Growth performance, population dynamics and floristic diversity in willow cultivations

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Cover: The field experiment at Ultuna, Sweden. One-year-old shoots of willow clone Tordis surrounded by senesced flowers of *Taraxacum officinale* L. and blooming flower of *Papaver dubium* L.
(photo: M. Welc)

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Abstract

In commercial practice, willows are propagated from dormant cuttings. Use of non-dormant propagules could reduce the establishment costs. The overall objective of this study was to assess the effects of propagule phenology (non-dormant versus dormant), planting system (cuttings versus billets) and planting time on establishment and biomass production of willows grown in controlled and field experiments. Willows are susceptible to weed competition and weeds are a major constraint in willow establishment and biomass production. The potential risk of long-lasting weed infestations in crops cultivated after willow termination is also of concern. Thus the effects of weeds on growth performance, and long-term development and floristic diversity of the weed flora in crops established after termination of a willow cultivation were investigated in controlled and field experiments.

Willow growth was significantly affected by propagule phenology, planting system, planting time, clone, and weed treatment. Non-dormant cuttings planted early in the season sprouted earlier and had similar aboveground biomass production as dormant cuttings. Willow grown from non-dormant cuttings planted later in the season had lower competitive ability than dormant cuttings. Survival was not affected by propagule phenology, but was higher for willows grown from cuttings (91%) than from billets (39%), and in weeded (69%) than in unweeded (54%) plots. More aboveground biomass was produced from dormant than non-dormant cuttings planted later in the growing season (59%), from cuttings than from billets (52%), and from willows in weeded than unweeded plots (64% and 83% for the bucket and field experiment, respectively). For a given diameter, total and stem mass were larger for willows grown without than grown with weeds. Specific leaf area was higher for leaves formed on stems from non-dormant than dormant cuttings, and lower for willows grown without than with weeds. Impact of planting time and willow clone on willow performance parameters was also observed. Higher weed flora species richness, cover, diversity and composition was observed in willow than in cereal stands in the long-term field experiment.

Willow establishment costs can be reduced by using non-dormant propagules planted early in the growing season but not by using billets instead of cuttings. Higher weed flora species richness, cover, diversity and composition in willow compared with cereal stands does not impose risk of weed infestation to succeeding crop. Therefore, willow cultivations may contribute to floristic diversity within the agricultural landscape by hosting diverse weed flora, without compromising subsequent crop yields.

Keywords: billet, cutting, dormant, non-dormant, Salix, weed competition, yield

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Dedication

To everyone who in one or another way supported me in this adventure.

We are by nature observers, and thereby learners. That is our permanent state.
Ralph Waldo Emerson

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Welc M*., Lundkvist A. & Verwijst T. (2017). Effects of cutting phenology (non-dormant versus dormant) on early growth performance of three willow clones grown under different weed treatments and planting dates. BioEnergy Research, 10:1094–1104, DOI: 10.1007/s12155-017-9871-2.

II Welc M., Lundkvist A. & Verwijst T. (2017). Effects of propagule phenology (non-dormant versus dormant) and planting system (vertical versus horizontal) on growth performance of willow clones grown under different weeding regimes (manuscript).

III Verwijst T. & Welc M. (2017). Effects of weed treatment, willow clone, planting time and cutting phenology on allometric relations in willow (manuscript).

IV Welc M*., Lundkvist A., Nordh N-E. & Verwijst, T. (2017). Weed community trajectories in cereal and willow cultivations after termination of a willow short rotation coppice. Agronomy Research (https://doi.org/10.15159/AR.17.040).

Papers I and IV are reproduced with the permission of the publishers.

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The contribution of Monika Welc to the papers included in this thesis was as follows:

I In collaboration with the co-authors participated in study planning, performed the experimental work, evaluated the data and wrote the main part of the manuscript, and was responsible for correspondence with the journal.

II In collaboration with the co-authors participated in study planning, performed the experimental work, evaluated the data, and wrote the main part of the manuscript.

III In collaboration with the co-author participated in paper planning, performed the experimental work, evaluated the data and wrote part of the manuscript.

IV Participated in paper planning in collaboration with the co-authors, performed main part of the experimental work, evaluated the data 2009-2012, collected and evaluated the data 2013-2015, wrote the main part of the manuscript, and was responsible for correspondence with the journal.

Abbreviations

CA	Correspondence analysis
CCA	Canonical correspondence analysis
cm	Centimetre
DW	Dry weight
DW_A	Total aboveground dry weight
DW_L	Dry weight of leaves (entire plant)
DW_S	Dry weight of stems (entire plant)
GDD	Growing-degree days
g	Gram
ha	Hectare
L	Litre
LA	Leaf area (individual leaf)
LDW	Leaf dry weight (individual leaf)
m	Metre
m^2	Square metre
NMDS	Non metric multidimensional scaling
SLA	Specific leaf area
SRC	Willow short rotation coppice
t	Ton
WSA	Weed suppressive ability
WT	Weed tolerance
yr	Year

1 Willow SRC in Sweden

Willow (*Salix*) is a relatively new perennial crop cultivated on approximately 10.500 ha in Sweden (Jordbruksverket, 2017). Willow short rotation coppice (SRC) is a source of renewable energy that can replace fossil fuels to generate electricity, transport energy and heat (WMO, 2016). Thus it can contribute to alleviation of greenhouse gas concentrations in the atmosphere and to improvement of energy security and sustainable development (McCormick & Kåberger, 2005). Furthermore, following the trends for preservation of biological diversity on global and local scale (EU, 2016) and within the new concept of multifunctional landscape (Ssegane *et al.*, 2015), willow SRC is regarded as an important component of the agricultural system, contributing to preservation of biodiversity on the agroecosystem level (Hjulfors & Hjerpe, 2012; Augustson *et al.*, 2006) and providing numerous ecosystem functions, including carbon and nutrient sequestration and cycling (Bressler *et al.*, 2017; Ens *et al.*, 2013; Rowe *et al.*, 2013; Rytter, 2012).

1.1 Biomass production in willow SRC

Due to its inherent regeneration ability, willow can be propagated vegetatively by means of fragmented wooden shoots acting as propagules (*e.g.* cuttings (Figure 2A) and billets (Figure 2B)). This method of willow propagation was probably developed for osier production back in ancient times (Prance & Nesbitt, 2004). It was revived in experimental willow cultivations in the early 1950s (Stott, 1956) and has been continuously used since then (Iserbrands & Richandson, 2014). Propagules also serve as a source of carbohydrates and hormones (and to some extent water and nutrients), which are indispensable for initiation of willow growth until the shoot reaches sunlight and begins to rely on carbohydrates produced photosynthetically.

Most commonly, willow SRC is established from approximately 20 cm long cuttings (Figure 2A), planted vertically in soil. Cuttings are prepared from one-year-old shoots harvested from the field after growth cessation, and stored at sub-zero temperatures in order to retain vigour and vitality until planting in spring (Nordh, 2005).

Since around 2000, when willow SRC planting was mechanised, willows are commonly planted in double rows at a density 15.000-18.000 plants ha^{-1}, which minimises intraspecific competition and self-thinning, but also facilitates mechanised management such as weeding, fertilisation and harvest (Larsson, 1998).

The expected productive lifetime of willow SRC ranges from 20 to 25 years. The stand is coppiced 4-7 times at 3- to 5-years intervals, depending on willow biomass increment, harvesting conditions and demand for wood chips. The mean biomass yield in commercial Swedish willow SRC is usually <5 t DW ha^{-1} yr^{-1} and can increase to >10 t DW ha^{-1} yr^{-1} with efficient weed control and regular fertilisation (Mola-Yudego *et al.*, 2015; Larsson & Lindegaard, 2003).

1.2 Floristic diversity preservation in willow SRC

In the agricultural landscape, established willow SRC is less frequently disturbed by management practices than neighbouring cropland. Therefore it provides a unique habitat in which flora, mycoflora and fauna can establish, reside and propagate (Romano *et al.*, 2013; Baum *et al.*, 2012; Campbell *et al.*, 2012; Vonk, 2008; Reddersen, 2001). Numerous studies report the role of willow SRC in floristic diversity preservation in agroecosystems. For example, Gustafsson (1987), Baum *et al.* (2012) and Augustson (2004) frequently observed tree species, perennials and rare or endangered plant species in willow SRC that were not present on surrounding arable land. However, as willow SRC cultivations also host many species considered weeds, their actual influence on neighbouring cropland is still strongly debated among willow growers. Some view willow SRC as a floristic diversity hotspot and refuge for plant species eliminated from arable areas by management operations, while others suspect willow SRC of being an incubator of weed propagules that may spread to and infect neighbouring cropland. Moreover, there are unanswered questions about the risk of long-lasting weed infestations in crops established after willow SRC is terminated and whether weeds developed in the willow understorey hamper willow growth. Answering these questions would not only resolve the concerns of farmers, but also help to determine whether willow SRC can simultaneously provide economic values (*i.e.* biomass production) and environmental values (*i.e.* floristic diversity preservation).

2 Willow SRC establishment

This thesis focuses on biological aspects of willow cultivation, but these also have significant consequences for the economics of willow production. The intention of this thesis was to obtain novel information that could be communicated to commercial willow growers, in order to advance willow SRC in Sweden, and improve its economic and environmental value in current and future agriculture.

Cultivation of willow is a long-term commitment, but the greatest financial investments are required at the phase of willow SRC establishment and early growth (Hollsten *et al.*, 2012). The current economic prospects for willow cultivation in Sweden are rather poor and profits from production of biomass in willow SRC on commercial or private scale are considered low (Erik Holmén, ENA Energi, Enköping, Sweden, personal communication 2016).

In order to renew interest in willow cultivation and strengthen its economic viability, cost savings in establishment and management of willow SRC are required (Buchholz & Volk, 2011; Ericsson *et al.*, 2006; Toivonen & Tahvanainen, 1998). In general, the costs of willow SRC establishment are divided into planting material (propagule) costs and field operation costs, which comprise approximately 80% and 20% of the total, respectively (Caslin *et al.*, 2010). Field operation costs comprise the costs of planting and management in terms of weeding. In order to sketch the background for the research presented in this thesis, a short description of each cost component is presented below. The costs of fertilisation were beyond the scope of this thesis and are thus not considered.

2.1 Planting material

Propagules are routinely produced from dormant willow shoots, which are harvested after growth cessation (*i.e.* early winter-early spring) and stored at sub-zero temperatures (approximately -4 °C; (Gustafsson *et al.*, 2007)) for a period of few to several weeks. This step determines propagules vigour and vitality at planting. However, such cold storage is logistically demanding and approximately 3-5% of the total cost of the planting material can be attributed solely to the cold storage requirement (Lena Åsheim, Salixenergi Europa AB, Sweden, personal communication 2017). McCracken *et al.* (2010) concluded that reducing the cost of planting operations is still not sufficient to make willow SRC commercially viable, and suggested that savings in preparation of planting material could help to achieve this goal. Similarly, Buchholz and Volk (2011) and Volk *et al.* (2016) indicated that the cost of planting stock is still among the most critical variables influencing the profitability of willow SRC.

In Canada, successful establishment of willows from non-dormant rods was achieved by Teodorescu *et al.* (2011). However, willows were grown as green structures in an urban environment in that study, and not as willow SRC, so this planting method was selected due to its simplicity and not in an attempt to reduce the planting cost. However, willow clones V7534 and Olof grown from propagules prepared from actively growing one-year-old willow shoots established successfully and grew in experimental willow SRC in Ireland (McCracken *et al.*, 2010).

In order to evaluate the potential for use of non-dormant propagules for willow SRC establishment on the commercial scale, systematic research in this area is required. Thus far, there has been no published research comparing establishment and subsequent growth performance parameters of willows grown from non-dormant and dormant propagules in the same experiment, and under the conditions relevant to commercial willow SRC in Sweden. Therefore, one of the main aims of this thesis work was to evaluate growth performance of willow established from non-dormant propagules.

2.2 Planting operation

With technical developments and mechanisation, new methods for willow SRC planting have been developed during the past decade (Trzepieciński *et al.*, 2016; Gro & Culshaw, 2003). However, when designed for a certain purpose (*e.g.* willow planting or harvesting), machines become more specialised and more expensive to operate and maintain. In Sweden, willow SRC is conventionally established from cuttings that are cut and delivered into the soil during the same operation. A willow planter of the type Woodpecker 601 (Verwijst *et al.*, 2013) or Salix Maskiner 'Step Planter' (Caslin *et al.*, 2010) is commonly used during planting operations. The rental cost of a 'Step Planter' is roughly 1000 SEK ha^{-1} (Stig Larsson, European Willow Breeding, personal communication 2017), which accounts for approximately 10% of total planting costs per hectare (Rosenqvist, 2017).

The cost of planting operations can be reduced by using less specialised machines and/or less planting material. In Denmark, a sugarcane planter has been tested for horizontal planting of fragmented willow shoots in shallow trenches in the soil (Gro & Culshaw, 2003). It was found that willow SRC could be successfully planted from billets (*i.e.* short fragments of hardwood shoots that were chopped by the sugarcane harvester) using the sugarcane planter.

Gro and Culshaw (2003) suggested therefore that replacement of conventional vertical planting with horizontal planting can reduce the costs incurred during willow SRC establishment. However, in a study comparing vertical and horizontal planting systems, McCracken *et al.* (2010) showed that the amount of aboveground biomass produced from willows grown from billets was significantly lower than that produced from willows grown from cuttings. Therefore according to that study the potential for a cost reduction by using billets is poor (McCracken *et al.*, 2010).

However, Larsen *et al.* (2014a) showed that the difference in annual biomass production from cuttings and billets was only significant during first harvest rotation (8.6 and 5.6 t DW ha^{-1} yr^{-1} for cuttings and billets, respectively), and not during the second, suggesting convergence in biomass production in these two planting systems over time after willow SRC establishment. These results seem also to provide evidence that the planting system with billets can become economically viable, but requires further improvements and adaptations to specific environmental conditions.

However, any reduction in costs achieved by use of less specialised machines and/or use of less planting material at the stage of willow SRC establishment needs to be weighed against gains in the long-term perspective. Willow SRC established from vertically planted cuttings may be more uniform than that established from horizontally planted billets (*e.g.* no gaps in the stand caused by apical dominance), thus securing consistent yield (Edelfeldt *et al.*, 2015). Therefore savings in planting operations may not compensate for further losses due to uneven yield during consecutive harvests.

Against this background, the studies presented in this thesis were initiated to compare performance parameters of willows grown from cuttings and billets under conditions relevant to commercial willow SRC in central Sweden.

2.3 Weed control

At establishment and early growth, willows compete poorly with weeds for light, water and nutrients, and may be outcompeted during a relatively short period of time (Albertsson *et al.*, 2014b; Hollsten *et al.*, 2012; Sage, 1999). However, weed control, either mechanical or chemical, is relatively costly and is therefore often incompletely performed or omitted as a management practice in willow SRC (Nils-Erik Nordh, personal communication 2016). About 1.8-11.6% of the total costs of willow SRC establishment (willow clones introduced after 1990, low, medium or high harvest level) can be attributed to weeding (Rosenqvist, 2017). Weeding is usually required only at establishment and early growth (Hollsten *et al.*, 2012), but weed competition can have long-lasting effects on biomass production in willow SRC. This is because willow does not recover from damage due to weed competition in the establishment year (Clay & Dixon, 1997). Therefore if weed control is correctly performed, it secures a positive economic return from the willow SRC not only during the first harvest cycle, but during the entire lifespan of the willow SRC cultivation (Albertsson *et al.*, 2016).

Although the negative impact of weeds on willow growth performance is well-documented, the dynamics of willow-weed competition and the effect of weed pressure on the pattern of biomass partitioning in willow SRC have not been elucidated thus far. Therefore, this was one of the issues addressed in the present thesis.

3 Overall aims and objectives and specific hypotheses

The overall aim of this study was to assess the effects of propagule phenology (non-dormant versus dormant), planting system (vertical cuttings versus horizontal billets), planting time and willow clone on early growth performance of willow. Susceptibility of willows to weed competition is considered a major constraint in willow cultivation. The potential risk of long-lasting weed infestation in succeeding crops (after willow termination) may also be a concern. Therefore, additional aims of this thesis work were to evaluate: (i) effects of weeds on willow growth performance and (ii) long-term development and floristic diversity of the weed flora in crops established after termination of a willow cultivation.

Willow performance parameters and characteristics of weed flora developed in willow understorey were also investigated on different levels ranging from that of a single plant (Paper III), to buckets as an experimental unit (Paper I) and on to field level (Paper II) and landscape level (Paper IV).

The order of the studies described in Papers I-IV of this thesis was dictated by gradual accumulation of knowledge as the work progressed. Based on experience and knowledge gained from the bucket experiment (first experiment performed, Paper I), a field experiment was designed and conducted (Paper II). Gaps in knowledge identified in the bucket and field experiment were filled by further narrowing (Paper III) or broadening (Paper IV) the scale of investigation (Figure 1).

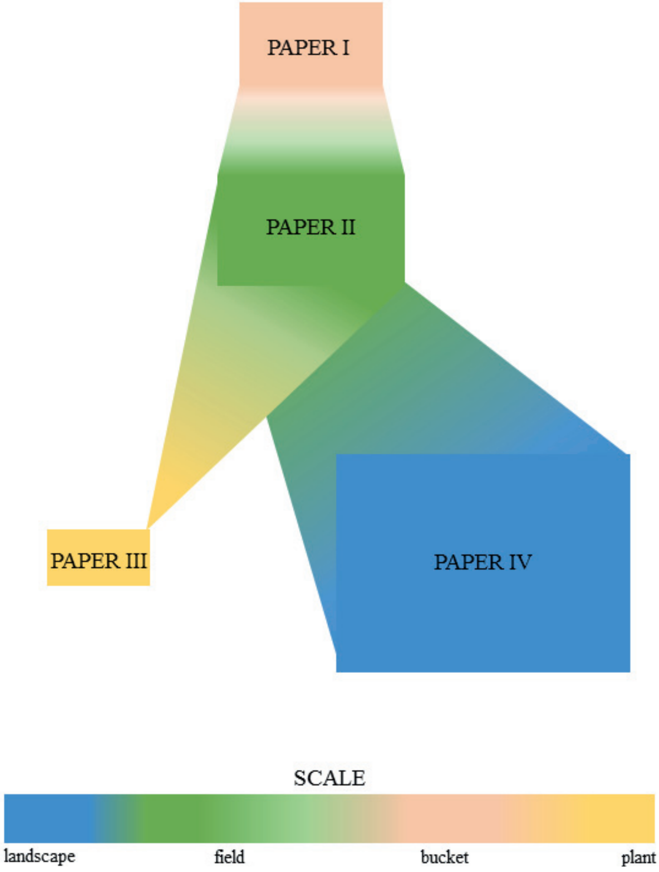

Figure 1. Schematic representation of the work in this doctoral thesis.

3. 1 Paper I

The objective of this study was to quantify the effects of cutting phenology (non-dormant versus dormant) on early growth performance parameters of three willow clones grown under different weed treatments and planting dates in a controlled bucket experiment. The following hypotheses were tested in the study: (1) phenological development in terms of bud burst: (i) will be faster for non-dormant cuttings compared with dormant cuttings at early planting dates, and (ii) will be clone-dependent; (2) subsequent performance (aboveground biomass increment) will dependent on cutting phenology, willow clone, planting date and weed treatment; (3) differences in willow growth performance will be in turn reflected in weed suppressive ability and weed tolerance of willow.

3. 2 Paper II

The objective of this study was to assess the effects of propagule phenology and planting system on growth performance of three willow clones grown under different weeding regimes. The following hypotheses were tested in the study: (1) phenological development, in terms of sprouting: (i) will be higher for non-dormant propagules compared with dormant ones, (ii) will be higher for cuttings than billets, and (iii) will be clone-dependent; (2) subsequent growth performance (survival and aboveground biomass production) over time will: (i) depend on propagule phenology and planting system, and (ii) will differ between weeding regimes and willow clones.

3. 3 Paper III

The objective of this study was to assess the effect of weed competition, willow clone, planting date and propagule phenology on allometric relations between weight (stem and leaf dry weight per shoot, total aboveground weight) and stem diameter in willows grown in a bucket and field experiment. The following hypotheses were tested in the study: (1) interspecific competition of weeds with willow will have a significant impact on the allometric relations in willow; (2) the effects of weeds on willow allometry will not be significant after the season in which willows were planted; and (3) competition from weeds will go along with an increased specific leaf area in willow.

3. 4 Paper IV

The objective of this study was to compare the development of the weed flora during six growing seasons in a long-term field experiment with willow and cereal crops established after termination of a 25-year-old willow SRC. The following hypotheses were tested in the study: (1) deep soil cultivation during willow termination will initially reduce weed species richness and cover in both willow and cereal crops compared with shallow soil cultivation; (2) weed species richness and cover will be initially similar in both willow and cereal crops; (3) weed species composition in willow and cereal crops will diverge over time; (4) this divergence will be due to the inherent impact of the cropping systems on their environment, as inferred from the Ellenberg index.

4 Materials and methods

4.1 Sites

The bucket experiment was conducted outdoors in 2013 (Papers I and III). The field experiment was planted in 2014, and the data presented in this thesis were collected during three consecutive growing seasons (2014-2016) (Papers II and III). The long-term field experiment was conducted in a 25-year-old willow SRC before its termination in winter 2009, and in willow SRC and cereal stands established thereafter during six growing seasons (2009-2015) (Paper IV). All experiments were performed at Ultuna near Uppsala, Sweden (59°48′N, 17°39′E).

4.2 Plant material

For the bucket experiment and field experiment, one-year-old willow shoots approximately 160 cm in length and with basal diameter of approximately 1 cm were randomly harvested either in late winter or in early spring prior to planting, from a willow nursery at Ultuna Uppsala, Sweden (59°48’N, 17°39’E) (Papers I and III) or at Pustnäs Nåntuna, Sweden (59°48’N, 17°40’E) (Papers II and III). Shoots harvested in late winter were stored at approximately -4 °C in polyethylene bags for 6-9 weeks until planting. Propagules denoted dormant were prepared from these shoots. Shoots harvested in early spring were stored at approximately +4°C for a maximum of week until planting. Propagules denoted non-dormant were prepared from these shoots. Prior to planting, a fragment of approximately 40 cm (bucket experiment) or 20 cm (field experiment) was cut from both the basal and apical part of each shoot to reduce the effect of drying and storage-related damage. From the remaining part of the shoot, four (bucket experiment) or six (field experiment) cuttings with the length of 20 ±0.3 cm

(Figure 2A), or twelve (field experiment) billets with the length of 10 ±0.3 cm (Figure 2B) were cut manually. In both the bucket and field experiment, willow cuttings and billets were planted manually. In the bucket and field experiment, the cuttings were planted vertically in the soil, leaving 1-2 cm of the cutting above the soil surface (Papers I-III). In the field experiment, the billets were placed in trenches at a depth of approximately 3 cm, pressed into soil and carefully covered with the soil (Papers II and III).

Studies presented in this thesis aimed to imitate a commercial planting system with cuttings and billets. However, it need to be emphasised that propagules were prepared and planted manually (*i.e.* selection of good quality material, high precision of cut and planting) therefore any possible damages (*e.g.* debarking, damage of buds) and irregularities in propagule size and distances between plants, typical for mechanical planting (Edelfeldt *et al.*, 2013), were minimised (Papers I-III).

Willow SRC in the long-term field experiment was established from dormant cuttings (Paper IV). Shoots used for cutting preparation were harvested after growth cessation (winter 2009) and stored at sub-zero temperatures (-4 °C) until planting. Willow SRC was planted mechanically on May 18, 2010 with a planting machine of the type Woodpecker 601 that cuts long willow shoots into approximately 20 cm long cuttings and delivers them into the soil at regular intervals.

Four different willow clones that are commercially available and which have been tested in numerous trials in Sweden were used in the experiments. These were: Jorr (*Salix viminalis* L.), Olof (*S. viminalis* × (*S. schwerinii* E. Wolf × *S. viminalis*), Tora (*S. schwerinii* × *S. viminalis*) and Tordis ((*S. schwerinii* × *S. viminalis*) × *S. viminalis*) (Caslin *et al.*, 2012). Clones Jorr, Olof and Tora were used in the bucket experiment (Papers I and III). Clones Jorr, Tora and Tordis were used in the field experiment (Papers II and III). Clone Tora was used in the long-term field experiment (Paper IV).

Figure 2. (A) Cuttings and (B) billets of willow clone Tora prepared for planting in the field experiment (Papers II-III). Photos: Monika Welc.

4.3 Experimental design

4.3.1 Bucket experiment (Papers I and III)

The bucket experiment was conducted outdoors during May to August 2013 in 12 L buckets filled with low-nutrient substrate. Overall, the experiment comprised 240 buckets, containing treatments comparing willow cutting phenology (two levels: non-dormant, dormant), weed treatment (two levels: with weeds, without weeds), willow clone (three levels: Tora, Jorr, and Olof) and planting date (five levels: May 3, 10, 24, and June 6, 16, 2013), all planted in four replicates. The planting dates May 3 and 10 were considered to be early growing season, the planting date May 24 was considered mid growing season and the planting dates June 6 and 16 were considered to be late growing season.

To avoid effects of competition for light between treatments, buckets were moved randomly within each planting date level during the first five weeks. To avoid damage of tall and branched shoots, random moving was not performed during weeks 6 to 8. In all buckets (designated willow monocultures and willow-weed mixtures), four 20 cm long cuttings were vertically planted (constant spacing between plants maintained) per bucket, giving a nominal planting density of 65 cuttings m^2. In half the buckets designated as willow-weed mixtures, 25 seeds of a model weed (spring barley, *Hordeum vulgare* L. var. Waldemar, Svalöf Weibull AB, Malmö, Sweden) were sown at a depth of approximately 2 cm (constant spacing between plants maintained) five days after willow planting, giving a nominal density of 400 plants m^2 (Figure 3). At each planting date, four buckets were sown with weed (spring barley) monocultures according to a same planting scheme, in order to assess weed suppressive ability and weed tolerance. Willows and weeds grew approximately 8 weeks (Figure 4) and were destructively harvested after this time. The data were analysed and presented in Paper I.

The allometric dataset collected from the bucket experiment was also used in the analyses in Paper III.

Figure 3. Three-weeks-old willow shoots (planting date May 3, 2013) of willow clone Tora grown in monocultures and in mixtures with spring barley in the bucket experiment (Paper I). Photo: Monika Welc.

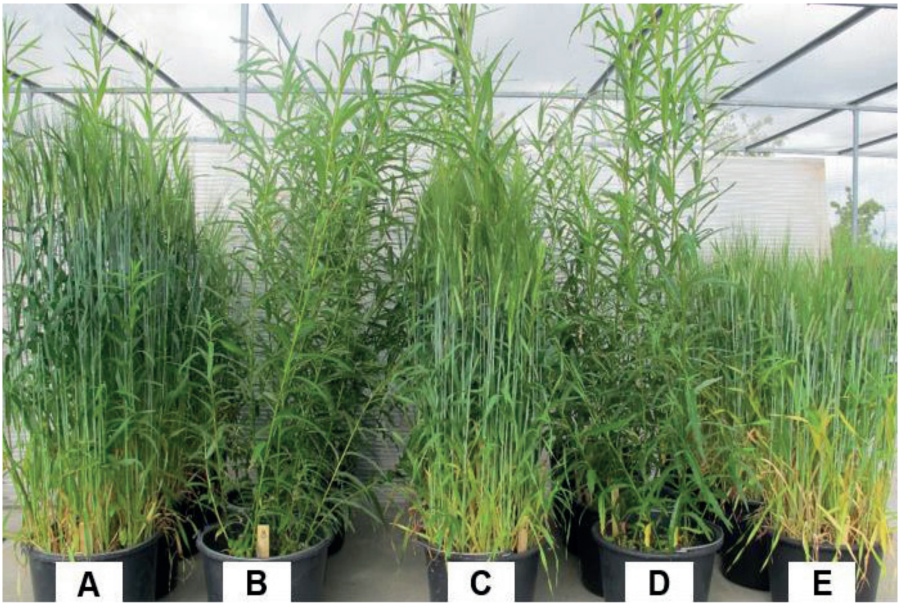

Figure 4. Eight-week-old shoots (planting date May 3, 2013) of willow clone Tora in the bucket experiment (Paper I). (A) Willow from non-dormant cuttings growing with spring barley; (B) willow from non-dormant cuttings; (C) willow from dormant cuttings growing with spring barley; (D) willow from dormant cuttings; and (E) spring barley. Photo: Monika Welc.

4.3.2 Field experiment (Papers II-III)

A split-split-plot field experiment was manually planted on an area of 0.6 ha on agricultural land at Ultuna on May 19-23, 2014. A set of four blocks were divided into 24 individual plots organised in four rows of six columns. In each block, weeding regime (weeded and unweeded) and planting system (cuttings and billets) were randomised to rows and willow clone (Tora, Tordis and Jorr) to columns, but the plots planted with non-dormant and dormant propagules of the same willow clone were set adjacent to each other. A double-row planting system was used, with 150 cm between double rows, 75 cm between rows within double rows and 70 cm or 35 cm between individual cuttings and billets within the row (Hollsten *et al.*, 2012). This resulted in a nominal density of approximately 13.000 and 26.000 plants ha^{-1} for cuttings and billets, respectively. A net plot of 4.5 m × 5.6 m was centrally designated in each plot, and measurements of willow and weed parameters (Table 1) were performed in these net plots, to avoid border interactions between willow clones and treatments during the growing seasons 2014-2016 (Figures 5-7).

Allometric datasets collected from the field experiment were also used in the analyses in Paper III.

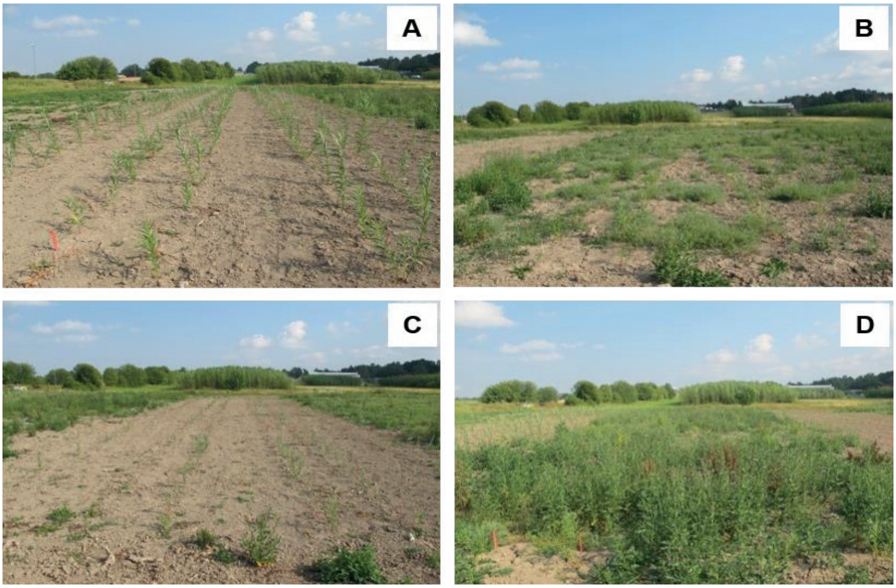

Figure 5. Four-month-old (September 20, 2014) willow plants in the field experiment (Paper II). (A) Willow from cuttings in weeded plots; (B) willow from cuttings in unweeded plots; (C) willow from billets in weeded plots; and (D) willow from billets in unweeded plots. Photos: Monika Welc.

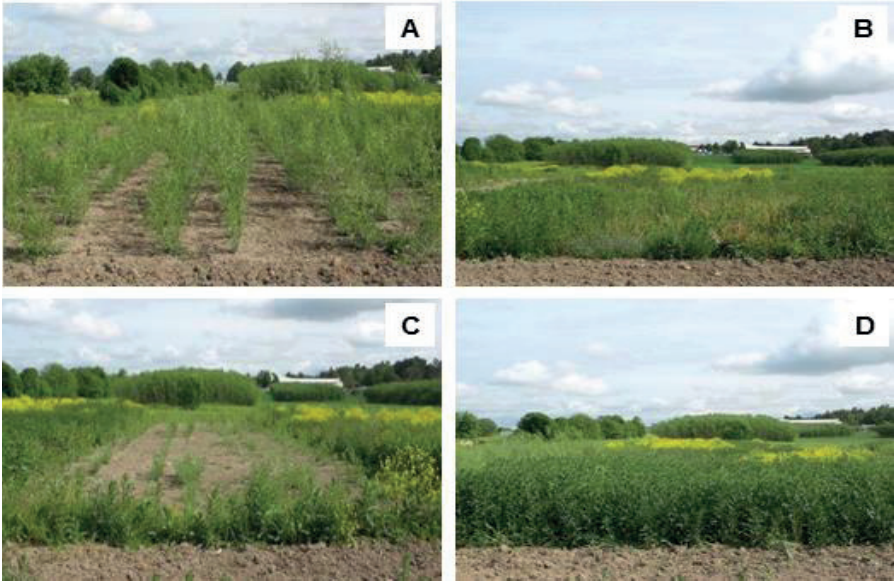

Figure 6. One-year-old (June 1, 2015) willow plants in the field experiment (Paper II). (A) Willow from cuttings in weeded plots; (B) willow from cuttings in unweeded plots; (C) willow from billets in weeded plots; and (D) willow from billets in unweeded plots. Photos: Monika Welc.

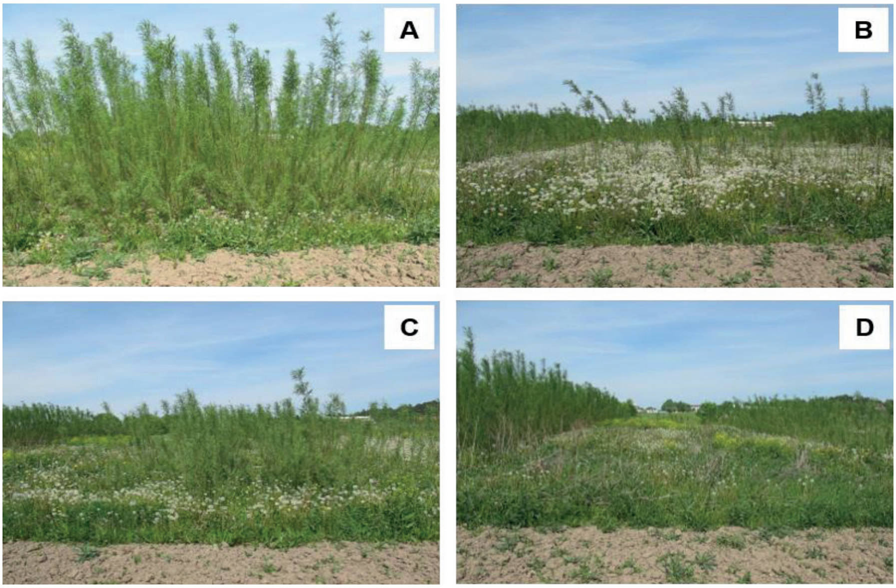

Figure 7. Two-year-old (June 1, 2016) willow plants in the field experiment (Paper II). (A) Willow from cuttings in weeded plots; (B) willow from cuttings in unweeded plots; (C) willow from billets in weeded plots; and (D) willow from billets in unweeded plots. Photos: Monika Welc.

4.3.3 Long-term field experiment (Paper IV)

The long-term field experiment was performed at Ultuna near Uppsala, Sweden (59°48'N, 17°39'E) on a 2.7 ha field for 25 years (1984-2009) grown by a willow crop (*Salix viminalis* L., clone 77683), on a 2.450 m^2 reference plot sown with annual crops (2004-2009), and in willow SRC and cereal stands established after termination of the willow SRC in 2009 (for details, see Paper IV). Termination was performed using the methods with either shallow or deep soil cultivation (Norberg & Nordh, 2012). Two areas of 100 m × 100 m were divided further into blocks of 50 m × 100 m, and subplots of 25 m × 100 m in a split-plot design were designated randomly to be planted either with willow SRC or sown with cereal (Figure 8). In subplots designated for willow SRC, the willow clone Tora was planted mechanically with a planting machine of the type Woodpecker 601. Planting density was approximately 20.400 plants ha^{-1}. Spacing between double rows was 150 cm, between rows within the double rows was 75 cm and that between individual cuttings in each row was 70 cm. In subplots designated for cereals, winter wheat (*Triticum aestivum* L., September 2009), spring barley (*Hordeum distichon* L., 2011, 2012, 2014) or spring wheat (*Triticum aestivum* L., 2013, 2015) was sown with a density of approximately 350-400 seeds m^{-2} (winter wheat and spring barley) or 500 seeds m^{-2} (spring wheat). Weed flora characteristics (for details, see section 4.4 Measurements) were recorded on the area of the 25-year-old willow cultivation (16 subplots intended to be planted anew with SRC or converted to cereal stands) before its termination (June 20-21, 2009), in a reference plot sown with annual crops (July 2, 2009), and in willow SRC and cereal stands established after termination of 25-year-old willow cultivation (July 26, 2010; June 28, 2012; July 14-15, 2013; July 28 and 30, 2014, July 27, 2015). No inventory was performed in 2011. Weed flora characteristics were recorded in a 4 m^2 sampling quadrants. In the 25-year-old willow cultivation and newly established willow SRC and cereal stands, five 4 m^2 quadrants were located 10, 30, 50, 70 and 90 m from the subplot headland, distributed along a transect centrally located in each subplot. In a reference plot sown with annual crops, five 4 m^2 sampling quadrants were distributed along a centrally located transect.

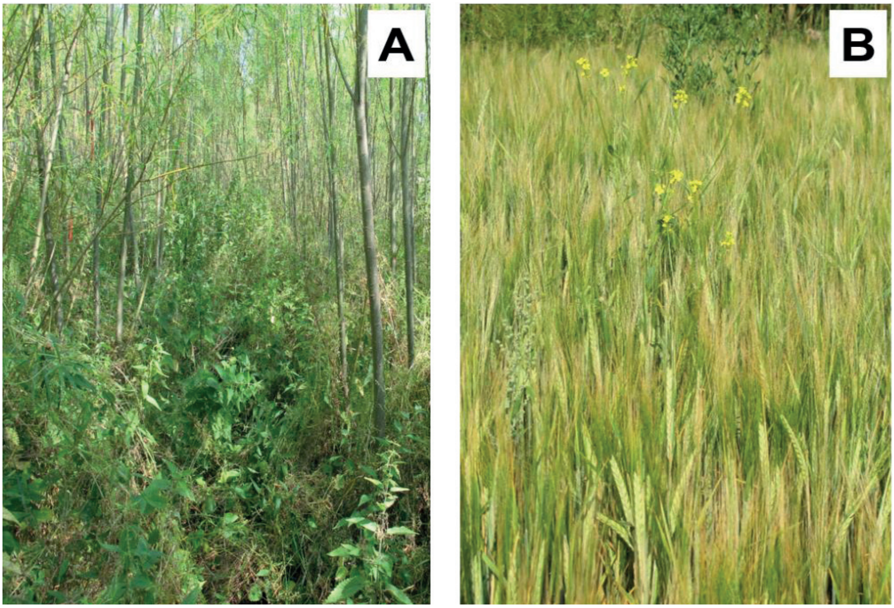

Figure 8. (A) Willow and (B) spring barley in the long-term field experiment (July 29, 2014). Photos: Monika Welc.

4.4 Measurements

Measurements performed in Papers I-IV are summarized in Table 1, and the methods described in separate paragraphs below.

4.4.1 Willow growth performance (Papers I-III)

Bud burst (Paper I)

Willow bud burst was assessed using a five-stage scale (Verwijst *et al.*, 2012). This scale classifies bud development based on bud swell, adhesion/protrusion of the bud tip from a shoot, the length of the bud tip and the length of leaves developed from the bud. Stages of the scale are: stage 1 - no sign of bud swelling, the tip of the bud is tightly pressed to the shoot; stage 2 - the tip of the bud starts to bend from the stem, bud scales are starting to open and the length of the shoot tip is 1-4 mm; stage 3 - the shoot tip is 5 mm or longer, protruding leaves are held together; stage 4 - new leaves start to bend from each other; stage 5 - one or more new leaves are perpendicular to the shoot axis. Bud burst was monitored daily from the day of planting until the most developed bud per cutting reached developmental stage 5 (on average for all planting dates, this occurred 22 days after planting).

Sprouting (Paper II)

Willow sprouting in the field experiment was assessed on June 2, 6, 13, 16, 26 and 30, 2014. Cuttings were regarded as sprouted when the scales on the most apical bud opened and the length of the shoot tip was 1-4 mm (developmental stage 2, Verwijst *et al.* (2012)). Billets were regarded as sprouted when the first leaves started to emerge and protrude above the soil surface. For each assessment date, percentage of sprouted individuals per net plot was recorded (for details, see section 4.3.2 Field experiment) and, based on the initial planting density (*i.e.* 13.000 cuttings ha^{-1} or 26.000 billets ha^{-1}), the value was scaled up to number of sprouted plants per hectare.

Survival (Paper II)

Willow survival was assessed on June 30, 2014, and on March 2, 2015 (after first growing season), on March 1, 2016 (after second growing season) and on December 5, 2016 (after third growing season). Each live cutting and billet in each net plot (in total 32 cuttings and 64 billets per plot) was inspected for survival. If there were no signs of bud development above the soil surface (assessment in first growing season) or no sign of live tissue under the shoot bark 10 cm above the ground level (second and third growing season), this was taken to indicate mortality of that willow plant. Based on the initial planting density (*i.e.* 13.000 cuttings ha^{-1} or 26.000 billets ha^{-1}), the value obtained for willow survival per net plot (for details, see section 4.3.2 Field experiment) was scaled up to willow survival per hectare and presented as a cumulative during the consecutive growing seasons.

Aboveground biomass production (Papers I-III)

Aboveground biomass production in the bucket experiment (Papers I and III) was assessed after destructive harvest. Destructive harvest for each planting date was performed approximately 60 days after willow planting and coincided with a stage of 60-66 expanded leaves of willow clone Tora grown from dormant cuttings without weeds. Each shoot (stem and leaves) per cutting was individually harvested, dried for 24 h at 90 °C and weighed. Aboveground biomass dry weight per bucket (DW, g) was then estimated (Paper III). In Paper I, shoot DW per bucket was calculated per growing-degree day (GDD °C) (McMaster & Wilhelm, 1997) and expressed as total aboveground dry weight per GDD (DW g GDD^{-1}).

Aboveground biomass production in the field experiment (Papers II and III) was assessed on March 2, 2015 (after first growing season), on March 1, 2016 (after second growing season) and on December 5, 2016 (after third growing season). Stem diameter (D) at 10 ±0.3 cm above the ground level was measured with a digital calliper for all shoots per stool in the net plot (for details, see section 4.3.2 Field experiment).

Furthermore, on March 2, 2015 (after first growing season), on March 1, 2016 (after second growing season) and on December 5, 2016 (after third growing season), a sample of 30 shoots from outside the net plots, stratified according to shoot diameter was harvested per weed treatment and willow clone. For each shoot, diameter (D) at 10 ±0.3 cm above ground level was measured and aboveground dry weight (72 h at 70 °C) was recorded after three consecutive growing seasons (March 2, 2015; March 1, 2016; December 5, 2016). As measurements in the field experiment were performed on dormant willows, aboveground dry weight includes only woody biomass and no leaves, and is therefore expressed as stem dry weight (DW_S) (Papers II and III).

The same dataset was used to establish the allometric relations between easy to measure willow parameters (for details, see Allometric relations below).

The sample of 30 shoots per growing season, weed treatment and willow clone was used to calculate DW of each shoot from its D, using an equation:

$$DW = b \times D^c \quad (1)$$

where DW is dry weight, D is diameter 10 ±0.3 cm above the ground level, b is constant and c is the exponent (Verwijst & Telenius, 1999).

The diameter values of the shoots from the net plots were used in the allometric equations (obtained as described above), and shoot dry weight per net plot was calculated and scaled up to dry weight t ha^{-1} (t DW ha^{-1}).

Allometric relations (Paper III)

In the bucket experiment, a sample of 12 shoots, stratified according to shoot height, was harvested per weed treatment, willow clone, planting date and cutting phenology. For each shoot, diameter (D) at the emergence point, stem dry weight (DW_S) (48 h at 90 °C), dry weight of leaves (DW_L), and total aboveground biomass (DW_A) were determined. From each shoot, leaves were harvested at 15 ±1 cm intervals, and leaf area (LA) (Leaf Area Meter AAM-5, Hayashi Denko Co Ltd., Tokyo, Japan) and dry weight (LDW) (24 h at 90 °C) of each individual leaf were recorded. Specific leaf area (SLA) was expressed as the ratio of leaf area to leaf dry mass (cm^2 g^{-1}) (Li *et al.*, 2005).

Prior to analyses, the LA and LDW values were averaged per shoot. The effects of the factors: weed treatment, willow clone, planting date and propagule phenology on allometric relations (DW_A, DW_S and DW_L versus D, DW_S versus DW_L, and LA versus LDW) were tested.

In the field experiment, allometric dataset was collected on March 2, 2015 (after first growing season), on March 1, 2016 (after second growing season) and on December 5, 2016 (after third growing season) according to the procedure described above (for details, see Aboveground biomass production in section 4.4.1 Willow growth performance). For the field experiment, the effects of the factors: weed treatment and willow clone on the allometric relation DW_S versus D were tested for three consecutive seasons (Paper III).

Weed suppressive ability and weed tolerance (Paper I)

Weed suppressive ability (WSA) was calculated according to Nelson *et al.* (2012):

$$WSA = 100 - \left(\frac{bw}{bt}\right) \times 100 \quad (2)$$

where bw denotes total aboveground weed biomass and bt is the total aboveground biomass (willow + weed) per experimental unit (bucket).

Weed tolerance (WT) was calculated according to Szumigalski and Van Acker (2005):

$$WT = \left(\frac{Cbw}{Cbwf}\right) \times 100 \tag{3}$$

where Cbw denotes the aboveground willow biomass in the presence of weeds and $Cbwf$ the aboveground willow biomass when grown without weeds.

Table 1. *Measurements performed in Papers I-IV of this thesis.*

	Paper			
	I	II	III	IV
Parameter				
	Willow growth performance			
Willow bud burst (GDD °C)	yes	no	no	no
Willow sprouting (plant ha^{-1})	no	yes	no	no
Willow survival (plant ha^{-1})	no	yes	no	no
Aboveground biomass production (DW g GDD^{-1} or ton DW ha^{-1})	yes	yes	no	no
Allometric relations:				
DW_A versus D	no	no	yes	no
DW_S versus D	no	no	yes	no
DW_L versus D	no	no	yes	no
DW_S versus DW_L	no	no	yes	no
LA versus LDW	no	no	yes	no
SLA (cm^2 g^{-1})	no	no	yes	no
WSA (%)	yes	no	no	no
WT (%)	yes	no	no	no
	Weed characteristics			
Weed species richness total	no	no	no	yes
Annuals	no	no	no	yes
Biennials	no	no	no	yes
Perennials	no	no	no	yes
Maximum weed height (cm)	no	no	yes	no
Cover abundance total (%)	no	no	yes	yes
Annuals (%)	no	no	no	yes
Biennials (%)	no	no	no	yes
Perennials (%)	no	no	no	yes
Species composition and divergence	no	no	no	yes
Habitat type, growth forms	no	no	no	yes
Grime's strategy	no	no	no	yes
Ellenberg index	no	no	no	yes
Indicator species	no	no	no	yes

4.4.2 Weed flora characteristics (Papers III-IV)

Weeds were identified to species/genus level (Lid & Lid, 2005) and the following weed flora characteristics were assessed:

Weed species richness (Paper IV), as a count of total weed species in a given plot/treatment or a count of weed species in a given life cycle category (annuals, biennials and perennials).

Maximum weed height (Paper III), measured for the tallest weeds in a given plot/treatment from the surface of the soil to the top of the measured plant (top of the shoot or spike).

Total *cover abundance (Paper III-IV)* and cover abundance in a specific life cycle category (annuals, biennials, and perennials), by using nominal transformation of the Braun-Blanquet scale (van der Maarel, 2007; Braun-Blanquet, 1964; Braun-Blanquet, 1932).

Species composition and divergence (Paper IV), investigated by non metric multidimensional scaling (NMDS) (ter Braak & Šmilauer, 2012; Lepš & Šmilauer, 1999).

Habitat type and growth forms (Paper IV), determined according to Chapin *et al.* (1996).

Grime's strategy (Paper IV), determined following Grime *et al.* (2007).

Ellenberg index (Paper IV), calculated following Ellenberg (1992) and Grandin (2004).

Indicator species (Paper IV), analysed following Dufrêne and Legendre (1997).

4.5 Statistical analyses

4.5.1 Descriptive statistics (Paper I-IV)

Descriptive statistical analyses were performed in STATISTICA (Dell Inc., version 13.2, 1984-2016, USA, STATISTICA (2015)).

4.5.2 Mixed models (Papers I, II and IV)

Mixed model ANOVA was used to detect differences in measured parameters depending on treatment. The data were fitted with linear models with random effects of bucket (Paper I) or block (Papers II and IV) using the PROC MIXED procedure in SAS 9.4 (Littell *et al.*, 2006; SAS). Before analyses, the data were inspected for normal distribution of residuals. If increasing residual dispersion with increasing values was observed, the data were square root-transformed (proportion data, McDonald (2014)) (Paper II) or logit-transformed (size data, Bithell *et al.* (2011)) (Paper II). Data remained untransformed if dispersal of residuals was similar for non-transformed and transformed data (Papers I, II and IV). Linear mixed models were fitted using the restricted maximum likelihood (REML) method with Kenward-Roger denominator degrees of freedom adjustment (Spilke *et al.*, 2005; Kenward & Roger, 1997). In all PROC MIXED analyses, post hoc multiple comparisons of the means were performed with Fisher's least significant difference test at a confidence level of 95%. Different order interactions between all fixed effects were tested in the analyses. Detail description of the statistical analyses are included in Papers I-IV in this thesis.

4.5.3 Allometric relations (Paper III)

Allometric relations between willow growth parameters in the bucket experiment and in the field experiment were described by a zero-intercept model:

$$Y = b_1 \times (1 + b_2 \times d) \times X^c \qquad (4)$$

where b_1 and b_2 are constants, c is the exponent and d is a dummy variable, separating between the effects of a factor (by coding d for different factor levels).

For all relations between features tested above, the significance (P_{b2}) with which b_2 deviated from zero (indicating a significant effect of the specific factor on the allometric relations investigated) was recorded. As the relations between the plant dimensions investigated (*e.g.* stem diameter is one-dimensional, leaf area is two-dimensional and weight and volume are three-dimensional) are intrinsically nonlinear, nonlinear models were used in the data analyses (Schabenberger & Pierce, 2001). Allometric datasets were analysed with the non-linear advanced models option with user-specified regression in STATISTICA (Dell Inc., version 13.2, 1984-2016, USA, STATISTICA (2015)).

4.5.4 Non metric multidimensional scaling (NMDS)(Paper IV)

Non metric multidimensional scaling was used to investigate gradients in weed species composition in willow SRC and cereal stands using the software package Canoco 5, Windows release version 5.02 (ter Braak & Šmilauer, 2012). The Bray-Curtis distance measure and NMDS solution were based on three axes without optimisation and perturbations, and the formula for stress type 2 (*i.e.* the sum of squared differences between individual distance values and their mean) was applied. Treatments of ties in distances were set as primary, *i.e.* different occurrences of the same distance value might be matched with different fitted values. The stress values (Clarke, 1993) and number of iterations for separate growing seasons were recorded.

4.5.5 Indicator species analysis (Paper IV)

Indicator species analysis revealing association of weed species to a specific cropping system was performed using RStudio version 0.98.501©2009-2013, package 'labdsv' version 1.6–1 (Roberts, 2010). The analyses were based on the algorithm consisting of species specificity (the proportion of sites of type *j* with species *i*) and fidelity (the proportion of the number of individuals of species *i* that are in a type *j* of site) (Dufrêne & Legendre, 1997).

4.6 Graphics (Papers I-IV)

The software STATISTICA (Papers I-III) or Excel (Paper IV) was used to present the results visually by means of scatterplots, column or pie charts.

The relations DW_A versus D in the bucket experiment (Paper III) and DW_S versus D in the field experiment (Paper III) were graphically presented in STATISTICA as scatterplots, using parameter estimates obtained from the zero-intercept fitted model (Dell Inc., version 13.2, 1984-2016, USA, STATISTICA (2015)).

Photographic documentation was performed with a digital camera Canon PowerShot G11 (Canon USA, Inc.). No modifications, apart from addition of scales (Figure 1 in Paper I, Figure 2 of this thesis) or picture designations (Figures 2, 4-8 of this thesis), were performed on the photographs used. Adobe Illustrator CC 2015 (version 19.1.0, 1989-2015, Adobe Systems Inc.) was used to draw Figure 1 in Paper IV and in Figure 1 in this thesis.

5 Results and discussion

The results of this doctoral project are presented and discussed below in separate paragraphs: 5.1 Growth performance in willow SRC, and 5.2 Floristic diversity in willow SRC.

The dates May 3 and 10 are referred to as early growing season, May 24 is mid-growing season and June 6 and 16 are late growing season only in the bucket experiment (Paper I). Although the field experiment (Paper II) was planted on May 19-23, 2014 (for details, see section 4.3.2 Field experiment), the terminology: early, mid- or late growing season is irrelevant to this experiment. To avoid confusion, the exact date is therefore provided in brackets when selected willow performance parameters are described and discussed below.

5.1 Growth performance in willow SRC (Papers I-III)

The main objective of the studies presented in this thesis was to assess the effect of propagule phenology (non-dormant versus dormant) and planting system (vertical cuttings versus horizontal billets) on willow establishment and growth. However, in practice willow performance parameters were affected not only by the propagule phenology and planting system, but also by other factors such as willow clone and weed competition. These factors are discussed in separate paragraphs below and are described in details in the paper/s indicated. Furthermore, some performance parameters assessed for willow in this thesis and under certain circumstances were affected by interactions between different factors. Impact of the most significant interactions of factors on selected performance parameters are described below. Impact of remaining significant interactions of factors on selected performance parameters are described in details in the Papers I-IV included in this thesis.

5.1.1 Propagule phenology (Papers I-III)

Propagule phenology had significant effect on willow bud burst, sprouting, aboveground biomass production per GDD, allometric relation (*i.e.* LA versus LDW), and WSA and WT.

Bud burst and sprouting (Papers I-II)

Propagule phenology had significantly effect on willow bud burst (Paper I) and sprouting (Paper II). When cuttings were planted early in the growing season (May 3 and May 10, 2013), bud burst of non-dormant cuttings occurred significantly earlier than for dormant cuttings. No such differences between cuttings of different phenology were found when planting was done in mid- to late growing season (May 24 to June 16, 2013) (Paper I).
From all propagules planted in the field experiment, significantly more (74%) of non-dormant than dormant (58%) propagules sprouted (Paper II).

Higher sprouting of non-dormant than dormant propagules and faster bud burst of non-dormant cuttings compared with dormant cuttings when planted early in the season give an advantage in willow establishment and significantly affects subsequent willow performance. However, earlier bud burst increases willow susceptibility to frosts and freezing in late spring in Sweden (Lantmännen, 2012).

It seems probable that willow bud burst and sprouting depend on the amount and activity of carbohydrate (Carpenter *et al.*, 2008) and/or hormone (Arora *et al.*, 2003) reserves contained in the propagule at planting. The results presented in Papers I and II showed:
(i) no difference in bud burst between non-dormant and dormant willow cuttings later in the growing season (Paper I), and therefore imply that the difference in the amount and activity of carbohydrate reserves was presumably insufficient to trigger these differences; and
(ii) as non-dormant and dormant propagules were planted in spring (May 19-23, 2014) in the field experiment (Paper II), the impact of carbohydrates on sprouting was negligible.
Thus, bud burst and sprouting of non-dormant and dormant willow propagules was mostly affected by hormonal activity (*i.e.* hormone amount and proportions), which varied in the very initial phase of willow growth (five weeks after planting, June 30, 2013) (Paper II) and throughout the growing season (May-June 2013) (Paper I). Assuming that carbohydrate reserves were not significantly different in non-dormant and dormant propagules when planted in early spring, willow bud burst (early season, Paper I) and sprouting (Paper II)

were more affected by the amount and activity of hormone reserves than carbohydrate reserves.

Aboveground biomass production (Paper I)

Willow aboveground biomass production per GDD can be assumed to be predominantly affected by the carbohydrate reserves rather than by the hormone reserves in non-dormant and dormant propagules (Paper I). This assumption was supported by the results, since willow propagated from non-dormant cuttings planted early in the season (May 3 and May 10, 2013) produced the same amount of aboveground biomass per GDD as willow propagated from dormant cuttings. Willows grown from dormant cuttings and planted in mid- to late season (May 24-June 16, 2013) produced approximately 59% more aboveground biomass per GDD than willows grown from non-dormant cuttings. As non-dormant cuttings were produced from growing shoots, their carbohydrate reserves were actively used for metabolism and growth (*i.e.* production of shoots), and were gradually depleted later in the growing season. In contrast, even when planted later in the season, carbohydrate reserves of dormant cuttings were only marginally used to maintain basic metabolic processes (*e.g.* respiration) and therefore were probably allocated to production of biomass. No published paper to date has concentrated exclusively on propagule phenology and its impact on subsequent willow performance. However, some evidence supporting this assumption has emerged in works focusing on other aspects of plant growth and development. For example, by planting willow cuttings over a period of a few months (May-November), Nordh (2011) showed that willow growth performance declines with later planting date during the year and concluded that cutting phenology is an important determinant of willow growth. Teodorescu *et al.* (2011) showed that aboveground biomass production from non-dormant shoots varied between willow clones planted from April to the end of May, but did not give an explanation for this trend. However, in studies aiming to identify the constraints in vegetative propagation of maple, Snow (1941) and Koelling (1968) suggested that the variability in growth performance and rooting ability of cuttings prepared and planted throughout the growing season may arise from differences in the content and type of carbohydrate reserves.

Practical implications from the results presented here are that willow planted early in the growing season can establish and grow from both non-dormant and dormant cuttings that can attain similar yield. This can have significant consequences for the economics of willow SRC production and implies that long-term cold storage of willow propagules is redundant and can be replaced by a system of planting cuttings that are produced from freshly harvested shoots. Prospects for cost reductions in this area are therefore promising.

Allometric relations (Paper III)

Cutting phenology had significant effect on the allometric relation between leaf area and leaf dry weight (LA versus LDW), and thus determined specific leaf area (SLA) of leaves produced from willows grown from non-dormant and dormant cuttings. The average SLA was higher for leaves formed on stems that have been produced from non-dormant (278 cm^2 g^{-1}) than dormant (249 cm^2 g^{-1}) cuttings (Paper III).

WSA and WT (Paper I)

Carbohydrate reserves in the propagule determine subsequent willow growth (including *e.g.* aboveground biomass production). This in turn may affect other willow performance parameters such as WSA and WT, as was confirmed by the results from the bucket experiment (Paper I). Non-dormant cuttings had significantly lower WSA (approximately 60%) and WT (approximately 52%) than dormant cuttings when planted mid- to late in the growing season (May 24-June 16, 2013). This can be explained by a decline in carbohydrate reserves in non-dormant cuttings later in the season, leading to a subsequent reduction in aboveground biomass production. As WSA and WT are simple proportions between biomass of the crop (willow in Paper I) and the weed (spring barley in Paper I) grown in mixtures and in monocultures (Nelson *et al.*, 2012; Szumigalski & Van Acker, 2005), WSA and WT estimates are directly affected by the amounts of aboveground biomass measured for crop and weed. Organisms at other trophic levels (*e.g.* symbionts, predators or pests) affecting biomass production or either crop or weed (or both) may thus indirectly affect WSA and WT. This was probably the case in the bucket experiment presented in Paper I. Spring barley planted on June 6 and 16, 2013 was infected with a pathogenic fungus (probably *Blumeria graminis* (DC.) Speer, powdery mildew) and the willows were infested with an herbivorous insect (probably *Phratora vulgatissima* L., willow leaf beetle).

5.1.2 Planting system (Paper II)

Survival and aboveground biomass production
Planting system had significant effected on willow survival and aboveground biomass production (t DW ha^{-1}) in the field experiment. Willow survival was significantly affected by planting system in the third (December 5, 2016) growing season (P=0.0004), and plants grown from cuttings had higher survival (91%) than plants grown from billets (32%). The low survival rate of billets was due to low sprouting during the first month after planting (unpublished data). A significant positive correlation between willow sprouting and willow survival was detected (P<0.026; r=0.5236). Furthermore, low willow survival led to a reduction in biomass production, as indicated by the correlation between willow survival and willow aboveground biomass production (P<0.018; r=0.1394). Biomass production was significantly lower for willows grown from billets than for willows grown from cuttings (Paper II), and after three growing seasons, willow propagated from cuttings had produced significantly higher biomass (11.710 t DW ha^{-1}) compared with willows originating from billets (6.131 t DW ha^{-1}).

The planting systems described in Paper II were defined by two general factors: propagule length (longer cuttings versus shorter billets) and planting orientation (vertical versus horizontal). However, each factor could be further described by a set of specific features possibly also affecting willow survival and aboveground biomass production to some extent. An attempt to disentangle these factors is made in the following paragraphs.

Propagule length
Propagule length, along with propagule diameter (thickness), are important determinants of propagule performance. This is because propagule length and diameter (*i.e.* volume) are proportional to amounts of carbohydrate and hormone reserves and the water content. For instance, Edelfeldt *et al.* (2015) showed that longer cuttings produced more biomass than shorter ones, and attributed it to larger carbohydrate reserves that could subsequently be invested in willow growth. Moreover, Burgess *et al.* (1990) concluded that length of cuttings determined the success of establishment of *Salix alba* L. Although no explanation of this trend was presented in this study, it seems probable that length affects cutting water content that accelerate rooting and sprouting (Tiller & John, 2012).

However, in the field experiment presented in Paper II, propagules had different lengths but: (i) had comparable diameter (ranged between 8 to 19 mm), and (ii) were planted in May 2014 (early spring), therefore the differences in the amounts of carbohydrate and hormone reserves were probably negligible. However, these propagules had a water content of approximately 52% (non-dormant propagules) and 46% (dormant propagules), therefore the survival and aboveground biomass production of willow in the field experiment (Paper II) were probably predominantly affected by water content varying between propagules of different length. This is in agreement with Burgess *et al.* (1990) who showed that cutting length, not diameter, was the most critical factor influencing willow survival and biomass production. It also supports findings by Jeżewski (1955) of a correlation between willow propagule length and subsequent survival and biomass production.

In addition, length of propagule defines the number of viable buds that can sprout (Edelfeldt *et al.*, 2015) and might in turn affect willow performance. In the field experiment (Paper II), approximately 3-4 and 4-6 buds were found on billets and cuttings, respectively (Figure 2), and number of viable buds seemed not to have significantly affected willow survival and production of biomass.

Planting orientation

The effect of planting orientation on sprouting and subsequent growth performance is determined by a combination of environmental factors (*i.e.* soil moisture and precipitation) and propagule characteristics (*i.e.* apical dominance and carbohydrate reserves). In Paper II, billets were planted horizontally at a depth of approximately 3 cm and cuttings were planted vertically to a depth of approximately 18 cm (usually 1-2 cm protruded above the soil surface) on a clayey soil determined as having relatively low permeability (Brady & Weil, 2007; Olsson & Samils, 1984). The amount of precipitation at early establishment (*i.e.* the first month after planting, May-June 2014) was very low (1.1 mm), whereas insolation (974 W m^{-2}) and wind speed (6 m s^{-1}) were relatively high (Anonymous, 2016). Thus less soil moisture was probably available for billets (planted approximately 3 cm below ground level) than for cuttings (having access to moisture already at the soil surface), leading to lower survival and lower aboveground biomass production of billets compared with cuttings. This explanation is consistent with previous studies suggesting that propagule length should be adapted to the type of soil on which willow SRC is established (McElroy & Dawson, 1986), and reporting higher susceptibility of billets than cuttings to drought (McCracken *et al.*, 2010).

Planting orientation affects some propagule characteristics. Successful establishment of vertically and horizontally planted cuttings may be affected by apical dominance. Edelfeldt *et al.* (2015) recorded: (i) higher sprouting and subsequent survival of willows grown from horizontally planted cuttings produced from apical than basal parts of the donor shoot, and (ii) higher sprouting of buds located at the apical end of each individual cutting, irrespective of its planting orientation. No such dependence was observed in the field experiment presented in Paper II, most likely because all propagules were prepared from a relatively short (*i.e.* 120 cm) middle fragment of each donor shoot (for details, see section 4.2 Plant material). Additionally, billets may be able to adapt to horizontal planting and sprout from the entire length, while in vertically planted cuttings uppermost buds may predominantly sprout. However, this was not reflected in willow survival and aboveground biomass production in either the field experiment (Paper II) or in other studies (Edelfeldt *et al.*, 2015). Finally, willow survival and subsequent aboveground biomass production may be defined by planting depth. Carbohydrate and hormone reserves initiate propagule sprouting and promote growth until the plant is not permanently exposed to light and obtains carbohydrates photosynthetically. A propagule that is buried deep in soil depletes its resources before shoots are able to reach the soil surface, and consequently dies (Edelfeldt *et al.*, 2015). However, as numerous billets reached the soil surface and established normally in the field experiment (Paper II), burial depth can be excluded as a factor influencing survival and biomass production in that experiment.

5.1.3 Planting date (Papers I-III)

Planting date significantly affects bud burst, aboveground biomass production (per GDD or t DW ha^{-1}), allometric relations (*i.e.* DW_L versus D and DW_S versus DW_L), and WSA and WT (Papers I-III). This factor is related to time. Time is one of the most frequently considered factors that affect willow growth and development. However, it is differently defined and expressed as *e.g.* 'year growth', 'rotation', 'growing season' or 'harvest cycle' (Albertsson *et al.*, 2014b; Nord-Larsen *et al.*, 2014; Abrahamson *et al.*, 2010; Clay & Dixon, 1997)

In this thesis, for willows planted in the bucket experiment on different dates in the same growing season (Paper I), planting date determines essentially the amounts of carbohydrate and hormone reserves available for willow growth, as discussed above (for details, see section 5.1.1 Propagule phenology). The differences in the amounts of carbohydrate and hormone reserves between planting dates are most likely factors having a direct impact on the trends in willow performance parameters observed in Paper I.

In the field experiment, time is defined as (assessment) date, and growth parameters were determined after consecutive growing seasons (2014-2016).

Bud burst (Papers I)

In the bucket experiment, bud burst differed significantly for willows planted on different dates, and was May 3 > May 10 > May 24 > June 16 > June 6, 2013 (Paper I).

Aboveground biomass production (Papers I-II)

In the bucket experiment, aboveground biomass production per GDD differed significantly for willows planted on different dates, and decreased in the order May 10 > May 3 > May 24 > June 16 > June 6, 2013 (Paper I).

In the field experiment, aboveground biomass production increased significantly after consecutive growing seasons from 0.287 to 8.920 t DW ha^{-1} (from the first to the third growing season, *i.e.* from March 2, 2015 to December 5, 2016 (Paper II).

Allometric relations (Papers III)

In the bucket experiment, planting date had significant effect on the relations which included DW_L (*i.e.* DW_L versus D and DW_S versus DW_L). The earlier the planting date, the more leaf mass per shoot, for a given D, was formed. Furthermore, for a given DW_S, larger DW_L was attained by willows planted early in the growing season (May 3 and 10, 2013) than by willows planted later in the growing season (June 6 and 16, 2013) (Paper III).

WSA and WT (Paper I)

The differences in WSA and WT dependent on planting date were detected in the bucket experiment. WSA and WT were significantly higher (approximately 30% and 66%, respectively) for willows planted on June 16, 2013 than planted on May 3-June 6, 2013. This was perhaps due to a fungal infection (probably *Blumeria graminis* (DC.) Speer; powdery mildew) and insect attack (probably *Phratora vulgatissima* L.; willow leaf beetle) observed for spring barley and willow planted on June 6 and 16, 2013 (Paper I).

5.1.4 Willow clone (Papers I-III)

Willow clone significantly affected bud burst, sprouting, aboveground biomass production (per GDD or t DW ha^{-1}), allometric relations (*i.e.* DW_L versus D, LA versus LDW in the bucket experiment, DW_S versus D in the field experiment), and WSA and WT (Papers I-III).

Bud burst and sprouting (Papers I-II)

In the bucket experiment, bud burst occurred earliest for willow clone Tora, followed by Jorr and Olof (Paper I). In the field experiment, willow clone Tordis had a significantly higher sprouting (97%) compared to willow clones Jorr (81%) and Tora (75%) (Paper II). Data on willow bud burst and sprouting as dependent on willow clone are scarce, but there is evidence that these parameters are determined genetically. For example, Hallingbäck *et al.* (2016) confirmed highly significant genetic effects on bud burst of *Salix viminalis* L. of different geographical origins.

Aboveground biomass production (Papers I-II)

Willow clone Olof was found to have lower aboveground biomass production per GDD (0.0058 ±0.00023 g GDD °C) compared with Tora (0.0074 ±0.00027 g GDD °C) and Jorr (0.0069 ±0.00026 g GDD °C) (Paper I). The results of previous studies (Sevel *et al.*, 2012) and reports from full-scale field trials (Caslin *et al.*, 2012) also confirm that Tora is still among the most high-yielding clones, especially in north-central Europe.

In the field experiment, aboveground biomass production (t DW ha^{-1}) differed significantly between willow clones, and decreased in the order Tordis > Tora > Jorr (Paper II). On average (March 2, 2015-December 5, 2016), aboveground biomass production was 11.480, 9.270 and 6.010 t DW ha^{-1} for Tordis, Tora and Jorr, respectively (Paper II). High yield of Tordis was previously observed also by Albertsson *et al.* (2014a) and Caslin *et al.* (2012) who listed this clone among those with the highest production of biomass.

The differences in aboveground biomass production between willow clones are determined genetically, but information about the genetic diversity of willow clones used in SRC is still scarce (Singh *et al.*, 2013; Barker *et al.*, 1999), especially when growth and other performance parameters are considered. However, in studies investigating selected growth traits of *Salix viminalis* L., Rönnberg-Wästljung (2001) have found that number of willow shoots and shoot mass is controlled genetically and shows a high amount of dominance genetic variance, independent of the origin of parents.

Allometric relations (Papers III)

In the bucket experiment, allometric relation DW_L versus D was significantly affected by willow clone. Clone Tora produced more leaf mass per shoot for a given diameter than clones Jorr and Olof. The allometric relation LA versus LDW was also clone-dependent, and clone Jorr had higher average SLA (284 cm^2 g^{-1}) than either Tora (255 cm^2 g^{-1}) or Olof (249 cm^2 g^{-1}) (Paper III).

In the field experiment, the effect of clone on relation DW_S versus D was significant. Tordis presented the highest DW_S for a given D, followed by Tora and Jorr (Paper III). Furthermore, this relation was clone-specific after the first (March 2, 2015) and third (December 5, 2016), but not after the second (March 1, 2016) growing season (Paper III).

Allometric relations between easy to measure features were repetitively found to be clone-specific, therefore should not be interchangeably used from one clone (or set of clones) to another (Mosseler *et al.*, 2016; Arevalo *et al.*, 2007; Heinsoo *et al.*, 2002; Telenius & Verwijst, 1995). This is because patterns of biomass production (Rönnberg-Wästljung, 2001), hence probably partitioning within each clone, is determined genetically. Furthermore, leaf parameters such as SLA differed between willow clones, as leaf characteristics are determined genetically (Tsukaya, 2013; Tsukaya, 2004).

To achieve continuous improvement by breeding, modern willow clones are usually crosses between clones presenting high yield and exhibiting other desired properties (*e.g.* resistance to frost, drought or pathogens). However, clone-dependent differences in productivity cannot be predicted only on the basis of clone genetic heritage. This is because clone-dependent differences have been reported for both genetically distant and closely related willow clones. For example, biomass production varied significantly between Tora and Jorr, and between Tordis and Jorr, which do not share genetic ancestors. Significant differences in biomass production were also recorded between Tora and Tordis, which are closely related (Tora is the female parent of Tordis) (Caslin *et al.*, 2012) (Papers I and II). These results support earlier findings published in the literature where the differences in biomass yield between different willow clones was recorded (Albertsson *et al.*, 2014a; Larsen *et al.*, 2014b). These observations and comparisons of willow clones obtained from various breeding programmes show therefore the significance of breeding efforts in improving the productivity of clones used in willow SRC (Vermerris, 2008; Lindegaard & Barker, 1996).

Clone-dependent differences in productivity cannot be predicted only on the basis of time of breeding and release to commercial use. This is because older clones may still achieve higher yield than newer clones. For example, Olof and Tordis were crossed in 1992 and 1995, respectively, and are characterised by high yields. However, Tora that was crossed in 1989, is still considered one of the high-yielding clone (Svalöf Weibull AB, Sweden; Stig Larsson, European Willow Breeding; Bo Gertsson, Lantmännen SW Seed; personal communication 2017). In fact, Tora is used as the yield control when biomass of numerous clones is compared simultaneously (Caslin *et al.*, 2012).

WSA and WT (Paper I)

Clone-dependent differences in WSA and WT were detected in the bucket experiment. The highest and the lowest WSA was recorded for Tora and Olof, respectively, and (ii) the highest WT was recorded for Tora (Paper I). Thus far, no study has focused specifically on the WSA and WT of various willow clones. However, the magnitude of biomass reduction due to weed competition is known to vary between willow clones (Albertsson *et al.*, 2014a), indicating slight differences in their competitive ability.

5.1.5 Weed competition (Papers I-III)

Weed competition significantly affected aboveground biomass production (per GDD or t DW ha^{-1}), survival and allometric relations (*i.e.* DW$_A$ versus D, DW$_S$ versus D, DW$_S$ versus DW$_L$, and LA versus LDW) (Papers I-III).

Survival (Papers II)

Survival was significantly higher for willows grown in weeded (69%) than in unweeded plots (54%). Weed pressure significantly affected willow survival in the field experiment in the second (March 1, 2015) and third (December 5, 2016) growing seasons ($P<0.0001$ and $P=0.0011$, respectively). Furthermore, cuttings in weeded plots had significantly higher survival (96%) than cuttings grown in unweeded plots (86%) (*i.e.* June 30, 2014-December 5, 2016). Corresponding values for billets were 42% (weeded plots) and 22% (unweeded plots), respectively (Paper II). This is consistent with findings by Albertsson *et al.* (2014b), who showed that due to weed competition only approximately 59% of initially planted cuttings in a field trial in Sweden survived after the first harvest cycle.

The negative impact of weeds on willow survival and growth is related to competition for light, rather than nutrients, according to Clay and Dixon (1997), Sage (1999) and Edelfeldt *et al.* (2016). The low ability of willow to compete with weeds for light is determined genetically, as willows are light-demanding pioneers predominantly invading habitats devoid of vegetation cover (Newsholme, 1992) (*e.g.* fallow areas, river banks, post-mine hips, screes, alluvial deposits). Support was found for this assumption in the present thesis, as WSA and WT of willow were significantly (*P*<0.0001) lower that the suppressive ability and tolerance of the spring barley used in the bucket experiment as a model weed (Figure 9).

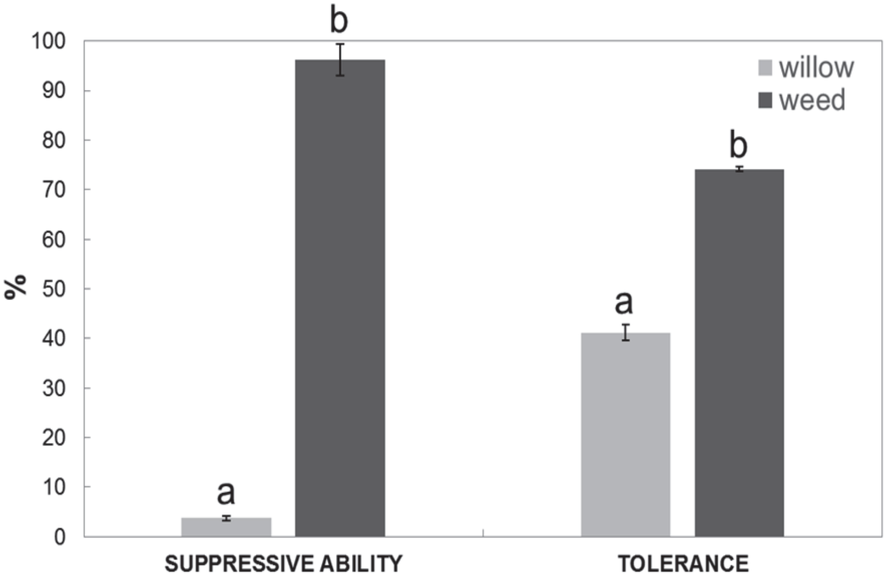

Figure 9. Mean (bars are standard error of the means, SE) suppressive ability (WSA) and mean weed tolerance (WT) of willow and model weed in the bucket experiment. Spring barley was used as the model weed. Statistically significant differences (*P*<0.05, ANOVA) in suppressive ability or tolerance between willow and weed are indicated by lower case letters. This graph is not included in any of Papers I-IV in this thesis.

Aboveground biomass production (Papers I-III)

When grown with weeds in the bucket experiment (Paper I), willow produced approximately 64% less aboveground biomass per GDD than willow grown without weeds. Aboveground biomass production in the field experiment was approximately 83% higher for willow grown in weeded compared with unweeded plots (Paper II).

These results of these studies confirm observations published in the literature. Weed pressure is an important determinant of willow performance parameters, especially at establishment and early growth. This is because willow does not recover from damage due to weed competition in the establishment year (Clay

& Dixon, 1997). For example, in the second year after willow establishment (after first coppicing), Sage (1999) reported an approximately 20% reduction in biomass for willow grown under weed pressure compared with willow grown in weed-free plots. Furthermore, Albertsson *et al.* (2014b) reported an approximately 85% reduction of biomass in experimental willow SRC in Sweden when willows grew under weed competition compared with weed-free conditions. Clay and Dixon (1997) showed that biomass production from willow was reduced by approximately 60-90% for plants competing with weeds compared with plants grown without weed competition.

Allometric relations (Papers III)

In the bucket experiment, weed treatment had significant effect on allometric relations DW_A versus D, DW_S versus DW_L, LA versus LDW and DW_S versus D (Paper III). For a given D, DW_A and DW_S were larger for stems of willows grown without weeds than these grown with weeds. The relation LA versus LDW was affected by weed treatment, and the average SLA was larger (296 cm^2 g^{-1}) for leaves produced on stems of willows grown with weeds than grown without weeds (238 cm^2 g^{-1}) (Paper III). Competition from weeds may be considered as environmental stress in terms of limited availability of light. Therefore, plants response to light limitation by changes in leaf parameters including SLA. In the bucket experiment, SLA for willows grown with weeds was presumably higher because plants tend to compensate low intensity of light by expansion of the area by which photosynthetic energy can be intercepted (Nobel, 1976).

In the field experiment, allometric relation DW_S versus D was affected by the weed treatment, but only after the second growing season (March 1, 2016) (Paper III). A possible explanation of this trend may be that during this growing season weed cover and maximum weed height peaked, and therefore willows still were negatively affected by weeds overshading them.

Reduction of willow biomass due to weed competition is well-documented, but knowledge about its impact on willow allometry is scarce. However, a study by Sage (1999) provides support for the assumption that biomass partitioning in willows varies under different levels of competition from weeds. It revealed that thinner, but taller, stems are produced from willow grown with weeds compared with willow grown without weeds.

Practical implications from the results presented in this thesis are that weed competition is a dominant factor affecting most of the performance parameters and subsequently leading to severe losses of willow yield. Therefore efficient weed control during willow establishment is essential, and may be prolonged also during the second growing season (Paper III). This has the economic implication. This is because a reduction in willow biomass is not merely a reaction to scarcity of resources taken over by weeds, but affects patterns of willow biomass partitioning on the level of the entire plant that may in turn affect willow yield. Therefore weed control should not be omitted for fear of economic losses or to reduce these losses, because the consequences of inappropriate weed control are reflected in low survival and subsequent low biomass production during entire lifespan of willow SRC.

5.2 Floristic diversity in willow SRC (Paper IV)

The aim of this part of the doctoral project was to compare weed flora in willow SRC and cereal stands, and to determine the contribution of willow SRC to floristic diversity preservation in the agricultural landscape (Paper IV). Numerous studies have reported higher species richness and diversity of plants, fungi, insects and animals in willow SRC compared with agricultural fields established within the same landscape (Romano *et al.*, 2013; Baum *et al.*, 2012; Campbell *et al.*, 2012; Vonk, 2008; Reddersen, 2001). Therefore, the role of willow SRC in increasing biodiversity on the level of agrarian ecosystems has been emphasised during the past decade by government institutions (*e.g.* Swedish Board of Agriculture, Hollsten *et al.* (2012)) and by farmers (Nils-Erik Nordh, personal communication 2016). However, although higher diversity of plants in willow SRC compared with cropland is an undeniable fact, farmers are concerned that flora developed in willow SRC planted on their farmland may become a reservoir of weeds that will spread to the surrounding crops, or may cause long-lasting weed infestations in crops established on land returned to cereal production after termination of willow SRC.

Detailed analyses of floristic data collected in the long-term field experiment (Paper IV) provided insights into flora richness, composition and divergence over time. Floristic data collected from the field experiment were not published or prepared for publication in any of Papers I-IV in this thesis. However, these data were also implemented and discussed in separate paragraph of this thesis as selected weed flora characteristics were significantly affected by the planting system.

5.2.1 Cropping system (Paper IV)

The results of weed flora monitoring in the long-term field experiment (Paper IV) confirmed higher richness and diversity of weed species in willow SRC compared with neighbouring cereal stands (Figure 8), thereby providing support for earlier findings (Baum *et al.*, 2012; Augustson *et al.*, 2006; Gustafsson, 1986). However, although these two cropping systems were established on land previously grown with willow for 25 years, meaning that they shared a similar management history and seed bank, flora development in the two cropping systems followed distinctive trajectories. This indicates that the differences in weed flora both over time and between cropping systems are related to the specific environmental conditions (*i.e.* light, soil moisture, nitrogen level and soil reaction, as inferred from the Ellenberg index) rather than to similarities in the initial seed bank. This has practical implications. The expression of the seed bank is determined by the cropping system, and therefore there is no risk of weed infestation other than that for the respective cropping system. Therefore land reclaimed after termination of a long-term willow SRC can be returned to agricultural production after termination of long-term willow SRC (Paper IV). Furthermore, willow SRC established in agricultural land can provide a habitat and refuge for flora eliminated from cropland by intensive management, and consequently contribute to floristic diversity preservation at the landscape scale (Paper IV). This has no significant consequence for the economics of willow production, but delivers numerous environmental, cultural and aesthetic values.

Characteristics such as weed species richness, ground cover, diversity, composition and trajectories of weed flora development differed between cropping systems (Paper IV), most likely in response to: (i) variable light conditions and (ii) management practices (*i.e.* disturbances), associated with individual cropping system.

In the long-term field experiment, seeding density of cereals defined the gaps within the cereal canopy available for weeds to establish (Figure 8B). Besides, cereals reach their maximal elongation of stems within a few weeks after sowing (Bleiholder et al., 1997) and the canopy was homogeneous in terms of height and architecture (*i.e.* distribution of shoots and leaves). Thus, in cereal stands light conditions over a season change according to a pattern of sowing-germination-establishment-growth that is followed every season. Although the gaps between individual cereal plants are relatively small, these gaps may be colonised by fast-growing competitive weeds that usually show earlier germination and faster growth than cereals (*e.g. Sinapis arvensis* L., *Elymus repens* (L.) Gould, *Cirsium arvense* (L.) Scop.) (Paper IV). When adapted to cereal canopy height and architecture, weeds can establish and outgrow cereals in competition for light (*e.g. Cirsium arvense*, *Elymus repens*) (Paper IV). The canopy of willow develops over a few growing seasons and light conditions are determined by willow growth. Penetration of light to the understorey at the stage of willow establishment is unlimited, as the canopy is discontinuous and scattered (so-called 'light phase' of willow canopy development, Figure 5), but it gradually decreases as the willow grows and forms a denser canopy (the so-called 'shade phase' of willow canopy development, Figures 7 and 8A) (Gustafsson, 1988). These temporal alterations in light penetration to the understorey are mirrored in weed species richness, diversity and composition, as shade-tolerate weed species (*e.g. Ranunculus repens* L., *Chelidonium majus* L., *Galium odoratum* (L.) Scop.) succeed light-demanding species (*e.g. Chenopodium album* L., *Fumaria officinalis* L. and *Sinapis arvensis*) upon gradual closure of willow canopy (Figures 7 and 8A) (Paper IV).

Regularly drilled (spring or autumn 2010-2015), harrowed (2010), tilled (2011-2015) and fertilised (2012-2015) cereal fields in the long-term field experiment (Paper IV) became a habitat for annuals or perennials that tolerate or are favoured by these disturbances (*e.g. Sinapis arvensis*, *Fumaria officinalis*, *Chenopodium album*, *Cirsium arvense*). However, no significant temporal differences in richness and composition of the weed flora in cereal stands were observed during consecutive growing seasons. This can be explained by the fact that:

(i) both mechanical and chemical disturbance act as an environmental filter, favouring weed species which are either resistant to these disturbances or have developed strategies to overcome them (Ryan *et al.*, 2010; Booth & Swanton, 2002; Håkansson, 1995), and

(ii) composition of weed flora due to frequent disturbances is usually very simple and contains species which can complete their life cycle within the short period between disturbance events (Matus & Tothmeresz, 1995).

In contrast, willow SRC in the long-term field experiment in Paper IV remained undisturbed by any management practices (apart from weeding at the stage of willow establishment, 2010). This promoted establishment of perennials and biennials (*e.g. Cirsium arvense*, *Deschampsia cespitosa* (L.) P. Beauv, *Elymus repens*, *Geranium robertianum* L.) and stimulated an increase in weed species richness and composition during consecutive growing seasons, in a similar manner as reported by Pučka *et al.* (2016), Norberg and Nordh (2012) and Augustson (2004). In response to management practices and light conditions, weed flora diversity and composition diverged already in the first growing season after establishment of willow SRC and cereal stands (Figure 10, growing season 2010), and followed different trajectories during consecutive seasons (Figure 10, growing seasons 2012 and 2015). A decrease in weed species richness was observed over time, presumably as a result of:

(i) adaptation of the seed bank to disturbances related to system management (*i.e*. more annuals) or intraspecific competition between weeds and crops (Zimdahl, 2004; Håkansson, 2003) in cereal stands, and

(ii) interspecific competition between weed species adapting differently to environmental conditions (*e.g*. shade-tolerant species dominate) affected by canopy development in willow SRC (Håkansson, 2003).

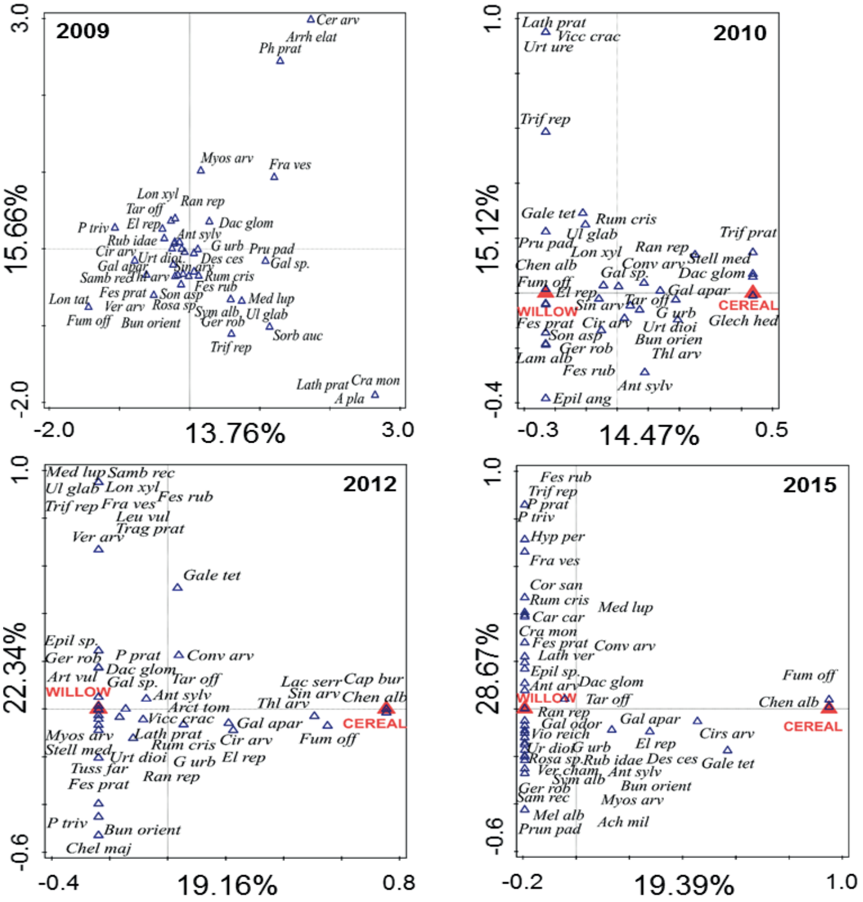

Figure 10. Correspondence analysis (CA) (2009) and canonical correspondence analysis (CCA) (2010, 2012, 2015) ordination diagrams presenting gradients in the weed species composition in 25-years-old willow SRC (June 20-21, 2009) before its termination, and in newly established willow SRC and cereal stands in consecutive growing seasons (July 26, 2010; June 28, 2012; July 27, 2015) (Canoco software; ter Braak and Šmilauer (2012). Weed species codes are explained in Table S1 (Appendix). This graph is not included in any of Papers I-IV in this thesis.

5.2.2 Planting system (field experiment)

The maximum weed height (*P*=0.0002) and cover abundance of perennial weed species (*P*=0.0217) differed significantly between planting systems in the field experiment (unpublished data), and it was higher under willow grown from billets than grown from cuttings. This was probably due to variable light conditions under willow grown from billets compared with willow grown from cuttings. In addition, composition of the weed flora differed in the understorey of willow grown from cuttings and willow grown from billets (*P*<0.0001 for growing season 2015 in which weeding ceased; Figure 11). Although no exact measurements of light were available for the field experiment, sunlight penetration through the canopy to the understorey was greater in plots with willow grown from billets than with willow grown from cuttings, mainly due to:
(i) differences in their architecture, as willow plants grown from billets tended to produce many thinner, but shorter shoots, while plants grown from cuttings produced fewer, but longer and thicker shoots, and
(ii) lower sprouting rate and subsequent survival of willow grown from billets compared with willow grown from cuttings, leading to larger gaps within the canopy available for weed establishment (Paper II).

Visual observations (Figures 5-7) and statistical analyses by means of correlations between willow survival and weed cover abundance, and willow canopy cover and weed cover abundance seem to support this assumption. Higher willow survival was negatively correlated with weed cover abundance (cuttings: *P*<0.0001, r=-0.272; billets: *P*<0.0001, r=-0.361). Greater cover by the willow canopy was negatively correlated with cover abundance of weeds developed in willow understorey (cuttings: *P*<0.0001, r=-0.543; billets: *P*<0.0001, r=-0.367) (unpublished data).

Regular weeding was carried out in half the plots in the field experiment, aimed at supporting establishment and early growth of willow (Paper II). However, floristic data collected in the course of this field experiment showed that weeding acted as a disturbance and significantly affected not only weed cover abundance, as expected and desired, but also weed flora richness, diversity, composition and trajectories of development (Figure 11 and unpublished data). When undisturbed by weeding (unweeded plots), establishment and development of flora continued from several days after willow planting throughout consecutive growing seasons (2014-2016; Figure 11), and reached relatively high weed species richness and composition already 4 months after willow planting (Figure 11, growing season 2014). Similar observations have been reported by Albertsson *et al.* (2014b) who found 47 weed species covering approximately 85% of the ground in willow

SRC cut-back after the first growing season. As intended, no weeds were present in weeded plots at the phase of willow establishment and early growth (May 2014, Paper II), but weed flora began to develop also in weeded plots following cessation of weeding (May 2015). However, the trajectories, composition and diversity of the flora developed in these plots seemed to differ from those of the flora in unweeded plots. The differences were due not only to temporal delay in weed flora establishment and development, but probably also due to alterations in spatial conditions (*e.g*. light conditions) as the willow canopy developed over time.

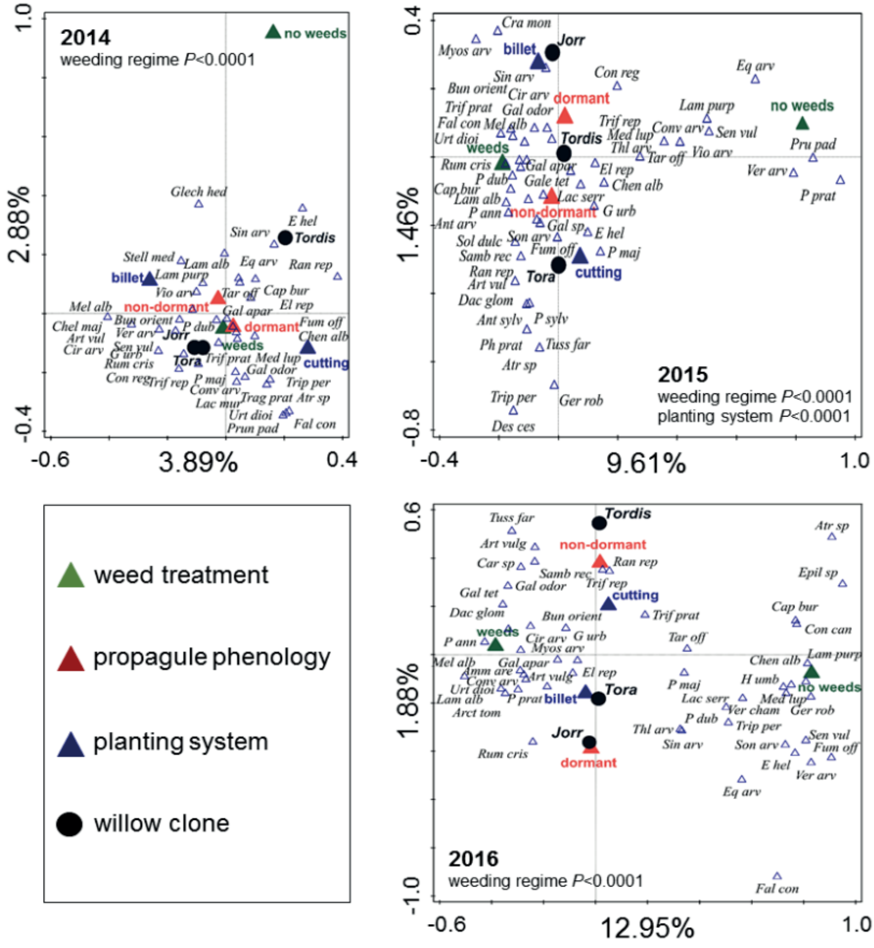

Figure 11. Canonical correspondence analysis (CCA) ordination diagrams presenting gradients in weed species composition in willow SRC (field experiment) in growing seasons 2014 (September, 16-17), 2015 (July, 22-23) and 2016 (August, 02-03) as dependent on weed treatment (green triangles; with weeds, without weeds), propagule phenology (red triangles, non-dormant, dormant), planting system (blue triangles, cutting, billet) and willow clone (black dots; Tora, Tordis and Jorr) (Canoco software; ter Braak and Šmilauer (2012)). The growing season and statistical significance of weed treatment (2014-2016) and planting system (2015) according to interactive forward selection of predictors is presented under the indication of the growing season in each graph. Weed species codes are explained in Table S1 (Appendix). This graph is not included in any of Papers I-IV in this thesis.

6 Conclusions

6.1 Growth performance in willow SRC

Propagule phenology

- Bud burst of willows grown from non-dormant cuttings occurred faster than bud burst of willows grown from dormant cuttings when they were planted early in the growing season.
- Sprouting of willows grown from non-dormant propagules (73%) was higher than sprouting of willows grown from dormant (58%) propagules.
- When planting was postponed to later growing season, willows grown from dormant cuttings produced significantly more aboveground biomass per GDD than those from non-dormant cuttings.
- SLA was higher for leaves formed on stems that have been produced from non-dormant than dormant cuttings.
- When planted later in the growing season, willows grown from non-dormant cuttings had lower WSA (approximately 60%) and WT (approximately 52%) than WSA and WT of dormant cuttings.

Planting system

- Higher number of initially planted cuttings (91%) compared with billets (32%) survived (June 30, 2014-December 5, 2016).
- Willows grown from cuttings (11.710 t DW ha^{-1}) produced significantly more aboveground biomass than willows grown from billets (6.131 t DW ha^{-1}).

Planting date

- Bud burst differed for willows planted at different dates, and was faster during early than during later growing season (May 3 > May 10 > May 24 > June 16 > June 6, 2013).

- Aboveground biomass production increased significantly over time (*i.e.* from 0.287 to 8.920 t DW ha^{-1} from March 2, 2015 to December 5, 2016).
- WSA (30%) and WT (66%) were significantly higher for willows planted later in the growing season (June 16, 2013) than planted early in the season (May 3-June 6, 2013).

Willow clone

- Bud burst occurred faster for Tora than for Jorr and Olof.
- Sprouting was higher for clone Tordis (97%) compared with Jorr (81%) and Tora (75%).
- Olof had significantly (16%) lower aboveground biomass production per GDD than Tora and Jorr.
- Tordis produced the highest aboveground biomass (11.480 t DW ha^{-1}), followed by Tora (9.270 t DW ha^{-1}) and Jorr (6010 t DW ha^{-1}).
- Tora produced more leaf mass (DW_L) per shoot for a given diameter (D) than clones Jorr and Olof.
- Jorr presented higher SLA (284 cm^2 g^{-1}) than Tora (255 cm^2 g^{-1}) and Olof (249 cm^2 g^{-1}).
- Tordis presented the highest stem mass (DW_S) for a given diameter (D), followed by Tora and Jorr.
- WSA was the highest for Tora, and the lowest for Olof, and WT was the highest for Tora.

Weed treatment

- Survival was significantly higher for willows grown in weeded plots (69%) compared to unweeded plots (54%).
- Higher survival (93%) was recorded for cuttings in weeded plots than in unweeded plots (49%), and for billets in weeded plots (49%) than in unweeded plots (39%) (June 30, 2014-December 5, 2016).
- Aboveground biomass production of willow grown without weeds was significantly higher than for willows grown with weeds.
- For a given diameter (D), total aboveground mass (DW_A) and stem mass (DW_S) were larger for stems of willows grown without weeds than these grown with weeds.
- The average SLA was larger (296 cm^2 g^{-1}) for leaves produced on stems of willows grown with weeds than grown without weeds (238 cm^2 g^{-1}).

6.2 Floristic diversity in willow SRC

Cropping system

- Weed species richness, cover, composition and diversity were higher in willow SRC than in cereal stands.
- Upon gradual closure of the willow canopy, there is no risk of competition for light between willow and weeds.
- Weeds did not infest cereal stands established after termination of a long-term willow SRC.

Planting system

- The maximum weed height and cover abundance of perennial weed species was higher in plots with willow grown from billets than in plots with willow grown from cuttings.
- Composition of the weed flora in the understorey was higher under willow grown from billets than willow grown from cuttings in growing season 2015 (weeding ceased).

The results and knowledge gained through the research presented in this thesis will be communicated to commercial willow growers, in order to increase biomass production in willow SRC in Sweden and thus improve its economic and environmental value in current and future agriculture.

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Appendix

Table S1. Weed species identified in correspondence analysis (CA) and canonical correspondence analysis (CCA) in the field experiment (Paper II) and in the long-term field experiment (Paper IV). Abbreviated names of plant species are used in Figures 10 and 12 that were not presented in any of the Papers I-IV in this thesis.

No.	Name of plant species	Abbreviation
1	*Acer platanoides* L.	A pla
2	*Achillea millefolium* L.	Ach mil
3	*Ammophila arenaria* (L.) Link	Amm are
4	*Anthemis arvensis* L.	Ant arv
5	*Anthriscus sylvestris* Hoffm.	Ant sylv
6	*Arctium tomentosum* Mill.	Arct tom
7	*Arrhenatherum elatius* (L.) P. Beauv. ex J. & C. Presl	Arrh elat
8	*Artemisia vulgaris* L.	Art vul
9	*Atriplex* sp. L.	Atr sp
10	*Bunias orientalis* L.	Bun orient
11	*Capsella bursa-pastoris* (L.) Medik.	Cap bur
12	*Carex sp.* L.	Car sp.
13	*Carum carvi* L.	Car car
14	*Cerastium arvense* L.	Cer arv
15	*Chelidonium majus* L.	Chel maj
16	*Chenopodium album* L.	Chen alb
17	*Cirsium arvense* (L.) Scop.	Cir arv
18	*Consolida regalis* Gray	Con reg
19	*Conyza canadensis* (L.) Cronquist	Con can
20	*Convulvulus arvensis* L.	Conv arv
21	*Cornus sanguinea* L.	Cor san
22	*Crataegus monogyna* Jacq.	Cra mon
23	*Dactylis glomerata* L.	Dac glom
24	*Deschampsia cespitosa* (L.) P. Beauv.	Des ces
25	*Elymus repens* (L.) Gould	El rep
26	*Epilobium angustifolium* (L.)	Epil ang
27	*Epilobium* sp. L.	Epil sp.
28	*Euphorbia helioscopia* L.	E hel
29	*Equisetum arvense* L.	Eq arv
30	*Fallopia convolvulus* (L.) Á. Löve	Fal con

31	*Festuca pratensis* Huds.	Fes prat
32	*Festuca rubra* L.	Fes rub
33	*Fragaria vesca* L.	Fra ves
34	*Fumaria officinalis* L.	Fum off
35	*Galeopsis tetrahit* L.	Gale tet
36	*Galium aparine* L.	Gal apar
37	*Galium odoratum* (L.) Scop.	Gal odor
38	*Galium* sp. L.	Gal sp.
39	*Geranium robertianum* L.	Ger rob
40	*Geum urbanum* L.	G urb
41	*Glechoma hederacea* L.	Glech hed
42	*Holosteum umbellatum* L.	H umb
43	*Hypericum perforatum* L.	Hyp perf
44	*Lactuca muralis* (L.) Fres	Lac mur
45	*Lactuca serriola* L.	Lac serr
46	*Lamium album* L.	Lam alb
47	*Lamium purpureum* L.	Lam purp
48	*Lathyrus pratensis* L.	Lath prat
49	*Lathyrus vernus* L. Bernh.	Lath ver
50	*Leucanthemum vulgare* Lam.	Leu vul
51	*Lonicera tatarica* L.	Lon tat
52	*Lonicera xylosteum* L.	Lon xyl
53	*Medicago lupulina* L.	Med lup
54	*Melilotus alba* Medik.	Mel alb
55	*Myosotis arvensis* (L.) Hill	Myos arv
56	*Papaver dubium* L.	P dub
57	*Phleum pratense* L.	Ph prat
58	*Plantago major* L.	P maj
59	*Poa annua* L.	P ann
60	*Poa pratensis* L.	P prat
61	*Poa trivialis* L.	P triv
62	*Poa sylvestris* A. Gray	P sylv
63	*Prunus padus* L.	Pru pad
64	*Ranunculus repens* L.	Ran rep
65	*Rosa* sp. L.	Rosa sp.
66	*Rubus idaeus* L.	Rub idae
67	*Rumex crispus* L.	Rum cris
68	*Sambucus racemosa* L.	Samb rec
69	*Senecio vulgaris* L.	Sen vul
70	*Sinapis arvensis* L.	Sin arv

71	*Solanum dulcamara* L.	Sol dulc
72	*Sonchus arvensis* L.	Son arv
73	*Sonchus asper* (L.) Hill	Son asp
74	*Sorbus aucuparia* L.	Sorb auc
75	*Stellaria media* (L.) Vill	Stell med
76	*Symphoricarpos albus* (L.) S. F. Blake	Sym alb
77	*Taraxacum officinale* F. H. Wigg.	Tar off
78	*Thlaspi arvense* L.	Thl arv
79	*Tragopogon pratensis* L.	Trag prat
80	*Trifolium pratense* L.	Trif prat
81	*Trifolium repens* L.	Trif rep
82	*Tripleurospermum perforatum* (L.) Sch.Bip.	Trip per
83	*Tussilago farfara* L.	Tuss far
84	*Ulmus glabra* Huds.	Ul glab
85	*Urtica dioica* L.	Urt dioi
86	*Urtica urens* L.	Urt ure
87	*Veronica arvensis* L.	Ver arv
88	*Veronica chamaedrys* L.	Ver cham
89	*Vicia cracca* L.	Vicc crac
90	*Viola arvensis* Murr.	Vio arv
91	*Viola reichenbachiana* Jord	Vio reich