be expected from the known relationship between V_{cmax} and N_{area} (Wilson et al. 2000, Grassi et al. 2005, Xu and Baldocchi 2003). Similarly high assimilation rates have been previously observed in poplar until the end of the growing season (Bernacchi et al. 2003). This pattern is also in agreement with the canopy level (eddy covariance) measurements of net ecosystem exchange in the same plantation showing a net ecosystem carbon uptake until the end of September (Zona et al. 2013b, Broeckx et al. 2013). This contrasts however with data reported in other temperate deciduous species for which photosynthetic uptake and photosynthetic capacity already showed a substantial decline by early-mid September (Wilson et al. 2000, Grassi et al. 2005, Dillen et al. 2012). Delayed senescence with sustained carbon uptake is most probably associated with the pioneering and fast-growth habit of poplar species (Eckenwalder 1996). However, RGR of the stem decreased from August onwards, and the production of new leaves also rapidly decreased towards the beginning of September. Above-ground biomass production was also slowed down at the end of the growing season, although not completely stopped in several genotypes (Skado and to a lesser extent Wolterson and Oudenberg). This suggested a shift in allocation of photosynthates to other (below-ground) tree compartments at the end of the growing season, at least for some genotypes (Broeckx et al. 2013). The highest photosynthetic performance was observed for the genotype with the lowest TDM (Wolterson); the lower photosynthetic rates were likely compensated by the larger leaf area in high yielding genotypes (such as Skado) (Tharakan et al. 2005, Marron et al. 2007). The lowest and highest total leaf area observed for genotypes Wolterson and Skado, respectively, at the experimental site among four genotypes included in this study (Broeckx et al. 2012b), is in line with this hypothesis.

In contrast to previous findings in oak (Wilson et al. 2000, Grassi et al. 2005), the correlation between V_{cmax} and N_{area} did not vary throughout the growing season (data not shown) but spring measurements were missing and the relationship was not significant at the end of September. The absence of seasonality indicates no change in N allocation to the photosynthetic apparatus, at least during the summer period (July to early September). The relationship was significant for all genotypes except Koster, suggesting N_{area} as a reliable estimator of photosynthetic capacity for most genotypes during a substantial period of the growing season.

Stomatal closure in response to low water availability is the primary limitation of carbon assimilation rate (Hogg et al. 2000, Regier et al. 2009, Chaves et al. 2002). Considering the temporal dynamics of soil water potential, the drop in gas exchange rates observed in early July could be attributed to seasonal drought. However, not all genotypes responded similarly indicating substantial genotypic variation in the degree of this response; genotypes Wolterson and Oudenberg showed no decreased A_{sat} and $g_{s\text{-}sat}$ at low soil water availability, although spring measurements were missing for genotype Oudenberg. Local measurements of soil water potential around the mast could not exclude the possibility of genotypic differences in soil water potential related to genotypic differences in total leaf area; bigger trees encountering rapid and more severe water shortage due to high transpiratory water loss. A reduction in g_m was observed with decreasing $\Delta^{13}C_{bl}$ and increasing $WUE_{i\text{-}sat}$ at low soil water potential (MC3). A constant N_{area} with SLA, $WUE_{i\text{-}sat}$ and $\Delta^{13}C_{bl}$ suggested that N_{area} was not responsive to water stress. The same amount of N per area was diluted over a higher mass per area in thicker leaves. Contrasting results on the effect of water availability on leaf N have been reported in literature (Reich et al. 1989, Poorter and Evans 1998, Grassi et al. 2005, Han 2011). The constant N_{area} during drought explains the absence of a reduction of V_{cmax} in response to low water availability, and indicates that there was no change in N allocation to the photosynthetic apparatus (Rubisco and chlorophyll). Previously, V_{cmax} was found unaffected by water stress in *Quercus* and *Fagus* spp. (Cano et al. 2013). These results indicated a reduction in A_{sat} due to stomatal and mesophyll conductance rather than biochemical limitation.

Seasonal variation of $WUE_{i\text{-}sat}$ is driven by a differential response of A_{sat} and $g_{s\text{-}sat}$ to the environment as a result of the differences in the physiological driving factors, although both parameters are not independent. This was reflected in the non-linear relationship, with A_{sat} reaching a genotype dependent plateau at high $g_{s\text{-}sat}$. While variation along the curve is mainly driven by soil water potential (Ψ_s), variation in the elevation of the curve is generally related to the intrinsic photosynthetic capacity, i.e. V_{cmax} and J_{max} (Farquhar et al. 1980). Hence, the same $WUE_{i\text{-}sat}$ might be observed for genotypes with low photosynthetic capacity operating at low stomatal conductance as for genotypes with high intrinsic photosynthetic capacity and high stomatal conductance (Gilbert et al. 2011). The latter contributed to differences in the genotypic ranking among $WUE_{i\text{-}sat}$, A_{sat} and $g_{s\text{-}sat}$, also changing throughout the growing season. The observed negative $\Delta^{13}C_{bl}$-$WUE_{i\text{-}sat}$ relationship varied significantly among genotypes and with timing throughout the growing season. This observation confirms the effects of both species and water availability on the relationship between $\Delta^{13}C_{bl}$ and $WUE_{i\text{-}sat}$ that were previously reported for poplar (DesRocher et al. 2007, Larchevêque et al. 2001, Xu et al. 2008). The absence of a correlation in genotype Wolterson could be most likely explained by the lower water stress experienced as a consequence of the low(er) total leaf area, hence the lower transpiration and soil water depletion, as mentioned above. The limited variation in $g_{s\text{-}sat}$ driving the variation in $WUE_{i\text{-}sat}$ caused the inability to detect a significant $\Delta^{13}C_{bl}$-$WUE_{i\text{-}sat}$ relationship. No correlation was found when soil water availability recovered after heavy rainfall, i.e. at the end of July (MC4), in this study. This potentially reflected the difference in integrated time considered between both parameters, where $\Delta^{13}C$ still reflected the effects of low soil water availability in previous weeks while WUE_i reflected non-limiting water conditions at that time. Both differences in time scale of integration between $\Delta^{13}C_{bl}$ and $WUE_{i\text{-}sat}$ and the dependence of $WUE_{i\text{-}sat}$ on A_{sat} caused the high degree of scatter in the $\Delta^{13}C_{bl}$-$WUE_{i\text{-}sat}$ relationship.

Substantial variation was found in $\Delta^{13}C_{bl}$ and $WUE_{i\text{-}sat}$ among the six genotypes studied, confirming earlier observations in poplar (Rasheed et al. 2013, Monclus et al. 2005 & 2006, Dillen et al. 2011). As expected from theory, $\Delta^{13}C_{bl}$ and $WUE_{i\text{-}sat}$ were signifcanlty and negatively related. By definition, variations in $WUE_{i\text{-}sat}$ can originate from either variations in A_{sat}, g_{sat} or both. The strong correlation between $\Delta^{13}C_{bl}$ en $g_{s\text{-}sat}$, both when considering data per date or per genotype, suggested stomatal conductance as the main driver of variation in $WUE_{i\text{-}sat}$, in line with previous results (Monclus et al. 2006). This was confirmed by the absence of a correlation between photosynthetic capacity (V_{cmax}) and $WUE_{i\text{-}sat}$. The contra-intuitive negative correlation between $WUE_{i\text{-}sat}$ and A_{sat} indicated a strong stomatal control of net assimilaton rates, $g_{s\text{-}sat}$ variations outperforming variations in A_{sat}. However, if $g_{s\text{-}sat}$ is assumed to drive variation in $WUE_{i\text{-}sat}$, a stronger correlation between $g_{s\text{-}sat}$ or $\Delta^{13}C_{bl}$ and $\delta^{18}O_{bl}$ would be expected, since oxygen isotope discrimination during transpiration has been shown to reflect genetic variation in g_s (Barbour 2007). The detection of any relationship might be hindered by missing oxygen isotope discrimination values ($\Delta^{18}O$) due to the missing values of source water $\delta^{18}O$, since $\delta^{18}O_{bl}$ on organic matter did not reflect xylem water $\delta^{18}O$ from the transpiration stream. Reduced assimilation rate caused by a decrease in stomatal conductance with constant N allocation increased the nitrogen cost per unit of carbon gain. This trade-off between $WUE_{i\text{-}sat}$ and PNUE suggested maximization of resource use efficiency depending on the most limited resource (Reich et al. 1989, Xu and Baldocchi 2003, Han 2011). Instead, no correlation was found between PNUE and neither N_{area} nor N_{mass}. The inverse relationship between $WUE_{i\text{-}sat}$ and PNUE emphasized stomatal conductance as the main driver of variation in $WUE_{i\text{-}sat}$ (Soolanayakanahally et al. 2009). This trade-off suggested a parallel variation of $g_{s\text{-}sat}$ and g_m (Warren and Adams 2006, Soolanayakanahally et al. 2009); although the correlation between stomatal and internal conductance was not very strong in this study, a parallel reduction of both parameters was found when soil water availability was limiting (MC3). The positive (or negative) correlation recorded between $\Delta^{13}C_{bl}$ (or $WUE_{i\text{-}sat}$) and SLA suggested thicker leaves when discrimination (water-use) was increased (decreased) in response to low water availability. The development of small and thick leaves is – for tree species – generally seen as an adaptation to drought and to high irradiance (Groom and Lamont 1997, Niinemets 2001). The absence of a correlation with g_m of either $\Delta^{13}C_{bl}$, $WUE_{i\text{-}sat}$ and SLA contradicts previous suggestions that internal conductance contributes to this variation in $WUE_{i\text{-}sat}$ with SLA (Monclus et al. 2006).

Understanding the relationship between WUE and growth is gaining the interest of breeders, as they want to optimize WUE when selecting for high yielding genotypes. Differences in the time scale of integration and in the physiological drivers, however, may obscure the detection of a relationship between both traits. Relative growth rate and $\Delta^{13}C_{bl}$ integrated a similar time period of several weeks; this explained the detection of a negative correlation in absence of a correlation between RGR and $WUE_{i\text{-}sat}$. When averaged over the growing season, no significant correlation was found between RGR and $\Delta^{13}C_{bl}$. Similarly, only seasonal average $\Delta^{13}C_{bl}$ was negatively correlated to final TDM at the end of the growing season, reconfirming the importance of the time scale of integration when the relationship between WUE and growth is addressed. The small contribution of A_{sat} to RGR and the absence of a correlation with SLA, suggested that RGR is mainly dependent on the allocation of biomass to the photosynthetic compartment (Shipley 2006). Both individual and total leaf area might be good indicators of biomass production in poplar (Larson and Isebrands 1972, Monclus et al. 2005, Marron et al. 2007, Broeckx et al. 2012b, Verlinden et al. 2013). The lack of a positive correlation between $WUE_{i\text{-}sat}$ and RGR is in line with the observation that $g_{s\text{-}sat}$ drives most of the variation in $WUE_{i\text{-}sat}$.

5. Conclusion

Substantial seasonal variation in photosynthetic parameters was observed in response to soil water availability, although the response was genotype dependent. Photosynthetic limitation at low soil water potential could be ascribed to stomatal rather than biochemical limitations. N_{area} was a reliable estimator of photosynthetic capacity for most genotypes in the experiment, at least during the summer period of the studied growing season. As could be predicted based on the theory behind, carbon isotope discrimination ($\Delta^{13}C$) scaled negatively with intrinsic water-use efficiency (WUE_i), but the relationship varied considerably among genotypes and throughout the growing season. The variation among genotypes was likely explained by the genotypic variation in stomatal response to low soil water availability. The variation throughout the growing season could be mainly ascribed to the difference in the temporal scale $\Delta^{13}C$ versus WUE_i. Stomatal conductance was the main driver of variation in WUE_i. We found no straightforward relationship between water-use efficiency – estimated as $\Delta^{13}C$ and WUE_i – and growth determinants. The results suggested a considerable effect of the time scale of integration. A negative correlation between stem biomass production and $\Delta^{13}C$ was observed when similar time scales were considered.

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