Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2016.1173384

57 (2016) 22743–22763
October

Taylor & Francis
Taylor & Francis Group

Pollutant removal efficiency of a pilot-scale Horizontal Subsurface Flow in Sicily (Italy) planted with *Cyperus alternifolius* L. and *Typha latifolia* L. and reuse of treated wastewater for irrigation of *Arundo donax* L. for pellet production—Results of two-year tests under Mediterranean climatic conditions

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Received 13 June 2015; Accepted 25 February 2016

ABSTRACT

Constructed wetland systems (CWs) are developed biological technologies for the treatment and reuse of wastewater. The aims of this study were to evaluate the treatment performance of a pilot Horizontal Subsurface Flow system (HSSFs), to evaluate the reuse of treated wastewater (TWW) from CWs for the irrigation of giant reed (*Arundo donax* (L.)) and to assess the effects of TWW on the biomass yield of giant reed grown for pellet production. The research was carried out in Sicily (Italy) in a pilot-scale HSSFs which was fed with urban-treated urban wastewater following secondary treatment from an activated sludge wastewater treatment plant. Giant reed plots were irrigated with TWW from plantedunits, TWW from an unplanted-unit and with freshwater (FW). The pilot system was found to have excellent removal efficiency for all the parameters examined. The different treatment levels of irrigation did not affect significantly the growth and yield of giant reed ecotypes. Differences between heating values of above-ground biomass irrigated with FW and TWW were negligible. TWW from CWs can be used for the irrigation of species grown for energy purposes, thereby providing an alternative source of water, particularly in areas where water deficit in the agricultural sector is significant.

Keywords: Urban treated wastewater; Horizontal Subsurface Flow constructed wetland; Water balance; Giant reed; Pellet

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Presented at EuroMed 2015: Desalination for Clean Water and Energy Palermo, Italy, 10–14 May 2015. Organized by the European Desalination Society.

1. Introduction

In recent years, the problem of energy has been pushed to the forefront on all decision-making and operational levels due to a significant rise in consumption, increasing difficulties in the supply of oil and gas, and the consequent sharp rise in fossil fuel product prices on international markets. Italy, which is highly dependent upon imported energy, is undergoing huge changes in its energy systems, focussing on low-impact, secure supply energy. Energy saving issues and a rise in environmental awareness have led to increased interest in renewable sources of energy. Renewable energy, in a relatively short period of time, has become a matter of strategic interest for the agricultural sector, which is interested in agricultural and forestry biomass as a form of energy [1]. The development of energy crops is considered by many as beneficial both to the environment, primarily through the reduction of greenhouse gas emissions, and to the agricultural sector by helping to solve the problem of food surpluses and huge areas of crop land left abandoned [2]. Energy crops can provide farmers with an opportunity to supplement their income and diversify production, an aspect sustained by energy and environmental policies which encourage and support their use [3]. The success of energy crops is closely linked to the production costs, so it is preferable to grow perennial crops and to adopt extensive cultivation systems [4]. Among the perennial crops, giant reed (*Arundo donax* L. Poaceae family) is considered one of the most promising energy crops in the arid and semi-arid areas of the Mediterranean region due to high lignocellulosic biomass yields and low input requirements [5–10]. Giant reed is a perennial rhizomatous C_3 grass naturalised in the Mediterranean region. It can adapt to various types of soils and tolerate severe drought conditions due to an abundant root system which contributes to high water adsorption efficiency [3,11]. Previous research activity highlighted the high production potential of giant reed in various Mediterranean environments. Christou et al. [12] reported yields of 26 t ha^{-1} of dry matter for giant reed in Greece. Mantineo et al. [9] found a maximum yield of 38.8 t ha^{-1} of dry matter for giant reed in Italy. Lewandowski et al. [5] reported yields ranging from 3 to 37.7 t ha^{-1} of dry matter for this species in southern Europe. Several studies confirmed the high long-term productivity of giant reed under wet conditions and in soils characterised by water and nutrient availability [13,14]. Water and nutrients represent the major resources needed for biomass production. Irrigation and fertilisation are, therefore, the most important agronomic practices for achieving high productivity of the species [15]. In the southern regions of Italy, water is often a limited resource due to long periods of water shortage in the summer period. In recent years, a fall in rainfall levels and an increase in air temperatures have had a significant impact on water resources with a decrease in irrigation water availability in agriculture. Water shortage has strongly influenced the agricultural sector despite the fact that the demand for water in this sector has also increased yearly, both in order to satisfy the water requirements for crops which were once only rain fed, and as a result of climate change severe over the last few years. With regard to nutrients, Nassi o di Nasso et al. [16] stated that the nitrogen demand of giant reed could be significant and highlighted that in some experiments the biomass yield and nitrogen content of the species increased with rising nitrogen rates [17] while in other investigations nitrogen rates did not affect the biomass yield [18].

In the Mediterranean areas characterised by severe water scarcity, the reuse of treated wastewater (TWW) would reduce freshwater consumption, reduce pollution in water bodies and retain better quality water for human consumption [19–21]. TWW would increase crop yields due to the fact the water contains significant concentrations of organic and inorganic micro and macronutrients which are necessary for crop growth [22] and in order to maintain fertility and productivity levels of the soil [23]. Moreover, TWW would enhance the economic benefits for farmers due to reduced need for fertiliser [15,24]. One of the most important biological technologies for pollution control in wastewater and its reuse is constructed wetland systems (CWs). They are engineered systems designed to treat wastewater and are an alternative to the more widespread conventional treatment technologies using higher energy inputs [25]. As reported in various studies, CWs may play an important role in the treatment and reuse of wastewater, particularly in those areas where agriculture is highly dependent upon irrigation [26–32]. There is little literature in the Mediterranean region regarding the reuse of TWW from CWs for the irrigation of giant reed [4,33].

The aims of the study were: (i) to evaluate the pollutant treatment performance of a pilot-scale Horizontal Subsurface Flow system (HSSFs) and calculate the water balance, (ii) to assess the effects of irrigation with TWW from a pilot-scale HSSFs on chemical and physical soil properties compared to irrigation with FW, (iii) to assess the effects of irrigation with TWW from a pilot-scale HSSFs on above-ground biomass yield of giant reed compared to irrigation with FW and (iv) to assess the effects of irrigation with TWW from a pilot-scale HSSFs on giant reed pellet production compared to irrigation with FW.

2. Materials and methods

2.1. Test site

Tests on the reuse of urban wastewater for the irrigation of giant reed (*A. donax* (L.)) were carried out from 2013 to 2014 in the experimental area of the pilot HSSFs in Piana degli Albanesi, a rural community (6,000 inhabitants) in the west of Sicily (37˚59´56´´40 N - 13˚16´50´´16 E, 740 m a.s.l.). The climate of the area is humid with a mean annual rainfall of about 800 mm, mainly distributed between October and April. With reference to time series 2002–2014, the annual average temperature was 15.3˚C, average maximum temperature was 19.9˚C and average minimum temperature was 10.5˚C. The summer drought was severe and the dry period was between June and September.

2.2. Description of the pilot HSSF system

The system was designed by the Department of Agricultural and Forest Sciences at the University of Palermo (Italy) in 2004 and was located downhill from the town's sewage plant (Fig. 1). The system included 3 separate parallel units (A, B and C) each 33 m long and 1 m wide, providing a total surface filter bed area of 99 m^2 (Fig. 2). Filter bed depth was 0.5 m, to allow for greater root development and to create a larger rhizosphere. The slope was 1.5%, needed to obtain regular flow. The walls of the three units were made of concrete and the floor was levelled with fine sand. The units were filled with a substrate of evenly sized 20–30 mm silica quartz river gravel (Si 30.32%; Al 5.23%; Fe 6.87%; Ca 2.79%; Mg 1.01%). Each unit was lined with sheets of IDROEVA. In March 2012, Units A and B were planted with *Cyperus alternifolius* L (umbrella sedge) and *Typha latifolia* L. (reedmace), respectively, while unit C was unplanted. The treated urban wastewater from the outflow tank of the municipal sewage plant was initially fed into a reinforced storage tank. This water was pumped through a 1-m wide perforated pipe into each of the three units to ensure even distribution of the wastewater throughout the filter bed section, reducing the risk of hydraulic short-circuiting. In each unit, the pipe was placed 10 cm from the surface of the substrate. The homogeneous distribution of wastewater in each unit was ensured by the use of a timer-controlled pumping system. The flow inlet was measured by a flow meter in each unit. Pumping was continuous throughout the day without variations in time. The outflow tanks, located downhill from the three units, were installed with a filter grill between the tanks and the substrate in order to avoid blockage. The outflow wastewaters flowed downhill into three 64 m^3 storage tanks, one

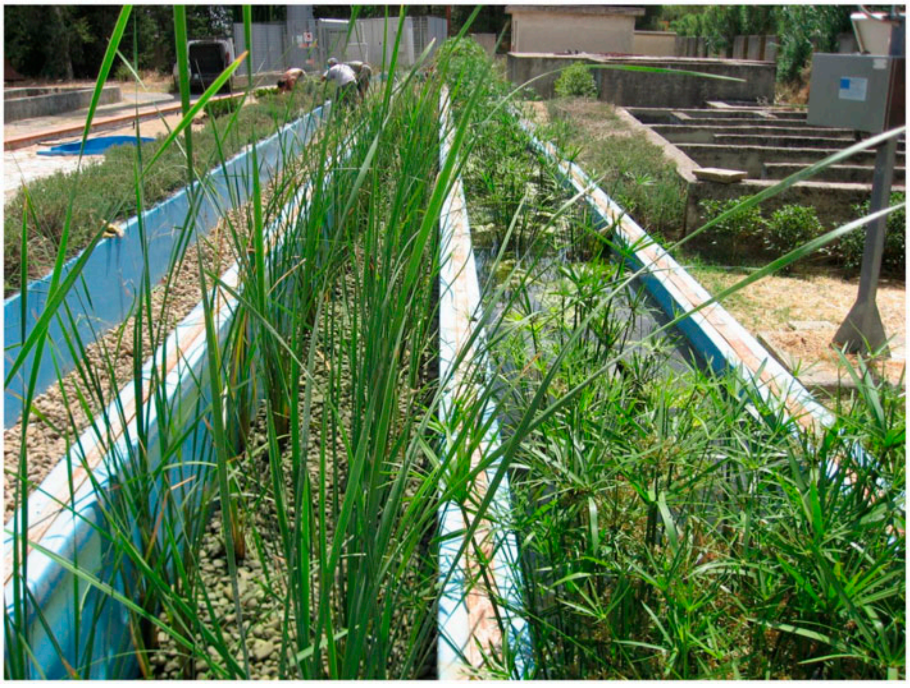

Fig. 1. A view of pilot-scale HSSF system located downhill from the sewage plant in Piana degli Albanesi (Sicily, Italy).

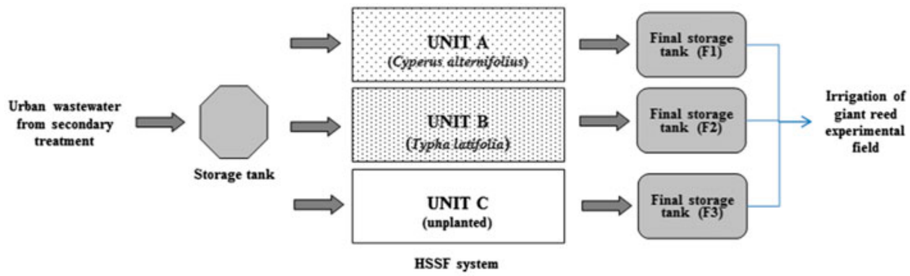

Fig. 2. Layout of pilot-scale HSSF system in Piana degli Albanesi (Sicily, Italy).

for unit, which were connected to sprinkler systems and used to irrigate giant reed. The units operated under the same hydraulic conditions and were tested under a hydraulic loading rate (HLR) of 12 cm d^{-1}.

2.3. Urban wastewater analysis

Urban wastewater samples were taken twice per month during the period April–September 2013 and 2014, amounting to a total of 24 times. The samples were collected at the inflow (0 m) and at the outflow (33 m) of each unit. A litre of wastewater was collected from each of the two points during each sampling. There was only one influent sampling point for each unit. The influent sample was taken close to the pipe, while the effluent sample was collected at the mouth of the outflow pipe. The influent and effluent samples were instantaneous samples. The pH value, electrical conductivity (EC), temperature (T) and dissolved oxygen (DO) levels were determined directly on site using a portable Universal meter (Multiline WTW P4), following the calibration protocol for each of the four parameters being studied. Using Italian water analytical methods [34], total suspended solids (TSS), biochemical oxygen demand (BOD_5), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN), ammonia nitrogen (NH_4-N) and total phosphorus (TP), potassium (K), calcium (Ca), magnesium (Mg) and chloride (Cl) levels were determined. Total coliform (TC), faecal coliform (FC), faecal streptococci (FS), *Escherichia coli* (EC) and *Salmonella* spp. levels were determined by membrane filter methods, based on standard methods for water testing [35]. Removal efficiency (RE) of a pilot HSSFs was calculated based on pollutant concentrations according to [36]:

$$\mathrm{RE} = \frac{C_i - C_0}{C_i} \times 100 \qquad (1)$$

where C_i and C_0 are the mean concentrations (mg/L) of the pollutants in the influent and effluent.

2.4. Water balance

The FAO Penman–Monteith method was used to calculate ET_0 [37]. The Penman–Monteith equation was used to calculate daily ET_0 (mm d^{-1}) based on microclimate data taken from an automatic weather station belonging to the Sicilian Weather and Climate Service located near to the pilot system:

$$ET_0 = \frac{0.4008\,\Delta(R_n - G) + \gamma(900/T + 273))u_2(e_s - e_a)}{\Delta + \gamma(1 + 0.34\,u_2)} \qquad (2)$$

where R_n is net radiation at the crop surface (MJ m^2 d^{-1}), G is soil heat flux density (MJ m^2 d^{-1}), T is average air temperature (℃), u_2 is wind speed at 2 m height (m s^{-1}), e_s is the saturation vapour pressure (kPa), e_a is the actual vapour pressure (kPa), $e_s - e_a$ is the saturation vapour pressure deficit (kPa), Δ is the slope of the vapour pressure curve (kPa ℃$^{-1}$) and γ is the psychrometric constant (kPa ℃$^{-1}$). The ET_0 values were calculated using the cool season turfgrass *Festuca arundinacea* Schreb. The water balance for each unit was determined separately every 10 d from April to November for both years. This period was chosen according to the growth dynamics of the two species. For the planted-units, an estimate of the water balance was calculated, in agreement with [36], using the following equation (Eq. (3)): $Q_o = Q_i + (P - ET_c)A$, where Q_o = output wastewater flow rate (m^3 d^{-1}), Q_i = wastewater inflow rate (m^3 d^{-1}), P = precipitation rate (mm d^{-1}), ET_c = crop evapotranspiration (mm d^{-1}) and A = wetland top surface area (m^2).

For the unplanted-unit, the water balance was calculated using the following equation: $Q_o = Q_i + (P - ET_{con})A$, where ET_{con} = evapotranspiration from unplanted control (mm d^{-1}).

The amount of water at the inflow and outflow of each unit was determined using a volumetric flow meter. Rainfall was determined with a pluviometer. ET_c was estimated using Eq. (3): $ET_c = Q_i + P(A) - Q_o$. ET_{con} was estimated using Eq. (3):

$$ET_{con} = Q_i + P(A) - Q_o \tag{3}$$

Crop coefficients (K_c) values for *C. alternifolius* and *T. latifolia* were calculated, in agreement with [37,38], using the following equation

$$K_c = ET_c/ET_0 \tag{4}$$

Crop coefficients were calculated every 10 d for each growth stage of the two macrophytes.

2.5. Description of the experimental field and main cultivation practices

The experimental field of giant reed was set up close to the pilot HSSFs. Two local ecotypes of 1-year-old plants were used for the tests. Planting was carried out in March 2012 using rhizomes taken from plants of two ecotypes. The rhizomes were planted at a depth of 0.15–0.20 m. The plots were 9 m^2 and were spaced 100 cm apart. The plant density was 4 plants m^{-2}. The experimental field was equipped with a micro-irrigation system. Irrigation was applied from April to September two times per week both with FW and TWW. The water need of giant reed was defined by the difference between the amount of water lost by evapotranspiration and the rainfall rates. Crop evapotranspiration (ET_c) of giant reed was calculated according to [39] using the equation: $ET_c = K_c\ ET_0$, where K_c is the crop coefficient of giant reed and ET_0 is the reference evapotranspiration (Penman–Monteith equation). In 2012, plots received 120 kg N ha^{-1}, 150 kg P_2O_5 ha^{-1} and 150 kg K_2O ha^{-1}. In 2013–2014, the FW-irrigated plots were managed with 120 kg N ha^{-1} of nitrogenous fertiliser (urea). In TWW-irrigated plots, we estimated the amounts of N, supplied by irrigating with TWW, which should be taken into consideration for the N fertilisation programme of giant reed, based on previous analyses. No insecticide and fungicide treatments were carried out during the test period.

2.6. Plant and soil analysis

Plant height, stem diameter and above-ground dry biomass were the main biometric and productive parameters of giant reed which were determined in the tests. Plant height and stem diameter were randomly measured monthly, while stem density was determined once per year, in August, in a sample area of 2 m^2. At the end of the growing period of each year, the plants were cut back to a height of 10 cm above the soil surface. Plants in 10 m^2 area were harvested and weighed to determine above-ground (leaves and stems) fresh weight. The above-ground dry weight was calculated by drying the collected plant material in an oven at 62°C for 72 h. Dry samples were successfully analysed for total nitrogen by Kjeldahl apparatus.

The soil parameters were: pH, electrical conductivity (EC), organic matter (OM), total nitrogen (TKN), assimilable phosphorus (P), assimilable potassium (K), active calcareous ($CaCO_3$), magnesium (Mg) and sodium (Na) content. The soil measurements were carried out at a depth of 0.60 m close to the rhizosphere of giant reed. Before the planting, three soil samples was randomly collected in each replicate and analysed. At the end of the tests, one soil sample was collected in each subplot for each replicate and analysed. Soil samples were air-dried, ground and sieved to pass through a 2-mm sieve screen and then analysed for chemical and physical characteristics. The samples were analysed for pH and EC in the ratio of 1:2 dry soil:water extract, pH was determined with a calibrated pH meter, EC with a calibrated conductivity meter, OM with the Walkley and Black method [40], TKN by the Kjeldahl procedure [41], assimilable P by the Olsen method [42] and active calcareous using the Drouineau method [43]. The K, Mg and Na contents were determined by atomic absorption spectrophotometer. All the analyses were carried out at the Corissia Research Centre of Palermo.

2.7. Physical and energetic characterisation of crop residues and giant reed pellet

The moisture content of the ash was determined in accordance with UNI EN 14774-2:2010 Italian standards [44], specifically for sawn timber and other wood products, where the moisture content is determined from the dry weight of the sample. The Gross Calorific Value (GCV) for the ash-free dry matter was determined on homogenised, moisture-free samples placed in a Berthelot-Mahler bomb calorimeter, in accordance with UNI EN 14918:2010 Italian standards [45].

The ash content was determined, in accordance to UNI EN 14775:2010 Italian standards [46]. Dry, homogeneous 500 mg samples were placed in a porcelain crucible (previously weighed and oven-dried at 105°C) and then placed in a muffle furnace at 500°C for approx. 2 h, with a temperature gradient of 4 ℃ min^{-1}. The samples were left to cool in the drier before weighing. The crop residues were subsequently tested for pellet making, in accordance with UNI EN 15103:2010 Italian standards [47]. The pellet was obtained using shredded residues which were fed directly into the pellet machine. The residue was forced through a rotating die-hole press to form pellets which were then cut into 5-cm lengths. We determined the bulk density and the mechanical durability of the pellet. The bulk density is a parameter to evaluate the compatibility of the biomass to combustion, transport and storage systems. The mechanical durability (DU) is the difference (expressed as a percentage) between pellet weight before and after a cycle of stress which simulates, for example, transport. This parameter was determined using the Lignotester New Holmen Tester TekPro and was calculated using the following formula:

$$\mathrm{DU} = \frac{\mathrm{MA}}{\mathrm{ME}} \times 100 \tag{5}$$

where MA is the pellet weight after treatment and ME is the pellet weight before treatment. All the analyses were carried out at the Department of Agricultural and Forest Sciences of University of Palermo.

2.8. Climatic data

Data on rainfall, temperature and potential evapotranspiration were collected from a meteorological station belonging to the Sicilian Agro-Meteorological Information Service situated close to the pilot HSSF system. The station was synchronised with GMT in order to operate using synoptic forecast models. It was equipped with a MTX datalogger (model WST1800) and various sensors: wind speed sensor MTX (model Robinson cup VDI with an optoelectronic transducer), global radiation sensor (model PHILIPP SCHENK—8102 thermopile pyranometer) to measure cumulative direct and diffuse solar irradiance, temperature sensor MTX (model TAM platinum PT100 thermoresistance with anti-radiation screen), relative humidity sensor—MTX (model UAM with capacitive transducer with hygroscopic polymer films and anti-radiation screen), rainfall sensor MTX (model PPR with a tipping bucket rain gauge) and leaf wetness sensor MTX (model BFO with PCB). This equipment provided data on the wind speed (m s^{-1}), minimum daily relative moisture levels (%), average daily soil temperature (℃), average daily air temperature (℃), total daily solar irradiance (MJ m^{-2}), total daily rainfall-frequency (d mm > 1) (%) and rainy days per year (d mm > 1) (%). In addition, using the Penman–Monteith equation, the potential evapotranspiration (PET) was calculated.

2.9. Experimental design and statistical analysis

A split plot design for a two-factor experiment [48] was used with four replications. The main plot factor was irrigation (I) with four treatment levels: (1) I1 with FW; (2) I2 with TWW from the umbrella sedge-unit; (3) I3 with TWW from the reedmace-unit; (4) I4 with TWW from the unplanted-unit. The subplot factor was giant reed ecotype (AD) with two treatments levels: (1) AD1; (2) AD2. Statistical analysis was performed with the package MINITAB Release 14 for Windows and included analysis of variance (one-way ANOVA). The difference between means was carried out using the Tukey test. All the representative values were presented using mean ± standard error calculations.

3. Results and discussion

3.1. RE of pollutants in the pilot-scale HSSFs

Data showing the chemical–physical variations and pollutant removal relating to the urban wastewater are shown in Tables 1 and 2, respectively. Average ± standard error values highlighted differences in pH, T, ECw and DO levels between the planted-units. These differences were not high. The pH value at the inflow pipe was slightly alkaline, but at the outflow it was more alkaline in both of the planted-units with an even higher increase in the unplanted-unit. In the unplanted-unit, the absence of vegetation stimulated greater atmospheric aeration in the substrate and, in some cases, the growth of algae, with consequences on the hydrogenisation of the water, as found by [49]. Differences were found regarding electrical conductivity when comparing the planted-units with the unplanted-unit. The EC was found to be higher in the planted-units. The highest level (713.8 ± 6.8 μS cm^{-1}) was recorded on average at the outflow of the reedmace-unit due to evapotranspiration processes which determined a greater loss of water and an increase of the solute in the solution. The DO levels at the outflow of the planted-units were similar and equal to 0.9 ± 0.01 mg L^{-1} consistent with values found in other HSSFs [36]. Moreover, in contrast to figures reported by [50], they did not vary with a decrease in the wastewater temperature. At the outflow the removal of chemical–physical pollutants was found to be

Table 1
Variation in pH, T, DO and ECw in the pilot HSSFs units from April to September in both 2013 and 2014. Average (±standard error), minimum and maximum values are shown (n = 24)

Parameters	Main inlet	Unit A *Cyperus alternifolius*		Unit B *Typha latifolia*		Unit C Unplanted		Threshold values for Italian Ministerial Decree 185/2003
		Outlet	Variation (%)	Outlet	Variation (%)	Outlet	Variation (%)	
pH	7.5 ± 0.01 (7.3 − 7.7)	7.7 ± 0.02 (7.4 − 7.7)	−2.6	8.0 ± 0.03 (7.5 − 8.2)	−6.6	8.1 ± 0.01 (7.8 − 8.3)	−8.0	6 − 9.5
T (°C)	22.1 ± 0.1 (21.2 − 23.2)	22.3 ± 0.1 (21.7 − 23.4)	−0.9	22.4 ± 0.1 (21.7 − 23.2)	−1.3	22.7 ± 0.2 (22.4 − 23.4)	−2.7	–
EC (μS cm^{-1})	540.3 ± 9.7 (485.5 − 616.7)	616.8 ± 5.9 (609.3 − 683.4)	−14.1	713.8 ± 6.8 (677.6 − 772.3)	−32.1	560.5 ± 11.1 (504.6 − 651.2)	−3.7	3,000
DO (mg L^{-1})	1.4 ± 0.07 (1.2 − 1.4)	0.9 ± 0.01 (0.8 − 1.1)	35.7	0.9 ± 0.02 (0.9 − 1.1)	35.7	1.2 ± 0.01 (1.1 − 1.2)	14.2	–

Table 2
Main chemical and physical composition of the urban wastewater from the inflow and outflow of the pilot HSSFs units. RE from April to September in both 2013 and 2014. Average (±standard error), minimum and maximum values are shown (n = 24)

Parameters	Main inlet	Unit A *Cyperus alternifolius*		Unit B *Typha latifolia*		Unit C Unplanted		Threshold values for Italian Ministerial Decree 185/2003
		Outlet	RE (%)	Outlet	RE (%)	Outlet	RE (%)	
Colour	NP[a]	NP	–	NP	–	NP	–	–
Odour	NU[b]	NU	–	NU	–	NU	–	–
Coarse matter	–	–	–	–	–	–	–	–
TSS (mg L^{-1})	30.3 ± 0.4 (26.0 − 35.3)	10.2 ± 0.2 (9.0 − 12.1)	66.1	8.9 ± 0.2 (7.9 − 10.2)	70.6	21.8 ± 0.5 (19.3 − 27.8)	28.4	10
BOD_5 (mg O_2 L^{-1})	23.8 ± 0.3 (18.8 − 29.9)	7.6 ± 0.7 (6.8 − 10.1)	68.1	7.0 ± 0.4 (5.8 − 9.2)	70.6	12.5 ± 0.4 (11.2 − 13.8)	47.5	20
COD (mg O_2 L^{-1})	50.9 ± 3.7 (32.1 − 77.1)	12.5 ± 0.2 (9.8 − 16.9)	74.3	10.9 ± 0.1 (8.3 − 13.3)	77.4	27.3 ± 1.1 (21.8 − 37.4)	44.2	100
TKN (mg N L^{-1})	17.7 ± 0.1 (14.1 − 22.9)	9.1 ± 0.1 (9.1 − 10.8)	43.9	8.1 ± 0.1 (7.1 − 9.4)	52.8	15.2 ± 0.1 (12.7 − 18.5)	13.2	15
N-NH_4 (mg NH_4 L^{-1})	12.5 ± 0.2 (11.1 − 17.5)	7.1 ± 0.1 (6.2 − 9.2)	43.2	6.5 ± 0.3 (5.1 − 7.3)	48.0	6.5 ± 0.3 (9.2 − 11.7)	11.2	2
TP (mg P L^{-1})	7.5 ± 0.01 (7.1 − 8.2)	4.7 ± 0.08 (4.0 − 4.7)	37.8	4.4 ± 0.01 (3.9 − 5.5)	42.1	6.8 ± 0.02 (6.7 − 7.2)	9.2	2

[a]Not perceptible.
[b]Not unpleasant.

higher in both of the planted-units compared to the unplanted-unit. The improvement in the quality of the wastewater at the outflow in the planted-units was mainly due to the direct uptake of nutrients by the macrophytes and the action of the aerobic micro-organisms located close to the rhizosphere. Removal levels for TSS, BOD_5, COD, TKN and TP were on an average higher in the reedmace-unit than umbrella-sedge unit. Reedmace showed a greater level of adaptability to the climatic and substrate conditions of the CWs area. In both of the planted-units, TSS removal percentages were consistent with values found in literature and can be explained by filtration and sedimentation mechanisms at work in the medium and in the root system of the plants [51]. The high removal rate found in the two units allowed the wastewater to flow easily throughout the study without causing blockages or preferential flow channels. Throughout the two test years, the wastewater did not come to the surface nor did hydraulic short-circuiting occur. The marked differences found between the planted- and unplanted-units highlight the fact that the combined action of the macrophyte root systems with the substrate influences, the TSS reduction process to a greater extent than the substrate alone. BOD_5 RE varied on average from 70.6% (reedmace-unit) to 68.1% (umbrella sedge-unit). COD RE at the outlet varied on average from 77.4% (reedmace-unit) to 74.3% (umbrella sedge-unit). RE of BOD_5 and COD was similar for both the planted-units throughout the two test years. In general, organic matter RE stayed within a range consistent with previous HSSFs studies using urban wastewater, and was facilitated mainly by the high root density of the two macrophytes. The high organic matter RE in the two planted-units was due to aerobic biodegradation through heterotrophic bacteria close to the root system of the two macrophytes and to an additional anaerobic biodegradation processes in the pores of the substrate saturated by wastewater. When comparing the planted-units, RE for TSS, BOD_5 and COD were on an average higher than those of nitrogen and phosphorous. The lower nitrogen RE was due to low oxygen levels in the system which hindered ammonia nitrification significantly, a process which most literature maintains is the most important organic nitrogen removal mechanism. The influence of oxygen levels on the intensity of the nitrification process is maintained by [52], who observed a reduced or absent nitrification rate at dissolved oxygen levels below $0.5\ \mathrm{mg\ L^{-1}}$. Phosphorous removal was never found to be particularly high (lower than 45%) in either of the planted-units during the study, probably due to a range of factors, such as the gradual filling of the sorption sites by the plant root systems, by the non-regular harvesting of the plants, by the presence of undecomposed plant material around the substrate surface and by the adsorption properties intrinsic to the substrate itself.

On a microbiological level (Table 3), the three units showed marked differences for all the parameters in the study. Both of the planted-units produced pathogen levels which were lower at the outflow than the unplanted-unit. The TWW at the inflow and outflow pipes of the pilot HSSFs did not contain *Salmonella* spp. At the outflow, the reedmace-unit had lower FC, TC, FS and *E. coli* levels compared to umbrella sedge-unit. In the planted-units, RE of pathogens was very high for each microbial parameter in the study and consistent with international literature. For example, the RE of *E. coli* was on an average higher than 85% in the umbrella sedge-unit and higher than 90% in the reedmace-unit. Consistent with previous studies, the high bacteria removal capacity of the pilot system may be attributed to a combination of physical, chemical and biological mechanisms [53] such as filtration and adsorption, chemical oxidation, sedimentation, predation by nematodes and protists, and viral and bacterial activity [54,55]. It is important to highlight that the greater atmospheric air circulation in the substrate and the translocation of oxygen from the root system of the macrophytes to the substrate eased the production of a greater bacteria biofilm and promoted pathogen load removal than the unplanted-unit, as claimed by [56]. In our research, the average values of the chemical and microbiological parameters at the outflow of the pilot HSSFs were not all within the legal limits of the Italian Ministerial Decree 185/2003 regarding the reuse of treatment wastewaters for irrigation purposes. The age of the pilot HSSFs influenced significantly the concentration of TP at the outflow of planted and unplanted-units. Consistent with international literature, in the future we would expect to see a further reduction in phosphorous removal mostly due to the gradual saturation of most of the substrate sorption sites where these processes were active. During the test period, the microbiological data obtained for *E. coli* (on average $119\ \mathrm{CFU\ 100\ ml^{-1}}$ for reedmace-unit and $147\ \mathrm{CFU\ 100\ ml^{-1}}$ for umbrella sedge-unit) were not always found to be within these legislative limits ($10\ \mathrm{CFU\ 100\ ml^{-1}}$ in 80% of the samples and $100\ \mathrm{CFU\ 100\ ml^{-1}}$ maximum levels), however, a high micro-organism removal capacity was observed, which must be taken into consideration as most conventional treatment systems in Sicily, such as activated sludge or trickling filter systems, demonstrate low removal capacity of these pollutants due to the fact that often not all three treatment processes are effectuated. It is evident to find an adequate solution to

Table 3
Main microbiological composition of the urban wastewater from the inflow and outflow of the pilot HSSFs units. RE from April to September in both 2013 and 2014. Average (± standard error), minimum and maximum values are shown (n = 24)

Parameters	Main inlet	Unit A *Cyperus alternifolius*		Unit B *Typha latifolia*		Unit C Unplanted		Threshold values for Italian Ministerial Decree 185/2003
		Outlet	RE (%)	Outlet	RE (%)	Outlet	RE (%)	
Log_{10} (MPN 100 ml-1—CFUs 100 ml^{-1})								
Total coliforms (MPN 100 ml^{-1})	4.20 ± 0.07 (4.25 − 4.50)	3.48 ± 0.01 (3.52 − 3.66)	80.5	3.24 ± 0.03 (3.20 − 3.41)	88.7	4.10 ± 0.02 (3.97 − 4.24)	35.4	–
Faecal coliforms (MPN 100 ml^{-1})	4.34 ± 0.02 (4.11 − 4.26)	3.55 ± 0.03 (3.36 − 3.64)	83.5	3.31 ± 0.01 (3.05 − 3.55)	90.6	3.95 ± 0.02 (3.84 − 4.10)	44.5	–
Faecal streptococci (MPN 100 ml^{-1})	3.93 ± 0.03 (3.81 − 4.10)	3.30 ± 0.01 (3.13 − 3.50)	76.6	3.15 ± 0.01 (2.91 − 3.39)	83.1	3.68 ± 0.02 (3.57 − 3.87)	43.2	–
Escherichia coli (CFUs 100 ml^{-1})	3.10 ± 0.02 (2.95 − 3.24)	2.19 ± 0.02 (2.11 − 2.28)	87.3	2.04 ± 0.04 (1.87 − 2.13)	91.3	2.83 ± 0.02 (2.67 − 3.02)	46.6	10 (80% of samples) and 100 (maximum value point)
Salmonella spp. (MPN 100 ml^{-1})	–	–		–		–		–

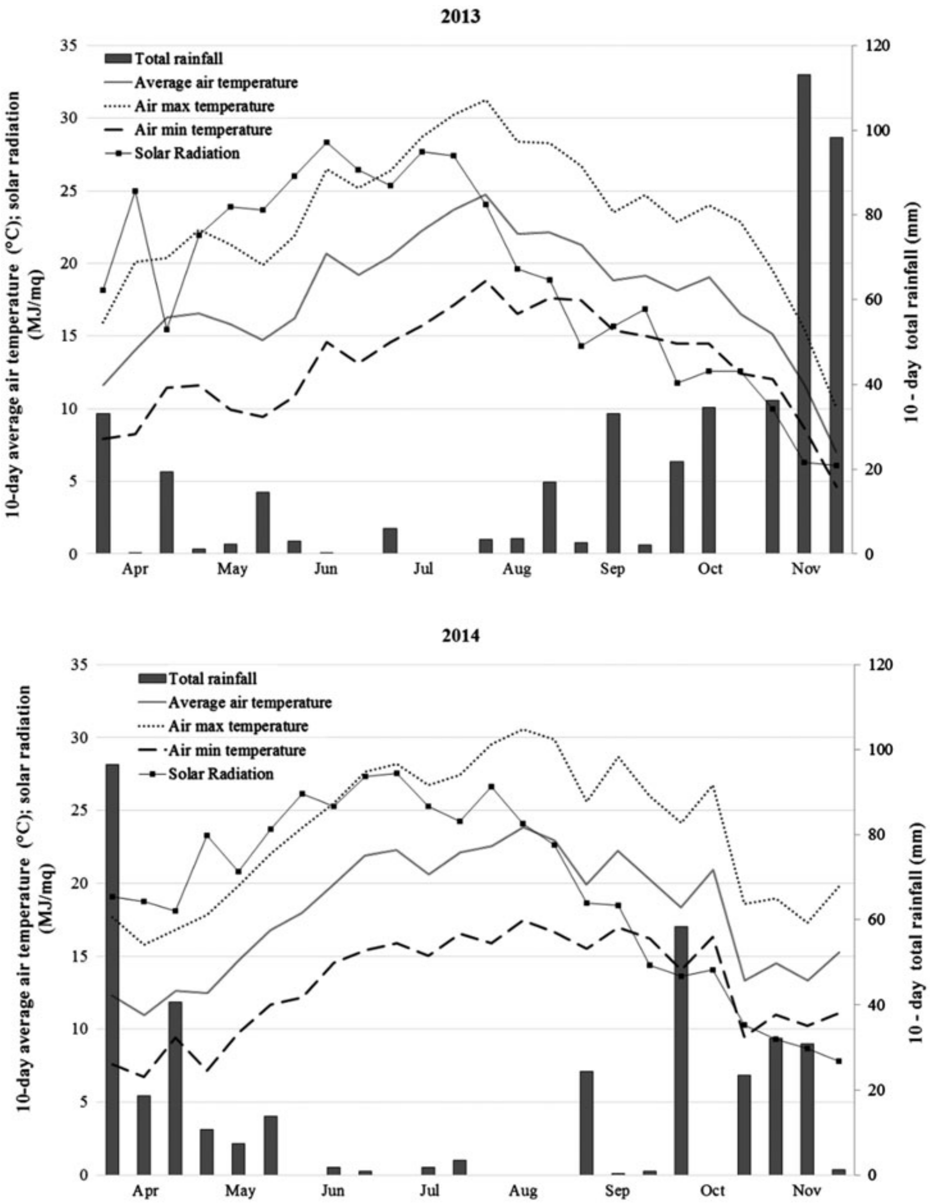

Fig. 3. Trends of 10-d minimum, maximum and average air temperature, solar radiation, and total rainfall during the test period.

improve the RE of bacteria and respect the legal limits of the law. A possible solution may be that of repeating the process by pumping the water at the outflow through the system again in order to obtain further microbial decontamination. Another interesting hypothesis might be the use of a combined HSSF-VSSF systems to remove pathogens with higher efficacy, as demonstrated in other Mediterranean areas [26,57–59]. The different retention times of the wastewater—greater in a HSSFs than a VSSFs—would determine a change in the general aerobic/anaerobic conditions and affect the chemical oxidation mechanisms regarding pathogens.

3.2. HSSFs water loss

Trends on maximum air temperature, minimum air temperature, average air temperature, solar radiation and total rainfall are shown in Fig. 3. At the outflow of the units, the amount of TWW was significantly influenced by the evapotranspiration processes. In both of the planted-units, cumulative ET_c was found to be

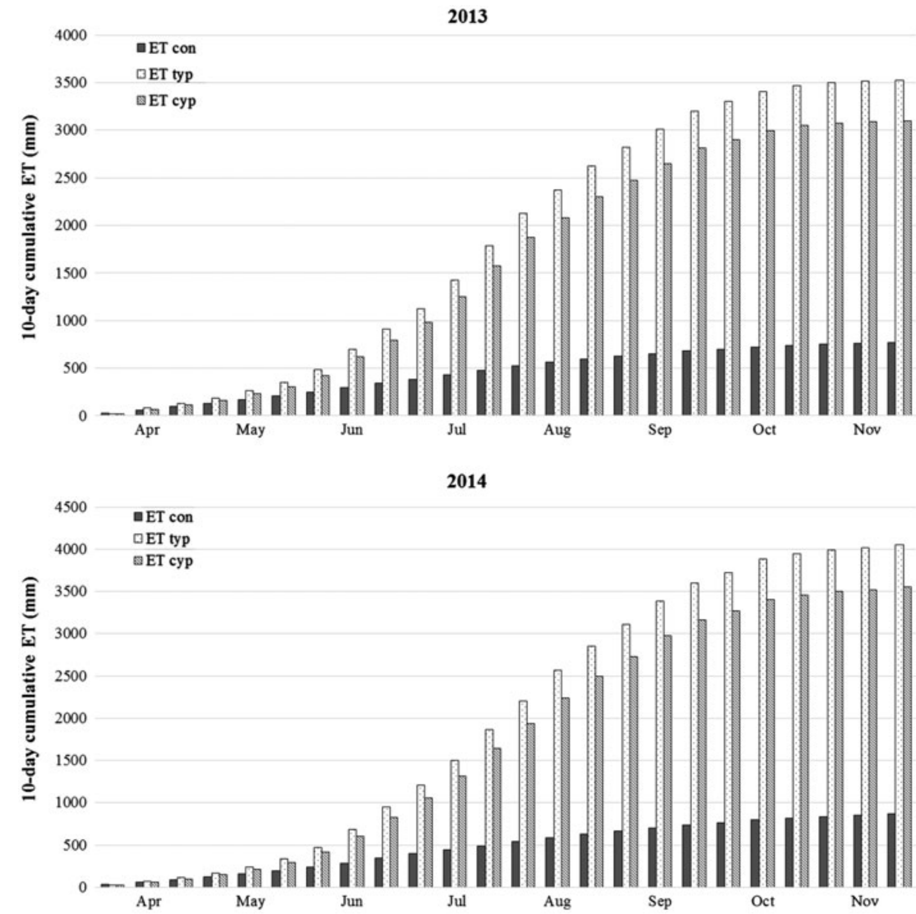

Fig. 4. 10-d cumulative evapotranspiration (ET_{con}, ET_{typ} and ET_{cyp}) in 2013 and 2014.

higher than total rainfall for both 2013 and 2014, taking into consideration the period April–November of each year (Fig. 4). In Fig. 5, Q_o trends relative to Q_i, cumulative ET_{typ}, cumulative ET_{cyp}, cumulative ET_{con} and total rainfall are shown. If we consider the reedmace-unit, between April and September of each year, average 10 d Q_o was found to be 51.51 m^3 in 2013 and 50.62 m^3 in 2014. As Q_i was constant for all of the 10-d periods, water loss was on average 8.49 and 9.38 m^3/10 d in the first and second year of tests, respectively. In the umbrella sedge-unit, water loss was on average lower at 7.42 m^3/10 d in 2013 and 8.17 m^3/10 d in 2014. In the unplanted-unit, average 10 d Q_o was found to be 58.36 m^3 in 2013 and 58.42 m^3 in 2014: maximum 10-d Q_o was 63.19 m^3 (1st 10 d April 2014) and minimum 10 d Q_o was 57.23 m^3 (2rd 10 d August 2014). The higher levels of water loss found in the two planted-units were mostly due to higher ET_c values found for both species in 2013 and 2014 (Fig. 6). Taking the different growth stages into consideration, greatest water loss in the two planted-units occurred during crop development stage and mid-season stage (spring–summer seasons), whereas the lowest values occurred during late-season stage (autumn season). In particular, in August 2014, with a complete absence of rainfall, water loss in the reedmace-unit was on average 16.54 m^3/10 d, equivalent to 27.5% of Q_i. Despite identical growth, climatic and hydraulic conditions in the system, the greater water loss occurred in the reedmace-unit and it was due to greater growth of reedmace compared to umbrella sedge (average leaf surface and foliar density) of the reedmace, as sustained by [60]. It is important to highlight that reedmace consumed more water but used water with greater efficiency than umbrella sedge, also due to a preliminary greater above-ground biomass production. Furthermore, the reedmace's greater above-ground biomass dry matter production at harvesting undoubtedly influenced water loss in the unit in as much as a considerable amount of water was used by the species to help growth above- and below-ground. A further amount was used in order to compensate the difference between the leaf surface vapour pressure and the ambient air vapour pressure, as the leaf surface of the

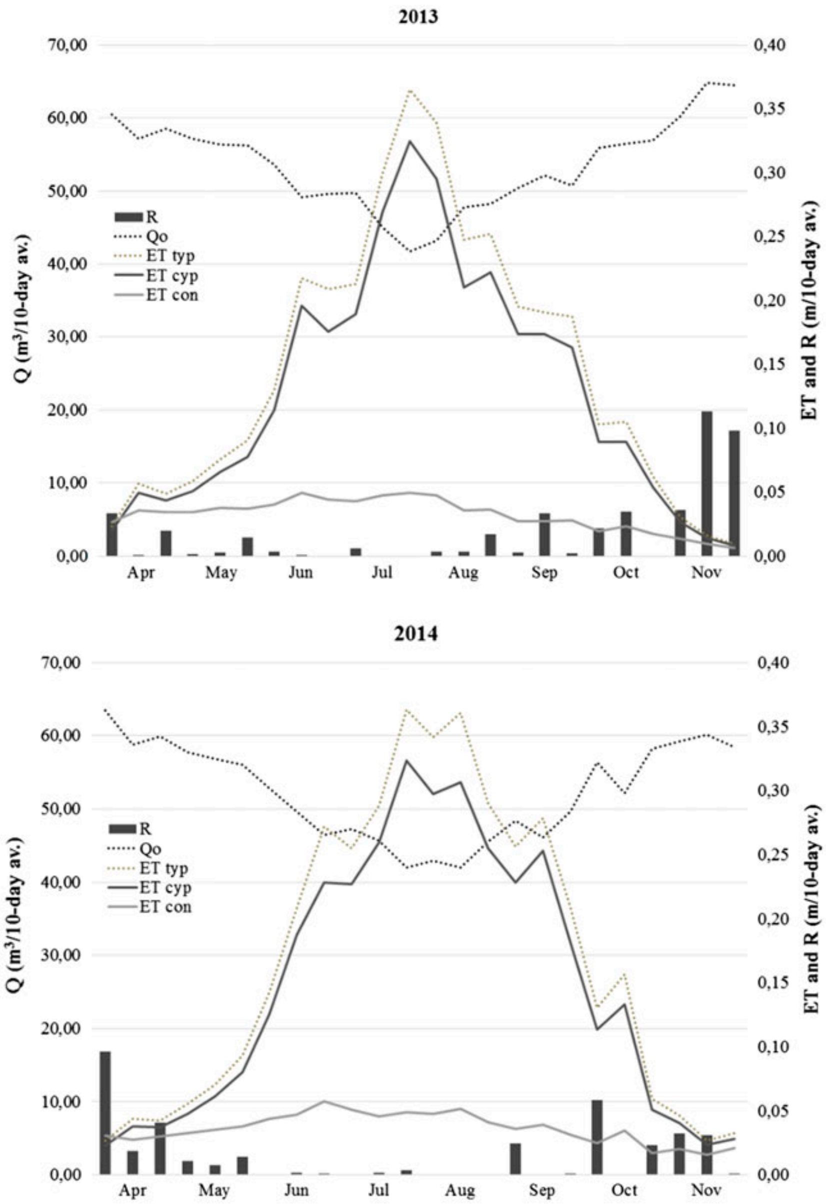

Fig. 5. Q_0 trends relative to Q_i, cumulative ET_{con}, cumulative ET_{typ}, cumulative ET_{cyp} and total rainfall in 2013 and 2014.

reedmace is larger than umbrella sedge. In arid and semi-arid regions of the Mediterranean the high water loss levels due to evapotranspiration must not be undervalued, but it is indubitable that CWs represent an innovative approach which could guarantee continuity for irrigation, even with large water losses during summer months.

3.3. FW and TWW characteristics

The chemical characteristics of TWW and FW are shown in Table 4. The composition of the two types of water varied significantly over the tests period. TWW had on average higher values of OM, EC, N, P, K and other alkali metals than FW. With comparing the TWW and FW, the lowest variations in nutrient and salt concentrations were found during the summer months of each year of tests. In this period, the growth of above- and below-ground biomass of the two macrophytes was higher than in other seasons and significantly affected the removal rates of pollutants in the planted-units of the pilot HSSFs significantly reducing the concentrations of chemical and microbiological parameters at the outflow. The water

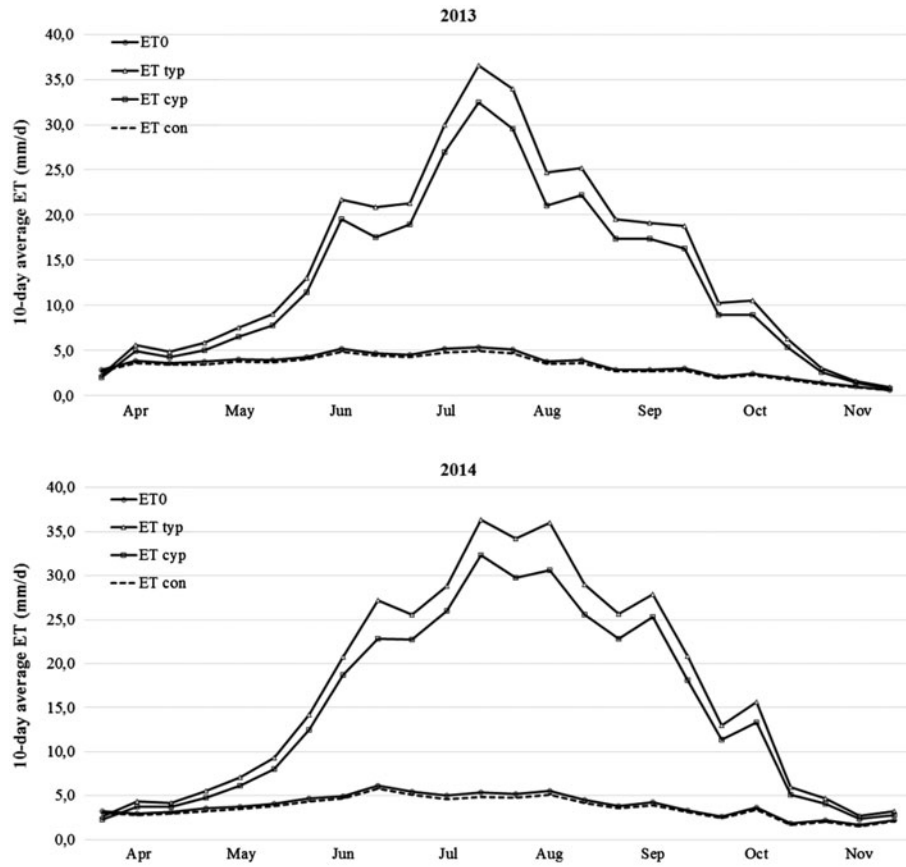

Fig. 6. 10 d- average ET_0, ET_{typ}, ET_{cyp} and ET_{con} in 2013 and 2014.

quality for the irrigation of giant reed was evaluated using the guidelines edited by [61] (Table 5). The limits of use of TWW and FW are shown in Table 6. Observing the nutrient contents in the effluents of the pilot HSSFs, we found that the concentration of TKN was on an average below the recommended guidelines, while the concentrations of Na and Cl showed a degree of slight to moderate restriction on use for irrigation. The average values of EC for FW (0.27 ± 1.1 dS m^{-1}), TWW from the umbrella sedge-unit (0.62 ± 5.9 dS m^{-1}), TWW from the reedmace-unit (0.71 ± 6.8 dS m^{-1}) and TWW from the unplanted-unit (0.56 ± 11.1 dS m^{-1}) were not critical for giant reed growth. All the values of EC may be considered with no degree of restriction on use for irrigation according to the recommended guidelines.

3.4. Effects of TWW irrigation on soil

The soil was sandy, clay, loam (Aric Regosol, 54% sand, 23% silt and 23% clay) with a pH of 7.9, OM of 1.91%, EC of 0.52 dS m^{-1}, total calcareous of 5.81%, active calcareous of 3.71%, TKN of 1.30 g kg^{-1}, assimilable P of 18.11 ppm, assimilable K of 152.20 ppm, Mg and Na content of 138.31 and 84.78 ppm, respectively. In Table 7 the chemical characteristics of the freshwater irrigated-soils and TWW-irrigated soils are reported. Given the quality of FW and TWW, and the nature of the soil, we did not observe significant changes in soil quality during a two-year irrigation period. No significant variations in pH were recorded between FW-irrigated soils and TWW-irrigated and the main reason was probably the short-term tests of TWW application. This was consistent with other studies which highlighted that the application of TWW significantly affected the soil pH only in long-term tests due to high content of elements such as Ca, Mg and Na in the wastewater or the oxidation of organic compounds and nitrification of ammonium [62–64]. The higher organic matter content in the plots irrigated with TWW-irrigated soils was found to be related to higher nutrient content and organic compounds. The application of TWW to soils without high water holding capacity, such as sandy clay loam soils,

Table 4
Chemical composition of freshwater and treated wastewater that were applied for irrigation of giant reed. Average values (± standard error) of two-year-tests are shown

Parameters	Freshwater	Treated wastewater from the *Cyperus alternifolius*-planted unit	Treated wastewater from the *Typha latifolia*-planted unit	Treated wastewater from the unplanted-unit
pH	7.0 ± 0.01	7.7 ± 0.02	8.0 ± 0.03	8.1 ± 0.01
EC (μS cm^{-1})	273.2 ± 1.1	616.8 ± 5.9	713.8 ± 6.8	560.5 ± 11.1
DO (mg L^{-1})	Not available	0.9 ± 0.01	0.9 ± 0.02	1.2 ± 0.01
BOD_5 (mg O_2 L^{-1})	1.5 ± 0.3	7.6 ± 0.7	7.0 ± 0.4	12.5 ± 0.4
COD (mg O_2 L^{-1})	2.0 ± 0.2	12.5 ± 0.2	10.9 ± 0.1	27.3 ± 1.1
TSS (mg L^{-1})	Not detected	10.2 ± 0.2	8.9 ± 0.2	21.8 ± 0.5
NO_3-N (mg N L^{-1})	0.3 ± 0.5	2.5 ± 0.1	2.4 ± 0.3	4.0 ± 0.1
TP (mg P L^{-1})	0.4 ± 0.3	4.7 ± 0.08	4.4 ± 0.01	6.8 ± 0.02
Cl (mg Cl L^{-1})	22.2 ± 0.5	111.1 ± 0.7	113.8 ± 0.7	119.2 ± 0.2
Ca (mg Ca L^{-1})	22.1 ± 0.7	60.1 ± 0.4	57.9 ± 0.4	71.1 ± 0.2
K (mg K L^{-1})	2.9 ± 1.2	73.1 ± 0.8	67.9 ± 0.3	84.8 ± 0.3
Mg (mg Mg L^{-1})	14.8 ± 1.0	20.5 ± 0.2	19.8 ± 0.3	23.3 ± 0.2
Na (mg Na L^{-1})	10.6 ± 0.4	139.1 ± 0.2	138.2 ± 0.4	148.2 ± 1.5
SAR (meq L^{-1})	0.9 ± 0.3	3.9 ± 1.3	4.0 ± 1.1	4.1 ± 1.1

did not contribute significantly to the accumulation of salts in the soil. The higher amounts of total dissolved salts in TWW with respect to FW were the main reason which explains the higher salinity in TWW-irrigated plots. However, differences between the treatments were not significant and this was consistent with other findings in international literature. In two-year-tests, EC varied on average from 0.65 to 0.60 dS m^{-1} in TWW-irrigated soils, while in FW-irrigated soils the value of EC was on an average 0.58 dS m^{-1}. TWW irrigation increased the N, P and K concentrations in the soil, but we did not find significant differences for N content compared to the FW-irrigated soils due to leaching process and plant uptake of nitrogen. Ca, Mg and Na also increased between the start and the end of the application of TWW. Of the alkali metals, Na was of great interest because of its negative effects on soil properties. Wastewater can be an adverse source of Na for the soils and their uses must be controlled especially when irrigation is applied on clay soils and in the long-term. In our research, we observe significant differences between the treatments due to higher concentration of Na in TWW than FW. According to international literature, the continuous use of TWW with high Na content can increase the concentration of this element in the soil. To avoid an excess of sodium in a long-term period, the level of Na in the soil needs to be checked periodically and carry out agronomic practices such as the application of good quality irrigation water for sodium removal purposes.

3.5. Effects of TWW irrigation on giant reed growth and yield

The different treatment levels of irrigation did not significantly affect the above-ground dry weight of the two ecotypes of *A. donax* (Table 8). The interactions ecotype x irrigation were found to be not significant for all parameters examined. The above-ground dry biomass was on an average 30.48 t ha^{-1} y^{-1} for AD1 and 30.75 t ha^{-1} y^{-1} for AD2. In this research, the maximum above-ground dry biomass yield of *A. donax* (32.12 t ha^{-1}) was much lower than the maximum value of biomass yield (64.30 t ha^{-1}) reported by [15], who investigated the effects of irrigation with urban wastewater on biomass yield of giant reed in southern Calabria (Italy). The difference was probably due to the lower plant density (3–4 plants m^{-2} compared to 6 plants m^{-2}). The effect of plant density on biomass yield was observed by [65] in similar climatic conditions. However, our findings were found to be similar to [11], who reported that dry biomass yield of giant reed was affected by soil water availability and nitrogen fertilisation rates. The fact that we did not observe significant differences in terms of N content and above-ground biomass yields was probably due to different N fertilisation management programmes. In the FW-irrigated plots we managed the growth of two ecotypes with 120 kg N ha^{-1} y^{-1} using a nitrogenous fertiliser (urea). In the second year of growth of giant reed, we used a higher rate of N than the commonly used N fertilisation programme. The dry

Table 5
Guidelines for interpretation of water quality for irrigation [61]

Item		No problems	Increasing problem	Severe problem
Salinity				
ECw	(mmhos cm^{-1})	<0.75	0.75–3	>3
Permeability				
ECw	(mmhos cm^{-1})	>0.5	0.5–0.2	<0.2
Specific ion toxicity				
Sodium (adj. SAR)	(meq L^{-1})	<3	3–9	>9
Chloride	(meq L^{-1})	<4	4–10	>10
Boron	(mg L^{-1})	<0.75	0.75–2	>2
Miscellaneous effects				
NO_3-N or NH_4-N	(mg L^{-1})	<5	5–30	>30
HCO_3	(meq L^{-1})	<1.5	1.5–8.5	>8.5
pH		Normal range 6.5–8.4		

Table 6
Restrictions on use for irrigation with freshwater and urban-treated wastewater from pilot HSSFs [22]

Item	Freshwater	Treated wastewater from the *Cyperus alternifolius*-planted unit	Treated wastewater from the *Typha latifolia*-planted unit	Treated wastewater from the unplanted-unit
Salinity	None	None	None	None
Infiltration (SAR)	None	None	None	None
Specific Ion Toxicity				
Sodium	None	Slight to moderate	Slight to moderate	Slight to moderate
Chloride	None	Slight to moderate	Slight to moderate	Slight to moderate
Miscellaneous effects				
Nitrogen (NO_3-N)	None	None	None	None

above-ground dry biomass yield increased with higher N rates in agreement with the previous studies [17,66,67]. In the TWW-irrigated plots we exploited the nutrient content in TWW to integrate the demand of N of giant reed. An additional application of nitrogenous fertiliser (urea) was made to sustain suitable plant growth in TWW-irrigated plots from planted-units of HSSFs (Table 9). Furthermore, the application of urea was not made in the TWW-irrigated plots from unplanted-unit. It was evident that irrigation with TWW provided combined fertilisation for giant reed because of the N supply. These results confirm that irrigation with TWW can decrease the need for N fertilisation while maintaining high productive performance of giant reed. The different treatment levels of irrigation did not significantly affect the growth of giant reed in terms of plant height and stem diameter (Table 8). The two ecotypes showed the highest values of biometric parameters in summer when the air temperatures and other climatic factors were more favourable for giant reed growth. Comparing the two ecotypes, AD2 performed better than AD1 in terms of plant height and stem diameter. Particularly, the highest average value of plant height (2.61 m) was recorded for AD2 in August 2013. Our results were found to be different to [15] who reported that growth and productivity were higher in FW-irrigated plants than TWW-irrigated plants. In addition [59] stated that the effect of fertigation on giant reed was not significant. In our research, the use of FW was not better than TWW and vice versa to obtain the best growth and yield performances of giant reed due to the different N fertilisation programmes. Therefore, if we consider the TWW as a

Table 7
pH, EC, OM, TKN, assimilable P, active calcareous, assimilable K, Mg and Na content in freshwater-irrigated soils and treated wastewater-irrigated soils. Average values of two-year-tests are shown

	pH	EC (dS m^{-1})	OM (%)	TKN (g kg^{-1})	TP (ppm)	Active $CaCO_3$ (%)	K (ppm)	Mg (ppm)	Na (ppm)
Giant reed ecotype									
Ecotype 1	7.93 a	0.63 a	1.96 a	1.60 a	18.26 a	3.79 a	149.10 a	137.11 a	93.22 a
Ecotype 2	7.92 a	0.64 a	1.97 a	1.64 a	18.29 a	3.83 a	147.89 a	138.23 a	94.55 a
Irrigation									
FW	7.91 a	0.58 a	1.92 a	1.55 a	18.24 a	3.73 a	150.34 a	136.65 c	84.88 c
TWW (1)	7.89 a	0.64 a	1.98 a	1.64 a	18.34 a	3.81 a	149.77 a	139.66 b	95.23 a
TWW (2)	7.87 a	0.65 a	1.99 a	1.63 a	18.33 a	3.82 a	150.23 a	140.11 a	96.12 a
TWW (3)	7.85 a	0.60 a	1.94 a	1.60 a	18.29 a	3.76 a	149.66 a	137.22 c	92.33 b
Ecotype x Irrigation	n.s.	n.s.	n.s.	*	n.s.	*	n.s.	*	*

Notes: Means followed by the same letter are not significantly different according to the Tukey test ($p \leq 0.05$).
*Significant, n.s. not significant. FW: freshwater-irrigated soils; TWW (1): treated wastewater-irrigated soils from the *C. alternifolius*-planted unit; TWW (2): treated wastewater-irrigated soils from the *T. latifolia*-planted unit; TWW (3): treated wastewater-irrigated soils from the unplanted unit.

Table 8
Main biometric and yield parameters of giant reed determined during the study. Average values of two-year-tests are shown

	Plant height (m)	Stem diameter (mm)	Above-ground dry weight (t ha^{-1})
Giant reed ecotypes			
Ecotype 1	2.49 a	13.3 a	30.48 a
Ecotype 2	2.54 a	13.7 a	30.75 a
Irrigation			
FW	2.48 a	13.91 a	30.79 a
TWW (1)	2.54 a	13.82 a	31.04 a
TWW (2)	2.53 a	13.62 a	30.65 a
TWW (3)	2.51 a	13.73 a	30.33 a
Ecotype x Irrigation	n.s.	n.s.	n.s.

Notes: Means followed by the same letter are not significantly different according to the Tukey test ($p \leq 0.05$).
*significant, n.s. not significant. FW: freshwater-irrigated plots; TWW (1): treated wastewater-irrigated plots from the *C. alternifolius* planted-unit; TWW (2): treated wastewater-irrigated plots from the *T. latifolia* planted-unit; TWW (3): treated wastewater-irrigated plots from the unplanted-unit.

source of water and fertiliser, the use of TWW can permit savings in water and fertiliser consumption with respect to traditional giant reed agronomic management, and this can represent an excellent way to cultivate giant reed in arid and semi-arid regions.

3.6. Effects of TWW irrigation on crop residues and pellet production

Results of the physical and energy characteristics of crop residues of giant reed are shown in Table 10. Ecotype, irrigation factors and the interactions ecotype x irrigation variations were not found to be significant for all parameters examined. The moisture content of crop residues at the time of harvest was found to be within the range of 40–60% as showed in several researches carried out in the Mediterranean area. The highest value of moisture content was recorded for TWW-irrigated plots from the *T. latifolia* planted-unit (58.73%), while the lowest was found (57.95%) for TWW-irrigated plots from the *C. alternifolius* planted-unit. Ash content ranged from 6.07 (TWW-irrigated

Table 9
Agronomic management of nitrogen fertilisation programme of giant reed in the freshwater-irrigated plots and treated wastewater-irrigated plots

Nitrogen (kg ha^{-1})	Freshwater-irrigated plots	Treated wastewater-irrigated plots (1)	Treated wastewater-irrigated plots (2)	Treated wastewater-irrigated plots (3)
2013				
March	60.00	22.08	27.92	0.00
July	60.00	22.08	27.92	0.00
N fertilisation	120.00	44.16	55.84	0.00
N water	Trace	75.84	64.16	120.00
Total N	120.00	120.00	120.00	120.00
2014				
March	60.00	21.20	28.00	0.00
July	60.00	21.20	28.00	0.00
N fertilisation	120.00	42.40	56.00	0.00
N water	Trace	77.60	64.00	120.00
Total N	120.00	120.00	120.00	120.00

Notes: (1) TWW from the *C. alternifolius*-planted unit; (2) TWW from the *T. latifolia*-planted unit; (3) TWW from the unplanted-unit.

Table 10
Main physical and energetic parameters of the crop residues of giant reed and physical parameters of pellet. Average values of two-year-tests are shown

	Moisture content (%) at the time of harvest	Ash content (%)	GCV (MJ kg^{-1})	Bulk density (kg m^{-3})	Mechanical durability of pellet (%)
Giant reed ecotypes					
Ecotype 1	58.71 a	5.87 a	15.00 a	114.25 a	92.58 a
Ecotype 2	58.15 a	5.97 a	14.88 a	115.75 a	92.33 a
Irrigation					
FW	58.72 a	5.78 a	14.79 a	116.00 a	92.66 a
TWW (1)	57.95 a	5.91 a	15.03 a	113.66 a	92.16 a
TWW (2)	58.73 a	6.07 a	15.02 a	114.50 a	92.50 a
TWW (3)	58.35 a	5.92 a	14.92 a	115.83 a	92.50 a
Ecotype x Irrigation	n.s.	n.s.	n.s.	n.s.	n.s.

Notes: Means followed by the same letter are not significantly different according to the Tukey test ($p \le 0.05$).
*significant, n.s. not significant. FW: freshwater-irrigated plots; TWW (1): treated wastewater-irrigated plots from the *C. alternifolius* planted-unit; TWW (2): treated wastewater-irrigated plots from the *T. latifolia* planted-unit; TWW (3): treated wastewater-irrigated plots from the unplanted-unit.

plots from the *T. latifolia* planted-unit) to 5.78% (FW-irrigated plots). No significant differences for ash content were recorded between FW-irrigated plots and TWW-irrigated plots due to the identical N rates used in the tests period. Previous studies [5,17] showed the influence of crop management on biomass ash content of giant reed. In particular [14,16] reported that an increase in fertilisation rates could reduce ash content and improve the biomass combustion quality. The same authors highlighted that higher nutrient availability in fertilised crops could lead to a higher translocation rate of nutrients from the above-ground parts to the rhizomes, thus decreasing the ash content of the biomass. Our findings were found to be similar to [8] who stated that fertilised crops gave a higher yield with a lower ash content. In addition [14] reported that air temperature, water availability and rain distribution can affect the biomass ash content. In our research we did not observe a significant influence of climatic factors on ash content probably due to constant irrigation water availability during the tests period. GCV was found to be between 15.03 (TWW-irrigated plots from the *C. alternifolius* planted-unit) and 14.79 MJ kg^{-1} (FW-irrigated plots). Differences between heating values of above-ground biomass irrigated with FW and TWW were negligible, as also found by [8,17,68]. The GCV values from the second to third year of growth were significantly lower than values found by [5,15,68] in similar climatic conditions. [8,68] reported that GCV is affected by fertilisation or by plant density or harvest time. In our research the effects of N fertilisation and plant density on GCV of giant reed ecotypes were the same in the different treatment levels of irrigation. In fact we used the same plant density of giant reed in both the FW-irrigated plots and the TWW-irrigated plots, and we applied the same N rates in the different irrigated-plots even if two diverse N fertilisation programme. Relatively to pellet of giant reed, the bulk density was on average 114.99 kg m^{-3}. Our findings were found to be similar to [69] who evaluated the combustion characteristics of four perennial energy crops. However, the bulk density of giant reed was much lower than wood crops (about 640 99 kg m^{-3}). The mechanical durability of the pellet was on an average very high for the two giant reed ecotypes (92.45%) and ranged from 92.66 (FW-irrigated plots) to 92.16% (TWW-irrigated plots from the *C. alternifolius* planted-unit). A high value of mechanical durability means high quality standard of the pellet. So, the greater the value of the mechanical durability the more the pellet can remain intact as a result of mechanical stress that may occur typically during the transport. By comparing the two ecotypes, AD1 was found to have the best energy properties with a highest GCV, lowest ash content and highest mechanical durability of the pellet.

4. Conclusions

TWW is an important source of water and nutrients needed to maintain high fertility and productivity levels of the soil, and improve the growth and yield of plant species. HSSFs constructed wetlands is an engineered system that permits the removal of the main chemical, physical and microbiological pollutants of wastewaters, and the reuse of the TWW for irrigation purposes. The results of this research suggest the interest in the use of HSSFs effluents for the irrigation of *A. donax*, which is one of the most promising energy crops in the Mediterranean region. The reuse of TWW contributes to obtaining savings in terms of freshwater and nitrogen compared to the commonly used agronomic management of giant reed. This represents an alternative agronomic strategy, in terms of irrigation and fertilisation, to increase the biomass yield of giant reed under specific climatic conditions. In this research, irrigation with TWW did not affect the chemical/physical characteristics of the soil, and the growth and yield of giant reed. Moreover, we did not observe significant differences for physical and chemical characteristics of crop residues and pellet production between TWW-irrigated plots and freshwater-irrigated plots. This highlights that the reuse of TWW can produce pellet of giant reed reducing the costs of freshwater and nitrogenous fertiliser, and enhancing the economic benefits for farmers. It is evident that further research is needed to evaluate the effects of TWW on biomass yield and quality in the long-term. However, the reuse of TWW represents a profitable solution to the management of *A. donax* for energy purposes and HSSFs constructed wetlands can play a strategic role in the treatment and reuse of wastewater in arid and semi-arid areas of the Mediterranean region.

Acknowledgements

The authors would like to thank the Sicilian Regional Ministry of Food and Agricultural Resources, the Corissia Research Centre and the University of Palermo for having provided the funds for the study. A special thanks also goes to Branwen Hornsby for her continuing support to the research and linguistic contribution.

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