Influence of soil texture and crop management on the productivity of miscanthus (*Miscanthus × giganteus* Greef et Deu.) in the Mediterranean

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Abstract

Biomass productivity is the main favorable trait of candidate bioenergy crops. *Miscanthus × giganteus* is a promising species, due to its high-yield potential and positive traits including low nutrient requirements and potential for C sequestration in soils. However, miscanthus productivity appears to be mostly related to water availability in the soil. This is important, particularly in Mediterranean regions where the risk of summer droughts is high. To date, there have been no studies on miscanthus responses under different soil conditions, while only a few have investigated the role of different crop managements, such as irrigation and nitrogen fertilization, in the Mediterranean. Therefore, the effects of contrasting soil textures (i.e. silty-clay-loam vs. sandy-loam) and alternative agricultural intensification regimes (i.e. rainfed vs. irrigated and 0, 50, 100 kg ha^{-1} nitrogen fertilization), on miscanthus productivity were evaluated at three different harvest times for two consecutive years. Our results confirmed the importance of water availability in determining satisfactory yields in Mediterranean environments, and how soil and site characteristics strongly affect biomass production. We found that the aboveground dry yields varied between 5 Mg ha^{-1} up to 29 Mg ha^{-1}. Conversely, nitrogen fertilization played only a minor role on crop productivity, and high fertilization levels were relatively inefficient. Finally, a marked decrease, of up to −40%, in the aboveground yield occurred when the harvest time was delayed from autumn to winter. Overall, our results highlighted the importance of determining crop responses on a site-by-site basis, and that decisions on the optimal harvest time should be driven by the biomass end use and other long-term considerations, such as yield stability and the maintenance of soil fertility.

Keywords: biomass, energy crop, harvest time, irrigation, leaf area index, leaf litter, nitrogen agronomic efficiency, nitrogen fertilization, yield

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Introduction

Perennial rhizomatous grasses are promising energy crops due to their high productivity, low nutrient requirements, ecosystem services and great potential for C mitigation (Lewandowski *et al.*, 2003a; Rowe *et al.*, 2009; Chum *et al.*, 2011). Miscanthus (*Miscanthus x giganteus* Greef et Deuter), is a high-yielding C4 perennial grass native to Asia and is characterized by vigorous growth and high adaptability in a wide range of European conditions (Clifton-Brown *et al.*, 2001; Lewandowski *et al.*, 2003a; Mantineo *et al.*, 2009). In fact, dry yields of miscanthus without irrigation range from 10 to 15 Mg ha^{-1} in northern and central Europe to more than 25 Mg ha^{-1} in southern Europe (Lewandowski *et al.*, 2003a; Angelini *et al.*, 2009; Zub & Brancourt-Hulmel, 2010).

However, productivity of miscanthus is largely related to precipitation and the soil moisture available (Kahle *et al.*, 2001; Richter *et al.*, 2008). This is an important issue, particularly in Mediterranean regions where there is a high risk of summer droughts. Irrigation could thus be an important technique to increase miscanthus yields at sites with poor water availability (Heaton *et al.*, 2004; Zub & Brancourt-Hulmel, 2010). Nevertheless, accessibility to water and its quality is likely to decrease in the near future and much attention is needed on how to allocate water for different uses (Wallace, 2000).

To date, in the Mediterranean there has been no research on miscanthus responses under different soil conditions, and particularly on poor, marginal lands, while few studies have evaluated crop productivity in

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response to irrigation. Results from these latter studies suggest that irrigation influences miscanthus yields, but often with among-year variation (Ercoli *et al.*, 1999; Cosentino *et al.*, 2007; Mantineo *et al.*, 2009). Some research has found a positive interaction between irrigation and nitrogen fertilization (Ercoli *et al.*, 1999; Cosentino *et al.*, 2007). However, those results probably depended on the high levels of nitrogen tested (120 and 200 kg N ha^{-1}), as other authors, using lower nitrogen fertilization rates (50 and 100 kg N ha^{-1}), did not find a significant interaction between the two factors (Mantineo *et al.*, 2009). In addition, the majority of European trials have highlighted little or no influence of nitrogen fertilization on miscanthus productivity (Arundale *et al.*, 2013).

However, the importance of determining miscanthus yield responses to applied N on a site-by-site basis has been highlighted, since excessive fertilization can lead to a negative environmental impact, e.g. increased GHG emissions, N leaching and uncertainties when economic assessments are required (Cadoux *et al.*, 2012; Davis *et al.*, 2013; Larsen *et al.*, 2013).

The potential harvest window of miscanthus is wide (Lewandowski *et al.*, 2003a).The optimal harvest period depends on the environmental conditions of the site which influences the start of the crop senescence, field accessibility etc., as well as the conversion process adopted (combustion, bioethanol, etc.) (Karp & Shield, 2008; Zegada-Lizarazu *et al.*, 2010). When considering the optimal harvest time, trade off has to be made between yield and quality optimization (Lewandowski & Heinz, 2003). This is particularly relevant for combustion processes, for which biomass losses due to a delayed harvest are preferred in return for a lower mineral content and a lower level of humidity of the biomass itself (Lewandowski & Kicherer, 1997).

Developing processes for separating, refining and converting lignocellulosic biomass to produce biofuels and biomaterials could make early harvest material attractive (Ragauskas *et al.*, 2006; Karp & Shield, 2008). From an agronomical perspective, early harvest requires careful analysis, as rhizome nutrient stocks and soil nutrient contents may become depleted, which in turn will affect the biomass production in the long run (Strullu *et al.*, 2011).

Overall, biomass production entails investigating the appropriate agronomic practices for specific environmental conditions, as crop management is a key factor in sustainable biomass production systems (Zegada-Lizarazu *et al.*, 2010). The aim of this study was thus to deepen the understanding of the influence of contrasting soil textures and alternative agricultural intensification regimes (i.e. irrigation and nitrogen fertilization) on miscanthus productivity at different harvest times.

Materials and methods

Site and trial set up

The research was carried out at the Interdepartmental Centre for Agro-Ecological Research (CIRAA) in the Pisa coastal plain (central Italy; latitude 43°68′ N, longitude 10°35′ E; 1 m a.s.l. and 0% slope) for two consecutive years, i.e. 2011 and 2012. The area, having originated from land reclamation, is characterized by heterogeneous soil textures, with different soils located within few hundred meters one another, and thus provides a particularly suited site for comparing soil effects under the same environmental conditions (e.g., meteorological conditions, water table depth, etc.).

In spring 2010, two adjacent fields characterized by two contrasting soil textures, i.e. silty-clay-loam (SiC) and sandy-loam (SL), were used to carry out two experiments:

- Experiment 1: three main plots were arranged in the SiC soil and three in the SL soil. Within each main plot, three nitrogen fertilization levels [0 (N_0), 50 (N_{50}), 100 (N_{100}) kg ha^{-1}] were randomly assigned as subplots (size 6.5 × 5.0 m).

- Experiment 2 was set up on the SiC soil. Main plots (three replicates) were assigned to two irrigation regimes: 0% (ET_0) vs. 75% (ET_{75}) of the potential evapotranspiration. Within each main plot, three nitrogen fertilization levels [0 (N_0), 50 (N_{50}), 100 (N_{100}) kg ha^{-1}] were randomly identified as subplots (size 6.5 × 5.0 m).

Meteorological data were obtained from the closest weather station (<500 m from the experimental site). Soil samples were collected within 0–60 cm of the surface in April 2010 and analyzed for texture, pH, organic matter, total nitrogen, available phosphorus, exchangeable potassium, cation-exchange capacity (CEC) and soil water characteristics (Table 1). The soil was a *Typic Xerofluvent*, representative of the lower Arno river plain, characterized by a shallow water table never below a 2.5 m depth even during the driest periods.

Table 1 Soil characteristics at the experimental sites (0–60 cm; April 2010)

	SiC	SL
Sand (2–0.05 mm) (%)	18.1	78.9
Silt (0.05–0.002 mm) (%)	46.4	11.0
Clay (<0.002 mm) (%)	35.5	10.1
pH (1 : 1 w/v)	8.2	7.7
Organic matter (Walkley–Black) (%)	1.8	0.9
Total nitrogen (Kjeldahl) (g kg^{-1})	1.4	0.7
Available phosphorus (Olsen) (mg kg^{-1})	69.3	116.9
Exchangeable potassium (Dirks and Scheffer) (mg kg^{-1})	223.7	151.7
CEC (meq 100 g^{-1})	22.3	8.5
Field capacity (%w)	35.3	9.7
Wilting point (%w)	16.1	2.0

Tillage was conducted in the autumn of 2009 by ploughing, followed by rotary harrowing immediately before planting. Crop establishment was carried out on April 22, 2010 using rhizomes, at a density of two plants per m^{-2} (1×0.5 m spacing). No preplant fertilizer was required. Plants were watered throughout the first growing season to get them established. By the end of the first year, the establishment rate was close to 100 percent in all plots. Weeding and pest control were never necessary at any point during the trial.

In both Experiments 1 and 2, nitrogen (urea) fertilization treatments were always performed in the spring, when crops were 0.20–0.30 m tall. In Experiment 2, water was distributed by drip irrigation. Drip irrigation pipes were placed 1 m apart and positioned in the inter-rows. The distance of the drippers was 30 cm with a dripper flow rate of 1 l per hour. Daily potential evapotranspiration (ETP) was estimated through the FAO Penman–Monteith equation (Allen *et al.*, 1998) from daily climatic data. Irrigation was scheduled every 2 days.

Table 2 summarizes the principal cultivation phases (emergence, fertilization and harvest dates) and the amount of water (precipitation and irrigation) the crops received.

Measurements

In both Experiments 1 and 2, productive measurements for the second and third year of growth (i.e. 2011 and 2012 respectively) were collected in three different periods: early autumn (A_1) (i.e. mid October), late autumn (A_2) (i.e. mid November) and winter (W) (i.e. end of January – early February).

At each harvest time, the aboveground biomass was sampled in a 4 m^2 area (2×2 m^2 subreplicates) and fresh weight was determined. Border plants from the outer rows were not included in the sampling area. Plant subsamples were partitioned into leaves, stems and inflorescence. After partitioning, subsamples were dried at 60 °C until constant weight to determine the dry matter content and dry biomass yield. Aboveground dry yield was derived as the sum of leaves, stems and inflorescence components.

The agronomic efficiency of the nitrogen fertilization (A_E, kg kg_N^{-1}) was calculated according to Delogu *et al.* (1998), as:

$$A_E = \frac{Y_{NX} - Y_{N0}}{N_X}$$

where Y_{Nx} represents the aboveground dry yield at N_x fertilization level, Y_{N0} the aboveground dry yield receiving no N fertilization and N_x the applied nitrogen fertilization rate. Thus, A_E represents the ability of the crop to increase yield in response to N applied.

In addition, in both years leaf litter on top of the ground was collected at each harvest date in a 2 m^2 area. Litter subsamples were dried at 60 °C until constant weight to obtain the amount of dry leaf litter per unit of area.

Finally, during the growing seasons, the crop canopy development was measured. The leaf area index (LAI) was estimated using a leaf canopy analyzer (SunScan, Delta-T Devices Ltd., Cambridge, UK).

Statistical analysis

Data were analyzed by a split-split plot ANOVA to evaluate the effect of the main plot (i.e. soil texture in Experiment 1, irrigation in Experiment 2), of the subplot (nitrogen fertilization) and of the sub-subplot (time of harvest) on the aboveground dry yield and the amount of litterfall per unit of area (Gomez & Gomez, 1984). Tukey's HSD *post hoc* test was used to separate means. The statistical analysis was performed with R software (R Core Team, 2013) and the *agricolae* package (de Mendiburu, 2013).

Table 2 Summary of the principal crop cultivation phases, precipitation, irrigation and N fertilization dates in Experiment 1 [silty-clay-loam (SiC) vs. sandy-loam (SL)] and Experiment 2 [0% (ET_0) vs. 75% (ET_{75}) of the potential evapotranspiration] in the second (2011) and third (2012) growing years. A_1, A_2 and W represent early autumn, late autumn and winter harvests, respectively

	2011		2012	
	SiC, SL, ET_0	ET_{75}	SiC, SL, ET_0	ET_{75}
DOE*	01 April		02 April	
DOH*				
A_1	12 October		2 October	
A_2	10 November		26 November	
W	25 January		20 February	
N fertilization date	19 April		17 April	
Precipitation (mm)†				
A_1	164.2		395.8	
A_2	223.0		695.8	
W	373.8		1155.6	
Irrigation (mm)	–	390	–	342
Start of irrigation	–	14 June	–	21 June
End of irrigation	–	02 November	–	19 September

*DOE and DOH represent the day of emergence and day of harvest respectively.
†Precipitation are cumulated daily values calculated from DOE to DOH.

Results

Meteorological data

The study site represents a typical Mediterranean climate, with warm dry summers, cool winters and precipitation events concentrated in the autumn and spring. Figure 1 reports the meteorological trend of the second (2011) and third (2012) growing seasons. Compared to the 20-year term (1986–2005) precipitation trend (876 mm yr^{-1}), 2011 was a dry year, with 30% less precipitation than the long-term average (627 mm), while 2012 was in line with the long-term trend, with a slightly higher precipitation (+7%, 936 mm). In accordance with Bagnouls & Gaussen (1957), dry periods occurred in 2011 and 2012 during the spring-summer periods. As a general trend, the air temperatures increased from March to August. In the period of investigation, the average yearly temperature was 14.7 °C, temperatures lower than 0 °C were recorded in February 2012 only, and maximum values over 30 °C were quite frequent in July and August.

Experiment 1 – Contrasting soil textures

In the second year of growth (2011) crops growing in SiC soil showed a significantly higher aboveground dry yield (Table S1) compared to crops growing in SL soil (19.1 vs. 10.9 Mg ha^{-1}) (Fig. 2a). In addition, maximum yields were recorded at the A$_1$ harvest (17.4 Mg ha^{-1}). On average, switching from A$_1$ to W harvest led to a significant reduction in dry biomass yield (−5.7 Mg ha^{-1}, -32.8%) (Fig. 2b). General trends in biomass productivity were amplified in the third growing year (2012), when miscanthus growing in SL soil was

severely influenced by the summer drought which led to premature aboveground senescence, leaf loss and inhibition of flowering. Hence, averaged over the three harvest dates, dry biomass yield in the SL soil was one order of magnitude lower than in the SiC soil (24.6 vs. 3.9 Mg ha^{-1}). A significant interaction between soil and date of harvest was observed in 2012. In fact, productivity in the SL soil did not significantly decrease from the A$_1$ to W harvest (i.e. values remained low, though stable), while miscanthus yield in the SiC soil did decrease (−11.9 Mg ha^{-1}, −40.5%) (Fig. 2c).

On the other hand, in both years of investigation no effect of the nitrogen fertilization level on the aboveground dry yield was recorded (Table S1). Averaged over the 2 years, the increase in the aboveground dry yield stood at around 2 Mg ha^{-1} every 50 kg N ha^{-1} in the SiC soil, while it was negligible in the SL soil. As a consequence, the agronomic efficiency of the N fertilization (A$_E$) reflected mostly differences between soils, rather than among nitrogen fertilization rates (Table 3).

In both years, delayed harvest at W time significantly ($P < 0.001$) increased miscanthus dry matter concentration compared to autumn (A$_1$ and A$_2$) harvests (+31% on average). In W, crops in SiC and SL soils had similar aboveground dry matter concentration in 2011 (805 g kg^{-1} on average), while the following year (2012) miscanthus growing in the SL soil reached a higher dry matter concentration (around 710 g kg^{-1}) compared to the SiC (Table 4).

As expected, litterfall on soil surface increased ($P < 0.001$) steadily from A$_1$, reaching maximum values in W for the 2 years of investigation (3.4 Mg ha^{-1} on average in 2011 and 4.6 Mg ha^{-1} on SiC soil in 2012). The only exception was the 2012 litterfall on SL soil when plants senesced early in the season and maximum leaf
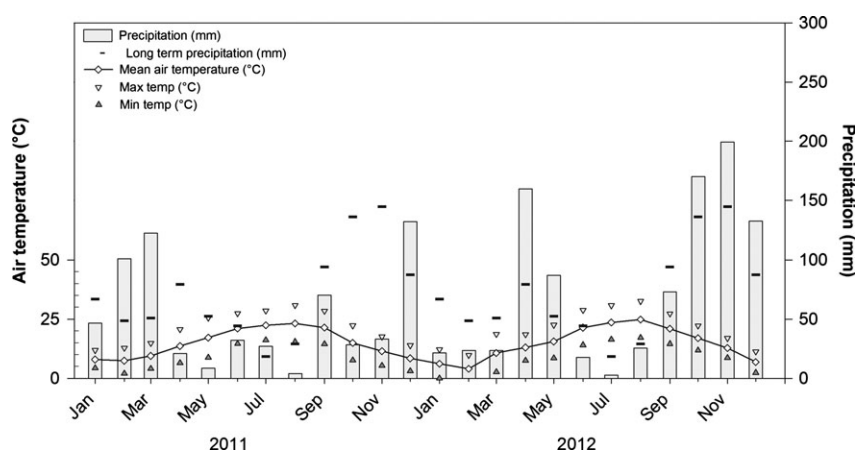

Fig. 1 Monthly and long-term (1986–2007) precipitation, minimum, maximum and mean air temperature in San Piero a Grado (Pisa) in 2011 and 2012. The graph is presented as a Bagnouls & Gaussen (1957) diagram, to identify dry months, i.e. when precipitation (P) is equal to or less than twice the monthly mean air temperature value (T) (P ≤ 2T).
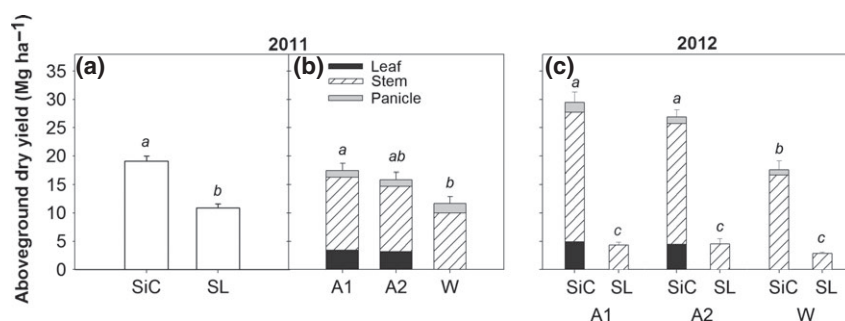
Fig. 2 Aboveground dry yield in the second (2011) and third (2012) growing seasons in Experiment 1. Data are presented according to the ANOVA: (a) significant differences between the two soil textures in 2011 (SiC and SL stand for silty-clay-loam and sandy-loam soils respectively); (b) significant differences among the three harvest dates in 2011 (A_1, A_2 and W represent early autumn, late autumn and winter harvests, respectively); (c) significant interaction between soil texture and harvest time in 2012. Different letters indicate significant differences at $P < 0.05$. Vertical bars represent the SE ($n = 3$).

Table 3 Effect of the nitrogen fertilization rate on the aboveground dry yield and agronomic efficiency of the N fertilization (A_E) in Experiment 1. Data are averaged over the three harvest dates (A_1, A_2 and W) and the two growing years (i.e. 2011 and 2012). SiC and SL represent the silty-clay-loam and the sandy-loam soils. N_0, N_{50}, N_{100} represent 0, 50 and 100 kg N ha^{-1}, respectively. Standard errors are given in brackets ($n = 3$)

Soil texture	N fertilization rate	Aboveground dry yield (Mg ha^{-1})	Agronomic efficiency (kg kg_N^{-1})
SiC	N_0	19.7 (± 2.2)	
	N_{50}	21.6 (± 2.2)	38.1
	N_{100}	24.2 (± 2.0)	44.6
SL	N_0	7.1 (± 1.7)	
	N_{50}	8.0 (± 1.4)	18.9
	N_{100}	7.1 (± 1.6)	0.4

Table 4 Dry matter concentration ($\pm$SE, $n = 3$) as a function of the harvest time in silty-clay-loam (SiC) and sandy-loam (SL) soils (Experiment 1) for the second (2011) and third (2012) growing seasons. A_1, A_2 and W represent early autumn, late autumn and winter harvests, respectively. For each year, different superscript letters indicate a significant ($P < 0.05$) interaction between soil texture and harvest time

	Dry matter concentration (g kg^{-1})	
	SiC	SL
2011		
A_1	521.3 (± 6.1)[b]	487.9 (± 8.6)[b]
A_2	525.6 (± 5.1)[b]	494.8 (± 7.8)[b]
W	788.9 (± 13.1)[a]	821.1 (± 11.7)[a]
2012		
A_1	493.5 (± 11.8)[c]	529.7 (± 37.4)[bc]
A_2	528.1 (± 7.0)[bc]	442.5 (± 35.5)[c]
W	609.3 (± 19.6)[b]	708.2 (± 10.9)[a]

litter was attained already during the A_1, decreasing progressively to W, owing to litter decomposition (Fig. 3).

In terms of the leaf area index (LAI), Fig. 4 shows a comparison of miscanthus grown in SiC and SL soils and receiving N_0, N_{50} and N_{100} fertilization rates. In 2011, the LAI pattern of crops grown in SiC and SL was similar, i.e. LAI increased until a maximum around July/August, 4.6 and 3.1 m m^{-2} in SiC and SL respectively, and then decreased steadily. In the following year, there was a marked difference between the miscanthus grown in SiC and SL soils: crops in the SiC soil showed an increasing trend until August, reaching maximum LAI values of 6.8, while crops in SL soil exhibited decreasing LAI values starting as early as mid-May, and had completely senesced in August. In the SiC soil, increasing levels of N fertilization led overall to higher LAI values during the midseason (i.e. June–August), thus obtaining increases in maximum LAI around 1 m^2 m^{-2} every 50 kg N ha^{-1}.

Experiment 2 – Contrasting irrigation regimes

In both the second (2011) and third (2012) years of growth, no aboveground yield differences were recorded between the two irrigation treatments (around 20 Mg ha^{-1} in 2011 and 25 Mg ha^{-1} in 2012), while harvest time significantly influenced the aboveground dry yield (Table S2). Maximum aboveground dry yields were observed at the A_1 stage (23.2 and 29.0 Mg ha^{-1} in 2011 and 2012 respectively), then values decreased until the W harvest to 16.0 and 18.1 Mg ha^{-1} in 2011 and 2012 respectively (Fig. 5). Yield losses ranged from 31 to 38% from A_1 to W.

The effect of nitrogen fertilization was observed for the second growth year (2011), when crops receiving 50 and 100 kg N ha^{-1} yielded significantly (Table S2) higher (21.6 Mg ha^{-1} on average) aboveground dry biomass compared to the unfertilized treatment (16.9
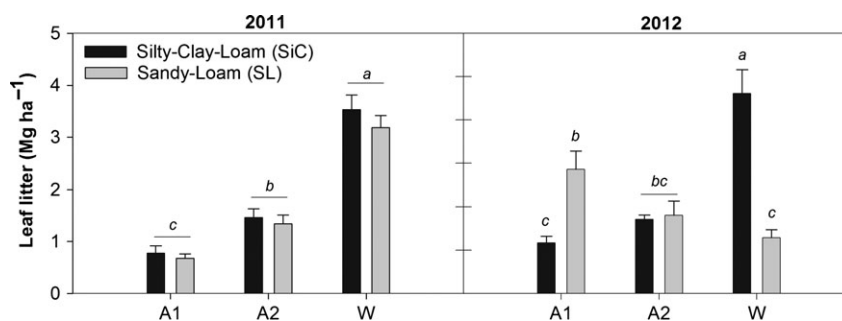
Fig. 3 Leaf litter during the second (2011) and third (2012) year of growth under contrasting soil textures (Experiment 1). A_1, A_2 and W represent early autumn, late autumn and winter harvests, respectively. Different letters indicate significant differences at $P < 0.05$. Vertical bars represent the standard error ($n = 3$).
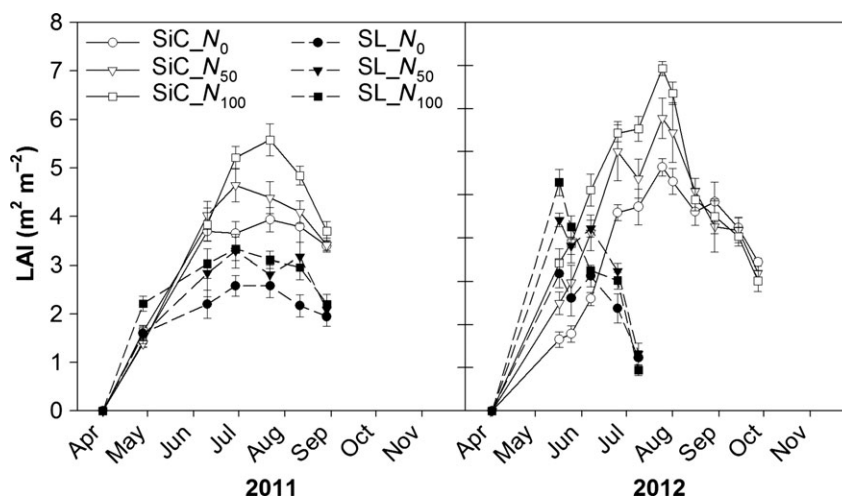

Fig. 4 Dynamics of the leaf area index (LAI) for the second (2011) and third (2012) growing seasons in miscanthus grown on a silty-clay-loam (SiC) soil and on a sandy-loam (SL) soil and receiving 0 (N_0), 50 (N_{50}) and 100 (N_{100}) kg N ha^{-1}. Vertical bars represent the standard error ($n = 3$).

Mg ha^{-1}) (Table 5). Table 5 also reports the agronomic efficiencies of the N fertilization (A_E). Averaged over the irrigation regimes, in 2011 when the crop was supplied with 50 kg ha^{-1} of nitrogen, an increase in dry yield around 93 kg per kg of nitrogen was recorded. On the other hand, a higher level of N fertilization, up to 100 kg N ha^{-1}, reduced its agronomic efficiency nearly by half, showing increases in dry yield of around 48 kg per kg of applied nitrogen. Conversely, in 2012 the effect of nitrogen fertilization on the aboveground dry yield was not significant, and differences in the A_E between the N_{50} and N_{100} treatments were much smaller (Table 5).

While no differences were recorded in terms of dry matter concentration between irrigation regimes and among nitrogen fertilization levels, switching from an early to late harvest drastically reduced the moisture content of the crop, thus significantly ($P < 0.001$ in both years) increasing its dry matter concentration from

around 480–510 g kg^{-1} up to 800 g kg^{-1} in 2011 and 630 g kg^{-1} in 2012 (Table 6).

Concerning the leaf litter, no significant differences were recorded between the two irrigation levels ($P = 0.678$ in 2011 and $P = 0.453$ in 2012). Conversely, the amount of leaf litter retrieved on the soil surface increased significantly ($P < 0.001$ in both years) from A_1 to W. Litterfall at W time accounted for around 3.3 and 4.7 Mg ha^{-1} in 2011 and 2012 respectively (Fig. 6).

Leaf area index dynamics throughout the season were similar in the second (2011) and third (2012) years and matched the trend already reported for miscanthus grown in SiC soil: on average, maximum LAI was observed around 100 days after emergence, on July 22 and 25 in 2011 and 2012 respectively (Fig. 7). The statistical analysis performed on maximum LAI revealed no influence of the irrigation regime, while a significant effect of nitrogen fertilization was observed in both years (Table S3), with the highest values recorded in
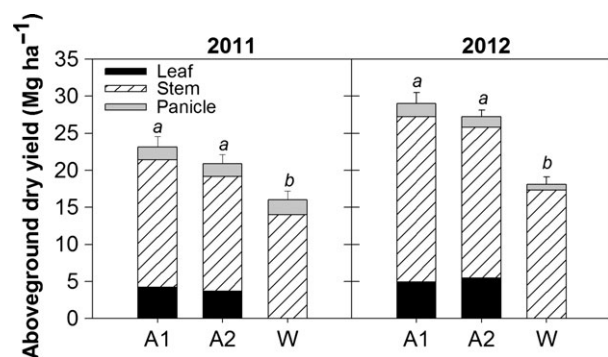
Fig. 5 Aboveground dry yield in the second (2011) and third (2012) growing seasons in Experiment 2. Data are presented according to the ANOVA and report the effect of the harvest time. A_1, A_2 and W represent early autumn, late autumn and winter harvests, respectively. Different letters indicate significant differences at $P < 0.05$. Vertical bars represent the SE ($n = 3$).

Table 5 Effect of the nitrogen fertilization rate on the aboveground dry yield and agronomic efficiency of the N fertilization (A_E) in Experiment 2 for the second (2011) and third (2012) growing seasons. Data are averaged over the two irrigation regimes (ET_0 and ET_{75}) and the three harvest dates (A_1, A_2 and W). N_0, N_{50}, N_{100} represent 0, 50 and 100 kg N ha^{-1}, respectively. Different superscript letters indicate significant differences at $P < 0.05$. Standard errors are given in brackets ($n = 3$)

Year	N fertilization rate	Aboveground dry yield (Mg ha^{-1})	Agronomic efficiency (kg kg$_N$$^{-1}$)
2011	N_0	16.9 (± 2.2)[b]	
	N_{50}	21.5 (± 2.2)[a]	93.3
	N_{100}	21.7 (± 2.5)[a]	48.2
2012	N_0	23.5 (± 2.9)	
	N_{50}	25.2 (± 2.5)	34.8
	N_{100}	25.6 (± 3.0)	20.9

miscanthus receiving 50 and 100 kg N ha^{-1} (around 4.7 m m^{-2} in 2011 and 7.7 m m^{-2} in 2012).

Discussion

Our main objective was to investigate the aboveground productivity of miscanthus in response to different soil textures (silty-clay-loam vs. sandy-loam), and crop management (irrigation and nitrogen fertilization), at different harvest times. The rationale for an early autumn harvest (A_1) was based on the fact that, in Mediterranean environments, the full plant flowering phase occurs in September/October, and flowering corresponds to the maximum harvestable aboveground biomass yield (Petrini *et al.*, 1996; Cosentino *et al.*, 2007;

Table 6 Dry matter concentration ($\pm$SE, $n = 3$) as a function of the harvest time in Experiment 2 for the second (2011) and third (2012) growing seasons. Data are averaged over the two irrigation regimes (ET_0 and ET_{75}) and the three N fertilization levels (N_0, N_{50} and N_{100}). A_1, A_2 and W represent early autumn, late autumn and winter harvests, respectively. For each year, different superscript letters indicate significant differences at $P < 0.05$

	Dry matter concentration (g kg^{-1}) 2011	2012
A_1	511.2 (± 4.6)[b]	476.2 (± 9.0)[c]
A_2	517.0 (± 5.1)[b]	528.8 (± 4.3)[b]
W	803.0 (± 9.1)[a]	631.6 (± 13.5)[a]

Nassi o Di Nasso *et al.*, 2011). On the other hand, late autumn (A_2) and winter (W) harvests provide useful comparisons with literature data, as well as explaining variations in biomass yield and dry matter concentration with delayed harvests (Zub & Brancourt-Hulmel, 2010).

Miscanthus yields may vary considerably depending on the environmental conditions of the study site, the management practices and harvest time (Brosse *et al.*, 2012). Studies in Europe and United States and summarized by Heaton *et al.* (2010) showed how the harvestable biomass of miscanthus ranges between 5 and 55 Mg ha^{-1}. In southern Europe, under rainfed conditions, miscanthus aboveground dry yields are generally around 25–30 Mg ha^{-1} (Angelini *et al.*, 2009), while irrigated conditions led to yields of around 36 Mg ha^{-1} in Portugal (Clifton-Brown *et al.*, 2001), 34–38 Mg ha^{-1} in Italy (Ercoli *et al.*, 1999; Cosentino *et al.*, 2007) and 38–44 Mg ha^{-1} in Greece (Danalatos *et al.*, 1996, 2007).

The results obtained in our experiments confirmed the importance of water availability in determining satisfactory miscanthus yields in a Mediterranean environment. In fact, miscanthus plantations in soils characterized by a poor water holding capacity (i.e. SL soil) were severely affected after three growing years, with harvestable dry yields lower than 5 Mg ha^{-1}. This is consistent with our observations regarding LAI throughout 2012 when crops grown in SL soil ended as soon as mid–July, leading to premature senescence. In miscanthus, drought conditions may cause a decrease in LAI, even at higher latitudes (Clifton-Brown *et al.*, 2000), and a significant reduction in belowground biomass (Clifton-Brown & Lewandowski, 2000). Since aboveground resources are not mobilized until midsummer, stress conditions during growth considerably affect the remobilization of nutrients and carbohydrates from above- to belowground organs, in turn affecting
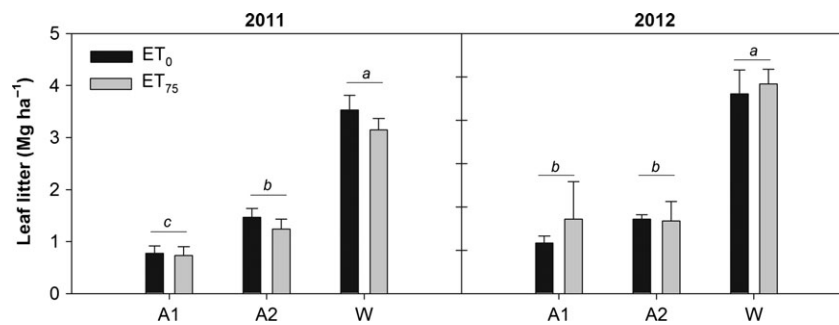
Fig. 6 Leaf litter during the second (2011) and third (2012) year of growth under contrasting irrigation regimes (Experiment 2). A_1, A_2 and W represent early autumn, late autumn and winter harvests, respectively. Different letters indicate significant differences at $P < 0.05$. Vertical bars represent the SE ($n = 3$).
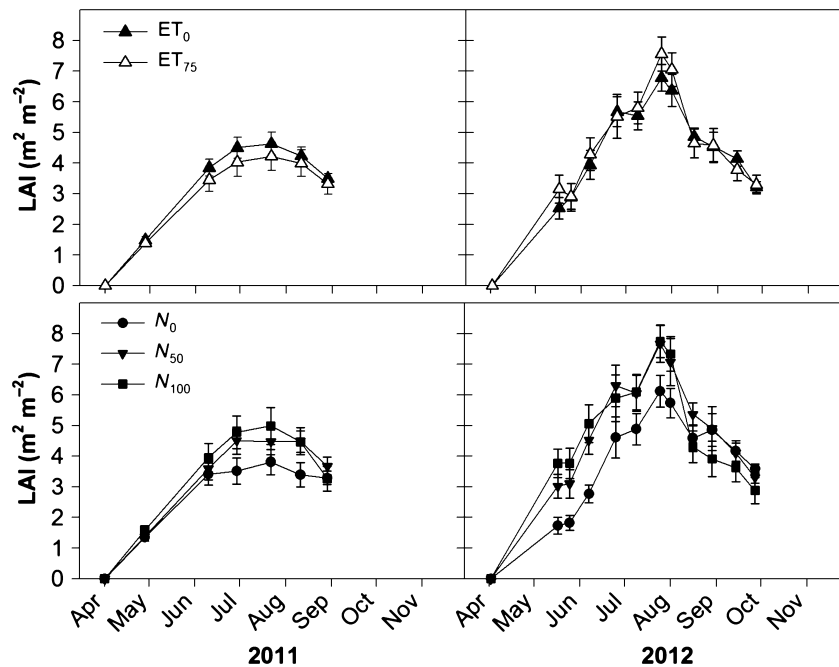

Fig. 7 Dynamics of the leaf area index (LAI) in miscanthus as function of the irrigation regime (upper panels) and the nitrogen fertilization rate (lower panels) for the second (2011) and third (2012) growing seasons. ET_0 and ET_{75} represent the two irrigation regimes, i.e. 0% and 75% of the potential evapotranspiration respectively. N_0, N_{50}, N_{100} represent 0, 50 and 100 kg N ha^{-1}, respectively. Vertical bars represent the standard error ($n = 3$).

biomass accumulation in the next years (Beale & Long, 1997; Himken *et al.*, 1997; Strullu *et al.*, 2011; Dohleman *et al.*, 2012).

The detrimental effects of drought conditions on miscanthus plants lacking a well established root system have been recognized (Mann *et al.*, 2013a). Mantineo *et al.* (2009) stated how irrigation in the first 3 years after the establishment affected miscanthus belowground growth and size, and the same authors found good aboveground yields during the fourth and fifth years (around 27 and 18 Mg ha^{-1}) when no irrigation was given. These findings are corroborated by Mann *et al.* (2013b) who investigated the root system dynamics

of miscanthus in response to rainfed and irrigated conditions, and highlighted no roots development below a depth of 1.2 m under rainfed conditions, while given supplemental irrigation during the establishment, miscanthus was able to develop roots 3 m down. Therefore, miscanthus growth patterns in sandy-loam soil (Experiment 1) highlighted the importance of supplying irrigation water also during the years following the establishment.

However, in soils characterized by a good water holding capacity (Experiment 2), revealed that irrigation water had no influence on crop productivity. Previous studies conducted in the Mediterranean (central and

southern Italy) comparing irrigated and rainfed miscanthus crops gave ambiguous results. In fact, in southern Italy, two- and three-year old crops responded to irrigation only when water supply exceeded 440 mm (Cosentino *et al.*, 2007) or when precipitation during the growing season was rather limited (around 400 mm) (Mantineo *et al.*, 2009). The importance of precipitation for miscanthus grown in Mediterranean was confirmed by Petrini *et al.* (1996) who compared rainfed and irrigated miscanthus in two different locations in central Italy. In 2-year old crops no differences in the aboveground yield were recorded at the site with a higher precipitation (>420 mm), while a 58% increase in aboveground dry yield was observed in irrigated miscanthus at the site with a lower precipitation (around 313 mm). Finally, in our experimental site, Ercoli *et al.* (1999), when comparing the effect of irrigation and nitrogen fertilization on miscanthus yield, observed an increase in about 20% (+4.5 Mg ha^{-1}) in irrigated vs. rainfed plots harvested in autumn. This is consistent with our results: when precipitation during the growing season was rather low (~164 mm) and similar to that reported by Ercoli *et al.* (1999) (~173 mm), plots receiving irrigation increased their dry yield by around 15% compared to rainfed plots. Conversely, in 2012 when precipitation was much greater (~400 mm) miscanthus under ET_0 and ET_{75} yielded nearly the same.

Overall, our study and the literature stress the importance of determining miscanthus yield responses to N fertilization rates on a site-by-site basis, as many factors can confound the fertilization effect (e.g. soil texture, soil organic matter and nutrient contents, mineralization rate, crop rooting depth, harvest time, crop age, etc.) (Zub & Brancourt-Hulmel, 2010; Anderson *et al.*, 2011; Cadoux *et al.*, 2012; Maughan *et al.*, 2012). Most of the trials have been conducted in continental climates. In these environments crop responses to modest N fertilization rates (40–100 kg N ha^{-1}) have been highlighted by Acaroğlu & Şemi Aksoy (2005) and Boehmel *et al.* (2008). Through the boundary line approach, Lewandowski & Schmidt (2006) reported increasing miscanthus yields up to 110 kg N ha^{-1}. Similarly, Schwarz *et al.* (1994) found that miscanthus yields increased significantly from 0 to 90 kg N ha^{-1}, and decreased with 120 and 180 kg N ha^{-1}. In a article by Arundale *et al.* (2013) who summarized multiyear trials in Illinois (USA) a positive, though small, yield response to N fertilization was highlighted with high rates (around 200 kg N ha^{-1}). Several studies have, however, found no influence of N fertilization on crop yield at harvest (Himken *et al.*, 1997; Heaton *et al.*, 2004; Christian *et al.*, 2008; Strullu *et al.*, 2011; Behnke *et al.*, 2012; Kering *et al.*, 2012; Maughan *et al.*, 2012; Larsen *et al.*, 2013).

Our results therefore confirmed a general trend reported in the literature. Although in the Mediterranean only a few trials have investigated the N fertilization effect on miscanthus yields, our results agree with those of Danalatos *et al.* (2007) and Mantineo *et al.* (2009) who found no yield increase in young miscanthus stands (1–5 years old). Conversely, positive responses to N fertilization were observed by Cosentino *et al.* (2007) and Ercoli *et al.* (1999), particularly when water was not limited. However, these results may differ from ours due to the lower N availability in the soil (Cosentino *et al.*, 2007) or to the higher fertilization rates (Ercoli *et al.*, 1999). On the other hand, our results corroborate the importance of N fertilization for LAI development, as observed by Wang *et al.* (2012), at least for the first few years after the establishment, and, in addition by the findings of Strullu *et al.* (2013) who reported how LAI expansion in miscanthus depended on the nitrogen accumulated in the aboveground biomass.

Our results also highlighted that, although N fertilization can lead to higher dry yield (i.e. two-year old crop in Experiment 2), whenever fertilizer application is not limited a significant reduction in the agronomic efficiency of the fertilization (A_E) is likely to occur. These findings are consistent with other studies that found a positive effect of N fertilization on miscanthus yields. For instance, when calculating the A_E from data reported by Arundale *et al.* (2013) values are around 45 kg kg$_N$$^{-1}$ when 67 kg N ha^{-1} are applied, while they drop to 30 kg kg$_N$$^{-1}$ when 134 and 202 kg N ha^{-1} are distributed. In addition, the observed differences in terms of the A_E between the 2 years of investigation (Experiment 2) confirmed how miscanthus yield responses to N fertilization may be affected by the amount and the distribution of precipitation during the growing season.

The aboveground biomass yield of miscanthus normally peaks between August and October, and then decreases to lower values in winter (Heaton *et al.*, 2008; Miguez *et al.*, 2008; Zub & Brancourt-Hulmel, 2010). This reduction is mainly due to the translocation of assimilates, leaf detachment and shoot-tip fall (Beale & Long, 1997; Kahle *et al.*, 2001; Strullu *et al.*, 2011). Overall, in our study the decrease in aboveground biomass due to delayed harvest (from A_1 to W) stood around 30–40%. This basically concurs with the range reported in the literature. In central and northern European conditions, a 25–35% yield reduction has been generally observed (Schwarz *et al.*, 1994; Himken *et al.*, 1997; Clifton-Brown *et al.*, 2000; Kahle *et al.*, 2001; Strullu *et al.*, 2011). However, it appears that the largest proportional decrease in dry yield from early to late harvest takes place at lower latitudes which have a greater yield potential and a higher amount of leaves at early harvest

(Miguez *et al.*, 2008). For instance Lewandowski *et al.* (2003b) observed miscanthus yield losses up to 35% in Portugal compared to 24% and 27% in Germany and England, respectively. Our results support this hypothesis.

When accounting for yield losses in delayed harvests, the number of days between autumn and winter harvests is also fundamental (Lewandowski *et al.*, 2003b). Our results highlighted a steady decline in harvestable biomass of 0.27–0.31% per day, which is consistent with data reported by Lewandowski *et al.* (2003b) and Clifton-Brown *et al.* (2004, 2007), i.e. 0.30–0.39% per day. The recorded decline corresponds to a yield decrease of around 0.07 Mg ha^{-1} per day of delay, which is the same figure as reported by Heaton *et al.* (2004) for miscanthus.

Our results confirmed an increase in dry matter content due to delayed harvests (Lewandowski & Kicherer, 1997; Lewandowski *et al.*, 2003b; Smith & Slater, 2011). However, if we assume a dry matter threshold for combustion equal to 25–30% (Lewandowski *et al.*, 2003a) only a winter harvest would be suitable, unless the biomass is left to dry or forcibly dried. For other uses (i.e. bioethanol) early harvests may also be appropriate, although this negates carbon cycling to soil through litterfall (around 3–5 Mg ha^{-1}) (Kahle *et al.*, 2001; Amougou *et al.*, 2012) and may negatively affect the storage of belowground resources (Strullu *et al.*, 2011).

In conclusion, in the Mediterranean, (i) soil and site characteristics, such as soil texture, soil hydraulic properties, presence and depth of the water table, appear to be the main drivers affecting miscanthus yield and management; (ii) the choice of N fertilization should be driven by economic and environmental factors, through the evaluation of nutrient removal with the harvest; (iii) the harvest window of miscanthus is wide and despite the optimal harvest time being influenced by the biomass end use, other long-term considerations should be taken into account, such as the yield stability and the maintenance of soil fertility.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Results of the ANOVA in Experiment 1 for the aboveground dry yield in 2011 and 2012 (i.e. second and third growing seasons respectively).

Table S2. Results of the ANOVA in Experiment 2 for the aboveground dry yield in 2011 and 2012 (i.e. second and third growing years respectively).

Table S3. Results of the ANOVA in Experiment 2 for maxi-mum LAI in 2011 and 2012 (i.e. second and third growing seasons, respectively). Maximum LAI was achieved on 22 July 2011 and 25 July 2012.