Role of C3 plant species on carbon dioxide and methane emissions in Mediterranean constructed wetland

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Abstract

C3 plant species are widely used to vegetate constructed wetlands (CW), but so far no information is available on their effect on CW $CO_{2(eq)}$ balance in the Mediterranean climate. The aim of this research was to study carbon dioxide (CO_2) and methane (CH_4) emissions and $CO_{2(eq)}$ budgets of CW horizontal sub-surface flow pilot-plant beds vegetated with *Arundo donax* L. and *Phragmites australis* (Cav.) Trin. ex Steud. compared with an unvegetated bed in Sicily. The highest total plant biomass production was measured in the bed vegetated with *A. donax* (17.0 kg m^{-2}), whereas *P. australis* produced 7.6 kg m^{-2}. CO_2 and CH_4 emissions and showed significant correlation with average air temperature and solar radiation for each bed. The CO_2 emission values ranged from 0.8±0.1 g m^{-2} d^{-1}, for the unvegetated bed in April, to 24.9±0.6 g m^{-2} d^{-1} for the bed with *P. australis* in August. The average CO_2 emissions of the whole monitored period were 15.5±7.2, 15.1±7.1 and 3.6±2.4 g m^{-2} d^{-1} for *A. donax*, *P. australis* and unvegetated beds respectively. The CH_4 fluxes differed significantly over the monitored seasons, with the highest median value being measured during spring (0.963 g m^{-2} d^{-1}). No statistical differences were found for CH_4 flux among the studied beds. Cumulative estimated CH_4 emissions during the study period (from April to December) were 159.5, 134.1 and 114.7 g m^{-2} for *A. donax*, *P. australis* and unvegetated beds respectively. $CO_{2(eq)}$ balance showed that the two vegetated beds act as $CO_{2(eq)}$ sinks, while the unvegetated bed, as expected, acts as a $CO_{2(eq)}$ source. Considering only the above-ground plant biomass in the $CO_{2(eq)}$ budgets, *P. australis* and *A. donax* determined uptakes of 1.30 and 8.35 kg $CO_{2(eq)}$ m^{-2} respectively.

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Introduction

C3 plant species are widely used to vegetate constructed wetlands (CWs), which are common natural-like systems (Vymazal, 2010) adopted to treat different wastewaters: landfill leachate (Bulc, 2006), agriculture drainage and animal wastewater (Borin and Tocchetto, 2007; Borin *et al.,* 2013a), textile wastewater (Bulc and Ojstršek, 2008), urban wastewater (Barbera *et al.,* 2009), pesticides (O'Geen *et al.,* 2010). In these systems the organic carbon fraction content in the wastewaters is mainly removed through volatilization, determining a positive flux of greenhouse gases (GHGs), such as carbon dioxide (CO_2) and methane (CH_4) into the atmosphere. However, the atmospheric CO_2 uptake by plant photosynthesis can balance $CO_{2(eq)}$ fluxes (Barbera *et al.,* 2014a). In general plants through their root systems (Lai *et al.,* 2011) influence CO_2 production (Ström *et al.,* 2005) and CH_4 production and consumption (Segers, 1998; Ström *et al.,* 2005; Wang *et al.,* 2008), mainly through roots oxygen (Griess *et al.,* 1990) and exudate release (Brix *et al.,* 2001). In particular, plant species with different physiology and therefore different magnitude of oxygen (Wigand *et al.,* 1997) and exudate release (Ström *et al.,* 2003) can determine a different CO_2:CH_4 ratio emission and global warming potential (GWP) given that CH_4 has a GWP 25 times higher than CO_2 (IPCC, 2007). In the Mediterranean basin, where temperatures in late spring and summer are high, the use of C3 species in CWs could determine a different environmental C balance compared with the CWs located at higher latitude.

Several studies on GHG emission, at different latitudes, include CW beds vegetated with *Phragmites australis*, which is the most widely studied C3 species. Only a few studies have been carried out in a Mediterranean environment on GHG emission from pilot-plant CWs (Garcia *et al.,* 2007; Barbera *et al.,* 2014a) or a full-scale CW (Barbera *et al.,* 2014b) vegetated with *P. australis* or C4 plant species. To our knowledge no studies have focused on GHG emission from CWs vegetated with *Arundo donax* (C3 species), either in a Mediterranean environment or at other latitudes.

The question if wetlands act as sinks or sources of GHGs has recently gained increasing importance to understand more thoroughly the role of the ecosystem services that they provide (Mitsch *et al.,* 2013) and address their management. Since vegetation is the key component of CWs, its role needs to be definitely better understood.

In this contest the aim of this research was to compare CO_2 and CH_4 emissions and $CO_{2(eq)}$ budgets of CW horizontal sub-surface flow (HSSF) pilot-plant beds unvegetated or vegetated with *A. donax* L. and *P. australis* (Cav.) Trin. ex Steud.

Materials and methods

Study sites

The research was conducted from April 1st to December 20th 2012 in a pilot plant located in San Michele di Ganzaria (Eastern Sicily – lat. 37°30′ N, long. 14°25′ E, alt. 370 m asl). The area has a typical Mediterranean climate (Köppen climate classification) with medium rainfall (approximately 500 mm y^{-1}) mainly in winter. The pilot plant consisted of three parallel HSSF beds, two vegetated with *P. australis* (Cav.) Trin. ex Steud. (common reed) and *A. donax* L. (giant reed) and one unvegetated control bed. Each bed was rectangular in shape with a surface area of 4.5 m^2 (1.5×3.0 m) and was built of concrete, partially buried, and lined with an impermeable membrane. The beds were filled to an average depth of 0.6 m, with 10-15 mm volcanic gravel; during the monitoring period, water depth was about 0.55 m. System details are available in Toscano *et al.* (2009). Wastewater inflow was 40 L h^{-1}. Beds were planted in December 2011 at a density of 5.5 plants m^{-2}.

Studied C3 species

P. australis is the most frequently used macrophyte to vegetate CWs. It is a tall perennial grass of the Poaceae family found in natural wetlands across temperate and tropical regions of the world. It commonly forms extensive stands (known as reed beds) and is capable of reproduction by seed, but primarily asexually multiplication via rhizomes. *P. australis* is a C3 plant species characterised by aerating tissues (aerenchyma channels and pith cavities) which play a crucial role in this species and generally in aquatic plants by providing oxygen to the submerged organs and often to the rhizosphere (Engloner, 2009).

A. donax is a tall perennial herbaceous plant of the same family occurring in grasslands and wetlands over a wide range of climatic habitats. It is classified as an emergent aquatic plant (Cook, 1990). Like *P. australis*, it is a C3 species, with an unusually high photosynthetic capacity (Rossa *et al.*, 1998) and productivity similar to that of C4 species (Christou *et al.*, 2001; Borin *et al.*, 2013b).

Meteorological variables

A CR510 automatic weather station (Campbell Scientific, Logan, UT, USA) was installed close to the experimental plant to measure rainfall, air temperature, wind speed and global radiation. Evapotranspiration rates were estimated using a water balance method, measuring for each bed the influent wastewater flow rate, the water increase due to precipitation and the discharged wastewater volume.

Gas sampling and analyses

CO_2 and CH_4 sampling and analysis were performed from April 1st (vegetative regrowth) to December 20th 2012 (end of vegetative cycle). The gas samplers did not cover growing plants.

CO_2 emissions were estimated *in situ* using the static-stationary chamber technique. The cylindrical chambers, of PVC, were 35 cm high and 12.5 cm wide. The bottom part (20 cm) was permanently inserted in the gravel substrate and the chamber was sealed with a lid in which the CO_2 emitted from the bed was absorbed in a sodium hydroxide (NaOH) solution trap following modifications reported in Barbera *et al.* (2014b) to reduce experimental error (Jensen *et al.,* 1996). The CO_2 traps, two in each bed, were placed in the inner part of the beds to reduce the border effect. They were replaced every ten days, therefore the monthly total bed respiration (respiration of bed microbes, roots and rhizomes) was calculated based on a decadal dataset.

CH_4 flux was measured using the static non-stationary chamber technique (Barbera *et al.*, 2014a) three times a month in two microsites in order to replicate the measures. The flux cylindrical chamber, 42 cm high and 20 cm wide, was inserted into the gravel substrate using a permanent ring inserted into the substrate before the beginning of measurements to prevent soil disturbance in each site. The surface CH_4 flux was determined by measuring the temporal change in CH_4 concentration inside the chamber using a portable flame ionisation detector (Crowcon Gas-Tec®; Crowcon Detection Instruments Ltd., Abingdon, UK) detecting CH_4 concentrations down to parts per million levels.

Biomass sampling and analyses

In December 2012 above-ground and below-ground biomasses were sampled from three points in the inner part of each bed in order to minimize the border effect. The below-ground biomass was collected at three depths (0-20, 20-40 and 40-60 cm) and was divided in roots and rhizomes. Biomass sub-samples were homogenized for quality analysis: biomass dry weight was determined using a thermo-ventilated oven at 65°C until constant weight was reached. Biomass C content was determined by CNS Macrovario combustion analyzer (Elementar Analysensysteme GmbH, Hanau, Germany).

Growing season $CO_{2(eq)}$ balance

Carbon environmental balance was calculated considering CO_2 and CH_4 emissions and the storage of fixed carbon in plant biomass in terms of $CO_{2(eq)}$ using the following formula:

$$CO_{2(eq)} = CO_2 + (CH_4 * 25) + C_{biomass} * (44/12) \tag{1}$$

where:
- CO_2 and CH_4 were the GHG emissions measured during the growing season;
- the CH_4 emission for $CO_{2(eq)}$ budgets was computed as 25 times CO_2 (IPCC, 2007);
- 44 represents the molecular weight of CO_2, and 12 is the C atomic weight.

As indicated by Mander *et al.* (2008), the above-ground plant biomass respiration was not considered, assuming that respired C was previously assimilated by plant gross photosynthesis.

Statistical analysis

The normality of data was tested using the Kolmogorov-Smirnov, Lilliefors, and Shapiro-Wilk tests. CO_2 and CH_4 emissions from the study sites did not show a normal distribution, so the Kruskal-Wallis non-parametric test was used to check the significance of differences (accepted at the level of $P \leq 0.05$) using a combination of replications in time and space rather than through independent experimental units. Correlations between average air temperature and solar radiation with CO_2 and CH_4 emissions were evaluated using Spearman Rank correlation. The range distribution of biomass and emission values was expressed in terms of standard deviation.

Results and discussion

Environmental parameters

Meteorological data recorded at the site during the monitoring period (April-December 2012) are reported in Figure 1. Cumulative rainfall was 105.8 mm; the air temperature reached its maximum value on July 13th (43.4°C) and its minimum value on December 13th (0.2°C). The highest monthly average solar radiation value (27.6 MJ m^{-2} d^{-1}) was recorded in July and the average wind speed was generally below 1 ms^{-1}. The correlation between average air temperature and solar radiation with CO_2 and CH_4 emissions showed a specific response for each bed (Table 1). CO_2 emission was positively correlated with average air temperature in the bed vegetated with *P. australis* ($P<0.001$), instead no correlation was found for *A. donax*. For all beds CH_4 emission was positively correlated with solar radiation ($P<0.001$), whereas only the unvegetated bed showed a positive correlation between CH_4 emission and average air temperature ($P<0.05$). These results are undoubtedly interesting, but caution must be used due to the short monitoring period of about nine months and considering that the study was carried out during the first operating year and so the systems did not reach the below-ground biomass turnover with a low amount of sludge accumulated in the beds. Both conditions could determine, in the following years, different emission ratios between the two species and different responses to environmental conditions.

Plants biomass production

At the end of the vegetative season, *A. donax* showed the higher total plant biomass yield (16.8 kg m^{-2}), while *P. australis* produced 7.5 kg m^{-2}. Considering the plant organs, giant reed has always scored the highest biomass production, namely 1.8, 5.1 and 3.3 times higher than the common reed in terms of biomass of above-ground portion, roots and rhizomes respectively. Roots density declined with depth in the bed vegetated with *A. donax*, whereas for *P. australis* higher density was found in the 20-40 cm gravel layer. Rhizomes were distributed only in the first 20 cm of substrate for *A. donax* and in the 0-40 cm layer for *P. australis* (Table 2). At the end of the study period, *P. australis* showed a higher above-ground/below-ground ratio (2.7) than *A. donax* (1.5; Table 2). The reported high above-ground production confirms the high productive potential of the two species cultivated under optimal water and nutritional conditions (Idris *et al.,* 2012a; Borin *et al.,* 2013b).

Greenhouse gases emissions

The average CO_2 emission was significantly lower in spring for all studied CW beds (Figure 2A) with the lowest monthly CO_2 average daily emission recorded in April with 5.2±1.6, 6.1±1.0 and 0.8±0.1 g m^{-2} d^{-1} for common reed, giant reed and unvegetated beds respectively. The highest monthly average daily CO_2 emissions were recorded in August for common reed (24.9±0.6 g m^{-2} d^{-1}) and in September for giant reed (24.3±2.7 g m^{-2} d^{-1}) and the unvegetated bed (6.6±1.1 g m^{-2} d^{-1}). With respect to season averages, no significant differences were found in CO_2 emission between *A. donax* and *P. australis* beds, instead a significantly lower emission was recorded for the unvegetated bed (Figure 2B), with a median value 4.3 times lower than the average median value of vegetated beds. Bed respiration did not show any species-specific effect, but there was a significantly higher emission from vegetated beds than the unvegetated one, confirming that the presence of vegetation is very important for the respiration of CW total ecosystems (Ström *et al.,* 2007). Nevertheless the effect of different species may be present, as suggested by Maltais-Landry *et al.* (2009) who reported significant differences among HSSF CW mesocosms vegetated with *P. australis*, *Phalaris arundinacea* and *Typha angustifolia*.

In our study the average respiration of the bed throughout the entire monitored period was 15.1±7.1, 15.5±7.2 and 3.6±2.4 g CO_2 m^{-2} d^{-1} for *P. australis*, *A. donax* and unvegetated beds respectively, in agreement with Barbera *et al.* (2014b) who reported, in the same area, higher CO_2 emissions from vegetated sites than an unvegetated one in a full scale HSSF CW. Even at higher latitudes in Southern Sweden, Ström *et al.* (2007) reported an average CO_2 flux of 25.1±4.7 and 4.3±0.7 g m^{-2} d^{-1} from a zone vegetated with *P. australis* and an unvegetated zone respectively in a peat-based CW site. Søvik *et al.* (2006) reported an average CO_2 emission of 2.5±0.2 and 10.6±0.6 g m^{-2} d^{-1} during winter and summer respectively in a comparative study conducted in northern Europe (Estonia, Norway and Poland) on GHG emissions from vegetated HSSF CWs (*P. australis*, *Iris pseudocorus*, *Typha latifolia*, and *Scirpus lacustris*). Picek *et al.* (2007) reported CO_2 emissions varying between 0.4 and 27.2 g m^{-2} d^{-1} during summer and fall in an HSSF CW bed vegetated with *P. australis* that treated combined sewage and stormwater runoff.

In our study common and giant reed showed similar CO_2 cumulative emissions, with about 3.98 and 4.08 kg m^{-2} respectively, whereas the

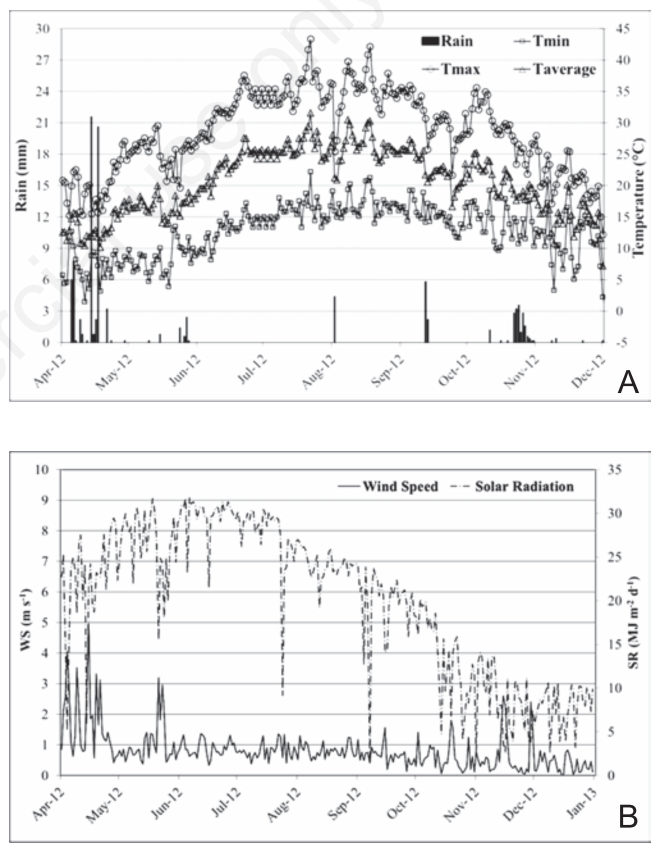

Figure 1. A) and B) Meteorological data recorded in San Michele di Ganzaria during the study period.

Table 1. Correlation of CO_2 and CH_4 bed emissions with temperature and solar radiation.

Correlation	*P. australis*	*A. donax*	Unvegetated
CO_2 *vs* solar radiation	0.108 n.s.	–0.311 n.s.	-0.541**
CO_2 *vs* average air temperature	0.789***	0.293 n.s.	0.196 n.s.
CH_4 *vs* solar radiation	0.760***	0.818***	0.798***
CH_4 *vs* average air temperature	0.191 n.s.	0.341 n.s.	0.477*

*P<0.05; **P<0.01; ***P<0.001. n.s., not significant;

unvegetated bed reported 0.94 kg m^{-2}.

The fluxes of CH_4 were significantly different among the studied seasons (Figure 3A). The highest median value was measured during spring (0.963 g m^{-2} d^{-1}), followed by summer (0.399 g m^{-2} d^{-1}) and fall (0.018 g m^{-2} d^{-1}). The highest CH_4 spring emission was probably due to the plant settlement phase characterised by: i) a fast development of root system resulting in high release of exudates that improved the activity of methanogen microorganisms (Brix *et al.*, 2001; Ström *et al.*, 2003; Saarnio *et al.*, 2004) in vegetated beds. This is supported by the positive correlation between CH_4 emissions and solar radiation, which influenced root exudates by photosynthesis activity (Grayston *et al.* 1997); ii) the incomplete root system development that determined less oxygen presence in the bed and so lower CH_4 oxidation rate. Considering the unvegetated bed, the significant ($P \leq 0.001$) positive correlation between CH_4 emission and solar radiation can be supported by the indirect effect of the latter on substrate and water temperature. Furthermore only for the unvegetated bed CH_4 emissions were also correlated ($P \leq 0.05$) with average air temperature, in agreement with Tanner *et al.* (1997) who found a correlation between air temperature and CH_4 emissions, and with Sorrel *et al.* (1997) who reported significantly lower methanogenesis at 12°C than 30°C. Johansson *et al.* (2004) studied CH_4 emission from ponds where urban wastewater was treated and reported that sediment and water temperatures explained a large proportion of the flux variations (33-43%).

No statistical differences were found for the CH_4 emissions from the three studied beds (Figure 3B). Inamori *et al.* (2007) reported that CH_4

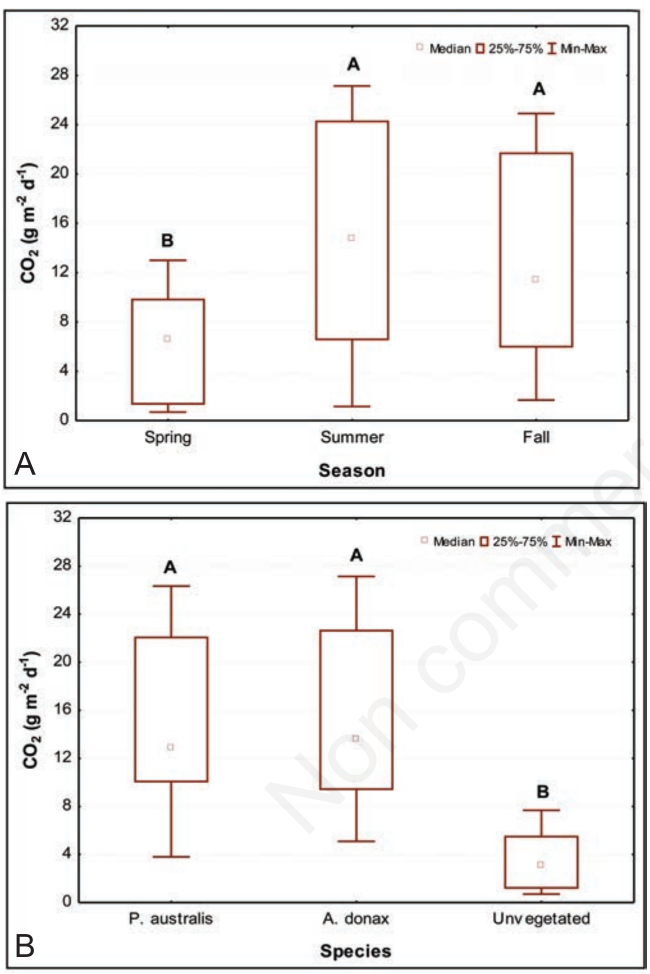

Figure 2. Emission of carbon dioxide beds: A) in different seasons and B) with different species.

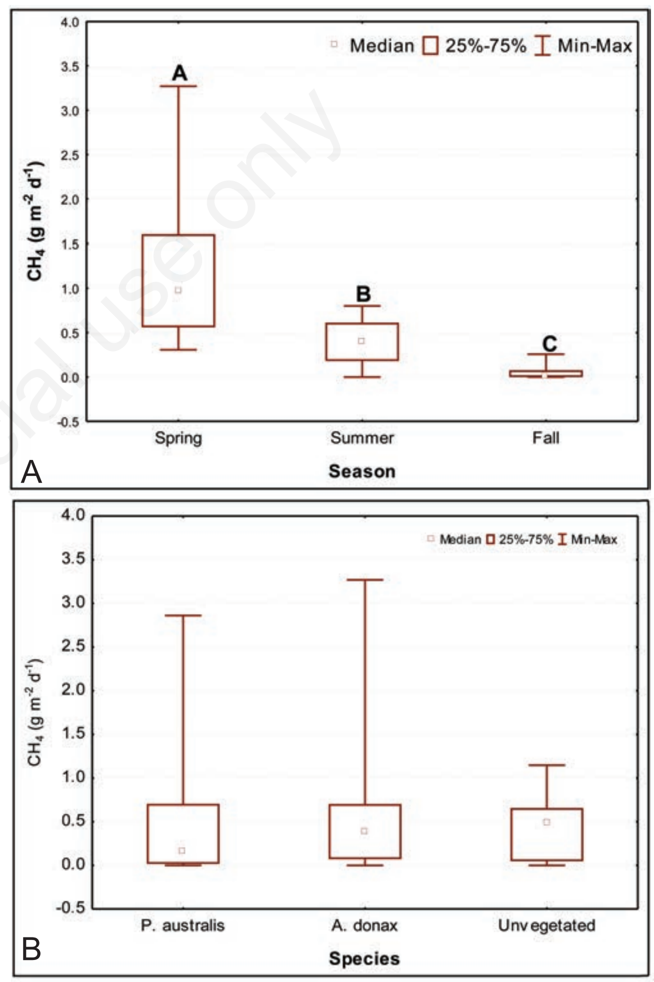

Figure 3. Emission from methane beds: A) in different seasons and B) with different species.

Table 2. Above-ground and below-ground biomass production (±SD) at the end of the study period and plant fraction incidence.

Species	Above-ground biomass (Mg ha^{-1})	Below-ground biomass (Mg ha^{-1})					Roots:Rhizomes ratio	Above-ground: Below-ground ratio
		Roots 0-20 cm	Roots 20-40 cm	Roots 40-60 cm	Rhizomes 0-20 cm	Rhizomes 20-40 cm		
P. australis	55.0±3.9	0.24±0.01	0.78±0.06	0.12±0.02	12.89±0.62	6.02±0.34	0.06	2.7
A. donax	99.3±6.9	3.25±0.16	2.01±0.13	0.57±0.08	62.40±3.63	-	0.09	1.5

emission from plant units is the net balance between CH_4 production by methanogens and oxidation to CO_2 by methanotrophs. Given that macrophytes' aerenchymatous tissues transport and release oxygen into the rhizosphere, they increase CH_4 oxidation in the anaerobic bed layers (Griess *et al.,* 1990; Jespersen *et al.,* 1998; McDonald *et al.,* 2002).

Cumulative estimated CH_4 emission during the study period was 159.5, 134.1 and 114.7 g m^{-2} for *A. donax*, *P. australis* and the unvegetated bed respectively.

The higher bed respiration and methane emissions from vegetated beds can be also attributed to more labile carbon being accessible via plant exudates (Zemanovà *et al.*, 2010), estimated as 20% of the above-ground biomass produced during the growing season (Picek *et al.*, 2007), that intensified bacterial activity (Gagnon *et al.*, 2007). Ström *et al.* (2003) reported that CH_4 emission rates and potential CH_4 production are dependent on substrate quality and the linkage between root exudation of labile carbon, *e.g.* acetate and CH_4 formation.

Growing season $CO_{2(eq)}$ balance

The highest monthly average daily $CO_{2(eq)}$ emission was calculated for all beds in June and ranged from 25.9 g m^{-2} d^{-1} (unvegetated bed) to 60.7 g m^{-2} d^{-1} (bed vegetated with *A. donax*). At the end of the trial period, the two vegetated beds had similar $CO_{2(eq)}$ cumulative emission values and trends (Figure 4) with 7.34 and 8.07 kg $CO_{2(eq)}$ m^{-2} for common and giant reed respectively. The unvegetated bed instead had a cumulative $CO_{2(eq)}$ emission of 3.81 kg m^{-2}.

Considering the plant biomass C content (Table 3) and the beds biomass yield (Table 2), *P. australis* and *A. donax* fixed 11.61 and 27.03 kg $CO_{2(eq)}$ m^{-2} respectively, showing a positive balance, while the unvegetated bed, as expected, had a negative balance (Table 4).

Since CWs are multiyear wastewater depuration systems, where, after the settlement phase, C fixed in the plants below-ground biomass remains stable due to the root systems turnover, we can exclude it (Table 4, column 3) from the $CO_{2(eq)}$ balance. Moreover assuming a yearly above-ground biomass cut agronomy management, the $CO_{2(eq)}$ balance for the two vegetated beds showed that during the trial period (about 9 months) they acted as $CO_{2(eq)}$ sinks, with an atmosphere $CO_{2(eq)}$ uptake equal to 1.30 and 8.35 kg m^{-2} for *P. australis* and *A. donax* respectively (Table 4). The above-ground biomass could be used to produce renewable energy, in fact estimating the higher heating values (HHV) using C fixed in the biomass, in accordance with Demirba (1997) formula HHV = 0.196 * %C + 14.119, *A. donax* and *P. australis* have an HHV respectively of 22.96 and 22.51 MJ kg^{-1}.

The C3 plants studied in the Mediterranean environment determined a positive $CO_{2(eq)}$ balance. *A. donax* that has a high photosynthetic rate and productivity similar to those of C4 species (Christou *et al.,* 2001) fixes more than six times $CO_{2(eq)}$ in its above-ground biomass than *P. australis*. Considering that the depuration efficiency, in terms of wastewater pollutant abatement, is not significantly different between the two plant species (Idris *et al.,* 2012a), *A. donax* could be used in CW in the Mediterranean environment. Nevertheless, *A. donax* is a perennial plant that produces a high quantity of rhizomes concentrated in the bed first layer and could lead after several years of operation to a possible decrease of its efficiency and/or an increase in maintenance costs. Although the result showed interesting prospects for *A. donax*, so far only a few studies have been carried out on this plant and in small experimental CW beds (Calheiros *et al.,* 2010, 2012; Idris *et al.,* 2012a, 2012b); therefore long terms study are needed to validate the effects of this species on CW depuration efficiency and GHG emission prior to disseminate information for technology transfer.

Conclusions

CWs are natural-like systems widely used to treat different wastewaters, where depuration processes determine greenhouse gas emis-

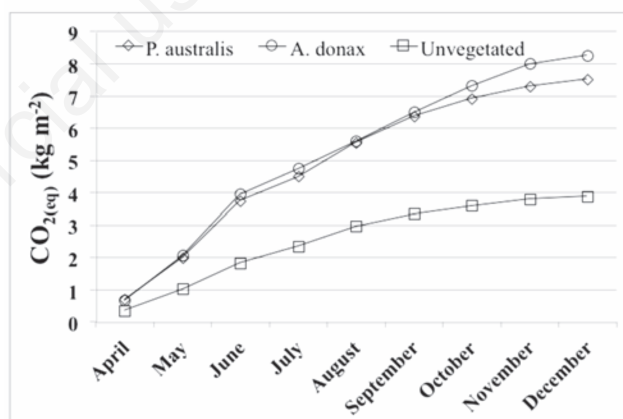

Figure 4. Cumulative $CO_{2(eq)}$ emission trends for the three beds.

Table 3. Percentage carbon content in biomass fractions.

Species	Above-ground biomass (%carbon)	Below-ground biomass (%carbon) Roots 0-20 cm	Roots 20-60 cm	Rhizomes 0-20 cm	Rhizomes 20-40 cm
P. australis	42.83	34.31	38.53	41.06	39.33
A. donax	45.09	35.89	40.47	42.81	-

Table 4. Beds $CO_{2(eq)}$ balance (kg m^{-2}).

Species	$CO_{2(eq)}$ emitted	$CO_{2(eq)}$ fixed total biomass	$CO_{2(eq)}$fixed above-ground biomass	$CO_{2(eq)}$ total balance*	$CO_{2(eq)}$ partial balance°
P. australis	7.34	11.61	8.64	4.27	1.30
A. donax	8.07	27.03	16.42	18.96	8.35
Unvegetated	3.81	-	-	–3.81	–3.81

*$CO_{2(eq)}$ total balance = $CO_{2(eq)}$ fixed total biomass - $CO_{2(eq)}$ emitted; °$CO_{2(eq)}$ partial balance = $CO_{2(eq)}$ fixed aboveground biomass - $CO_{2(eq)}$ emitted.

sion. With this in mind Søvik *et al.* (2006) reported that the question then arises if CWs, used to protect freshwater ecosystems, are a solution to an environmental problem or if they substitute one problem with another by reducing water pollution, yet increasing greenhouse gas emissions.

The results achieved in this study confirm the role of plants in CO_2 and CH_4 emissions from CWs that with respect to CO_2 determine significantly higher emission from vegetated beds than unvegetated ones.

Nevertheless the $CO_{2(eq)}$ balance needs to be considered in order to have a more complete view to answer the question posed by Søvik *et al.* (2006). Both vegetated beds showed a positive balance (CO_2 sink), whereas the unvegetated one gave a negative value (CO_2 source) confirming that vegetation in CWs contributes to enhance the environmental value of this system of wastewater depuration.

Although *A. donax* fixed in the above-ground biomass 1.9 more times $CO_{2(eq)}$ than *P. australis,* it had a positive balance of $CO_{2(eq)}$ 6.4 times greater than *P. australis*. These positive preliminary results should encourage further studies to confirm the *A. donax* promising role as vegetation in Mediterranean CWs.

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