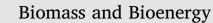
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Research paper

## Biomass yield, nutrient concentration and nutrient uptake by SRC willow cultivars grown on different sites in Denmark



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#### ABSTRACT

Yield and nutrient uptake of willow cultivars are important factors for long-term feasibility and sustainability of willow short rotation coppice (SRC). This study investigated biomass yield of eight willow cultivars (Inger, Klara, Linnea, Resolution, Stina, Terra Nova, Tora, Tordis) during two three-year harvest rotations on four Danish sites. Also, concentration and uptake of N, P and K was measured in harvested biomass in 2<sup>nd</sup> harvest rotation on two of the sites.

Dry matter (DM) yield differed significantly between sites in both 1<sup>st</sup> and 2<sup>nd</sup> harvest rotation, but the relative difference between sites decreased from 106 to 54 %. Mean DM yield across cultivars and sites increased 67 % from 1<sup>st</sup> to 2nd harvest rotation but ranging from 44 to 108 % between sites. There were certain significant differences in yield ranking of cultivars between sites and harvest rotations but, overall, ranking was rather consistent. Across all four sites and all six growth years, there were four cultivar groups differing significantly in DM yield, with highest yield in Tordis and Tora, second highest in Klara and Resolution followed by Terra Nova and Inger and lowest yield in Linnea and Stina, with Stina having 39 % lower yield than Tordis.

Concentration and uptake of N, P and K in harvested biomass in  $2^{nd}$  harvest rotation differed significantly between cultivars and between sites. Across cultivars and sites, nutrient concentration decreased linearly with increasing DM yield, whereas nutrient uptake increased linearly. These results have implications for nutrient balance and fertilization strategies for willow SRC.

#### 1. Introduction

Willow (*Salix* spp). production is considered a relatively sustainable source of feedstock for bioenergy e.g. compared to straw which is a commonly used biomass for bioenergy in Denmark, with willow having low carbon footprint and eutrophication potential and high energy output to input ratio [1]. Willow production has also been shown to be more profitable than poplar (*Populus* spp.) or locust (*Robinia pseudoacacia* L.) production [2]. However, the evaluations of both the sustainability and the economic feasibility rely on assumptions regarding the biomass yield across different sites, and yield is often one of the main factors for economic success in SRC [3]. For instance, a 10 % increase in biomass yield has been shown to increase the economic revenue of willow production by 13–20 % [4]. Therefore, it is very relevant to optimize the practically achievable yield by proper management of factors potentially influencing yield.

Choice of robust and high-yielding willow cultivars is one of the management factors that the willow grower can actively affect, and which can have significant economic impact [4]. A number of studies in

Europe and North America have shown significant differences in biomass yield between willow cultivars or genotypes [5–13], demonstrating the importance of the genetic material used in willow production. The yield level of a willow plantation generally increases from first to subsequent harvest rotations [10,14]. However, willow cultivars may also differ in their yield development over time [6,9,10,15], and this emphasizes the need for testing of willow cultivars over more than one harvest rotation.

A range of environmental factors may also affect the yield level for willow, and the environmental effects have been shown to account for the majority of the yield variation compared to genetic effects [5]. In some cases, willow cultivars have been found to respond differently to site-specific growth conditions, e.g. related to soil characteristics, climate and management [7,9], soil type [13] and growing degree days [9]. Hence, to improve the economic feasibility of willow production, it is important to choose cultivars with a documented high yield over multiple harvest rotations and within various growth conditions.

Fertilization practice is another management factor by which the willow grower can potentially affect the yield and economic feasibility

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of willow production. Fertilization is considered as one of four dominating cost components, along with establishment, harvest and road transport [16], and the economic effect of fertilization depends on both the yield increase, the biomass price and the fertilization costs [17]. However, the effect of fertilization varies markedly between studies, with fertilization resulting in very large yield increases in certain studies [17], moderate increases in some studies [18-21] and no significant effect in other studies [22,23]. The variability in fertilization effect complicates the development of general recommendation for fertilization of willow. The lack of consistency of the fertilization effect in willow suggests that nutrients should be applied at a rate which balances nutrient removal by harvest in order to maintain growth in the long term [24]. The quantity of harvested nutrients, however, depends on both the biomass yield and the concentration of nutrients in the biomass, and both parameters are highly variable. The concentration of e.g. N, P and K are typically 3-4 fold higher in the bark fraction than in the wood fraction of willow [25]. Since the bark proportion decreases with increasing age and yield [26], the average concentration of N, P and K also decreases with yield, e.g. as exemplified for various shoot ages [27,28]. Hence, the nutrients in the biomass are 'diluted' at high biomass yields. Moreover, since willow cultivars may differ both in yield [7] and nutrient concentration [27,28], it is relevant to study the resulting nutrient removal across a range of willow cultivars. This is of importance both in relation to nutrient balance and fertilization recommendations for general willow production as well as in relation to use of willow as a bioremediation medium [29]. Another aspect of nutrient uptake in willow is the nutrient use efficiency (NUE). This has implications in terms of crop productivity and environmental problems caused by enhanced use of fertilizers [30], and for bioenergy production it is generally desirable with a high biomass production per unit of applied or removed nutrient. However, if willow is grown for phytoremediation purposes, willow clones with low NUE and high nutrient removal may be more desirable [31]. Willow cultivars have been found to differ in their NUE [31,32], but little is known about potential differences in NUE among willow cultivars grown commercially for bioenergy in Northern Europe.

We conducted four willow cultivar trials on four different sites in Denmark, each including eight willow cultivars. Biomass yield in the 1<sup>st</sup> three-year harvest rotation was reported in Larsen et al. [7], and here we report the biomass yield over both the 1<sup>st</sup> and 2<sup>nd</sup> three-year harvest rotations. Moreover, we analysed the concentration and uptake of N, P and K in harvested willow biomass in 2<sup>nd</sup> rotation for two of the trials. The paper focuses on the following research questions: i) How is willow biomass yield affected by cultivar, site and harvest rotation as well as their interacting effects? ii) How is nutrient concentration and nutrient uptake affected by willow cultivar and site and how is nutrient concentration and uptake related to biomass yield?

#### 2. Materials and methods

#### 2.1. Trial sites and management of trials

Five cultivar trials were established in Denmark in May 2010 as described by Larsen et al. [7] who reported yield data from the 1<sup>st</sup> harvest rotation. Four of these trials were continued during the 2<sup>nd</sup> three-year harvest rotation, namely the trials Højmark and Foulum on loamy sand and Foersom and Jyndevad on coarse sand. The trials Foulum and Jyndevad are located on experimental stations affiliated with Aarhus University, whereas the trials Højmark and Foersom are located on commercial willow fields. Details on climate and management are shown in Table 1, and details on soil type, ground water level and previous land-use are shown in Supplementary Material Table 1 and in Larsen et al. [7].

Climatic conditions were measured at meteorological stations within 1 km from the trials in Foulum and Jyndevad, and for the trials in Højmark and Foersom, weather data were obtained from the meteorological station Boris, located approx. 17 and 14 km from the two trials, respectively. Missing data were replaced by grid-data (0.5–11.6 % of the observations) provided by The Danish Meteorological Institute. The most pronounced climatic difference between sites was the relatively lower precipitation in Foulum.

Planting of willow was done in May 2010, using cuttings of 20 cm length. Weed control and fertilization differed considerably between trials, with the trials Foulum and Jyndevad managed more intensively with more frequent weed control during the establishment phase and with annual fertilization from second to sixth growth year whereas the trials Højmark and Foersom were only fertilized once (Table 1). The mean annual quantity of applied nitrogen was 100 and 106 kg ha<sup>-1</sup> y<sup>-1</sup> in Foulum and Jyndevad but only 35 and 13 kg ha<sup>-1</sup> y<sup>-1</sup> in Højmark and Foersom (Table 1). For the application of organic fertilizer in Højmark (mink slurry), the calculated N rate is based on the typical mineral N mass fraction which is ca. 0.6 % whereas the total N mass fraction is ca. 0.9 %. The shoots from first growth year were not cut back in any of the trials.

#### 2.2. Experimental design

Eight willow cultivars were included in all four trials with six cultivars from Swedish breeding programs (Inger, Klara, Linnea, Stina, Tora and Tordis) and two cultivars from English breeding programs (Resolution and Terra Nova). The pedigree of the cultivars is described in Larsen et al. [7], and the genetic background is further described by Lindegaard et al. [10] and Caslin et al. [33]. All four trials were designed as a randomized block design with four replicate blocks, giving a total of 32 clonal plots per trial. However, the trials Foulum and Jyndevad included two and four additional treatments concerning harvest frequency and row distance, but these data are not reported here.

The trials were established according to standard practice for willow production with double rows with an internal distance of 0.75 m within double rows and 1.50 m distance between double rows. Intended area plant density was  $1.2 \text{ m}^{-2}$ , corresponding to 0.74 m plant distance within rows. The width of the gross plot was 4 double rows corresponding to 9.0 m, and the length varied from 12.5 to 20.0 m between sites. The net plot consisted of the central part of the gross plot with the two central double rows corresponding to a width of 4.5 m and with a length varying between 6.67 and 11.5 m. Hence, the gross plot area varied between 112.5 and 180.0 m<sup>2</sup>, whereas net plot area varied between 30.0 and  $51.75 \text{ m}^2$ . With an intended plant density of 12,000 plants ha<sup>-1</sup>, this would result in a plant number in the gross plot area ranging between 36 and 62 plants. See Larsen et al. [7] and Supplementary Material Table 1 for details on experimental design.

#### 2.3. Measurements

As an indicator of weed pressure during the establishment phase, the weed cover was monitored by visual evaluation of the fractional area cover in each plot on 25<sup>th</sup> August 2010 and 22<sup>nd</sup> September 2011 in the trials Højmark and Foersom. In Foulum and Jyndevad, the weed pressure was low (generally below 10 % cover) and weed cover was not monitored. In all four trials, plant density and biomass yield were measured after third and sixth growth year, i.e. representing 1<sup>st</sup> and 2<sup>nd</sup> three-year harvest rotation. The specific harvest dates differed between the trials (Table 1). The most pronounced difference was a very late harvest in the spring 2016 in Højmark and Foersom which was due to very wet conditions during the winter 2015 to 2016 which delayed the harvest until 19<sup>th</sup> of May. All willow cultivars had leaves at this point of time and, in general, it is undesirable to harvest willow during the growth season. From an experimental point of view, however, we assume that the late harvest of these two trials did not significantly affect the yield differences between the cultivars, since they were all harvested at the same time. Moreover, we assume that the mean annual

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