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RESEARCH ARTICLE

Effects of supplement with sanitary landfill leachate in gas exchange of sunflower (*Helianthus annuus* L.) seedlings under drought stress

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Abstract Sanitary landfill leachate is one of the major problems arising from disposal of urban waste. Sanitary landfill leachate may, however, have use in agriculture. This study, therefore, aimed to analyze initial plant growth and gas exchange in sunflower seedlings supplemented with sanitary landfill leachate and subjected to drought stress through variables of root fresh mass (RFM), shoot fresh mass (SFM), total fresh mass (TFM), relative chlorophyll content (CL), stomatal conductance (g_s), transpiration rate (E), net photosynthetic rate (A), ratio of internal to external CO_2 concentration (Ci/Ca),water use efficiency (EUA), instantaneous carboxylation efficiency (A/Ci), and electron transport rate (ETR). The experimental design was a completely randomized 2 (irrigated and non-irrigated) × 4 (sand, sand + 100 kg N ha^{-1} organic fertilizer, sand + 100 kg N ha^{-1} sanitary landfill leachate, and sand + 150 kg N ha^{-1} sanitary landfill leachate) factorial with five replicates. Under drought stress conditions, leachate treatment supplemented with 100 kg N ha^{-1} exhibited higher plant fresh weights than those of the treatment containing 150 kg N ha^{-1}. Increases in fresh mass in plant treatments supplemented with 100 and 150 kg N ha^{-1} sanitary landfill leachate were related to higher photosynthetic rates.

Keywords Drought stress · Gas exchange · Growth analysis · *Helianthus annuus* L. · Photosynthesis · Sanitary landfill leachate

Introduction

Climate, drought, and salinity are among the main factors responsible for the limitations of plant growth, development, and yield (Krasensky and Jonak 2012). This way, studies assessing the physiological responses of plants to different types of stress have been conducted with the goals of formulating management strategies and selecting more tolerant genotypes in different cultures (Otto et al. 2013). Moreover, it is common for crops to be influenced by factors such as nutritional order, drought, and temperature in tropical regions (Silva et al. 2012).

Water availability is the most common factor that influences the spatial distribution and plant survivability to different environments (Pou et al. 2012). According to Alvarenga et al. (2011), plant drought stress occurs due to a reduction in water availability in its thermodynamically appropriate state for the plant itself. Drought stress can cause closing of stomata, senescence, leaf abscission, and, ultimately, productivity loss.

In addition, studies show that drought causes a negative impact on the different aspects of plant growth (anatomical, morphological, physiological, biochemical, and molecular aspects), varying in different species according to duration, severity, and stage of development (Bezerra et al. 2003; Vitorino and Martins 2012). The closing of stomata is the initial physiological response of plants under drought stress that aims to

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reduce the transpiration rate for cell turgor maintenance. Besides that, plants are capable of promoting a reduction in the osmotic potential of the roots, which enables the reduction of hydric potential and maintenance of cellular turgor (Guimarães et al. 2011; Ferrari et al. 2015).

However, the closing of stomata may cause direct effects on photosynthetic activity through decreasing the CO_2 availability to the Calvin–Benson cycle. Thus, there is a decrease in NADPH oxidation produced in the photochemical phase of photosynthesis, raising, then, the $NADPH/NADP^+$ ratio. In this case, reduced ferredoxin produced during electron transfer in the photochemical phase can transfer its electrons to O_2 at photosystem I, leading to the formation of superoxide radicals ($\cdot O_2^-$). Consequently, this phenomenon may cause oxidative stress resulting from excessive formation of reactive oxygen species, causing damage to the photosynthetic apparatus (Sharma et al. 2012; Barbosa et al. 2014).

The sunflower (*Helianthus annuus* L.) plant is adapted to different environmental conditions and exhibits beneficial agronomic characteristics compared to most species grown in Brazil, such as resistance to drought and to low and high temperatures (Dutra et al. 2012). Due to its drought tolerance, short life cycle, good yield, and oil productivity, sunflower is emerging as a good option for cultivation in the Brazilian semiarid region (Viana et al. 2012).

In semiarid conditions, due to irregular rainfall and low soil fertility, plants often are subjected to situations of drought and nutritional stress (Selmar and Kleinwächter 2013). One of the alternatives for agriculture in these regions has been the use of water with inferior quality or residual irrigation (Lira et al. 2015). In addition, the presence of organic matter and other elements in wastewater is an alternative to supplying nutrients and to promoting improvement in plant acclimation to semiarid conditions (Firmino et al. 2015).

Sanitary landfill leachate is an example of highly contaminated effluent that is difficult to treat, and its final destination is currently one of the biggest environmental problems in the management of solid waste (Riguetti et al. 2015). The sanitary landfill leachate characteristics and its composition depend on the type of deposited residue, climatic conditions, mode of operation, and the age of the landfill (Panizza and Martinez-Huitle 2013). However, the presence of macronutrients and micronutrients in the leachate suggests the possibility for its use in agriculture (Matos et al. 2013), mainly for the cultivation of biomass and/or biofuels.

Lavagnolo et al. (2016) and Garbo et al. (2016) found positive correlations between the extraction capacity of macronutrients and micronutrients and the increase in plant biomass in sunflower plants treated with sanitary landfill leachate. Similarly, Matos et al. (2013) verified increases in dry masses and protein contents in Tifton 85 (*Cynodon* ssp.) grass plants using sanitary landfill leachate as a source of organic matter and nitrogen. Nevertheless, in none of the papers are described the possible effects of the effluent on plant physiology. Thus, this study aimed to analyze the initial growth and gas exchange parameters in sunflower plants supplemented with sanitary landfill leachate and subjected to water deficit through assessments of fresh weight parameters (root, shoot, and total), relative chlorophyll content, stomatal conductance, transpiration rate, net photosynthetic rate, ratio of internal to external CO_2 concentration, water use efficiency, instantaneous carboxylation efficiency, and electron transport rate.

Material and methods

Collection and characterization of sanitary landfill leachate

The leachate used in the experiment was collected in August 2015 at the *Aterro Sanitário Metropolitano Oeste de Caucaia* (*ASMOC*) from the third stabilization pond (aerobic optional) near the spillway located in the municipality of Caucaia, Ceará, Brazil. The effluent sample followed storage, maintenance, and transport regulations according to the physicochemical parameters analyzed (Table 1).

Experimental conditions, treatments, harvests, and analysis of data

The experiment was conducted in a greenhouse in Maracanaú, Ceará, Brazil, from September to October 2015. The average values of temperature and relative humidity during the day were 32.1 °C and 52%, respectively.

Sunflower (*Helianthus annuus* L. "BRS 323") seeds were provided by *Embrapa Produtos e Mercados—Escritório Dourados*, Mato Grosso do Sul, Brazil. After selection and disinfection with 0.7% sodium hypochlorite solution, seeds were sown in 5-L plastic vases filled with one of the following mixtures: (1) fine-grain sand (negative control); (2) sand + mixed commercial organic fertilizer (Fértil Vida®) (11.8% nitrogen [N]) applied proportionally corresponding to 100 kg N ha^{-1} (positive control); (3) sand + sanitary landfill leachate applied in proportion corresponding to 100 kg N ha^{-1}; and (4) sand + sanitary landfill leachate applied in proportion corresponding to 150 kg N ha^{-1}. The landfill leachate concentrations employed were defined in agreement with the N recommendations (60–80 kg N ha^{-1}) and the possible negative effects of higher values (100 and 150 kg N ha^{-1}). During the experiment, the plants were subjected to daily water of near 70% of the field capacity of the substrate (Casaroli and Lier 2008), with the maintenance of the daily replenishment of evaporated water by daily weighing of the vessels and replacement of the mass lost with water.

Table 1 Physicochemical characterization of sanitary landfill leachate used in the experiment

mg L^{-1}											
N-t	NH_4	NO_{3-}	NO_{2-}	P-t	Fe^{+2}	Zn	Mn	Cu	P_2O_5	C/N	TOC
504	323	153	19	7.9	16.1	22.3	24.5	1.5	18.1	1.39	660
mg L^{-1}									mS/cm		
K_2O	K^+	Na^+	Ca^{2+}	Mg^{2+}	Cl^-	CO_3^{-2}	HCO_3^-		pH	EC	SAR
2.196	1.800	234.6	54	58.5	2.428	96	231.8		7.8	7.6	5.37

N-t total nitrogen, *P-t* total phosphorus, *C/N* carbon/nitrogen ratio, *TOC* total organic carbon, *EC* electrical conductivity, *SAR* sodium adsorption ratio

Sixteen days after sowing, half of each group of plants was subjected to suspension of irrigation, being thus distributed into two groups: control (irrigated) and drought-stressed plants (non-irrigated). Therefore, the experimental design was completely randomized, arranged in a 2 (irrigated and non-irrigated) × 4 (sand, sand + 100 kg N ha^{-1}organic fertilizer, sand + 100 kg N ha^{-1} sanitary landfill leachate, and sand + 150 kg N ha^{-1} sanitary landfill leachate) factorial, and two harvest times (5 and 7 days after suspension of irrigation) with five replicates containing two plants each, totaling 80 experimental units.

Five days after the suspension of irrigation, the first fully expanded leaf was used to analyze gas exchange using an LI-6400XT portable infrared gas analyzer (LI-COR). The measurements occurred between 09:00 a.m. and 11:00 a.m. The following parameters were analyzed: stomatal conductance (g_s), transpiration rate (E), net photosynthetic rate (A), internal ratio to external CO_2 concentration (Ci/Ca), water use efficiency (EUA) (A/E), instantaneous carboxylation efficiency (A/Ci), and electron transport rate (ETR).

At 5 and 7 days after suspension of irrigation, relative chlorophyll content (CL) was estimated with a SPAD-502 portable meter (Minolta) using the first fully expanded leaf. Thus, the plants were harvested and separated into roots, stem, and leaves for the determination of root fresh mass (RFM), shoot fresh mass (SFM), and total fresh mass (TFM). Data of each harvest period were independently subjected to analysis of variance (ANOVA) and the means compared through Tukey's test at 5% probability using SigmaPlot 11.0 software.

Results and discussion

Production of fresh matter and relative contents of chlorophyll

ANOVA showed an interaction between the substrate factors used in cultivation and irrigation (T × I) that was significant at 1% probability with respect to the variables RFM, SFM, TFM, and CL (Table 2).

In general, there were greater increases in fresh mass in treatments with sanitary landfill leachate (sand + 100 kg N ha^{-1}sanitary landfill leachate, and sand + 150 kg N ha^{-1} sanitary landfill leachate) in both irrigated and non-irrigated conditions (Fig. 1). There were significant differences among 100 kg N ha^{-1}, sand, and organic fertilizer treatments in the two harvest periods and water regimes.

Table 2 Summary of the analysis of variance (ANOVA) for root fresh mass (RFM), shoot fresh mass (SFM), total fresh mass (TFM), and relative chlorophyll content (CL) of sunflower plants in different substrates (sand, sand + 100 kg N ha^{-1} mixed commercial organic fertilizer, sand + 100 kg N ha^{-1} sanitary landfill leachate, and sand + 150 kg N ha^{-1} sanitary landfill leachate) under control and water stress conditions at 5 and 7 days after suspension of irrigation

		Medium square							
		RMF		SFM		TFM		CL	
Variation factor	GL	5 days	7 days	5 days	7 days	5 days	7 days	5 days	7 days
Treatment (T)	3	2.71*	3.08*	86.95*	100.43*	154.58*	133.98*	648.48*	499.36*
Irrigation (I)	1	1.21*	4.77*	8.48*	92.63*	0.67*	155.79*	72.09*	33.23*
T × I	3	0,07*	0.83*	3.49*	44.81*	18.80*	50.35*	13.35*	27.10*
Error	32	0.008	0.02	0.050	0.127	0.056	0.151	1.284	1.474
Total corrected	39	–	–	–	–	–	–	–	–
CV (%)	–	6.73	8.99	4.20	5.61	3.26	4.89	3.81	4.20

RFM root fresh mass, *SFM* shoot fresh mass, *TMF* total fresh mass, *CL* relative chlorophyll content

$^*P \leq 0.001$

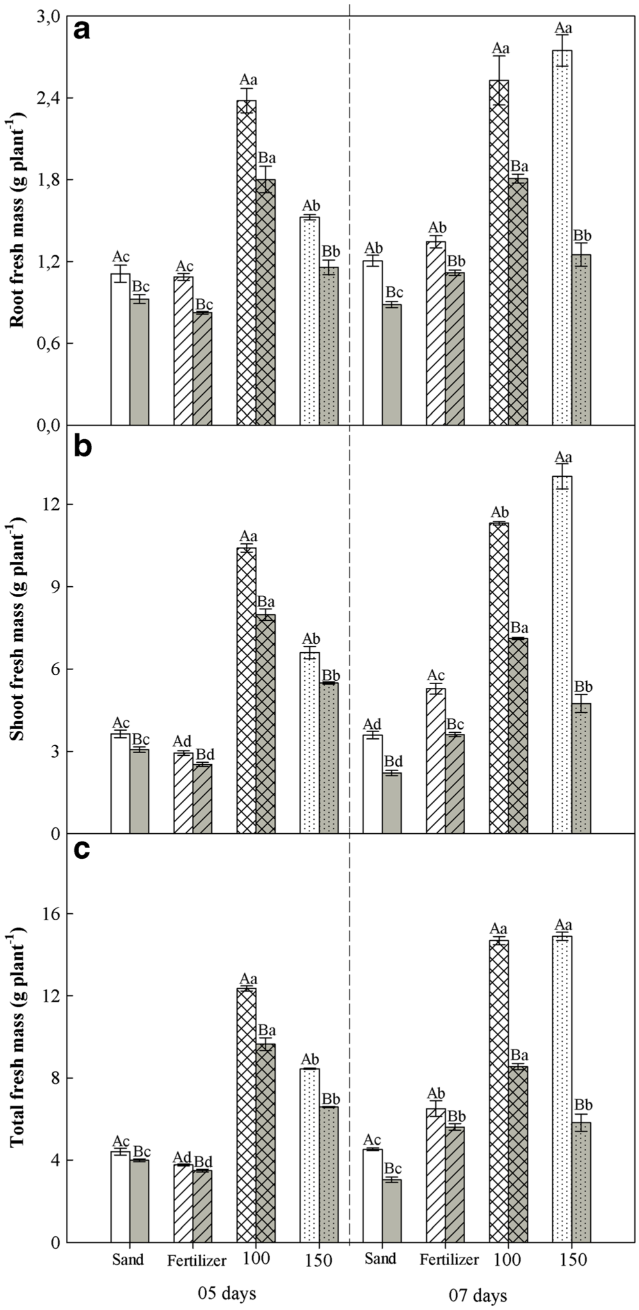

Fig. 1 Root (**a**), shoot (**b**), and total (**c**) fresh masses of sunflower plants under control (white bars) or drought stress (gray bars) conditions at 5 and 7 days after suspension of irrigation. Different capital letters indicate significant differences due to irrigation (control and stress), whereas different lowercase letters indicate statistical differences among the substrates (sand, sand + 100 kg N ha^{-1} organic fertilizer, sand + 100 kg N ha^{-1} sanitary landfill leachate, and sand + 150 kg N ha^{-1} sanitary landfill leachate) according to Tukey's test ($P \leq 0.05$). Statistical analyses were performed independently for each harvest. Bars represent the values of the means ± standard error of five replicates

At 7 days under water stress conditions, the sand + 100 kg N ha^{-1} sanitary landfill leachate treatment showed higher fresh mass relative to sand and organic fertilizer treatments with significant differences of 105 and 61.6% in RFM (Fig. 1a); 222 and 97.5% in SFM (Fig. 1b); and 181% and 52.3 in TFM (Fig. 1c), respectively.

The ANOVA results obtained in this study of fresh mass were similar to those reported by Guedes Filho et al. (2011) with sunflower plants under different irrigation and N regimes. According to the authors, increases in the supply of N and application of different irrigation levels influenced growth parameters and productivity of sunflower plants.

Among nutrients, N is one of the most required for sunflower plant growth. N fertilization and limited availability of water are important factors that affect agricultural productivity (Freitas et al. 2012; Alves et al. 2016). In the present study, the results of fresh mass corroborated with those of the aforementioned authors and demonstrated that the source of N (organic or leachate fertilizer) and the concentration (absence, 100 or 150 kg N ha^{-1}) influenced biomass accumulation under control or drought stress conditions.

According to Lobo et al. (2012), plants absorb N through mass flow, in which the element is transported to the roots through water. The results of Fig. 1 show that it is possible that the sanitary landfill leachate provided a more adequate N source and other nutrients compared to the commercial organic fertilizer. This may be because the leachate is a liquid effluent and, in this state, favors the flow of nutrients to the root system. Additionally, another hypothesis is that it could be the high concentrations of nitrate (NO_3^-) in the leachate (Table 1). The nitrate can be taken up easily by plants.

Regarding CL (Fig. 2), similar to the results of fresh mass, there were high increases in treatments with the sanitary landfill leachate (100 and 150 kg N ha^{-1}) under both conditions (control and stress). On the other hand, when compared to irrigated and non-irrigated conditions, higher values were found in CLs in non-irrigated conditions at both harvest times.

N is the constituent of a large amount of biomolecules, such as proteins, coenzymes, nucleic acids, chlorophyll, and phytochrome (Lobo et al. 2014). The chlorophyll content is related to the absorption of light for its use in the photochemical stage. Thus, it is expected that the higher contents were found in plants that had higher fresh mass—in this case, the irrigated plants (control).

In contrast, there were higher chlorophyll contents in plants under stress conditions. These results are similar to those of Paixão et al. (2014) when comparing sunflower genotypes with different tolerance to drought stress, in which the authors observed higher chlorophyll contents in plants under drought stress. However, the authors affirmed that this could be detrimental to plants because it would lead to higher absorption of light, which can cause photooxidation and damage to membrane integrity and the photosynthetic apparatus. In addition, Araújo and Deminicis (2009) affirmed that in most cases, photooxidation occurs as a secondary event and it is slow, but it could progressively lead to a decrease in photosynthesis (photoinhibition).

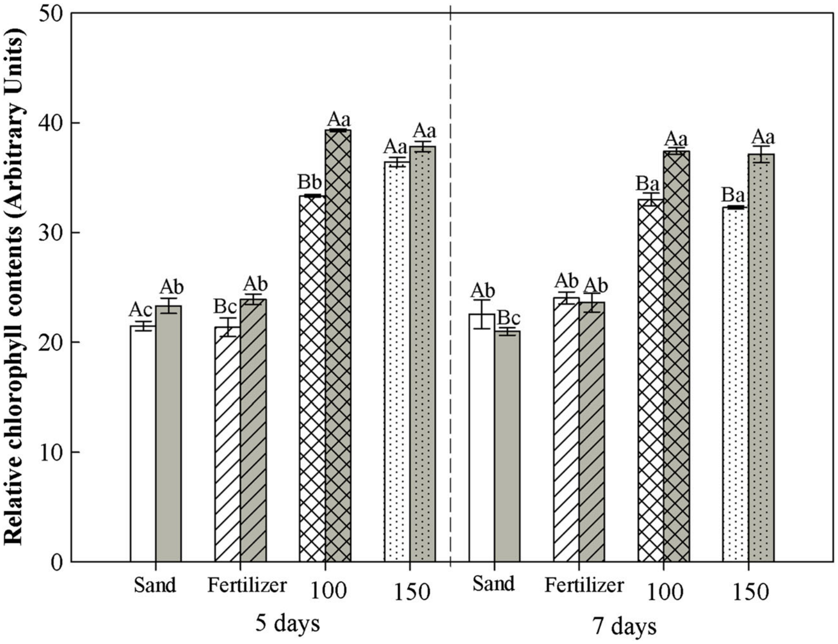

Fig. 2 Relative chlorophyll contents of sunflower plants under control (white bars) or drought stress (gray bars) conditions at 5 and 7 days after suspension of irrigation. Different capital letters indicate significant differences due to irrigation (control and stress), whereas different lowercase letters indicate statistical differences among the substrates (sand, sand + 100 kg N ha^{-1} organic fertilizer, sand + 100 kg N ha^{-1} sanitary landfill leachate, and sand + 150 kg N ha^{-1} sanitary landfill leachate) according to Tukey's test ($P \leq 0.05$). Statistical analyses were performed independently for each harvest. Bars represent the values of the means ± standard error of five replicates

Another possibility is that the method used (SPAD meter) facilitated the detection of higher chlorophyll content due to further dehydration of tissues. In addition, leaves reduced growth in the presence of drought stress, favoring an increase in the chlorophyll content per unit area.

Gas exchange

ANOVA showed that there were interactions among the factors, substrates used in the cultivation and irrigation (T × I) that were significant at 1% probability with respect to g_s, E, Ci/Ca, A, instantaneous EUA, instantaneous A/Ci, and ETR parameters (Table 3).

The results of g_s, E, Ci/Ca, and A of sunflower plants at 5 days after onset of drought stress are shown in Fig. 3.

In all treatments, seedlings subjected to drought stress had lower g_s values (Fig. 3b) with significant differences relative to irrigated plants. In addition, regarding to drought stress, g_s values observed in the sand + 100 kg N ha^{-1} sanitary landfill leachate treatment were 80% less than those of the commercial fertilizer treatment.

Table 3 Summary of the analysis of variance for stomatal conductance (g_s), transpiration rate (E), ratio of internal to external CO_2 (Ci/Ca), net photosynthetic rate (A), instantaneous water use efficiency (EUA), instantaneous carboxylation efficiency (A/Ci), and electron transport rate (ETR) of sunflower plants in different substrates (sand, sand + 100 kg N ha^{-1} organic fertilizer, sand + 100 kg N ha^{-1} sanitary landfill leachate, and sand + 150 kg N ha^{-1} sanitary landfill leachate) under control or drought stress conditions at 5 days after the suspension of irrigation

		Medium square						
Variation factor	GL	g_s	E	Ci/Ca	A	EUA	A/C_i	ETR
Treatment (T)	3	0.034*	7.065*	0.099*	277.9*	5.568*	0.006*	12240.7*
Irrigation (I)	1	0.496*	127.5*	0.074*	344.9*	3.220*	0.002*	5354.4*
T × I	3	0.131*	7.705*	0.007*	47.71*	0.418*	0.0003*	2222.2*
Error	32	0.095	0.198	0.0007	0.254	0.069	0.00001	86.17
Total corrected	39	–	–	–	–	–	–	–
CV (%)	–	6.8	6.97	4.05	2.70	8.27	6.23	5.52

*$P \leq 0.001$

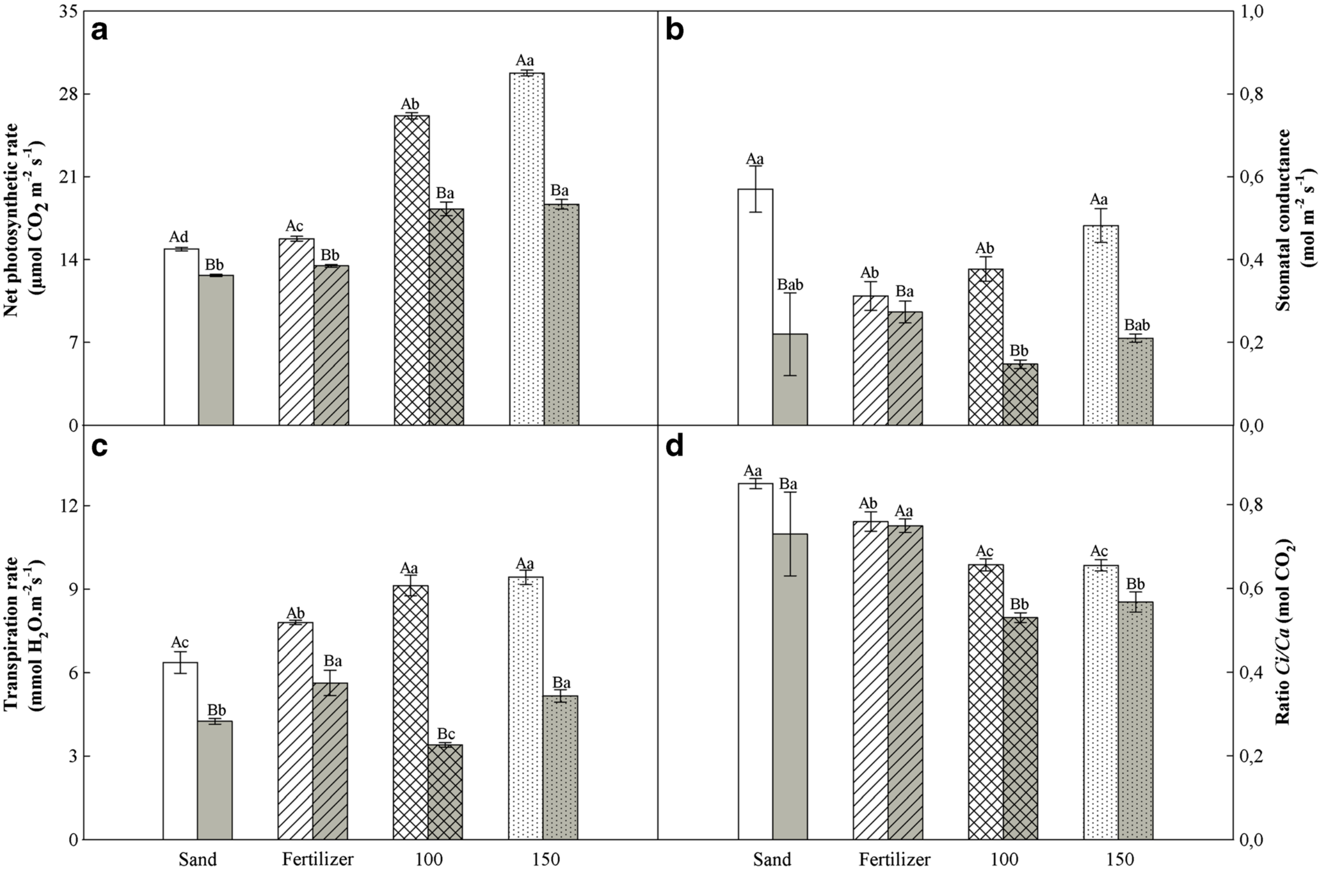

Fig. 3 Net photosynthetic rate (*A*) (**a**), stomatal conductance (g_s) (**b**), transpiration rate (*E*) (**c**), and ratio of internal to external CO_2 concentration (*Ci*/*Ca*) (**d**) of sunflower plants under control (white bars) or drought stress conditions (gray bars) at 5 days after suspension of irrigation. Different capital letters indicate significant differences due to irrigation (control and stress), whereas different lowercase letters indicate statistical differences among the substrates (sand, sand + 100 kg N ha^{-1} organic fertilizer, sand + 100 kg N ha^{-1} sanitary landfill leachate, and sand + 150 kg N ha^{-1} sanitary landfill leachate) according to Tukey's test ($P \leq 0.05$). Statistical analyses were performed independently for each harvest. Bars represent the values of the means ± standard error of five replicates

Values of *E* (Fig. 3c) showed similar behavior to g_s under water stress conditions. The sand + 100 kg N ha^{-1} landfill leachate treatment showed values 65% lower than those of the treatment with commercial fertilizer. Similarly, Cechin et al. (2010) also found reductions in g_s and *E* values in sunflower plants under drought stress.

The processes of g_s and *E* are directly related since they correspond to initial responses of plants to drought stress (Otieno et al. 2005; Albuquerque et al. 2013). Reductions in g_s, while inhibiting water loss by *E*, can decrease the entry of CO_2 and, thus, availability of CO_2 for photosynthesis (Araújo and Deminicis 2009).

With respect to *Ci*/*Ca* and *A* (Fig. 3a, d), in a general way, under drought stress conditions the plants exhibited a reduction in both parameters compared to plants under control conditions. However, seedlings that received sanitary landfill leachate (treatments 100 and 150 kg N ha^{-1}) exhibited the lowest *Ci*/*Ca* values with higher photosynthetic rates compared to those of sand and commercial fertilizer treatments.

According to Lemos et al. (2012), maintenance of the photosynthetic rate under conditions that reduce g_s is only possible with more efficient internal carbon fixation in the leaf mesophyll. Moreover, it is possible that this factor was responsible for the *Ci/Ca* values and *A* found in treatments with sanitary landfill leachate under drought stress conditions.

In addition, it was found that plants under drought stress conditions supplemented with sanitary landfill leachate had more effective control of stomatal closure; the inability of this implies a reduction of photosynthetic rate at the same magnitude. This reinforces the hypothesis that there was higher efficiency in CO_2 fixation in mesophyll cells in these treatments.

Given the results observed in Fig. 3, we analyzed the EUA, *A*/*Ci*, and ETR of sunflower seedlings (Fig. 4). In general, drought stress increased the EUA values (Fig. 4a), but reduced *A*/*Ci* (Fig. 4b) and ETR (Fig. 4).

Increases in EUA values (Fig. 4a) were similar to those found by Duarte et al. (2012) also in sunflower plants under suspension of irrigation. The authors found that EUA

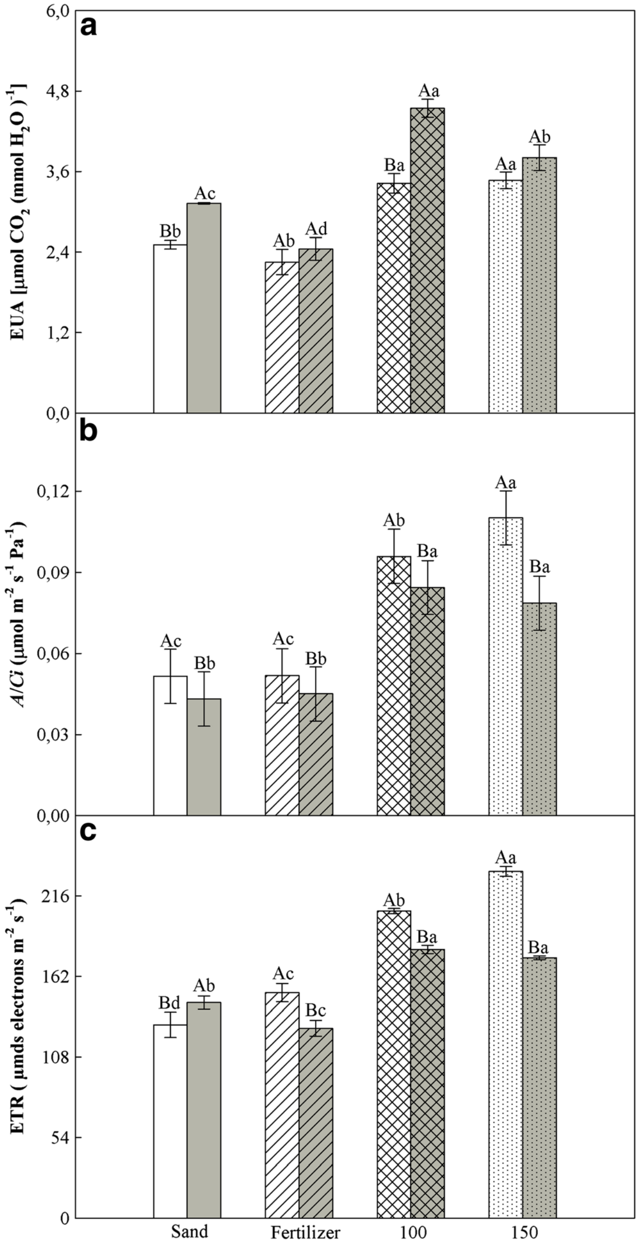

Fig. 4 Instantaneous water use efficiency (EUA) (**a**), instantaneous carboxylation efficiency (*A*/*Ci*) (**b**), and electron transport rate (ETR) (**c**) of sunflower seedlings under control (white bars) or drought stress (gray bars) conditions at 5 days after suspension of irrigation. Different capital letters indicate significant differences due to irrigation (control and stress), whereas different lowercase letters indicate statistical differences among the substrates (sand, sand + 100 kg N ha^{-1} organic fertilizer, sand + 100 kg N ha^{-1} sanitary landfill leachate, and sand + 150 kg N ha^{-1} sanitary landfill leachate) according to Tukey's test ($P \leq 0.05$). Statistical analyses were performed independently for each harvest. Bars represent the values of the means ± standard error of five replicates

increases, and they related the results to higher yields of achenes and oil in drought-stressed plants. The relationship between the increase in EUA and productivity in plants is also described by other authors using different plant cultures (Melo et al. 2010; Oliveira et al. 2013). Furthermore, Mantovani et al. (2013) emphasized that the relationship between EUA and biomass production could be used as a reference for strategic management in order to increase productivity. Therefore, it is suggested that the increase in this parameter was positive due to the use of sanitary landfill leachate, which is associated with lower *E* values in plants under drought stress.

With respect to *A*/*Ci* (Fig. 4b) and ETR (Fig. 4c) reductions, it is suggested that the results are related to the internal CO$_2$ concentration and photosynthetic assimilation rate, as described by Machado et al. (2005). The observed reductions in these parameters may be related to indirect responses (non-stomatal) to drought stress stemming from changes in biochemical and photochemical phases of photosynthesis, such as reductions in photosynthetic metabolism enzyme (e.g., Rubisco) activities and damage to photosystem II from photoinhibition (Kiani et al. 2008; Araújo and Deminicis 2009). In addition, these modifications can cause an imbalance between the production of NADPH in the photochemical phase and its use in the Calvin–Benson cycle (Sanda et al. 2011). This phenomenon is similar to results reported by Bertolli et al. (2015) in *Beaucarnea recurvata* Lem. under drought stress conditions. It is possible that the reduction in *A*/*Ci* is related to reduced energy requirements for the biochemistry phase of photosynthesis, resulting in a decreased ETR. Finally, we can infer that the results found in treatments with sanitary landfill leachate for the variables in Fig. 4 under drought stress conditions were mainly due to reductions in g_s and internal CO$_2$ concentration.

Conclusions

- Under drought stress conditions, there was a higher acclimation capacity of sunflower seedlings that received sanitary landfill leachate as a source of nutrients.
- The sanitary landfill leachate promoted the maintenance of photosynthetic capacity of sunflower seedlings under drought stress conditions, even under reduced g_s values.
- In general, under drought stress conditions, the treatment supplemented with 100 kg N ha^{-1} leachate showed higher plant fresh weights compared to the treatment containing 150 kg N ha^{-1}.
- Increases in fresh mass in plant treatments supplemented with 100 and 150 kg N ha^{-1} sanitary landfill leachate are related to higher photosynthetic rates in both under control or drought stress conditions.

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