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1 Energy of biomass sorghum irrigated with reclaimed wastewaters

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26 **Abstract**

27 The sustainability of biomass sorghum (*Sorghum bicolor* L. Moench.) in the Mediterranean
28 environments is linked to the potential to increasing the crop productivity using irrigation water of
29 different qualities: fresh and wastewater. An experiment was conducted in Southern Italy during
30 2012 and 2013 growing seasons to determine the biomass production and to estimate the yielded
31 energy from sorghum irrigated with fresh water and municipal wastewaters. Two stages of
32 wastewater reclamation process were compared: tertiary and secondary treatments.

33 During the growing seasons, the crop growth (biomass and LAI) was surveyed on sorghum crops
34 irrigated with three water qualities. In order to determine the effects of the irrigation water qualities
35 on the final energy yielded, on the harvested biomass, structural components (cellulose,
36 hemicellulose and lignin contents for deriving the ethanol production) and high heating value were
37 analyzed. The data obtained during two crop seasons showed that, sorghum irrigated with municipal
38 wastewater plant produced more dry biomass (23.3 vs 20.3 t ha⁻¹), energy yield (383 vs 335 GJ ha⁻¹),
39 and ethanol (6824 vs 6092 L ha⁻¹) than sorghum biomass with fresh water. As a consequence, the
40 water efficiency for producing bioenergy increased when the waste waters were supplied in
41 substitution of fresh waters. Different indices were calculated for comparing the effect of the water
42 quality on the water use efficiency (WUE) of biomass sorghum crops.

43

44 **1. Introduction**

45 Biomass crops have been studied for two decades as the most promising renewable energy sources
46 that can be used for the production of energy obtained by co-generation and fermentation processes.
47 The production of bioethanol using lignocellulosic feedstocks allow a series of benefits derived
48 from the reduction greenhouse gas emissions, as required by the Kyoto Protocol (Heaton et al.,
49 2004 and Zhao at al., 2009). From an economic point of view, the replacement of traditional crops

50 with non-food species represent a new opportunity for the rural development, when incomes food
51 crops are no more sustainable (McKendry, 2002); Moreover new vegetal material and biomass are
52 required for the self-energy production by farms and by forestry companies for accumulating
53 biomass commodities (Barbera et al., 2009). Finally the biomass crops allow the reassessment of
54 marginal land from farmers (Zema et al., 2012).

55 One of the prime sources investigated as energy is the biomass obtained from ‘dedicated’ crops of
56 sorghum (*Sorghum bicolor* L. Moench.). Sorghum, a C4 species (uses the “malate” cycle) of
57 tropical origin, is the fifth most important cereal crop in the world and can be used as green fodder,
58 straw and silage as well as to produce syrup and fuel (ethanol). It is grown in 99 countries around
59 the world on 44 million ha, mainly in those areas which are too dry for growing maize (FAOSTAT,
60 2014).

61 Sorghum is more environmentally sustainable (Dalianis, 1996) compared to other energy crops
62 (maize, sunflower, and soybean) particularly because of its relatively lower water requirements
63 (Steduto et al., 1997; Mastroilli et al., 1999; Vasilakoglou et al., 2011; Garofalo and Rinaldi, 2013)
64 and of its high efficiency to transform the evapotranspired water and the intercepted energy in dry
65 matter.

66 This is a crucial point under the Mediterranean climates, where high temperatures and solar
67 radiation are beneficial to sorghum eco-physiology, but the scarcity of water resources limits its
68 cultivation. Therefore, anomalous waters (saline or waste) could represent an important contribution
69 to solving the ever-increasing problems of water scarcity, particularly in the Mediterranean areas.
70 As an example, in southern Italy (Apulia region), more than 65% of the water resources are
71 allocated to irrigation (Disciglio et al., 2014). In these conditions water re-use for agriculture needs
72 to be a top priority, mainly in producing non-food crops.

73 In order to reduce the pressure of irrigated cropping systems on the water resources, alternative
74 irrigation strategies, as the use of wastewater (WW) to replace fresh water, require to be tested
75 under field conditions for the biomass crops. The literature shows that the irrigation with WW is a
76 promising solution for crops in semi-arid environments. In these areas, the use of WW allows
77 preservation of the natural water resources and maintains the soil fertility levels and the soil
78 productivity (Lopez et al., 2010).

79 A large number of studies (Bastos and Mara, 1995; El Hamouri et al., 1996; Lopez et al., 2006;
80 Palese et al., 2009; Ndiaye et al., 2011; Petterson et al., 2011) have shown that the chemical and
81 microbiological contamination (viruses, bacteria and protozoa pathogens) remains a crucial issue to
82 ensure the safe use of WW in agriculture. The European directives laws are restrictive for chemical
83 and microbiological parameters and are not in line with the most recent approaches proposed and
84 recommended by the World Health Organization (WHO, 2006). The reuse of tertiary wastewater is
85 encouraged as a general principle, although the actual laws do not differentiate the wastewater
86 according to the risk associated with the different types of reuse (Alcalde-Sanz and Gawlik, 2014).
87 The irrigated energy crops represent a typical case of a relatively low level of risk because they do
88 not require the same water quality than food crops.

89 In comparison with an intensive depuration, the use of secondary WW in agriculture reduces: the
90 treatment costs (Angelakis et al., 1999; Paranychianakis et al., 2006; Aiello et al., 2007; Andiloro et
91 al., 2010); disposal of polluting effluents into surface water bodies (Tamburino et al., 1999; Aiello
92 et al., 2007; Andiloro et al., 2010); cultivation cost due to the reduced need for fertilizers
93 (Tamburino et al., 1999; Paranychianakis et al., 2006, Bedbabis et al., 2010). Several experimental
94 evidence underline the improvement of crop growth due to WW irrigation for food species (Meli et
95 al., 2002; Bedbabis et al., 2010; Borin et al., 2013; Vivaldi et al., 2013; Disciglio et al., 2014) and
96 perennial energy crops. (Zema et al, 2012; Molari et al., 2014). Regarding the effect of WW

97 irrigation on the sorghum productivity, recent results on biomass yield are reported by Campi et al.
98 (2014) but not on the energy yielded. Likewise, studies about the effect of WW on the sorghum
99 energy yield are missing in literature.

100 To assess if a reduced level of WW treatment is consistent with the energy yielded by sorghum
101 grown in a the Mediterranean environment (southern Italy), this research reports the results on the
102 growth dynamics and energy (ethanol and heat) yielded from the biomass sorghum in relation to the
103 irrigation water quality (urban WW and fresh water), after 2-year of cultivation.

104

105 **2. Materials and methods**

106 *2.1 Experimental site*

107 The study was conducted in southern Italy (Trinitapoli, lat: 41°21', long: 16°03', altitude 0 m a.s.l.),
108 close to municipal WW treatment plant which supplied different qualities of reclaimed water for
109 irrigation during the two growing seasons (2012 and 2013). The area was characterized by a
110 Mediterranean climate with warm and dry summers: a maximum air temperature ranging from 32
111 °C to 43 °C and a minimum relative humidity ranging from 15% to 40% (Campi et al., 2009). The
112 annual average precipitation was of 560 mm from 1977 to 2011, with rainfall events mainly
113 concentrated in the autumn and late winter seasons and greatly reduced or absent in the spring-
114 summer season. The agrometeorological data (daily rainfall, minimum and maximum temperatures,
115 relative air humidity, solar radiation, wind speed) necessary to calculate the reference
116 evapotranspiration, according to Allen et al. (1998), were recorded at an agro-meteorological station
117 located at a short distance from the experimental site. During the 2012 growing season, mean values
118 of air temperature were similar to those recorded during 2013. The total rainfall was higher in 2013
119 than in 2012 (150 mm more) and it was concentrated in the last part of the sorghum cultivation
120 cycle, in September (Fig. 1).

121 The soil in north Apulia is predominately clay in all horizons with low percentage of stones. Before
122 the 2012 sowing, the soil was sampled of the experimental plots at two profile depths (0-0.20 and
123 0.21-0.40 m) in five replicates. The texture and hydrologic constants (field capacity, FC, and
124 wilting point, WP) were determined in the soil-sieved samples. Soil texture was classified as clay-
125 loam (USDA Soil Survey Staff, 1975) with an average content of 318 g kg⁻¹, 355 g kg⁻¹ and 327 g
126 kg⁻¹ of sand, silt and clay, respectively (Table 1), determined using the hydrometer method. Soil
127 water content in volume at FC (-0.03 MPa) and WP (-1.5 MPa) were 38 mm mm⁻¹ and 26 mm mm⁻¹,
128 respectively (measured using the Richards chambers). The soil water reserve was moderate (180
129 mm), because the root system does not develop below 1.5 m in this soil. The soil chemical fertility
130 was good (Table 1), with adequate content of total nitrogen (1.5 g kg⁻¹), soil organic carbon (14 g
131 kg⁻¹), and available phosphorous (71 mg kg⁻¹).

132 In late winters of both growing seasons (2012 and 2013), the soil was prepared by ploughing to a
133 depth of 0.25-0.30 m. Immediately before sowing (May), the soil was tilled using a double-disking
134 harrow and finally a field cultivator was used to prepare the seedbed. The sorghum experimental
135 plots were grown within the crop sequence 'wheat - broad bean - sorghum' in 2012; 'sugar beet -
136 broad bean - sorghum' in 2013'.

137

138 *2.2 Irrigation water quality*

139 During both sorghum growing seasons, irrigation water was sampled nine times randomly (in 4
140 replications) from the dripping lines corresponding to the three water quality treatments, using a 1 L
141 sterile glass bottles and stored at 4 °C before chemical analysis.

142 The supplied waters were analyzed in triplicate, according to the Italian standard methods (APAT,
143 IRSA-CNR, 2003) referencing the common international methods (APHA, AWWA, WEF, 2005),
144 for the following parameters: electrical conductivity (EC; dS m⁻¹), biological oxygen demand over 5

145 days (BOD_5 ; $\text{mgO}_2 \text{ L}^{-1}$), chemical oxygen demand (COD; mg L^{-1}), chlorine (active Cl^- , mg L^{-1}),
146 ammonium-nitrogen ($\text{NH}_4\text{-N}$; mg L^{-1}), nitrate nitrogen ($\text{NO}_3\text{-N}$; mg L^{-1}), phosphorus ($\text{PO}_4\text{-P}$; mg L^{-1}),
147 sodium (Na^+ ; mg L^{-1}), calcium (Ca^{2+} ; mg L^{-1}), magnesium (Mg^{2+} ; mg L^{-1}), potassium (K^+ ; mg L^{-1}),
148 sodium adsorption ratio (SAR), chlorides (mg L^{-1}) and fluorides (F^- ; mg L^{-1}).

149 The pH was measured with a GLP 22+ pH and Ion-Meter, CRISON, and the EC with a GLP 31+
150 EC Meter, CRISON. The sodium, calcium, magnesium and potassium levels were determined using
151 ion-exchange chromatography (Dionex ICS-1100; Dionex Corporation, Sunnyvale, CA, USA). The
152 sodium adsorption ratio (SAR) was calculated according to Richards 1954.

153

154 *2.3 Crop management and irrigation treatments*

155 The sorghum hybrid 'KWS Bulldozer' was cultivated during two growing seasons (2012 and 2013)
156 in the experimental field. This hybrid is characterized by medium-late vegetative cycle, increased
157 height with good tolerance to bending, and high yielding in dry and green biomass (Campi et al.,
158 2014). Sorghum was sown on May 28, 2012 and on May 21, 2013 with a plant density of 18 plants
159 m^{-2} and it was grown following the agro-techniques aiming at reducing the energy inputs: weed
160 control at the initial crop stage and nitrogen fertilization of 100 kg N ha^{-1} after 30 days from
161 sowing. Pest control was not required during the crop cycle due to the absence of phytosanitary
162 problems (with the exception of sporadic presence of aphid colonies).

163 Sorghum crops were submitted to the following irrigation treatments:

- 164 • Fresh water (FW), withdrawn from the water network of the "Consorzio di Bonifica della
165 Capitanata" and directly from the dam "Marana Capacciotti";
- 166 • Secondary-treated municipal wastewater (SW) originated from the public plant located near
167 the experimental site. After screening and grit removal, the WW flows to primary clarifiers
168 followed by a partial aerobic stabilization of the sludge (Vivaldi et al., 2013).

169 • Tertiary-treated wastewater (TW) produced by the same public plant cited above, where the
170 WW is treated in two subsequent phases. Primarily, water is collected in a tank of 180 m³
171 and pumped to the sand filter section, including five tanks with the following filling
172 materials: anthracite, quartz sand and gravel support with different diameters. The second
173 phase was represented by ultra-filtration module equipped with hollow fiber membranes
174 with cellulose triacetate double wall. Periodically all the lines were automatically cleaned by
175 back flushing (Vivaldi et al., 2013). This purification process allows to obtain WW with a
176 lower content of suspended solids and nutrients for plants respect to the secondary-treated
177 municipal wastewater (SW);

178 The irrigation water was scheduled for supplying the amount of water lost by evapotranspiration
179 (ETc) calculated according the FAO-56 methodology (Allen et al., 1998).

180 WW was reused under a controlled flow rate and distribution conditions specifically aimed to avoid
181 contamination of bordering fields (a dripping irrigation system was adopted) and the underlying
182 groundwater irrigation volumes were scheduled in order to maintain the soil water content lower
183 than field capacity. The root depth of the sorghum is over 2 m, however when the soil profile is
184 moist, most of the water is taken up from the top one-fifth of the root zone (Steduto et al., 2012).
185 Consequently a depth of 0.5 m was adopted for dimensioning the irrigation volumes.

186 Irrigation treatments were arranged in a complete randomized experimental design, where blocks of
187 400 m² were replicated 3 times. Soil water content in the whole soil profile (mm d⁻¹) was monitored
188 by capacitance probes. Capacitance probes (10HS, Decagon Devices Inc., USA) were installed
189 horizontally into the soil at three depths (-0.3, -0.6 m and -1.2 m) and connected to a data logger
190 (Grillo MMS, Tecno.El, Italy).

191 The irrigation volumes were 300 mm during 2012 season and 340 mm during 2013 season.

192

193 *2.4 Biomass analysis.*

194 At regular intervals (every 7-10 days), leaf area index (LAI) and dry biomass were measured. The
195 LAI was measured by an area meter (LAI-2000 Plant Canopy Analyzer, Li-Cor, USA) and the dry
196 matter was determined on sampled plants by using a dry-oven (at 65°C for 48 h). At the end of the
197 sorghum cycle (the second week of September) all plants were harvested from 20 m² plots, and then
198 the aboveground biomass production was determined.

199 Plant tissue samples were accurately milled into fine pieces (1-2 mm) and stored until were required
200 for the determination of higher heating value, lignin, cellulose and hemicellulose content.

201 The higher heating value (HHV; MJ kg⁻¹) of sorghum was measured using a LECO AC500 bomb
202 calorimeter (LECO Corp., St. Joseph, MI, USA) with 1g of dried sample. Neutral detergent fiber
203 (aNDFom) content was determined without sodium sulfite and with heat stable amylase (Van Soest
204 et al., 1991). Acid detergent fiber (ADFom) and acid detergent lignin [lignin (sa)] were determined
205 by sequential analysis of the residual aNDFom and expressed as exclusive residual ash (Van Soest
206 et al., 1991). Hemicellulose was calculated as aNDFom–ADFom, and cellulose as ADFom–lignin
207 (sa), and both were assayed sequentially on the same sample. An Ankom apparatus (Ankom220,
208 Fairport, NY, USA) was used for extraction and filtering.

209

210 *2.5 Calculation and statistical analysis*

211 To asses the energy yield per hectare of sorghum (GJ ha⁻¹), the HHV obtained with the bomb
212 calorimeter was multiplied by biomass production.

213 A theoretical calculation of ethanol yield from cellulose and hemicellulose was formulated as
214 follows.

215 Ethanol yield from cellulose and hemicellulose (L ha⁻¹) = cellulose and hemicellulose content (%)
216 in dry matter x dry biomass (t ha⁻¹) x 1.11 (conversion factor of sugar from cellulose and

217 hemicellulose) x 0.85 (process efficiency of sugar from cellulose and hemicellulose) x 0.51
218 (conversion factor of ethanol from sugar) x 0.85 (process efficiency of ethanol from sugar) x
219 1000/0.79 (specific gravity of ethanol, g mL⁻¹) (Institution of Japan Energy, 2006).

220 The seasonal irrigation volumes (I), and biomass (B), energy yield (ey - MJ m⁻²) and ethanol
221 production (eth) by sorghum were used to determine the water use efficiency indices (WUE) for the
222 different treatments, according to the following formula:

$$223 \quad bWUE(kg \cdot m^{-3}) = \frac{B(Kg \cdot m^{-2})}{I(m^3 \cdot m^{-2})} \quad (1)$$

224 bWUE (biomass-water use efficiency) indicates the amount of biomass (in kg) produced by
225 sorghum using 1 m³ of irrigation water;

$$226 \quad eyWUE(MJ \cdot m^{-3}) = \frac{EY(MJ \cdot m^{-2})}{I(m^3 \cdot m^{-2})} \quad (2)$$

227 eyWUE (energy yield-Water use efficiency) indicates the amount of energy (in MJ) produced in
228 sorghum using 1 m³ of irrigation water.

$$229 \quad ethWUE(L \cdot m^{-3}) = \frac{Ethanol(L \cdot m^{-2})}{I(m^3 \cdot m^{-2})} \quad (3)$$

230 ethWUE is ethanol-Water use efficiency that indicates the amount of ethanol (in L) produced by
231 sorghum using 1 m³ of irrigation water.

232 Data were analyzed using the statistical package Statgraphics Plus 5.1 (StatPoint Technologies Inc.,
233 Warrenton, VA). Duncan's multiple range test was used to separate treatment means when ANOVA
234 results indicated significant differences.

235

236 **3. Results**

237

238 *3.1 Wastewater properties*

239 The analysis of the main chemical properties of WW utilized for crop irrigation (Table 2) showed a
240 slight alkalinity (mean pH value = 7.4) and a moderate EC (average = 1.4 dS m⁻¹, indicating a
241 moderate salt content). The chemical characteristics varied considerably among the three sources of
242 irrigation water used. The values of most of the chemical parameters, such as EC, COD, BOD₅,
243 Na⁺, K⁺, Ca²⁺, Mg²⁺, NH₄⁺-N, NO₃⁻-N, PO₄²⁻-P were significantly higher in SW compared to TW
244 (Table 2). The other analyzed parameters showed similar values in all the three irrigation waters.
245 Moreover, the main chemical parameters for TW and SW meet the Italian standard for WW re-use,
246 except for NO₃⁻-N, PO₄²⁻-P, BOD₅ and COD (Table 2). The high value of PO₄²⁻-P in 2013 FW was
247 a consequence of 2 outlier values measured on the two samples collected consecutively in July.
248 Neglecting these exceptional values, probably due to a temporary pollution of the conventional
249 water, the average PO₄²⁻-P value during the 2013 sorghum season was of 9 mg L⁻¹, corresponding to
250 the average value measured in the previous year.

251 The contents of N/P/K compounds in both WW (TW and SW) indicate its appreciable potential as
252 fertilizer, in particular for nitrogen (in forms NO₃⁻-N and NH₄⁺-N) which represent one of the most
253 important nutrients for plants.

254

255 *3.2 Crop growth and biomass yield*

256 In both years (2012 and 2013), the irrigation scheduling prevented that the water content values in
257 the soil profile exceeded the Readily Available Water (p) threshold (0.32 mm mm⁻¹) to avoid water
258 stress (Fig. 2). In particular, at depth of 0.3 m, the soil water content was close to FC following each
259 event of rainfall or irrigation, while at depth of 0.6 m, the soil water content was always close to
260 FC, and at depth of 1.2 m, the soil water content was close to saturation. The high values of the soil
261 water content at a depth greater than 0.6 m were due to the presence of a shallow groundwater,

262 while the moisture variations during the sorghum crop cycle were detected on the 0.5 m top soil
263 (Fig. 2). This 0.5 m soil layer was considered for dimensioning the water amount of each irrigation.
264 The quality of the irrigation water affected the crop growth: the trends of LAI (Fig. 3) and dry
265 biomass (Fig. 4) were consistent in both growing seasons (2012 and 2013). In particular, differences
266 in crop growth started in July, from the stem elongation phase of the sorghum until the crop harvest.
267 During the 2012 growing season (Fig. 3a), the WW treatments (TW and SW) showed an average
268 increase in maximum LAI values by 10% in comparison with the FW treatment, while during the
269 2013 growing season (Fig. 3b) an increase of 8% and 15% of the maximum LAI values was
270 induced by the TW and SW treatments, respectively, in comparison with the FW treatment.
271 Data reported in table 3 shows that the highest dry biomass productions were observed on the crops
272 irrigated with the WW (TW or SW) in both growing seasons, being significantly higher than
273 biomass production of sorghum irrigated with FW by 2.2 t ha⁻¹ and 3.4 t ha⁻¹, respectively. The
274 statistical analysis showed a significant interaction between the irrigation water treatments and the
275 growing season. In the 2012, the biomass yielded from the FW treatment was lower (by 2.1 t ha⁻¹)
276 than that of the sorghum irrigated with WW (TW or SW). In 2013, the SW treatment led to a final
277 production of sorghum biomass significantly higher respect to the TW and FW treatments, by 2.7
278 and 5.4 t ha⁻¹, respectively (Fig. 5a).

279

280 *3.3 Energy yield*

281 The HHV of sorghum was not influenced by the quality of the irrigation water (Table 3). These data
282 allowed the calculation of the energy yield, which could be obtained from the biomass harvested per
283 hectare of sorghum irrigated with different water qualities. The best performance in energy yield
284 was attained by the sorghum irrigated with WW and the higher values of this parameter were
285 estimated in 2013. (Table 3 and Fig. 5c). Statistical analysis indicated an interaction between

286 irrigation water treatments and growing seasons (Fig. 5c).. In particular, during the 2012 season,
287 irrigation with WW led to a significant increase in energy yield, corresponding to + 10%: from 372
288 GJ ha⁻¹ (both WW treatments) to 336 GJ ha⁻¹ (FW treatment). During the 2013 season, the energy
289 yield in the SW treatment (423 GJ ha⁻¹) was significantly higher than that obtained by the sorghum
290 irrigated with TW (364 GJ ha⁻¹) or FW (333 GJ ha⁻¹).

291

292 *3.4 Calculated ethanol yield*

293 Table 4 shows the structural components of sorghum (lignin, cellulose and hemicellulose content).
294 They were not significantly affected by the quality of the irrigation waters. Calculating the
295 production of ethanol by the formula suggested by the Institut of Japan Energy (2006), the WW
296 provided a better conversion of biomass sorghum in ethanol. On average, a significantly higher
297 production of calculated ethanol production was obtained from sorghum grown in 2013 season
298 (Table 4). In particular, during the 2012 season, irrigation with WW (TW or SW) led to a
299 significant increase in ethanol of 500 L ha⁻¹, compared to the FW treatment (Fig. 5e). During the
300 2013 season the significant increase of calculated ethanol production occurred especially with
301 sorghum irrigated with SW, resulting an increase of 560 and 1440 L ha⁻¹, compared to the TW and
302 FW treatments (Fig. 5e). This was due to the higher irrigation volume supplied during 2013 which
303 caused a reduction of the ratio between ethanol production and irrigation.

304

305 *3.5 Water use efficiency indices*

306 The water use efficiency (WUE) for biomass (bWUE), energy yield (eyWUE) and ethanol
307 (ethWUE) were significantly affected by both the irrigation treatment and the growing season.
308 These three indicators were consistent, with higher values of WUE in sorghum irrigated with SW
309 (Table 5) and cultivated during 2013 season (with the exception of ethWUE).

310 The statistical analysis showed an interaction between the irrigation treatment and the growing
311 season (Table 5) for the three WUE indices. The analysis *per* season (Fig. 5b, d and f) showed in
312 2012, the highest values of WUE indices in both treatments with WW (TW and SW) respect to the
313 irrigation with FW, while in the 2013, the highest WUE indices values were only obtained by the
314 SW treatment respect to TW and FW treatments.

315

316 **4. Discussion**

317 The trends of leaf development in sorghum obtained with the use of fresh water were consistent
318 with those reported by other authors (Gherbin et al., 1996; Mastroilli et al., 2011; Cosentino et al.,
319 2012), while the results describing the effects of WW on the bio-energy produced by biomass
320 sorghum can be considered as a novelty.

321 The increase in plant growth under the WW treatments can be attributed mainly to the nutrients
322 availability, since they are the critical factors responsible of improveing growth of irrigated
323 sorghum in Mediterranean area (Zema et al., 2012). By examining the contributions of nutrients
324 (nitrogen, phosphorus and potassium) during the two seasons (Table 6), the average N supplied
325 (nitrate and ammonium) with irrigation during the growing seasons (2012 and 2013) was
326 corresponding to the supply of 6, 86 and 112 kg ha⁻¹, for the FW, TW and SW treatments
327 respectively.

328 Through the irrigation waters, crop was also supplied by 7, 12 and 17 kg of P ha⁻¹, and 10, 68 and
329 67 kg of K ha⁻¹. The analysis of water gave the same results reported by Vivaldi et al. (2013) and
330 Disciglio et al. (2014) for other crops tested on the same experimental site.

331 In particular, the N content in WW varied with the quality of the treated waters or with the
332 efficiency of the treatment plant (Table 6): in 2012 the nitrogen supply was similar for the two types
333 of WW (100 kg ha⁻¹ for TW and 114 kg ha⁻¹ to SW), while in 2013 the irrigation with SW provided

334 a greater quantity of nitrogen (110 kg ha^{-1}) respect to TW (71 kg ha^{-1}). As a consequence in the
335 2012, dry biomass yield of sorghum under SW treatment was similar to TW, while in the 2013 dry
336 biomass yield of SW was significantly higher ($p < 0.05$) respect TW treatment. Studies of the
337 nitrogen level on the productivity of sorghum carried on the same environment (Cosentino et al.,
338 2012; Palumbo et al., 2014) indicated that yield was more affected by the soil water status during
339 the sorghum cycle than by the nitrogen level. In our study, should be considered that the distribution
340 of nitrogen is similar to a fertigation and several studies (Steduto, 1984, Boman, 1996; Kafkafi and
341 Tarchitzky, 2011) show that this technique improves crop productivity, but technically it cannot be
342 proposed in the extensive open field crops requiring the irrigation supply. In the case of sorghum,
343 the literature about the effects of fertigation of nitrogen are missing.

344 There are a few research about effect of WW on productivity of energy crops. In particular, Day
345 and Tucker (1977) with the use of WW and Mendoza et al. (2006) with the use of sludge, show the
346 increase in productivity of sorghum. Zema et al. (2012) performed a study on the use of WW on
347 other herbaceous perennial energy plants (*Typha latifolia*, *Arundo donax* and *Phragmites australis*).
348 Best results were found on *Typha latifolia*. These plants irrigated with WW showed an increase in
349 growth compared to plants irrigated with FW, indicating higher average values of LAI (+86.7%),
350 height (+25.6%) and higher average (+54.5%) and maximum biomass (+146%) yield: this may be
351 ascribed to the indirect fertilizing role of WW. Molari et al. (2014) show that *Arundo donax*
352 represents a real opportunity to produce amounts of high biomass, for energy purposes, in marginal
353 land with marginal irrigation water.

354 Regarding the structural components of sorghum, we underline that the proportions of cellulose and
355 lignin in biomass are important in biochemical conversion processes for the ethanol production. The
356 biodegradability of cellulose is greater than that of lignin, hence the overall conversion of the plant
357 material containing carbon as cellulose is greater than for plants with a higher proportion of lignin.

358 For the production of ethanol, a biomass feedstock with a high cellulose/hemi-cellulose content is
359 needed to provide a high yield. While the lignin content represents a potentially large energy
360 source. However, the current techniques involving hydrolysis/enzymatic systems are not performed
361 to convert the lignin (McKendry, 2002). Cellulose and hemicellulose contents in sweet sorghum
362 have rarely been documented. In this study, we observed that cellulose, hemicellulose and lignin
363 contents of sorghum were 32.5%, 24.8% and 7.6 % in dry matter. Our results show a higher content
364 of cellulose and hemicellulose compared to those reported by Zhao et al. (2009) in China, who
365 showed that sorghum contained a range of 19-27% of cellulose and 16-23% hemi-cellulose in
366 relation to different cultivars and phenological phases. Of course, this depends on the genetic
367 component of sorghum and different environment. WW did not affect significantly the structural
368 component of sorghum, only the cellulose content was significantly higher in the 2013 season,
369 probably due to meteorological trend during the sorghum cycle (290 mm of rain in 2012 season and
370 140 mm in 2013 season) which was favorable to the accumulation of cellulose.

371 The HHV shown in table 3 is in according to those reported by Bludau (1992b) and Fernando et al.
372 (2010b), which used fresh water for irrigation, while the negligible effects of WW on HHV were
373 also demonstrated by Angelini et al. (2005) and Zema et al. (2012) for other crops.

374 The increase in biomass yield of biomass sorghum, due to the effect of WW, determined a
375 significant difference of the estimated production of ethanol, energy yield and the WUE indices
376 (bWUE, ethWUE and eyWUE).

377 Values of bWUE can be retained high, if compared to those reported in literature. As shown by the
378 field results, the efficiency of sorghum in converting water into biomass was higher mainly when
379 WW were applied, even if in the literature are reported (Farrè and Faci, 2006) values of bWUE,
380 between 2.89 and 3.75 kg m⁻³, obtained by irrigating with fresh water.

381 Field trials and data from the literature clearly showed that bWUE in sorghum grown in the
382 Mediterranean area can vary remarkably from 5.84 to 22.81 kg m⁻³, even with similar water
383 amounts of irrigation (Mastrorilli et al., 1998; Mastrorilli et al., 2011; Palumbo et al., 2014). This
384 can be mainly explained by the rainfalls amount and distribution (Garofalo and Rinaldi, 2013). The
385 bWUE values calculated in the 2012 growing season were significantly higher because of greater
386 amount of rainfalls (Fig. 1).

387 Based on these results we recommend to irrigate with WW, because it allowed high values of WUE,
388 raw biomass production, energy yield and calculated ethanol yield.

389 The risk of contamination of groundwater from the wastewater is high, if irrigation is not well
390 managed. The monitoring of soil water content shows that a precise irrigation scheduling allows to
391 wet the layer of soil where the roots are more active and to avoid drainage of nutrients into
392 groundwater. In particular, sorghum crop demonstrated a quite good aptitude to uptake nutrients
393 and heavy metals from contaminated soil, by accumulating Pb in leaves and Cd, Zn and Cu in stems
394 (Barbanti et al., 2006; Zhuang et al., 2009).

395 About the possible contamination of biomass energy crops by microorganism, the problem does not
396 exist because, first of all the soil has a great ability to neutralize and immobilize microorganisms
397 and harmful elements and moreover the microorganisms on the vegetation do not survive to the
398 drastic transformation (cogeneration or fermentation) of biomass. In addition, Disciglio et al. (2014)
399 working in the same area and with WW from the same water treatment plant, showed that there was
400 no soil contamination, due to the high soil capacity to break down the bacterial load and to the high
401 temperature at the soil surface that contributes to creating an environment unfavorable for the
402 microorganisms.

403

404 **5. Conclusion**

405 This research shows original results regarding the application of WW on dedicated energy crops in
406 the Mediterranean area and indeed has considered possible ways to irrigate energy sorghum in a
407 sustainable way via the use of WW.

408 In general, biomass yield of sorghum was increased by irrigation with WW and, as consequence,
409 also the ethanol and the energy production per unit of cultivated area.

410 The indices of calculated WUE show how the use of WW increases the efficiency conversion of
411 water in biomass and in energy. This is an important aspect for a semi-arid climate where the fresh
412 waters are designated primarily for the civil sector.

413 Even though the results achieved require further verification by mid- or long-term research, the
414 present investigation showed that sorghum crops irrigated with WW can produce appreciable
415 biomass and energy yields.

416

417 **6. Acknowledgements**

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589 **Table 1.** Main physical-chemical characteristics of the soil

Parameter	depth				590
	0–0.20 m		0.21–0.40 m		591
	average	sd	average	sd	592
Clay (g kg ⁻¹)	325	36	329	32	593
Silt (g kg ⁻¹)	361	23	350	17	594
Sand (g kg ⁻¹)	329	3	321	4	595
E.C. (dS m ⁻¹)	1.1	0.1	1.4	0.2	596
pH	8.1	0.2	8.3	0.1	597
total Limestone (g kg ⁻¹)	172	111	202	73	598
active Limestone (g kg ⁻¹)	9.8	0.5	9.9	0.6	599
C (g kg ⁻¹)	14.0	0.6	13.8	0.6	600
N (g kg ⁻¹)	1.5	0.05	1.4	0.07	601
P (mg kg ⁻¹)	79.75	5.3	62.21	13.73	602
Ca (mg kg ⁻¹)	3286	40.4	3283	67.1	603
Na (mg kg ⁻¹)	180.2	12.3	231.6	32.4	604
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622 **Table 2.** Main chemical properties of the three irrigation treatments (fresh water-FW, tertiary
 623 water-TW and secondary water-SW). Average values and standard deviation (in parentheses) of
 624 water sampled from May to August in 2012 and 2013. The Italian threshold values for wastewater
 625 irrigation reuse (MD 152/06) are also reported.

Chemical properties		MD 152/06	2012			2013		
			FW	TW	SW	FW	TW	SW
CE	dS/m	3	0.64 (0.01)	1.47 (0.09)	1.52 (0.11)	0.62 (0.05)	1.31 (0.07)	1.33
pH		6-9.5	7.65 (0.22)	7.63 (0.08)	7.57 (0.09)	7.30 (0.26)	7.44 (0.43)	7.30
BOD ₅	mgO ₂ L ⁻¹	20	6 (0.06)	22 (0.18)	40	3 (0.05)	6 (0.08)	49 (5.8)
COD	mgO ₂ L ⁻¹	100	0 (0)	135 (12.4)	166 (22.1)	10 (0.11)	43 (0.62)	103 (1.8)
Na ⁺	mg L ⁻¹		48 (9.1)	119 (7.9)	128 (10.9)	31 (0.41)	104 (10.1)	103 (11.1)
K ⁺	mg L ⁻¹		1 (0.11)	23 (4.0)	22 (4.9)	7 (0.9)	29 (3.1)	28 (3.9)
Ca ²⁺	mg L ⁻¹		51 (15.2)	92 (27.1)	83 (8.6)	48 (5.8)	116 (22.8)	122 (20.1)
Mg ²⁺	mg L ⁻¹		12 (2.7)	20 (3.3)	21 (3.5)	8 (0.9)	21 (3.7)	16 (2.4)
NH ₄ ⁺ -N	mg L ⁻¹		2 (0.08)	41 (5.8)	46 (6.1)	0.5 (0.06)	26 (3.4)	37 (4.1)
NO ₃ ⁻ -N	mg L ⁻¹	2	5 (0.11)	3 (0.21)	11 (1.80)	3 (0.39)	4 (0.05)	10 (1.3)
PO ₄ ²⁻ -P	mg L ⁻¹	10	0 (0)	25 (4.8)	23 (5.1)	18 (4.8)	10 (3.8)	22 (4.6)
Free Chlorine	mg L ⁻¹	1200	53 (9.8)	197 (51.1)	182 (49.1)	25 (3.5)	138 (13.9)	143 (15.1)
F ⁻	mg L ⁻¹	1.5	1 (0.09)	0 (0)	1 (0.10)	0.5 (0.08)	1.6 (0.11)	2 (0.28)
SAR		10	2 (0.14)	3 (0.31)	3 (0.40)	1 (0.16)	2 (0.31)	2 (0.40)

626 The values for each trait were determined on 8 samples for each season and each irrigation water.
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637 **Table 3.** Effects of the growing season (S), irrigation treatment (I) and their interaction (SxI) in the
 638 biomass production (t ha^{-1}), higher heating value (HHV-MJ kg^{-1}) and energy yield (GJ ha^{-1}).

Treatments		Biomass	HHV	Energy yield
Season (S)	2012	22.1 a ^b	16.33 a	360 b
	2013	22.5 a	16.58 a	373 a
Irrigation water (I)	FW	20.39 b	16.40 a	335 c
	TW	22.57 a	16.45 a	371 b
	SW	23.87 a	16.52 a	395 a
p ^a	S	ns	ns	**
	I	*	ns	***
	SxI	*	ns	***

639 ^a *, **, *** and ns denote statistical significance at the 0.05, 0.01 and 0.001 levels and the absence
 640 of significance, respectively.

641 ^b Different letters in the columns indicate significant differences between treatments within the same
 642 factor, according to the Duncan's test ($P < 0.05$).

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658 **Table 4.** Effects of growing season (S), irrigation treatment (I) and their interaction (SxI) in the
 659 proportion of lignin, cellulose and hemicellulose (%) and ethanol production (L ha⁻¹).

Treatments		Lignin (%)		Cellulose (%)		Hemicellulose (%)		Ethanol (L ha ⁻¹)	
Season (S)	2012	7.80	a ^b	30.2	b	24.9	a	6262	b
	2013	7.34	a	35.1	a	24.7	a	6898	a
Irrigation water (I)	FW	7.23	a	33.8	a	24.5	a	6092	c
	TW	7.61	a	31.8	a	24.8	a	6533	b
	SW	7.87	a	32.8	a	25.1	a	7115	a
p ^a	S	ns		*		ns		***	
	I	ns		ns		ns		***	
	SxI	ns		ns		ns		*	

660 ^a *, *** and ns denote statistical significance at the 0.05 and 0.001 levels and the absence of
 661 significance, respectively.

662 ^b Different letters in the columns indicate significant differences between treatments within the same
 663 factor, according to the Duncan's test (P < 0.05).
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680 **Table 5.** Effects of growing season (S), irrigation treatment (I) and their interaction (SxI) in the
 681 biomass water use efficiency (bWUE-kg m⁻³), energy yield-water use efficiency and (eyWUE-MJ
 682 m⁻³) ethanol-water use efficiency (ethWUE-L m⁻³).

Treatments		bWUE	eyWUE	ethWUE
Season (S)	2012	7.3 a ^b	120 a	2.03 a
	2013	6.8 b	110 b	2.09 a
Irrigation water (I)	FW	6.5 c	105 c	1.91 c
	TW	7.2 b	116 b	2.06 b
	SW	7.6 a	123 a	2.22 a
p ^a	S	*	***	ns
	I	**	***	***
	SxI	*	***	*

683 ^a *, **, *** and ns denote statistical significance at the 0.05, 0.01 and 0.001 levels and the absence
 684 of significance, respectively.

685 ^b Different letters in the columns indicate significant differences between treatments within the same
 686 factor, according to the Duncan's test (P < 0.05).
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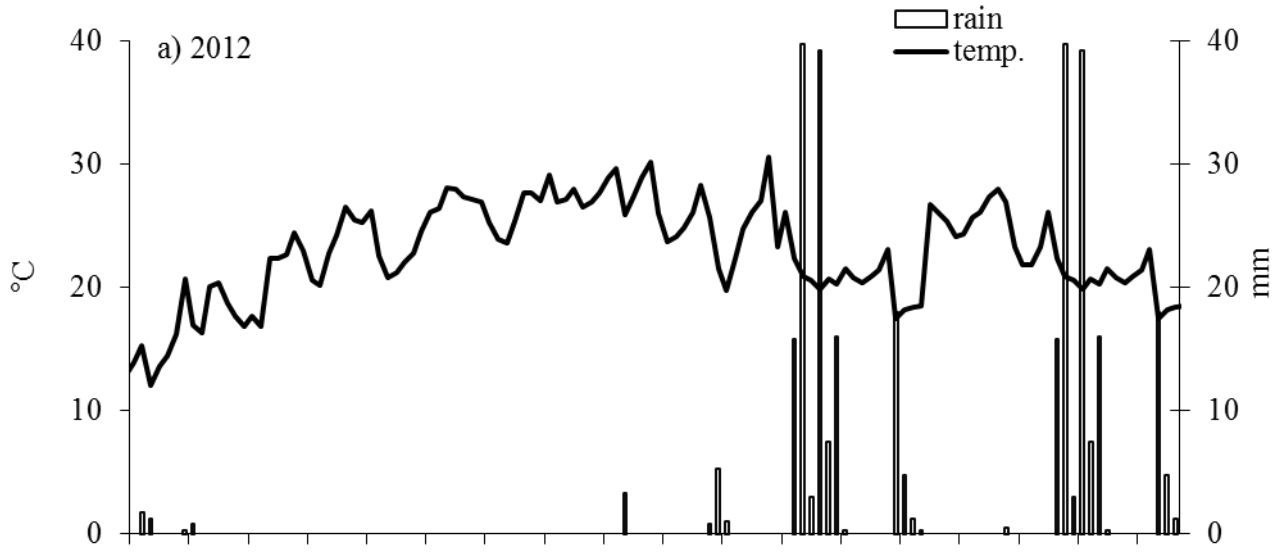
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702 **Table 6.** Average nitrogen, phosphorus and potassium supplied (kg ha^{-1}) with irrigation for the
 703 three irrigation treatments (fresh water-FW, tertiary water-TW and secondary water-SW), and
 704 during the two growing seasons (2012 and 2013).
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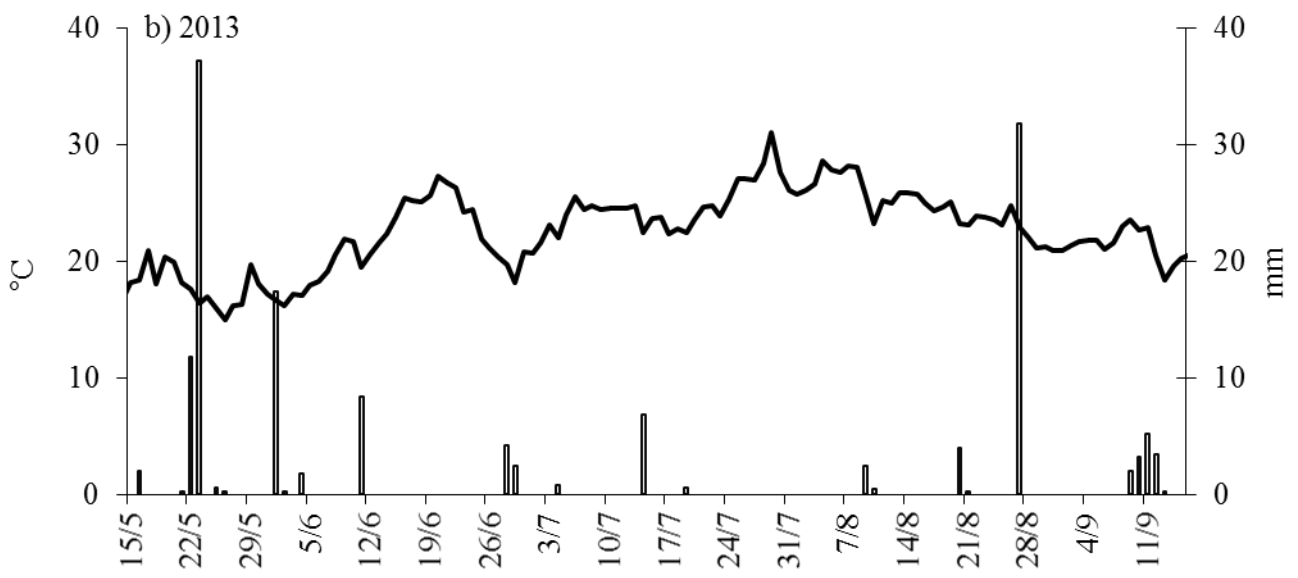
Season	FW	TW	SW
Nitrogen (kg ha^{-1})			
2012	8	100	114
2013	4	71	110
average	6	86	112
Phosphorus (kg ha^{-1})			
2012	0	17	16
2013	14	8	18
average	7	12	17
Potassium (kg ha^{-1})			
2012	0	56	55
2013	20	80	79
average	10	68	67

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711 **Fig. 1.** Rain and air temperature during 2012 and 2013 growing seasons

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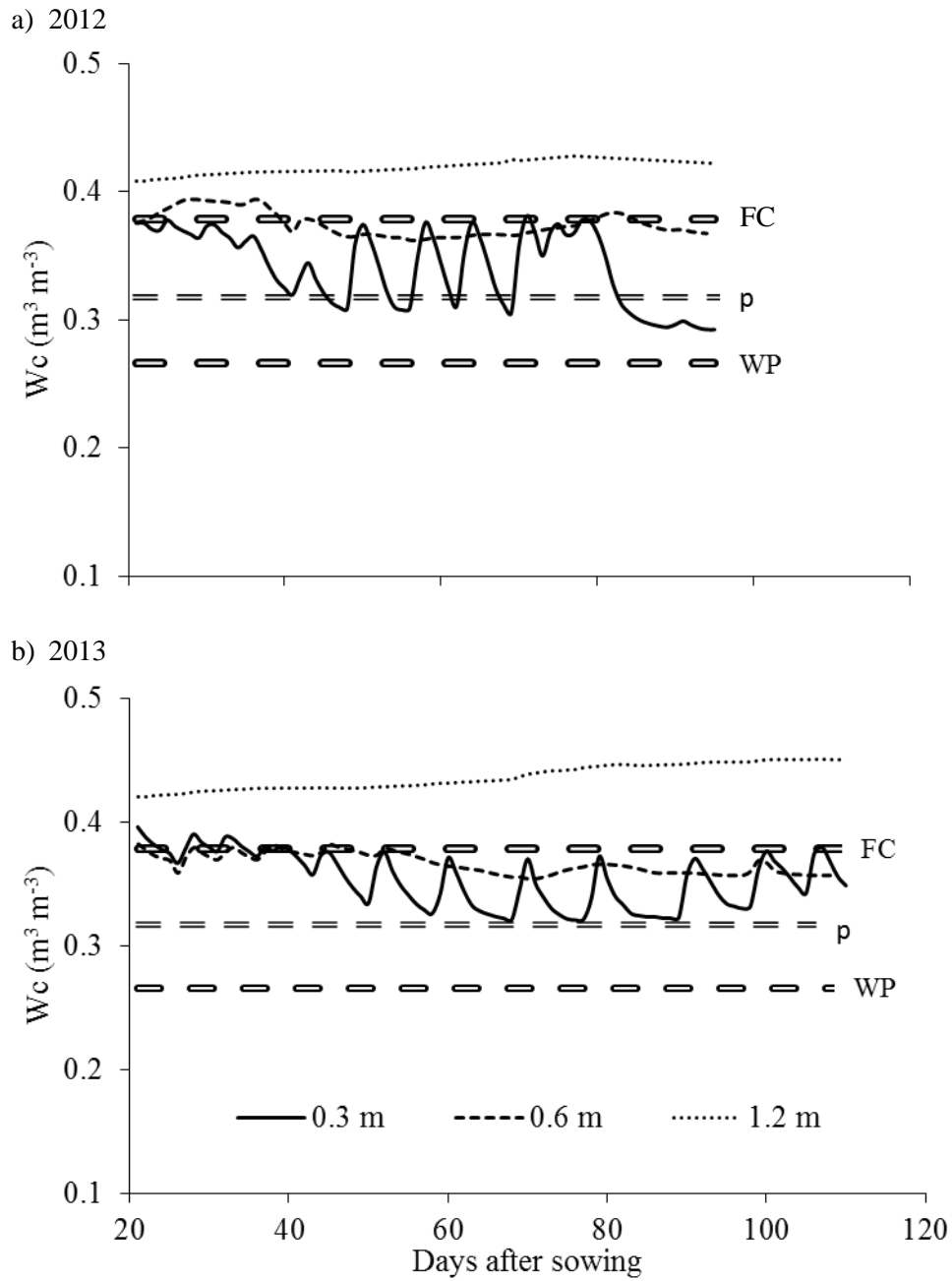
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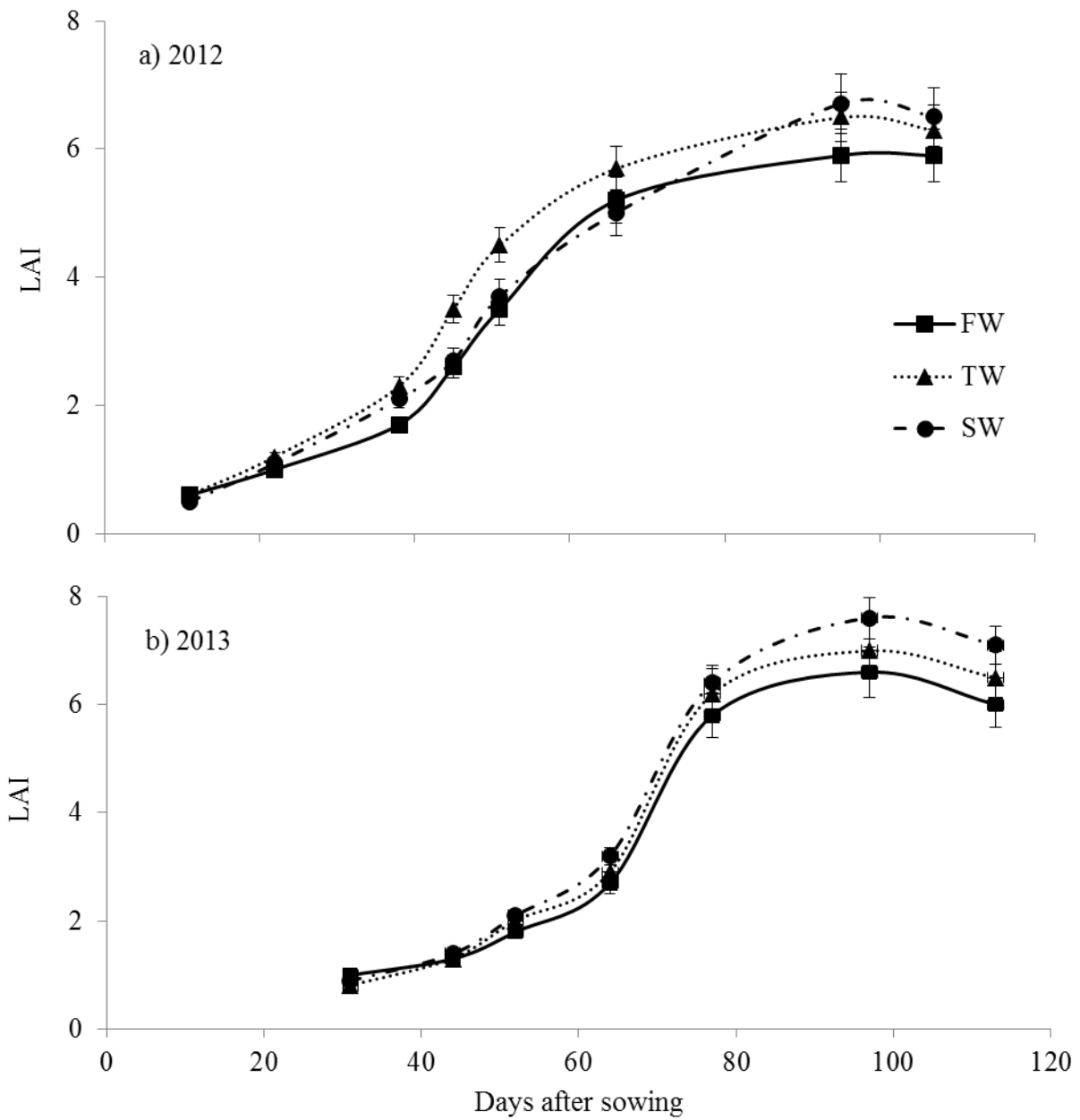
719 **Fig. 2.** Water contents (W_c) at three soil depths (0.3, 0.6 and 1.2 m) of the soil during 2012 and
 720 2013 growing seasons. The dashed lines correspond to the water contents of the soil at field
 721 capacity (FC) and at wilting point (WP). p = Readily Available Water threshold.

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728 **Fig. 3.** LAI of sorghum for three irrigation treatments (fresh water-FW, tertiary water-TW and
 729 secondary water-SW), and two growing seasons (2012-a and 2013-b).

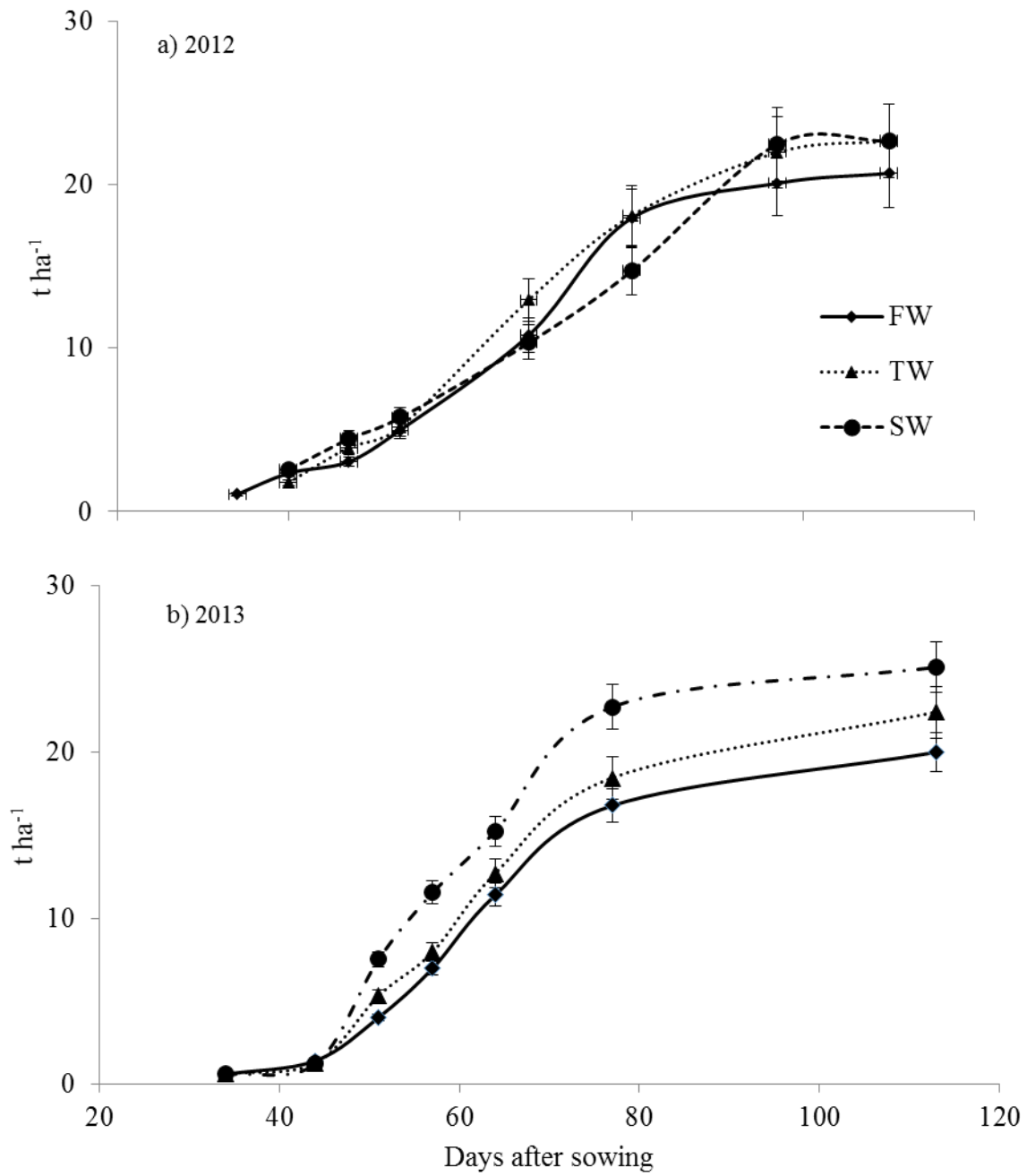
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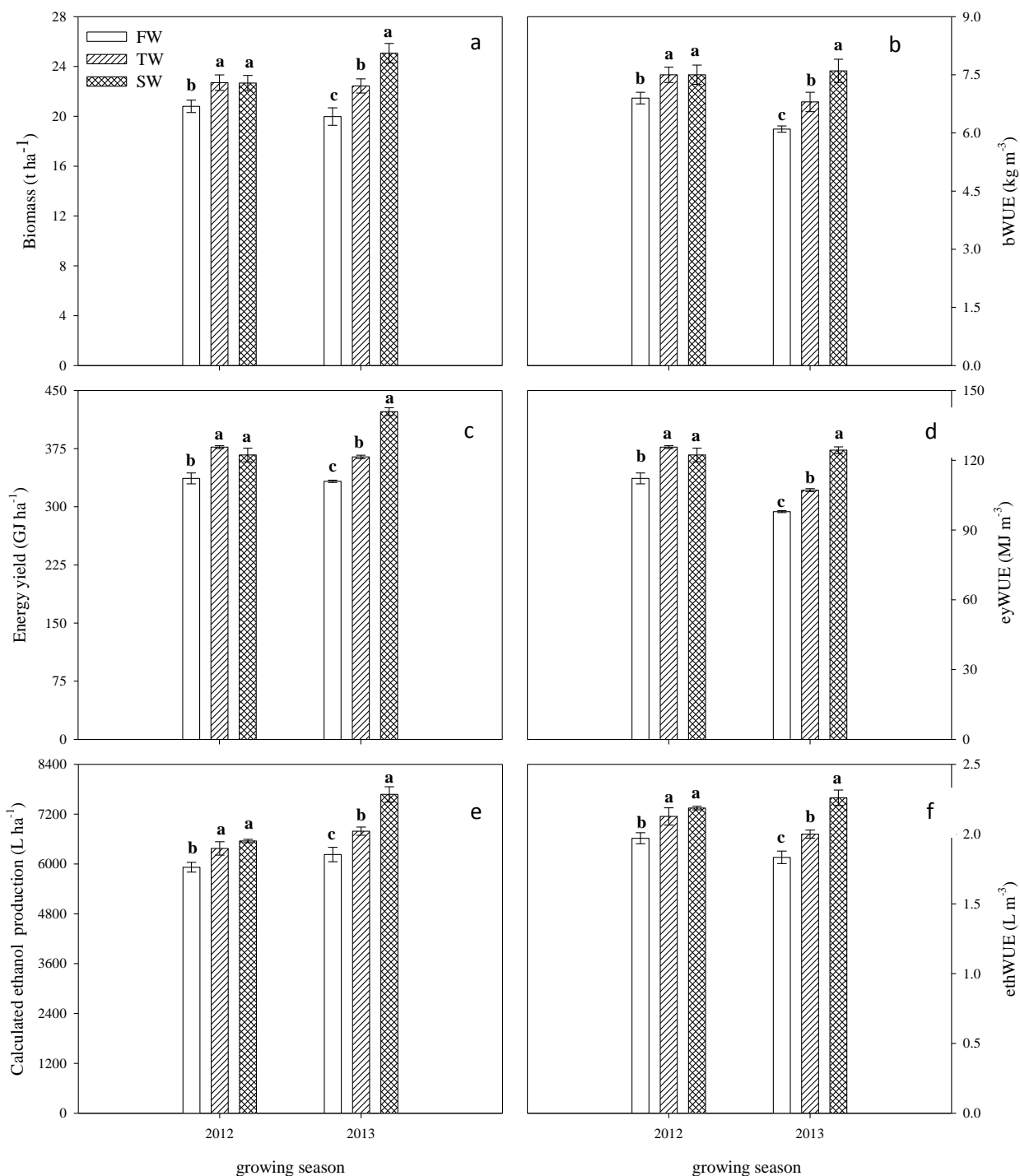
737 **Fig. 4.** Dry matter ($t\ ha^{-1}$) of sorghum for three irrigation treatments (fresh water-FW, tertiary
 738 water-TW and secondary water-SW), and two growing seasons (2012-a and 2013-b).

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744 **Fig. 5.** Biomass production (a; t ha⁻¹), biomass water use efficiency (b; bWUE-kg m⁻³), energy yield
 745 (c; GJ ha⁻¹), energy yield-water use efficiency (d; eyWUE-MJ m⁻³) calculated ethanol production (e;
 746 L ha⁻¹) and ethanol-water use efficiency (f; ethWUE-L m⁻³) for three irrigation treatments (fresh
 747 water-FW, tertiary water-TW and secondary water-SW), and two growing seasons (2012 and 2013).
 748 Different letters indicate significant differences among irrigation water qualities for the same
 749 growing season, according to the Duncan's test (P < 0.05).