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Seasonal nutrient dynamics and biomass quality of giant reed (*Arundo donax* L.) and miscanthus (*Miscanthus* x *giganteus* Greef et Deuter) as energy crops

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Abstract

The importance of energy crops in displacing fossil fuels within the energy sector in Europe is growing. Among energy crops, the use of perennial rhizomatous grasses (PRGs) seems promising owing to their high productivity and their nutrient recycling that occurs during senescence. In particular, nutrient requirements and biomass quality have a fundamental relevance to biomass systems efficiency. The objective of our study was to compare giant reed (*Arundo donax* L.) and miscanthus (*Miscanthus* × *giganteus* Greef et Deuter) in terms of nutrient requirements and cellulose, hemicellulose and lignin content. Hence, the aim was to identify, in the Mediterranean environment, the optimal harvest time that may combine, besides a high biomass yield, high nutrient use efficiency and a good biomass quality for second generation biofuel production. The research was carried out in 2009, in San Piero a Grado, Pisa (Central Italy; latitude 43°41' N, longitude 10°21' E), on seven-year-old crops in a loam soil characterised by good water availability. Maximum above-ground nutrients content were generally found in summer. Subsequently, a decrease was recorded; this suggested a nutrient remobilisation from above-ground biomass to rhizomes. In addition, miscanthus showed the highest N, P, and K use efficiency, probably related to its higher yield and its C4 pathway. Regarding biomass quality, stable values of cellulose (38%), hemicellulose (25%) and lignin (8%) were reported from July onwards in both crops. Hence, these components appear not to be discriminative parameters in the choice of the harvest time in the Mediterranean environment. In conclusion, our results highlighted that, in our environment, a broad harvest period (from late autumn to winter) seems suitable for these PRGs. However, further research is required to evaluate the role of rhizomes in nutrient storage and supply during the growing season, as well as ecological and productive performances in marginal lands, in particular where water availability may be a limiting factor.

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Key words: giant reed, miscanthus, PRGs, nutrient concentration, nutrient content, nutrient use efficiency, second generation biofuel.

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Introduction

The increasing petroleum price and negative impact of fossil fuels on the environment are encouraging the use of lignocellulosic materials to help meet energy needs (Lemus *et al*., 2009; Amougou *et al*., 2010). Among energy crops, the use of perennial rhizomatous grasses (PRGs), such as miscanthus (*Miscanthus* × *giganteus* Greef et Deuter) and giant reed (*Arundo donax* L.), seems promising owing to their high productivity and to the nutrient recycling that occurs during growth and senescence (Angelini *et al*., 2009; Heaton *et al*. 2009; Zub *et al*., 2009; Smith and Slater, 2010). In addition, these lignocellulosic crops are thought to have positive effects on soil properties, biodiversity, energy balance, greenhouse gas mitigation and carbon footprint, especially when their cultivation is compared with arable crops (Rowe *et al*., 2009). Although several studies have been conducted on mineral fertilisation responses of these two crops (Angelini *et al*, 2005; Cosentino *et al*., 2007b; Smith and Slater, 2010; Cadoux *et al*., 2011), little information is available about differences in nutrient dynamics of miscanthus and giant reed. Smith and Slater (2011) have demonstrated that, in mature miscanthus crops, harvest time critically influences nitrogen dynamics. Moreover, a delayed harvest can reduce nitrogen concentration in biomass feedstock, dropping nitrogen fertiliser requirements (Heaton *et al*., 2009). Nonetheless, biomass losses should also be taken into account in delayed harvests. In the literature, it is found that the highest nutrient content occurs in late summer. Thereafter, nutrients are effectively translocated to rhizomes and then remobilised during shoot elongation the following year (Beale and Long, 1997; Himken *et al*., 1997; Smith and Slater, 2011). Moreover, Himken *et al*. (1997) and Beale and Long (1997) highlighted that miscanthus low input requirements could be associated with its C4 pathway. On the other hand, little is known about giant reed nutrient dynamics under European conditions. Most of the researchers have concentrated on natural plant communities dominated by giant reed (Sharma *et al*., 1999). In the Mediterranean environment, preliminary studies were conducted in Central Italy, generally observing low nutrient requirements (Nassi o Di Nasso *et al*., 2009a).

Lignocellulosic biomass from miscanthus and giant reed can produce heat, electricity or transportation fuels using several conversion technologies. Currently, biomass-based transportation fuels are identified as first and second generation biofuels (Sassne *et al*., 2008). In particular, second generation biofuels can be produced from a variety of lignocellulosic feedstocks (Cherubini and Jungmeier, 2010), such as crop residues and energy crops. Cellulose, hemicellulose and lignin are the three major components of lignocellulosic raw materials; these are closely associated in a complex crystalline structure. Following some processing steps (pretreatment, hydrolysis and fermentation), cellulose and hemicellulose are transformed into bioethanol, while lignin remains largely unconverted. Lignin, though, can be reused within the bioethanol production system as an energy source or can be used for synthesising high-value chemicals (Keshwani and Cheng, 2009). For this reason, the study of lignocellulosic component dynamics and their variation among different species is important in improving knowledge on overall bioenergy chain performances.

Our study used an experimental framework of miscanthus (*M.* × *giganteus*) and giant reed (*A. donax*) field trials carried out in Pisa (Central Italy). The following research questions were addressed:

1. Does the N, P and K concentration in the above-ground biomass differ between miscanthus and giant reed during the growing season?
2. How much N, P and K are removed by miscanthus and giant reed crops harvested at different times?
3. How much does biomass quality (referring to cellulose, hemicellulose and lignin percentage) differ between miscanthus and giant reed during the cropping cycle?

Gathered knowledge could be useful in identifying, in the Mediterranean area, optimal harvest times that may combine elevated biomass yield, high nutrient use efficiency and good biomass quality for second generation biofuel production.

Materials and Methods

A field trial was established in 2003 at the Enrico Avanzi Interdepartmental Centre for Agro-Environmental Research (CIRAA) of the University of Pisa, comparing miscanthus (*M.* × *giganteus*) and giant reed (*A. donax*). The soil was a typical Xerofluvent, representative of the lower Arno river plain (sand 41%, silt 38.5%, clay 20.5%, organic matter 2%, total nitrogen 1.1 g/kg, assimilable phosphorus 6.2 mg/kg, exchangeable potassium 138.8 mg/kg), characterised by a shallow water table. The experimental design was a randomised block with three replications (plots 7 m × 7 m each). Tillage was conducted in autumn of 2002 after wheat harvesting, and consisted of medium-depth ploughing (30-40 cm). Seedbed preparation was conducted in spring, immediately before planting. For both crops, establishment was performed using rhizomes of about 500 g, with at least a couple of buds. Rhizomes were planted at 10-20 cm of soil depth, at 0.50 × 1 m spacing (20,000 plants/ha). Taking soil nutrient availability into account and following fertiliser doses reported by Beale and Long (1997), fertilisers were distributed at a rate of 100 kg N/ha (urea), 100 kg P_2O_5/ha (triple superphosphate) and 100 kg K_2O/ha (potassium sulphate). Nitrogen fertiliser was broadcasted in the establishment year: 50% as preplant and 50% as side dressing when plants were 0.30-0.40 m tall. In the following years, P_2O_5 and K_2O fertilisers were applied during winter, while N was applied entirely in spring at the beginning of the growing season. Plots were kept weed-free by hoeing. No crop diseases were detected during the experimental period and irrigation treatment was never necessary.

The experiment began when the crop stands were seven years old, an age when they are generally considered mature (Christian *et al*., 2008; Angelini *et al*., 2009). Total above-ground biomass was measured at ten dates spread across the annual crop production cycle during 2009. At each sampling date, an area of 1 m^2 from each plot was collected and fresh-weighed. Subsamples were dried to constant mass at 60°C. Hence, crop dry matter percentage was calculated. Afterwards, each dried sample was milled to powder in a Retsch SM1 rotor mill (particle size <297 mm) for subsequent chemical analysis. Nitrogen concentration was determined by the Kjeldahl method, while P and K concentrations were determined by spectrophotometric analysis and flame photometry, respectively. Nutrient content was calculated as the product of nutrient concentration and dry yield. Nutrient use efficiency (NUE) was expressed as the ratio between dry matter production and nutrient content (g/g), according to Beale and Long (1997). Therefore, NUE indicates the total biomass produced per unit of nutrient absorbed (Cosentino *et al*., 2007a). From July to January, cellulose, hemicellulose and lignin contents of miscanthus and giant reed were determined using the Van Soest method (1991). Data were analysed by one-way ANOVA in order to evaluate differences, at each sampling date, between the two species during the growing season. A post-hoc test was performed to compare results from each sampling date, using the LSD test (α=0.05). An arcsine transformation was applied to all data expressed as a percentage before performing ANOVA (Gomez and Gomez, 1984).

Results and Discussion

Seasonal monthly mean air temperature and rainfall are shown in Figure 1. Air temperature increased from March to August with maximum values (>30°C) in July and August. Although the total amount of rainfall was similar to the long-term average (933 *vs* 902 mm, respectively), its distribution was quite different. Compared to the long term, higher rainfall was recorded during 2009 in early spring, September and winter (+60%). On the other hand, a very dry period was recorded during late spring and summer (Figure 1).

Figure 2A reports the above-ground biomass accumulation of giant reed and miscanthus. After the end of June, differences between the two species became significant. Miscanthus maximum above-ground dry yield was attained at the end of August (39 t/ha) and declined progressively to 28 t/ha (-30%), as a consequence of leaf loss (data not shown). Giant reed maximum above-ground dry yield was achieved at the end of September, with a value of 32 t/ha, which remained stable until February. Dry matter percentage increased almost linearly over the course of the experiment (Figure 2B). Maximum values (about 60%) were achieved in February, when no significant differences

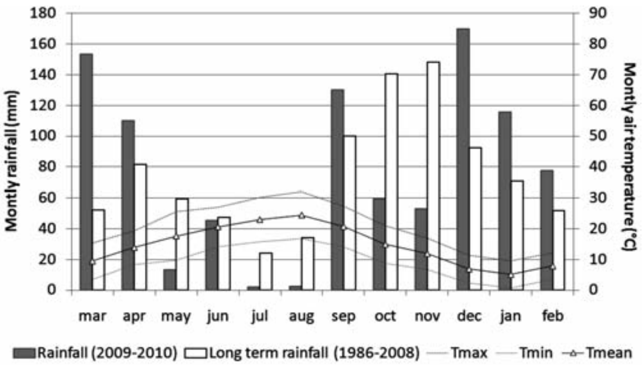

Figure 1. Meteorological data during the 2009 growing season in comparison with the long-term average (1986-2008) at the field experimental site (Pisa, Italy, 43°40' N, 10°19' E).

between the two species were recorded. A high dry matter percentage of the lignocellulosic material is considered advantageous in terms of reduced costs in drying plant material prior to combustion (Himken *et al*., 1997) and bioethanol production (Öhgren *et al*., 2006).

Macronutrient (N, P and K) concentration dynamics showed the same pattern in both species, with progressively decreasing values during the growing season (Figure 3A-C). This confirmed previous findings reported by several authors, for either miscanthus or giant reed cultivated under different management and environmental conditions (Beale and Long, 1997; Himken *et al*., 1997; Sharma *et al*., 1999; Christian *et al*., 2008; Heaton *et al*., 2009; Nassi o Di Nasso *et al*., 2009a). Indeed, nutrient concentrations in the above-ground material appear to become diluted as the above-ground dry matter increased, and then declined further as the canopy senesced (Beale and Long, 1997). In fact, throughout the growing season, nutrient concentration decline was most pronounced in the period of active growth (June-August), while from late summer to winter the change was negligible. Purely in terms of N, P and K concentrations, little benefits would thus be obtained by harvesting either in autumn or in winter. In general, nitrogen concentration declined steadily from a high of >1% in young shoot tissue in spring to <0.5% in late autumn, confirming results reported by Heaton *et al*. (2009). Significantly higher values of N were recorded in miscanthus from May to July. From summer onwards, no difference was found between crops. The overall N reduction throughout the growing season was -77% and -88% for giant reed and miscanthus, respectively (Figure 3A). Our miscanthus values were in agreement with those of Beale and Long (1997), Kahle *et al*. (2001) and Heaton *et al*. (2009), while they were lower than those reported by Himken *et al*. (1997). Phosphorus concentration was much lower than that for N and K. Giant reed and miscanthus showed significantly different values in early May and from July to September. Afterwards, crops did not differ in P, both ranging from 0.12% to 0.08% (Figure 3B). Potassium concentration was the highest among the three macronutrients. Potassium was the only mineral element that showed significantly different values between giant reed and miscant hus during the whole season, except for June and July (Figure 3C). In young shoots, K declined from 3.4% to 0.7% in giant reed and from 2.8% to 0.5% in miscanthus. For both species, P and K results confirmed those reported by some authors for miscanthus (Beal and Long, 1997; Himken *et al*., 1997; Kahle *et al*., 2001) and giant reed (Sharma *et al*., 1999; Nassi o Di Nasso *et al*., 2009a). In addition, when these two PRGs are compared with annual graminaceous crops (i.e. maize and wheat), a lower nutrient concentration is revealed (Beale and Long, 1997).

The above-ground nutrient content is defined as the product of dry matter and nutrient concentration. Results showed that P content was characterised by the lowest values, while K content was the highest (Figure 4). Overall, N, P and K contents presented a similar dynamic in both species. They reached maximum values in late July to early August and then they decreased until winter, confirming trends reported by Beale and Long (1997) and Heaton *et al*. (2009). Only P content in miscanthus obtained its maximum in October. The rapid increase in nutrient content of shoots between May and July suggests that giant reed and miscanthus are highly efficient in nutrient acquisition. A possible explanation could be related to their deep rooting system (Monti and

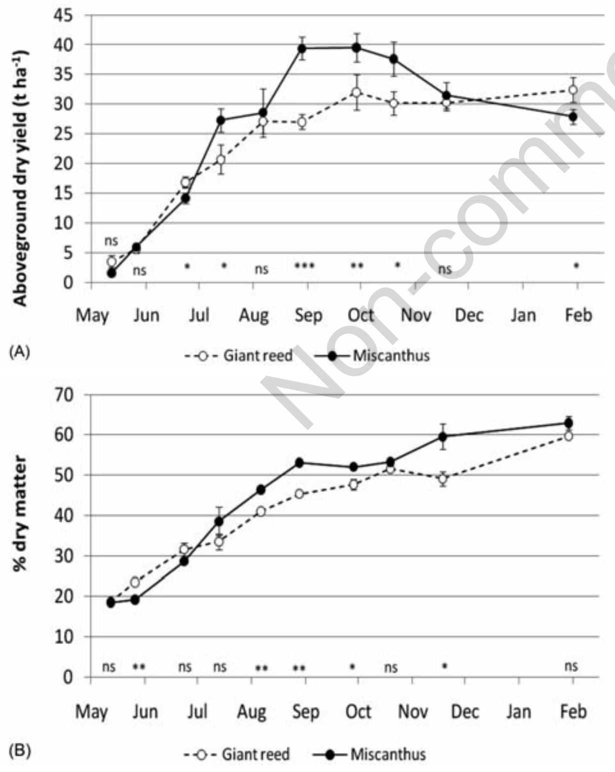

Figure 2. Seasonal variation (2009) in above-ground biomass accumulation (A) and dry matter percentage (B) of giant reed and miscanthus in Pisa, Italy (43°40' N, 10°19' E). ○, giant reed; ●, miscanthus; bars represent the standard deviation; *, **, *, significant differences at P<0.05, P<0.01, P<0.001, respectively; ns, no significant differences.**

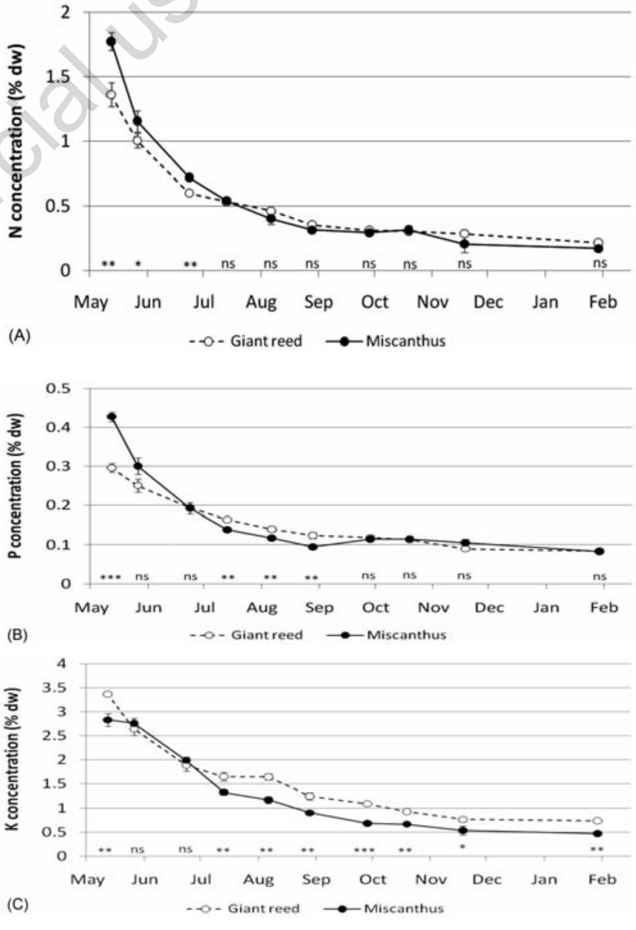

Figure 3. Seasonal variation (2009) in N (A), P (B) and K (C) concentrations of giant reed and miscanthus in Pisa, Italy (43°40' N, 10°19' E). ○, giant reed; ●, miscanthus; bars represent the standard deviation; *, **, *, significant differences at P<0.05, P<0.01, P<0.001, respectively; ns, no significant differences.**

Zatta, 2009), which remains dormant during winter but can respond quickly to a rapid increase in the plant's demand for nutrients at the start of growth in spring (Himken *et al*., 1997). Some authors have underlined that macronutrient content decline is consistent with nutrient cycling in perennial rhizomatous grasses and it represents a survival strategy for those species (Himken *et al*., 1997; Sharma *et al*., 1999; Heaton *et al*., 2009; Nassi o Di Nasso *et al*., 2009a). Furthermore, macronutrient translocation towards the rhizome may represent an environmental friendly strategy that could reduce fertiliser application (Rowe *et al*., 2009). Concerning N content, significant differences were recorded between the species, although no clear pattern could be identified. Nevertheless, miscanthus N content was significantly higher than that of giant reed in late autumn, while it was significantly lower in winter. Maximum above-ground content was reached in summer, with about 130 kg/ha in both crops, while in November values decreased to 86 and 64 kg/ha in giant reed and miscanthus, respectively. At the end of the growing season (February), N content dropped further to 69 and 53 kg/ha (Figure 4A). This agrees with the results of Heaton *et al*. (2009) for miscanthus. For giant reed, Nassi o Di Nasso *et al*. (2009a) reported lower values during the whole growing season, probably owing to the crop age (15 years old) and to lower yields (about 20 t/ha). Phosphorus above-ground content did not differ significantly between both species. Maximum P content was about 38 kg/ha and 45 kg/ha in giant reed and miscanthus, respectively. Afterwards, it decreased to about 25 kg/ha in both crops at the end of January (Figure 4B). In miscanthus, lower values were reported by Beale and Long (1997) and Himken *et al*. (1997) consistent with their lower dry yields. Significant differences were recorded between species in K content. In summer, K content reached its maximum value (440 and 360 kg/ha in giant reed and miscanthus, respectively) (Figure 4C). The decrement rate, until late November, was nearly the same in both crops (-30%), reaching values of about 280 kg/ha. Subsequently, K content maintained stable values, confirming results reported by Beale and Long (1997) for miscanthus crops. On the other hand, Himken *et al*. (1997) showed lower values in four-year-old miscanthus, probably owing to diverse environmental conditions and fertilisation level. Regarding giant reed, our values were higher than those observed by Nassi o Di Nasso *et al*. (2009a) with no fertilisation and dry yield of about 20 t/ha; hence, this information suggests a strong influence of crop yield and crop management on macronutrient content.

Finally, nutrient use efficiencies (NUEs) were taken into account in order to evaluate suitable harvest times that may combine high yields and low nutrient contents. For all three macronutrients and for both crops, NUEs increased progressively from October to January. Nitrogen NUE (N_{NUE}) achieved minimum values of 330 and 316 g/g in October and maximum values of 467 and 522 g/g in late January, for giant reed and miscanthus, respectively. Average N_{NUE} was significantly higher for miscanthus (442 *vs* 382 g/g). As suggested by Long (1983), this may be a result of the photosynthetic pathway of C4 crops (miscanthus) that is more efficient in its use of N than the C3 pathway (giant reed). However, a high N_{UE} does not necessarily mean that the whole production system is efficient; in fact, N_{UE} does not take into account losses from the system. In particular, N can easily be lost as gaseous NO_X, by

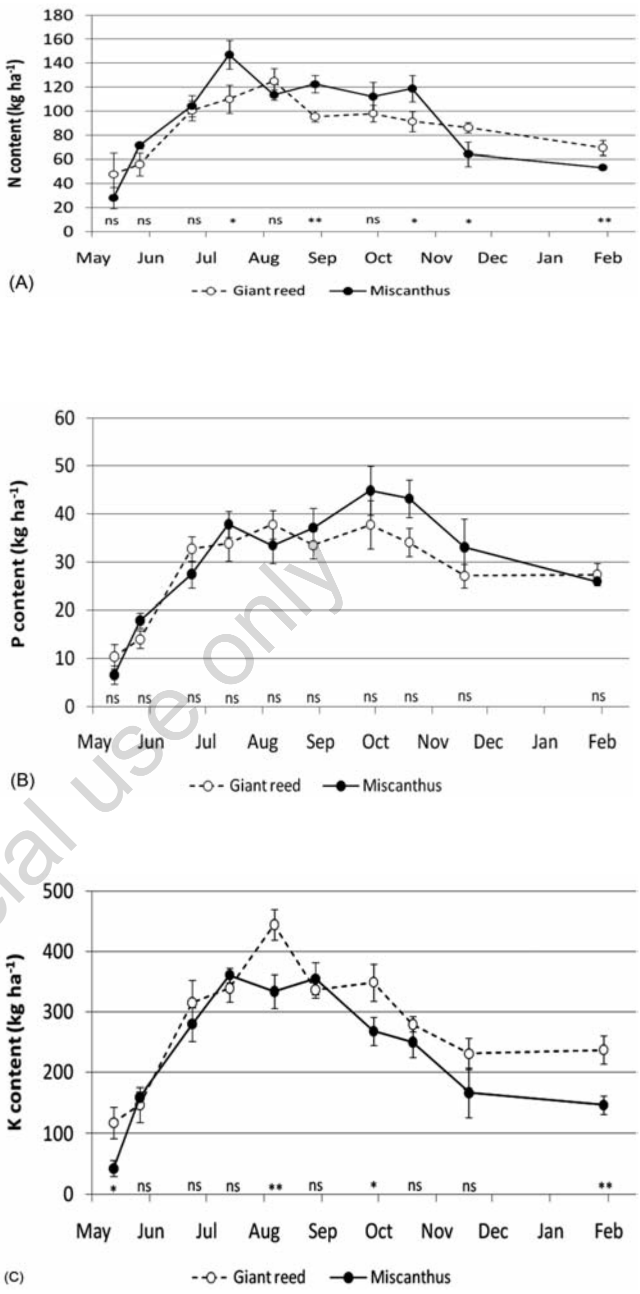

Figure 4. Seasonal variation (2009) in N (A), P (B) and K (C) contents of giant reed and miscanthus in Pisa, Italy (43°40' N, 10°19' E). ○, giant reed; ●, miscanthus; bars represent the standard deviation; *, **, *, significant differences at $P<0.05$, $P<0.01$, $P<0.001$, respectively; ns, no significant differences.**

Table 1. Nutrient use efficiencies of giant reed and miscanthus crops during 2009 growing season in Pisa (43°40' N, 10°19' E), Italy.

	N_{NUE}, g/g		P_{NUE}, g/g		K_{NUE}, g/g	
Date	Giant reed	Miscanthus	Giant reed	Miscanthus	Giant reed	Miscanthus
19 October	330 a	316 a	884 a	870 a	108 a	150 a
18 November	351 b	489 a	1115 a	952 a	131 b	188 a
23 January	467 b	522 a	1183 a	1073 a	136 b	189 a
Mean	*383 b*	*442 a*	*1061 a*	*965 a*	*125 b*	*176 a*

NUE values, followed by the same letter across species, are not significantly different (P=0.05).

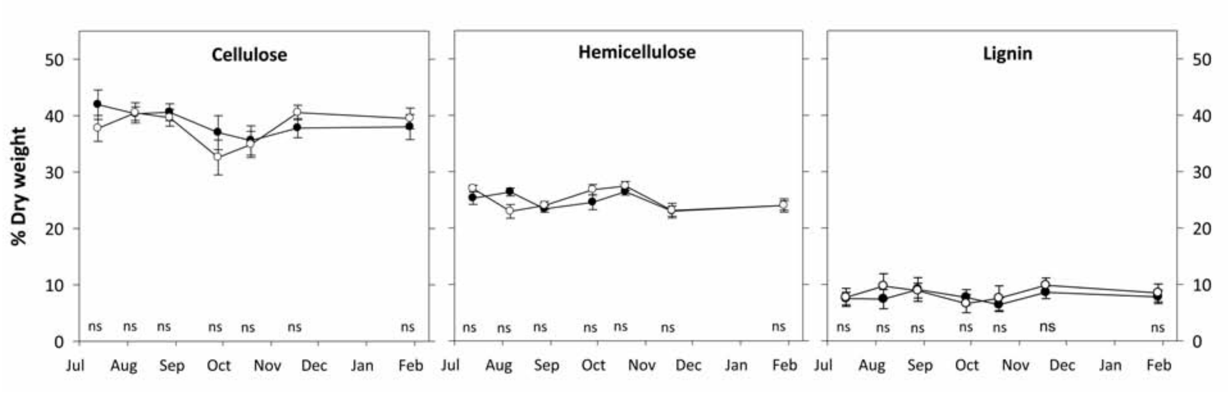

Figure 5. Seasonal variation (2009) in cellulose, hemicellulose and lignin content of giant reed and miscanthus in Pisa, Italy (43°40' N, 10°19' E). ○, giant reed; ●, miscanthus; bars represent the standard deviation; *, **, *, significant differences at P<0.05, P<0.01, P<0.001, respectively; ns, no significant differences.**

surface runoff or leached as nitrate (Jorgensen & Mortensen, 1997). Mean P_{NUE} values (October-January) were slightly higher in giant reed, showing averages of 1062 g/g, while miscanthus P_{NUE} was around 965 g/g, with no significant differences between species. On the other hand, miscanthus showed significantly higher mean P_{NUE} values (176 g/g) compared to giant reed (125 g/g). With regard to K_{NUE}, our results may have been affected by high soil K availability. In fact, as suggested by Cadoux *et al*. (2011), high soil K availability may have led to potassium luxury uptake by these two crops.

Tables 1 and 2 provide evidence that giant reed and miscanthus species show higher nutrient use efficiencies than arable crops (i.e. maize and wheat). Moreover, when compared with woody crops, PRGs confirm their high N and P NUEs. Above-ground cellulose, hemicellulose and lignin content in giant reed and miscanthus showed little variation during the growing season; average values were about 38%, 25% and 8%, respectively. No significant differences between crops were recorded (Figure 5). For comparison, cellulose, hemicellulose, and lignin content in common agricultural residues are listed in Table 3. For switchgrass, Keshwani and Cheng (2009) have reported lower cellulose content (33%), slightly higher hemicellulose content (26%) and more than twice lignin content (18%). For cellulose and hemicellulose, our giant reed results were higher than those reported by Shatalov and Pereira (2002) and Cosentino *et al*. (2007b), and in agreement with those of Neto *et al*. (1997). Regarding miscanthus, Cosentino *et al*. (2007b) have observed higher values of cellulose and similar values of hemicellulose. In addition, Scordia *et al*. (2009) reported similar cellulose content while hemicellulose and lignin contents were higher. For lignin, an increasing trend was observed from October to November in giant reed and from September to November in miscanthus (Figure 5). This behaviour should probably be linked to a progressive loss of leaves, as leaves are characterised by a lower lignin content than stems (Cosentino *et al*., 2007b; Nassi o Di Nasso *et al*., 2009b). The overall observed differences could be related to genetic variability (e.g. local ecotype used), as well as crop management and crop age, which seem to affect biomass quality (Nassi o Di Nasso *et al*., 2010). Moreover, the dissimilarity revealed in cellulose, hemicellulose and lignin contents could be because of the adoption of different methods for characterising the biomass. For this reason, we believe there is a need to define international methods that would facilitate comparisons among the available data (Nassi o Di Nasso *et al.*, 2010).

Table 2. Nutrient use efficiencies of some woody and arable crops.

	N_{NUE}, g/g	P_{NUE}, g/g	K_{NUE}, g/g	Source
Poplar	145-370	1000-2000	256-370	Jug *et al.*, 1999
Willow	152-244	909-1429	323-500	Jug *et al.*., 1999
Eucalyptus	219	3477	427	Lodhiyal and Lodhiyal, 1997
Maize	66-111	333-556	86-161	Beale and Long, 1997
Wheat	83-87	-	117-133	Jorgensen, 2000

Table 3. Cellulose, hemicellulose and lignin contents in common agricultural residues.

Lignocellulosic materials	Cellulose, %	Hemicellulose, %	Lignin, %
Hardwoods stems	40-55	24-40	18-25
Softwood stems	45-50	25-35	25-35
Corn cobs	45	35	15
Wheat straw	30	50	15
Grasses	25-40	35-50	10-30
Leaves	15-20	80-85	0

Source: Sun and Cheng, 2002.

Conclusions

Firstly, both species showed a decline in N, P and K concentrations throughout the season. Between the species no significant differences were recorded for N and P, while giant reed was characterised by significantly higher K concentration than miscanthus from July onwards.

Secondly, N, P and K nutrient contents generally reached their maximum values in summer. Supposing an autumnal harvest, we could expect nutrient contents of about 90 kgN/ha, 30 kgP/ha and 260 kgK/ha for giant reed and about 90 kgN/ha, 40 kgP/ha and 210 kgK/ha for miscanthus. Besides, supposing a winter harvest, nutrient contents could be reduced to 70 kgN/ha, 30 kgP/ha and 240 kgK/ha for giant reed and about 50 kgN/ha, 20 kgP/ha and 130 kgK/ha for miscanthus. In addition, although both crops show evident nutrient cycling, miscanthus appears to be able to recycle macronutrients to a higher extent than does giant reed. The miscanthus C4 pathway could explain its higher ability in reducing macronutrient uptakes compared to giant reed. Furthermore, both PRGs seem able to achieve higher dry matter production per unit of nutrient uptake (i.e. higher NUE) than arable crops.

Thirdly, from October to January, our results reported little variation of cellulose, hemicellulose and lignin percentage with no significant differences between the two species (38% cellulose, 25% hemicellulose and 8% lignin). Therefore, in our environment, cellulose, hemicellulose and lignin contents appear not to be discriminative parameters in the choice of the harvest time.

In conclusion, our results highlighted that, under the environmental conditions of the Mediterranean area, a broad harvest period is possible (from autumn to winter). Nonetheless, the choice of harvest time cannot detract from environmental, economical and logistic considerations that can strongly affect the whole bioenergy chain sustainability.

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