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Soil water uptake and root distribution of different perennial and annual bioenergy crops

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Keywords

bioenergy; energy crops; soil water; root system; miscanthus; switchgrass

Abstract

Background and aims

Bioenergy crops are expected to provide biomass as a replacement for fossil resources, but their impact on the water cycle is still under question. This study aimed at both quantifying the ability of bioenergy crops to use soil water and analysing the relationship between their root systems and soil water uptake.

Methods

Water content was monitored continuously for seven years (2007-2013) under perennial (*Miscanthus × giganteus* and *Panicum virgatum*), semi-perennial (*Festuca arundinacea* and *Medicago sativa*) and annual (*Sorghum bicolor* and *× Triticosecale*) bioenergy crops. Root distribution was characterized in 2010 down to 3 meters depth. Soil water deficit (SWD) was calculated as the difference between field capacity and actual water content.

Results

Maximal SWD (0-210 cm) during the growing season was higher for semi-perennials, despite a lower biomass production than perennials. Water capture in deep soil layers was greater under perennials and semi-perennials than under annual crops. A curvilinear asymptotic relationship was found between water capture and root density and described by a model the parameters of which varied between crops, indicating a variable soil water capture for a given root density.

Conclusions

This study provides quantitative information required to simulate the impact of bioenergy crops on drainage and aquifer loading.

47

48 **Abbreviations**

49 I: irrigation

50 P: precipitation

51 PET: potential evapotranspiration

52 PWC: proportional water capture

53 RID: root intersection density

54 RLD: root length density

55 RMSE: root mean square error

56 SWC: soil water content

57 SWD: soil water deficit

58

59 **Introduction**

60 In response to the challenges of climate change and depletion of fossil resources, biomass is
61 expected to contribute significantly to the energy transition by providing renewable carbon for
62 bioenergy, biomaterials and biochemicals (IPCC 2011; Ragauskas et al. 2006). Among
63 biomass resources, dedicated bioenergy crops have large technical potential and will probably
64 be a major player in the increase of bioenergy production (Bentsen and Felby 2012; Chum et
65 al. 2011). The wide range of conversion technologies leads to a large diversity of candidate
66 crops: short rotation coppices, perennial crops, semi-perennial forage crops and annual crops
67 (Karp and Shield 2008; Lewandowski et al. 2003; Sanderson and Adler 2008; van der Weijde
68 et al. 2013; Zegada-Lizarazu and Monti 2011).

69 Perennial C4 rhizomatous crops like *Miscanthus* × *giganteus* (hereafter referred to as
70 miscanthus) or *Panicum virgatum* (hereafter referred to as switchgrass) are considered as
71 promising energy crops because of their high biomass production, low nutrient requirements

and low greenhouse gas emissions (Cadoux et al. 2014; Don et al. 2011; Somerville et al. 2010). However, large deployment of these crops could modify their regional environment. One particular concern is the effect on the water cycle through modifications in evapotranspiration (McIsaac et al. 2010; Vanloocke et al. 2010). Several authors have suggested that perennial bioenergy crops consume more water than annual crops, because of their higher biomass production, longer growing period and deeper root system (Heaton et al. 2010; Powlson et al. 2005; Rowe et al. 2009). Using soil moisture measurements over four growing seasons in central Illinois, McIsaac et al. (2010) estimated that miscanthus had higher evapotranspiration than switchgrass and maize-soybean rotation. Hickman et al. (2010) found similar results at the same site during one growing season by using a micrometeorological method. Using a model-based approach, Le et al. (2011) predicted an average 58% and 36% increase of total seasonal evapotranspiration for miscanthus and switchgrass respectively, compared to maize in the Midwest United States. This higher water consumption during crop growth will reduce the amount of water drained during winter. Vanloocke et al. (2010) predicted with a dynamic global vegetation model a decrease in drainage ranging from 50 to 250 mm yr⁻¹ with miscanthus cultivation instead of current land cover for the Midwest United States. This decrease of drainage is likely to impact aquifers in case of large-scale land conversions.

Among factors influencing crop water use, morphology and distribution of roots within the soil profile are of prime importance because they define the amount of water that can be supplied to the crop (Jackson et al. 2000). Deep rooting (> 2 m) has been reported by several authors for miscanthus and switchgrass (Ma et al. 2000; Neukirchen et al. 1999; Riche and Christian 2001). This extensive root system may allow these crops to maintain their growth in case of drought period but is also likely to lead to a greater soil water deficit (SWD) than annual crops at the end of the growing season (Riche and Christian 2001). To our knowledge,

only one study has compared soil water uptake and root distribution of different bioenergy crops (Monti and Zatta 2009). However this study was restricted to one growing season and the soil sampling depth was only 120 cm. There is a need to compare a wide range of candidate bioenergy crops over multiple seasons to take into account climate variability and in a deep soil to maximize the differences in root distribution between crops.

We hypothesized that perennial C4 crops use deep soil water resources because of their extensive root system and high biomass production, leading to a higher SWD than with other crops. The aim of this study was (1) to quantify soil water utilization for perennial, semi-perennial and annual bioenergy crops using a long term and continuous monitoring of soil water, and (2) to study the relationship between the root system of the crops and soil water uptake.

Materials and methods

Study site and experimental design

This study is based on an ongoing long-term experiment carried out by INRA at the experimental station of Estrées-Mons, northern France (49.872°N, 3.013°E), on a Haplic Luvisol (IUSS Working Group WRB 2006). The experiment was initiated in 2006 and six crops were compared, representing a wide range of bioenergy crop types: two perennial C4 crops, two semi-perennial forage crops and two annual crops. The chosen crops were miscanthus (*Miscanthus×giganteus* Greef & Deuter ex Hodkinson & Renvoize), switchgrass (*Panicum virgatum* cv. Kanlow), fescue (*Festuca arundinacea* cv. Dulcia from 2006 to 2008, Noria from 2009 to 2010 and Bariance after 2010), alfalfa (*Medicago sativa* cv. Alpha from 2006 to 2008, Orca from 2009 to 2010 and Salsa after 2010), fibre sorghum (*Sorghum bicolor* (L.) Moench cv. H133) and triticale (*× Triticosecale* Wittmack cv. Triskell from 2006 to 2008, Amarillo from 2009 to 2011 and Tarzan after 2011). The annual crops were grown in

rotation (triticale grown after sorghum and *vice-versa*) as well as the semi-perennial crops (alfalfa grown after fescue and *vice-versa*) and all crops were present each year. A catch crop was sown every year in August or early September between triticale and sorghum (rye in 2007, mustard in 2008, oat-vetch mixture in 2009 and mustard-clover mixture from 2010 to 2013). The perennial crops were established in 2006 and the semi-perennial crops were sown in 2006 (first rotation), 2009 (second rotation) and 2011 (third rotation). Two harvest dates were compared for miscanthus and switchgrass: an early harvest in October and a late harvest in February. The experiment also included two nitrogen treatments for each crop except alfalfa, with a plot size of 360 m² and three replicates per treatment. Details about crop management and experimental treatments are given by Cadoux et al. (2014). In this study, we selected experimental treatments maximizing plant growth and thus water consumption: the late harvest for miscanthus and switchgrass and the highest nitrogen treatment for all crops. During the period 2007-2013, the mean N fertilization rates for the selected treatments were 120 kg ha⁻¹ yr⁻¹ for miscanthus, switchgrass, sorghum and triticale, 170 kg ha⁻¹ yr⁻¹ for fescue and 0 for alfalfa. The experiment did not receive irrigation, except in May 2011 for fescue, alfalfa, sorghum and triticale (58 mm in total) to facilitate crop establishment during a drought period.

Climatic data

Climatic data were obtained from an automatic weather station situated on the experimental site. Over the period 2007-2013, mean temperature was 10.6 °C, annual rainfall (P) and Penman potential evapotranspiration (PET) were 686 and 714 mm respectively and annual global radiation was 4154 MJ m⁻². The water balance (P-PET) during the growing season displayed a rather large variability between years (Table 1). We considered March 1 as the beginning of the growing season because it corresponds approximately to the time when

winter crops like triticale start growing again and PET begins running higher than 1 mm d⁻¹. We considered November 1 as the end of the growing season because all annual and semi-perennial crops have been harvested and perennials are close to total senescence. The wettest year was 2008 and 2009 was the driest, with only 116 mm of precipitation from June to September. Springs 2010 and 2011 were drier than the seven-year average.

Biomass production

The aboveground biomass at harvest was estimated for each crop. At each harvest date, plants were harvested manually and weighed. Miscanthus and switchgrass were harvested in February or early March. Fescue and alfalfa were harvested in two or three cuttings depending on the years, with the last cut in October. Sorghum was harvested in late September and triticale in late July or early August. Details about sampling methodologies are given by Cadoux et al. (2014). The dry matter content was determined after drying representative subsamples at 65°C for 96 h. The biomass production was expressed in tons of dry matter per hectare and per year for all crops.

Soil and soil water monitoring

Measurements

We used water content reflectometers (Campbell Scientific CS616) to monitor the soil moisture profile continuously from July 2007 to November 2013. Probes were installed in May 2007 in six plots (one plot per crop), inserted horizontally into the soil at 15, 45, 75, 105, 135, 165 and 195 cm depth (three replicates at 15 cm depth and two replicates at the other depths). Temperature sensors (Campbell Scientific 107) were also placed at 15 and 195 cm depth. Data were recorded at an hourly time step using CR1000 Campbell Scientific data

loggers. Probes placed at 15 cm depth were removed for soil tillage and reinserted as soon as possible.

Soil cores taken down to 210 cm from the six plots in 2006 were analysed to determine soil characteristics which were very homogeneous in the six plots (Table 2). Bulk density was measured at each CS616 depth in May 2007 using steel cylinders of 98 cm³ (5 cm diameter, three replicates) and measurements were repeated for the upper depth (15 cm) in 2010 and 2011 or 2012 with six replicates. Gravimetric water content was measured in the three blocks twice a year (in mid-March and early November) from 2007 to 2013. At each date of measurement, soil cores were collected down to a depth of 150 cm with a driller 18 mm in diameter. The cores were divided into five layers (0-30, 30-60, 60-90, 90-120 and 120-150 cm). In each soil layer, one soil sample was formed by mixing five soil cores for each plot. In the instrumented plots, additional measurements were made during summer 2009 and from November 2011 to November 2013 down to 210 cm, with three individual cores per plot divided into seven layers. Only gravimetric measurements made in the instrumented plots were used to calibrate CS616 probes.

Data processing and calculations

Data from CS616 probes need proper correction and calibration in order to obtain accurate soil moisture measurements (Rudiger et al. 2010). First of all, data were regularly collected in a database managed with PostgreSQL and eventual outliers were eliminated. Secondly, the soil temperature was simulated at each CS616 depth using a script developed with R software (R Core Team 2014). We used Fourier's law to simulate heat conduction transfer through the soil profile. Soil temperature at 15 and 195 cm depth were taken as boundary conditions and the initial temperature along the soil profile was determined by linear interpolation between the two depths. Depth and time increments as well as thermal diffusivity (*alpha*) were optimized using two other plots of the same experiment with supplementary soil temperature

measurements at 75 and 135 cm depth. The optimized value for *alpha* was $24 \text{ cm}^2 \text{ h}^{-1}$ and the root mean square error (RMSE) was 0.2°C over a period of 434 days. Thirdly, period measurements from the CS616 probes were corrected for measured or simulated soil temperature, using the temperature correction equation provided by Rudiger et al. (2010) with the slope coefficient for silt loam. The fourth step consisted in deriving a relationship between corrected period measurements and volumetric soil water contents, obtained from gravimetric water contents and bulk density measurements. A covariance analysis was applied with R software for the two or three replicates of each plot and depth in order to choose (with a 95% confidence level) between an individual calibration with a specific linear regression equation for each replicate, a common calibration or an individual calibration of the intercept with a common slope. The mean coefficient of determination was 0.86 for the 90 probes ($n=17$) and the mean RMSE was $0.016 \text{ cm}^3 \text{ cm}^{-3}$. Finally, all corrected period measurements were converted into volumetric and gravimetric water content and the two or three replicates were averaged. Missing data were filled in by linear interpolation and data were aggregated to obtain daily measurements. Standard deviation between replicates was, on average over the period 2007-2013, $0.019 \text{ cm}^3 \text{ cm}^{-3}$ for the first layer (3 replicates) and $0.007 \text{ cm}^3 \text{ cm}^{-3}$ for the other layers (2 replicates).

The soil water content (SWC, in mm) was calculated in each 30 cm soil layer and summed up over the monitored soil profile (0-210 cm). For each crop, SWC calculated over the three replicated plots with gravimetric measurements were compared to SWC calculated in the single instrumented plot using CS616 probes. We found a good, unbiased relationship between the two estimates ($y = 1.005 x$; $R^2=0.94$; $n=65$), which indicated that SWC assessed with CS616 probes were representative of the whole field. The soil water deficit (SWD, in mm) was defined as the difference between SWC at field capacity and the measured SWC (Beale et al. 1999) for each soil layer or over the monitored soil profile. For each soil layer,

the proportional water capture (PWC, in %) was calculated as the fraction of potentially available water that had been captured by plant roots (Monti and Zatta 2009):

$$PWC = \frac{SWC_{FC} - SWC}{SWC_{FC} - SWC_{WP}} \cdot 100 \quad (1)$$

where SWC_{FC} is the water content at field capacity (in mm) and SWC_{WP} the water content at permanent wilting point (in mm). SWC_{FC} was calculated as the median of the gravimetric measurements made in winter (March) over the period 2007-2013. SWC_{WP} was measured with Richard's pressure plates at -1.5 MPa water potential.

Root mapping

We collected data on root distribution during the year 2010 for each plot in which soil water was monitored using a modified trench profile method (Tardieu 1988). First of all, a trench 300 cm deep was dug into the plot. The observed vertical profile (180 cm wide, 300 cm deep) which was perpendicular to the crop row was then prepared. After the working surface had been smoothened, roots were made visible by removing approximately 1 cm of soil with a knife. Next, roots were mapped on three adjacent 60 * 300 cm grids with cells of 1.9 * 1.9 cm. Since root counting was a very time-intensive operation, the number of root impacts in each cell was measured only on 20% of the cells for each 60 cm wide grid (the seven cells at the right of the grid), and the presence or absence of root impact was noted for the other cells. The distribution of roots was recorded on 11 and 25 June 2010 for miscanthus and switchgrass respectively (4-year-old crops), on 14 and 21 September 2010 for fescue and alfalfa respectively (1.5-year-old crops), on September 9, 2010 for sorghum (at the beginning of anthesis) and on July 13, 2010 for triticale (ten days before physiological maturity).

Relationship between root density and proportional water capture

In studies dealing with roots and water uptake, the root distribution is often described using the root length density (RLD), i.e. the total root length per unit of soil volume (Gregory 2006). Experimental measurement of RLD by extracting soil cores or soil monoliths can be extremely labour-intensive. Mapping and counting root impacts on a vertical soil profile has the advantage of being easier to do in the field but provides no direct measurement of RLD. However, the root intersection density (RID), i.e. the mean number of root impacts per cm² (Chopart et al. 2008), can be calculated from such measurements and linear relationships between RID and RLD have been found for various crops (Chopart et al. 2008; Chopart and Siband 1999; Dusserré et al. 2009). We therefore assumed that RID could be used as an indicator of RLD. Indeed, we also determined RLD for miscanthus and switchgrass (Ferchaud et al. 2012) and verified the linear relationship between RLD and RID for these two crops.

RID was calculated in each 60 * 300 cm grid for each layer of 30 cm thickness.

The relationship between RID and water capture was studied in 2010 using PWC calculated at the date of the maximal soil water deficit (over 0-210 cm). The relationship was described for each crop with a model derived from King et al. (2003):

$$PWC = (a - y_0) \cdot (1 - e^{-k \cdot RID}) + y_0 \quad (2)$$

where k is a “resource capture coefficient” which summarizes details of water uptake physiology and soil water transport. A higher k value leads to a faster increase in water extraction when root density increases. Compared to the original model of King et al. (2003), we added two parameters: a which is the highest PWC achievable by the crop ($a = 100\%$ in the original model) and y_0 which is the PWC obtained in free root soil layers due to possible water capillary rise ($y_0 = 0$ in the original model). A common value for all crops was chosen for y_0 . The parameter optimization for a and k was realised with the Excel solver using the GRG nonlinear method. The minimized criterion was the RMSE.

Statistical analysis

All statistical analyses were performed with R (R Core Team 2014). The effect of the crop on highest and lowest SWC of each year was evaluated by one-factor analysis of variance (ANOVA), using the different probes as replicates. We used a two-factor ANOVA without replication with crop and year as factors in order to test the crop effect on mean SWD for each soil layer and for 0-210 cm. For PWC, we included the soil layer as third factor. For RID, the effect of the crop was evaluated in each layer by one-factor ANOVA, using the three adjacent grids as replicates. Differences between crops were evaluated with Tukey's HSD (honest significant difference) test for all variables. The assumptions of ANOVA were checked by visual examination of the residuals against predicted values and using Shapiro-Wilk and Levene's tests. If necessary, we used square root transformation or arcsine square root transformation to satisfy these assumptions.

Results

Soil water content

SWC over the soil profile (0-210 cm, measured with CS616 probes) fluctuated over the seven-year period with a regular pattern for all crops (Fig. 1). It was at its highest level in winter (above 700 mm), decreased every year during spring and summer and rose in autumn. SWC in winter was rather stable between years and crops. It was close to the estimated SWC at field capacity (mean = 730 mm) and peaked from time to time at approximately 750 mm. Differences in maximum SWC between crops were only significant three years out of six. In 2009-2010 and 2010-2011, SWC did not reach field capacity for several crops (miscanthus and switchgrass in 2009-2010, miscanthus, switchgrass, fescue and alfalfa in 2010-2011). Indeed, in these cases, autumn and winter precipitations were not sufficient for the deeper layers to reach field capacity. The timing of SWC decrease depended on the crops, with an

earlier decline for triticale, fescue and alfalfa (except in 2009 and 2011 when these two crops were newly sown) than for the other crops. The lowest SWC over a year were observed on average over the period 2007-2013 in mid-October for miscanthus, in mid-September for switchgrass, fescue, alfalfa and sorghum and in early July for triticale. Minimal SWC were more variable between years and crops than maximal SWC. Differences in minimal SWC between crops were significant every year, with a range of variation between crops higher than 100 mm five years out of seven. The lowest SWC over the period 2007-2013 were observed in 2009 for all crops except for triticale, for which the lowest SWC was observed in 2013. These values (433 mm for alfalfa, 451 mm for fescue, 495 mm for switchgrass, 507 mm for sorghum, 526 mm for miscanthus and 528 mm for triticale) were significantly higher than the estimated SWC at permanent wilting point (mean = 318 mm). The timing of SWC increase after this minimal point was more stable between crops than the timing of SWC decrease in spring, but generally faster for annual crops than for the other crops.

Maximal soil water deficit

In order to quantify soil water utilization by the crops, we calculated the maximal SWD each year over the soil profile (0-210 cm), corresponding to the minimal SWC for the year. Maximal SWD ranged from 61 mm for triticale in 2007 to 294 mm for alfalfa in 2009. For each crop, the variability of the maximal SWD between years was large, with standard deviations of 41 to 59 mm. Nevertheless, alfalfa had the largest maximal SWD six years out of seven and fescue always had the second largest maximal SWD. The ranking of other crops was more variable between years. On an average, over the period 2007-2013, the differences between crops were significant (Fig. 2). Alfalfa had a higher maximal SWD (218 mm) than the other crops except fescue. Maximal SWD was lowest for sorghum (142 mm) and intermediate for triticale, miscanthus and switchgrass (156, 157 and 171 mm respectively).

320 At maximal SWD, SWD calculated in each soil layer was higher in the topsoil (Fig. 2). On an
 321 average, 47% of the total SWD was located in the upper two layers (0-60 cm), 38% in the
 322 three intermediate layers (60-150 cm) and only 15% in the two deeper layers (150-210 cm).
 323 However, this distribution was crop-dependent: annual crops had a higher proportion of the
 324 total SWD in the 0-60 cm layer (56%) and a lower proportion in the 150-210 cm layer (11%)
 325 than the other crops. SWD in the 150-210 cm layer was 16 mm for sorghum and triticale,
 326 significantly lower than alfalfa (46 mm). SWD in the first layer (0-30 cm) was significantly
 327 lower for miscanthus and switchgrass (44 and 35 mm respectively) than for fescue, alfalfa and
 328 triticale (66, 56 and 56 mm respectively).
 329 We examined the influence of climate conditions on maximal SWD. Using data of
 330 precipitation (P), irrigation (I) and potential evapotranspiration (PET), the water balance (P+I-
 331 PET) was calculated each year and for each crop from March 1 to the date of maximal SWD.
 332 The water balance was -238 mm on an average and was very similar for most crops (-256 ± 5
 333 mm), except for triticale (-148 mm). It was negatively correlated with maximal SWD ($r = -$
 334 0.63 ; $p < 0.001$; Fig. 3).
 335 The influence of aboveground biomass production on maximal SWD was also investigated.
 336 No significant correlation was found between biomass production and maximal SWD (Fig. 4).
 337 This was true not only when all crops were grouped together but also for each crop
 338 independently, despite large differences in biomass production between crops and years for
 339 some crops. Miscanthus, switchgrass and triticale had a rather stable biomass production over
 340 the period 2007-2013 but fescue, alfalfa and sorghum displayed a higher variability with low
 341 biomass production ($< 10 \text{ t ha}^{-1}$) some years. Surprisingly, the highest maximal SWD (-294
 342 and -275 mm for alfalfa and fescue respectively) was observed for low biomass production
 343 (3.2 and 6.4 t ha^{-1}) during the year 2009, which was the driest year and when fescue and
 344 alfalfa were newly sown. The biomass production of catch crops grown between triticale and

sorghum was not taken into account because it was very low (0.5 ± 0.2 t DM ha⁻¹ on average over the period 2007-2013).

Proportional water capture

PWC was calculated for each year and each soil layer at the date of the maximal SWD. Crops, soil depth and their interaction significantly affected PWC. On average, PWC decreased with depth from 74% in the 0-30 cm layer to 19% in the 180-210 cm layer, and never reached 100% below 30 cm. However, differences between crops were significant for all soil layers (Fig. 5). For the first soil layer (0-30 cm), PWC was significantly lower for miscanthus and switchgrass than for other crops. The highest PWC observed in the 0-30 cm layer over the period 2007-2013 was only 76% and 57% for miscanthus and switchgrass respectively, compared to 100% for the other crops. The differences between crops were smaller in the 30-60 and 60-90 cm layers, with a higher PWC for fescue and alfalfa. PWC was smaller for sorghum and triticale below 90 cm. Alfalfa had the highest PWC (42% and 33% on average) in the 150-180 and 180-210 cm layers respectively. It was significantly higher than for sorghum and triticale (15% and 16% in the 150-180 cm layer; 12% and 11% in the 180-210 cm layer, respectively). The variability of PWC between years was smaller in deeper than in upper layers for annual crops, but not for other crops.

PWC calculated at the date of maximal SWD were compared to PWC calculated at the end of the growing season, i.e. November 1 (data not shown). PWC changed very slightly for the three deeper layers (only 1% on average) but was reduced in the upper layers. This means that there was no or very little additional water retrieval in the deeper layers after the date of maximal SWD, even for crops for which maximal SWD was observed on average more than one month before the end of their growing period (switchgrass, fescue, alfalfa).

Root distribution and root intersection density

Fig. 6 shows the root distribution of each crop observed in 2010 on the trench profiles. Miscanthus, switchgrass and alfalfa had a particularly deep root system: the maximum rooting depth was 300, 288 and 276 cm respectively. Sorghum had a more superficial root system (maximum rooting depth = 128 cm) and fescue and triticale were intermediate with a maximum rooting depth of 200 cm. The proportion of cells including roots decreased with depth, more or less according to the crops. This proportion decreased from 79% (sorghum) to 100% (fescue) in the 0-30 cm layer down to 0% (sorghum) to 10% (miscanthus) in the 180-210 cm layer. For the crops with a maximum rooting depth exceeding 210 cm, the proportion of cells including roots in the 210-300 cm layer was 7, 1 and 4% respectively for miscanthus, switchgrass and alfalfa.

Root intersection density (RID) decreased drastically with depth for all crops (Table 3). It varied from 0.57 (sorghum) to 1.17 roots cm⁻² (fescue) in the 0-30 cm layer, and became lower than 0.04 roots cm⁻² for all crops in the 180-210 cm layer. The crop effect was significant in all soil layers. RID was significantly higher for fescue than sorghum, alfalfa and miscanthus in the first layer and higher for fescue, alfalfa and switchgrass than for miscanthus and sorghum in the 30-60 and 60-90 cm layers. In the 150-180 and 180-210 cm layers, miscanthus, switchgrass and alfalfa had a significantly higher RID than sorghum and triticale. Over 0-210 cm, the mean RID was 0.15 and 0.18 roots cm⁻² respectively for sorghum and miscanthus, 0.24 and 0.26 roots cm⁻² respectively for triticale and alfalfa and 0.30 and 0.32 roots cm⁻² respectively for switchgrass and fescue. Only miscanthus, switchgrass and alfalfa produced roots below 210 cm, with a significantly lower RID for switchgrass.

Relationship between root distribution and water uptake

We studied the relationship between RID measured in 2010 and PWC calculated for each soil layer at the date of the maximal SWD in 2010. Maximal SWD occurred on July 11 for triticale, on August 15 for switchgrass, fescue, alfalfa and sorghum and on October 31 for miscanthus. The year 2010 was the second driest year during the growing season, after 2009. PWC were therefore higher during year 2010 than the average for all crops at almost all depths.

A positive linear correlation was found between RID and PWC for all species ($r = 0.72$; $p < 0.001$). However, the relationship was not strictly linear but rather curvilinear asymptotic and differences appeared between crops (Fig. 7). The highest PWC found in the 0-30 cm layer varied widely between crops, from 53% for switchgrass to 100% for fescue and triticale whereas the corresponding RID was similar for most crops. This was also true for the deeper soil layers where a large variability in PWC was observed for identical RID. The model derived from King et al. (2003) was fitted for each crop independently (Fig. 7). Water capture was observed for sorghum in the layers 150-180 cm and 180-210 cm (PWC = 14 and 10% respectively), although no visible root was found in these layers. We used the mean of these two values (12%) for the y_0 parameter for all crops. Simulated PWC were in good agreement with observed data, with a mean RMSE of 7% (Table 4). The goodness of fit was equivalent for all crops except for fescue, which had the highest RMSE. The parameter values obtained after optimization were rather variable between crops (Table 4). The “resource capture coefficient” k was highest for fescue, followed by switchgrass and alfalfa and smaller for sorghum and triticale. Annual crops were characterized by an a value (highest PWC achievable by the crop) greater than for other crops, particularly perennials.

Discussion

Soil water deficit and water capture

Few studies have compared the evolution of soil water content during the growing season for different bioenergy crops. For example, alfalfa has been compared to annual crops (Entz et al. 2001) but not to fescue or perennial C4 crops. Our study is the first one comparing perennial, semi-perennial and annual bioenergy crops. Entz et al. (2001) found that soil water content during summer (over 0-150 cm) was always lower for alfalfa than for annual crops during five years of cultivation. This is consistent with our study showing that the highest maximal SWD was observed for alfalfa six years out of seven. The differences in maximal SWD over 0-210 cm between miscanthus, switchgrass and annual crops were small and not significant in our study with a different ranking according to the year. McIsaac et al. (2010), who monitored soil moisture under miscanthus, switchgrass (cv. Cave-In-Rock) and a maize-soybean rotation, also found contrasting results between growing seasons. They found that the minimal soil moisture under miscanthus and switchgrass was either equal (two years out of four) or lower (the other two years) than under annual crops. However, in contrast to our results, they observed that miscanthus resulted in lower minimal soil moisture than switchgrass during the four growing seasons.

We found that maximal SWD was correlated to the water balance (P+I-PET) but not to the aboveground biomass production. The biomass production of the semi-perennial crops was only 57% of the miscanthus production and 64% of the switchgrass production but their maximal SWD was higher. In fact, maximal aboveground biomass of perennial crops (in October) was even higher than the biomass at harvest (in February) due to leaf fall during winter (only for miscanthus) and carbon transfer from aboveground to belowground parts in autumn (Dohleman et al. 2012; Strullu et al. 2011). This suggests that miscanthus and switchgrass had higher water use efficiency than fescue and alfalfa. Beale et al. (1999) calculated water use efficiencies for miscanthus in UK with the maximal aboveground

biomass reached by the crop during the growing season. They found comparable values to other C4 crops, such as maize, and higher values than C3 crops such as willow. Furthermore, it is possible that the timing of the growing periods of the crops affects water use efficiency due to differences in climate conditions. The lack of correlation between maximal SWD and aboveground biomass production observed during the seven years for each crop independently indicates that SWD was much more sensitive to the water balance than biomass production.

Our results also indicate that perennial C4 crops and semi-perennial forage crops, and particularly alfalfa, have the ability to take up significant amounts of water in deep soil layers (150-210 cm). Water uptake is even likely to occur below 210 cm for miscanthus, switchgrass and alfalfa since these crops have roots deeper than 210 cm. Our results are in agreement with Campbell et al. (1994) and Dardanelli et al. (1997) who showed that alfalfa growing in deep soil can withdraw water to a depth of 250 cm, and Finch and Riche (2008) who found significant soil water depletion down to 170 cm with miscanthus at two sites in England.

However, the ability of miscanthus and switchgrass to take up deep soil water did not lead to a significantly higher maximal SWD than annual crops because it was compensated by a lower SWD in the 0-30 cm layer. This lower SWD near the soil surface, which had not been emphasized in previous studies, was at least partly due to lower soil evaporation. Indeed, these two crops have a high and dense canopy during summer, when PET is maximal, which is likely to limit soil evaporation. Furthermore, the fallen leaves of miscanthus accumulating at the soil surface form a 2-4 cm thick mulch (Amougou et al. 2012) which enhances the reduction of soil evaporation.

Root distribution

We assumed that our protocol was appropriate to compare the root systems of the different crops. The root systems of annual crops measured at anthesis or post-anthesis were probably close to maximal development (Hoad et al. 2001; Zhang et al. 2004). Root distribution of perennial and semi-perennial crops was recorded at two periods during the year 2010 (June and September respectively). Root extension of perennial crops had probably reached steady state since the crops were 4 years old. Neukirchen et al. (1999) did not find any effect of the sampling date on root density when comparing three dates of measurements in a 5-year-old miscanthus. Semi-perennials were at the end of their second year of growth at the time of measurement: the recorded root distribution corresponded to well-established crops and was probably close to the maximal root development achieved during the 2010 growing season although we do not know if it was at steady state. The climate conditions in 2010, with a first part of the growing season (March-June) drier than the average, might have affected root development of annual and semi-perennial crops. However, this is unlikely because the available soil water was high in March (240 mm over 0-120 cm) and the biomass of these crops in 2010 was similar to the average (104% of the 2007-2013 mean biomass production). In our conditions, i.e. in a deep loamy soil with no obstacles to rooting, crops exhibited large differences in rooting depth. Miscanthus, switchgrass and alfalfa had a particularly deep root system. The maximal rooting depth of miscanthus (≥ 300 cm) was deeper than that recorded in other field experiments: 200 cm for a 6-year-old crop in a silty clay loam in England (Riche and Christian 2001) and 250 cm for a 3-year-old crop in a sandy loam in Germany (Neukirchen et al. 1999). The difference with our study might result from differences in soil characteristics or maximum depth of observation. The maximum rooting depth of switchgrass measured in our experiment (288 cm) was intermediate between that observed by Riche and Christian (2001) for a 6-year-old crop in England (240 cm) and that reported by Ma et al. (2000) in a sandy loam in Alabama for a 7-year-old crop (330 cm). Evidence of a deep root

system for alfalfa was also found in several studies (Campbell et al. 1994; Dardanelli et al. 1997). The maximum rooting depth of 200 cm observed for triticale was consistent with the highest values reported for winter cereals such as winter wheat (Hoad et al. 2001; King et al. 2003; Zhang et al. 2004). Sorghum had the shallowest root system in our conditions, with only 128 cm depth. This value was lower than that found by Robertson et al. (1993) for various grain sorghum cultivars in a sub-tropical environment in Australia (190 cm). Monti and Zatta (2009) found roots of fibre sorghum in Italy down to 120 cm depth. In our conditions, sorghum had a short growing period because it was sown after mid-May (May 21 in 2010) due to its susceptibility to low temperatures and this may have limited root development.

Root density has frequently been found to decrease exponentially with depth (Gregory 2006). This exponential decrease is more or less verified for most crops, but not for alfalfa or miscanthus (Table 3). The root distribution observed for miscanthus, i.e. a rather low density in the upper layers with a drastic decrease below 30 cm and a constant and rather high density in the deeper layers, is consistent with the results of Neukirchen et al. (1999) and Riche and Christian (2001). However, it differs from the study of Monti and Zatta (2009) who found a surprisingly very low root density for miscanthus below 90 cm.

Relationship between root distribution and water capture

PWC was correlated to RID, meaning that root density was a limiting factor for extracting water, at least in deep layers. Indeed, soil-root water transfer occurs mainly in the few centimetres surrounding the roots, due to limitations in soil and/or root hydraulic conductivity (Garrigues et al. 2006). However, the shape of the relationship (curvilinear asymptotic) showed that deep roots with a low density were relatively more efficient for recovering water than shallower, denser roots. Robertson et al. (1993) found the same type of relationship

between water consumption and RLD for grain sorghum with a plateau above an RLD threshold. Zhang et al. (2004) also reported higher water uptake per unit of root length in depth than near the soil surface for rain-fed and irrigated winter wheat. When root density increases, competition between neighbouring roots is enhanced, reducing their relative efficiency.

Water capture equivalent to 15 mm in the 150-210 cm layer was observed for sorghum which had no roots in this layer. The deepest roots of sorghum might have not been observed due to spatial variability. It is also possible that upward soil water transfer occurred in these free root layers due to a hydraulic gradient caused by root uptake in the upper layers. Adding the y_0 parameter (PWC in free root layers) to the model allowed us to take this observation into account and increase the goodness of fit for annual crops (RMSE was divided by 2.5 on an average) with no impact for the other crops. We chose to take a common value for y_0 although it is likely that the upward water transfer depends on soil characteristics and decreases with depth when the distance to the deepest root increases.

The optimized parameters were rather variable between crops. The differences observed for a (highest PWC achievable by the crop), with lower values for perennial crops, could be partly explained by differences in soil evaporation between crops. However, the same tendency was observed when the model was fitted on data excluding the first soil layer. Very different values obtained for the “resource capture coefficient” k suggest large differences in water capture efficiency between crops. Monti and Zatta (2009) also found differences between crops but their ranking (miscanthus > sorghum > switchgrass) was different from ours (switchgrass > miscanthus > sorghum). In fact, their data on root density were very different from ours: low root density of miscanthus in depth compared to switchgrass and sorghum. We hypothesize that this discrepancy was due to a warmer climate, favourable to sorghum, in

Italy and to the presence of a water table close to the soil surface which could penalize root growth of miscanthus.

Differences between crops for k can result from factors relative to root system or crop evaporative demand (growing period, morphological factors, etc.). The root system of annual crops like cereals grows simultaneously to the canopy and achieves its maximal development at anthesis (King et al. 2003), contrary to perennial crops. Consequently, the deep roots of annual crops have less time than shallower roots to take up soil water. Robertson et al. (1993) hypothesized that incomplete water extraction in depth for grain sorghum under severe drought was due not only to lower root density but also to the lack of time for deeper roots to extract water between the arrival of roots in the soil layer and crop maturity. An extraction front travelling down the soil profile with time has been observed for annual crops (Dardanelli et al. 1997; Dardanelli et al. 2004; Robertson et al. 1993) and is generally attributed to the growth of the root system. In 2009, the driest year of our experiment, an extraction front clearly appeared for sorghum: water depletion started approximately late June in the 30-60 cm layer (one month after sowing), late July in the 60-90 cm layer, mid-August in the 90-120 and 120-150 cm layers and late August for the two deeper layers. This difference in the timing of water extraction with depth could explain low values of k and high values of a observed for annual crops. Among root characteristics, root spatial arrangement could also explain differences between crops. For example, the degree of root clustering can significantly change soil water uptake for a given root density (Beudez et al. 2013). Physiological properties such as root hydraulic conductivity could also influence the ability of roots to extract soil water (Nippert et al. 2012).

Conclusions

This study provides an original monitoring of soil water utilization by different perennial and annual energy crops during seven years. As expected, perennial and semi-perennial crops were characterized by proportional water capture in deep soil layers higher than annual crops. Conversely, PWC was lower in the upper soil layer for miscanthus and switchgrass than for the other crops. Semi-perennial crops lead to a greater soil water deficit than the other crops, due to an important water uptake both in surface and deep layers. Contrary to our initial hypothesis, perennial C4 crops resulted in similar SWD than annual crops whereas perennials were more productive and had a deeper root system.

Our study also highlights the relationship between water uptake and root distribution of the crops. PWC was correlated to root density with a curvilinear asymptotic function but its parameters were crop-dependent. Therefore root density was not the only factor determining maximal water uptake. Since aboveground biomass was not correlated to SWD, other factors such as the timing and length of the growing period are likely to affect water use.

In the perspective of predicting the effect of bioenergy crops on water drainage, a complete water balance will have to be made in order to quantify the ratio between evapotranspiration and drainage for each crop. Our results already indicate that the risk of drainage reduction compared to annual crops is probably higher with semi-perennial crops like alfalfa than with perennial C4 crops. The impact of the crops on drainage will also depend on soil and climate characteristics. Areas with deep soils and low winter rainfall are probably more susceptible to exhibit large differences in drainage between crops. Further studies are needed to explore this effect of soil and climate variability. Finally, our dataset will be useful to test and improve soil-crop models in order to simulate the impact of bioenergy crops on drainage and aquifer loading under various environmental conditions.

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752

Fig. 1 Evolution of the soil water content (SWC, 0-210 cm) over time (from July 2007 to November 2013) for each crop (Mis = miscanthus; Swi = switchgrass; Fes = fescue; Alf = alfalfa; Sor = sorghum; Tri = triticale). For fescue, alfalfa, sorghum and triticale, changes between plots were made the first day of March. Asterisks indicate significant differences between crops for the highest and lowest SWC of each year (° = NS; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

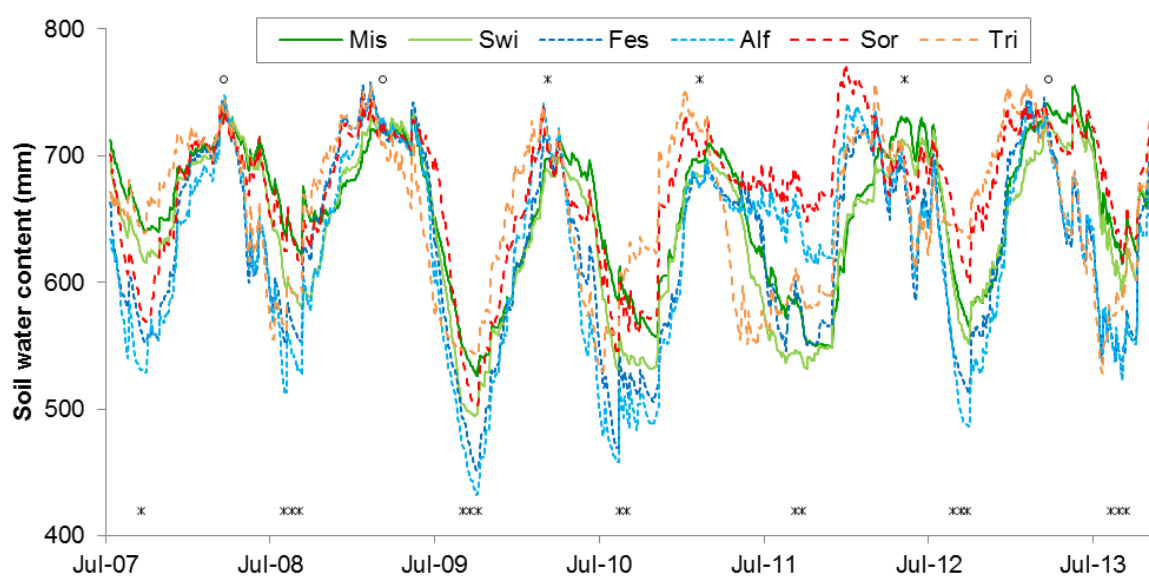


Fig. 2 Soil water deficit (SWD) calculated for each crop over the period 2007-2013. SWD is the mean (over 7 years) of the maximal values of SWD (0-210 cm) encountered during each year. Different letters indicate significant differences ($p < 0.05$) between crops. The seven soil layers (from 0-30 cm to 180-210 cm) are represented with a grey gradient from white to dark grey.

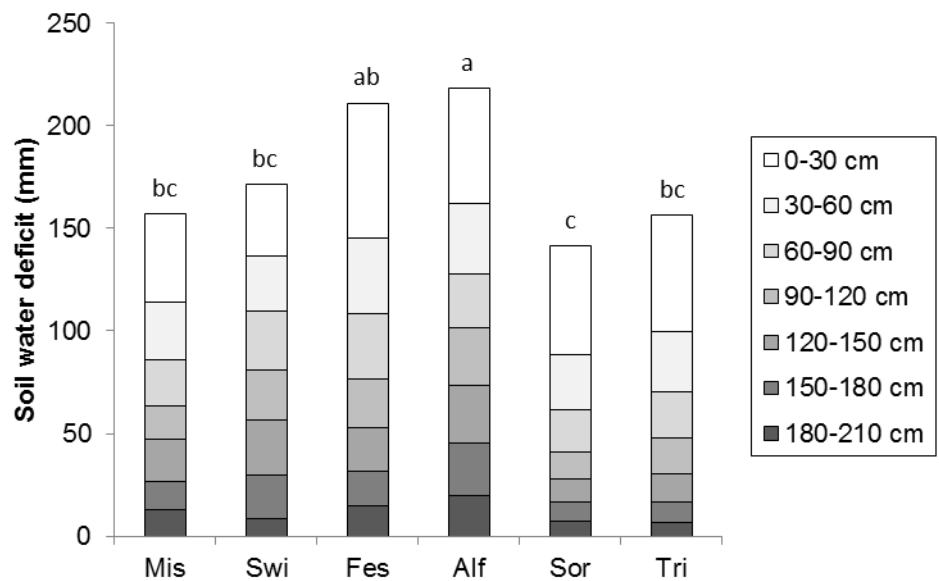


Fig. 3 Relationship between the maximal soil water deficit and the water balance ($P+I-PET$) observed each year for each crop

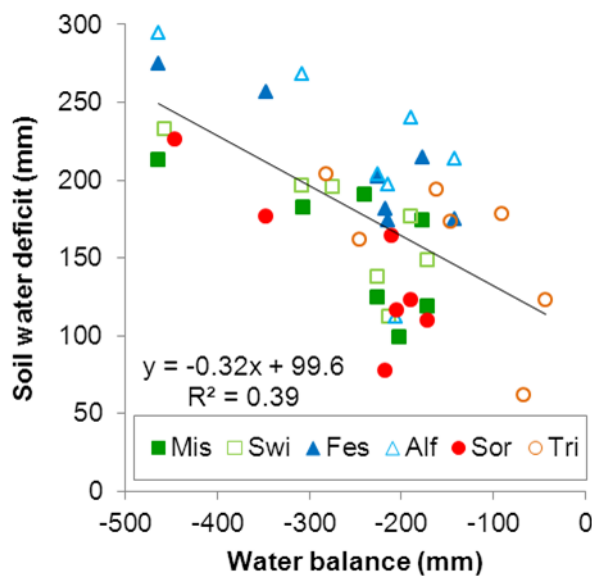


Fig. 4 Relationship between the maximal soil water deficit and the aboveground biomass production observed each year for each crop

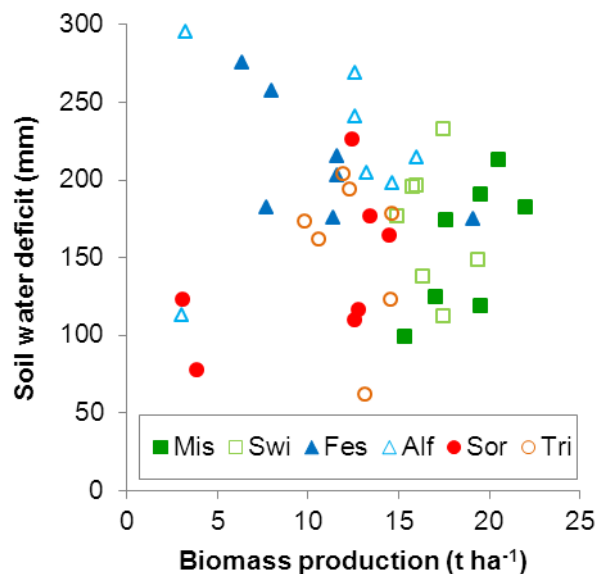


Fig. 5 Proportional water capture (PWC) versus depth for each crop (Mis = miscanthus; Swi = switchgrass; Fes = fescue; Alf = alfalfa; Sor = sorghum; Tri = triticale). PWC is calculated at the date of the maximal soil water deficit (mean value over the period 2007-2013). Horizontal bars represent the range between the minimal and the maximal values found over the seven-year period. Asterisks indicate significant differences between crops in each soil layer (* = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

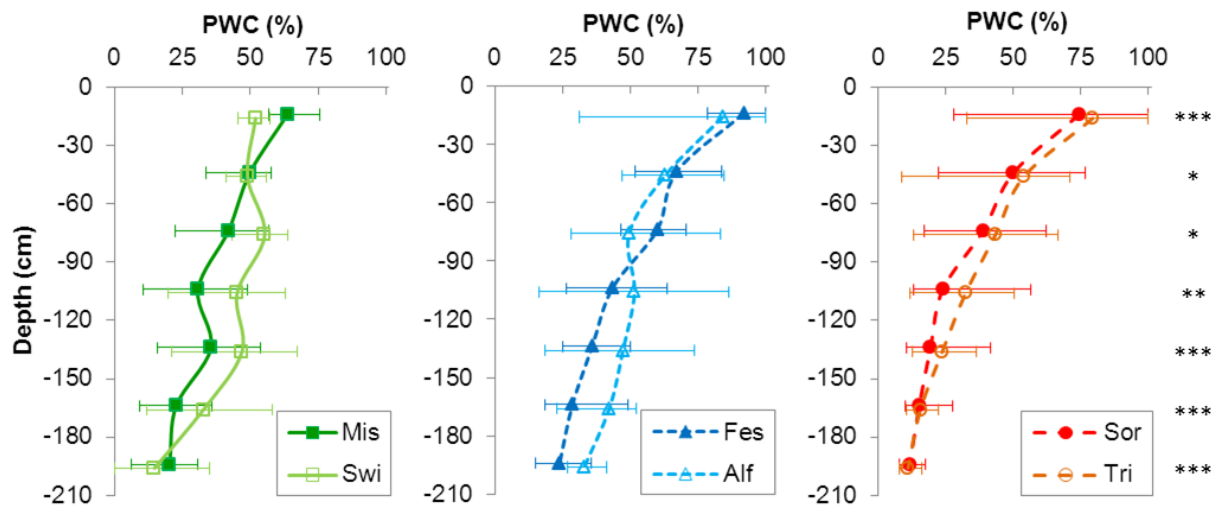


Fig. 6 Spatial distribution of roots observed on the vertical trench wall (180 cm * 300 cm) for each crop in 2010. Each black dot represents a grid cell (1.9 * 1.9 cm) containing at least one root.

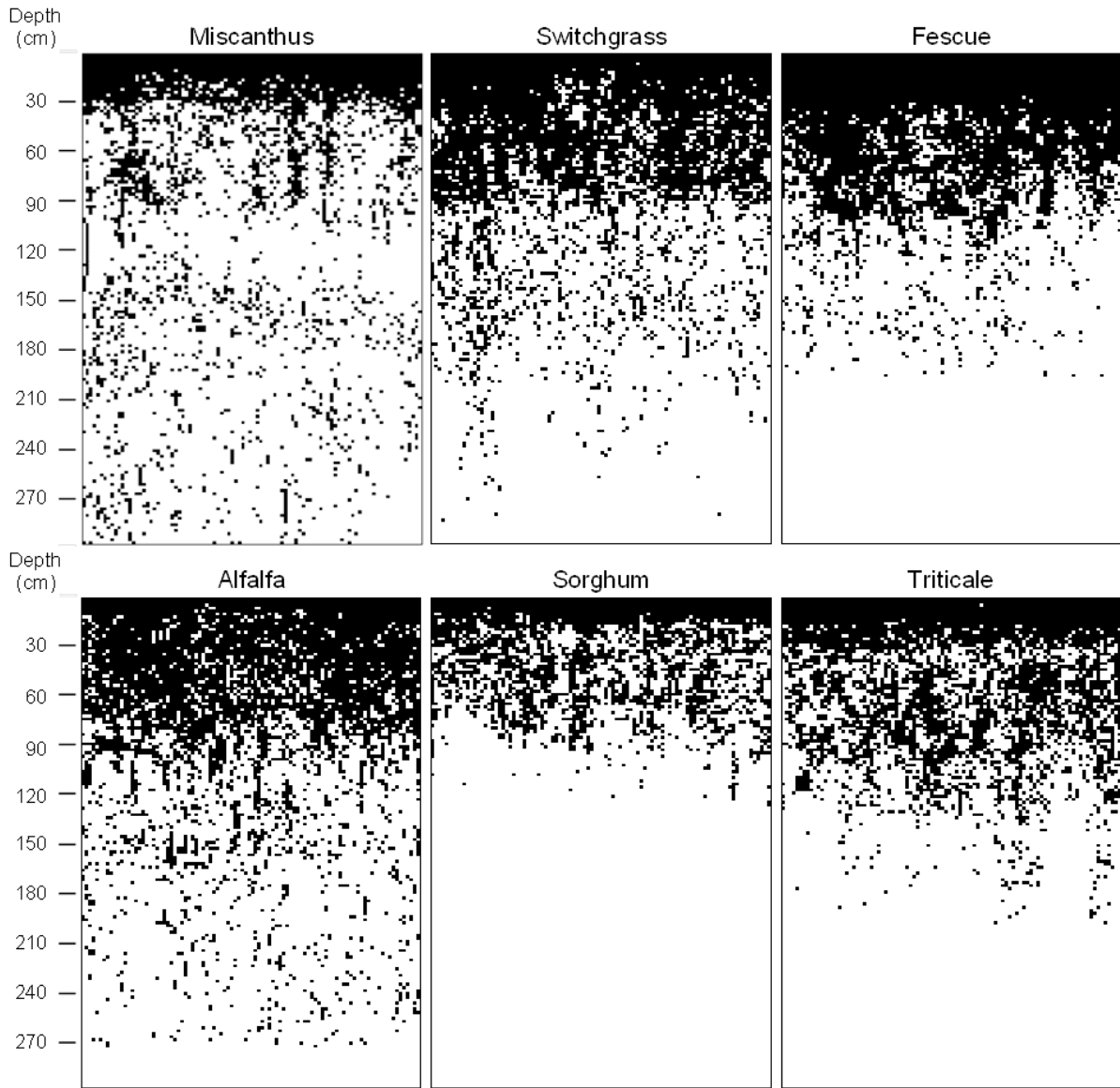
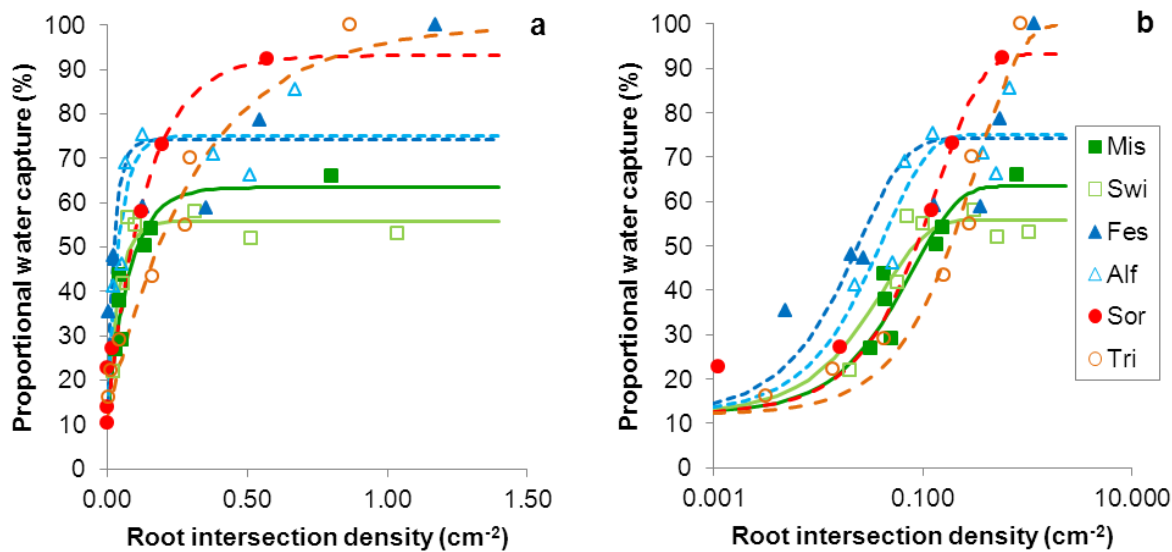


Fig. 7 Relationship between proportional water capture (PWC) and root intersection density (RID) for each crop. PWC was calculated at the date of maximal soil water deficit in 2010: a) RID expressed in linear scale; b) RID expressed in logarithmic scale. Symbols are experimental data and lines are modelled data. Miscanthus (Mis) is represented by a dark continuous line, switchgrass (Swi) by a light continuous line, fescue (Fes) by a dark dotted line, alfalfa (Alf) by a light dotted line, sorghum (Sor) by a dark broken line and triticale (Tri) by a light broken line.



797 **Table 1** Meteorological data: P = precipitation (mm), PET = Penman potential
798 evapotranspiration (mm) recorded at Estrées-Mons over the period 2007-2013

Year	March-June			July-October			March-October		
	P	PET	P-PET	P	PET	P-PET	P	PET	P-PET
2007	290	364	-73	216	327	-110	507	690	-184
2008	281	350	-69	317	318	-1	598	668	-70
2009	200	341	-142	145	432	-288	344	773	-429
2010	136	374	-239	270	346	-76	406	720	-315
2011	108	328	-219	243	294	-52	351	622	-271
2012	282	294	-13	219	346	-127	501	640	-139
2013	202	293	-91	321	345	-24	523	637	-114
Mean	214	335	-121	247	344	-97	461	679	-217

799

800

801 **Table 2** Soil characteristics measured in the experimental plots (mean $\pm$ standard deviation)

	Soil layers (cm)						
	0-30	30-60	60-90	90-120	120-150	150-180	180-210
Clay (g kg ⁻¹)	169 $\pm$ 25	227 $\pm$ 33	267 $\pm$ 22	243 $\pm$ 11	222 $\pm$ 12	190 $\pm$ 13	234 $\pm$ 21
Fine silt (g kg ⁻¹)	320 $\pm$ 17	305 $\pm$ 21	283 $\pm$ 44	277 $\pm$ 20	275 $\pm$ 13	272 $\pm$ 29	305 $\pm$ 10
Coarse silt (g kg ⁻¹)	459 $\pm$ 16	418 $\pm$ 19	410 $\pm$ 22	440 $\pm$ 16	467 $\pm$ 23	489 $\pm$ 28	404 $\pm$ 5
Fine sand (g kg ⁻¹)	38 $\pm$ 9	43 $\pm$ 18	37 $\pm$ 21	36 $\pm$ 15	35 $\pm$ 13	46 $\pm$ 13	52 $\pm$ 15
Coarse sand (g kg ⁻¹)	13 $\pm$ 3	6 $\pm$ 2	2 $\pm$ 1	2 $\pm$ 1	1 $\pm$ 1	1 $\pm$ 1	3 $\pm$ 1
Organic C ^a (g kg ⁻¹)	9.8 $\pm$ 0.3	6.0 $\pm$ 0.3	3.0 $\pm$ 0.2	2.3 $\pm$ 0.2	2.1 $\pm$ 0.3	1.7 $\pm$ 0.3	2.6 $\pm$ 0.7
pH in water	7.7 $\pm$ 0.2	7.8 $\pm$ 0.2	7.8 $\pm$ 0.1	8.0 $\pm$ 0.1	8.0 $\pm$ 0	8.2 $\pm$ 0.1	8.2 $\pm$ 0.1
CaCO ₃ (g kg ⁻¹)	2 $\pm$ 1	2 $\pm$ 2	1 $\pm$ 1	2 $\pm$ 2	1 $\pm$ 1	1 $\pm$ 2	3 $\pm$ 4
WFC ^b (g kg ⁻¹)	243 $\pm$ 3	221 $\pm$ 3	219 $\pm$ 3	217 $\pm$ 4	222 $\pm$ 2	228 $\pm$ 6	238 $\pm$ 3
WWP ^c (g kg ⁻¹)	90 $\pm$ 12	102 $\pm$ 18	107 $\pm$ 11	104 $\pm$ 9	95 $\pm$ 13	89 $\pm$ 6	104 $\pm$ 5
Bulk density (g cm ⁻³)	1.47 $\pm$ 0.05	1.55 $\pm$ 0.02	1.57 $\pm$ 0.03	1.58 $\pm$ 0.01	1.55 $\pm$ 0.03	1.49 $\pm$ 0.03	1.51 $\pm$ 0.03

802

803 ^a Anne Method (AFNOR X 31-109)

804 ^b Water content at field capacity (median of field measurements made in March over the
805 period 2007-2013), corresponding to ca. -20 kPa water potential

806 ^c Water content at permanent wilting point (measured with Richard's pressure plates at -1.5
807 MPa water potential)

808

809 **Table 3** Root intersection density (mean number of root impacts per cm²) measured for each soil layer and crop (mean $\pm$ standard deviation).

810 Different letters indicate significant differences ($p < 0.05$) between crops in each soil layer.

Soil layer (cm)	Miscanthus	Switchgrass	Fescue	Alfalfa	Sorghum	Triticale
0-30	0.800 $\pm$ 0.159 <i>bc</i>	1.038 $\pm$ 0.107 <i>ab</i>	1.173 $\pm$ 0.169 <i>a</i>	0.669 $\pm$ 0.066 <i>c</i>	0.571 $\pm$ 0.056 <i>c</i>	0.867 $\pm$ 0.140 <i>abc</i>
30-60	0.155 $\pm$ 0.037 <i>b</i>	0.513 $\pm$ 0.082 <i>a</i>	0.546 $\pm$ 0.009 <i>a</i>	0.510 $\pm$ 0.113 <i>a</i>	0.194 $\pm$ 0.030 <i>b</i>	0.295 $\pm$ 0.084 <i>b</i>
60-90	0.134 $\pm$ 0.055 <i>b</i>	0.314 $\pm$ 0.018 <i>a</i>	0.352 $\pm$ 0.095 <i>a</i>	0.379 $\pm$ 0.035 <i>a</i>	0.123 $\pm$ 0.013 <i>b</i>	0.278 $\pm$ 0.057 <i>a</i>
90-120	0.043 $\pm$ 0.016 <i>bc</i>	0.101 $\pm$ 0.035 <i>ab</i>	0.127 $\pm$ 0.071 <i>ab</i>	0.126 $\pm$ 0.042 <i>ab</i>	0.016 $\pm$ 0.010 <i>c</i>	0.160 $\pm$ 0.058 <i>a</i>
120-150	0.043 $\pm$ 0.034 <i>a</i>	0.072 $\pm$ 0.039 <i>a</i>	0.027 $\pm$ 0.013 <i>a</i>	0.067 $\pm$ 0.039 <i>a</i>	0.001 $\pm$ 0.001 <i>b</i>	0.042 $\pm$ 0.017 <i>a</i>
150-180	0.050 $\pm$ 0.006 <i>ab</i>	0.057 $\pm$ 0.017 <i>a</i>	0.020 $\pm$ 0.012 <i>bc</i>	0.051 $\pm$ 0.014 <i>ab</i>	0 $\pm$ 0 <i>d</i>	0.014 $\pm$ 0.011 <i>c</i>
180-210	0.031 $\pm$ 0.022 <i>a</i>	0.020 $\pm$ 0.004 <i>ab</i>	0.005 $\pm$ 0.002 <i>bc</i>	0.022 $\pm$ 0.004 <i>ab</i>	0 $\pm$ 0 <i>c</i>	0.003 $\pm$ 0.004 <i>c</i>
210-240	0.023 $\pm$ 0.010 <i>a</i>	0.010 $\pm$ 0.004 <i>b</i>	0 $\pm$ 0 <i>c</i>	0.020 $\pm$ 0.002 <i>a</i>	0 $\pm$ 0 <i>c</i>	0 $\pm$ 0 <i>c</i>
240-270	0.020 $\pm$ 0.012 <i>a</i>	0.003 $\pm$ 0.002 <i>b</i>	0 $\pm$ 0 <i>b</i>	0.016 $\pm$ 0.009 <i>a</i>	0 $\pm$ 0 <i>b</i>	0 $\pm$ 0 <i>b</i>
270-300	0.022 $\pm$ 0.016 <i>a</i>	0.001 $\pm$ 0.001 <i>b</i>	0 $\pm$ 0 <i>b</i>	0.001 $\pm$ 0.000 <i>b</i>	0 $\pm$ 0 <i>b</i>	0 $\pm$ 0 <i>b</i>

811

812 **Table 4** Parameter and statistical criterion values obtained for each crop after fitting the
 813 model describing the relationship between root intersection density and proportional water
 814 capture (see Eq. 2 in text)

		Miscanthus	Switchgrass	Fescue	Alfalfa	Sorghum	Triticale
<i>a</i>	(%)	63.5	55.9	74.2	75.1	93.2	100.0
<i>k</i>	(cm ²)	12.6	24.4	39.6	23.9	7.3	3.1
RMSE	(%)	5.4	4.9	13.8	7.3	4.6	5.7