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Agronomic and environmental evaluation of perennial herbaceous plants fertilized with slurry to obtain biomass for bioenergy

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Giulia Florio, January 31st 2014

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Riassunto

La gestione delle acque reflue da allevamenti zootecnici è uno dei temi principali del sistema agricolo ed ambientale, in particolare nei paesi europei, soggetti alle forti restrizioni date dalla Direttiva Nitrati. Quest'ultima si propone di proteggere la qualità delle acque impedendone la contaminazione di nitrati da risorse agricole. Purtroppo, però, il contesto zootecnico produce una vasta quantità di acque reflue, che non sempre è facile gestire.

Allo stesso tempo la Commissione Europea, attraverso la Direttiva sulle Energie Rinnovabili, ha fissato alcuni importanti obbiettivi nel quadro bioenergetico europeo. I 27 stati membri, infatti, devo raggiungere il 20% del loro consume finale di energia ed il 10% dell'energia usata nel settore dei trasporti da fonti rinnovabili entro il 2020. Recentemente il settore di ricerca sulle bioenergie ha messo in risalto l'importanza della sostenibilità ed in questo caso le biomasse lignocellulosiche possono giocare il loro ruolo, in quanto non competono con la produzione di cibo e fibre. Molti studi sottolineano le potenzialità di questi carburanti di seconda generazione nell'essere considerati bioenergie sostenibili. A differenza dei biocarburanti di prima generazione, quelli di seconda potrebbero essere ottenuti in aree marginali, evitando la competizione di terreno. Inoltre alcune piante potrebbero essere cultivate con ridotti input ed irrigate con acque di scarsa qualità. Per questa ragione lo scopo di questo lavoro è stato quello di interrogarsi su nuove potenziali specie erbacee perenni in grado di dare elevati produzioni di biomassa adatta alla bioenergia, al contempo idonee a crescere irrigate con liquami zootecnici.

Le seguenti specie sono state studiate: *Artium lappa* L., *Arundo donax* L., *Canna indica* L., *Carex acutiformis* L., *Carex pseudocyperus* L., *Carex riparia* Curtis, *Glyceria maxima* (Hartman) Holmb., *Helianthus tuberosus* L., *Iris pseudocorus* L., *Lythrum salicaria* L., *Mischantus x giganteus* Greef et Deu., *Phalaris arundinacea* L. var. *picta*, *Scirpus sylvaticus* L. e *Symphytum x uplandicum* Nyman. Sono state coltivate in cassoni ed irrigate con liquame simulato. La ricerca si è focalizzata su consumo idrico, resa e costituzione della biomassa, asportazioni di azoto e fosforo, resa e potenziale energetico in etanolo e metano ed, infine, qualità delle acque di percolazione.

Complessivamente *A. donax* ha dato le rese in biomassa più elevate, incrementandole annualmente (26.2, 62.8, 95.1 e 140.1 t/ha, dal 2010 al 2013 rispettivamente) ed è risultata statisticamente diversa da tutte le altre specie, a parte nel 2011, quando non erano presenti differenze significative tra essa e *M. x giganteus* (55.2 t/ha). *A. donax* ha riportato anche il miglior input energetico. Per quanto concerne l'aspetto ambientale, è sempre questa specie che ha dato le più alte asportazioni, superando la quantità di azoto immessa con la fertilizzazione. Infine, confrontando le acque di percolazione ad inizio e fine prova, i contenuti mediani di azoto totale e nitrico risultano più bassi a fine sperimentazione per tutte le specie vegetali.

Summary

Animal wastewater management is becoming one of the central topics in agronomic and environmental systems, especially in the European countries that are subject to the severe restrictions of the Nitrates Directive. This Directive aims to protect water quality by preventing the loss of nitrates from agricultural sources, but livestock farms produce vast quantities of wastewater that are not easy to handle.

At the same time the European Commission, with the Renewables Directive, set an important goal for European bioenergy: The EU 27 has to meet 20% of its gross final energy consumption and 10% of the energy used in the transport sector from renewable sources by 2020. Bioenergy research has recently stressed the importance of sustainability and in this case ligno-cellulosic biomasse can play a role because it doesn’t compete with food and fibre production. Many studies underline the potential of second-generation biofuels as sustainable bioenergy. Unlike first-generation biofuels, the second-generation ones might be obtained in marginal areas, avoiding the land competition for food and fibre. In addition some plants could be cultivated with reduced inputs and irrigated with poor quality water.

The aim of this work was therefore to find new perennial herbaceous plants able to give high biomass productivity suitable for bioenergy and to grow under irrigation with livestock wastewater.

The following species were studied: *Artium lappa* L., *Arundo donax* L., *Canna indica* L., *Carex acutiformis* L., *Carex pseudocyperus* L., *Carex riparia* Curtis, *Glyceria maxima* (Hartman) Holmb., *Helianthus tuberosus* L., *Iris pseudocorus* L., *Lythrum salicaria* L., *Mischantus x giganteus* Greef et Deu., *Phalaris arundinacea* L. var. *picta*, *Scirpus sylvaticus* L. and *Symphytum x uplandicum* Nyman. They were cultivated in growth boxes and irrigated with simulated slurry. The research focussed on their water consumption, biomass production, nitrogen and phosphorus content, different constituents of fibres (hemicellulose, cellulose, lignin), ethanol and methane yield and energy output and the quality of percolation water.

Overall *A. donax* gave the highest biomass yields, increasing yearly (26.2, 62.8, 95.1 and 140.1 t/ha, from 2010 to 2013 respectively) and was significantly different from all the

other species, apart from in 2011, when it was not significantly different from *M. x giganteus* (55.2 t/ha). *A. donax* also supplied the best energy output (624 GJ/ha). Regarding the environmental aspect, *A. donax* again showed the highest nitrogen uptake, exceeding the input of 400 kg/ha. Finally, comparing the initial and final percolation water during the experiment, lower total nitrogen and nitrate nitrogen median contents were found and variability among species decreased over the years.

1 General background and objectives of the thesis

Introduction

Animal wastewater management is one of the central topics in agronomic and environmental systems, especially in European countries (Martinez et al., 2009). On the traditional farm manure was considered an essential and cheap source of fertilizer but nowadays, with the evolution in stockbreeding, livestock produce a huge quantity of wastewater that is difficult to handle. Nitrogen and phosphorus are two nutrients with the greatest potential to create water pollution (EEC, 1991) and, at saturation, they are lost to either surface or ground waters (Martinez et al., 2009). The negative effects on both soil and water of excess spreading on arable land are well-known (Smith et al., 2000, Martinez et al., 2009). Thus, even if nitrogen is an important and vital nutrient that helps plants and crops to grow, high concentrations are harmful to people and nature. Generally, farming remains responsible for over 50% of the total nitrogen discharge into surface waters (EC, 2010).

For this reason the European Commission (EC) imposed severe restrictions on its use through the Nitrate Directive (91/676/EEC), in order to protect water quality across Europe and reduce water pollution caused or induced by nitrates from agricultural sources by promoting the use of good farming practices. The EC imposes the designation as Nitrate Vulnerable Zones (NVZs): “areas of land which drain into polluted waters or waters at risk of pollution and which contribute to nitrate pollution” (EEC, 1991). The Directive allows a maximum of 170 kg per hectare per year from animal wastes on these areas. In 2011 the EC granted Regions of the Padana Plain (Veneto, Lombardy, Piedmont and Emilia Romagna) a derogation (No. L 287/36) raising this limit to 250 kg during the 2012-2015 period for crops with high nitrogen demand and long growing season (Official Journal of the European Union, 2011). This area of Italy is characterized by a very high nitrogen input farming system and accounts for 7 million livestock units. Furthermore it has one of the largest aquifers in Europe and 67% of the utilised agricultural area (UAA) is defined as NVZs (Perego et al., 2012). In Veneto Region, where this thesis is inserted, NVZs cover about 87% of the UAA (ISTAT, 2011).

Two reports from the Commission to the Council and European Parliament, COM(2010)47 and COM(2013) 683, indicate good progress towards cleaner water overall from 2004 to 2011, but further improvements have to be made (EC, 2010 and 2013).

Consequently creating new or additional treatments is necessary to observe this European Directive (Henkens and van Keulen, 2001, Harrington and Scholz, 2010). There are currently several methods to abate excess nitrogen to comply with the Nitrate Directive, among these the most used are: mechanical separation of liquid and solid manure, aeration of slurry and biogas production, which is expanding in several countries (Burton and Turner, 2003; Anon., 2010; Petersen et al., 2013).

In this thesis the term slurry is taken as a wastewater with its negative consequences but with the possibility to be exploited as a fertilizer. For this reason the research carried out for this PhD thesis deals with the production of biomass fertilized with wastewater from livestock to produce bioenergy.

Renewable energy

The increasingly important role of bioenergy is underlined in many studies (Nijsen et al., 2012, Dornburg et al., 2010, Van Vuuren et al., 2007) and in numerous climate change mitigation policies the importance is stressed of replacing fossil fuels with renewable energy sources (RES) (Dandres et al., 2012).

On a world-wide scale in 2011, the latest year for which data are available, RES provided 19% of total energy, in comparison with only 7% in 2004, of which 9.7% came from modern renewable sources (REN21, 2013), such as solar, wind and geothermal energy, biomass and biofuels (Figure 1).

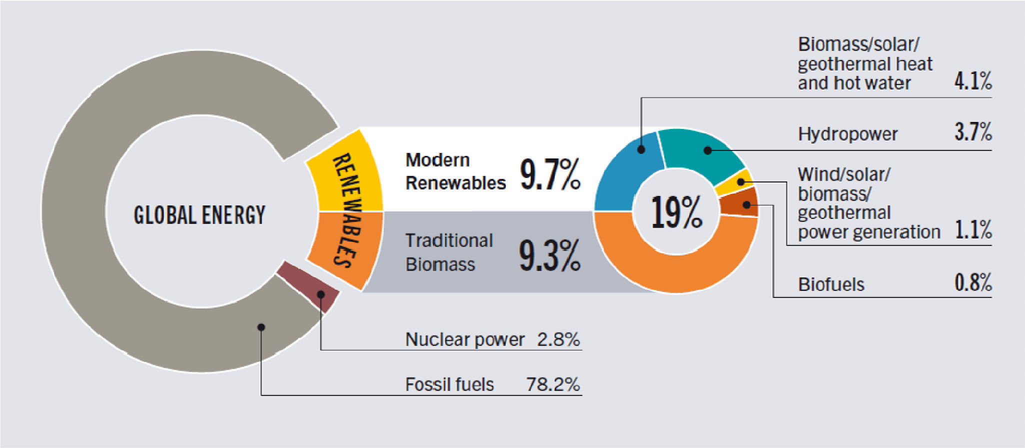

Figure 1 Estimated RES share of global final energy consumption in 2011 (Ren, 2013).

In Europe the climate and energy package is a set of binding legislation which aims to ensure that the European Union (EU) meets ambitious climate and energy targets for 2020 (EC, 2009a and b). These targets, known as the "20-20-20" targets, set three key objectives for 2020:

- A 20% reduction in EU Greenhouse Gases (GHG) emissions from 1990 levels;
- Raising the share of EU energy consumption produced from RES to 20%;
- A 20% improvement in the EU's energy efficiency.

Effectively, according to the Renewable Energy Directive (RED) (European Directive 2009/28/EC), the EU member states have to reach a 20% share of energy from renewable sources by 2020 (each member state has its own percentage) and 10% of renewable energy specifically in each EU member state transport sector. The EC also established a long-term target to cut GHG in the Strategy Plan 2020.

The substitution of fossil fuels by RES is thus a key issue and many studies are focused on the benefits they can provide to the environment (Hill et al., 2006, Ragauskas et al., 2006), particularly on the GHG reduction (Koonin, 2006; Dhillon and von Wuehlisch, 2013).

The EU targets rely on the major use of biomass in all energy sectors and it is expected to account for 56% of the RES supply by 2020 (Beurskens et al., 2011; Bentsen and Felby, 2012).

In the EU, as a result of all these policies and measures, the RES share increased from 8.5 to 12.7% in 2005-2010 (EC, 2013).

Italy, according to RED, which was adopted by Legislative Decree No 28/2011, has to achieve 17% energy from RES by 2020. In 2010 it reached 10.4%, overstepping its first interim target of 7.6% (EC, 2013).

RES production in Italy has been continuously growing recently. In effect the gross internal consumption of renewable energy has increased between 2000 and 2010 from 423 PJ (5.8% of gross energy consumption) to 755 PJ (10.3%). In 2010 the dominant source of RES was biomass (43.2%), followed by geothermal (26.4%), hydro (24.4%), wind (4.4%) and solar (1.7%) (Scarlat et al., 2013).

Renewable energy and biomass

RES biomass has a wide range of different sources, such as forest biomass (woody species in short rotation forestry such as poplar, willows, eucalyptus and robinia), agricultural residues, post processed biomass wastes (i.e. sewage sludge, municipal solid waste, manure) and energy crops of annual or multiannual species (Bentsen and Felby, 2012; Elbersen et al., 2012).

The International Energy Agency (IEA) (2006) reported that energy from biomass is mainly derived from cultivated crops, which nowadays represent the most common energy agro-system worldwide. 3.2% of total cropping area in the EU (about 5.5 Mha) presently grows energy crops. Most of this land is cultivated for biofuel production, covering 82% of energy crops; the remainder is used for the production of first generation (1st gen.) bio-ethanol crops (10%), biogas (7%), and perennial species go mostly into electricity and heat generation (1%) (Dworak et al., 2009; Elbersen et al., 2012).

In general energy crops are crops grown specifically for energy, in terms of biofuels or electricity and heat by combustion. They are based on intensive agricultural systems, characterized by high density plants and mechanization, high energy inputs, short rotation (1-4 years) and plant cycles usually less than 20 years (Fiorese and Guariso, 2010; Fazio and Monti, 2011; Wichtman and Wichtman, 2011).

Currently they mainly include traditional food crops such as rapeseed, sugarbeet, sorghum, wheat, sunflower and silage maize (Krasuska et al., 2010).

According to the first Italian progress report on Directive 2009/28/EC (EC, 2013), the domestic biomass supply for energy purposes was estimated at about 19 million tonnes in 2010. The main source of biomass was wood, which derived from direct (forests and other wooded land) and indirect supply (residues and co-products from wood and paper industry), accounting for about 10.8 million tonnes. Agricultural biomass, including crops, by-products and residues, amounted to about 3.0 million tonnes. Biomass was also taken from waste (municipal, industrial, etc.), which was quantified as about 5 million tonnes. In addition, in 2010 a significant amount of biomass (4.5 million tonnes) was imported as wood, wood pellets and residues (Scarlat et al., 2013).

Bioenergy thus forms a crucial element of the agriculture and energy policy in many countries (Nijsen et al., 2012).

Furthermore the FAO (2008) stated that it is feared that the introduction of energy crops in a scenario of decreasing food stocks will compete for land with food crops, in turn leading to food price increases.

Consequently the rapid expansion of energy crops at large-scale and the socio-environmental cascade impacts recently led to the identification of some sustainability criteria for biomass production (Elbersen et al., 2005; Cramer et al., 2007; Wichtmann and Wichtmann, 2011). GHG balance, including the whole bioenergy production chain, must be positive and therefore fewer emissions must be produced than on average with fossil fuels (Searchinger et al., 2008). Biomass production must not directly or indirectly induce negative effects on biodiversity at any level (genes, species, and ecosystems), and possibly improve biodiversity conservation in the area (IUCN, 2006). Biomass production should economically sustain local development and social well-being of the population, by giving a positive contribution towards local prosperity (Cramer et al., 2007). Finally energy crops have to face little or no competition with food production and local biomass application. The entire biomass production cycle must maintain the quality of soil, surface and ground water and air, which implies minimizing fertilizer and pesticide use, and, at the same time, implementation of “best practices” in agricultural systems.

In addition, the European Commission recently published a proposal (COM(2012) 595) to limit to 5% the use of food-based biofuels to meet the 10% renewable energy target of the precedent Directive (EC, 2012).

For these reasons, even if dedicated crops for energy uses represent a means for reducing the dependence on fossil fuels, there is a need to adopt an integrated and multifunctional approach for biomass production, and ligno-cellulosic biomass may play a useful role in this context.

Non-food biomass

Nowadays sustainability of biomass is receiving great attention, so growing interest is being focused on the use of non-food biomass to produce biogas and biofuels, the latter are also known as second generation (2nd gen.) biofuels (Sims et al., 2010).

A lot is known on the production of 1st gen. liquid biofuels derived from agricultural produce, such as maize, sugarbeet, rapeseed and soybean, and therefore their potential to offset GHG and mitigate global warming and environmental pollution (Mabee, 2006). On the other hand, these crops are also fat and sugar sources so compete with food production and might be a cause of enhancing of price provisions (Tan et al., 2008).

On the contrary, 2nd gen. bioenergy uses ligno-cellulosic raw materials from non-food biomass, which is abundant and easily available throughout the world. The sources of 2nd gen. biomass are divided in three main categories by Tan et al. (2008). Firstly forest residues, such as woods, straws from pulp and paper industries and logging activities. Then secondary waste, including municipal solid waste, animal manure and food processing industries waste (Houghton et al., 2006). Lastly dedicated agricultural crops, like grasses, or short rotation crops. In this last category the most used species are Miscanthus (*Miscanthus x giganteus* L.), switchgrass (*Panicum virgatum* L.), and short rotation coppice poplar (*Populus spp*.) and willow (*Salix spp*.) (Eisentraut, 2010). These energy crops will still probably be grown on land that could be used for food and fibre production, like the 1st gen. ones, but their energy yields (in terms of GJ/ha) are higher than those of crops grown to produce 1st gen. biofuels on the same land (Sims et al., 2010) and they can also be grown on poorer quality soil.

The same consideration can be made regarding biogas production, which is a well-established technology based on anaerobic digestion of organic materials. These feedstocks can derive from different sources, as mentioned above for biofuels, but a more sustainable production might be achieved using non-food biomass. For that reason, in Italy, legislation provides subsidies to support the use of this kind of biomass (Ministerial Decree of 6 July 2012).

Effectively, Nijsen et al. (2012) reported that several studies have argued that the growing of perennial grass on degraded soils for the production of energy crops would not only make them less susceptible to soil degradation compared with (annual) food crops but will also significantly increase the productivity of these lands (Samson and Omielan, 1994; Parrish and Fike, 2005; Tilman et al., 2006; Campbell et al., 2008; Fargione et al., 2008; Sanderson and Adler, 2008; Sexton and Zilberman, 2008).

It could also be a way of avoiding competition for land with food production and at the same time improve the soil quality of land considered inadequate for arable crops (Fazio and Monti, 2011; Fahd et al., 2012; Kallioinen et al., 2012). Additionally, there is also a real need to devote marginal land to cultivate non-food energy crops, since arable lands are not sufficient to meet the energy demand (Tan et al., 2008). Certainly marginal and degraded areas could be used but crops need adequate inputs to maintain high yields over the longer term (Luoma, 2009).

Regarding the cultivation, sustainable agriculture can be achieved adopting vigorous and perennial plants to minimize the yearly costs of sowing and soil tillage and using organic sludges and/or wastewater to apply nutrients. In fact animal effluents are rich in organic matter, nitrogen and phosphorus and the fertilizing properties of wastewaters have been proved in many studies (e.g. Tamburino et al., 1999; Lopez et al., 2006; Morari and Giardini, 2009). On the other hand the distribution of animal effluents and wastewaters on fields can often be a source of environmental concern.

In this context, the utilisation of perennial herbaceous plants suitable for wetland treatment systems may offer an interesting solution to achieve the targets of huge biomass availability and allocation of organic wastes and poor quality waters at the same time. In fact wetland plants are able to tolerate high pollutant loads and ameliorate the water quality, also providing 50-60 t/ha per year of biomass (Kadlec and Knight, 1997).

Consequently the allocation of this kind of plant species to produce bioenergy on marginal lands and the use of poor quality water and animal wastes as fertilisers might allow a low cost chain of biomass production to be implemented in which the harvest is the only operation. At the same time this strategy contributes to the protection of surface water bodies from pollution.

To combine all the above-mentioned positive aspects it is hence necessary to identify and characterise appropriate plant species and develop sustainable systems for cultivation and transformation of plants suitable for being irrigated with wastewater and bioenergy production.

Research structure and objectives

The aim of this PhD research is to study and characterize perennial herbaceous species for their composition and potential production of 2nd gen. ethanol and biogas in order to increase the possibility of wastewater reuse and to create an alternative renewable energy chain that is sustainable.

The sub-objectives for achieving the main goal are:

1. To study potential perennial herbaceous species and determine their water consumption, biomass production, nitrogen and phosphorus uptakes and percolated water quality;
2. To acquire biomass characterization of the studied species;
3. To test and compare ethanol and methane production of the studied species;
4. To environmentally assess a scenario of a studied species.

2 Growth boxes trial

Materials and methods

Site description

The experiment began in 2010 on the “Lucio Toniolo” experimental farm of the University of Padova at Legnaro (Figure 2), near Padova (45° 21’ N; 11° 58’ E; 6 m a.s.l.), north-east Italy, and ended in 2013. In this part of the Veneto Region, the climate is sub-humid, mean annual rainfall is about 810 mm and is moderately uniformly distributed throughout the year, with a higher variability from September to November. Mean annual average temperature is about 12.5 ºC. The reference evapotranspiration (ETo), calculated with the Penman–Monteith formula, is 945 mm in the median year and increases during the summer.

In this work data regarding the period June 2010- October 2013 are taken in account.

Figure 2 Location of the experimental site in Italy.

Experimental set up and management

The experimental site consisted of 48 concrete growth boxes (2x2 m sided), laid out in two parallel lines of 24 boxes. They were installed with the top at 1.3 m above ground level, to avoid water table influence, and the bottom open, to allow water percolation (Figure 3). They were filled with fulvi-calcaric Cambisol (CMcf) soil, according to FAO-UNESCO classification (Table 1).

Figure 3 View of the experimental site.

Table 1 Main physical and chemical characteristics of the soil, April 2006.

Parameter	**0-50 cm**	**50-140 cm**
Sand (%)	31	35
Silt (%)	49	45
Clay (%)	20	15
pH	8.1	8,1
ECe (mS/cm)	0.28	0.26
Total carbonate (%)	20.1	17.3
Soluble Carbonate (%)	4.1	3.9
Organic carbon (%)	0.82	0.66
Organic matter (%)	1.4	1.1
C to N ratio	7.5	6.6
Total nitrogen(%)	1.1	1
Available P (mg/L)	50	16
Available K (mg/L)	135	128
water content -10 kPa (%)	36	33
water content -1500 kPa (%)	20	13

A porous ceramic plate (**Ø** 27 cm) was placed at 0.90 m depth in 16 boxes. The plates had air-entry suction of 50 kPa, saturated hydraulic conductivity of $1.25*10^{-5}$ cm/s. They were connected to a suction system by a network of Rilsan plastic thin (**Ø** 2 mm) pipes, protected by bigger (**Ø** 20 mm) and more rigid PVC pipes. This system consented the conduction of vacuum and the collection of percolation water samples. The central components were placed in a small building close to the growth boxes and consist of: (Figure 4):

- 1 electric vacuum pump (power 0.37 KW) provided with a mechanical vacuum gauge. The pump was connected to a tank (50 L), provided with 2 pressure switches that allow the regulation of minimum and maximum thresholds;
- 1 pair of 5 L bottles to collect overflows;

- 16 pairs of 1 L bottles to collect samples; each pair was connected to one ceramic plate by a plastic pipe;
- 1 panel to control distribution of the vacuum, each ceramic plate was handled separately by means of a valve.

The system was started by manual activation of the pump, which, once it reached the set power, stabilized the suction intensity and began samples collection in the bottles.

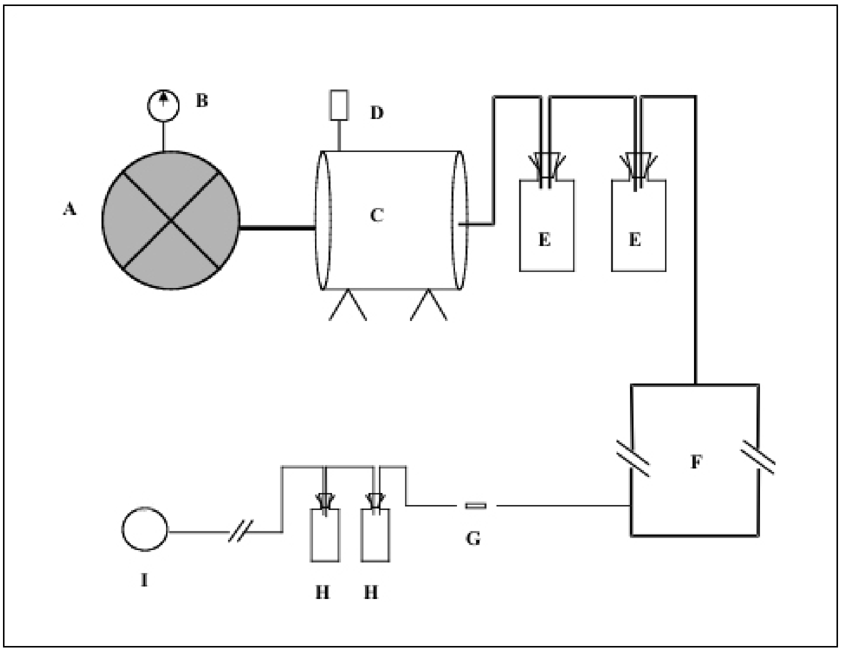

Figure 4 Layout of the suction system to collect percolated water samples. A: electric vacuum pump; B: mechanical vacuum gauge; C: tank; D pressure switches; E: bottles for overflows; F panel to control distribution of the vacuum; G: valve; H: bottles to collect samples; I: porous ceramic plate

The experimental site was activated in June 2010 with plant species transplanting, following adequate soil preparation. There were four growth boxes for each species with 4 plants/m^2 density in a randomized block design.

Fourteen species were cultivated during the research (Figure 5, Table 4).

The plants were fertilized in May of every year, from 2010 to 2012, with an equal amount of pellet manure Biorex (Italpollina, Italy) (Table 2), equivalent to 400 kg N/ha. In spring 2013 the quantity corresponded to 250 kg N/ha and the sludge of anaerobic digester feed with silage maize + bovine slurry was used. Controlled irrigations were applied from May to September, corresponding to 40 mm of water, twice per week. Fertilizer and irrigation application simulated slurry supply.

Plots were kept free of weeds manually the first year of growth, then weed control was no longer needed. During the 4-year experiment no crop diseases were detected. *P. arundinacea* did not react positively to the transplantation and had a limited growth during the season, moreover at the beginning of 2013 crop season two *S. uplandicum* replicates were transplanted, due to the death of plants.

Table 2 Fertilizer composition.

Composition	(%)
Organic nitrogen (N)	2.8
Total phosphoric anhydride (P_2O_5)	3
Water-soluble potassium oxide (K_2O)	2
Total Organic Carbon (C)	38
Organic matter	65

Every year plants were harvested at the end of the season when stems were dead, as in Christian et al. (2008), by cutting the stems at a height of 5 cm. In 2010, being start-up year, there was a single harvest (SH) for all four replicates, while in both following years multiple harvests (MH) were also done for 2 replicates, scheduled as in Table 3. MH were done after plant flowering. The last year there was again only the SH.

Table 3 Harvesting schedule during the trial.

Year		MH		SH and last MH
2010	-	-	-	03 November
2011	11 May	15 June	26 July	05 November
2012	18 May	-	23 July	12 October
2013	-	-	-	23 October

Table 4 List of all cultivated species with scientific name, common name, cultivation year and harvesting type.

N.	Scientific name	Acronym	Common name	Family	2010	2011	2012	2013
1.	*Arctium lappa* L.	AL	Greater Burdock	Asteraceae	-	SH	SH	SH
2.	*Arundo donax* L.	AD	Giant Reed	Poaceae	SH	SH MH	SH MH	SH
3.	*Canna indica* L. [1]	CI	Indian Shot	Cannaceae	SH	-	-	-
4.	*Carex acutiformis* Ehrh.	CA	Lesser Pond Sedge	Cyperaceae	SH	SH MH	SH MH	SH
5.	*Carex pseudocyperus* L. [1]	CP	Cyperus-Like Sedge	Cyperaceae	SH	-	-	-
6.	*Carex riparia* Curtis	CR	Great Pond Sedge	Cyperaceae	SH	SH MH	SH MH	SH
7.	*Glyceria maxima* (Hartm.) Holmb.	GM	Reed Sweetgrass	Poaceae	-	SH	SH MH	-
8.	*Helianthus tuberosum* L.	HT	Jerusalem Artichoke	Asteraceae	-	SH	SH	SH
9.	*Iris pseudacorus* L.	IP	Yellow Flag	Iridaceae	SH	SH MH	SH MH	SH
10.	*Lythrum salicaria* L. [1]	LS	Purple Loosestrife	Lythraceae	-	-	SH	SH
11.	*Miscanthus x giganteus* Greef et Deu.	MG	Giant Miscanthus	Poaceae	SH	SH MH	SH MH	SH
12.	*Phalaris arundinacea* L. var. *picta* L. [1]	PA	Ribbon Grass	Poaceae	-	SH	-	-
13.	*Scirpus sylvaticus* L.	SS	Woodland Bulrush	Cyperaceae	SH	SH MH	-	-
14.	*Symphitum x uplandicum* Nyman	SU	Comfrey	Boraginaceae	SH	SH MH	SH MH	SH

[1] These species were only cultivated for 1 year

-: not cultivated, SH: single harvest, SH MH: single harvest and multiple harvests.

1. 2. 3. 4.

5. 6. 7.

Figure 5 Images of all cultivated species, numbers refer to Table 4.

8.

9.

10.

11.

12.

13.

14.

Figure 4 (continued)

Selected species

All 14 species considered in this study grow naturally in wetland or moist conditions (Cook, 1996). They are all herbaceous and perennial, with the exception of *Artium lappa* L., which is biannual. The vast majority are not cultivated. A brief description of their botanic features follows; uses and biomass productivity are also given, when available.

1) *Artium lappa* L., greater burdock, is diffused in Northern American and temperate European regions (Pignatti, 1982). It is a biennial plant which can reach 2 m the second year of growth. It has large, alternating, cordiform leaves with a long petiole and pubescent on the underside. The flowers are purple and grouped in globular capitula, which are surrounded by an involucre made up of many bracts, each curving to form a hook in order to be carried long distances on the fur of animals. It flowers in mid-summer, from June to September. *A. lappa* is among the most popular plants in traditional Chinese Pharmacopoeia and is associated to several biological effects (Spignoli et al., 1999), related to inflammatory disorders (Ferracane et al., 2010). Thus studies in the literature are focussed on the metabolic profile of its bioactive compounds. A study was recently carried out in Latvia on its potential biogas production (Dubrovskis et al., 2011).

2) *Arundo donax* L., giant reed, is native to East Asia but is now widely diffused. In Mediterranean areas it is frequent in riparian habitats and throughout the United States it is an emergent aquatic plant (Angelini et al., 2009). It is one of the tallest herbaceous grasses, grows in dense clumps and the stems can reach a height of up to 8–9 m. It flowers with a dense, erect panicle in summer. The better propagation is by rhizomes (Christou et al., 2000.). *A. donax* can grow in different soil types and tolerates drought, salinity and flood (Nassi o Di Nasso et al., 2013). It is one of the most studied crops for energy purposes due to its huge productivity. Yields reported in Spain showed 45.9 t/ha on average, ranging from 29.6 to 63.1 t/ha (Hildago and Fernandez, 2001). In Italy Mantineo et al. (2009) obtained yields from 6.1 to 38.8 t/ha in a semi-arid Mediterranean environment, while Angelini et al. (2009) reported that *A. donax* fields reached 49 t/ha during its maturity phase, from 3rd to 8th year of growth, while production higher than 100 t/ha has been recorded at plot level (Molari et al., 2010; Borin et al., 2013).

3) *Canna indica* L., commonly named Indian shot, is a very popular ornamental plant because it has very decorative leaves and flowers. It's ramifications grow from a thick, branching, underground rhizome and reach 150-250 cm in height forming a compact mass. The green leaves are large, the inflorescence is in terminal clusters with groups of flowers. The fruits are ellipsoid capsules with large amounts of black and very hard seeds. Propagation is by seeds or by rhizome subdivision. Indian shot can be grown from sea level to 900 m a.s.l. and is often used in constructed wetlands for its capacity to treat wastewater (Calheiros, 2007).

4, 5, 6) Carex acutiformis Ehrh., *Carex pseudocyperus* L. and *Carex riparia* Curtis. *Carex,* or sedge, is one of the largest plant genera, including more than 3000 species and represents one of the most common vascular plant groups in the world. Sedges are evergreen, form compact bushes, flower in spring with flowers at the top of the green stems. Propagation is by seeds or by subdivision of rhizhomes. They occur in very different habitats: in wet and moist locations such as peat bogs, fens, meadows and pasture communities as well as their peripheries. They also grow in dry and extremely dry habitats, including xerothermic and psammophilous grasslands among others (Bogucka-Kockaa and Janyszekb, 2010). The genus Carex is important for wetlands and is commonly used (Van Acker et al., 2005). *C. elata*, which is smaller than *C. riparia*, can provide a yearly production of 60 t/ha (Borin and Salvato, 2012).

7) Glyceria maxima (Hartm.) Holmb., reed sweetgrass, is native to Europe and temperate Asia (Clarke et al., 2004). It has unbranched stems that can reach 115 cm in height (Tanner, 1996). The leaf sheaths are rough in texture and have a reddish-brown band at the junction with the leaf. The leaf blades are shallowly grooved, with prominent midribs (Howard. 2012). In dense stands reproduction seems to be entirely by vegetative means rather than by seed (Howard, 2012). Tanner (1996) reported aboveground biomass of 33 t/ha. *G. maxima* is used for sewage treatment in artificial wetlands (Tylova-Munzarova et al., 2005).

8) Helianthus tuberosum, L., Jerusalem artichoke, is native of the central regions of North America and arrived in Europe in the 16th century (Cosgrove et al., 1991). In Europe it can be found in uncultivated areas such as roadsides, stream banks, wasteland and abandoned farmsteads. The plants grow well under a wide range of climates but it maximises its production under moderate temperature and adequate water supply (Parameswaran, 1999). Jerusalem artichoke has stout, pubescent stems and can grow to 3 m tall, leaves are opposite on the upper part of the stem but alternate below. The flowers are yellow and produced in 5–10 cm diameter capitate flower heads, The tuber is elongated and uneven, typically 5–10 cm long and 3–5 cm thick, rich in inulin (Pignatti, 1982). Baldini et al. (2011) underline that *H. tuberosum* has been used mainly for its tubers, so as a sugar and dietary fibre crop, but it has recently been studied as a biomass crop for energy uses, particularly for bioethanol production (Curt et al., 2006), methane from anaerobic digestion (Lehtomaki et al., 2008) and gas from pyrolysis (Encinar et al., 2009). In Sweden maximum yields of 16 t/ha were obtained (Gunnarson et al.,1985), in Australia Jerusalem artichoke irrigated with wastewater gave above-ground part yields from 16 to 80 t/ha (Parameswaran, 1999). In spite of its good performance, harvesting is a difficult task, due to the irregular shape and small size of the tubers, but if the economic produce were the stems, most of the crop's drawbacks would be overcome (Curt et al., 2006).

9) Iris pseudacorus L., commonly named yellow iris, is native to Europe, western Asia and northwest Africa. It has robust rhizomes, erect leaves, bright yellow flowers and dry capsule fruits, containing numerous pale brown seeds, by which it spreads quickly but it also propagates by rhizomes. *I. pseudacorus* is common in wetlands, where it tolerates submersion, low pH, and anoxic soils but it can survive prolonged dry conditions (Yousefi and Mohseni-Bandpei, 2010). It has primarily been used as an ornamental plant in water gardens, but has also been widely planted for erosion control and in sewage treatment ponds (Sutherland, 1990). The highest total both above-ground and below-ground biomass reached was 17 t/ha, in planted microcosm units (Haiming et al., 2011).

10) Lytrum salicaria L., or purple loosestrife, is of Eurasian origin, but is now widespread in freshwater wetlands (Brown et al., 2006). It develops a strong taproot, and may have up to 50 stems arising from its base. Its leaves are sessile, opposite or whorled, lanceolate with rounded to cordate bases. Inflorescence is spike-like (10-40 cm long), and each plant may have numerous rose-purple inflorescences (Ling Cao, 2012). Purple loosestrife is used in treatment wetlands (Zhang et al., 2007). Yields of 7-8 t/ha were obtained in Italy at plot scale (Molari et al., 2010).

11) *Miscanthus x Giganteus* Greef et Deu. The genetic origin of *Miscanthus* is in East-Asia (Greef and Deuter, 1993). As a consequence of its triploidy, *M. × giganteus* is sterile and cannot form fertile seeds (Linde-Laursen, 1993) so it is propagated by rhizome division or in vitro cultures (Clifton-Brown and Lewandowski, 2002). The canopy of *M.× giganteus* can reach a height of 4 m (Angelini et al., 2009). Most yields reported for miscanthus in Europe have been assessed using the 'standard' genotype *M. × giganteus*. The stands need 3–5 years to become fully established and reach the maximum yield, yields in general are very variable (Lewandowsi et al, 2000). For locations in southern Europe yields above 30 t/ha are reached only with irrigation, and 10–25 t/ha in central and northern Europe, which are more typically without irrigation (Lewandowsi et al., 2000). In the United Kingdom a research on 14 successive harvests showed a range of yields from 1.46 to 18.33 t/ha (Christian et al., 2008). In Italy Cosentino et al. (2007) observed mean yields from 3.9 to 24.6 t/ha during a 2-year trial., Angelini et al. (2009) of 29.4 t/ha from the 3rd to 8th year and Mantineo et al. (2009) from 2.5 to 26.9 t/ha during a 5-year trial.

12) *Phalaris arundinacea* L., or reed canary grass, is a rhizomatous perennial grass that can grow more than 2 m tall. It has green, broad flat leaves and a hollow stem, single flowers occur in dense clusters in summer, they are green to purple at first and change to beige over time. This plant reproduces by seed or creeping rhizomes. It establishes in constructed or restored wetlands (Waggy, 2010) and is used in treatment wetlands (Hurry and Bellinger., 1990). A well irrigated and fertilized reed canary grass can give 48 t/ha of dry matter (Borin and Salvato, 2012).

13) *Scirpus sylvaticus* L., known as club-rush or bulrush or grassweed, has grass-like leaves, and clusters of small spikelets, often brown in colour and can be from 0.3 to 3 m tall. The leaves are long, keeled, broad and flat, and the corymbose flowers, which appear in summer, are very branched (Cook, 1996). It is an evergreen rhizomatous sedge characteristic of infertile wetlands (Crick and Grime, 1987). Biomass yields from 5 to 37 t/ha were reported by Kuusemets and Lõhmus (2005).

14) *Symphytum x uplandicum* Nyman, Russian comfrey, is a crossbreed between *Symphytum officinale* and *S. asperum* (Culvenor et al., 1980). It is a perennial herb known as comfrey, gum plant or boneset, and is employed topically as anti-

inflammatory, emollient and mild anaesthetic in phytotherapy, due to allantoin found in the underground organs and leaf (Toledo, 2006). It has a taproot up to 3m in length and a fleshy and extensive root system. It is propagated mainly through root cuttings and offsets. It prefers wet soil and a sunny position, so often grows along ditches (Hills, 1976). Once plants are well established, plenty of vegetative material can be harvested by cutting several times during the year; the plants regenerate quickly because of the large food reserves in the roots and can produce two to five crops per year (Bremness, 1998). Yields of 6.9 t/ha were registered in the United Kingdom (Wilkinson, 2003).

Soil moisture measurement

Over the growing season soil moisture content was measured every 10 cm to 100 cm with a Diviner 2000 device (Sentek, Stepney, Australia) which consists of a probe and hand-held data logging display unit, allowing measures onsite. Data were collected from July to September in 2010, from May to October in 2011, from March to June in 2012 and from July to August in 2013. Measurements in the last two years couldn’t be continuing, due to the device being damaged.

Vegetation sampling and analysis

Vegetation was harvested as scheduled in Table 3, considering a sampling area (50 x 50 cm) in the middle of each growth box surface, cut at 5 cm height. The collected aboveground biomass was weighed onsite for total fresh weight, while 100 g ca. samples were dried in a force draught oven at 65 °C for 36 hours, milled at 2 mm (Cutting Mill SM 100 Comfort, Retsch, Germany); in addition, 1 g powdered sub-samples were dried at 130 °C to measure the residual moisture content. The 65 °C dry samples were then analysed to determine:

- total Kjeldahl nitrogen (N) and phosphorus (P) content, using the FAO official method (FAO, 2011) - one replicate;
- carbon (C), hydrogen (H) and oxygen (O) content, with the Elemental Analyser EA 1100 CHNS-O (CE Instruments Ltd., Lancashire, UK). These analyses were performed every year for each harvest, both SH and MH - two replicates;

- potassium (K), magnesium (Mg) and calcium (Ca), using the inductively coupled plasma atomic emission spectroscopy (ICP-AES) technique (Hou and Jones, 2000) by Spectrometer Optima 2000DV (PerkinElmer Inc., Massachusetts, USA) - two replicates;
- the different constituents of fibre (hemicellulose, cellulose, lignin and ashes, determined sequentially according to Van Soest’s scheme analysis (Fan et al., 1987) through Fibre Analyser FIWE 6 (VELP Scientifica, Usmate, Italy) - three replicates.

N and P analyses were performed every year for each harvest, both SH and MH, from all growth boxes from 2010 to 2012, giving a total of 176 samples. The remaining analyses were conducted only for the first harvest of each species, with two or three replicates as mentioned above. The results were then expressed in dry matter.

During the 2012 growing season plant were also monitored according to the BBCH scale (Hack et al., 1992). From the beginning of April to the end of October 2012, BBCH stages (Table 5) were ascribed to plants twice a week to check their growth and development.

Table 5 BBCH scale stages

Stage	Description
0	Germination / sprouting / bud development
1	Leaf development (main shoot)
2	Formation of side shoots / tillering
3	Stem elongation or rosette growth / shoot development (main shoot)
4	Development of harvestable vegetative plant parts or vegetatively propagated organs/booting (main shoot)
5	Inflorescence emergence (main shoot) / heading
6	Flowering (main shoot)
7	Development of fruit
8	Ripening or maturity of fruit and seed
9	Senescence, beginning of dormancy

Water sampling and chemical analysis

During the entire trial percolation water samples were taken once a month from November to April, when percolation occurred. The water was thus collected as follows:

- winter 2010/2011: from November 2010 to March 2011;
- winter 2011/2012: November and December 2011 for a reduced number of species. This was due to an unusually dry autumn and winter;
- winter 2012/2013: from October 2012 to April 2013.

A total of 196 percolation water samples were collected and analysed to detect total nitrogen (TN), nitric nitrogen (NO_3-N), total phosphorus (TP) and orthophosphate (PO_4-P). All samples were frozen immediately after collection and stored until laboratory analysis. TN and TP were determined using Valderrama method (Valderrama, 1981), PO_4-P with Olsen method (Olsen et al., 1954) and NO_3-N by modified Cataldo method (Cataldo at al., 1975).

Soil sampling and analysis

Soil samples were taken before the beginning of the trial, at the end of March 2010, and at the end of the monitoring period in April 2013. Sampling involved the top 0-20 cm soil layer and a deeper layer at 20-50 cm. After collection soil samples were air-dried, crushed using a rolling pin and manually sieved, first at 2 mm and then at 500 µm. Organic carbon, total nitrogen and sulphur were measured by Springer and Klee method (Springer and Klee, 1954).

Data elaboration

All statistical analyses were performed using the computer software package STATISTICA 7.0 (Statsoft Inc., 2004). The data series of nitrogen, phosphorus and fibre content and water parameters didn’t follow normal distribution. Thus, statistical analyses were implemented with the Kruskal-Wallis non parametric test and box-plots were used to present the data. Different letters indicate significant differences at $p < 0.05$ by Kruskal-Wallis test. Result regarding yields and uptake respected normal distribution so ANOVA analyses were conducted, followed by Fisher's Least Significant Difference (LSD) test, where different letters indicate significant differences at $p < 0.05$ by LSD test.

Results and discussion

Meteorological data and water balance

In Legnaro the long-term (1995-2000) average precipitation corresponds to 840 mm/year while the average annual temperature is 13.5 °C. In 2010 the annual precipitation surpassed the long-term average (1141 mm), while in 2011 and 2012 it was almost half that (601 and 603 mm, in 2011 and 2012 respectively) with lower amounts especially during spring and summer. In 2013 the data refer to the period until 31st October 2013 but the trend was nevertheless higher than the long-term one, mainly in the first 6 months of the year and with particularly heavy rainfall in March 2013 (Figure 6a).

During the four-year experiment the monthly temperature trends were similar to the long-term average with higher values from May to September (Figure 6b).

Irrigation supplied during years is shown in Figure 7, in total the plants received 1646 mm in 2010, 1571 mm in 2011, 1954 mm in 2012 and 970 mm in 2013.

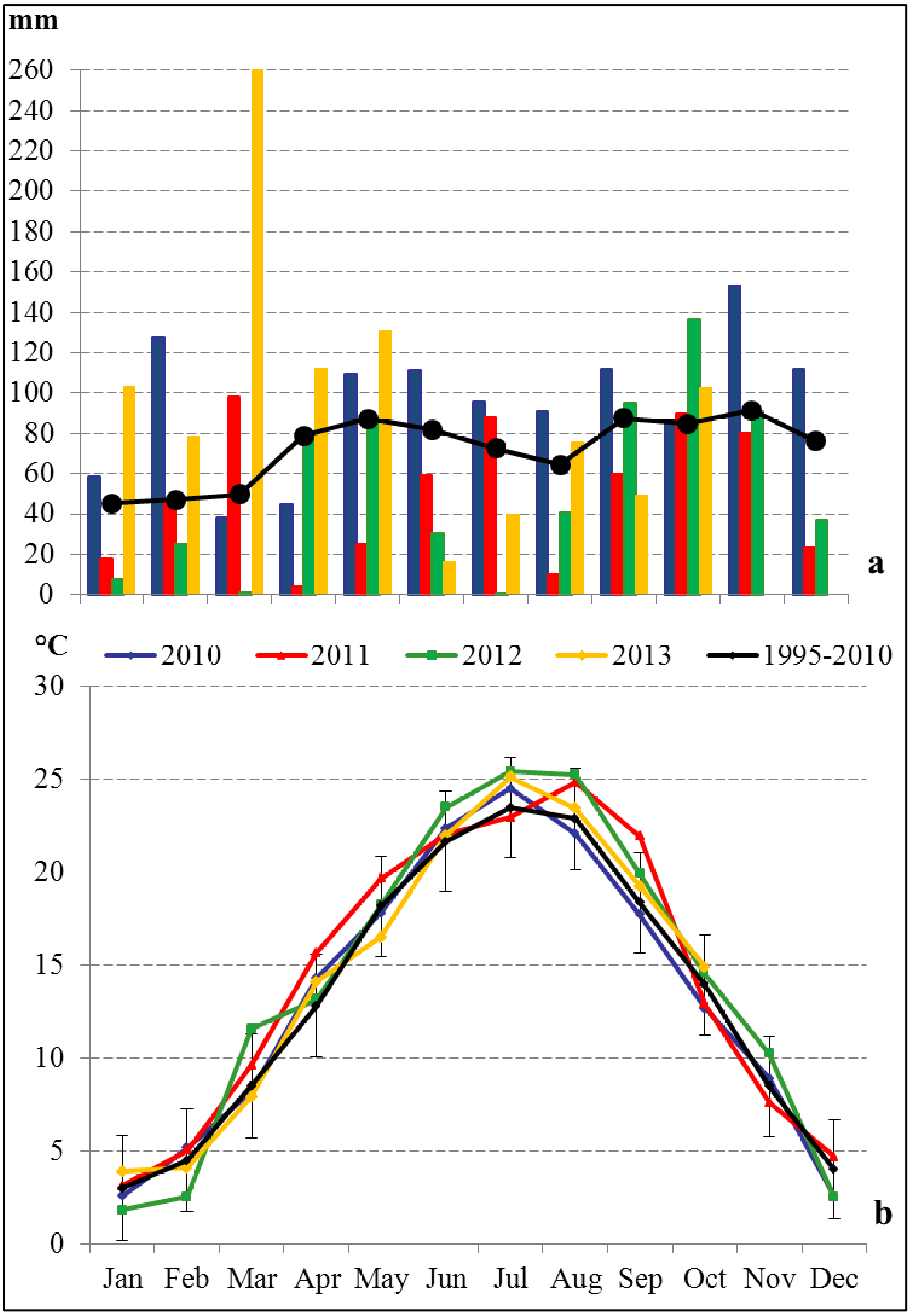

Figure 6 Meteorological data during the trial in Legnaro (PD): a) monthly precipitation, b) monthly average temperature. Data refer to the period from 1st January 2010 to 31st October 2013 (ARPAV, modified data).

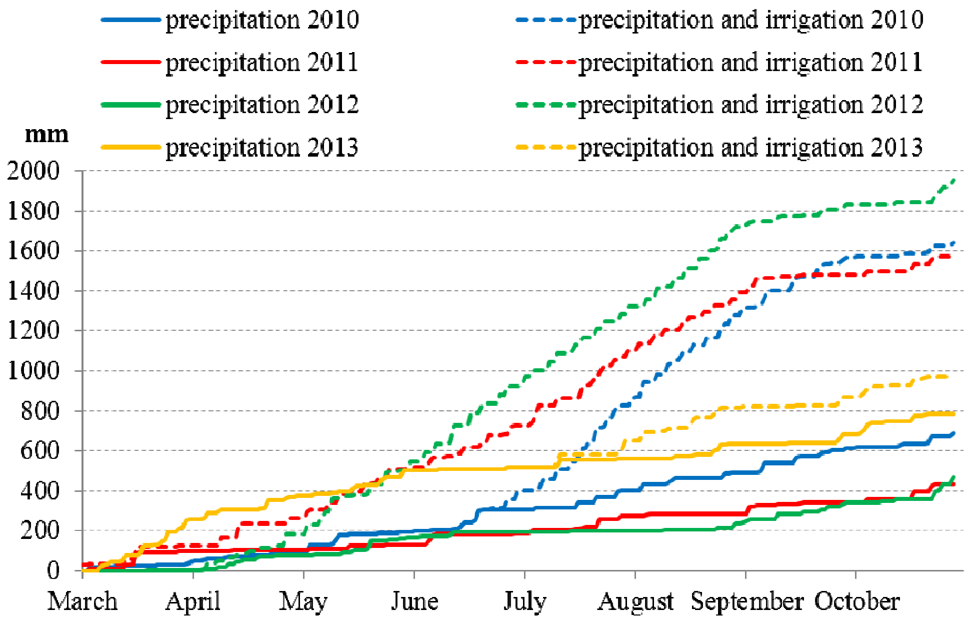

Figure 7 Cumulative water volumes supplied to the trial from March to October 2010, 2011, 2012 and 2013.

Soil moisture

Evapotranspiration and crop coefficients

Evapotranspiration (ET) is the loss of water from surface to atmosphere through liquid water vaporization from both soil and plants. In this research ET was estimated by the following equation based on the water balance of a drainage basin:

$$ET=P+I+\Delta H \quad (1)$$

Where P is precipitation, I is irrigation and ΔH is soil moisture variation. Groundwater, run-off and leaching were not taken into account because considered minimal.

At a later stage the reference evapotranspiration (ET_0) of the experimental site was calculate with the Penman-Monteith equation (Allen et al., 1998), in order to obtain the crop coefficient (K_c) for each species through the following formula:

$$K_c= ET/ET_0 \quad (2)$$

ET varied vastly among both species and years. In general values were higher than ET_0, with the exception of October and 2013 measurements. These last had low ET because of reduced precipitation and irrigation supply.

In 2010 this parameter did not show a high level of variation, ranging from 423 to 453 mm in July and August and from 246 to 260 mm in September. Comparable values among species were probably due to the initial development of the plants, which were all transplanted in June (Table 6).

Table 6 ET of the studied plant species in 2010

Species	Jul mm	Aug mm	Sep mm
A. donax	444	435	264
C. indica	431	436	275
C. acutiformis	449	446	262
C. pseudocyperus	449	423	256
C. riparia	453	442	262
I. pseudacorus	432	429	246
M. x iganteus	444	457	269
S. sylvaticus	440	441	256
S. x uplandicum	440	440	264
ET_0	218	176	125

In 2011 measurements could be done for longer so it was possible to observe a better trend in ET, which increased from May (minimum 233, maximum 335 mm) to August (minimum 303, maximum 467 mm) and then reduced until October (minimum 111, maximum 22 mm). Among plants it was not possible to identify a species which had the highest or the lowest ET overall, but there was a monthly specificity (Table 7).

Table 7 ET of the studied plant species in 2011

Species	May mm	Jun mm	Jul mm	Aug mm	Sep mm	Oct mm
A. lappa	252	230	335	336	269	42
A. donax	335	263	322	339	284	63
C. acutiformis	245	237	347	303	290	111
C. riparia	270	227	337	309	285	30
G. maxima	225	257	360	467	281	56
H. tuberosus	310	266	337	322	273	44
I. pseudacorus	264	235	344	305	284	99
M. x giganteus	295	266	291	382	274	63
P. arundinacea	233	240	377	467	281	52
S. x uplandicum.	250	273	336	318	273	22
S. sylvaticus	255	249	349	314	290	46
ET_0	173	171	192	184	137	82

In 2012, ET showed an increase from April to June. In the first month *A. donax* registered the lowest value (126 mm) and *L. salicaria* the highest (178 mm) but in July the situation was the contrary (527 and 388 mm, respectively). Instead *S x uplandicum* maintained lower ET during the entire monitoring (135, 141 and 436 mm) (Table 8).

Table 8 ET of the studied plant species in 2012

Species	Apr mm	May mm	Jun mm
A. lappa	146	284	443
A. donax	126	116	527
C. acutiformis	144	436	461
C. riparia	147	313	470
G. maxima	149	27	466
H. tuberosus	153	440	511
I. pseudacorus	155	329	506
L. salicaria	178	515	388
M. x giganteus	141	356	518
S. x uplandicum	135	141	436
ET_0	117	183	199

In the last year species could only be observed for two months, in which ET was lower than ET_0. A specific trend could not be identified among species (Table 9).

Table 9 ET of the studied plant species in 2013

Species	Jul mm	Aug mm
A. lappa	167	152
A. donax	137	123
C. acutiformis	175	149
C. riparia	153	149
G. maxima	167	129
H. tuberosus	82	177
I. pseudacorus	169	139
L. salicaria	163	180
M. x Giganteus	139	141
S. x uplandicum	130	147
ET_0	219	196

The majority of species in this study are wetland macrophytes. Since ET estimates are hard to obtain for wetlands, even in research systems (USEPA, 2000), ET rates have not been thoroughly investigated for most of them. Some data can be found in the literature regarding miscanthus (Hickman et al., 2010). Moreover ET rates, also in the same species, differ significantly due to different meteorological conditions and latitudes. However, it is important to point out that under the same environmental conditions, plants provided very different ET values, as observed by Salvato and Borin (2010).

Kc values reflected ET values so the same considerations can be made. In Table 14 the mean Kc were calculated from the values collected in the 4-year trial to give an overview. Kc were compared with those of maize which are: 0.3-0.5 during first month of growing, 0.7-0.85 in the development stage, 1.05-1.2 at mid-season, 0.8-0.9 during the late season and finally 0.55-0.6 at harvest (FAO, 2013). Thus in general it was observed that all species had higher Kc than maize.

Table 10 K_c of the studied plant species in 2010

Species	Jul	Aug	Sep
A. donax	2.04	2.46	2.11
C. indica	1.98	2.47	2.19
C. acutiformis	2.06	2.53	2.09
C. pseudocyperus	2.06	2.40	2.04
C. riparia	2.08	2.50	2.09
I. pseudacorus	1.98	2.43	1.96
M. x Giganteus	2.04	2.59	2.14
S. sylvaticus	2.02	2.50	2.04
S. x uplandicum	2.02	2.50	2.11

Table 11 K_c of the studied plant species in 2011

Species	May	Jun	Jul	Aug	Sep	Oct
A. lappa	1.5	1.3	1.7	1.8	2.0	0.5
A. donax	1.9	1.5	1.7	1.8	2.1	0.8
C. acutiformis	1.4	1.4	1.8	1.7	2.1	1.4
C. riparia	1.6	1.3	1.8	1.7	2.1	0.4
G. maxima	1.3	1.5	1.9	2.5	2.1	0.7
H. tuberosus	1.8	1.6	1.8	1.8	2.0	0.5
I. pseudacorus	1.5	1.4	1.8	1.7	2.1	1.2
M. x giganteus	1.7	1.6	1.5	2.1	2.0	0.8
P. arundinacea	1.4	1.4	2.0	2.5	2.1	0.6
S. x uplandicum	1.4	1.6	1.8	1.7	2.0	0.3
S. sylvaticus	1.5	1.5	1.8	1.7	2.1	0.6

Table 12 K_c of the studied plant species in 2012

Species	Apr	May	Jun
A. lappa	1.3	1.6	2.2
A. donax	1.1	0.6	2.7
C. acutiformis	1.2	2.4	2.3
C. riparia	1.3	1.7	2.4
G. maxima	1.3	0.1	2.3
H. tuberosus	1.3	2.4	2.6
I. pseudacorus	1.3	1.8	2.5
L. salicaria	1.5	2.8	2.0
M. x Giganteus	1.2	1.9	2.6
S. x uplandicum	1.2	0.8	2.2

Table 13 K_c of the studied plant species in 2013

Species	Jul	Aug
A. lappa	0.8	0.8
A. donax	0.6	0.6
C. acutiformis	0.8	0.8
C. riparia	0.7	0.8
G. maxima	0.8	0.7
H. tuberosus	0.4	0.9
I. pseudacorus	0.8	0.7
L. salicaria	0.7	0.9
M. x Giganteus	0.6	0.7
S. x uplandicum	0.6	0.7

Table 14 Mean K_c of the studied species.

Species	Apr	May	Jun	Jul	Aug	Sep	Oct
A. lappa	1.3	1.5	1.8	1.3	1.3	1.9	0.5
A. donax	1.1	1.3	2.1	1.4	1.6	2.1	0.8
C. indica	-	-	2.0	2.5	2.2	-	-
C. acutiformis	1.2	1.9	1.9	1.5	1.6	2.0	1.4
C. riparia	1.3	1.6	1.9	1.5	1.7	2.0	0.4
C. pseudocyperus	-	-	2.1	2.4	2.0	-	-
G. maxima	1.3	0.7	2.0	1.6	1.9	2.0	0.7
H. tuberosus	1.3	2.1	2.1	1.4	1.7	2.0	0.5
I. pseudacorus	1.3	1.7	2.0	1.5	1.6	2.0	1.2
L. salicaria	1.5	2.8	2.0	0.7	0.9	-	-
M. x giganteus	1.2	1.8	2.1	1.4	1.8	2.0	0.8
P. arundinacea	-	1.4	1.4	2.0	2.5	2.1	0.6
S. x uplandicum	1.2	1.1	1.9	1.5	1.7	2.0	0.3
S. sylvaticus	-	1.5	1.5	1.7	2.1	2.1	0.6

Biomass production

Biomass characteristics

Mineral composition is important for potential energy crops because it allows to evaluate which technology is more suitable for the conversion of plants into biofuels (Monti et al., 2008).

Nitrogen and phosphorus contents were measured for every harvest (both SH and MH) for each growth box from 2010 to 2012. Significant differences were observed among species but not among years (Figure 8).

Nitrogen median content ranged from 2.31 to 0.50%, the best result was given by *S. x uplandicum* (2.31%) but also by *P. arundinacea* (2.20%) and *A. lappa* (2.05%), while provided by *H. tuberosum* (0.50%) and *M. x giganteus* (0.70%) were lower.

It is well known that biomass collected at the end of the growing season displays lower nitrogen than in spring, mainly due to the translocation to belowground biomass (Beale and Long, 1997; Christian et al, 1998; Kadlec and Wallace, 2009). Furthermore Kadlec and Wallace (2009) underline that different plant parts may show differences in nitrogen content and seasonal variability may also be very wide.

Phosphorus median content showed values from 0.067 to 0.503%. Again *S. x uplandicum* gave the highest result (0.503%), followed by *L. salicaria* (0.0278%), *A. lappa* (0.261%), *G. maxima* (0.255%) and P. *arundinacea* (0.230%). Lower P percentages were found in *C. pseudocyperus* (0.067%), *M. x giganteus* (0.70%), *A. donax* (0.085%), *C. indica* (0.095%) and *H. tuberosum* (0.096%).

Results were similar to those reported in the Italian literature for miscanthus and giant reed (Cosentino et al., 2007; Monti et al., 2008; Nassi o Di Nasso et al., 2010; Borin et al., 2013).

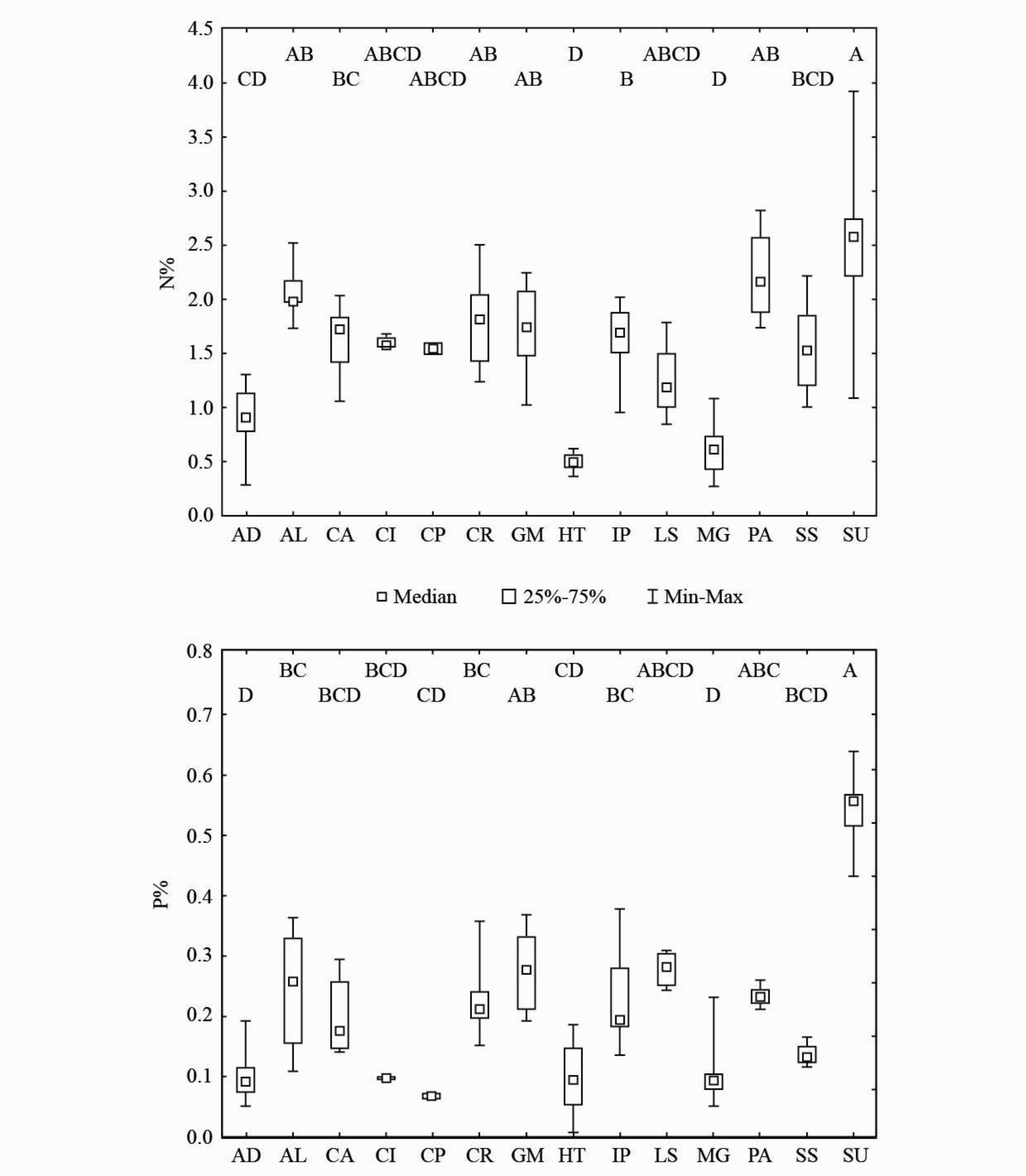

Figure 8 Box-plots of nitrogen (N) and phosphorus (P) content in the biomass of the studied species from 2010 to 2012. Different letters indicate significant differences at $P < 0.05$ by Kruskal-Wallis test.

Biomass element concentrations (C, H, O, Ca, K and Mg) were analysed with two replicates so mean values are reported in Table 15. C content showed values from 42.2 (*C. pseudocyperus*) to 50.6% (*P. arundinacea*). With regard to H, *C. pseudocyperus* again gave the highest content (7.59%) while *S. sylvaticus* the lowest (4.47%). For O, *C.*

pseudocyperus instead showed the lowest value (40.3%) while the highest was reported by *C. indica*. The latter presented a very low Ca content (0.275%) while *C. pseudocyperus* was again the species with highest value (2.701%). Finally *M. x giganteus* determined low values for both K and Mg (0.271 and 0.050%) while *S. x uplandicum* and *C. indica* reported the highest, 8.436 and 0.845% respectively. Monti et al. (2008) stated slightly higher values for miscanthus while data for giant reed were very similar to those presented here. Nassi o di Nasso et al. (2010) also gave similar data for *A. donax*, with the exception of H, which was double the values here.

Table 15 Mean values of biomass element quality of the studied species.

Species	C %	H %	O %	Ca %	K %	Mg %
A. lappa	-	-	-	-	-	-
A. donax	42.2	5.26	51.5	0.115	0.823	0.048
C. indica	40.6	5.01	52.5	0.275	3.244	0.845
C. acutiformis	43.1	5.01	50.9	0.595	2.136	0.204
C. pseudocyperus	50.6	7.59	40.3	2.701	1.154	1.119
C. riparia	46.9	5.70	44.2	0.633	2.593	0.211
G. maxima	45.7	4.66	49.5	0.580	1.772	0.315
H. tuberosum	44.4	5.60	49.7	0.557	1.865	0.298
I. pseudacorus	43.4	5.17	50.1	2.407	3.684	0.344
L. salicaria	46.2	4.86	48.9	0.432	0.372	0.0153
M. x giganteus	44.5	5.53	49.7	0.280	0.271	0.050
P. arundinacea	42.2	5.13	47.2	0.554	1.793	0.316
S. sylvaticus	43.8	4.47	51.4	0.716	2.880	0.151
S. x uplandicum	44.1	4.87	50.6	2.269	8.436	0.446

-: not determined.

Cellulose content had median values ranging from 23.1% (*S. x uplandicum*) to 45.4% (*M. x giganteus*), hemicellulose from 17.4% (*S. x uplandicum*) to 36.8% (*S. sylvaticus*) and lignin from 2.6% (*G. maxima*) to 14.5% (*H. tuberosum* and *L. salicaria*). As a comparison the fibre characteristics of some feedstocks (Whright, 2008) are reported in Table 17. It is also worth mentioning that lignin content in woods can vary from 15% to 40% (Sarkanen and Ludwig, 1971).

Cellulose and hemicellulose results for miscanthus and giant reed were comparable to ones found in the literature while lignin percentages were lower (Pascoal Neto et al., 1997; Shatalov and Pereira, 2001; Ververis et al., 2004; Shatalov and Pereira, 2005; Scordia et al., 2012; Di Girolamo et al., 2013). With regard to *A. donax* Pascoal Neto et al. (1997) reported that lignin content was highly dependent on the stage of maturity of the plant and decreased gradually from the older parts to the younger parts, such as foliage.

Furthermore ligno-cellulosic biomass has a very complex and rigid structure, made of hemicellulose, cellulose and lignin in a proportion depending on plant species and cropping factors (Di girolamo et al., 2011).

Table 16 Median fibres value of the studied species. Different letters indicate significant differences at P< 0.05 by Kruskal-Wallis test.

Species	Cellulose %	Hemicellulose %	Lignin %
A. lappa	-	-	-
A. donax	38.7 ab	31.7 ab	6.7 ab
C. indica	31.1 ab	31.7 ab	5.6 ab
C. acutiformis	29.7 ab	36.0 ab	7.9 ab
C. pseudocyperus	26.7 b	29.5 ab	7.4 ab
C. riparia	29.1 ab	33.1 ab	6.0 ab
G. maxima	31.3 ab	36.8 a	2.6 b
H. tuberosum	28.5 ab	16.8 ab	14.5 a
I. pseudacorus	28.2 ab	9.4 b	7.2 ab
L. salicaria	45.4 a	18.6 ab	14.5 a
M. x giganteus	43.4 a	30.5 ab	5.8ab
P. arundinacea	28.9 ab	33.9 ab	5.4 ab
S. sylvaticus	36.5 ab	32.0 ab	13.3 ab
S. x uplandicum	23.1 b	17.4 b	8.0 ab

-: not determined.

Table 17 Mean fibre characteristics of feedstocks (Whright, 2008, modified)

Feedstock	Cellulose %	Hemicellulose %	Lignin %
Bamboo	41 - 49	24 - 28	24 - 26
Hardwood	45	30	20
Hybrid poplar	39 - 46	17 - 23	21 - 8
Maize stover	30 - 38	19 - 25	17 - 21
Sugarcane bagasse	32 - 43	19 - 25	23 - 28
Sweet sorghum	27	25	11
Switchgrass	31 - 34	24 - 29	17 - 22

BBCH scale

The studied species revealed different growth rates according to the BBCH scale (Table 18). At the beginning of April all plants had already started leaf development, with the exception of *C. riparia*. Both *Carex* showed early inflorescence emergence while giant reed and miscanthus had late flowering. Beginning of senescence occurred at the end of May for *C. acutiformis*, followed by *C. riparia* in June. Again giant reed and miscanthus showed the most tardive behaviour.

Table 18 BBCH scale results of the studied species in 2012 crop season. Numbers refer to Table 5.

Species	03/04/2012	05/04/2012	12/04/2012	17/04/2012	26/04/2012	03/05/2012	10/05/2012	23/05/2012	29/05/2012	01/06/2012	08/06/2012	11/06/2012	18/06/2012	26/06/2012	03/07/2012	10/07/2012	18/07/2012	25/07/2012	05/08/2012	14/08/2012	21/08/2012	27/08/2012	12/09/2012	21/09/2012	09/10/2012
A. lappa	3	3	3	3	3	4	4	4	5	5	5	5	5	5	6	6	6	8	8	8	8	8	/	/	/
A. donax	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3/5	3/5	3/5	3/5	3/5	3/5	3/5	6
C. acutiformis	3	5	6	7	7	7	7	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
C. riparia	0	1	5	5	6	6	7	7	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
G. maxima	1	3	3	3	3	3	3	3	3	5	6	6	6	6	6	6	6	8	8	8	8	8	8	8	8
I. pseudacorus	1	3	3	3	5	6	6	7	7	7	7	7	7	7	7	8	8	8	8	8	8	8	8	8	8
L. salicaria	/	/	/	/	/	/	/	/	/	/	1	1	3	5	6	6	6	6	6	6	6	6	6	8	8
M. x Giganteus	1	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3/5	3/5	3/5	3/5	3/5	3/5	6	6
S. x uplandicum	1	3	3	3	5	5	6	7	7	7	7	7	7	7	7	7	7	7	7	8	8	8	8	8	8

Biomass yield

Biomass yield was assessed by ANOVA test within the year; differences among species were reported so means were then compared by Fisher's Least Significant Difference (LSD) Test (Table 19). In 2010 the highest biomass production was given by *A. donax* (26.2 t/ha), followed by *M. x giganteus* (13.1 t/ha); all the other species had yields between 3.1 and 6.8 t/ha. The second year species gave values ranging from 0.2 to 62.8 t/ha, higher than the previous year. *A. donax* and *M. x giganteus* again gave better results while the lower were obtained by *P. arundinacea* (0.2 t/ha), *S. sylvaticus* (3.5 t/ha), *A. lappa* (3.9 t/ha) and *G. maxima* (4.5 t/ha). In 2012 not all the species produced more biomass than in 2011, with yields from 9.9 to 95.1 t/ha. During the last year all yields decreased, apart from *A. donax* and *M. x giganteus*, going from 3.3 to 140.5 t/ha. The reduction was probably due to the lower fertilizer and water supply.

From 2010 to 2013 *A. donax* gave the highest biomass yields. It increased yearly (26.2, 62.8, 95.1 and 140.1 t/ha, respectively) and was significantly different from all the other species, with the exception of 2011, in which there was no significant difference from miscanthus (62.8 and 55.2 t/ha, respectively). The maximum dry biomass yield of giant reed was higher than the maximum results found in the literature (Hildago and Fernandez, 2001; Lewandowski et al., 2003; Christou et al., 2005; Angelini et al., 2009; Mantineo et al., 2009; Nassi o Di Nasso et al., 2013; Borin et al., 2013), probably because of the notable quantity of nitrogen and water supplied during this experiment, as reported by Zema et al. (2012). Furthermore, a small scale trial could provide higher yields, as in Molari et al. (2010). The same considerations can be made for *M. x giganteus,* which showed higher yields than values provided by other authors (Lewandowski et al., 2000; Cosentino et al., 2007; Christian et al., 2008; Angelini et al., 2009). In this sense Petrini et al. (1996) obtained yields of 41 t/ha in miscanthus growing in the best nitrogen and water conditions and also Zub et al. (2009) underlined that biomass production responses to nitrogen depend on available water. *H. tuberosum* and *S. x uplandicum* also gave higher results than reported in the literature (Gunnarson et al., 1985; Parameswaran, 1999; Wilkinson, 2003). All the other species had lower results than expected and found in the literature (Tanner, 1996; Kuusemets and Lohmus, 2005; Tylova-Munzarova et al., 2005; Haiming et al., 2011; Borin and Salvato, 2012).

Table 19 Mean biomass yields of the studied plant species in 2010, 2011, 2012 and 2103. Different letters indicate significant differences at P< 0.05 by LSD test.

Species	2010			2011			2012			2013		
	mean	s.d.		mean	s.d.		mean	s.d.		mean	s.d.	
	t/ha			t/ha			t/ha			t/ha		
A. lappa	-			3.9	1.7	c	30.1	5.8	bc	13.7	9.3	c
A. donax	26.2	17.1	a	62.8	43	a	95.1	55.3	a	140.5	20.5	a
C. indica	6.8	1.2	bc	-			-			-		
C. acutiformis	4.8	1	bc	15.7	4.1	bc	14.6	2.4	b	6.9	2	c
C. pseudocyperus	6	2.3	bc	-			-			-		
C. riparia	4.8	0.4	bc	14.7	2.3	bc	9.1	6.2	b	5.8	1.3	c
G. maxima	-			4.5	1.3	c	9.7	4.4	b			
H. tuberosum	-			28.6	7.2	bc	40.1	14.9	bc	18.9	4	c
I. pseudacorus	6.2	3.3	bc	13.2	3.3	bc	9.9	3.1	b	6.5	0.6	c
L. salicaria	-			-			11.9	6.6	b	11.8	1.9	c
M. x giganteus	13.1	1.6	b	55.2	7.9	a	46.2	25.6	b	51.6	27.1	b
P. arundinacea	-			0.2	0.1	c	-			-		
S. sylvaticus	3.8	0.6	c	3.5	0.6	c	-			-		
S. x uplandicum	5.1	0.6	bc	16.1	2.3	bc	25.1	30	bc	3.3	1.5	c

-: non cultivated.

A. donax, *C. acutiformis*, *C. riparia*, I. *pseudacorus*, *M. x giganteus* and *S. x uplandicum* were grown for 4 successive years so data were analysed by a two-way ANOVA then the LSD test for the means separation was applied to show significant differences. Species, year and also interaction species-year were significantly different at P< 0.001 (Figure 9).

Finally the different harvest management was assessed for each species with a two-way ANOVA. Interaction year-harvest was not significantly different for the six species. *A. donax*, *I. pseudacorus* and *S. x uplandicum* had no significant differences among year nor harvest, *C. riparia* had significantly higher yields in 2010, while the remaining species through MH.

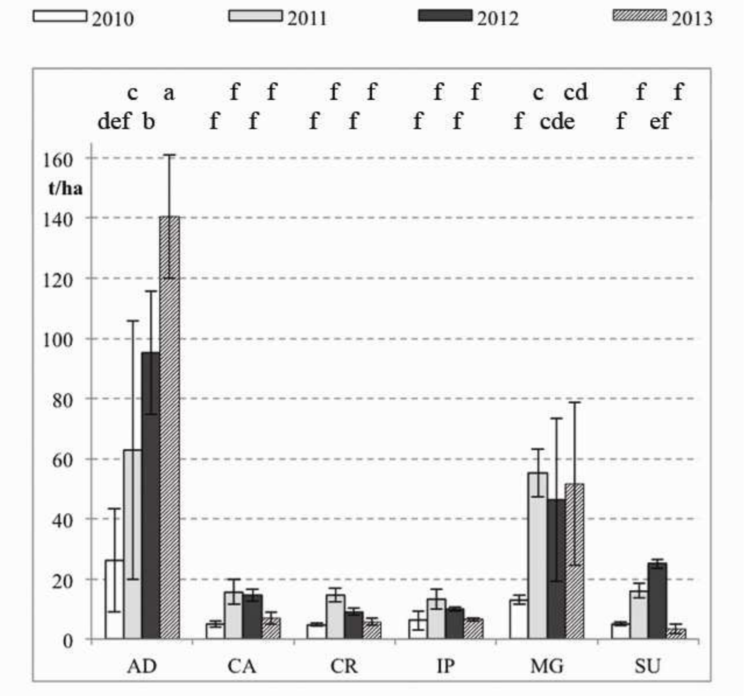

Figure 9 Mean biomass yields of the plant species grown for 4 successive years. AD: *A. donax*, CA: *C. acutiformis*, CR: *C. riparia*, IP*: I. pseudacorus*, MG: *M. x giganteus*, SU: *S. x uplandicum*

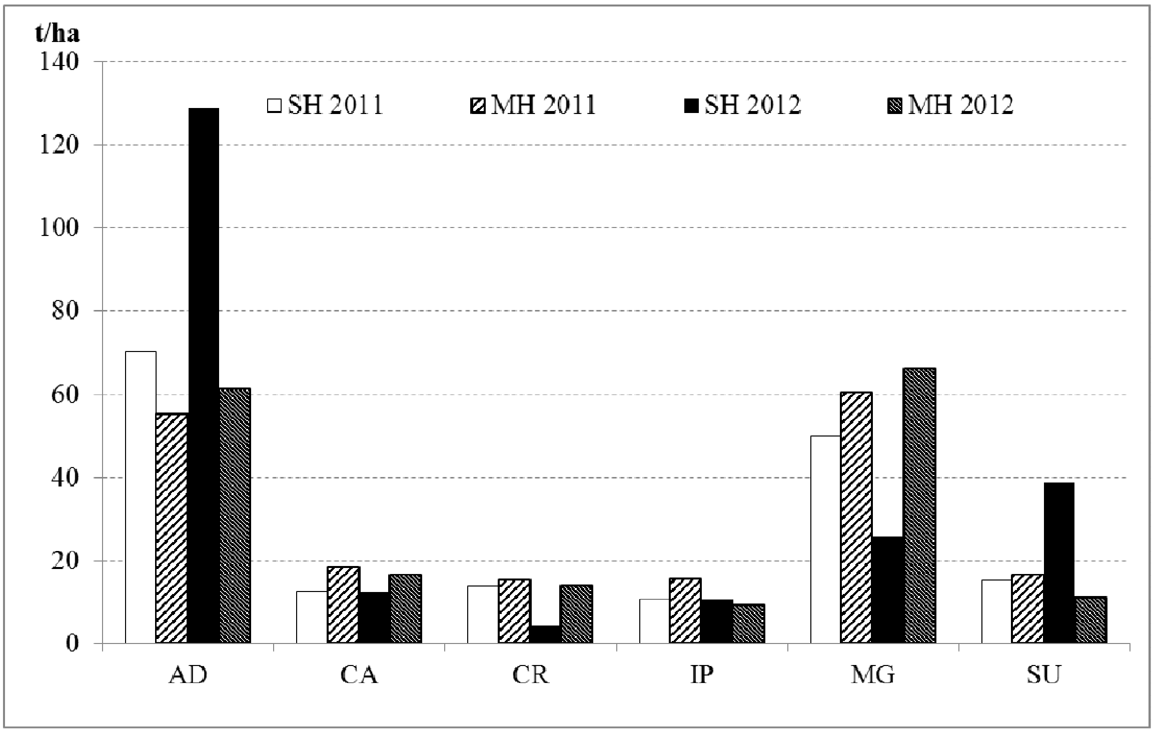

Figure 10 Comparison of single harvest (SH) and multiple harvests (MH): mean biomass yields of the studied plant species in 2011 and 2012. AD: *A. donax*, CA: *C. acutiformis*, CR: *C. riparia*, IP*: I. pseudacorus*, MG: *M. x giganteus*, SU: *S. x uplandicum*

Table 20 Significant differences by two-way ANOVA in Figure 10.

Species	Year	Harvest	Interaction
A. donax	n.s.	n.s.	n.s.
C. acutiformis	n.s.	*	n.s.
C. riparia	*	*	n.s.
I. pseudacorus	n.s.	n.s.	n.s.
M. x giganteus	n.s.	*	n.s.
S. x uplandicum	n.s.	n.s.	n.s.

n.s.: non-significant; *: $p<0.05$; **: $p<0.01$;***: $p<0.001$.

Nitrogen and Phosphorus uptake

Uptakes were calculated multiplying yields by the corresponding annual nutrient content, with the exception of 2013 for which the 2010-2012 mean N and P percentage values were used. Plant uptakes were assessed by ANOVA test within the year. Since differences were found among species, the means were then compared by LSD test (

Table 21 and Table 22).

In 2010 *A. donax* gave the highest value of N uptake (305.9 kg/ha), all the other species had yields between 65.3 and 118.2 kg/ha. The following year *A. donax* again showed best uptake (548.2 kg/ha), followed by *S. x uplandicum* (374.7 kg/ha), *M. x giganteus* (246.7 kg/ha) and *C. riparia* (244.6 kg/ha), which reached a high result but not significantly equal to the previous ones. The others had values under 190 kg/ha. In the third year uptakes slightly increased with values ranging from 137.2 to 589.7 kg/ha. During the last year all N uptakes increased, going from 83.8 to 1143.6 kg/ha, with the exception of *A. donax* and *M. x giganteus*. The reduction was probably due to the lower fertilizer and water supply but also because the 3-year mean N contents were used. *A. donax* registered maximum N uptake during the entire experiment, significantly different from all other species, apart from in 2012 when it was not different from *A. lappa*. Its values rose from 305.9 to 1143.6 kg/ha and from the second year of cultivation it removed more nitrogen than the amount introduced by fertilization. Christian et al. (1997) also reported a plant uptake greater than the quantity supplied as input and arising from the soil nitrogen apart from miscanthus. The latter showed a good result from the second year. *A. lappa* also considerably raised its uptake in 2012 (from 81.4 to 590.0 kg/ha) but the year after it decreased again (290.5 kg/ha). Similarly *S. x uplandicum* rose from 118.2 to 374.7 and 362.1 kg/ha and then diminished to 83.8 kg/ha. This fall was mainly due to a low yield following transplantation. Both giant reed and miscanthus had maximum N uptakes higher than the maximum results found in the literature (Christian et al., 2008; Nassi o Di Nasso et al., 2013; Borin et al., 2013). Vymazal (2011) reported aboveground N uptake in the range of 220-880 kg/ha for species in natural wetlands (Vymazal, 1995) and of 53-587 N kg/ha for constructed wetlands (Vymazal and Kröpfelová, 2008), thus our macrophytes data were marginally lower.

During the first growing year *S. x uplandicum* and *A. donax* gave the highest P uptakes (14.6 and 14.2 kg/ha, respectively), all the other species had values under 7.5 kg/ha. In 2011 *S. x uplandicum* again showed the best result (69.3 kg/ha) followed by *A. donax* (60.6 kg/ha) and *M. x giganteus* (43.0 kg/ha). Similar results were obtained in 2012: *S. x uplandicum* with the highest P uptake (115.8 kg/ha), then *A. donax* (102.4 kg/ha) and *A. lappa* (99.3 kg/ha). The last year showed reduced results, going from 13.3 to 155.6 t/ha, apart from *A. donax* and *M. x giganteus*, which remained stable. Also for P uptake the increased trend is related to the lower inputs. From 2010 to 2012 *S. x uplandicum* showed an unexpectedly high value, due to its high P tissue concentration, increasing yearly (14.6, 69.3 and 115.8 t/ha, respectively) and was significantly different from all the other species, apart from 2010, when it was not significantly different from *A. donax* (14.2 kg/ha). *A. donax* and *M. x giganteus* also demonstrated good P uptakes, mainly due to their high yields. In the literature, macrophytes generally have results in the range of 1-110 kg/ha (Vymazal, 2011).

Table 21 Mean nitrogen uptake of the studied plant species in 2010, 2011, 2012 and 2103. Different letters indicate significant differences at P< 0.05 by LSD test.

Species	2010				2011				2012				2013			
	mean		s.d.		mean		s.d.		mean		s.d.		mean		s.d.	
	kg/ha				kg/ha				kg/ha				kg/ha			
A. lappa					81	±	32	def	590	±	114	a	291	±	196	bc
A. donax	306	±	200	a	548	±	286	a	528	±	265	ab	1144	±	167	a
C. indica	106	±	19	b												
C. acutiformis	84	±	17	b	245	±	117	bc	219	±	60	cd	111	±	32	d
C. pseudocyperus	89	±	34	b												
C. riparia	100	±	9	b	220	±	42	cd	159	±	115	cd	99	±	21	d
G. maxima					80	±	25	def	154	±	62	cd				
H. tuberosum					140	±	48	cdef	202	±	71	cd	95	±	20	d
I. pseudacorus	108	±	57	b	190	±	81	cdef	137	±	57	d	104	±	9	d
L. salicaria									142	±	74	cd	148	±	24	cd
M. x giganteus	91	±	11	b	247	±	119	def	272	±	142	cd	309	±	162	bc
P. arundinacea					5	±	4	f								
S. sylvaticus	65	±	10	b	49	±	14	ef								
S. x uplandicum	118	±	15	b	375	±	103	b	362	±	335	bc	84	±	38	d

Table 22 Mean phosphorus uptake of the studied plant species in 2010, 2011, 2012 and 2103. Different letters indicate significant differences at P< 0.05 by LSD test.

Species	2010				2011				2012				2013			
	mean		s.d.		mean		s.d.		mean		s.d.		mean		s.d.	
	kg/ha				kg/ha				kg/ha				kg/ha			
A. lappa					6.8	±	2.5	ef	99.3	±	19.1	ab	28.4	±	19.1	cd
A. donax	14.2	±	9.3	a	60.6	±	37.4	ab	102.4	±	38.0	ab	155.6	±	22.7	a
C. indica	3.1	±	0.6	bcd												
C. acutiformis	4.6	±	1.0	bcd	22.6	±	6.2	de	36.1	±	10.2	bc	13.7	±	3.9	c
C. pseudocyperus	4.0	±	1.5	bcd												
C. riparia	7.5	±	0.6	b	29.4	±	4.6	cd	24.1	±	16.7	c	13.3	±	2.9	c
G. maxima					8.5	±	2.4	ef	30.8	±	15.4	c				
H. tuberosum					11.6	±	6.8	def	55.6	±	9.3	abc	18.2	±	3.8	c
I. pseudacorus	2.8	±	1.5	cd	22.6	±	3.9	de	25.6	±	10.5	c	14.3	±	1.3	c
L. salicaria									34.0	±	22.0	c	32.9	±	5.4	c
M. x giganteus	6.7	±	0.8	bc	43.0	±	21.3	bc	51.5	±	21.1	abc	55.2	±	29.0	b
P. arundinacea					0.5	±	0.3	f								
S. sylvaticus	1.4	±	0.2	d	5.1	±	1.2	d								
S. x uplandicum	14.6	±	1.8	a	69.3	±	15.6	a	115.8	±	146.1	a	17.7	±	8.1	c

Furthermore, *A. donax*, *C. acutiformis*, *C. riparia*, I. *pseudacorus*, *M. x giganteus* and *S. x uplandicum* were analysed by a two-way ANOVA, being cultivated for 4 successive years. N and P uptake means were then separated by LSD test. Species, year and also interaction species-year were significantly different at P< 0.001 (Figure 11).

The different harvest management was also tested for each species with a two-way ANOVA. Regarding N uptake, interaction year-harvest was significant only for *M. x giganteus*, which was also influenced by both main effects. *A. donax*, *I. pseudacorus* and *S. x uplandicum* had no significant differences among year nor harvest. The latter was significant for all the remaining species, which had higher N uptake with MH. *C. riparia* had significantly higher yields in 2010, while the remaining species through MH. Interaction year-harvest was significant only for *C. riparia*, as concerns P uptake. *A. donax*, *I. pseudacorus* and *S. x uplandicum* had no significant differences among year nor harvest. *C. acutiformis* obtained higher P uptake in 2011 and with MH. Harvest was significant also in *C. riparia* and *M. x giganteus*.

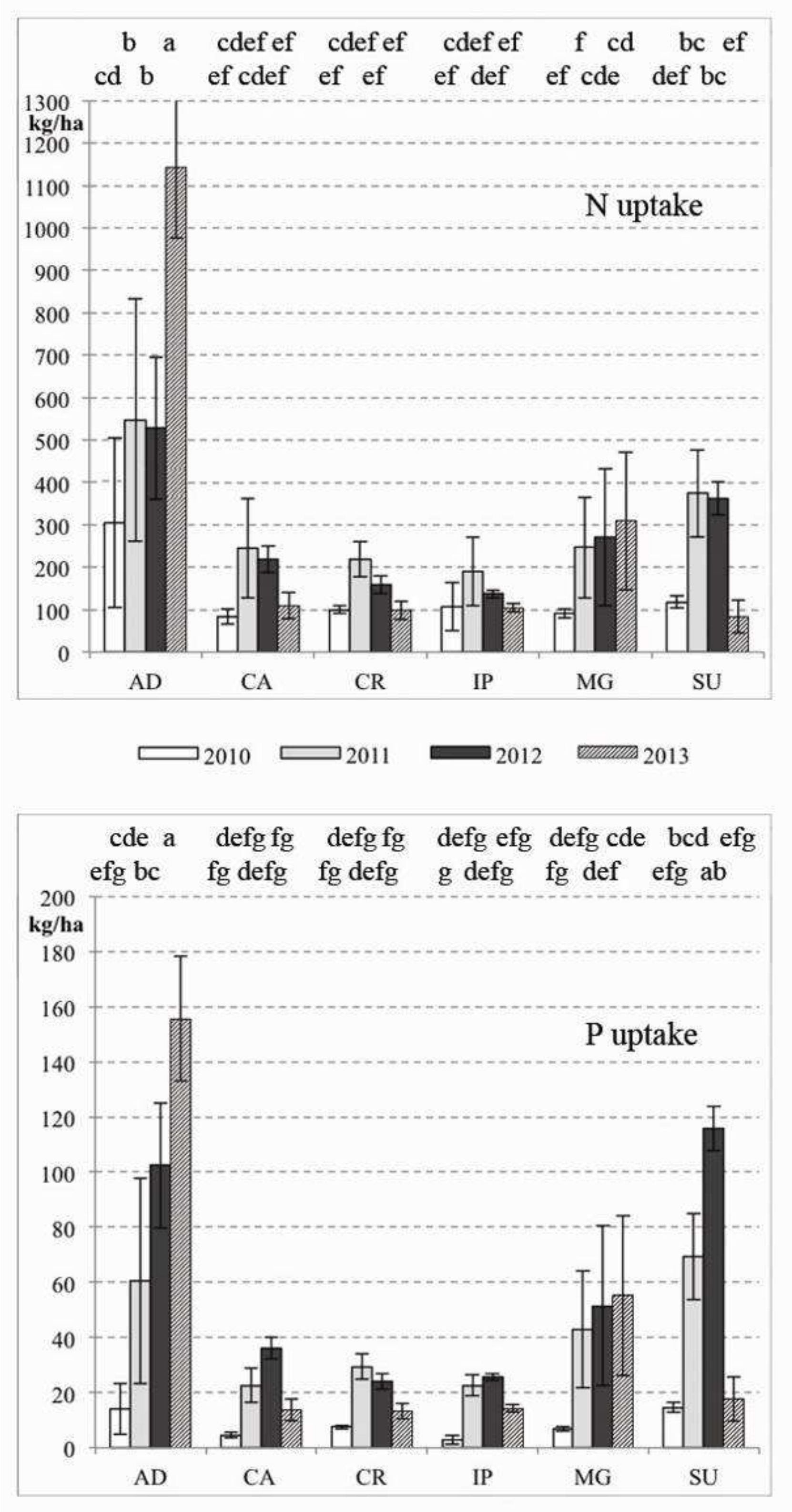

Figure 11 Nitrogen and phosphorus uptakes of the plant species grown for 4 successive years, from 2010 to 2103. AD: *A. donax*, CA: *C. acutiformis*, CR: *C. riparia*, IP*: I. pseudacorus*, MG: *M. x giganteus*, SU: *S. x uplandicum*

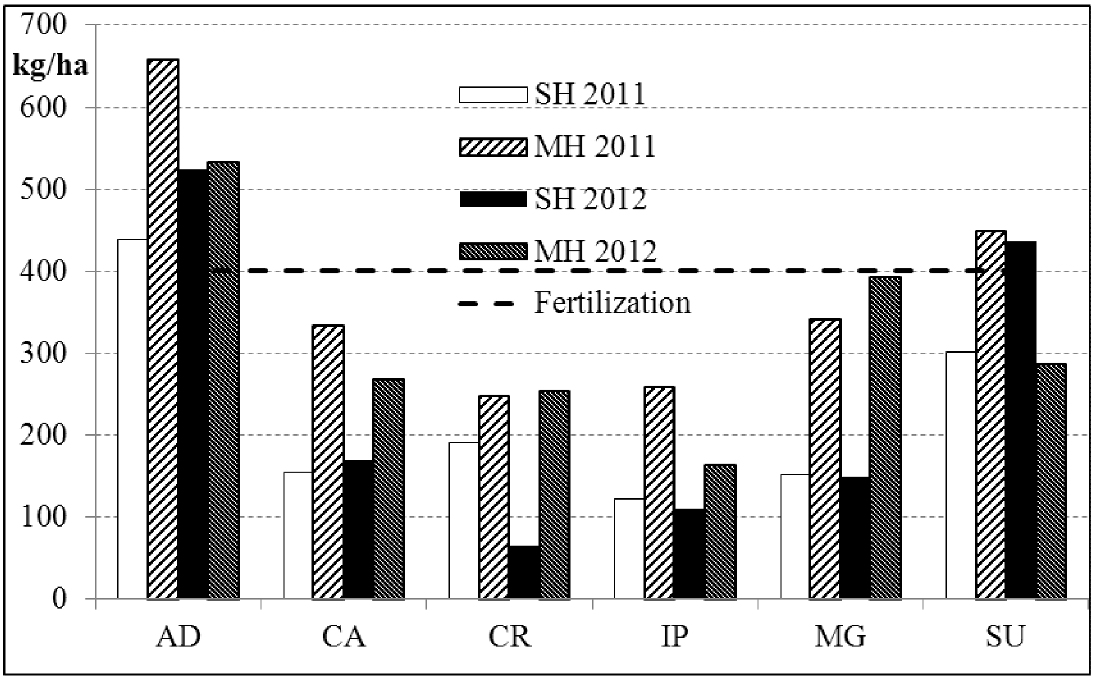

Figure 12 Comparison of single harvest (SH) and multiple harvests (MH): mean nitrogen uptake of the studied plant species in 2011 and 2012. AD: *A. donax*, CA: *C. acutiformis*, CR: *C. riparia*, IP*: I. pseudacorus*, MG: *M. x giganteus*, SU: *S. x uplandicum*

Table 23 Significant differences by two-way ANOVA in Figure 12

Species	Year	Harvest	Interaction
A. donax	n.s.	n.s.	n.s.
C. acutiformis	n.s.	*	n.s.
C. riparia	n.s.	**	n.s.
I. pseudacorus	n.s.	*	n.s.
M. x giganteus	***	*	***
S. x uplandicum	n.s.	n.s.	n.s.

n.s.: non-significant; *: $p<0.05$; **: $p<0.01$;***: $p<0.001$.

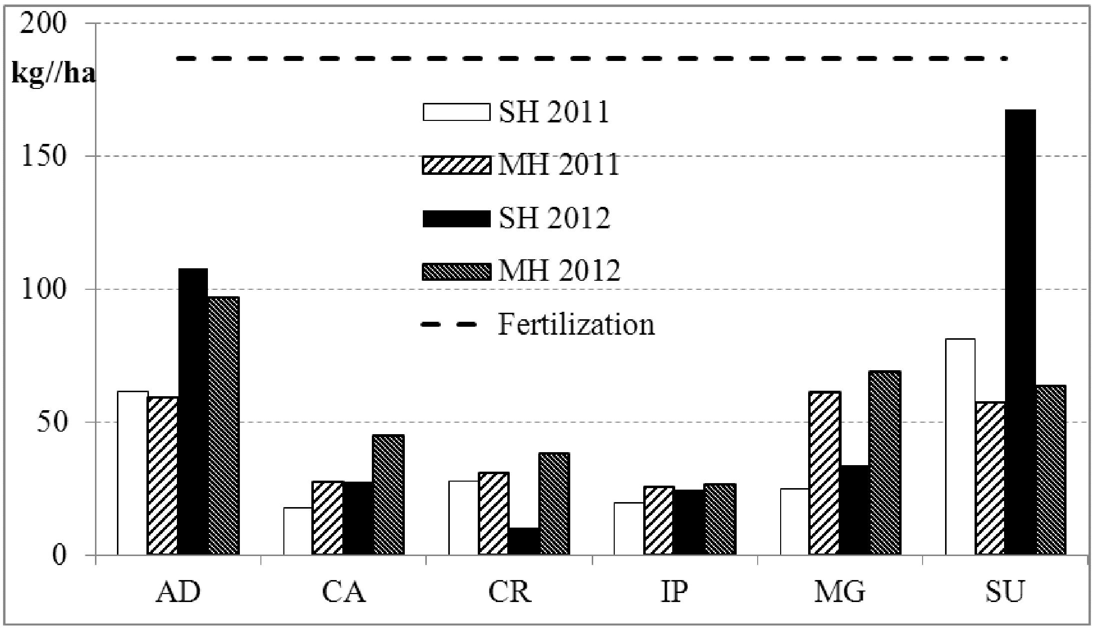

Figure 13 Comparison of single harvest (SH) and multiple harvests (MH): mean phosphorus uptake of the studied plant species in 2011 and 2012. AD: *A. donax,* CA: *C. acutiformis,* CR: *C. riparia,* IP*: I. pseudacorus,* MG: *M. x giganteus,* SU: *S. x uplandicum*

Table 24 Significant differences by two-way ANOVA in Figure 13

Species	Year	Harvest	Interaction
A. donax	n.s.	n.s.	n.s.
C. acutiformis	**	**	n.s.
C. riparia	n.s.	*	*
I. pseudacorus	n.s.	n.s.	n.s.
M. x giganteus	n.s.	**	n.s.
S. x uplandicum	n.s.	n.s.	n.s.

n.s.: non-significant; *: p<0.05; **: p<0.01;***: p<0.001.

Lastly cumulative N and P apparent balances were carried out from 2010 to 2013 for the plant species grown for 4 successive years. Nitrogen showed negative values only for *A. donax*. This means that on average only giant reed recovered all the N applied and also adsorbed it from the soil, as reported by Borin et al. (2013). On the other hand, the remaining species and overall P apparent balance reported positive values.

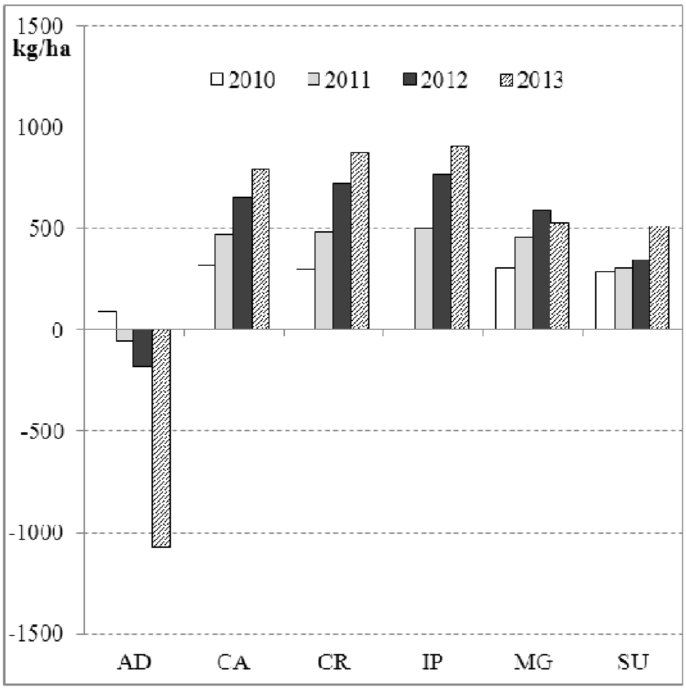

Figure 14 Cumulative N apparent balance from 2010 to 2013 for the plant species grown for 4 successive years. AD: *A. donax*, CA: *C. acutiformis*, CR: *C. riparia*, IP*: I. pseudacorus*, MG: *M. x giganteus*, SU: *S. x uplandicum*

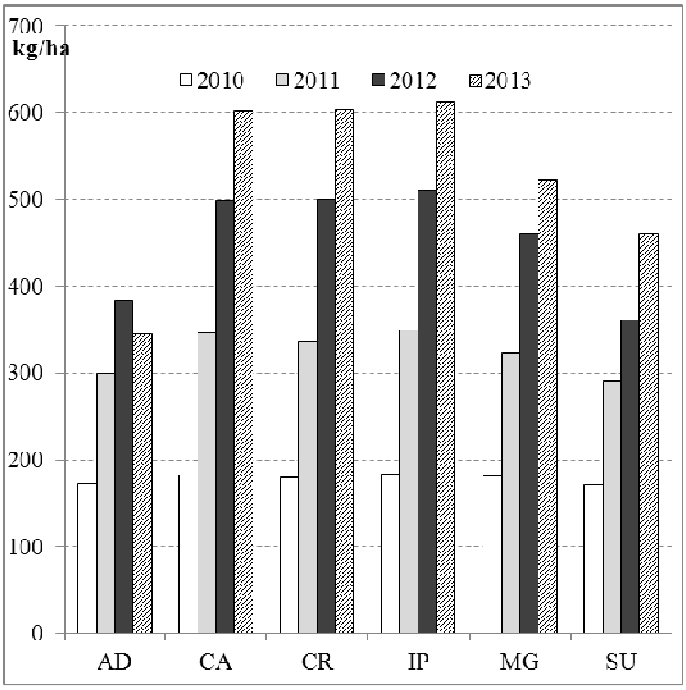

Figure 15 Cumulative P apparent balance from 2010 to 2013 for the plant species grown for 4 successive years. AD: *A. donax*, CA: *C. acutiformis*, CR: *C. riparia*, IP*: I. pseudacorus*, MG: *M. x giganteus*, SU: *S. x uplandicum*

Percolation water

The three winters can be distinguished by their trends (from Figure 16 to Figure 19). Water in winter 2010/2011 showed the higher values with a peak in December and a gradual decline over the successive months. Winter 2011/2012 did not allow an adequate number of samples to be collected but it was anyway possible to note that December had lower concentration than October. In the last season (winter 2012/2013), percolation waters presented the lowest values, apart from NO_3-N, which fluctuated widely (Figure 17).

To compare the initial and final percolation water during the experiment, data were presented in box-plots and the medians were contrasted within the year by Kruskal-Wallis test at $p < 0.05$.

Total nitrogen showed higher median concentrations in 2010 (from 7.50 to 38.46 mg/L) than 2013 (from 1.52 to 4.33 mg/L). Among species *C. acutiformis* had the highest median values for both years while the lowest were obtained by *S. x uplandicum* and *I. pseudacorus*, in 2010 and 2013 respectively (Figure 20). Variability also decreased over the years.

The NO_3-N medians also decreased, ranging from 0.39 to 7.08 mg/L during the first years and from 0.00 to 2.25 mg/L in 2013. In 2010 nitric nitrogen showed the highest concentration in *C. acutiformis* and the lowest in *M. x giganteus*. In 2013 no significant differences were found among species (Figure 20).

Total phosphorus showed higher and more variable concentrations in 2013, when median TP varied from 0.060 to 0.145 mg/L, than in 2010 (from 0.025 to 0.034 mg/L) (Figure 21).

PO_4-P values diminished, varying from 0.009 to 0.019 mg/L the first winter and from 0.001 to 0.010 mg/L in 2013.

For both phosphorus parameters no significant differences were detected among species.

Data were also compared overall by Kruskal-Wallis test at $p < 0.05$ and significant differences were found for TN, NO_3-N and PO_4-P, for which water samples were significantly higher in winter 2010/2011 than in 2012/2013.

In general it is worth noting that median percolated water values were lower than the corresponding supply for each parameter. Regarding nitrate nitrogen, Bonaiti and Borin

(2010) with yearly input of 240 kg N/ha, in a field trial with the same soil, reported average values in the groundwater ranging from 0 to 90 mg/L. Studies in the Po Valley with similar input showed higher N content in agricultural water (Perego et al., 2012).

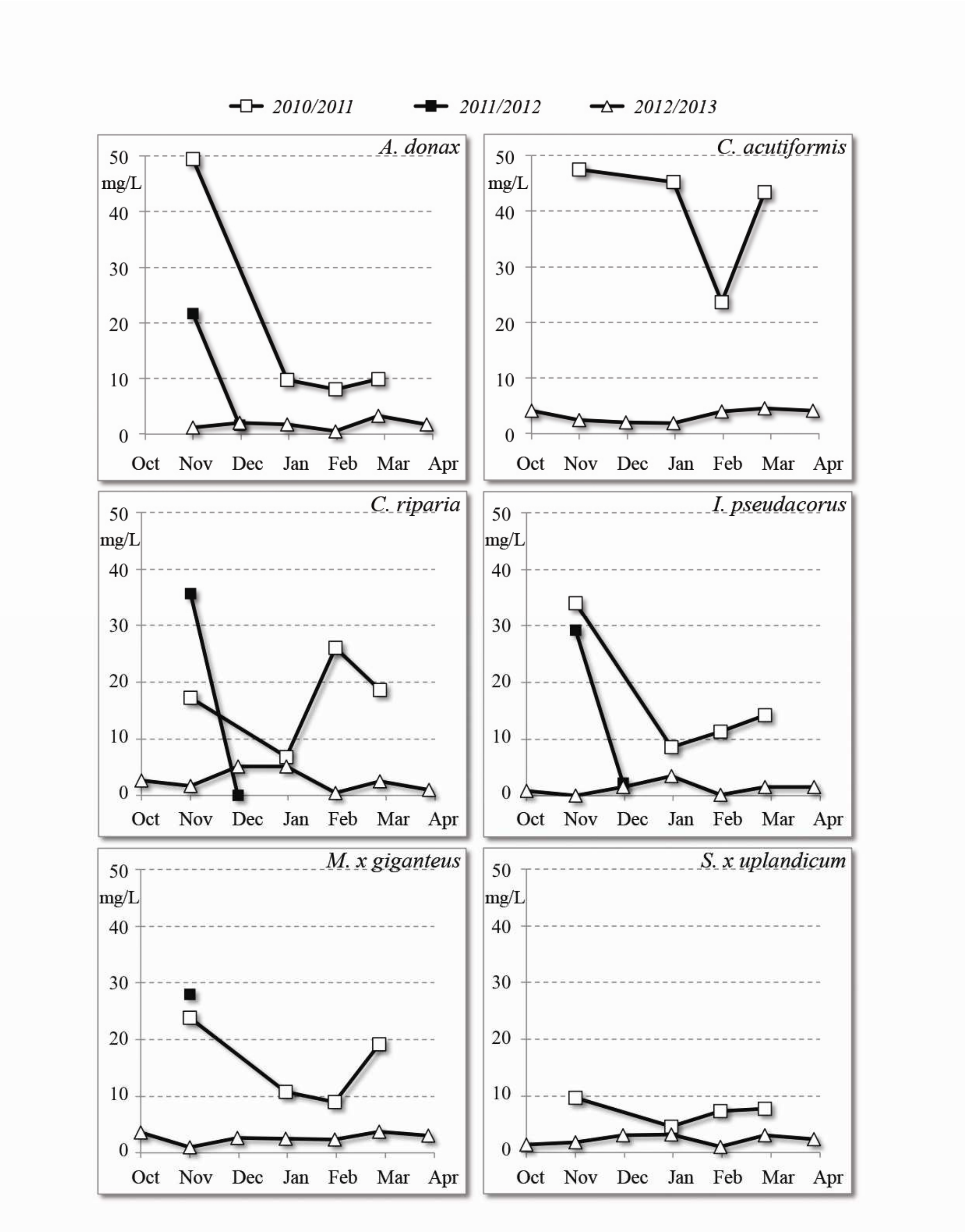

Figure 16 Comparison of total nitrogen concentration in percolated water, from 2010 to 2103.

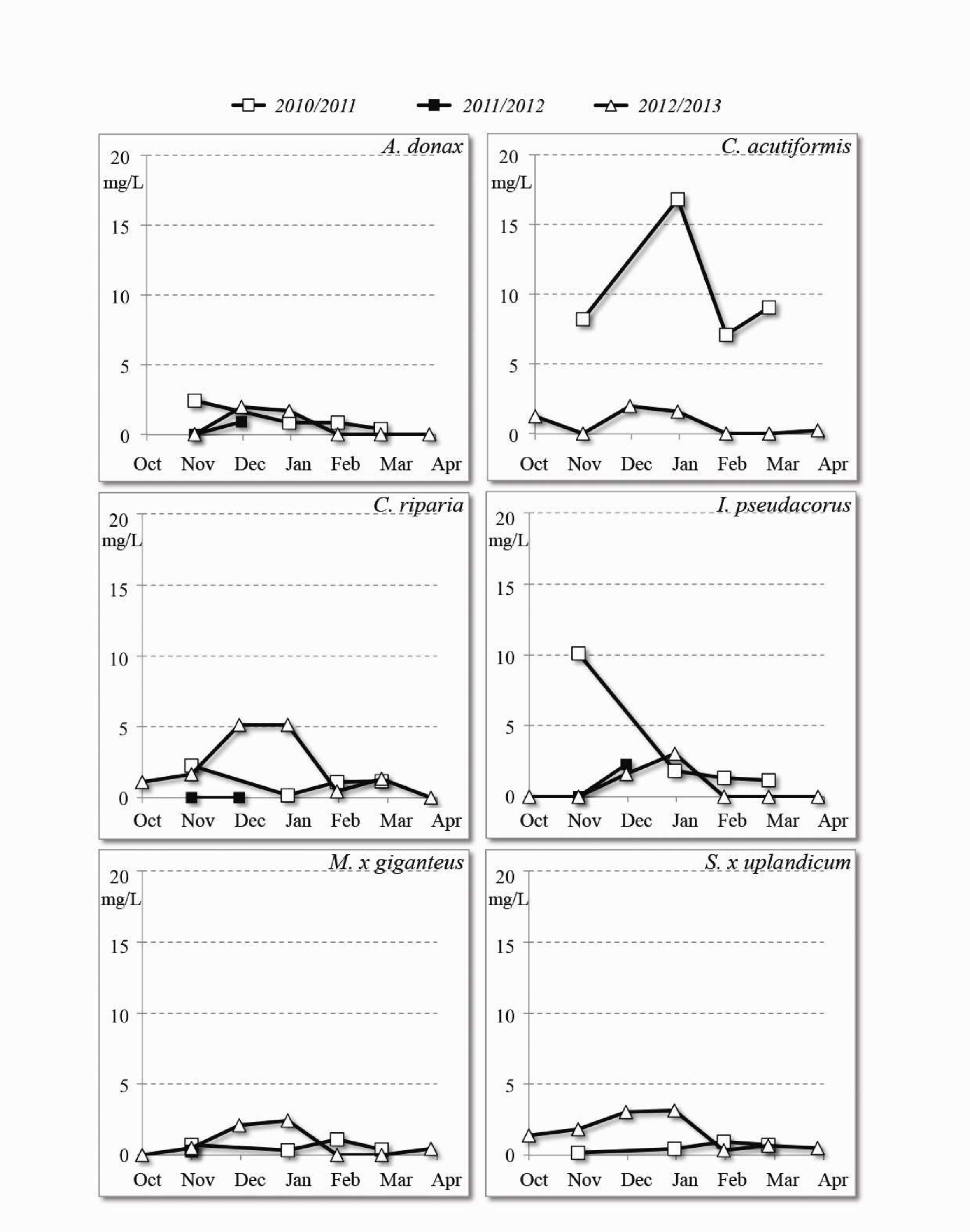

Figure 17 Comparison of nitrate nitrogen concentration in percolated water, from 2010 to 2103.

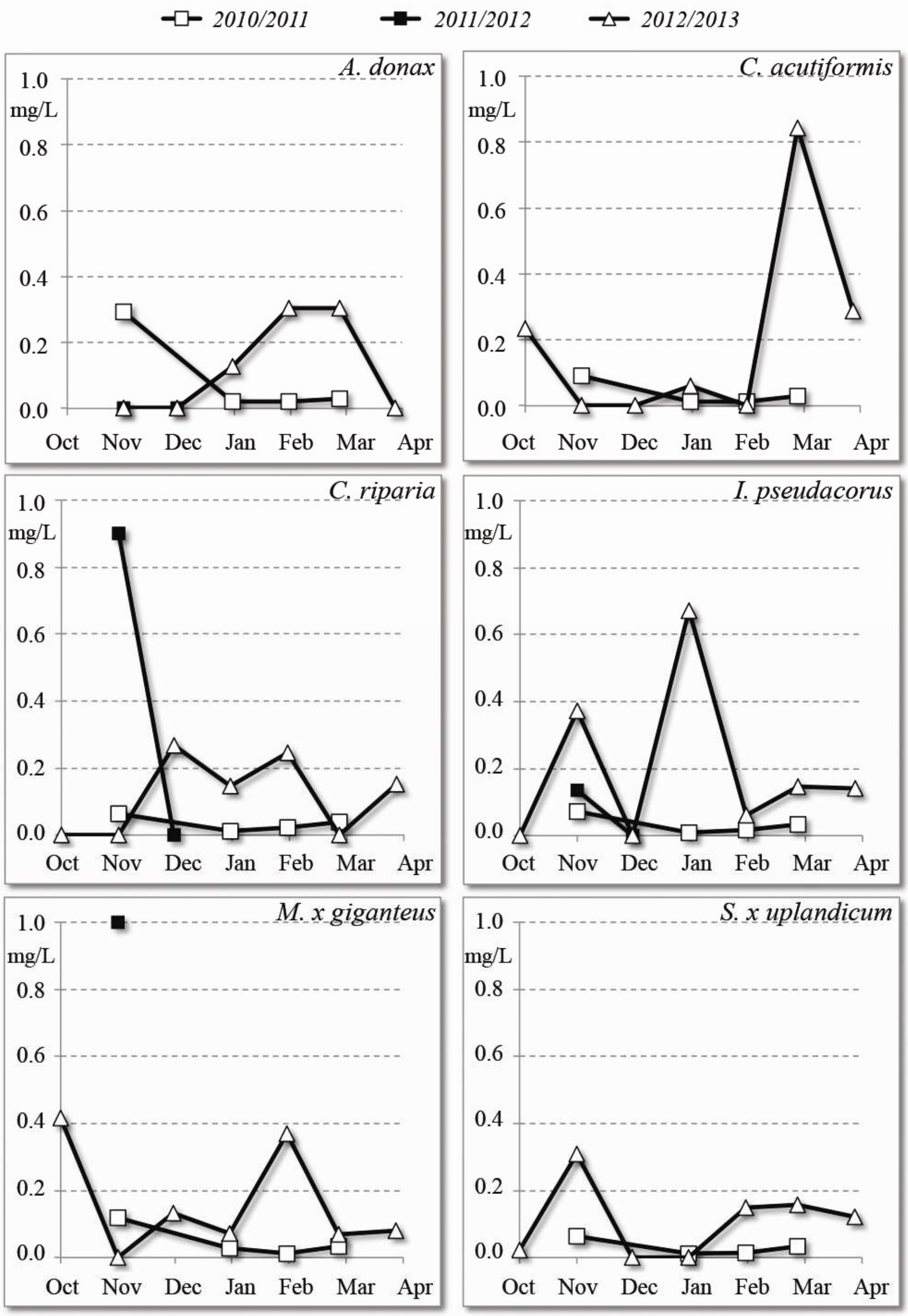

Figure 18 Comparison of total phosphorus concentration in percolated water, from 2010 to 2103.

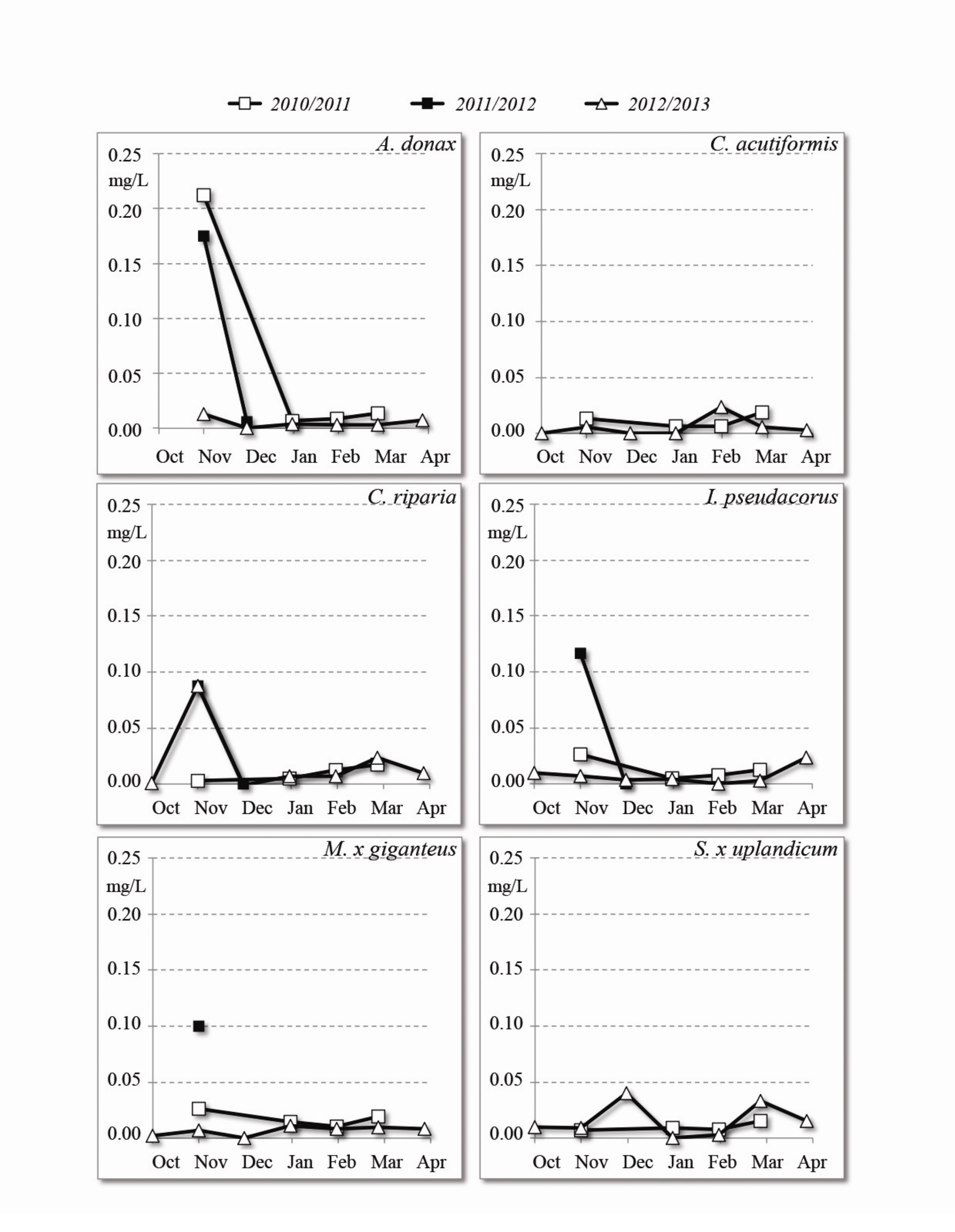

Figure 19 Comparison of orthophosphate concentration in percolated water, from 2010 to 2103.

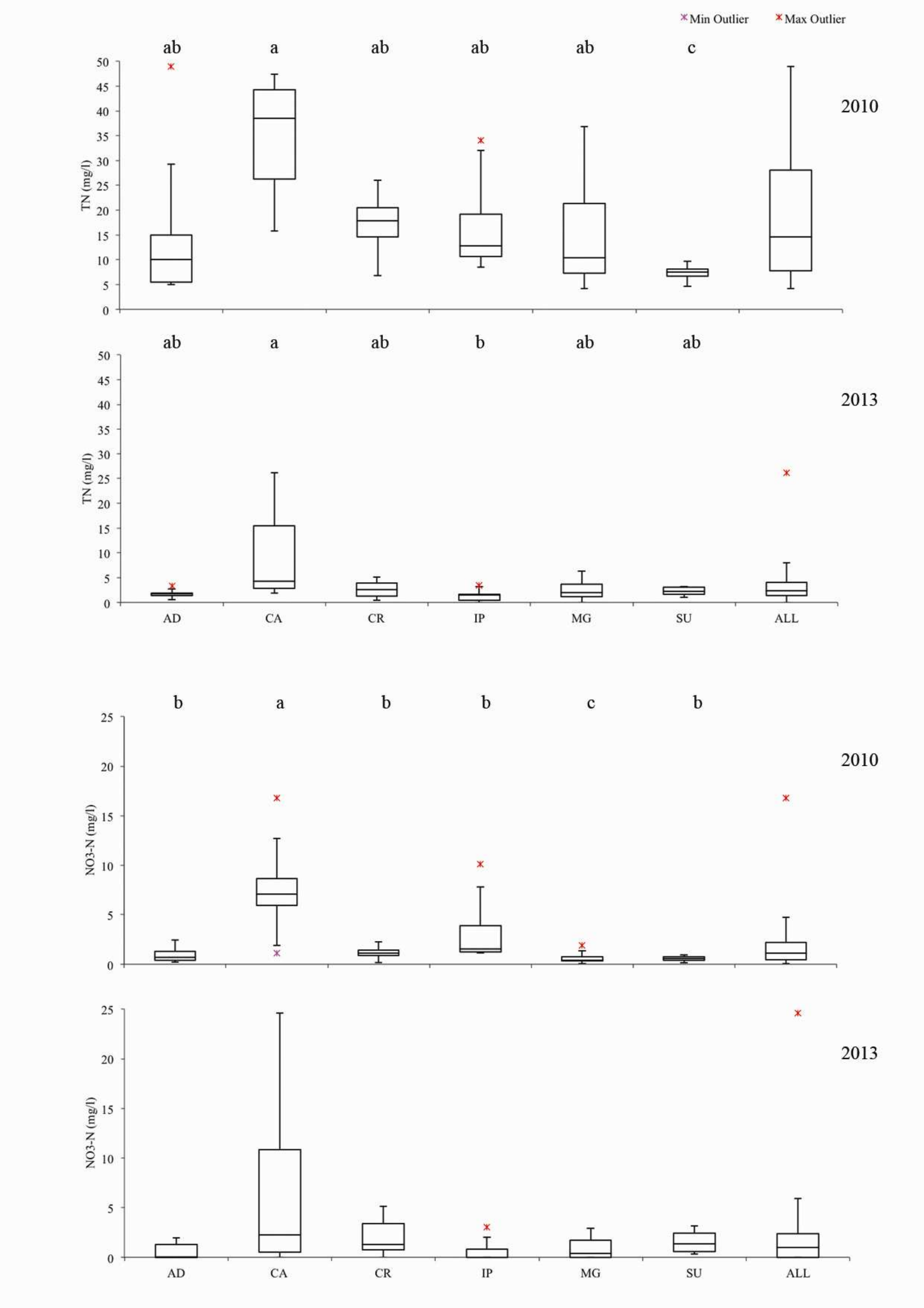

Figure 20 Box-plots of nitrogen forms concentration in 2010 and 2013. AD: *A. donax*, CA: *C. acutiformis*, CR: *C. riparia*, IP: *I. pseudacorus*, MG: *M. x giganteus*, SU: *S. x uplandicum*, ALL: all species

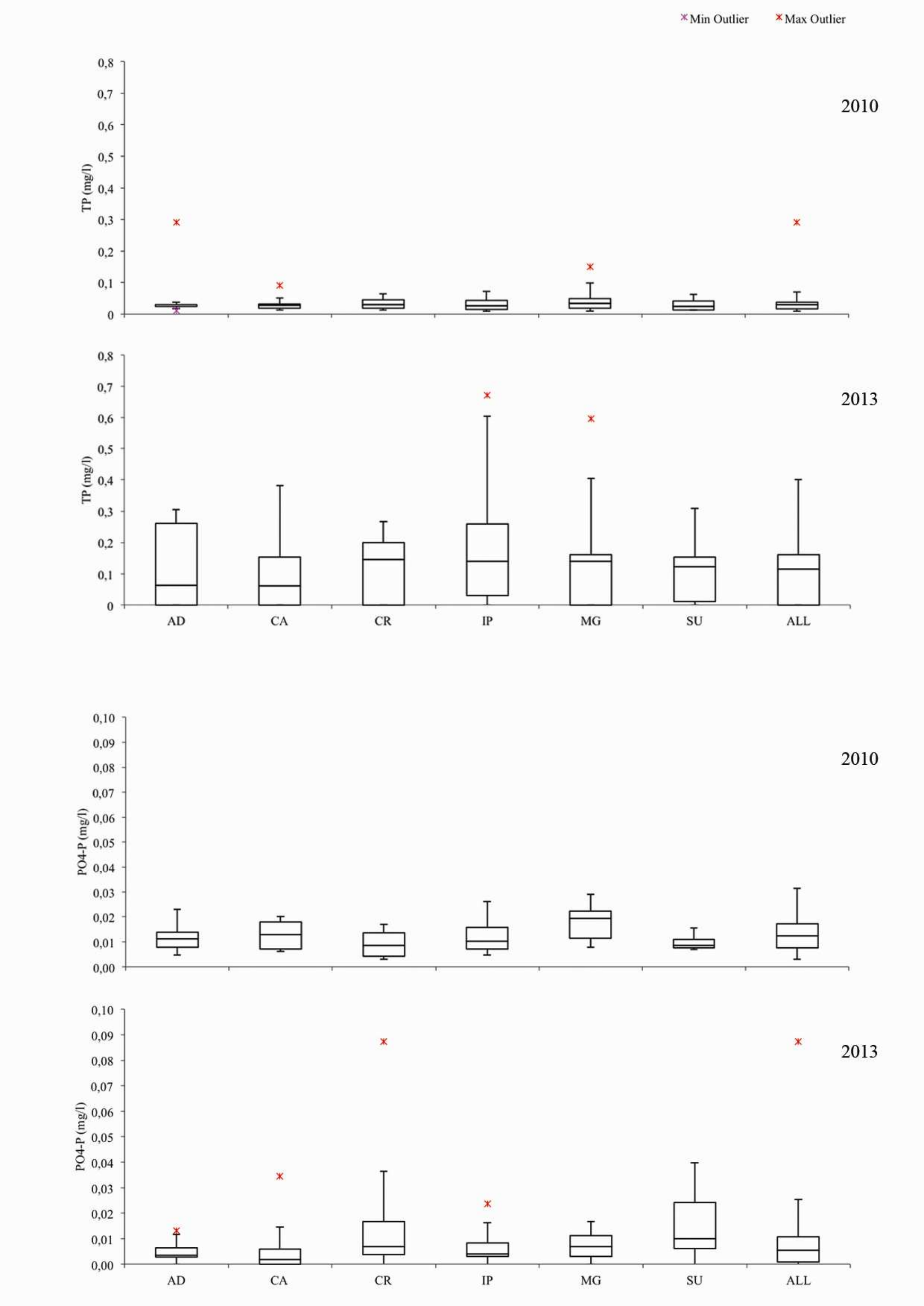

Figure 21 Box-plots of phosphorus forms concentration in 2010 and 2013. AD: *A. donax*, CA: *C. acutiformis*, CR: *C. riparia*, IP*: I. pseudacorus*, MG: *M. x giganteus*, SU: *S. x uplandicum*, ALL: all species

Soil

From 2010 to 2013 organic carbon measured in the soil showed mean values varying from 1.6 to 1.2% for the top layer (0-20 cm) and from 1.5 to 0.9% in the 20-50 cm layer (Figure 22).

Top layer soil showed higher nitrogen content. It did not vary noticeably: from 2010 to 2013 a slight decrease (0.02%) was detected in the mean values for both layers (Figure 23).

Unlike the previous ones, the sulphur content increased during the trial (Figure 24).

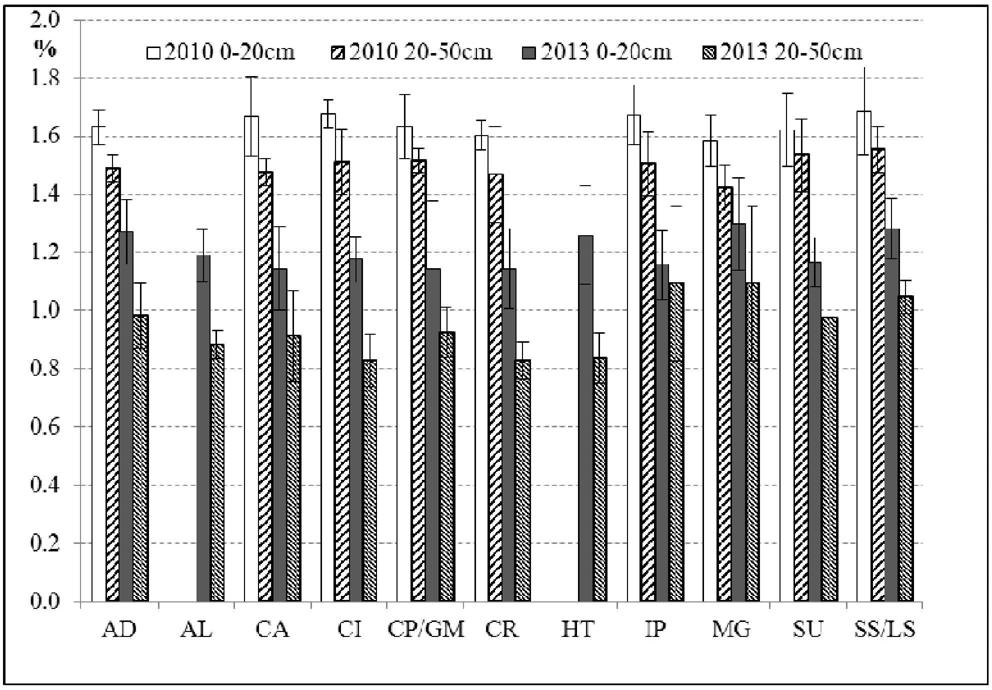

Figure 22 Organic carbon content in two soil layers in 2010 and 2013. AD: *A. donax,* AL: *A. lappa,* CA: *C. acutiformis,* CP/GM: *C. pseudocyperus/G. maxima,* CR: *C. riparia,* HT*: H. tuberosus,* IP*: I. pseudacorus,* MG: *M. x giganteus,* SU: *S. x uplandicum,* SS/LS: *S. sylvaticus/L. salicaria*

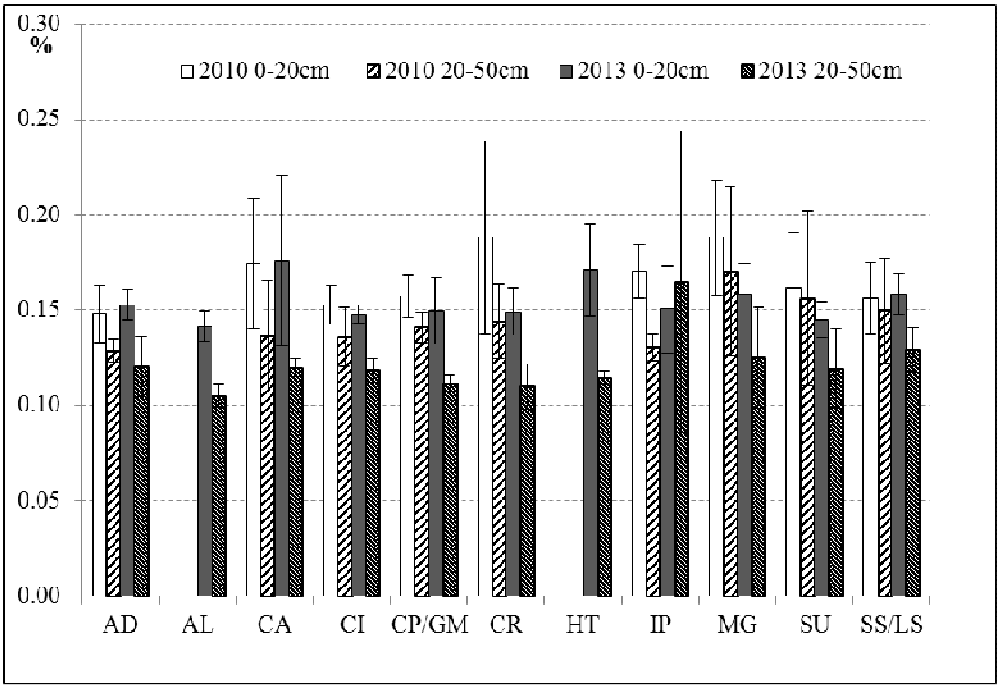

Figure 23 Nitrogen content in two soil layers in 2010 and 2013. AD: *A. donax,* AL: *A. lappa,* CA: *C. acutiformis,* CP/GM: *C. pseudocyperus/G. maxima,* CR: *C. riparia,* HT*: H. tuberosus,* IP*: I. pseudacorus,* MG: *M. x giganteus,* SU: *S. x uplandicum,* SS/LS: *S. sylvaticus/L. salicaria*

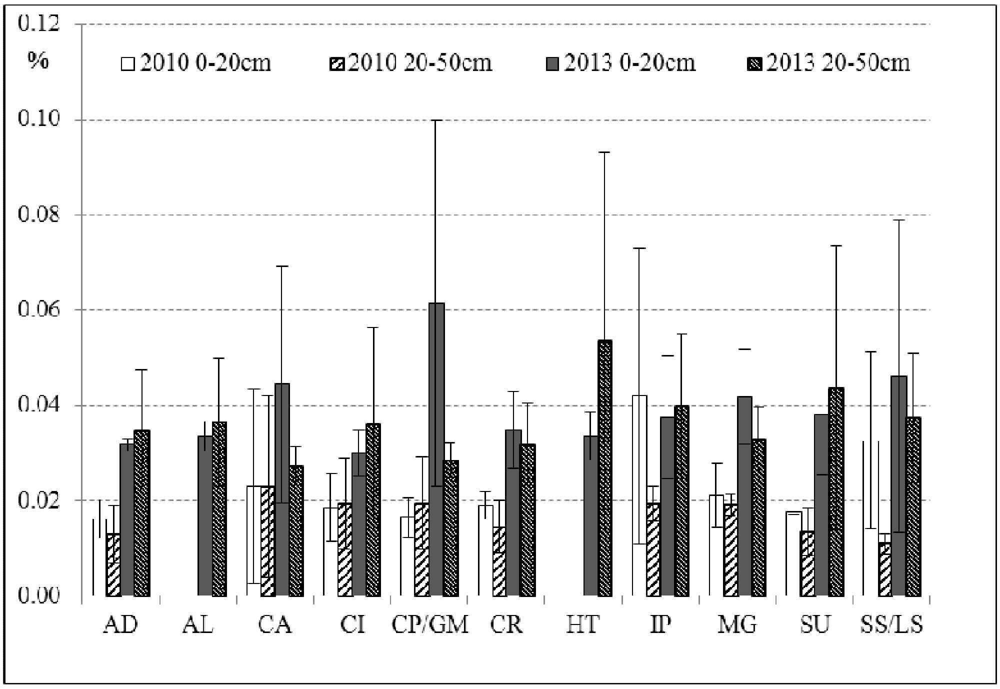

Figure 24 Sulphur content in two soil layers in 2010 and 2013. AD: *A. donax,* AL: *A. lappa,* CA: *C. acutiformis,* CP/GM: *C. pseudocyperus/G. maxima,* CR: *C. riparia,* HT*: H. tuberosus,* IP*: I. pseudacorus,* MG: *M. x giganteus,* SU: *S. x uplandicum,* SS/LS: *S. sylvaticus/L. salicaria*

Conclusions

From 2010 to 2013, 14 species were evaluated in total, 6 of them were grown for 4 consecutive years. These plants are perennial so further experimenting might provide more exhaustive conclusions, but from the data obtained, the following observations can be made.

All the species received the same amount of water but their ET differed in the analysed months and was generally higher than ET_0. Soil moisture was lower in May and October, due to the reduced rainfall and water supply . In general moisture at 10 cm depth had a range of values from 5 to 20 mm then it increased until stabilizing at 60 cm depth with 40 mm for all species. Kc results reflected ET ones: higher Kc were obtained by *C. indica*, *C. pseudocyperus* and *P. arundinacea* while *A. lappa riparia* and *S.* x *uplandicum* gave the lowest values. In general it was observed that all species had higher Kc than maize.

The studied species revealed different growth rates according to the BBCH scale. Both *Carex* showed early behaviour. Beginning of senescence started at the end of May for *C. acutiformis*, followed by *C. riparia* in June, followed gradually by the other species. Overall *A. donax* and *M. x giganteus* showed the most tardive behaviour.

The biomass characterization in terms of elements and fibre varied among species, but results were similar to those reported in the literature, when available.

It is worth reporting that the best results in nitrogen and phosphorus median content were given by *S. x uplandicum* while the lowest were provided by *H. tuberosum*, *A. donax* and *M. x giganteus.*

Nevertheless the highest uptakes were obtained by the latter, due to their remarkable biomass yields. Effectively *A. donax* gave the highest biomass yields, increasing yearly (26.2, 62.8, 95.1 and 140.1 t/ha, from 2010 to 2013 respectively) and was significantly different from all the other species, apart from in 2011, when it was not significantly different from *M. x giganteus* (55.2 t/ha). Overall the most productive species were *A. donax*, *M. x giganteus* and *H. tuberosus*. Similarly 2010-2013 higher mean N and P uptakes were obtained by *A. donax*, *A. lappa* and *S. x uplandicum* but it must be said that the cumulative N apparent balance showed negative values only for *A. donax*. This means that on average only giant reed recovered all the N applied and also adsorbed it from the soil, as reported by Borin et al. (2013).

In general yields and uptake increased from 2010 to 2012 and then decreased in the last year, due to the lower fertilizer and water supply. However the increasing trends have been retained for the entire trial by *A. donax* and *M. x giganteus*. Their maximum dry biomass yields and uptake were higher than the maximum results found in the literature (Lewandowski et al., 2000; Hildago and Fernandez, 2001; Lewandowski et al., 2003; Christou et al., 2005; Angelini et al., Cosentino et al., 2007; 2009; Christian et al., 2008; Angelini et al., 2009; Mantineo et al., 2009; Nassi o Di Nasso et al., 2013; Borin et al., 2013), probably for the reason that a notable quantity of nitrogen and water were supplied during this experiment, as reported by Zema et al. (2012) and the small-scale trial that might positively affect the productivity. The different harvest management did not significantly affect the 6 species grown for 4 successive years. In particular MH gave higher yield in *C. acutiformis* and *M. x giganteus* and better uptakes for *M. x giganteus*, *C. acutiformis* and *C. riparia*.

It should be noted that moderate variability was found in yield and uptake results. This might again be due to the small-scale trial. Furthermore the different heights of species sometimes promoted growth of taller plants at the expense of the smaller species. Thus in further experiments an adequate positioning of species must be taken into account, besides amplifying the growing surface.

Comparing the initial and the final percolation water during the experiment, lower TN and NO_3-N median contents were found and variability among species decreased over the years. On the contrary TP showed higher and more variable concentration in 2013 but PO_4-P values diminished. In general the literature on N and NO_3-N with similar inputs in Northern Italy showed higher N content in agricultural water (Bonaiti and Borin, 2010; Perego et al., 2012).

Finally soil samples taken before and after the trial did not vary noticeably.

3 Ethanol and methane production

Introduction

The objectives of the work described in this chapter were:

- to characterize the species described in Chapter 2 in terms of potential ethanol and methane production per unit of dry matter (DM);
- to describe the potential ethanol and methane production in regard to the biomass yields obtained in Chapter 2;
- to estimate and compare the potential ethanol and methane production with SH and MH management for a selected number of species grown in Chapter 2;
- to calculate and compare the energy output potential of the produced ethanol and methane.

Since dried biomasses are easier to store, manage and transport, dry samples were used for both lines of energy production, in prospect of an application of those technologies.

Experimentation on ethanol production was done at the National Agency for New Technologies, Energy and Sustainable Economic Development (ENEA) - Biotechnical Laboratory Trisaia (Rotondella, Matera, Italy) and the methane trial (Marchetti et al., 2013) at the Agricultural Research Council (CRA) - Research Unit for Swine Husbandry (San Cesario sul Panaro, Modena, Italy).

Ethanol production

Ethanol production from ligno-cellulosic biomass consists of three steps: pretreatment, hydrolysis and fermentation.

Unlike the 1st-gen. materials, which come from available glucose sources, e.g. maize and sugar beet, the ligno-cellulosic ones, composed mainly of cellulose, hemicellulose and lignin, have to be pretreated before being hydrolysed. In fact cellulose chains interact with hemicellulose and lignin to form a lignin–carbohydrate complex, making it difficult to depolymerize them into fermentable sugars (Karim and Ryu, 2011). This is a very important phase of the process because it is a necessary stage (Alvira et al., 2010) and also represents the most expensive step of the entire procedure (Chiaramonti et al., 2012;

Ge et al., 2011). In fact the pretreatment should separate the main biomass components and make this material more accessible to the subsequent enzymatic reactions (Monsier et al., 2005a). A number of pretreatment options have been developed for the production of ligno-cellulosic ethanol, which can be divided into the following main categories (Chiaramonti et al., 2012):

1. Physical process (Size reduction);
2. Physico-chemical process (Autohydrolysis, Steam explosion, SO_2-added steam explosion, CO_2 explosion and Ammonia fibre explosion);
3. Chemical pretreatment (Acid hydrolysis, Alkaline hydrolysis and Organosol process);
4. Biological pretreatment.

In our experiment a bio-chemical pretreatment was chosen, according to the research aim of taking the sustainable aspects into account. Pretreatments using diluted acids to solubilize hemicellulose have been adopted for a wide variety of ligno-cellulosic biomasses (Alvira et al., 2010). Among acids, sulphuric acid (H_2SO) resulted as the most effective (Monsier et al., 2005b). Alkaline pretreatments, which allow lignin structure breakage (Taherzadeh and Karimi, 2008), have also been studied on this kind of biomass (Liang et al., 2010; Park et al., 2010; Hendricks et al., 2009; Hu et al., 2008). Sodium hydroxide (NaOH), calcium hydroxide (CaOH), potassium hydroxide (KOH) and ammonia (NH_3) can be used, but NaOH has been particularly studied (MacDonald et al., 1983; Soto et al., 1994; Zhao et al., 2008; Zhu et al. 2010).

As a second step, cellulose hydrolysis occurs, which transforms cellulose in fermentable sugars (Duff and Murray, 1996), through cellulase enzymes activity (Bhat and Bhat, 1997; Lynd et al., 2002). Cellulolysis yield is affected by temperature, pH (Saddler and Gregg 1998), residence time (Tengborg et al., 2001) and enzyme dosage (Sun and Cheng, 2002). The latter, due to its high price, sometimes represents an obstacle for ethanol commercialisation (Wyman, 2007). In our experiment a reduced amount of enzymes was applied.

Lastly glucose is fermented to ethanol. The microorganism mainly used for ligno-cellulosic hydrolysed biomasses fermentation is *Saccharomyces cerevisiae* (Olsson and Haan-Hagerdal, 1993) and its optimum temperature is reported to be 37 °C at a PH of 5 (Alfani et al., 2000). An ENEA selected strain (Picco et al., 2012) was used in this trial.

Separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) are two principal process configurations for the production of bioethanol from ligno-cellulosic biomass (Ask et al., 2012). In the first, enzymatic hydrolysis and fermentation are carried out separately, allowing each process to be run under its optimum conditions. In SFF, on the other hand, the glucose produced can be converted rapidly into ethanol, reducing time and cost (Olofsson et al., 2008), but all processes are carried out in suboptimal conditions.

Materials and methods

The dry biomass samples of each species were processed with a three-step chemical pretreatment to recover as much cellulose as possible, the cellulose was then hydrolysed with a mix of commercial enzymes to obtain glucose that was lastly fermented to obtain ethanol. The latter was first carried out for all species in Erlenmeyer flasks; in a second phase the fermentation was carried out on a selection of species (*A. donax*, *C. riparia*, *M. x giganteus* and *S. x uplandicum*) in 5 L bioreactors. Since hydrolysis and fermentation were executed at different temperatures, 40 and 30 °C respectively, both trials can be considered as SHF processes. Analyses and experiments were repeated three times in flasks and once in bioreactors. The ethanol yield per hectare was lastly calculated by multiplying the biomass yield, obtained in Chapter 2, with the ethanol yield. Finally SH and MH management in terms of ethanol production were compared for *A. donax, C. riparia, M. x giganteus* and *S. x uplandicum*.

Erlenmeyer flasks screening

For each species the milled biomass (5% DM) was treated first with diluted H_2SO (2%) at 80 °C for 24 hours, secondly with diluted NaOH (1%) at 40 °C for 24 hours, finally concentrated H_2O_2 was added until 1% concentration at 25 °C for 24 hours. This pretreated material (5 g) was then hydrolysed in an Erlenmeyer flask (liquid volume 500 ml) shaken at 100 rpm with an adequate liquid substrate (Albergo et al., 2013) and a mix of commercial enzymes: 20 FPU/g of Celluclast 1.5L and 30 CBU/g of Novozym 188, 60% and 22% dosage respectively, as used in Bauer and Gibbons (2012). The experiments were conducted at 40 °C and pH 5 for 72 hours and glucose was monitored afterwards by HPLC Varian (SpectraLab Scientific Inc., USA). The theoretical glucose productivity percentage was calculated for each studied plant as follows:

$$G_t = CEL \times G_s \quad (3)$$

$$G_y = G_m / G_t \times 100 \quad (4)$$

Where G_t is theoretical measured glucose, CEL is measured cellulose, G_s is glucose stoichiometric yield (1.111) and G_m is measured glucose.

NaOH was then added to the Erlenmeyer flasks with hydrolysed matter and 1 g/l of *Saccharomyces cerevisiae* M861/10a was inoculated. The fermentation was at 30 °C and 110 rpm for 24 hours. At the end glucose residuals and ethanol product were measured by HPLC.

The theoretical ethanol productivity percentage for each studied plant was calculated as follows:

$$E_t = G_m \times E_s \quad (5)$$

$$E_y = E_m / E_t \times 100 \quad (6)$$

Where E_t is theoretical ethanol, E_s is ethanol stoichiometric yield (0.511) and E_m is measured ethanol.

Analyses and experiments were repeated three times.

Bioreactor scale trial

The experiment was done for *A. donax*, *C. riparia*, *M. x giganteus* and *S. x uplandicum* in a similar vein to the Erlenmeyer flasks, but with 150 g of biomass in 5 L BIOSTAT B bioreactors (Sartorius BBI Systems GmbH, Germany). The experiment was repeated once.

Results and discussion

Pretreatment allowed a cellulose recovery, which varied from 63 (*H. tuberosum*) to 90% (*I. pseudacorus*). Hemicellulose solubilisation was very similar among species, between 90 and 95 %, a part for *I. pseudacorus*, *L. salicaria* and *M. x giganteus*, (79, 79 and 87% respectively). On the contrary lignin solubilisation data had a wide variability, going from 49 (*L. salicaria*) to 95 (*S. sylvaticus*) (Table 25).

Glucose yield varied a lott, ranging from 21 (*L. salicaria*) to 83% (*S. x uplandicum*).

Ethanol yields in flasks gave different values among species, going from 3.5 to 17.4 g/L. Best results were achieved by *C. riparia* and *G. maxima*, followed by *P. arundinacea* while lower yields were obtained by *L. salicaria*, *H. tuberosum* and *A. donax* (Table 26).

As expected, generally ethanol data were lower than values reported in the literature for 1st gen. ethanol, which can reach 99% of the theoretical maximum (Patzek, 2006; Quintero et al., 2008; Pin et al., 2008; Davila-Gomez et al., 2011). For maize, sugar beet, sugar cane and sweet sorghum ethanol, annual yields can vary from 4,700 to almost 10,000 L/ha (Zhang et al., 2010; Sánchez and Cardona, 2008; Agrocadenas, 2006; Poitrat, 1999).

This was due to the lignin presence, which is the most recalcitrant component to biodegradation (Taherzadeh and Karimi, 2008) and also hampers cellulose biodegradability and its availability to enzymes reactions (Smith et al., 2010). But at the same time cellulose percentage in the biomass was not correlated to ethanol yields (data not shown), as reported by other authors (Corredor et al., 2009; Capecchi et al., 2013) Effectively some species, even if the fibre content was similar after pretreatment (data not shown), produced different amounts of ethanol during fermentation.

Generally higher ethanol yields from ligno-cellulosic biomasses are reported in the literature, varying from 300 to 450 kg ethanol/t DM due to different pretreatment or fermentation (Ge et al., 2011; Scordia et al., 2010; Kallioinen et al., 2012; Scordia et al., 2013).

Ethanol yield per hectare was assessed by ANOVA test within the year. There were differences among species, so means were compared by Fisher's Least Significant Difference (LSD) Test (Table 27). In 2010 ethanol production varied from 0.36 (*A.*

donax) to 1.25 t/ha (*S. sylvaticus*). The second year species gave values ranging from 0.04 to 3.71 t/ha, higher than the previous year; *M. x giganteus* showed the highest mean yield. Not all species produced more ethanol in 2012 than in 2011, with yields from 0.38 to 4.75 t/ha; no significant differences were found among species. During the last year yields varied from 0.38 to 6.70 t/ha. *A. donax* showed the best result, followed by *M. x giganteus* (3.88 t/ha), all the other species had values lower than 1.50 t/ha. From 2010 to 2013, mean ethanol yields of species cultivated for more than one year, varied from 0.35 (*S. sylvaticus*) to 3.87 t/ha (*A. donax*).

The SH and MH ethanol yields of *A. donax, C. riparia*, *M. x giganteus* and *S. x uplandicum* were then assessed with a two-way ANOVA. Interaction year-harvest was not significantly different for all the species. *A. donax* and *S. x uplandicum* had no significant differences among year nor harvest, *C. riparia* had significantly higher yields in 2011, while *M. x giganteus* through MH (Table 28).

The bioreactor only gave considerably better results for *A. donax* and *M. x giganteus* (Table 29) so bioreactor transformation values will be considered for these two species in the following. These higher results were obtained because the use of bioreactors promoted the fermentation. In fact it allowed a better reagents mixing especially at the beginning of the hydrolysis, avoiding stratification and vortexes. Furthermore during fermentation there is an accumulation of the chemical reaction products, such as carbon dioxide and acetic acid, which decrease pH and inhibit yeast activity (Viola et al., 2004). Bioreactor, instead, maintaining a constant pH, reduced this negative effect.

Table 25 Cellulose recovery, hemicellulose and lignin solubilisation for the different species after pretreatment

Species	Cellulose Recovery %	Hemicellulose Solubilisation %	Lignin Solubilisation %
A. lappa	-	-	-
A. donax	75	90	54
C. indica	75	91	88
C. acutiformis	78	95	82
C. pseudocyperus	86	95	86
C. riparia	82	95	88
G. maxima	80	93	62
H. tuberosum	63	91	53
I. pseudacorus	90	79	69
L. salicaria	78	79	49
M. x giganteus	78	87	64
P. arundinacea	82	95	77
S. sylvaticus	66	94	95
S. x uplandicum	79	92	86

Table 26 Ethanol yields obtained for the different species in the flask trial

Vegetal species	Glucose yield (% of theoretical maximum)		Ethanol yield (% of theoretical maximum)		Ethanol yield (t EtOH)/ha)							
					2010		2011		2012		2013	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
A. lappa	-		-		-		-		-		-	
A. donax	32	1	19	2	1.25	0.82	2.99	2.05	4.54	2.64	6.70	0.98
C. indica	73	12	52	1	1.17	0.21	-		-		-	
C. acutiformis	61	6	50	2	0.83	0.17	2.67	0.70	2.49	0.41	1.17	0.33
C. pseudocyperus	55	1	42	9	0.80	0.30	-		-		-	
C. riparia	81	9	72	2	1.21	0.10	3.71	0.57	2.30	1.56	1.46	0.32
G. maxima	70	10	69	22	-		1.02	0.29	2.20	1.00	0.00	0.00
H. tuberosum	24	2	16	5	-		1.60	0.40	2.25	0.83	1.06	0.22
I. pseudacorus	79	9	52	10	0.95	0.50	2.04	0.52	1.53	0.47	1.01	0.09
L. salicaria	21	3	17	3	-		-		0.38	0.21	0.38	0.06
M. x giganteus	47	4	27	4	0.98	0.12	4.15	0.59	3.47	1.92	3.88	2.04
P. arundinacea	65	8	61	5	-		0.04	0.03	-		-	
S. sylvaticus	61	4	28	9	0.36	0.06	0.34	0.06	-		-	
S. x uplandicum	83	1	55	6	0.97	0.12	3.05	0.45	4.75	6.59	0.63	0.29

-: not determined.

Table 27 Mean ethanol yields per hectare of the studied species in 2010, 2011, 2012 and 2103. Different letters indicate significant differences at P< 0.05 by LSD test.

Species	Ethanol yield (t EtOH)/ha										
	2010			2011			2012		2013		
	mean	s.d.		mean	s.d.		mean	s.d.	mean	s.d.	
A. lappa	-			-			-		-		
A. donax	1.25	0.82	a	2.99	2.05	bc	4.54	2.64	6.70	0.98	a
C. indica	1.17	0.21	a	-			-		-		
C. acutiformis	0.83	0.17	ab	2.67	0.70	bcd	2.49	0.41	1.17	0.33	c
C. pseudocyperus	0.80	0.30	ab	-			-		-		
C. riparia	1.21	0.10	a	3.71	0.57	ab	2.30	1.56	1.46	0.32	c
G. maxima	-			1.02	0.29	ef	2.20	1.00	-		
H. tuberosum	-			1.60	0.40	de	2.25	0.83	1.06	0.22	c
I. pseudacorus	0.95	0.50	a	2.04	0.52	cde	1.53	0.47	1.01	0.09	c
L. salicaria	-			-			0.38	0.21	0.38	0.06	c
M. x giganteus	0.98	0.12	a	4.15	0.59	a	3.47	1.92	3.88	2.04	b
P. arundinacea	-			0.04	0.03	f	-		-		
S. sylvaticus	0.36	0.06	b	0.34	0.06	f	-		-		
S. x uplandicum	0.97	0.12	a	3.05	0.45	abc	4.75	6.59	0.63	0.29	c

-: not determined.

Table 28 Comparison of single harvest event (SH) and multiple harvest events (MH): mean ethanol yields of the studied species in 2011 and 2012.

Species	Ethanol yield (% of theoretical maximum)		Ethanol yield (t EtOH)/ha			
			2011		2012	
	SH	MH	SH	MH	SH	MH
A. donax	19 ± 2	39 ± 13	3.37	5.75	6.18	6.39
C. riparia	72 ± 2	48 ± 3	3.54	2.48	1.06	2.27
M. x giganteus	27 ± 4	50 ± 1	3.74	9.14	1.94	10.03
S. x uplandicum	55 ± 6	41 ± 4	2.91	1.76	7.38	1.18

Table 29 Comparison of flask and bioreactors trial on 4 selected species.

Species	Ethanol yield (kg EtOH)/t DM	
	Flasks	Bioreactors[1]
A. donax	48 ± 4	135
C. riparia	253 ± 6	289
M. x giganteus	75 ± 4	107
S. x uplandicum	190 ± 27	156

1: Experimentation on bioreactors had only one replicate.

Methane production

The use of wetland biomasses for biogas production is not well investigated in the literature but has recently received growing attention (Alvinge, 2010; Dipu et al., 2011; Comino et al, 2012). A possible limit to their use for the production of biogas is due to their composition, since ligno-cellulosic plant tissues are more difficult for the anaerobic reactors microflora to attack, but this is not mandatory as in 2nd gen. ethanol production. Some authors have recently studied different pretreatments, reporting risen yields when lignin demolition pretreatment was applied (Alvinge, 2010; Di Girolamo et al. 2013).

Materials and methods

The milled biomass of each species was used as substrate for anaerobic digestion to obtain biogas in reactors. The reactions were carried out in 118.5 mL serum bottles closed with butyl rubber stoppers and aluminium seals. The mixture, giving a total weight of 50 g, consisted of

- 1.25 g dry samples;
- 23.75 mL of a specific synthetic medium for methanogens without energy sources;
- 25 mL inoculum.

The substrate concentration was chosen on the basis of preliminary tests (Vismara et al., 2012), phosphate buffered basal medium (PBBM) was used as medium (Kenealy & Zeikus, 1981) and pig slurry as inoculum source. The reactors were left to incubate for 70 days at 35 °C and pH 7. Biomethanation potential (BMP) was measured according to Owen et al. (1979) by means of 100-mL glass syringes. The reactors were shaken at each measurement date. Methane concentration in the biogas was determined by gas chromatograph Micro GC Agilent 3000 (Agilent Technologies, USA). Experiments and analyses were repeated three times.

The methane yield per hectare was then calculated by multiplying the biomass yield, obtained in Chapter 2, with the methane yield.

Lastly SH and MH management in terms of methane production were compared for *A. donax*, *C. riparia, M. x giganteus* and *S. x uplandicum*.

Results and discussion

Regarding methane yields, species reported different BMP data, expressed as ml of methane in standard conditions of temperature and pressure (at 273K and 760 mm Hg; STP) per g of volatile solids ($CH_{4(STP)}$/ g VS) (Figure 25). Higher values, between 200 and 250 mL $CH_{4(STP)}$/ g VS, were given by *G. maxima*, *C. riparia, S. sylvaticus* and *A. lappa* (Marchetti et al., 2013). These amounts are overall lower than those reported in the literature for ligno-cellulosic feedstocks, such as cereal straws and non-food biomass, which varied from 276 to 620 mL $CH_{4(STP)}$/ g VS (Bauer et al., 2010; Dubrovskis et al. 2011; Chandra et al., 2012; Di Girolamo et al., 2013). Only in Dinuccio et al. (2010) there were some agroindustrial wastes that were comparable, specifically in ascending order grape stalk/marc, rice straw, tomato skin and seeds and barley straw (mean yields from 98 to 229 mL $CH_{4(STP)}$/ g VS). However no data was found in the literature for any of the species treated in this thesis but, as stated by Di

Girolamo et al. (2013), there is great variability in CH_4 production among biomass crops, due to specific differences among plants and their associated characteristics (plant stages, origin, time of harvest, etc.). Generally results obtained here might lead to a pretreatment being considered in further experiments.

Methane yield per hectare was assessed by ANOVA test within the year, differences among species were compared by LSD Test (Table 30). During the first year, methane production of the species ranged from 563 to 2922 m^3/ha. The two following years methane yield increased, ranging from 31 to 7007 m^3/ha and from 1666 to 10617 m^3/ha. In 2013 all species had lower yields, with the exception of *A. donax* and *M. x giganteus*, varying from 901 to 15679 m^3/ha.

The SH and MH ethanol yields of *A. donax, C. riparia*, *M. x giganteus* and *S. x uplandicum* were later assessed with a two-way ANOVA. Interaction year-harvest was not significant for all the species. *A. donax* and *S. x uplandicum* had no significant differences among year nor harvest, while *C. riparia* and *M. x giganteus* had significantly higher yields through MH (Table 31). *A. donax* gave the best result every year, going from almost 3000 to more than 15000 m^3/ha, statistically different from all other species, followed by *M. x giganteus* (from 1360 to 5750 m^3/ha) and *H. tuberosum* (4156 to 5823 m^3/ha). These results were related to the high biomass yields of these species.

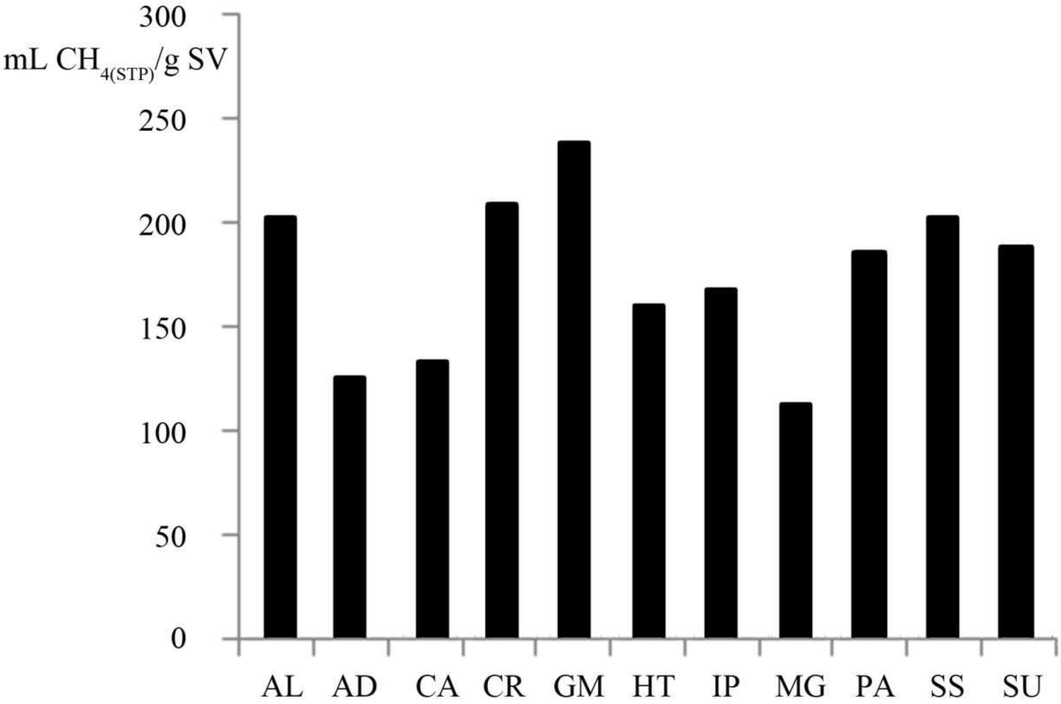

Figure 25 BMP of the studied species biomass, expressed as ml of methane in standard conditions of temperature and pressure per g of volatile solids ($CH_{4(STP)}$/ g VS). BMP values are net of methane produced endogenously by the inoculum (Marchetti et al., 2013, modified). AL: *A. lappa,* AD: *A. donax,* CA: *C. acutiformis,* CR: *C. riparia,* GM: *C. G. maxima,* HT*: H. tuberosus,* IP*: I. pseudacorus,* MG: *M. x giganteus,* PA: *P. arundinacea,* SU: *S. x uplandicum,* SS: *S. sylvaticus*

Table 30 Mean methane yields per hectare of the studied species in 2010, 2011, 2012 and 2103. Different letters indicate significant differences at P< 0.05 by LSD test.

Species	Methane yield (m^3 CH$_4$/ha)											
	2010			2011			2012			2013		
	mean	s.d.		mean	s.d.		mean	s.d.		mean	s.d.	
A. lappa	-			580	253	ef	4092	553	bc	2040	1377	cd
A. donax	2922	1913	a	7007	4797	a	10617	6176	a	15679	2291	a
C. indica				-			-			-		
C. acutiformis	563	117	b	1819	478	def	1696	278	c	798	228	cd
C. pseudocyperus				-			-			-		
C. riparia	877	75	b	2683	415	cd	1666	1133	c	1055	230	cd
G. maxima	-			899	257	def	1941	879	bc			
H. tuberosum	-			4156	1046	bc	5823	2162	b	2743	575	c
I. pseudacorus	849	449	b	1818	460	def	1363	420	c	901	80	cd
L. salicaria	-			-			-			-		
M. x giganteus	1360	162	b	5750	822	ab	4810	2665	bc	5379	2822	b
P. arundinacea	-			31	20	f	-			-		
S. sylvaticus	662	105	b	615	104	def	-			-		
S. x uplandicum	704	88	b	2211	323	cde	3448	4778	bc	459	210	d

-: not determined.

Table 31 Comparison of single harvest event (SH) and multiple harvest events (MH): mean methane yields of the studied species in 2011 and 2012. Values are not net of methane produced endogenously by the inoculum.

Species	Methane yield (m^3 CH_4/t DM)		Methane yield (m3 CH4/ha) 2011		2012	
	SH	MH	SH	MH	SH	MH
A. donax	131	196	9204	10860	16865	12074
C. riparia	201	250	2809	3833	839	3508
M. x giganteus	124	153	6160	9232	3201	10134
S. x uplandicum	157	144	2407	2412	6095	1619

Ethanol and methane energy comparison

After the two laboratory trials on ethanol and methane production, an energy comparison was conducted on the energy content of the two types of bioenergy produced by the studied species. Thus the response of species was evaluated energetically in term of ethanol or methane production, expressed as GJ/t DM and afterwards as GJ/ha.

Materials and methods

The ethanol and methane energy outputs per hectare were calculated by multiplying the biomass yields with the calorific value of ethanol and methane, as in Bauer et al. (2010). Specifically 1 Nm^3 of methane corresponds to 39.79 MJ (Beitz and Küttner, 1987) and 1 kg of ethanol to 26.8 MJ (KTBL, 2005).

Results and discussion

Energy yields reflected the corresponding ethanol and methane yields. For both, the annual mean range increased from 2010 to 2012 and declined in the last year, apart from *A. donax* and *M. x giganteus* (Table 32). Thus in 2010 ethanol energy outputs varied from 10 to 95 GJ/ha and the following years from 1 to 227, from 10 to 334 and from 10 to 508 GJ/ha. Regarding methane, the ranges sequence from 2010 to 2013 was 26-116, 1-279, 54-422 and 18-624 GJ/ha. *A. donax* and *M. x giganteus* gave the best results for both transformations and all years.

A more immediate comparison can be seen in Figure 26, where 2010-2013 mean energy output of species that received both types of laboratory processing are reported. Furthermore they were analysed by a two-way ANOVA then the LSD test was applied for the means separation to show significant differences. Methane transformation was significantly higher than the ethanol one ($P< 0.05$). Species were significantly different at $P< 0.001$, while interaction species-transformation had no significant difference. *A. donax* gave the highest mean energy output (327 GJ/ha/y), followed by *M. x giganteus* (146 GJ/ha/y) and *H. tuberosum* (106 GJ/ha/y). The lowest results were shown by *S. x uplandicum* (17 GJ/ha/y) and *C. acutiformis* (60 GJ/ha/y). Even though ethanol and methane yields were lower than values reported in the literature, energy outputs per hectare were generally comparable regarding *A. donax* and *M. x giganteus*, due to their high biomass production in this experiment. In effect, several authors reported *A. donax* energy output varying from 240 to 600 GJ/ha/y, and *M. x giganteus* from 180 to 350 GJ/ha/y (Cosentino et al., 2008; Mantineo et al., 2009). Of course data obtained here were lower than 1st gen. biofuels output, which can reach 800 GJ/ha/y for sugarcane for example (Larson, 2006). But it is worth noticing that the energy efficiency, which is expressed by the ratio between the entire energy content of biomass yield (output) and the energy utilised in the cropping system (input), is lower in 1st gen. biofuels (from 1 to 5) than in 2nd gen. ones (that can also reach 50) (Angelini et al., 2005; Cosentino et al., 2007; Sims et al., 2010).

Table 32 Comparison of energy yields obtained from ethanol and methane for the studied species from 2010 to 2013.

Species	Ethanol energy yield (GJ/ha)								Methane energy yield (GJ/ha)							
	2010		2011		2012		2013		2010		2011		2012		2013	
	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
A. lappa	-		-		-		-		-		23	10	178	34	81	55
A. donax	95	62	227	156	344	200	508	74	116	76	279	191	422	246	624	91
C. indica	31	6	-		-		-		-		-		-		-	
C. acutiformis	22	5	71	19	67	11	31	9	22	5	72	19	67	11	32	9
C. pseudocyperus	21	8	-		-		-		-		-		-		-	
C. riparia	32	3	99	15	62	42	39	9	35	3	107	16	66	45	42	9
G. maxima	-		27	8	59	27	-		-		36	10	77	35	-	-
H. tuberosum	-		43	11	60	22	28	6	-		165	42	232	86	109	23
I. pseudacorus	26	14	55	14	41	13	27	2	34	18	72	18	54	17	36	3
L. salicaria	-		-		10	6	10	2	-		-		-		-	
M. x giganteus	37	4	158	23	132	73	148	78	54	6	229	33	191	106	214	112
P. arundinacea	-		1	1	-		-		-		1	1	-		-	
S. sylvaticus	10	2	9	2	-		-		26	4	24	4	-		-	
S. x uplandicum	26	3	82	12	127	153	17	8	28	4	88	13	137	164	18	8

-: not determined.

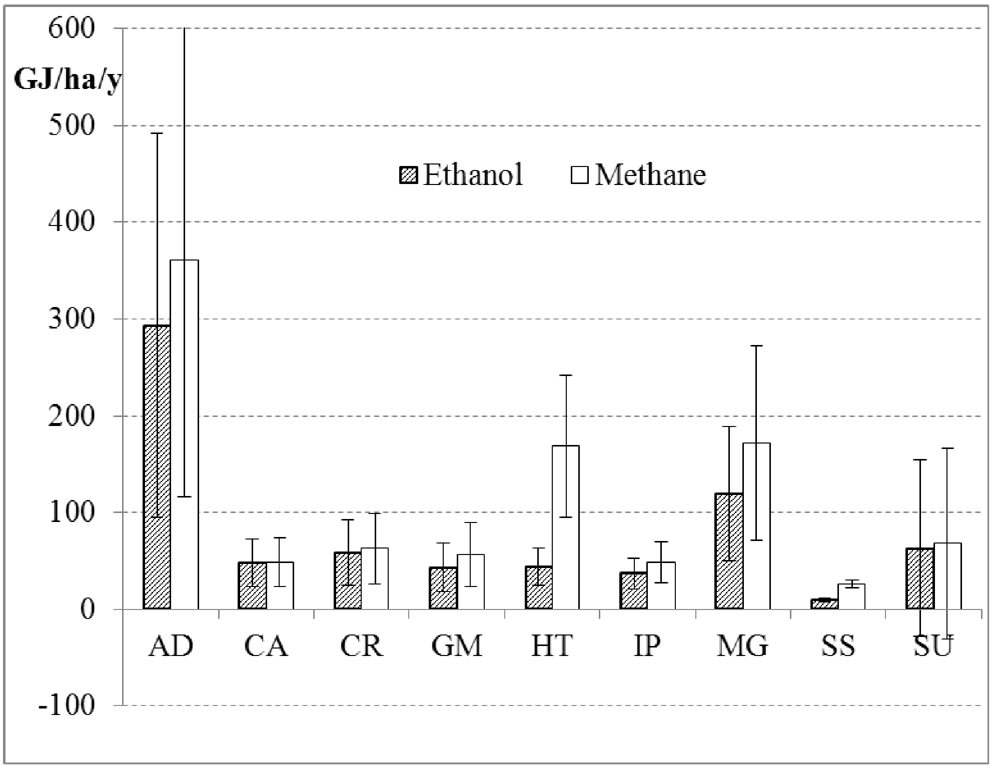

Figure 26 Comparison of energy yields obtained from ethanol and methane: mean value 2010-2013 of the studied species. AD: *A. donax,* CA: *C. acutiformis,* CR: *C. riparia,* GM: *C. G. maxima,* HT*: H. tuberosus,* IP*: I. pseudacorus,* MG: *M. x giganteus,* SS: *S. sylvaticus,* SU: *S. x uplandicum*

Conclusions

Overall both ethanol and methane trials obtained lower yields (138 kg ethanol/ t DM and 140 m^3 $CH_{4(STP)}$/ t VS) than the maximum values found in the literature, which varied from 300 to 450 kg ethanol/t DM and from 276 to 620 m^3 $CH_{4(STP)}$/ t VS (Patzek, 2006, Quintero et al., 2008; Pin et al., 2008; Davila-Gomez et al., 2011; Bauer et al., 2010; Chandra et al., 2012; Di Girolamo et al., 2013). But, when reported on a per hectare scale, their yields were considerable, especially for *A. donax* and *M. x giganteus,* which reached mean values of 3.87 t ethanol/ha and 9056 m^3 $CH_{4(STP)}$/ t VS, and 3.12 t ethanol/ha and 4325 m^3 $CH_{4(STP)}$/ t VS, respectively. Consequently these two species also had the highest mean energy outputs, 360 GJ/ha for *A. donax* and 172 GJ/ha *M. x giganteus,* respectively. *A. donax* thus gave the best result on energy yield.

The energy comparison also showed that methane outputs are energetically higher than ethanol ones and demonstrated that *A. donax* and *M. x giganteus,* even if showing low ethanol and methane yields, are the most suitable for the transformation.

Regarding harvest management, only *M. x giganteus* showed a positive reaction to multiple harvests in both cases.

Further analysis, particularly the environmental impacts from production, transport and transformation might give an additional key to compare the ethanol and methane production scenario for the studied species. But this has to be evaluated by Life Cycle Assessment studies and the scope of this research was to compare the species and provide initial data.

4 Environmental assessment on Veneto scale

Introduction

This chapter describes an environmental assessment that was conducted, focusing on the N balances in the soil under different crops. As stated by Dobermann (2001), nitrogen budgeting approaches are often applied to valuate and understand nitrogen use efficiency and cycling by mass balance. One of the most promising species, in terms of biomass yield and energy output, was chosen among all the plants cultivated in the growth boxes in Chapter 2. Since on average only *A. donax* recovered all the N applied and also adsorbed it from the soil, as reported by Borin et al. (2013), *M. x Giganteus* was selected. This species was compared with maize (*Zea Mays* L.), being the most widespread crop in the North-East of Italy and a key crop for intensive agricultural production (Grignani et al., 2007). The aim of the study was to assess the N leaching of the two crops at different N levels, with particular attention to the Nitrates Directive limits and derogation (EEC, 1991; Official Journal of the European Union, 2011), and consequently to investigate what the best use of N fertilizer was in this context.

Material and methods

In order to obtain potential yields with different amount of nitrogen, a *M. x Giganteus* and a *Z. Mays* nitrogen response curve were created from the Mitscherlich-Baule equation (Frank et al., 1990):

$$Y= \beta_0 [1 - \exp(-\beta_1 (\beta_2 + N))] \qquad (7)$$

Where Y is crop yield (t DM/ha/y), N is applied nitrogen (kg N/ha/y) and the β are parameters. The model parameters for the response curves were calibrated by minimizing the residual sum of squares, using the Generalized Reduced Gradient (GRG) Nonlinear Solving Method (Frontline Systems, Inc., Incline Village, NV, USA). For the Miscanthus response curve, yield data were taken from our trial (Cf. Chapter 2) and from Lewandowski and Schmidt (2006) while for the maize one, they were extracted from Giardini et. al (1997). The latter trial was carried out on the same Experimental farm as our trial.

The SimDen 2.0 model (Vinther, 2005) was then used to simulate total denitrification (N_{den}) and N_2O emissions (N_{em}) at different amount of nitrogen input in the soil. The model was set as follows:

a) Soil type: Clay loam soil (soil type 8: 25-45% clay, 0-45 silt and 10-75% total sand), according to the Italian experimental trial soil texture (Cf. Table 1);

b) Nitrogen Input: Animal manure/slurry N - surface applied and incorporated, because of the simulated use of slurry;

c) Pre-history with respect to input of organic matter or precipitation: High - High precipitation or organic matter level, due to the large annual amount of wastewater distributed on the crops.

Afterwards the N leaching was calculated by mass balance as follows:

$$N_L=N_{in}-(N_{up}+N_{den}+N_{em}) \tag{8}$$

Where N_L is N leaching, N_{in} is N input by slurry, N_{up} is N uptake. The latter was obtained multiplying yield data by mean N content, from our (0.53%, cf. Chapter 2) and Giardini et. al (1997) (1.3%) trials, for Miscanthus and maize respectively.

Subsequently N leaching was expressed for the entire Veneto Region. The area devoted to Miscanthus was assumed to be the Non-cultivated Agricultural Area, i.e. Total Agricultural Area (TAA) minus Utilized Agricultural Area (UAA) (ISTAT, 2010), corresponding to 31867 ha, while maize area was assumed to be the area currently cropped with maize, i.e. 247927 ha (ISTAT 2013). Three different fertilization levels were then compared:

1. F_1: 170 kg N/ha – limit imposed by Nitrates Directive for vulnerable zones (EEC, 1991);
2. F_2: 250 kg N/ha – higher limit allowed on the Padana Plain by Nitrates Directive derogation for crops with high N demand and long growing season (Official Journal of the European Union, 2011);

3. F_3: 340 kg N/ha – limit imposed by Nitrates Directive for non-vulnerable zones (EEC, 1991).

Finally different amounts of N were allocated from maize to Miscanthus to assess the consequences in terms of N leaching and crop yield.

Results and discussion

The N response curves reported potential yields obtainable by slurry fertilization of from 50 to 400 kg/ha (Figure 27) and then allowed then to estimate the corresponding N leaching to be estimated. In our simulation it occurred for both crops with fertilizations higher than 150 kg/ha (Figure 28). In the three fertilization scenarios maize N_L varied from 7.3 to 107.3 kg N/ha (Table 33). Thus going from 170 to 340 kg N/ha of fertilization, N_L became almost 16 times higher. These values might be negligible when expressed per hectare but total N_L corresponded to 2128 t N in F_1 when reported on Veneto scale and reached 30000 t N in the case of F_3 (Table 34). It is worth mentioning that, even in F_1, with the lowest N_{in}, cultivating *M. x Giganteus* on Non-cultivated Agricultural Area, could allow to be spread almost 5000 tons of N to obtain biomass suitable for bioenergy (255000 t DM ca.) in Veneto Region, with minimal N leaching. Consequently the possibility to grow Miscanthus in non-cultivated areas might be taken into account as a possibility to manage slurry and complement agronomic production.

Furthermore, removing gradual quantities of N from maize and allocating them to Miscanthus, it was possible to see the consequences in terms of N_L and yield (Table 35). Since F_1 was best scenario in our simulation maize N_{in} were reduced of increasing 5 kg N/ha quota from 170 kg N/ha to 130 kg N/ha, assigning these allocation to miscanthus. Obviously maize yields decreased with the reduction of N_{in} so to maintain productivity higher than 8 t/ha maize N_{in} should be at least 150 kg N/ha. Thus it was possible to see that allocating part of maize N_{in} to Miscanthus allowed to be produced up to 20 t/ha of biomass suitable for bioenergy. Consequently this can be considered an environmentally good use of N both for N leaching and bioenergy purpose.

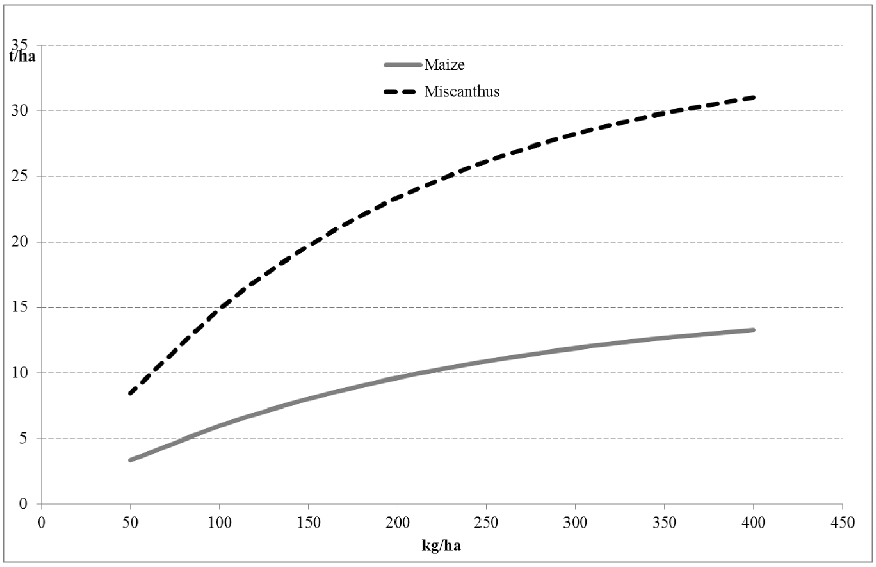

Figure 27 Maize and Miscanthus N response curves: crop yields at different N input

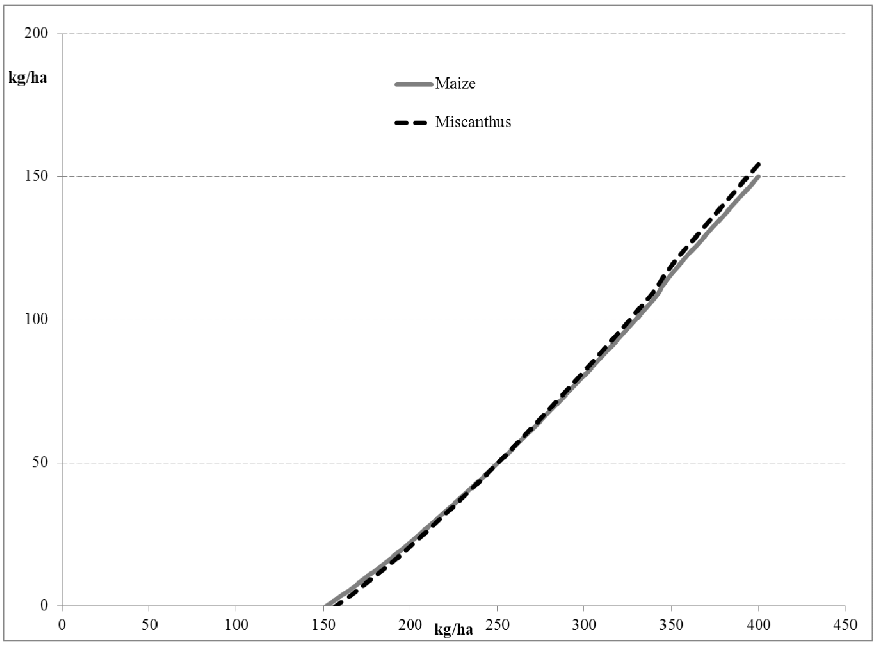

Figure 28 Maize and Miscanthus N leaching at different N inputs

Table 33 Maize and Miscanthus N leaching in the three different scenarios F_1: 170 kg N/ha, F_2: 250 kg N/ha, F_3: 340 kg N/ha

Species	N_L (kg N/ha)		
	F_1	F_2	F_3
Maize	7.8	49.8	107.3
Miscanthus	5.7	49.7	110.0

Table 34 Maize and Miscanthus N input, N leaching and yield in the three fertilization scenarios. F_1: 170 kg N/ha, F_2: 250 kg N/ha, F_3: 340 kg N/ha

			F_1			F_2			F_3		
Species	Surface	% of TAA	N_{in}	N_L	Yield	N_{in}	N_L	Yield	N_{in}	N_L	Yield
	ha	%	t N	t N	t DM/ha	t N	t N	t DM/ha	t N	t N	t DM/ha
Maize	247927	24.6	42148	1946	8.7	61982	12343	10.9	84295	26609	12.5
Miscanthus	31867	3.2	5417	182	21.3	7967	1585	26.1	10835	3504	29.8

Table 35 Maize and Miscanthus N input, N leaching and yield in different allocations.

N_{in}				N_L		Yields	
allocated to Maize	removed from Maize	allocated to Miscanthus	allocated to Miscanthus	Maize	Miscanthus	Maize	Miscanthus
kg N/ha	kg N/ha	t N	kg N/ha	kg N/ha	kg N/ha	t DM/ha	t DM/ha
170	0	0	0	8	≈0	8.7	0.0
165	5	1240	39	3	≈0	8.6	6.8
160	10	2479	78	≈0	≈0	8.4	12.2
155	15	3719	117	≈0	≈0	8.2	16.6
150	20	4959	156	≈0	≈0	8.0	20.2
145	25	6198	194	≈0	6	7.8	23.0
140	30	7438	233	≈0	21	7.7	25.3
135	35	8677	272	≈0	50	7.5	27.1
130	40	9917	311	≈0	82	7.3	28.6

Conclusion

In this simulation maize and Miscanthus resulted as having similar N leaching response at different N input. According to the obtained data, to best minimize N losses the maximum amount of N input should be 150 kg/ha. It should be mentioned that, even in this lowest fertilization scenario, cultivating *M. x Giganteus* on Non-cultivated Agricultural Area, could allow almost 5000 tons of N to be spread and more than 255000 t of biomass suitable for bioenergy obtained in Veneto Region, with minimal N leaching. Consequently the possibility of growing Miscanthus in non-cultivated areas might be taken into account as a possibility to manage slurry and complement agronomic production.

In addition, allocating part of maize N_{in} to Miscanthus allowed up to 20 t/ha of biomass suitable for bioenergy to be produced, reducing maize N leaching.

Furthermore these data lay the basis on *M. x Giganteus* N leaching for a potential future Life Cycle Assessment dealing with its cultivation.

5 General conclusions

Fourteen plant species were evaluated in growth boxes from 2010 to 2013. During the trial they were studied to obtain data on their suitability on biomass production and phytoremediation. These plants were perennial so further experimenting might provide more exhaustive conclusions, but from the data obtained, the following observations can be made.

In general, the biomass characterization varied among species, but results were similar to those reported in the literature, while biomass yields were higher than what reported by other authors, when both available.

Overall *A. donax* gave the best result, even if it was not very productive in ethanol or methane transformation. This dominance was due to its huge potential in biomass production which allowed it to reach very high bioenergy production per hectare. It was also the only one which recovered all the N applied and also adsorbed it from the soil. Also *M. x Giganteus* showed remarkable results but lower than giant reed ones.

Regarding percolation water, lower TN, NO_3-N and PO_4-P median contents were found comparing the initial and the final data during the experiment. On the contrary TP showed higher and more variable concentration in 2013.

But at the same time, through the environmental assessment, it was possible to see how different amount of fertilization can impact on N leaching.

Consequently further analysis, particularly on the environmental impacts from cultivation to ethanol/methane transformation might give additional interpretation keys to assess the production of bioenergy from *A. donax* irrigated by slurry. Thus data obtained in this dissertation might put the basis for a potential future Life Cycle Assessment dealing with this bioenergy scenario.

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References

Aerts R., Caluwe H. D. E., Konings H., 1992. Seasonal allocation of biomass and nitrogen in four Carex species from mesotrophic and eutrophics fens as affected by nitrogen supply. J. Ecol. 80, 1992, 653–664

Agrocadenas, 2006. Segundo informe de coyuntura maı´z 2006. Observatorio Agrocadenas Colombia, Ministry of Agricultural and Rural Development. http://www.agrocadenas.gov.co/home.htm In: Sánchez O., Cardona C., 2008. Trends in biotechnological production of fuel ethanol from different feedstocks. Bioresour Technol., 99, 13, 5270–5295

Akula, V.R., 2013. Wetland biomass - suitable for biogas production? Thesis, Halmstad University, Sweden.

Albergo R., Ambrico A., Balducchi R., Maccioni O., Palazzo S., Trupo M., 2013. Produzione di bioetanolo da biomasse provenienti da siti di fitodepurazione nell'ambito del progetto FITOPROBIO. Rapporto Tecnico 2013. ENEA - Laboratorio Biotecnologie, Centro Ricerche Trisaia. Available on: http://hdl.handle.net/10840/4698 accessed on 20 May 2013

Alfani F., Gallifuoco A., Saporosi A., Spera A., Cantarella M., 2000. Comparison of SHF and SSF processes for bioconversion of steam-exploded wheat straw. Jurnal of Industrial Microbiology & Biotechnology, 25, 2000, 184–192

Allen, R. G., Pereira L. S., Raes, D., Smith M., 1998. Crop evapotranspiration: guidelines for computing crop water requirements. In: Proceedings of the Irrigation and Drainage Paper, 56. Food and Agricultural Organization, United Nations, Rome, Italy

Alvinge S., 2010. Evaluation of emergent macrophytes as a source for biogas production after mechanical, alkaline and funga pretreatments

Alvira P., Tomàs-Pejò M., Ballesteros M., Negro M. J., 2010. Pretreatment technologies for an efficient bioethanol production process on enzymatic hydrolysis: a review. Bioresour. Technol., 101, 2010, 4851–4861

Angelini L. G., Ceccarini L., Nassi o Di Nasso N., Bonari E., 2009. Comparison of Arundo donax L. and Miscanthus x giganteus in a long-term field experiment in Central Italy: Analysis of productive characteristics and energy balance biomass and bioenergy, 33, 2009, 635–643

Anon, 2010. Biogas barometer – November 2010. Le journal des energies renouvelables, No. 200, 104–119. Available at: http://www.eurobserv-er.org/downloads.asp In: Petersen S. O., Blanchard M., Chadwick D., Del Prado A., Edouard N., Mosquera J. and Sommer S. G., 2013. Manure management for greenhouse gas mitigation. Animal, 7 s2, 2013, 266–282

AOAC, 1993. International. Methods of analysis of nutrition labeling. Arlington, Texas, 1993

ASCE-EWRI, 2004. The ASCE Standardized Reference Evapotranspiration Equation. Technical Committee Report to the Environmental and Water Resources Institute of the American Society of Civil Engineers from the Task Committee on Standardization of Reference Evapotranspiration, 2004

Ask M., Olofsson K., Di Felice T., Ruohonen L., Penttilä M., Lidén G., Olsson L., 2012. Challenges in enzymatic hydrolysis and fermentation of pretreated Arundo donax revealed by a comparison between SHF and SSF. Process Biochemistry, 47, 2012, 1452–1459

Baldini M., Danuso F., Rocca A., Bulfoni E., Monti A., De Mastro G., 2011. Jerusalem artichoke (Helianthus tuberosus L.) productivity in different Italian growing areas: a modelling approach. Italian Journal of Agronomy, volume 6, e20, 2011, 126–132

Barbagallo S., Cirelli G. L., Marzo A., Milani M., Toscano A., 2010. Hydraulics and removal in two HSSF constructed wetlands for wastewater reuse with different operational life. In: Proceedings of 12th IWA International Conference on Wetland Systems for Water Pollution Control. Island of San Servolo, Venice, Italy, 4–8 October 2010

Barbera A. C., Cirelli G. L., Cavallaro V., Di Silvestro I., Pacifici P., Castiglione V., Toscano A., Milani M., 2009. Growth and biomass production of different plant species in two different constructed wetland systems in Sicily. Desalination, 247, 130–137

Bauer A., Leonhartsberger C., Bösch P., Amon B., Friedl A., Amon T., 2010. Analysis of methane yields from energy crops and agricultural by-products and estimation of energy potential from sustainable crop rotation systems in EU-27. Clean Techn Environ Policy, 12, 2010, 153–161

Bauer N. A., Gibbons W. R., 2012. Saccharification versus simultaneous saccharification and fermentation of kraft pulp. Int. J. Agric. & Biol. Eng., 5 (1), 2012, 48–55

Beale C. V., Long S. P., 1995. Can perennial C4 grasses attain high efficiencies of radiant energy conversion in cool climates? Plant, Cell and Environment, 18, 1995, 641–50

Beale C. V., Long S. P., 1997. Seasonal dynamics of nutrient accumulation and partitioning in the perennial C4-grasses Miscanthus × giganteus and Spartina cynosuroides. Biomass Bioenergy, 12 (6), 1997, 416–428. In: Christian D. G., Riche A. B., Yates N. E., 2008. Growth, yield and mineral content of Miscanthus x giganteus grown as a biofuel for 14 successive harvests. Industrial Crops and Products, 28, 2008, 320–327

Beitz W., Küttner K. H., 1987. Dubbel pocket-book for engineering (Dubbel Taschenbuch für den Maschinenbau). Springer, Berlin. In: Bauer A., Leonhartsberger C., Bösch P., Amon B., Friedl A., Amon T., 2010. Analysis of methane yields from energy crops and agricultural by-products and estimation of energy potential from sustainable crop rotation systems in EU-27. Clean Techn Environ Policy, 12, 2010, 153–161

Bentsen N. S., Felby C., 2012. Biomass for energy in the European Union – a review of bioenergy resource assessments. Biotechnology for Biofuels, 2012, 5:25. Available on: http://www.biotechnologyforbiofuels.com/content/5/1/25 accessed on 20 January 2013

Berg C., 2004. Word Fuel Ethanol. Analysis and Outlook, Japan, 2004. Available on: http://www.distill.com/World-Fuel-Ethanol-A&O-2004.html accessed on 15 May 2011

Beurskens L. W. M., Hekkenberg M., Vethman P., 2011. Renewable Energy Projections as Published in the National Renewable Energy Action Plans of the European Member States. Covering all 27 EU Member States with updates for 20 Member States. ECN Policy Studies, ECN-E, 28 November 2011, 10–069

Bogucka-Kockaa A., Fatty M. J., 2010. Acids composition of fruits of selected Central European sedges, Carex L. (Cyperaceae). Grasas y Aceites, 61 (2), Abril–Junio 2010, 165–170

Bonaiti G., Borin M., 2010. Efficiency of controlled drainage and subirrigation in reducing nitrogen losses from agricultural fields. Agricultural Water Management, 98, 2010, 343–352

Boose A. B., Holt J. S., 1999. Environmental effects on asexual reproduction in Arundo donax. Weed Research, 39, 1999, 117–27

Borin M., Barbera A. C., Milani M., Molari G., Zimbone S. M., Toscano A., 2013. Biomass production and N balance of giant reed (Arundo donax L.) under high water and N input in Mediterranean environments. Europ. J. Agronomy, 51, 2013, 117– 119

Borin M., Florio G., Barbera A., Cirelli G. L., Albergo R., Palazzo S., 2011. Preliminary evaluation of macrophyte wetland biomasses to obtain second generation ethanol. 19th European Biomass Conference and Exhibition, 6–10 June 2011, Berlin, Germany, 642–648

Borin M., Molari G., Toscano A., Zimbone S. M., 2009. Production and energy transformation of herbaceous biomasses irrigated with treated wastewater – first results. In proceedings. 17th European Biomass Conference and Exhibition, 29 June – 3 July 2009, Hamburg, Germany 552–556

Borin M., Salvato M., 2012. Effetcs of five macrophytes on nitrogen remediation and mass balance in wetland mesocosms. Ecol. Engin. submitted

Boutwell J. E., 2002. Water quality and Plant growth evalutions of the floating islands in Las Vegas Bay, Lake Mead, Nevada. U.S., Department of the Interior, Bureau of Reclamation, Denver, Colorado, 24

Braun R., 2007. Anaerobic digestion: a multi-faceted process for energy, environmental management and rural development. In: Ranalli P. (Ed.) Improvement of crop plants for industrial end uses. Springer, 335–416

Bremness L., The Complete Book of Herbs. Dorling Kindersley, London, UK, 1988

Bridget L., Tait J. W., 2000. Papyrus. In: Nicholson T. P., Shaw I. (eds). Ancient Egyptian materials and technology. Cambridge University Press, Cambridge, 227–253

Brown C. J., Blossey B., Maerz J. C., Joule S. J., 2006. Invasive plant and experimental venue affect tadpole performance Biological Invasions, 8, Springer, 2006, 327–338

Burton C. H., Turner C., 2003. Manure management. Treatment strategies for sustainable agriculture, 2nd edition. Silsoe Research Institute, Bedford, UK. In: Petersen S. O., Blanchard M., Chadwick D., Del Prado A., Edouard N., Mosquera J. and Sommer S. G., 2013. Manure management for greenhouse gas mitigation. Animal, 7 s2, 2013, 266–282

Calheiros C. S. C., Rangel A. O. S. S., Castro P. M. L., 2007. Constructed wetland systems vegetated with different plants applied to the treatment of tannery wastewater. Water research, 41, 1790-1798.

Campbell J. E., Lobell D. B., Genova R. C., Field C. B., 2008. The global potential of bioenergy on abandoned agriculture lands. Environmental Science and Technology, 42, 2008, 5791–5794

Cao L., 2012. Lythrum salicaria. USGS Nonindigenous Aquatic Species Database, Gainesville, FL. Available on: http://nas.er.usgs.gov/queries/factsheet.aspx?SpeciesID=239 accessed on 8 May 2013

Capecchi L., Nissen L., Grigatti M., Mattarelli P., Barbanti L., 2013. Produzione di bioetanolo di seconda generazione da colture erbacee annuali e poliennali. In Proceeding of: XLII Convegno nazionale della Società Italian di Agronomia. Reggio Calabria 18–20 Settembre 2013, 272–274

Cataldo D.A., Haroon M., Schrader L.E., Youngs V.L., 1975. Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. Soil Science and Plant Analysis, 6, 1975, 71–80

Chandel A. K., Chandrasekhar G., Radhika K., Ravinder R., Ravindra P., 2011. Bioconversion of pentose sugars into ethanol: a review and future directions. Biotechnol Mol. Biol. Rev., 6 (1), 2011, 8–20

Chiaramonti D., Prussi M., Ferrero S., Oriani L., Ottonello P., Torre P., Cherchi F., 2012. Review of pretreatment processes for lignocellulosic ethanol production, and development of an innovative method biomass and bioenergy, 46, 2012, 25–35

Chong M. L., Sabaratnam V., Shirai Y., Hassan M. A., 2009. Biohydrogen production from biomass and industrial wastes by dark fermentation. Int. J. Hydrogen Energy, 34, 2009, 3277–3287

Christian D. G., Riche A. B., Yates N. E., 1998. Nutrient requirement and cycling in energy crops. In: El Bassam N., Behl R. K., Prochnow B. (Eds.). Sustainable Agriculture for Food, Energy and Industry. James and James, London, 799–804. In: Christian D. G., Riche A. B., Yates N. E., 2008. Growth, yield and mineral content of Miscanthus x giganteus grown as a biofuel for 14 successive harvests. Industrial Crops and Products, 28, 2008, 320–327

Christian D. G., Riche A. B., Yates N. E., 2008. Growth, yield and mineral content of Miscanthus x giganteus grown as a biofuel for 14 successive harvests. Industrial Crops and Products, 28, 2008, 320–327

Christou M., Mardikis M., Alexopoulou E., 2000. Propagation material and plant density effects on the Arundo donax yields. In: Biomass for energy and industry: proceeding of the First World Conference, Sevilla, Spain, June 5–9 2000, 1622–8. In: Angelini L. G., Ceccarini L., Nassi o Di Nasso N., Bonari E., 2009. Comparison of Arundo donax L.

and Miscanthus x giganteus in a long-term field experiment in Central Italy: Analysis of productive characteristics and energy balance biomass and bioenergy, 33, 2009, 635–643

Christou M., Mardikis M., Alexopoulou E., 2005. Biomass production from perennial crops in Greece. In: Proceedings of the 14th European Conference and Technology Exhibition on Biomass for Energy, Industry and Climate Protection, 17-21 October 2005, Paris, France

Ciria M. P., Solano M. L., Soriano P., 2005. Role of macrophyte Typha latifolia in a constructed wetland for wastewater treatment and assessment of its potential as a biomass fuel. Biosystems Engineering, 92 (4), 535–544

Clarke A., Lake P. S., O'Dowd D. J., 2004. Ecological impacts on aquatic macroinvertebrates following upland stream invasion by a ponded pasture grass (Glyceria maxima) in southern Australia. Marine and Freshwater Research, 55 (7), 2004, 709–713

Clifton-Brown J. C., 1997. The importance of temperature in controlling leaf growth of Miscanthus in temperate climates. Dissertation. University of Dublin, Trinity College, Dublin, 1997

Clifton-Brown J. C., Lewandowski I., 2002. Screening Miscanthus genotypes in field trials to optimise biomass yield and quality in Southern Germany. European Journal of Agronomy, 16, 2002, 97–110

Clifton-Brown J. C., Stampfl P.F., Jones M.B., 2004. Global Change Biol., 10, 2004, 509–518

Comino E., Riggio V. A., Rosso M., 2012. What we can do with the constructed wetland vegetable biomass? EcoSummit 2012 - Ecological Sustainability Restoring the Planet's Ecosystem Services. Available on: http://porto.polito.it/2504387/ accessed on 20 May 2013

Cook C. D. K., 1996. Aquatic Plants. SBP Academic Publishing, Amsterdam/New York, ISBN 90-5103-132-7, 228

Corredor D. Y., Salazar J. M., Hohn K. L., Bean S., Bean B., Wang D., 2009. Evaluation and Characterization of Forage Sorghum as Feedstock for Fermentable Sugar Production. Appl. Biochem. Biotechnol, 158, 2009, 164–179

Cosentino S. L., Patanè C., Sanzone E., Copani V., Foti S., 2007. Effects of soil water content and nitrogen supply on the productivity of Miscanthus×giganteus Greef et Deu. in a Mediterranean environment. Industrial Crops and Products, 25, 2007, 75–88

Cosentino S. L., Copani V., Patanè C., Mantineo M., D'Agosta G. M., 2008. Agronomic, energetic and environmental aspects of biomass energy crops suitable for Italian environment. Ital. J. Agron. 2008, 2:81:95

Cosgrove D. R., Oelka D. A., Doll D. J., Davis D. W., Undersander D. J., Oplinger E. S., 1991. Jerusalem artichoke. Alternative Field Crops Manual. University of Wisconsin Press, Wisconsin. Available on: http://www.hort.purdue.edu/newcrop/afcm/ accessed on 10 February 2011

Cramer J., Wissema E., de Bruyne M., Lammers E., Dijk D., Jager H., van Bennekom S., Breunesse E., Horster R., van Leenders C., Wonink S., Wolters W., Kip H., Stam H., Faaij A., Kwant K., 2007. Testing framework for sustainable biomass. Final report from the project group "Sustainable production of biomass". Commissioned by the Energy Transition's Interdepartmental Programme Management (IPM), March 2007, Available at: www.lowcvp.org.uk/assets/reports/070427-cramer-finalreport_en.pdf accessed on 12 January 2013

Crawford R. M. M., Brändle R., 1996. Oxygen deprivation stress in a changing environment. Journal of Experimental Botany, 47, 145–159

Crick J. C., Grime J. P., 1987. Morphological Plasticity and Mineral Nutrient Capture in Two Herbaceous Species of Contrasted Ecology. New Phytologist, Volume 107, No. 2, Oct. 1987, 403–414. Available on: http://www.jstor.org/stable/2433065?origin=JSTOR-pdf accessed on 15 September 2013

Culvenor C. C. J., Clarke M., Edgar J.A., Frahn J. L., Jago M.V., Peterson J.E., Smith L.W., 1980. Structure and toxicity of the alkaloids of Russian comfrey (Symphytum • uplandicum Nyman), a medicinal herb and item of human diet. Experientia, Volume 36, Fasc. 4, 15 April 1980, 377–502

Curreli N., Agelli M., Rescigno A., Sanjust E., Rinaldi A., 2002. Complete and efficient enzymic hydrolysis of pretreated wheat straw. Process Biochemistry, 37, 2002, 937–941

Curreli N., Fadda M. B., Rescigno A., Rinaldi A. C., Soddu G., Sollai F., Vaccargiu S., Sanjust E., Rinaldi A., 1997. Mild alkaline/oxidative pretreatment of wheat straw. Process Biochemistry, 32, 1997, 665–670

Dandresa T., Gaudreaultb C., Tirado-Secoa P., Samsona R., 2012. Macroanalysis of the economic and environmental impacts of a 2005–2025 European Union bioenergy policy using the GTAP model and life cycle assessment. Renewable and Sustainable Energy Reviews, 16, 2012, 1180–1192

Davila-Gomez F. J., Chuck-Hernandez C., Perez-Carrillo E., Rooney W. L., Serna-Saldivar S. O., 2011. Evaluation of bioethanol production from five different varieties of sweet and forage sorghums (Sorghum bicolor (L) Moench), Industrial Crops and Production, 33, 2011, 611–616

De Stefani G., 2011. Performance of floating treatment wetlands (FTW) with the innovative Tech-ia system. Università degli studi di padova, Scuola di dottorato in scienze delle produzioni vegetali, 2011

Dhillon R. S., von Wuehlisch G., 2013. Mitigation of global warming through renewable biomass. Biomass and Bioenergy, 48, 75–89

Di Girolamo G., Grigatti M., Barbanti L., Angelidaki I., 2013. Effects of hydrothermal pre-treatments on Giant reed (Arundo donax) methane yield. Bioresource Technology, 147, 152–159

Dinuccio E., Balsari P., Gioelli F., Menardo S., 2010. Evaluation of the biogas productivity potential of some Italian agro-industrial biomasses. Bioresource Technology, 101, May 2010, 3780–3783

Dipu S., Kumar A. A., Thanga V. S. G., 2011. Potential Application of Macrophytes Used in Phytoremediation. World Applied Sciences Journal, 13 (3), 2011, 482–486. Available on: http://www.idosi.org/wasj/wasj13(3)/15.pdf accessed on 12 January 2013

Dornburg V., van Vuuren D., van de Ven G., Langeveld H., Meeusen M., Banse M., van Oorschot M., Ros J., van den Born G. J., Aiking H., Londo M., Mozaffarian H., Verweij P., Lyseng E., Faaij A., 2010. Bioenergy revisited: Key factors in global potentials of bioenergy. Energy Environment Science, 3, 2010, 258–267

Dubrovskis V., Adamovics A., Plume I., Kotelenecs V., Zabarovskis E., 2011. Biogas production from greater burdock, largeleaf lupin and sosnovsky cow parsi. Proceeding of Engineering for Rural Development. 10th International Scientific Conference on Engineering for Rural Development, Jelgava, Latvia, 26 - 27 May 2011, 388–392

Duff S. J., Murray W. D., 1996. Bioconversion of forest products industry waste cellulosics to fuel ethanol: A review. Bioresource Technology, 55, 1996, 1–33

Dworak T., Elbersen B., van Diepen K., Staritsky I., van Kraalingen I., Suppit I., Berglund M., Kaphengst T., Laaser C., Ribeiro M., 2009. Assessment of inter-linkages between bioenergy development and water availability. Ecologic – Institute for International and European Environmental Policy Berlin/Vienna, July 2009

EC, 2009a. Directive 2009/28/EC of the European parliament and of the Council. On the promotion of the use of energy from renewable sources and amending and subsequently repealing Directives 2001/77/EC and 2003/30/EC. Official Journal of European Union. L 140, 2009, 16–62

EC, 2009b. Directive 2009/29/EC of the European Parliament and of the Council of 23 April 2009 amending Directive 2003/87/EC so as to improve and extend the greenhouse gas emission allowance trading scheme of the Community. Official Journal of the European Union. L 140, 2009, 63–87

EC, 2010a. Fact sheet The EU Nitrates. Available on: http://ec.europa.eu/environment/pubs/pdf/factsheets/nitrates.pdf accessed on 15 May 2011

EC, 2010b. Report from the commission to the Council and the European Parliament. On the implementation of Council Directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources based on Member State reports for the period 2004-2007. Available on: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=COM:2010:0047:FIN:EN:PDF accessed on 15 May 2011

EC, 2012. COM (2012) 595 Proposal for a directive of the European parliament and of the council amending Directive 98/70/EC relating to the quality of petrol and diesel fuels and amending Directive 2009/28/EC on the promotion of the use of energy from renewable sources. Available on: http://ec.europa.eu/clima/policies/transport/fuel/docs/com_2012_595_en.pdf accessed on 12 January 2013

EC, 2013. COM (2013) 175. Report from the commission to the European parliament, the council, the European economic and social committee and the committee of the regions. renewable energy progress report. REN21. 2013. Renewables 2013 Global Status Report. Available on: http://www.ren21.net/Portals/0/documents/Resources/GSR/2013/GSR2013_lowres.pdf accessed on 12 January 2013

EC, 2013. Report from the commission to the Council and the European Parliament. On the implementation of Council Directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources based on Member State reports for the period 2008–2011. Available on: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=COM:2013:0683:FIN:EN:PDF accessed on 20 May 2013

EEC, 1991. European Economic Community (EEC) Council Directive 91/676/EEC of 12 December 1991. Concerning the protection of waters against pollution caused by nitrates from agricultural sources. Official Journal L, 375, 1991, 1–8

Eisentraut A., 2010. SuStainable Production of Second-Generation biofuelS, Potential and perspectives in major economies and developing countries. Information paper, International Energy Agency 2010

Elbersen B., Andersen. E., Bakker R., Bunce R., Carey P., Elbersen W., van Eupen M., Guldemond A., Kool A., Meuleman B., Noij G., Roos Klein-Lankhorst J., 2006. Large-scale biomass production and agricultural land use – potential effects on farmland habitats and related biodiversity. Alterra report. Wageningen, 2006

EurObserv'ER, 2013. Available on: http://www.energies-renouvelables.org/observ-er/stat_baro/observ/baro216_en.pdf accessed on 20 May 2013

Fahd S., Fiorentino G., Mellino S., Ulgiati S., 2012. Cropping bioenergy and biomaterials in marginal land: the added value of the biorefinery concept. Energy, 37, 2012, 79–93

Fan L.T., Gharpuray M.M., Lee Y.H., 1987. Cellulose Hydrolysis. Springer Publisher

FAO, 2008. The State of Food and Agriculture. Biofuels: prospects risks and opportunities. ISSN 0081-4539

FAO, 2011. Quality assurance for animal feed analysis laboratories. FAO Animal Production and Health Manual No. 14. Rome.

FAO, 2013. Crop Water Information: Maize. Fao Water. Available on: http://www.fao.org/nr/water/cropinfo_maize.html accessed on 20 May 2013

Fargione J., Hill J., Tilman D., Polasky S., Hawthorne P., 2008. Land clearing and the biofuel carbon debt. Science, 319, 2008, 1235–1238. In: Nijsen M., Smeets E., Stehfest E., Detlef P. van Vuuren D. P., 2012. An evaluation of the global potential of bioenergy production on degraded lands. GCB Bioenergy, Volume 4, Issue 2, March 2012, 130–147

Fazio S., Monti A., 2011. Life cycle assessment of different bioenergy production systems including perennial and annual crops. Biomass and bioenergy, 35, 2011, 4868–4878

Ferracane R., Graziani G., Gallo M., Fogliano V., Ritieni A., 2010. Metabolic profile of the bioactive compounds of burdock (Arctium lappa) seeds, roots and leaves. Journal of Pharmaceutical and Biomedical Analysis. Natural Bioactive Compounds and Nutrigenomics, Volume 51, Issue 2, 20 January 2010, 399–404

Fiorese G., Guariso G., 2010. A GIS-based approach to evaluate biomass potential from energy crops at regional scale. Environmental modelling & software, 25, 2010, 702–711

Fisher B., Nakicenovic N., Alfsen K., Morlot J. C., de la Chesnaye F ., Hourcade J., Jiang K., Kainuma M., la Rovere E., Matysek A., Rana A., Riahi K., Richels R., Rose S., van Vuuren D., Warren R., 2007. Issues related to mitigation in the long-term context. In: Climate Change 2007. Mitigation of Climate Change. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Eds Metz B., Davidson O., Bosch P., Dave R., Meyer L., Cambridge University Press, New York, 2007, 169–250

Fonkou T., Ivo B. S., Lekeufack M., Mekontso T. F., Amougou A., 2011. Potential of Cyperus Papyrus in Yard-Scale Horizontal Flow Constructed Wetlands for Wastewater Treatment in Cameroon. Universal Journal of Environmental Research and Technology. Volume 1, Issue 2, 2011, 160–168. Available on: www.environmentaljournal.org accessed on 15 September 2013

Frank M. D., Beattie B. R., Embleton M. E. 1990. A Comparison of Alternative Crop Response Models. American Journal of Agricultural Economics, Vol. 72, No. 3 (Aug., 1990), 597-603.

Fu Y. J., Xin C., An C. L., 2009. Iron plaque formation on wetland plants and its influence on phosphorus, calcium and metal uptake. Aquat. Ecol., 43, 2009, 879–890

Ge X., Burner D. M., Xu J., Phillips G. C., Sivakumar G., 2011. Bioethanol production from dedicated energy crops and residues in Arkansas, USA. Biotechnology Journal, 6, 2011, 66–73

Giardini L., Berti A., Borin M. 1997. Concimazione azotata in mais e pomodoro in tre tipi di terreno (anni 1993 e 1994) in Agricoltura e ricerca, numero 168, marzo/aprile 1997. 93-100.

Greef J. M., Deuter M., 1993. Syntaxonomy of Miscanthus × giganteus GREEF et DEU. Angewandte Botanik, 67, 1993, 87–90

Grignani, C., Zavattaro, L., Sacco, D., Monaco, S., 2007. Production, nitrogen andcarbon balance of maize-based forage systems. Eur. J. Agron. 26, 442–453.

Gunnarson S., Malmberg A., Mathisen B., Theander O., Thyselius L., Wünsche U., 1985. Jerusalem artichoke (Helianthus tuberosus L.) for biogas production. Biomass, 7 (2), 1985, 85–97

Hack H., Bleiholder H., Buhr L., Meier U., Schnock-Fricke U., Weber E., Witzenberger A., 1992. Einheitliche Codierung der phänologischen Entwicklungsstadien mono - und dikotyler Pflanzen - Erweiterte BBCH - Skala, Allgemein - Nachrichtenbl. Deut. Pflanzenschutzd, 44, 1992, 265–270

Haiming W., Jian Z., Peizhi L., Jinyong Z., Huijun X., Bo Z., 2011. Nutrient removal in constructed microcosm wetlands for treating polluted river water in northern China. Ecological Engineering, 37, 2011, 560–568

Harrington C., Scholz M., 2010. Assessment of pre-digested piggery wastewater treatment operations with surface flow integrated constructed wetland systems. Bioresource Technology, 101, 2010, 7713–7723

Headley T. R., Tanner C. C., 2006. Applications of floating wetlands for enhanced stormwater treatment: a review. Auckland Regional Council, Technical Publication, Auckland

Hendriks A. T. W. M., Zeeman G., 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. Bioresource Technology, 100 (1), 2009, 10–18

Henkens P. L. C. M., van Keulen H., 2001. Mineral policy in The Netherlands and nitrate policy within the European Community. The Netherlands Journal of Agricultural Science, 49 (2–3), 2001, 117–134

Hickman G. C., Vanloockew A., Dohleman F. G., Bernacchi C. J., 2010. A comparison of canopy evapotranspiration for maize and two perennial grasses identified as potential bioenergy crops. Global Change Biology Bioenergy, 2, 2010, 157–168

Hidalgo M., Fernandez J., 2000. Biomass production of ten populations of giant reed (Arundo donax L.) under the environmental conditions of Madrid (Spain). In: Kyritsis S., Beenackers A.A.C.M., Helm P., Grassi A., Chiaramonti D., editors, 2000. Biomass for Energy and Industry: Proceeding of the First World Conference, Sevilla, Spain, 5–9 June 2000. London: James & James (Science Publishers) Ltd., 2001, 1881–1884

Hill J., Nelson E., Tilman D., Polasky S., Tiffany D., 2006. Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. Proceedings of the National Academy of Sciences of the United States of America, 103 (30), 2006, 11206-11210

Hills L. D., 1976. Comfrey: Past, Present and Future. Faber Finds

Houghton J., Weathermax S., Ferrell J., 2006. Breaking the biological barriers to cellulosic ethanol: a joint research agenda. US Department of Energy. Available at: http://genomicscience.energy.gov/biofuels/2005workshop/2005low_crosscutting.pdf accessed on 12 January 2013. In: Tan K. T., Lee K. T., Mohamed A. R., 2008. Role of energy policy in renewable energy accomplishment: The case of second-generation bioethanol. Energy Policy, 36, 2008, 3360– 3365

Howard V. M., 2012. Glyceria maxima. USGS Nonindigenous Aquatic Species Database, Gainesville, FL. Available on: http://nas.er.usgs.gov/queries/factsheet.aspx?SpeciesID=1120 accessed on 15 june 2007

Hu Z., Wang Y., Wen Z., 2008. Alkali (NaOH) pretreatment of switchgrass by radio frequency-based dielectric heating. Applied Biochemistry and Biotechnology, 148, 2008, 71–81

Hurry R. J., Bellinger E. G., 1990. Potential yield and nutrient removal by harvesting of *Phalaris arundinacea* in a wetland treatment system. In: Constructed Wetlands in Water Pollution Control, Cooper P. F., Findlater B. C. (eds.) Pergamon Press: Oxford, United Kingdom, 543–546

IEA, 2005. Bioenergy. Benefits of Bioenergy. Report.

IEA, 2006. World Energy Outlook 2006. International Energy Agency, Paris.

IPANE, 2001. University of Connecticut, Invasive Plant Atlas of New England (IPANE: Iris pseudacorus. Available on: http://webapps.lib.uconn.edu/ipane/browsing.cfm?descriptionid=59 accessed on 16 January 2003

Isci A., Murphy P. T., Anex R. P., Moore K. J., 2008. A rapid Simultaneous Saccharification and Fermentatio (SSF) technique to determine ethanol yields. Bioener, 1, 2008, 163–169

ISTAT, 2010. Available at: http://agri.istat.it/ accessed on 12 January 2013

ISTAT, 2013. Available at: http://agri.istat.it/sag_is_pdwout/jsp/dawinci.jsp?q=plC020000020000063200&an=2013&ig=1&ct=244&id=15A|18A|25A accessed on 12 November 2013

IUCN, 2006. The Future of Sustainability. Re-thinking Environment and Development in the Twenty-first Century. IUCN publishing. Available at: http://cmsdata.iucn.org/downloads/iucn_future_of_sustanability.pdf accessed on 12 January 2013

Johnston C. A., 1991. Sediments and nutrient retention by freshwater wetlands: effects on surface water quality. CRC Critical Reviews in Environmental Control, 21, 491–565. In:

Vymazal J., 2011. Plants used in constructed wetlands with horizontal subsurface flow: a review. Hydrobiologia, 674, 2011, 133–156

Kadlec R. H., Knight R. L., 1996. Treatment Wetlands, Lewis Publishers, CRC Press, Boca Raton, FL, USA, 893

Kadlec R. H., Wallace S. D., 2009. Treatment Wetlands, 2nd ed. CRC Press, Boca Raton, FL,272

Kallioinen A., Uusitalo J., Pahkala K., Kontturi M., Viikari L., von Weymarn N., Siika-aho M., 2012. Reed canary grass as a feedstock for 2nd generation bioethanol production. Bioresource Technology, 123, 2012, 669–672

Karim M.N., Ryu S., 2011. A whole cell biocatalyst for cellulosic ethanol production from dilute acid-pretreated corn stover hydrolyzates Appl Microbiol Biotechnol, 91, 2011, 529–542

Kenealy W., Zeikus J. G., 1981. Influence of corrinoid antagonists on methanogen metabolism. J. Bacteriol., 146, 133–140

Kirschner J., Balslev H., Brooks R. E., Clemants S. E., Ertter B., Hämet-Ahti L., Alvarez M. C. F. C., Novara L. J., Novikov V. S., Simonov S. S., Snogerup S., Wilson K. L., Zika P. F., 2002. Juncaceae 3: Juncus subg. Agathryon. Australian Biological Resource Study, Canberra, Australia, 2002, 1–192

Koonin S. E., 2006. Getting serious about biofuels. Science, 311 (5760), January 2006, 435

Krasuska E., Cadórniga C., Tenorio J. L., Testa G., Scordia D., 2010. Potential land availability for energy crops production in Europe. Biofuels, Bioprod, Biorefin, 4, 2010, 658–673

KTBL, 2005. Key data for agriculture (Faustzahlen für die Landwirtschaft). Kuratorium für Bauwesen in der Landwirtschaft e.V., Darmstadt. In: Bauer A., Leonhartsberger C., Bösch P., Amon B., Friedl A., Amon T., 2010. Analysis of methane yields from energy crops and agricultural by-products and estimation of energy potential from sustainable crop rotation systems in EU-27. Clean Techn Environ Policy, 12, 2010, 153–161

Kuusemets V., Lõhmus K., 2005. Nitrogen and Phosphorus Accumulation and Biomass Production by *Scirpus sylvaticu*s and *Phragmites australis* in a Horizontal Subsurface Flow Constructed Wetland. Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering, Volume 40, Issue 6-7, 2005, 1167–1175

Larson E. D., 2006. A review of life-cycle analysis studies on liquid biofuel systems for the transport sector. Energy for Sustainable Development Volume 10, Issue 2, June 2006, Pages 109–126

Lewandowski I. and Schmidt U. 2006. Nitrogen, energy and land use efficiencies of miscanthus, reed canary grass and triticale as determined by the boundary line approach. Agriculture, Ecosystems and Environment 112 (2006) 335–346.

Lewandowski I., Clifton-Brown J. C., Scurlock J. M. O., Huisman W., 2000. Miscanthus: European experience with a novel energy crop. Biomass and Bioenergy, 19, 2000, 209–27

Lewandowski I., Scurlock J. M. O., Lindvall E., Christou M., 2003. The development and current status of perennial rhizomatous grasses as energy crops in the US and Europe. Biomass and Bioenergy, 25, 335–361

Liang Y., Siddaramu T., Yesuf J., Sarkany N., 2010. Fermentable sugar release from Jatropha seed cakes following lime pretreatment and enzymatic hydrolysis. Bioresource Technology, 101 (16), 2010, 6417–6424

Linde-Laursen I. B., 1993. Cytogenetic analysis of Miscanthus ‘Giganteus’, an interspecific hybrid Hereditas, 119, 1993, 297–300

Lopez A., Pollice A., Lonigro A., Masi S., Palese A. M., Cirelli G. L., Toscano A., Passino R., 2006. Agricultural wastewater reuse in Southern Italy. Desalination, 187, 2006, 323–334

Luo A., Zhu J., Ndegwa P. M., 2002. Removal of carbon, nitrogen, and phosphorus in pig manure by continuous and intermittent aeration at low redox potentials. Biosyst. Eng., 82, 2002, 209–215

Luoma J. R., 2009. Hailed as a miracle crop, Jatropha falls short of hype. Yale Environment 360, Guardian Environment Network. Available on: http://www.guardian.co.uk/environment/2009/may/05/jatropha-biofuels-food-crops accessed on 15 September 2013

Lynn Wright Biomass Energy Data Book, 2010. Available on: http://cta.ornl.gov/bedb accessed on 15 May 2011

Mabee W., 2006. Economic Environment and Social Impact of 2nd Generation Biofuels in Canada. BIOCAP Research Integration Program. In: Tan K. T., Lee K. T., Mohamed A. R., 2008. Role of energy policy in renewable energy accomplishment: The case of second-generation bioethanol. Energy Policy, 36, 2008, 3360– 3365

MacDonald D. G., Bakhshi N., Mathews J. F., Roychowdhury A., Bajpai P., Moo-Young M., 1983. Alkaline treatment of corn stover to improve sugar production by enzymatic hydrolysis. Biotechnology and Bioengineering, 25 (8), 1983, 2067–2076

Mantineo M., D’Agosta G. M., Copani V., Patanè C., Cosentino S. L., 2009. Biomass yield and energy balance of three perennial crops for energy use in the semi-arid Mediterranean environment. Field Crops Research, 114, 2009, 204–213

Marchetti R., Vasmara C., Florio G., Borin M., 2013. Biomethanation potential of wetland biomasses. Bioresource Technology - submitted

Mariani C., Cabrini R., Danin A., Piffanelli P., Fricano A., Gomarasca S., Dicandilo M., Grassi F., Soave C., 2010. Origin, diffusion and reproduction of the giant reed (Arundo donax L.): a promising weedy energy crop. Annals of Applied Biology, 157, 2010, 191–202

Martinez J., Dabert P., Barrington S., Burton C., 2009. Livestock waste treatment systems for environmental quality, food safety, and sustainability. Bioresource Technology, 100 (22), 2009, 5527–5536

Michalski S. G., Durka W., 2012. Identification and characterization of microsatellite loci in the rush Juncus effusus (Juncaceae). American Journal of Botany, 2012, e53–e55

Ministerial Decre of 6 July 2012 - Incentivi per energia da fonti rinnovabili elettriche non fotovoltaiche Available on: http://www.sviluppoeconomico.gov.it/images/stories/normativa/DM_6_luglio_2012_sf.pdf accessed on 15 September 2012

Mohammad J., Taherzadeh M. J, Karimi K., 2008. Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review. Int. J. Mol. Sci., 9, 1621–1651

Molari G., Borin M., Milani M., Passoni M., Toscano A., Villani G., Zema D. A., Zimbone S. M., 2010. Energy transformation of herbaceous biomass irrigated with treated wastewater. Proc. of Int. Conf. AgEng 2010 “Towards environmental technologies”, Clermond Ferrand, France, 6-8 Sept., ISBN: 2853626849, 1–10

Monsier N., Handrickson R., Ho N., Sedlak N., Ladish M. R., 2005. Optimitation of pH controlled liquid hot water pretreatment of corn stover. Bioresour. Technol., 96, 2005, 673–686

Monsier N., Wyman C. E., Dale B. D., Elander R. T., Lee Y. Y., Holtzapplle M., Ladisch C. M., 2005. Feutures of promising technologies for pretreatment of lignocellulosic biomass. Bioresour. Technol., 96, 2005, 673–686

Monti A., Di Virgilio N., Venturi G., 2008. Mineral composition and ash content of six major energy crops. Biomass and Bioenergy, 32, 2008, 216–223

Morari F., Giardini L., 2009. Municipal wastewater treatment with vertical flow constructed wetlands for irrigation reuse. Ecological Engineering, 35, 643–653

Morari F., Lugato E., Polese R., Berti A., Giardini L., 2012. Nitrate concentrations in groundwater under contrasting agricultural management practices in the low plains of Italy Agriculture. Ecosystems & Environment, 147, 15 January 2012, 47–56

Nassi o Di Nasso N., Angelini L. G., Bonari E., 2010. Influence of fertilisation and harvest time on fuel quality of giant reed (Arundo donax L.) in central Italy. European Journal of Agronomy, 32, 2010, 219–227

Nassi o Di Nasso N., Roncucci N., Bonari E., 2013. Seasonal dynamic of aboveground and belowground biomass and nutrient accumulation and remobilization in giant reed (Arundo donax L.): a three-year study on marginal land. BioEnergy Research, Volume 6, Issue 2, June 2013, 725–736

Neto C. P., Seca A., Nunes A. M., Coimbra M. A., Domingues F., Evtuguin D., Silvestre A., Cavaleiro J. A. S., 1997. Variations in chemical composition and structure of macromolecular components in different morphological regions and maturity stages of Arundo donax. Industrial Crops and Products, 6, 1997, 51–58

Nijsen M., Smeets E., Stehfest E., Detlef P. van Vuuren D. P., 2012. An evaluation of the global potential of bioenergy production on degraded lands GCB Bioenergy, Volume 4, Issue 2, March 2012, 130–147

Official Journal of the European Union, 2011. Commission implementing decision of 3 November 2011 on granting a derogation requested by Italy with regard to the Regions of Emilia Romagna, Lombardia, Piemonte and Veneto pursuant to Council Directive 91/676/EEC concerning the protection of waters against pollution caused by nitrates from agricultural sources. Volume 54 L287/36. Available on: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2011:287:0036:0041:EN:PDF accessed on 20 May 2013

Ofoefule, A.U., Uzodinma, E.O., Onukwuli, O.D., 2009. Comparative study of the effect of different pretreatment methods on biogas yield from water Hyacinth (Eichhornia crassipes). Int. J. of Physical Sciences, 4, 535 - 539.

Olofsson K., Bertilsson M., Lidén G., 2008. A short review on SSF – an interesting process option for ethanol production from lignocellulosic feedstocks. Biotechnol Biofuels, 1, 2008, 7

Olsen S., Cole C., Watanabe F., Dean L., 1954. Estimation of available phosphorus in soils by extraction with sodium bicarbonate. U.S. Dept. of Agriculture, Washington D.C., 939

Olsson L., Hahn-Hägerdal B., 1993. Fermentative performance of bacteria and yeasts in lignocellulose hydrolysate. Proc Biochem, 28, 1993, 249–257

Owen W. F., Stuckey D. C., Healy J. J. B., Young L. Y., McCarty P. L., 1979. Bioassay for monitoring biochemical methane potential and anaerobic toxicity. Water Res., 13, 485–492

Parameswaran M., 1999. Urban wastewater use in plant biomass Production. Resources, Conservation and Recycling, 27 (1-2), 1999, 39–56

Park Y., Shiroma R., Al-Haq M. I., Zhang Y., Ike M., Arai-Sanoh Y., Ida A., Kondo M., Tokuyasu K., 2010. A novel lime pretreatment for subsequent bioethanol production from rice straw—calcium capturing by carbonation (CaCCO) process. Bioresource Technology, 101 (17), 2010, 6805–6811

Parrish D. J., Fike J. H., 2005. The biology and agronomy of switchgrass for biofuels. Critical Reviews in Plant Science, 24, 2005, 423–459

Patzek T. W., 2006. A Statistical Analysis of the Theoretical Yield of Ethanol from Corn Starch. Natural Resources Research, Vol. 15, No. 3, September 2006

Perdue R. E., 1958. Arundo donax–Source of musical reeds and industrial cellulose. Economic Botany, 12, 1958, 368–404

Perego A., Basile A., Bonfante A., De Mascellis R., Terribile F., Brenna B., Acutis M., 2012. Nitrate leaching under maize cropping systems in Po Valley (Italy). Agriculture, Ecosystems and Environment, 147, 2012, 57– 65

Petersen S. O., Blanchard M., Chadwick D., Del Prado A., Edouard N., Mosquera J. and Sommer S. G., 2013. Manure management for greenhouse gas mitigation. Animal, 7 s2, 2013, 266–282

Petrini C., Bazzocchi R., Bonari E., Ercoli L., Masoni A., 1996. Effect of irrigation and nitrogen supply on biomass production from Miscanthus in Northern-Central Italy. Agric. Mediterr., 126, 1996, 275–284. In: Cosentino S. L., Patanè C., Sanzone E., Copani V., Foti S., 2007. Effects of soil water content and nitrogen supply on the productivity of Miscanthus×giganteus Greef et Deu. in a Mediterranean environment. Industrial Crops and Products, 25, 2007, 75–88

Picco D., Pin M., Vecchiet A., Piscioneri I., Albergo R., Ambrico A., Maccioni O., Palazzo S., Trupo M. Ensilage Effect on the 2nd Generation Bioethanol Production from Sweet Sorghum Bagasse. In: Proceedings of 20th European Biomass Conference and Exhibition, 18–22 June 2012, Milan, Italy. 1652–1657

Pignatti S., 1994. Ecologia del paesaggio. Torino UTET (Unione Tipografico-Editrice Torinese)

Pin M., Jodice R., Albergo R., Balducchi R., 2008. La produzione del bioetanolo dal mais in Friuli Venezia Giulia tra criticità della filiera e margini di miglioramento tecnologico. Il bioetanolo dal mais: lo scenario attuale ed il ruolo del comparto agricolo. Notiziario ERSA, 2/2008

Politeo M., Borin M., Milani M., Toscano A., Molari G., 2011. Production and energy value of phragmites australis obtainedfrom two constructed wetlands. In: Faulstich M., Ossenbrink H., Dallemand J. F., Baxter D., Grassi A., Helm P. (Eds.): Proc. Of 19th European Biomass Conference and Exhibition, 6–10 June 2011, Berlin, Germany, ISBN 978-88-89407-55-7, 544–547

Poitrat, E., 1999. The potential of liquid biofuels in France. Renewable Energy 16, 1084–1089. In: Sánchez O., Cardona C., 2008. Trends in biotechnological production of fuel ethanol from different feedstocks. Bioresour Technol., 99, 13, 5270–5295

Prapa S., Suntud S., 2008. Feasibility of using constructed wetland treatment for molasses wastewater treatment. Bioresour. Technol. 99, 5610–5616

Prochnow, A., Heiermann, M., Plöchl, M., Linke, B., Idler, C., Amon, T., Hobbs, P.J., 2009. Bioenergy from permanent grassland - A review: 1. Biogas Bioresource Technology, 100, 4931 - 4944.

Quintero J. A., Montoya M. I., Sànchez O. J., Giraldo O. H., Cardona C.A., 2008. Fuel ethanol production from sugarcane and corn: Comparative analysis for a Colombian case. Energy, 33, 2008, 385–399

Ragauskas A. J., Williams C. K., Davison B. H., Britovsek G., Cairney J., Eckert C. A. Frederick Jr. W. J., Hallett J. P., Leak D. J., Liotta C. L., Mielenz J. R., Murph R., Templer R., Tschaplinski T., 2006. The path forward for biofuels and biomaterials. Science, 311 (5760), January 2006, 484–489

Rogner H. H., 2000. Energy Resources. Chapter 5. In: Goldemberg J. (Ed.), 2000. World energy assessment: energy and the challenge of sustainability. United Nations Development Programme Bureau for Development Policy, NewYork, September 2000

Saddler J. N., Gregg D. J., 1998. Ethanol production from forest product wastes. In: Bruce A., Palfreyman J. W. (eds) Forest products biotechnology. Taylor & Francis, London, 183–207

Salvato M., Borin M., 2010. Effect of different macrophytes in abating nitrogen from a synthetic wastewater. Ecological Engineering, 36, 2010, 1222–1231

Samson R. A., Omielan J. A., 1994. Switchgrass: a potential biomass energy crop for ethanol production. In: Proceedings of the Thirteenth North American Prairie Conference (eds Wickett R. G., Lewis P. D., Woodliffe A.), Windsor, Ontario, 253–258. In: Nijsen M., Smeets E., Stehfest E., Detlef P. van Vuuren D. P., 2012. An evaluation of the global potential of bioenergy production on degraded lands. GCB Bioenergy, Volume 4, Issue 2, March 2012, 130–147

Sánchez O., Cardona C., 2008. Trends in biotechnological production of fuel ethanol from different feedstocks. Bioresour Technol., 99, 13, 5270–5295

Sanderson M. A., Adler P. R., 2008. Perennial forages as second generation bioenergy crops. International Journal of Molecular Sciences, 9, 2008, 768–788. In: Nijsen M., Smeets E., Stehfest E., Detlef P. van Vuuren D. P., 2012. An evaluation of the global potential of bioenergy production on degraded lands. GCB Bioenergy, Volume 4, Issue 2, March 2012, 130–147

Sarkanen K. V., Ludwig C. H., 1971. Lignins: Occurrence, Formation, Structure and Reactions. John Wiley and Sons, New York

Scordia D., Cosentino S. L., Jeffries T.W., 2010. Second generation bioethanol production fromSaccharum spontaneum L. ssp. aegyptiacum (Willd.) Hack. Bioresour Technol., 101, 2010, 5358–5365

Scordia D., Cosentino S. L., Lee J., Jeffries T. W., 2012. Bioconversion of giant reed (Arundo donax L.) hemicellulose hydrolysate to ethanol by Scheffersomyces stipitis CBS6054. Biomass and Bioenergy, Volume 39, April 2012, 296–305

Searchinger T. D., Heimlich R., Houghton R. A., Dong F., Elobeld A., Fabiosa J., Tokgoz S., Hayes D., Yu T., 2008. Use of U.S. Croplands for Biofuels Increases Greenhouse Gases Through Emission from Land-Use Change. Science, 319, February 2008, 1238-1240

Sexton S. E., Zilberman D., 2008. Biofuel Impacts on Climate Change, the Environment and Food. Report to the Renewable Fuels Agency, Department of Agricultural and Resource Economics, University of California, Berkely. In: Nijsen M., Smeets E., Stehfest E., Detlef P. van Vuuren D. P., 2012. An evaluation of the global potential of bioenergy production on degraded lands. GCB Bioenergy, Volume 4, Issue 2, March 2012, 130–147

Shaojie L., 2012. Wetland Biomass - Chemical Benefits and Problems with Biogas Usage. Available on: http://hh.diva-portal.org/smash/record.jsf?pid=diva2:535260 accessed on 12 January 2013

Sharma K. P., Kushwaha S. P. S., Gopal B., 1998. A comparative study of stand structure and standing crops of two wetland species, Arundo donax and Phragmites karka, and primary production in Arundo donax with observations on the effect of clipping. Tropical Ecology, 39, 1, 1998, 39

Shatalov A. A., Pereira H., 2001. Arundo donax L. (giant reed) as a source of fibres for paper industry: perspectives for modern ecologically friendly pulping technologies. In: Kyritsis S., Beenackers A. A. C. M., Helm P., Grassi A., Chiaramonti D., editors, 2001. Biomass for Energy and Industry: Proceeding of the First World Conference, Sevilla, Spain, 5–9 June 2000. London: James & James (Science Publishers) Ltd., 2001, 1183–1186

Shatalov A. A., Pereira H., 2002. Influence of stem morphology on pulp and paper properties of Arundo donax L. reed. Industrial Crops and Products, 15, 2002, 77–83

Shatalov A. A., Pereira H., 2005. Kinetics of organosolv delignification of fibre crop Arundo donax L. Industrial Crops and Products, 21 (2), March 2005, 203–210

Shiralipour, A., Smith, P.H., 1984. Conversion of biomass into methane gas. Biomass, 6, 85 - 92.

Sims R. E. H., Mabee W., Saddler J. N., Taylor M., 2010. An overview of second generation biofuel technologies. Bioresource Technology 101 (2010) 1570-1580

Sivakumar G., Vail D. R., Xu J. F., Burner D. M., Lay J. O., Ge X., Weathers P. J., 2010. Bioethanol and biodiesel: Alternative liquid fuels for future generations. Engineering in Life Science, 10, 1, 2010, 8–18

Smith K. A., Charles D. R., Moorhouse D., 2000. Nitrogen excretion by farm livestock with respect to land spreading requirements and controlling nitrogen losses to ground and surface waters. Part 2: Pigs and poultry. Bioresource Technology, 71 (2), 2000, 183–194

Soto M. L., Dominguez H., Nunez M. J., Lema J. M., 1994. Enzymatic saccharification of alkali-treated sunflower rhulls. Bioresource Technology, 49 (1), 1994, 53–59

Spignoli G., Mercati V., Boncompagni E., 1999. S. S. Aboca (Eds.), Guida Bibliografica ai più noti fitoterapici, Italy, 1999, 37–42

Springer U., Klee J., 1954. Prüfung der Leistungsfähigkeit von einigen wichtigen Verfahren zur Bestimmung des Kohlenstoffs mittels Chromschwefelsaure sowie Vorschlag einer neuen Schnellmethode. Journal of Plant Nutrition and Soil Science, 64, 1954, 1–26

StatSoft, Inc., 2004. Statistica, Version 7.0, www.statsoft.com

Sun Y., Cheng J., 2002. Hydrolysis of lignocellulosic materialsfor ethanol production: a review. Bioresour. Thecnol., 83, 2002, 1–11

Sutherland W. J., 1990. Biological Flora of the British Isles: Iris pseudacorus L. No. 169. Journal of Ecology, 78, 1990, 833–848

Taherzadeh M. J., Karimi K., 2008. Pretreatment of lignocellulosic waste to improve ethanol and biogas production: A review. Int. J. Mol. Sci., 9, 2008, 1621–1651

Tan K. T., Lee K. T., Mohamed A. R., 2008. Role of energy policy in renewable energy accomplishment: The case of second-generation bioethanol. Energy Policy, 36, 2008, 3360– 3365

Tanner C. C., 1996. Plants for constructed wetland treatment systems - A comparison of the growth and nutrient uptake of eight emergent species. Ecological Engineering, 7 (1), 1996, 59–83

Tengborg C., Galbe M., Zacchi G., 2001. Influence of enzyme loading and physical parameters on the enzymatic hydrolysisof steam-pretreated softwood. Biotechnol. Prog., 17, 2001, 110–117

Terer T., Muasya A. M., Dadouh-Guebas F., Ndiritu G. G., Triest L., 2012. Integrating local ecological knowledge and management practices of an isolated semi-arid papyrus swamp (Loboi, Kenya) into a wider conservation framework. Journal of Environmental Management, 93, 2012, 71–84

Terer T., Triest L., Muasya M. A., 2012. Effects of harvesting Cyperus papyrus in undisturbed wetland, Lake Naivasha, Kenya Hydrobiologia, 680, 2012, 135–148

Tilman D., Hill J., Lehman C., 2006. Carbon-negative biofuels from low-input highdiversity grassland biomass. Science, 314, 2006, 1598–1600. In: Nijsen M., Smeets E., Stehfest E., Detlef P. van Vuuren D. P., 2012. An evaluation of the global potential of bioenergy production on degraded lands. GCB Bioenergy, Volume 4, Issue 2, March 2012, 130–147

Toledo A., 2006. Caracterização morfoanatômica de raiz e rizoma de Symphytum officinale L. (Boraginaceae) / Morpho-anatomical characterization of the root and rhizome of Symphytum officinale L. (Boraginaceae) - Rev. bras. Farmacogn, 16 (2), april–june 2006, 185–191

Tucker G. C., 1990. The genera of Arundinoideae (Gramineae) in the southeastern United States. Journal of the Arnold Arboretum, 71, 2, 1990, 145–77

Tylova-Munzarova E., Lorenzen B., Brix H., Votrubova O., 2005. The effects of NH4 and NO3 on growth, resource allocation and nitrogen uptake kinetics of Phragmites australis and Glyceria maxima Aquatic Botany, 81, 2005, 326–342

USEPA, 2000. Constructed wetlands treatment of municipal wastewaters. Washington, USA

Valderrama, J. C., 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. Mar. Chem., 10, 1981, 109–122

Van Acker J., Buts L., Thoeye C., de Gueldre G., 2005. Floating plant beds: BAT for CSO treatment? Book of abstracts from international symposium on wetland pollutant dynamics and control, Ghent, Belgium, September 4–8 2005, 186–187

Van Soest P. J., Robertson J. B., 1979. Systems of analysis evaluating fibrous feeds. Cornell University, Ithaca- N.Y.

Van Vuuren D. P., den Elzen M. G. J., Lucas P. L., Eickhout B., Strengers B. J., van Ruijven B., Wonink S., van Houdt R., 2007. Stabilizing greenhouse gas concentrations at low levels: an assessment of reduction strategies and costs. Climatic Change, 81, 2007, 119–159

Vasmara C., Florio G., Borin M., Orsi A., Marchetti R., 2012. Potential for methane and hydrogen production from wetland biomass. Environmental Engineering and Management Journal, 11, 2012, 3, Supplement, S121–S13, S130

Venendaal R., Jorgensen U., Foster C. A., 1997. European energy crops: a synthesis. Biomass and Bioenergy, 1997, 147–185

Ververis C, Georghiou K., Christodoulakis N., Santas P., Santas R., 2004. Fiber dimensions, lignin and cellulose content of various plant materials and their suitability for paper production. Industrial Crops and Products, 19, 2004, 245–254

Vinther F. P. 2005. SimDen – A simple empirical model for quantification of N_2O emission and denitrification. Paper at: Manure - an agronomic and environmental challenge. NJF-seminar no. 372, Nils Holgerssongymnasiet, Skurup, Sweden. Abstract of oral presentation, 5-6 September 2005.

Viola E., De bari I., Zimbardi F. Braccio G., 2004. Simulazione di un processo ed analisi dei costi per un impianto di produzione di etanolo da biomasse lignocellulosiche. Collana Rapporti tecnici Enea RT/2004/31/ENE ISSN/0393-3016

Vojtíšková L., Munzarová E., Votrubová O., Řihová A., Juřicová B., 2004. Growth and biomass allocation of sweet flag (Acorus calamus L.) under different nutrient conditions. Hydrobiologia, 518, 2004, 9–22

Vymazal J., 2004. Removal of phosphorus in constructed wetlands with sub-surface flow in the Czech Republic. Water, Air, and Soil Pollution: Focus, 4, 2004, 657–670. In:

Vymazal J., 2011. Plants used in constructed wetlands with horizontal subsurface flow: a review. Hydrobiologia, 674, 2011, 133–156

Vymazal J., 2007. Removal of nutrients in various types of constructed wetlands. Science of the Total Environment, 380, 2007, 48–65

Vymazal J., 2011. Plants used in constructed wetlands with horizontal subsurface flow: a review. Hydrobiologia, 674, 2011, 133–156

Vymazal J., Kröpfelová L., 2008. Wastewater Treatment in Constructed Wetlands with Horizontal Sub-Surface Flow. Springer, Dordrecht

Vymazal, J., 1995. Algae and Element Cycling in Wetlands. Lewis Publishers, Chelsea, MI, 1995. In: Vymazal J., 2011. Plants used in constructed wetlands with horizontal subsurface flow: a review. Hydrobiologia, 674, 2011, 133–156

Waggy M. A., 2010. Phalaris arundinacea. In: Fire Effects Information System, U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Fire Sciences Laboratory (Producer). Available on: http://www.fs.fed.us/database/feis/ accessed on 8 March 2012

Wichtman W., Wichtman S., 2011. Environmental, social and economic aspects of a sustainable biomass production. Journal of Sustainable Energy & Environment Special Issue, 2011, 77–81

Wilkinson A., 2003. A laboratory evaluation of comfrey (Symphytum officinale L.) as a forage crop for ensilage. Animal Feed Science and Technology, 104, 2003, 227–233

Wyman C. E., 2007. What is (and is not) vital to advancing cellulosic ethanol. Trends biotechnol., 25 (4), 2007, 153–157

Xiandeng H., Bradley T. J., 2000. Inductively Coupled Plasma/Optical Emission Spectrometry. Encyclopedia of Analytical Chemistry, R.A. Meyers (Ed.), John Wiley & Sons Ltd, Chichester, 2000, 9468–9485. Available on:

http://www.wfu.edu/chemistry/courses/jonesbt/334/icpreprint.pdf accessed on 15 September 2013

Yang B., Wyaman C. E., 2008. Pretreatment: the key to unlocking low cost lignocellulosics ethanol. Biofuels Bioprod. Bior., 2, 2008, 26–40

Yousefi Z., Mohseni-Bandpei A., 2010. Nitrogen and phosphorus removal from wastewater by subsurface wetlands planted with Iris pseudacorus. Ecological Engineering, 36, 2010, 777–782

Zema D. A., Bombino G., Andiloro S., Zimbone S. M., 2012. Irrigation of energy crops with urban wastewater: Effects on biomass yields, soils and heating values. Agricultural Water Management, 115, 2012, 55– 65

Zhang C., Xie G., Li S., Ge L., He T. D., 2010. The productive potentials of sweet sorghum ethanol in China. Appl. Ener 7, 2360-2368 In: Davila-Gomez F. J., Chuck-Hernandez C., Perez-Carrillo E., Rooney W. L., Serna-Saldivar S. O., 2011. Evaluation of bioethanol production from five different varieties of sweet and forage sorghums (Sorghum bicolor (L) Moench), Industrial Crops and Production, 33, 2011, 611–616

Zhang T., Wang L., He Z., Zhang D., 2011. A Growth inhibition and biochemical changes of cyanobacteria induced by emergent macrophyte Thalia dealbata roots. Biochemical Systematics and Ecology, 39, 2011, 88–94

Zhao Y., Wang Y., Zhu J. Y., Ragauskas A., Deng Y., 2008. Enhanced enzymatic hydrolysis of spruce by alkaline pretreatment at low temperature. Biotechnology and Bioengineering, 99 (6), 2008, 1320–1328

Zhu J., Wan C., Li Y., 2010. Enhanced solid-state anaerobic digestion of corn stover by alkaline pretreatment. Bioresource Technology, 101 (19), 2010, 7523–7528

Zub H. W., Brancourt-Hulmel M., 2010. Agronomic and physiological performances of different species of Miscanthus, amajor energy crop. A review. Agron. Sustain. Dev. 30, 2010, 201–214