The use of reclaimed water is a viable and safe strategy for the irrigation of myrtle plants in a scenario of climate change

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ABSTRACT

In this work, we irrigated myrtle plants with reclaimed waters (RWs) for 90 days with drainage. The treatments consisted of a control (0.8 dS m^{-1}) and two RWs: RW1 (2.0 dS m^{-1}) and RW2 (5.0 dS m^{-1}). In general, nutrients were accumulated in a greater proportion in shoots than in roots and increased in the RW treatments, with the exception of potassium and phosphorus. This behaviour produced a progressive decrease in the root water potential, which hindered the mobility of water to the leaves. This in turn caused a drop in leaf water potential and gas exchange parameters, especially in the RW2 treatment. The intrinsic water-use efficiency (WUE_i, P_n/g_s) did not show differences in any treatment. The RW2 treatment provoked a loss of biomass in the leaves but not in the stems and roots, resulting in more compact plants. Considering these results together, it is feasible to use RWs for plant irrigation, despite their high electrical conductivity. RWs are thus a viable alternative to scarce conventional water resources in a future scenario of climate change.

Key words | gas exchange, *Myrtus communis* L., ornamental plant, unconventional water resources

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INTRODUCTION

Regions with a Mediterranean climate characterised by high temperatures and low rainfall suffer from a permanent scarcity of conventional water resources. In addition, a future scenario of climate change involving extreme environmental conditions like drought compels us to look for new unconventional water sources in order to preserve natural fresh water resources. These situations have led us to consider the use of RW as an alternative water resource (Grattan *et al.* 2015). The use of RW is one current promising solution because it can include several plant nutrients and thus potentially decrease the use of external mineral fertilisers. In addition, using RW helps reduce the amount of pollutants entering natural water courses, particularly when the treated water is used for landscaping (Gómez-Bellot *et al.* 2014; Acosta-Motos *et al.* 2016, 2017a). Nevertheless, RW may contain high salt concentrations due to the fact that municipal wastewater treatment plants (WWTPs) located near the Mediterranean coast tend to produce wastewater with a high electrical conductivity (EC) (Intriago *et al.* 2018).

Salt stress is a well-known type of abiotic stress that produces malfunctions in many physiological and metabolic processes, resulting in decreased plant growth and productivity (Acosta-Motos *et al.* 2017b). The presence of good nutrients in RWs (Ca^{2+}, K^{+}, Mg^{2+} Mn and S), however, can offset or reduce the incidence of damage caused by salt associated with phytotoxic ions (Na^{+}, Cl^{-} and B^{3+}). Although B^{3+} is an essential element for plant growth, it

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can be toxic for plants when its concentration in the soil solution exceeds a given threshold value (Bañón *et al.* 2012).

Most revegetation and xeriscaping projects use plant varieties that show different levels of resistance (tolerance and avoidance) to salinity. In such projects, it is important to select salt-resistant species, including ornamental shrubs like myrtle (*Myrtus communis* L.), which is a bushy evergreen sclerophyllous plant of significant ornamental interest that is often used in revegetation and landscaping projects in arid and degraded lands (Navarro *et al.* 2009). It is important to keep in mind, however, that the salinity tolerance of most plants depends on the amount of saline water applied for plant production, particularly in plants grown in small commercial containers (Álvarez & Sánchez-Blanco 2014). Plants require a leaching fraction to control the salinity in the root zone (Bañón *et al.* 2011).

In this study, we evaluated the long-term effect of RW treatments with high ECs on the mineral nutrition, water relations, gas exchange and morphological parameters in *Myrtus communis* L. plants grown in controlled environmental conditions. We aimed to demonstrate that the use of RW as an unconventional resource for the irrigation of myrtle plants is viable and safe.

MATERIALS AND METHODS

Myrtle plants (108) were grown in 14 × 12 cm pots (1.2 L) filled with a composite of coconut fibre, sphagnum peat and perlite (8:7:1) and improved with Osmocote plus (2 g L^{-1} substrate) (14:13:13 N, P, K + microelements). The experiment was conducted in a controlled growth chamber, in which the temperature was set to 23 °C/18 °C (light period/darkness, respectively). Relative humidity (RH) values oscillated between 55% and 70%. A mean photosynthetic active radiation (PAR) of 350 $\mu mol\ m^{-2}\ s^{-1}$ at canopy height was provided during the light period (07:00–23:00 h).

At the beginning of the experimental period, three water samples from each irrigation water source were collected in glass bottles, transported in an ice chest to the laboratory and stored at 5 °C in order to characterise the irrigation water quality. A chemical analysis for each irrigation water was performed (Table 1). The EC was measured with a multi-range Cryson-HI8734 EC meter (Cryson Instruments, S.A., Barcelona, Spain). The pH was calculated with a Cryson-507 pH-meter (Cryson Instruments, S.A., Barcelona, Spain). The concentrations of B^{3+}, Ca^{2+}, K^+, Mg^{2+}, Mn, Na^+, P and S ions were determined using an inductively coupled plasma optical emission spectrometer (ICP-OES, IRIS Intrepid II XDL, Thermo Fisher Scientific Inc., Loughborough, UK). Chloride (Cl^-) ions were analysed by ion chromatography (Metrohm, Herisau, Switzerland). To ensure no significant differences in water composition during the experiment, the same respective source waters used for initial measurements were saved in 20 litre containers held at a temperature of 5 °C until required.

Table 1 | Chemical analyses of the water used in the different treatments

	Irrigation water		
Parameter	**Control**	**RW1**	**RW2**
EC (dS m^{-1})	0.80	2	5
pH (−log [H^+])	7.78	8.00	8.07
Na^+ (mmol l^{-1})	2.06	7.48	25.66
Cl^- (mmol l^{-1})	1.90	6.60	24.83
B^{3+} (mmol l^{-1})	0.02	0.04	0.12
Ca^{2+} (mmol l^{-1})	1.74	3.50	5.88
K^+ (mmol l^{-1})	0.43	0.40	1.58
Mg^{2+} (mmol l^{-1})	1.42	2.78	5.80
S (mmol l^{-1})	2.70	5.56	13.92
Mn (mmol l^{-1})	0.001	0.004	0.018
P (mmol l^{-1})	<0.004	0.02	0.07

Data are values collected at the beginning of the experimental period.

Myrtle plants (108) were initially classified in three groups (36) and irrigated as control plants for 2 weeks. Six plants of each group were randomly selected and harvested at the beginning of the experiment, and the remaining 30 plants were subjected to the respective irrigation treatments. The control treatment consisted of tap water with an EC of up to 0.8 dS m^{-1}. The RWs came from two WWTPs located in the Province of Murcia (Spain): RW1 (EC 2.0 dS m^{-1}) came from Jumilla and RW2 (EC 5.0 dS m^{-1}) from Campotejar. All myrtle plants were watered three times a week to above container capacity. At the beginning of the assay, the maximum water field capacity (WFC) of the substrate was calculated for each individual pot. Each pot with its plant (30 plants per treatment) was weighed before each irrigation event, and the volume of irrigation water required to refill the pot to its threshold level (i.e., its WFC plus its pre-determined level of leaching, depending on the treatment) was calculated and added to each plant. Specifically, the volume of irrigation applied was determined in each treatment as the point at which the leaching fraction reached 10% (control), 25% (RW1) and 45% (RW2) in v/v of applied water.

At the beginning and at the end of the experimental periods, the substrate was gently washed from the roots of six plants per treatment. Each harvested plant was divided into shoots (leaves and stem) and roots. The leaves and roots were then oven-dried at 80 °C until they reached a constant weight to measure their respective dry weights (DW). Total leaf area (TLA) was determined using a leaf area meter (AM 200; ADC BioScientific Ltd, Hoddesdon, UK). We then determined the shoot/root ratio, the leaf weight ratio (LWR), the inverse of the specific leaf area ($1/SLA$) (which links the total leaf dry weight (TLDW) with TLA), and the inverse of the leaf area ratio ($1/LAR$) (which links the total dry weight (TDW) with the TLA). The plant material, which we previously oven-dried at 80 °C until it reached a constant weight, was ground using a commercial grinder to obtain dry vegetable powder and used for nutrient analysis. The grinder was blown out with compressed air between samples to prevent cross-contamination. The level of Cl^- ions was analysed using a chloride analyser (Model 926; Sherwood Scientific Ltd, Cambridge, UK) in an aqueous extract obtained by mixing 100 mg of dry vegetable powder with 40 mL of water followed by shaking for 30 minutes and filtering. The amounts of B^{3+}, Ca^{2+}, K^+, Mg^{2+}, Mn, Na^+, P and S ions were determined in a digestion extract of 100 mg of tissue powder with 50 mL of a mix of HNO_3:$HClO_4$ (2:1, v/v) using an inductively coupled plasma optical emission spectrometer (ICP-OES, IRIS Intrepid II XDL, Thermo Fisher Scientific Inc., Loughborough, UK).

The leaf water potential (Ψ_l) was determined in six plants per treatment during the central hours of illumination in the experiment. The Ψ_l was measured with a pressure chamber (Model 3000; Soil Moisture Equipment Co., Santa Barbara, CA, USA) in which each leaf was placed within 20 s of collection and pressurised at a rate of 0.02 MPa s^{-1}. The leaf stomatal conductance (g_s) and net photosynthesis rate (P_n) were determined in attached leaves in six plants per treatment during the central hours of illumination in the experimental period using a gas exchange system (LI-6400; LI-COR Inc., Lincoln, NE, USA). The intrinsic water-use efficiency (WUE_i) was calculated as the P_n/g_s ratio. To calculate the soil water potential (Ψ_r), we used the following equation:

$$\Psi_r = (\Psi_{RW} - \Psi_C) \times g_s RW/g_s C$$

which assumes that $\Psi_r = 0$ for control plants. Ψ_C and Ψ_{RW} correspond to the mean value of Ψ_l in the control and RW treatments, respectively. The g_s C and g_s RW correspond to the mean value of g_s in the respective treatments.

All statistical analyses were performed using Statistical Package for the Social Sciences (IBM SPSS Statistics). Differences in the concentrations of nutrients between tissues (shoots and roots) were determined by means of a *t*-Student. The difference between treatments was analysed by one-way analysis of variance (ANOVA). Treatment means were separated using Tukey's Multiple Range Test ($P < 0.05$).

RESULTS AND DISCUSSION

Salts from RW2 induced a significant increase in Na^+, Cl^-, Ca^{2+}, B^{3+}, Mg^{2+}, S and Mn concentrations in shoots and a significant decrease in K^+ and P with respect to the control. In the case of the RW1 treatment, the B^{3+}, Mg^{2+}, S and Mn concentrations increased in the shoots. Furthermore, in the root systems of RW-treated plants, similar behaviour to that observed in shoots was found for all mineral concentrations with the exception of Na^+ and S in RW2 and P and S in RW1 (Table 2). Phytotoxic ions (Na^+, Cl^- and B^{3+}) were hindered from reaching the shoots in myrtle plants, restricting the build-up of toxic concentrations in leaves. Despite this avoidance mechanism, Cl^- reached the shoots in greater concentrations than the other phytotoxic ions (Table 2).

In general, an increase in external NaCl concentrations induces an increase in Na^+ and Cl^- in the roots and leaves of different ornamental plants. In a saline environment, the control of Na^+ and/or Cl^- concentrations in the aerial parts of plants is an important avoidance mechanism that allows

Table 2 | Concentrations of Na^+, Cl^-, B^{3+}, Ca^{2+}, K^+, Mg^{2+}, S, Mn and P in different tissues of *M. communis* plants at the end of the experimental period

Nutrients	Shoot (mmol kg^{-1} DW)	Root (mmol kg^{-1} DW)	*P*-Value
Na			
Control	200.91 B/b	358.99 AB/a	<0.001***
RW1	239.12 AB/a	288.37 B/a	>0.05 ns
RW2	453.73 A/a	458.26 A/a	>0.05 ns
	<0.05*	<0.05*	
Cl			
Control	343.66 B/a	200.94 B/b	<0.05*
RW1	405.63 B/a	255.40 B/b	<0.01**
RW2	668.54 A/a	398.12 A/b	<0.05*
	<0.01**	<0.001***	
B			
Control	36.15 B/a	15.68 B/b	<0.001***
RW1	74.16 A/a	33.96 A/b	<0.001***
RW2	77.84 A/a	37.83 A/b	<0.001***
	<0.001***	<0.001***	
Ca			
Control	321.05 B/a	158.54 B/b	<0.001***
RW1	351.14 B/a	147.30 AB/b	<0.001***
RW2	411.79 A/a	186.39 A/b	<0.001***
	<0.01**	<0.05*	
K			
Control	775.36 A/a	246.15 A/b	<0.001***
RW1	726.62 AB/b	208.34 AB/c	<0.001***
RW2	664.68 B/b	151.06 B/c	<0.001***
	<0.05*	<0.05*	
Mg			
Control	198.68 B/a	76.70 C/b	<0.001***
RW1	271.65 A/a	91.72 B/b	<0.001***
RW2	289.13 A/a	107.19 A/b	<0.001***
	<0.05*	<0.01**	
S			
Control	163.31 B/a	86.90 A/b	<0.001***
RW1	221.59 A/a	92.78 A/b	<0.001***
RW2	224.01 A/a	92.59 A/b	<0.001***
	<0.01**	>0.05 ns	
Mn			
Control	2.28 B/a	1.00 B/b	<0.001***
RW1	2.76 A/a	1.18 A/b	<0.001***
RW2	2.86 A/a	1.22 A/b	<0.01**
	<0.05*	<0.05*	
P			
Control	152.58 A/a	75.41 A/b	<0.001***
RW1	152.49 A/a	61.75 AB/b	<0.01**
RW2	84.82 B/a	52.16 B/b	<0.05*
	<0.05*	<0.05*	

Data are the means of six calculations ± standard error (SD). The differences between organs are shown horizontally (lowercase letters), while the differences between treatments are displayed vertically (capital letters).

Different letters per row or per column denote significant differences according to Tukey's Multiple Range Test. $*P < 0.05$, $**P < 0.01$ and $***P < 0.05$. $P > 0.05$ non-significant differences are indicated by 'ns'.

plants to survive and grow under salt stress conditions; the retention of these ions in the roots and lower stems restricts entry through the roots and limits transport to the shoots (Álvarez *et al.* 2018). According to Cassaniti *et al.* (2009), the composition of a salt solution can induce toxicity in plants due not only to the ions present (Na^+ and Cl^-), but also to a nutritional deficiency resulting from increased competition among cations and anions (Shannon & Grieve 1998). In our study, only K^+ and P decreased in all the tissues of myrtle plants subjected to RW2 treatment. The plants irrigated with RW had higher B^{3+} concentrations both in the aerial parts and in the roots, but Ca^{2+}, Mg^{2+}, Mn^{2+} and S could mitigate the negative effects (Bañón *et al.* 2012).

The greater solute concentrations in RW-treated plants produced a decrease in Ψ_r (osmotic stress), reaching values of −0.82 MPa in the RW2 treatment. This hindered the mobility of water to the leaves and caused a drop in Ψ_l (especially in the RW2 treatment), reaching values of −1.20 MPa (Figure 1(a) and 1(b)).

To avoid leaf water loss, a decrease in g_s took place, which supposes a decrease in P_n and therefore in CO_2 fixation (especially in the RW2 treatment) (Figure 2(a) and 2(b)). The WUE_i, expressed as the P_n/g_s ratio, was similar in all the treatments. The plants treated with RWs are therefore equally as efficient as those in the control treatment (Figure 2(c))

The decrease in g_s observed in our study suggests an adaptive and efficient control of transpiration by this species, limiting water loss (Hessini *et al.* 2008) or reducing the salt load of the leaves (Álvarez & Sánchez-Blanco 2015).

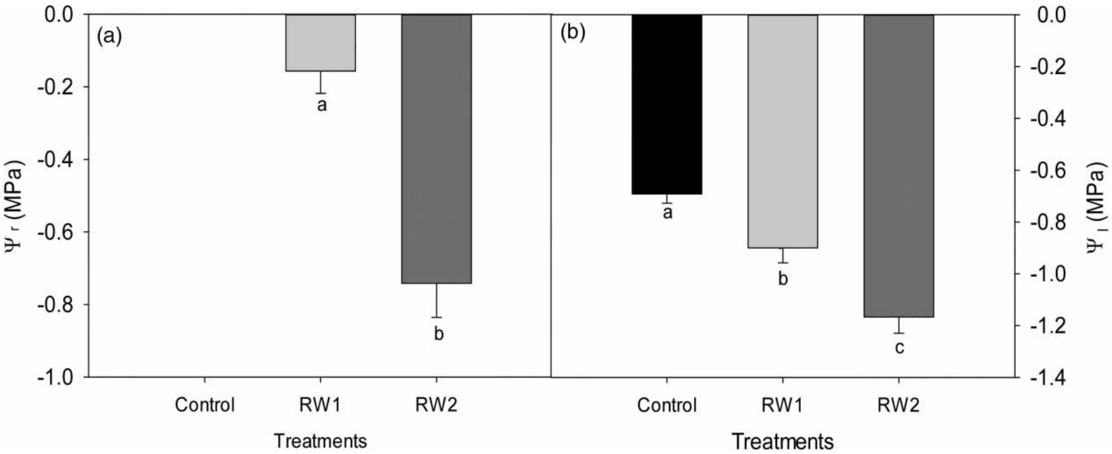

Figure 1 | Water relations parameters. The influence of different irrigation treatments on the soil water potential at the root surface (Ψ_r; a) and the leaf water potential (Ψ_l; b) of *M. communis* plants at the end of the experimental period. Data are the means of six calculations ± standard error (SD). Means within a parameter without a common letter are significantly different according to Tukey's Multiple Range Test.

As a result, P_n is inevitably reduced due to decreased CO_2 availability at the chloroplast level (Chaves *et al.* 2009), as seen in many other ornamental species subjected to water deficit and saline conditions (Navarro *et al.* 2009). The close association between P_n and g_s in salt-stressed plants suggests that, under these conditions, a decrease in P_n is largely a consequence of stomatal limitation (Flexas *et al.* 2004).

Therefore, g_s is strongly correlated with the absorption of water and its transport to the shoots. In general, plants show a tendency to reduce g_s levels in response to salt stress (Álvarez & Sánchez-Blanco 2014). The changes in g_s associated with salinity may be a consequence of reduced root hydraulic conductivity and a decrease in Ψ_l. However, P_n activity can remain high in spite of stomatal closure, leading to an increase in the $WUE_i = P_n/g_s$ ratio in response to salt stress (De Pascale *et al.* 2011).

In relation to morphological changes, we found that the RW1 and control plants had better growth in the aerial parts and showed the highest values in the number of leaves, TLA, TLDW; we observed a similar tendency (albeit not significant) in the shoot/root ratio (Table 3; Figure S1, available with the online version of this paper). On the other hand, the RW2 plants had worse growth in the aerial parts, compensated for by better development in the roots, leading to the highest values in *LWR*, and the lowest values of 1/*LAR* (Table 3, Figure S1). These morphological changes when RW was used did not affect the ornamental quality of the myrtle plants. Furthermore, RW treatments did not affect survival given that no plants died at the end of the experiment.

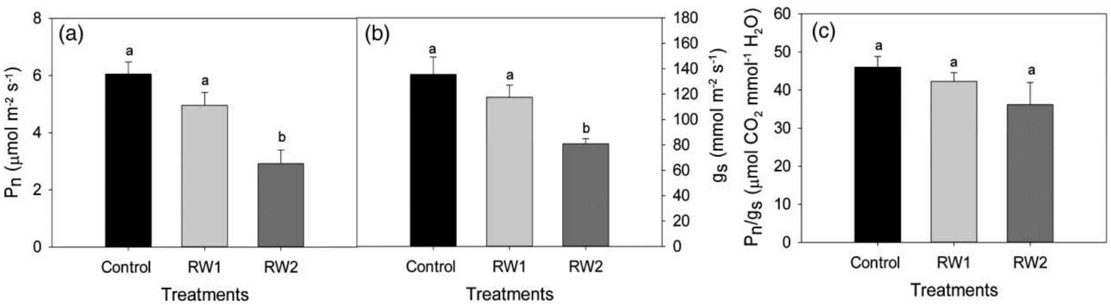

Figure 2 | Gas exchange parameters. The influence of different irrigation treatments on the net photosynthetic rate (P_n; a), leaf stomatal conductance (g_s; b) and intrinsic water-use efficiency (P_n/g_s; c) of *M. communis* plants at the end of the experimental period. Data are the means of six calculations ± standard error (SD). Means within a parameter without a common letter are significantly different according to Tukey's Multiple Range Test.

Table 3 | The influence of three irrigation treatments on the morphological parameters of *M. communis* plants at the end of the experimental period

	Treatments			
Morphological parameters	**Control**	**RW1**	**RW2**	***P*-Value**
Number of leaves	154.41 ± 10.40 a	165.98 ± 12.04 a	117.01 ± 3.78 b	0.006**
TLA	282.13 ± 22.33 a	285.30 ± 12.73 a	214.77 ± 22.37 b	0.038*
TLDW	2.21 ± 0.04 a	2.01 ± 0.06 b	1.98 ± 0.08 a	0.031*
TDW	11.41 ± 0.08 c	12.38 ± 1.42 b	13.38 ± 1.15 a	<0.001***
LWR (1/SLA)	60.79 ± 7.47 b	74.36 ± 3.24 ab	83.84 ± 3.85 a	0.022*
1/LAR	443.32 ± 37.72 ab	397.58 ± 33.83 b	524.12 ± 44.41 a	0.003**
Shoot/root ratio	1.04 ± 0.06 a	1.10 ± 0.11 a	0.89 ± 0.04 a	0.20 ns

Total leaf area (TLA) is given in (cm^2). Total leaf dry weight (TLDW) and total dry weight (TDW) are in given in (g $plant^{-1}$). Data are means of six calculations ± standard error (SD). Different letters per row or per column denote significant differences according to Tukey's Multiple Range Test. *P <0.05, **P <0.01 and ***P < 0.05. P > 0.05 non-significant differences are indicated by 'ns'.

The nutritional components of RW produce a beneficial effect on plant production and minimise the negative effects of the toxic ions present in this type of water, as has been observed in several ornamental species (Miralles *et al.* 2011; Valdés *et al.* 2012; Cassaniti *et al.* 2013; Gómez-Bellot *et al.* 2013a, 2013b, 2014). Furthermore, higher concentrations of nutrients like Ca^{2+}, Mg^{2+}, Mn and S promote improved growth and ornamental quality in these plants (Lubello *et al.* 2004). The reduction in TLA and the decrease in the shoot/root ratio can be viewed as adaptive mechanisms to salt stress. The decrease in TLA produces an indirect benefit, because plants can thus limit water loss by transpiration, which in turn can favour the retention of toxic ions in roots, limiting the accumulation of these ions in the aerial part of the plant (Munns & Tester 2008), as occurred in the RW2 treatments.

Nevertheless, a combination of stresses linked to experiments under field conditions can have a deleterious effect on plant productivity. Prolonged exposure of the plants to abiotic stresses such as extreme temperature, light stress or salinity favours greater weakness in the plants and therefore increased susceptibility to opportunistic biotic stresses. For this reason, under field conditions, myrtle plants could respond differently to salinity with respect to the behaviour observed under controlled conditions.

CONCLUSIONS

Myrtle plants irrigated with RW with high salinity levels (2.0–5.0 dS m^{-1}) avoided the accumulation of phytotoxic ions in the shoots, restricting the build-up of toxic concentrations in the leaves. In spite of this defence mechanism, we did observe a reduction in plant growth due to nutritional imbalance (increases in Na^+ and Cl^- ions, accompanied by a major decrease in P and K^+). Furthermore, the salts from the RW hindered the mobility of water to the leaves, reducing gas exchange. Nevertheless, these changes did not affect the survival of the myrtle plants. It is thus feasible to use reclaimed waters for myrtle plant irrigation despite their high EC, offering a viable alternative to scarce conventional water resources in a future scenario of climate change.

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