Agricultural Water Management 199 (2018) 11–21

Contents lists available at ScienceDirect

Agricultural Water Management

journal homepage: www.elsevier.com/locate/agwat

Using saline soil and marginal quality water to produce alfalfa in arid climates

F.J. Díaz[a,*], S.R. Grattan[b], J.A. Reyes[c], B. de la Roza-Delgado[d], S.E. Benes[e], C. Jiménez[a], M. Dorta[a], M. Tejedor[a]

[a] *Department of Animal Biology, Soil Science and Geology, University of La Laguna, La Laguna 38206, Canary Islands, Spain*
[b] *Department of Land, Air and Water Resources, University of California, Davis, CA 95616, USA*
[c] *Unit of Applied Botany, Canarian Institute for Agricultural Research, Puerto de la Cruz 38400, Canary Islands, Spain*
[d] *Department of Nutrition, Grasslands and Forages, Regional Institute for Research and Agro-Food Development, Villaviciosa 33300, Asturias, Spain*
[e] *Department of Plant Science, California State University, Fresno, CA 93740, USA*

ARTICLE INFO

Article history:
Received 12 July 2017
Received in revised form
10 November 2017
Accepted 2 December 2017
Available online 13 December 2017

Keywords:
Medicago sativa
Biosaline agriculture
Saline groundwater
Salinity tolerance
Forage quality

ABSTRACT

The gradual increase in the amount of land and water resources affected by salt in arid and semi-arid regions requires strategies to optimize the use of these marginal-quality resources. Recent field and greenhouse experiments have demonstrated the potential of growing certain 'pre-selected' varieties of alfalfa in highly saline conditions. A greenhouse study was conducted to determine the impact of irrigation with saline groundwater on alfalfa growth and production in saline-sodic soils. The sustainability of the system in terms of forage yield and quality was also evaluated. The study included three varieties of alfalfa (*Medicago sativa*, vars. SW8421S, PGI908S and WL656HQ) planted in pots filled with saline-sodic soil (Calcic Haplosalids) collected on the island of Lanzarote (Spain) and irrigated for 18 months with increasingly saline water. Although the yield of the alfalfa varieties was reduced by an average of 7, 20, 31 and 46% as the salinity of the irrigation water increased from 0.4 dS m^{-1} to 2.5, 5.0, 7.5 and 10.0 dS m^{-1}, respectively, their relative salt tolerance, based on the average electrical conductivity of the saturated soil extract (EC_e), was much higher than those established in the literature. Based on their nutritional quality, all alfalfa varieties are categorized as 'supreme' quality, with metabolizable energy (ME) values in excess of 10 MJ kg^{-1}. Moreover, no detriment to quality was observed at the higher levels of irrigation water salinity. Mineral composition analysis revealed S, K and B levels near or above the established maximum tolerable levels (MTLs) suggesting that this forage could only be safely consumed by ruminants over the long term if combined with other forages with lower mineral content.

1. Introduction

The gradual reduction in the quantity and quality of conventional water resources for agricultural use in arid and semi-arid regions, representing 40% of the world's 270 million irrigated hectares (Smedema and Shiati, 2002), has necessitated the supplementation of new water resources obtained from the desalination of saline groundwater and seawater (Díaz et al., 2013a,b; Martínez-Alvarez et al.,2017). In both cases, salt removal by reversible electrodialysis or reverse osmosis entails high energy costs with a correspondingly high CO_2 footprint that prevents the extensive use of this water for irrigation. Irrigation with such water is economically feasible only for high value agricultural crops (Beltran and Koo-Oshima, 2006). Moreover, even if desalinized water of this type is considered high quality water by farmers, initial experiences with desalinated water have not proven totally positive (Yermiyahu et al., 2007; Ben-Gal et al., 2009). In addition, desalination processes generate considerable amounts of brine which can negatively impact the quality of surface and groundwater sources and marine biological communities that develop naturally under saline conditions (Riera et al., 2012).

Along with desalination processes for providing additional water resources, alternative agricultural practices that rely on the use of marginal soil and water resources affected by salinity are needed (Rozema and Flowers, 2008). Biosaline agriculture (i.e. economically sustainable crop production using irrigation water and soils with a wide range of salinity levels) has gained popularity in recent years in arid and semiarid regions in various parts of the

---

[*] Corresponding author.
E-mail address: fjdiazpe@ull.edu.es (F.J. Díaz).

https://doi.org/10.1016/j.agwat.2017.12.003

Table 1
Average ionic composition of desalinated seawater and simulated groundwater treatments used for irrigation during the experiment; mean ± standard deviation; n = 35.

Treatment (EC target) dS m^{-1}	pH	EC dS m^{-1}	SAR (mmol L^{-1})$^{0.5}$	Ca^{2+} mmol$_c$ L^{-1}	Mg^{2+}	Na^+	K^+	CO_3^{2-}	HCO_3^-	SO_4^{2-}	Cl^-	NO_3^-	B mg L^{-1}
0.4	7.1 ± 0.6	0.4 ± 0.1	5.3 ± 0.8	0.4 ± 0.1	0.3 ± 0.2	3.0 ± 0.4	0.1 ± 0.0	0.1 ± 0.2	0.7 ± 0.5	0.2 ± 0.2	2.7 ± 0.3	0.0 ± 0.0	2.2 ± 0.2
2.5	8.2 ± 0.2	2.9 ± 0.2	16.9 ± 1.7	0.8 ± 0.2	2.0 ± 0.5	24.6 ± 1.5	0.3 ± 0.0	0.6 ± 0.6	1.6 ± 0.4	5.0 ± 0.8	18.6 ± 1.6	0.2 ± 0.0	2.2 ± 0.2
5.0	8.4 ± 0.3	5.3 ± 0.3	23.8 ± 1.7	1.3 ± 0.7	3.7 ± 0.4	47.4 ± 3.4	0.6 ± 0.1	1.0 ± 0.6	2.6 ± 0.6	9.9 ± 1.0	35.8 ± 4.2	0.5 ± 0.2	2.2 ± 0.1
7.5	8.4 ± 0.1	7.8 ± 0.2	29.2 ± 1.0	1.6 ± 0.7	5.8 ± 0.7	72.6 ± 2.3	0.9 ± 0.1	1.1 ± 0.5	4.2 ± 0.7	16.0 ± 1.8	56.1 ± 3.2	0.7 ± 0.2	2.2 ± 0.1
10.0	8.4 ± 0.1	9.9 ± 0.3	33.6 ± 0.7	2.0 ± 0.7	7.5 ± 0.3	95.1 ± 3.2	1.1 ± 0.1	1.2 ± 0.5	5.3 ± 0.7	21.5 ± 2.2	73.7 ± 3.9	0.9 ± 0.2	2.2 ± 0.1

world, due primarily to the limited supply of good-quality irrigation water (Masters et al., 2007). This type of farming, based on plants capable of growing in saline conditions, can lead to an economically viable market for salt-tolerant crops while also expanding crop production into marginal lands, thus alleviating pressure on conventional water resources (Díaz and Grattan, 2009). Potential benefits range from the production of food for human consumption, renewable energy (bioethanol and biodiesel) and materials for industrial use (fibre and oil), to a series of applications in revegetation, soil rehabilitation and carbon fixation projects (Grattan et al., 2008). One of the main potential benefits of biosaline agriculture is the production of forage for livestock (Masters et al., 2007).

The production of forage in biosaline agriculture, however, is not problem free. The most important problems include 1) the salinisation of the soils, 2) imbalances in the mineral composition of the forage and 3) the accumulation of trace elements in forage tissue to levels that can be toxic for livestock (Díaz and Grattan, 2009; Grattan et al., 2004a). The first of these problems requires control and application of management techniques, particularly adequate leaching, to prevent the continued accumulation of salt in the soil. In order to address nutritional imbalances and toxicity, it is important to control the mineral composition of the forages, choosing those that are not only tolerant to salinity but which also minimise the accumulation of toxic elements and offer a more balanced composition. Moreover, most potential nutritional disorders caused by biosaline agriculture forages can be avoided by using the latter only as a supplement to the overall animal diet, never as the sole forage (Robinson et al., 2004).

The present study evaluates the use of saline soils and saline groundwater to grow different varieties of alfalfa (*Medicago sativa* L.), which is one of the most important forages in arid and semiarid regions across the world (Djilianov et al., 2003). Alfalfa has historically been classified as moderately sensitive to saline conditions, with significant yield declines as the electrical conductivity of the saturated soil paste extract (EC_e) exceeds 2 dS m^{-1} (Ayers and Westcott, 1985). However, a series of recent studies have shown that some 'pre-selected' alfalfa varieties can thrive in much higher salt concentrations, both in soil and in irrigation water, without significant negative effects on productivity (Putnam et al., 2017). Given the potential interest of these results for arid and semiarid regions, it is important to confirm these findings in a broader range of soils and environmental conditions. In addition, although alfalfa is one of the most important forage crops regarding its high protein content, digestibility, palatability and milk production qualities, very few studies provide information concerning the effects of salinity on the nutritional quality of the crop (Ferreira et al., 2015).

The present greenhouse study used saline-sodic soils and saline groundwater, both of which are common in arid and semi-arid parts of the Canary Islands, to grow varieties of alfalfa marketed as tolerant to salinity. The primary objective was to determine the impact of different saline conditions on biomass production, nutritional quality and mineral composition of the selected varieties, and to evaluate the evolution of the soil salinity under the study conditions.

2. Material and methods

2.1. Experimental design

A two-way factorial experiment with four replications per treatment (3 alfalfa varieties * 5 irrigation water qualities * 1 soil type * 4 replications; n = 60) was conducted using mesocosms (soils placed in containers 40 cm in diameter and 50 cm in height, with an apparent soil bulk-density of approximately 1.2 g cm^{-3}). Pots were arranged using a completely randomized design. The experiment was conducted in a greenhouse belonging to the Canarian Institute for Agricultural Research on the island of Tenerife (Spain), where the air temperature during the study period (November 2014–April 2016) ranged from 9 to 47 °C (average ~21.5 °C) and relative humidity from 17 to 94% (average ~68.4%).

Five water quality treatments with salinity levels (EC_{iw}) of ~ 0.4, 2.5, 5.0, 7.5 and 10.0 dS m^{-1} were applied. The water with salinity of 0.4 dS m^{-1} – corresponding to seawater desalinated using reverse osmosis – was used as the control treatment. The other treatments simulated the quality of sodic chloride-dominated groundwater typically found in coastal wells on the islands of Lanzarote and Fuerteventura (Spain), with the EC_{iw} adjusted to between 2.5 and 10 dS m^{-1}, the salinity range frequently found in coastal groundwaters in the Canary Islands. The boron concentration was set at 2.5 mg L^{-1} for all treatments, representing the maximum concentration found in the groundwaters of these areas. The simulation of the irrigation waters was conducted using desalinated seawater, with NaCl, $MgSO_4$, $CaSO_4$, Na_2SO_4, KNO_3, $NaHCO_3$, and H_3BO_3 added in proper proportions to reflect the composition of the reference groundwater.

The different irrigation waters were analysed fortnightly to ensure salt concentration targets were met. The following parameters were tested: pH, EC_{iw}, cations (Ca^{2+}, Mg^{2+}, K^+, Na^+), anions ($CO_3{}^{2-}$, $HCO_3{}^-$, $SO_4{}^{2-}$, Cl^-, $NO_3{}^-$) and boron (B). The waters were analysed according to official methods (APHA, 1998). Water was applied using an automatic drip irrigation system, with four pressure-compensating emitters per container, each with a flow of 2 L h^{-1}. Water distribution uniformity was evaluated periodically using the low-quarter distribution uniformity method (DUlq; Burt et al., 1997), and values were consistently >0.98. Monitoring of volumetric water content with EC-5 sensors (Decagon Devices) allowed calculation of the required irrigation dosages to maintain the soils close to pot capacity (comparable to 'field capacity') throughout the whole study period. Table 1 shows the average chemical composition of the waters used for irrigation during the experiment. As can be seen, the salinity obtained with the different treatments (EC) differed slightly from the target concentrations (EC target). The pH of the waters obtained was slightly alkaline, varying between 7.8 and 8.7, as is common in the sodic chloride-dominated waters of coastal parts of the Canary Islands.

The soil used was a Calcic Haplosalids from the island of Lanzarote (Soil Survey Staff, 2006). Physicochemical characterisation of the soil was performed prior to application of fertilisers and irrigation in accordance with standard methods (Soil Survey Staff, 1996). The following parameters were analysed: texture, moisture

Table 2
Soil chemical properties for the initial soil (prior to leaching and sowing the alfalfa) and at the end of the experiment; mean ± standard deviation; n = 5-12; means with the same letter are not significantly different according to a one-way ANOVA and post hoc Tukey test ($p < 0.05$).

Parameter	Initial Soil	0.4	2.5	5.0	7.5	10.0
		Treatments (EC_{iw} target, $dS\,m^{-1}$)				
EC_e $dS\,m^{-1}$	54.5 ± 4.6 d	2.1 ± 1.1 a	12.6 ± 2.1 b	16.8 ± 1.8 c	19.1 ± 1.5 c	19.3 ± 2.0 c
pH_e	7.4 ± 0.1 a	8.4 ± 0.2 c	8.0 ± 0.1 b	8.0 ± 0.1 b	8.2 ± 0.2 b	8.1 ± 0.1 b
Exch. Ca $mmol_c\,kg^{-1}$	12.2 ± 1.3 a	18.7 ± 1.6 c	14.6 ± 1.3 b	14.6 ± 1.3 b	13.2 ± 0.9 ab	12.4 ± 1.4 a
Exch. Mg $mmol_c\,kg^{-1}$	4.0 ± 0.3 a	7.1 ± 0.4 c	4.9 ± 0.5 b	4.9 ± 0.3 b	5.1 ± 0.4 b	5.1 ± 0.4 b
Exch. K $mmol_c\,kg^{-1}$	3.7 ± 0.2 c	2.3 ± 0.4 a	2.7 ± 0.5 ab	2.9 ± 0.3 b	2.9 ± 0.2 b	2.9 ± 0.3 b
Exch. Na $mmol_c\,kg^{-1}$	9.0 ± 2.7 b	3.5 ± 1.7 a	11.2 ± 1.2 bc	12.0 ± 1.7 c	12.4 ± 2.2 c	12.3 ± 1.7 c
ESP_c %	39.8 ± 0.9 b	11.2 ± 2.6 a	38.5 ± 2.3 b	46.2 ± 3.3 c	48.5 ± 0.9 c	48.6 ± 2.0 c
$CaCO_3$ $g\,kg^{-1}$	169 ± 14 a	235 ± 36 b	245 ± 38 b	200 ± 41 ab	239 ± 69 b	232 ± 34 b
Organic C $g\,kg^{-1}$	6.8 ± 0.7 a	8.7 ± 0.9 b	8.8 ± 0.7 b	7.9 ± 0.8 ab	7.9 ± 1.4 ab	7.8 ± 1.0 ab
TN $g\,kg^{-1}$	0.8 ± 0.1 a	1.0 ± 0.1 b	1.0 ± 0.1 b	1.0 ± 0.1 b	0.9 ± 0.1 ab	0.9 ± 0.1 a
Olsen-P $mg\,kg^{-1}$	12.5 ± 2.8 a	73.7 ± 13.7 b	74.4 ± 7.3 b	79.9 ± 9.6 b	84.2 ± 12.9 b	82.7 ± 10.0 b
Fe $mg\,kg^{-1}$	11.4 ± 1.1 a	13.4 ± 1.6 ab	12.6 ± 1.8 ab	12.5 ± 1.2 a	13.7 ± 1.7 ab	15.1 ± 3.1 c
Mn $mg\,kg^{-1}$	11.4 ± 1.0 a	18.1 ± 3.4 abc	16.8 ± 4.5 ab	14.8 ± 4.0 ab	23.4 ± 8.7 bc	28.2 ± 14.4 c
Cu $mg\,kg^{-1}$	0.6 ± 0.1 a	0.7 ± 0.1 a	0.6 ± 0.1 a	0.6 ± 0.1 a	0.7 ± 0.2 a	0.7 ± 0.1 a
Zn $mg\,kg^{-1}$	0.6 ± 0.0 a	1.6 ± 0.4 b	1.4 ± 0.3 b	1.2 ± 0.3 b	1.4 ± 0.2 b	1.5 ± 0.2 b
HWSB $mg\,kg^{-1}$	7.3 ± 3.9 a	14.3 ± 4.0 c	12.8 ± 5.1 bc	11.3 ± 3.5 abc	8.6 ± 4.0 ab	6.4 ± 0.9 a
B_e $mg\,L^{-1}$	3.3 ± 1.8 b	6.8 ± 1.4 c	5.8 ± 0.6 c	4.0 ± 0.5 b	2.9 ± 0.3 ab	2.1 ± 0.2 a

content at field capacity and wilting point, electrical conductivity in saturated soil-paste extract (EC_e), pH in saturated soil-paste extract (pH_e), exchangeable cations (Ca^{2+}, Mg^{2+}, K^+, Na^+), calculated exchangeable sodium percentage (ESPc), carbonate content ($CaCO_3$), organic carbon (Organic C), total nitrogen (TN), available phosphorous (Olsen-P), micronutrients (Fe, Mn, Cu and Zn), hot water soluble boron (HWSB), and boron in saturated extract (B_e). Table 2 provides the results of the chemical parameters that were determined. The soil was clay loam in texture (380 ± 10 $g\,kg^{-1}$ clay, 388 ± 8 $g\,kg^{-1}$ loam, 232 ± 10 $g\,kg^{-1}$ sand), with gravimetric moisture content at pot capacity and wilting point of 27.3 and 15.7%, respectively, and high carbonation. It was extremely saline ($EC_e \sim 54 \pm 5\,dS\,m^{-1}$) and sodic ($ESP_c \sim 40 \pm 1\%$). The soil solution was dominated by Cl^- and Na^+ ions, reflecting the natural marine origins of the salt (i.e. seaspray). The values for B (both B_e and HWSB) indicate toxic levels for moderately sensitive species (Grattan et al., 2015). The B adsorption isotherm shows that retention of this element was moderate (data not shown). The soil had very low organic matter and nitrogen content, as is common of soils in arid regions. The low nutrient content necessitated the use of basal fertilisation (300 g of fertiliser NPK 21:10:10, 30 g gypsum and 500 g goat manure per container) to prevent masking the salinity effects with any nutritional deficiencies. The chemical parameters analysed at the beginning of the experiment were re-analysed at its conclusion to evaluate the impact of the application of the irrigation treatments. The bottom of each pot contained a 3-cm gravel layer to facilitate drainage.

Three non-dormant cultivars of alfalfa (*Medicago sativa*) were selected (varieties WL656HQ, SW8421S, PGI908S) based on their reported salt tolerance during germination and/or forage production. PGI908S has been classified as salt tolerant during germination and forage production, SW8421S as salt-tolerant only during forage production, and WL656HQ as tolerant only during germination (NAFA, 2016). In order to reduce the salinity of this highly saline soil near the surface of the pot, 2 Liters of desalinated seawater were added to each pot daily for 10 days prior to sowing. This provided a low-saline environment for the germinating seeds. After this minor reclamation of the surface soil, a total of 60 seeds were planted per container. To foster germination and crop establishment, only irrigation with desalinated seawater was applied to all pots in the first stage of the experiment. Following this initial period (60 days post seeding), the number of plants were thinned to approximately 28 plants per pot and the application of the treatments, which lasted for 430 days, commenced.

2.2. *Analysis of plant material*

A total of 15 harvests, also referred to as cuttings, were performed on forages throughout the treatment period to determine the total production of dry matter (DM). Harvest times were established when the control treatment plants reached approximately 10% flowering. The cutting height was established at 5–6 cm above the soil surface. The collected plant material was weighed, oven dried at 60 °C for 72 h, and weighed again to determine dry matter production expressed in grams per square meter. The cumulative biomass production of each of the treatments was calculated as the sum of the material obtained from the different cuts. The relative yield was expressed as the relationship between the biomass produced under the different treatments to that obtained under the control treatment (lowest salinity). These relative yields were used as an indicator of salinity tolerance (Díaz and Grattan, 2009).

Once dry, the plant material collected from each cut was finely ground to analyse the mineral composition and nutritional quality. From this, three combined samples were obtained by combining the plant material from every 5 consecutive cuts. Thus, the first sample was comprised of plant material from cuts 1–5, corresponding to the first 124 days from the commencement of the treatment application; the second sample was comprised of combined cuts 6–10, corresponding to days 124–269 from commencement; and the third sample consisted of combined cuts 11–15, corresponding to days 269–430.

The nutritional potential of the forages was evaluated on the basis of ash content, neutral detergent fibre (NDF), acid detergent fibre (ADF), crude protein (CP), enzymatic organic matter digestibility (EOMD), metabolizable energy (ME), and relative food value (RFV). A total of 180 tissue samples were analysed by near infrared spectroscopy (NIRS) using a FOSS NIRSystem 6500, scanning monochromator (Foss NIRSystem, Silver Spring, MD, USA) with a scanning range of 400–2500 nm at 2 nm intervals. The spectral data were recorded in reflectance mode (log 1/Reflectance) with the WINISI 2 software v.1.05 (Infrasoft International Inc., Port Matilda, PA, USA). One third of the samples randomly selected were also analysed by reference methods in duplicate and performed using traditional analytical methodologies: ash content after incineration to 550 °C and CP by Kjeldahl analysis (AOAC, 2000), NDF and ADF by Van Soest fractioning analysis (Van Soest et al., 1991), and EOMD following Roza and Argamentería (1992). These samples were used to establish equations for NIRS prediction models with the WINISI software using partial least squares

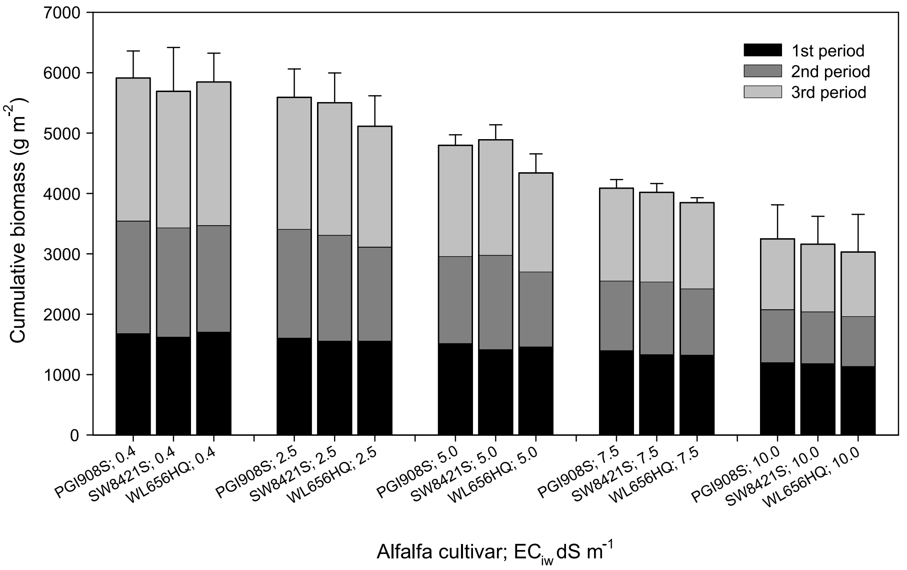

Fig. 1. Cumulative biomass production of three alfalfa cultivars under irrigation with different simulated groundwater quality; 1st period ~ 1–124 days after initial treatment application; 2nd period ~ 125–269 days after initial treatment application; 3rd period ~ 270–430 days after initial treatment application; bars represent mean and standard error; n = 4.

as regression tool. Selected equations were tested on an independent data base of ten samples, and were used to predict all reference values (ash content, NDF, ADF, CP and EOMD) for the rest of population. RFV was calculated from ADF and NDF as follow: RFV = [88.9-(0.779*ADF)]*[(120/NDF)/1.29] (Putnam et al., 2008). ME was calculated from ash content and in vivo organic matter digestibility (IVOMD) as follow: ME = K*(100-ash)*(IVOMD/100), where K = 0.16 for forages and IVOMD was predicted from EOMD values (ARC, 1984; Roza and Argamentería, 1992). The reference chemical analysis was performed in an accredited laboratory under the requirements of ISO/IEC (2005).

For the study of the mineral composition, the elements analysed using standard methods (USEPA, 1994) were P, K, Ca, Mg, S, Na, Cl, Fe, Mn, Cu, Zn and B. The concentrations of the different elements were compared with the established maximum tolerable levels (MTLs), defined as the maximum level of a particular element, present in the animal diet applied for a given period of time, which does not lead to a deterioration in the health or reduced yield in the animals (NRC, 2005).

2.3. Statistical analysis

Statistical methods were implemented using IBM SPSS Statistics V21.0. The level of significance for all tests was set to $P \leq 0.05$. Assumptions of normality (Kolmogorov Smirnov test) and homogeneity of variance (Levene test) were met for each analysis. Differences between initial soils and sampling after 430 days of irrigation under different treatments were assessed using an ANOVA and post-hoc Tukey's test. A univariate analysis of variance was used to determine the effect of time, water quality and alfalfa variety on biomass production, shoot mineral composition and forage quality.

3. Results

3.1. Impact of irrigation on soil chemical properties

The salinity, exchangeable cation content, nutrients and trace elements in the soils after 430 days of application of the different treatments are shown in Table 2. Soil EC_e decreased significantly compared to the initial soil, regardless of the treatment applied. Based on the stationary model described by Ayers and Westcot (1985) for the relationships between EC_{iw} (irrigation water salinity) and EC_e, our data indicate that the leaching fraction obtained was approximately 4% for the control treatment (0.4 dS m^{-1}), gradually increasing to 12% for the most saline treatments (10.0 dS m^{-1}). Levels of exchangeable Na and ESP_c increased slightly compared to the initial soil in the saline treatments above 2.5 dS m^{-1}. In general, there was a significant reduction in the levels of exchangeable K^+ compared to the initial soil and an increase in the Ca^{2+} and Mg^{2+} cations. A significant increase was also found in carbonate content for all treatments compared to the initial soil. Accumulation of extractable B in the soils was greater under the least saline treatments (0.4 and 2.5 dS m^{-1}). Regarding nutrients, significant increases in available P at the end of the experiment compared to the initial soil were observed. For the least saline treatments slight increases were also seen in organic C and TN. Micronutrient levels did not undergo significant changes with respect to initial levels, except for Zn, which increased compared to the initial soil regardless of the treatment applied (Table 2).

3.2. Biomass production

The cumulative production of biomass for the various alfalfa varieties under the different irrigation treatments is shown in Fig. 1. Statistical analysis reveals that production was affected by the treatment, variety and duration of the study period. Broadly speaking, the variety with the lowest production was WL656HQ, with an average of 1478 g m^{-2} per period, compared to the 1550 and 1575 g m^{-2} obtained with varieties SW8421S and PGI908S, respectively. Increased irrigation water salinity led to a significant decline in biomass production for all varieties. The average across all the varieties for the control treatment (0.4 dS m^{-1}) was 1938 ± 38 g m^{-2}, compared to 1800 ± 85, 1558 ± 98, 1327 ± 41 and 1048 ± 37 g m^{-2} for the 2.5, 5.9, 7.5 and 10.0 dS m^{-1} treatments, respectively. Plant age also significantly influenced production, with the third study period presenting higher biomass levels than the previous two (1773 g m^{-2} compared to 1443 and 1386 g m^{-2} for periods 1 and 2). The highest production rate expressed as kilogrammes of dry matter per hectare per day (kg DM ha^{-1} d^{-1}), was 137 (treatment 0.4 dS m^{-1}; var. PGI908S), while the lowest was 70 (treatment 10 dS m^{-1}; var. WL656HQ).

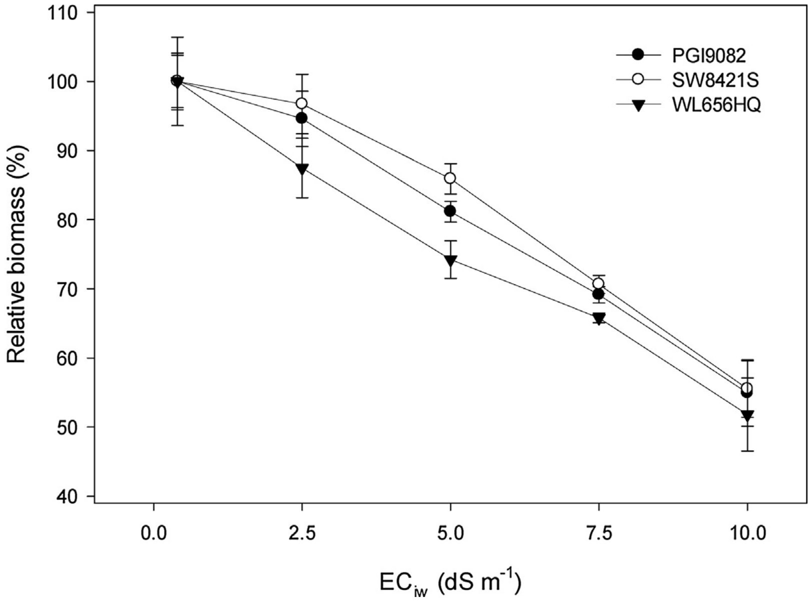

Fig. 2. Relative biomass of three alfalfa cultivars under irrigation with different simulated groundwater qualities; mean ± standard error; n = 4.

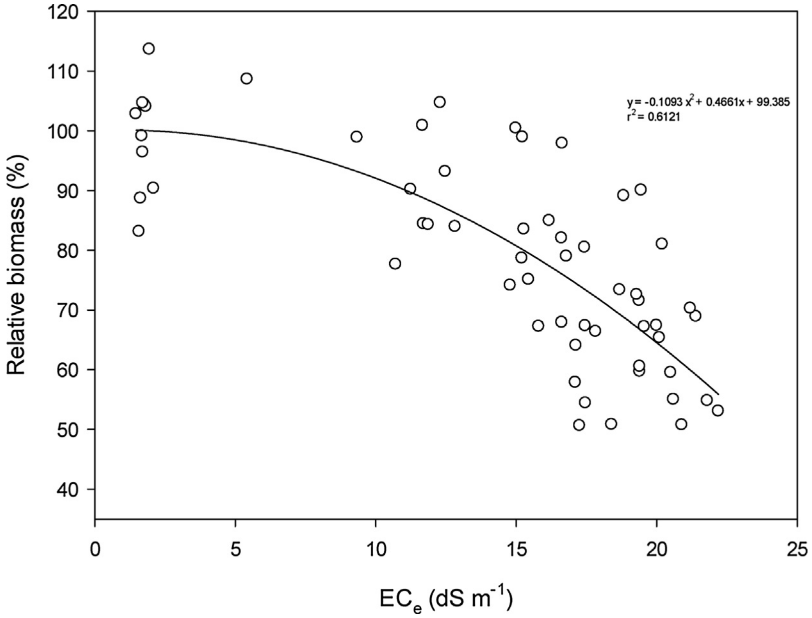

Fig. 3. Relative biomass of alfalfa at different salinity in the soil saturation extract (ECe) at the end of the experiment; n = 60.

The relative yield (relationship between the biomass produced under the different salinity treatments and the control treatment) according to the irrigation water salinity and soil salinity, are presented in Figs. 2 and 3, respectively. These data indicate that as irrigation water salinity increased i.e. 2.5, 5.0, 7.5 and 10.0 dS m^{-1}, alfalfa yield was reduced by 7, 20, 31 and 46%, respectively (Fig. 2). Statistical analysis indicates that this reduction is significant from 5.0 dS m^{-1}. As listed in Table 2, the treatments of 2.5, 5.0, 7.5 and 10.0 dS m^{-1} in the irrigation water produced average soil salinity (EC$_e$) of 12.6, 16.8, 19.1 and 19.3 dS m^{-1}, respectively, at the end of the study period. According to the equation shown in Fig. 3, the one that best describes our data, those EC$_e$ levels produced a decline in production by 12.1, 23.6, 31.6 and 32.3%, respectively.

3.3. *Forage quality*

The change in forage ash content, NDF, ADF, CP, RFV, and ME during the study period, for the different alfalfa varieties under the different water quality treatments, are illustrated in Figs. 4 and 5.

CP varied between 24.8 and 32.9% DM and was significantly affected by irrigation water salinity and the study period, but not by the variety or the interaction of the three factors (Fig. 4). The highest CP content was mostly found in the 7.5, 5.0 and 2.5 dS m^{-1} treatments, which were significantly higher than the control (0.4 dS m^{-1}) and the highest salinity (10 dS m^{-1}) treatments. CP levels varied over time, with a significant increase seen in the last period (mean ~30.2% DM), as compared to the two previous ones (mean ~29.7 and 27.7% DM, for periods 1 and 2, respectively).

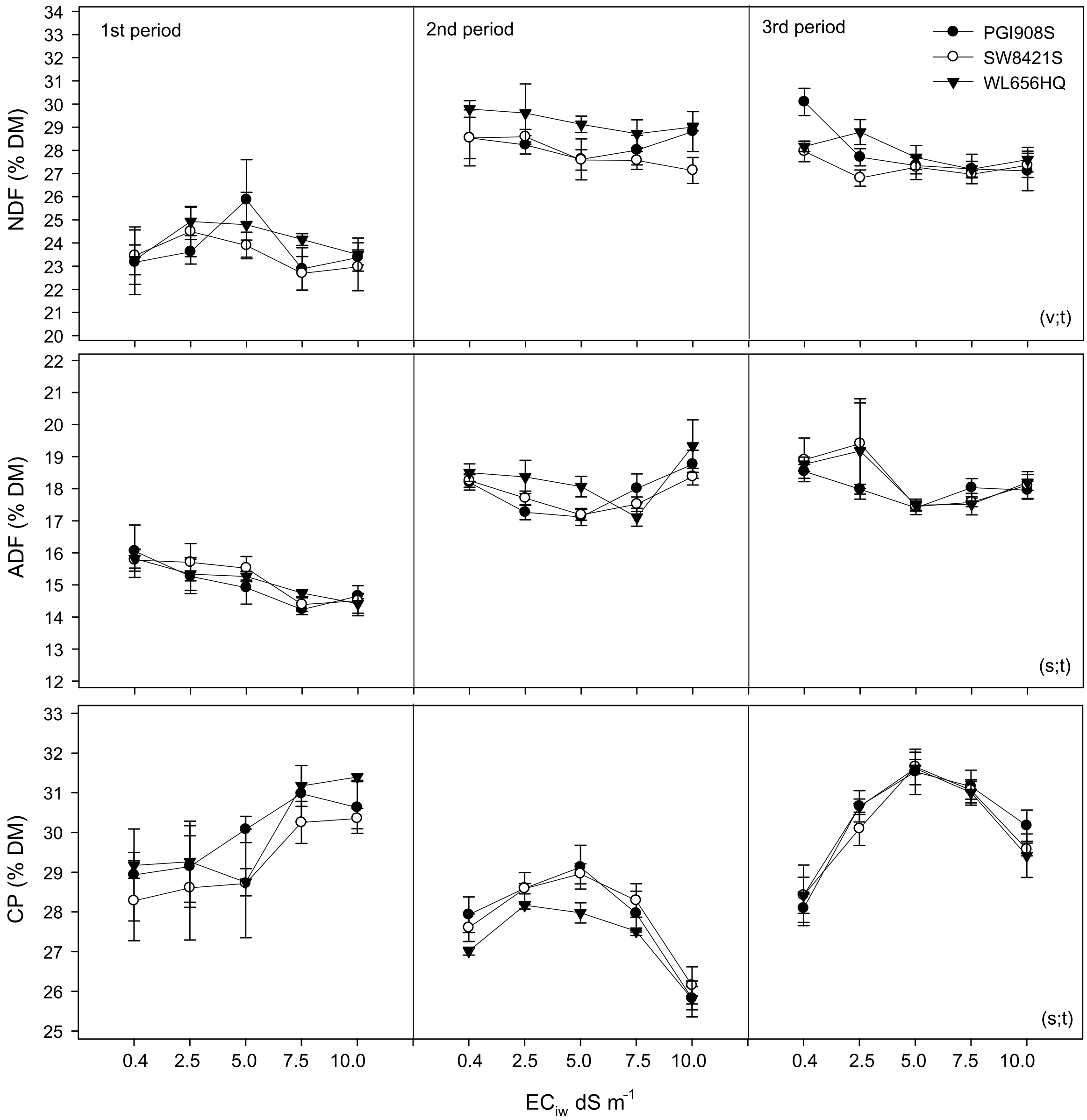

Fig. 4. Evolution of forage quality parameters of three alfalfa cultivars throughout the experiment; NDF, neutral detergent fibre; ADF, acid detergent fibre; CP, crude protein; 1st period ~ 1–124 days after initial treatment application; 2nd period ~ 125–269 days after initial treatment application; 3rd period ~ 270–430 days after initial treatment application; data represent means and standard error; n = 4; letters v, s and t, denote significant effects of variety, salinity treatment and time, respectively, according to a three-way ANOVA ($p < 0.05$) and post hoc Tukey test ($p < 0.05$).

NDF and ADF were significantly affected by the age of the plants (Fig. 4). Significant increases were seen between the first period and periods 2 and 3. For example, the average ADF value for all the varieties was 15.1% in period 1, compared to 18.0 and 18.2% for periods 2 and 3, respectively. The effect of the variety of alfalfa was significant only for NDF, with SW8421S presenting the lowest average value for the entire study period (26.2% DM) and WL656HQ the highest (27.1% DM). Only ADF was significantly influenced by the level of irrigation water salinity. In general, the 5 and 7.5 dS m^{-1} treatments showed the lowest ADF levels (mean ~16.6 and 16.7% DM, respectively), which were significantly lower than those of the least saline treatments (17.6 and 17.4% DM for 0.4 and 2.5 dS m^{-1}, respectively).

Ash content ranged from 10.4 to 16.2% DM throughout the study period and did not differ significantly between varieties, although it was affected by the salinity of the irrigation water and the age of the plant (Fig. 5). In general, the highest values were found in plants under the control treatment (mean ~13.3% DM) and the lowest in the 5.0, 7.5 and 10.0 dS m^{-1} treatments (mean ~12.6% DM). Older plants had significantly less ash content, decreasing from an average of 14.4% DM in the first period to 12.4% DM in the last.

Relative Feed Value (RFV) ranged from 213 to 367 and was significantly affected by the alfalfa variety and time of sampling, although not by the saline treatments (Fig. 5). The variety with the highest value was SW8421S (~271 ± 31), compared to WL656HQ which had the lowest (~262 ± 30). The youngest plants (first sample) had an average value of 303, which was significantly higher than those from the later sampling periods (245 and 251 for periods 2 and 3, respectively).

Metabolisable Energy (ME), the energy potential of the forage, varied between 9.9 and 10.8 MJ kg^{-1} DM (Fig. 5). In general, ME did not differ significantly between the varieties studied, nor did it change significantly over time; however it was affected by the saline treatments. The highest values were found under intermedi-

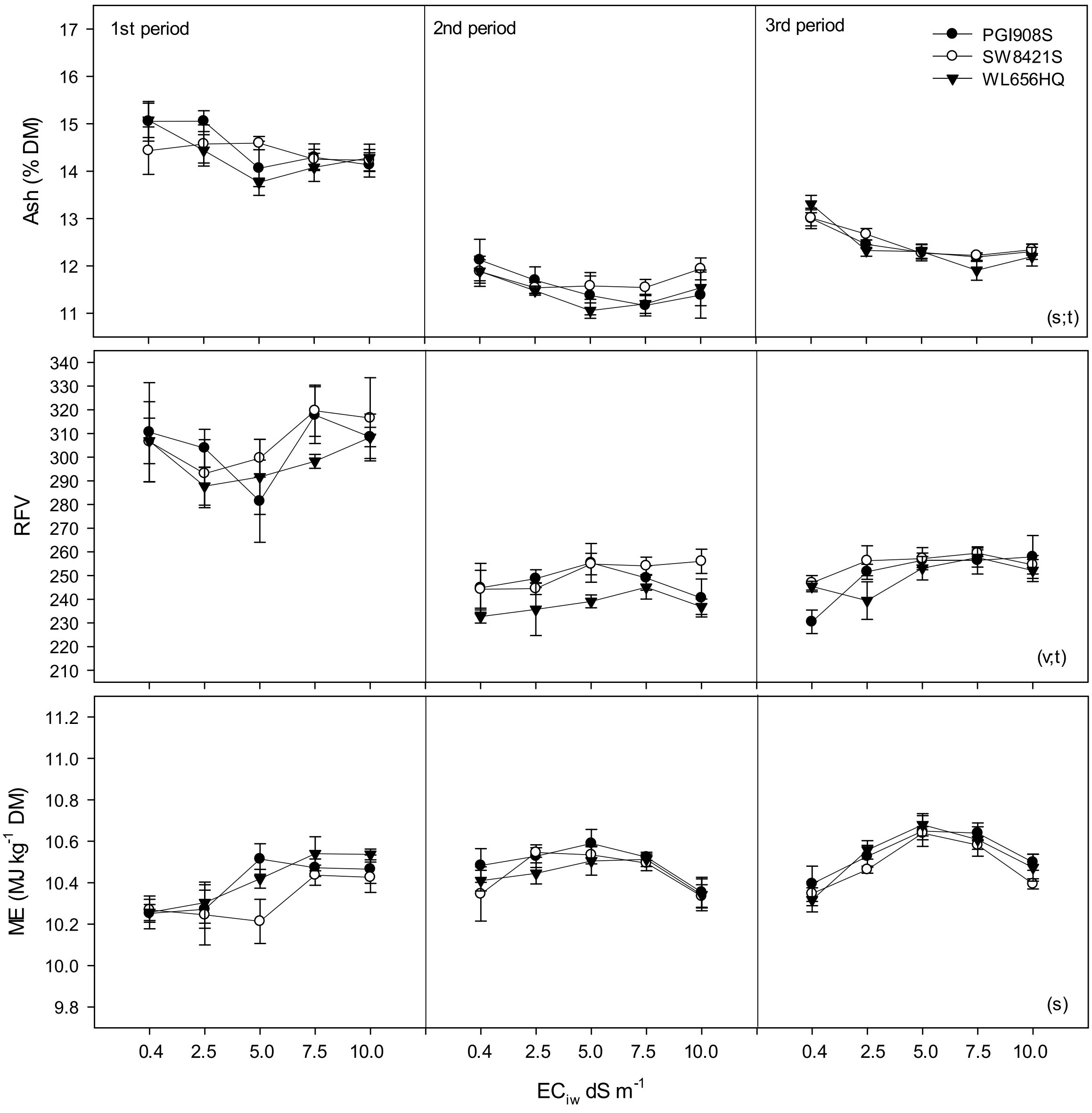

Fig. 5. Evolution of forage nutritional quality of three alfalfa cultivars throughout the experiment; ash content; RFV, relative feed value; ME, metabolisable energy; 1st period ~ 1–124 days after initial treatment application; 2nd period ~ 125–269 days after initial treatment application; 3rd period ~ 270–430 days after initial treatment application; data represent means and standard error; n = 4; letters v, s and t, denote significant effects of variety, salinity treatment and time, respectively, according to a three-way ANOVA ($p < 0.05$) and post hoc Tukey test ($p < 0.05$).

ate salinities of 5 and 7.5 dS m^{-1} (mean ~10.5 MJ kg^{-1} DM), while the control plants averaged 10.3 MJ kg^{-1} DM.

3.4. Forage mineral composition

Table 3 lists mean values for macronutrients and Na in the collected plant material for the three varieties under the different water quality treatments and for the three study periods. Statistical analysis shows that the concentrations of all elements, except P, were affected by salinity and by the degree of maturity of the plants, although no significant differences were observed between the varieties of alfalfa.

Levels of N ranged between 23.9 and 54.2 g kg^{-1}, with significant differences only between plants treated with 7.5 dS m^{-1} water and those in the control treatment (mean ~46.4 and 44.1 g kg^{-1} respectively). The lowest values were found in period 2 (mean ~43.2 g kg^{-1} compared to 46.1 and 46.4 g kg^{-1} for periods 1 and 3, respectively). Average P concentration in sampled forage during the study period was 4.5 g kg^{-1} and was significantly lower in period 2 (mean ~4.2 g kg^{-1}) than in periods 1 and 3 (mean ~4.5 and 4.7 g kg^{-1} respectively). Concentrations of K in the forage decreased gradually with increased salinity of the irrigation water, falling from an average of 37.8 g kg^{-1} in plants from the control treatment to 27.1 g kg^{-1} in plants from the 10 dS m^{-1} treatment. A decrease in forage K content with increased plant age was also observed; the mean of 38.5 g kg^{-1} for period 1 falling to 29.9 g kg^{-1} in period 3. Likewise K, Ca in the forage decreased gradually with increased salinity (control treatment mean ~19.8 g kg^{-1} vs. 10 dS m^{-1} treatment mean ~12.7 g kg^{-1}) and with plant age (period 1 mean ~20.8 g kg^{-1} vs. period 3 mean ~12.9 g kg^{-1}). Conversely, the Mg content in the forage increased with salinity, averaging 4.0 g kg^{-1} in plants from the control treatment and 5.0 g kg^{-1} from those in the 10 dS m^{-1} treatment, and Mg content also rose slightly with plant age. Concentrations of S increased with the salinity of the irrigation water (4.5 vs 5.6 g kg^{-1} in the control treatment and most saline treatment, respectively) and decreased with plant age (5.9 vs

Table 3

Tissue macronutrient concentration at different stages of the experiment: 1st period ∼ 1–124 days after initial treatment application, 2nd period ∼ 125–269 days after initial treatment application, and 3rd period ∼ 270–430 days after initial treatment application; mean ± standard deviation; n = 12; * asterisk denotes significant effects of variety, salinity treatment and time according to a three-way ANOVA and post hoc Tukey test ($p < 0.05$); ns denotes non-significant effects.

EC_{iw} target dS m^{-1}	N g kg^{-1}	P	K	Ca	Mg	S	Na
1st Period							
0.4	45.6 ± 2.9	4.9 ± 1.0	43.5 ± 2.7	22.4 ± 2.2	4.4 ± 0.5	6.1 ± 1.0	0.1 ± 0.4
2.5	45.3 ± 3.0	4.7 ± 0.9	40.9 ± 3.1	21.5 ± 2.0	4.3 ± 0.4	6.0 ± 1.0	2.4 ± 0.6
5.0	45.4 ± 2.3	4.3 ± 0.3	36.7 ± 2.3	20.1 ± 1.9	4.2 ± 0.3	5.8 ± 1.3	3.9 ± 0.7
7.5	47.1 ± 4.5	4.3 ± 0.4	35.8 ± 1.1	20.1 ± 1.6	4.5 ± 0.3	5.9 ± 0.3	4.6 ± 0.6
10.0	47.3 ± 1.9	4.4 ± 0.4	36.0 ± 2.1	19.8 ± 1.3	4.5 ± 0.3	5.9 ± 0.6	5.6 ± 0.6
2nd Period							
0.4	42.9 ± 1.2	4.5 ± 0.4	33.2 ± 1.8	17.7 ± 2.0	3.7 ± 0.3	3.0 ± 1.3	1.6 ± 0.4
2.5	44.9 ± 1.5	4.3 ± 0.4	30.5 ± 1.7	14.6 ± 1.1	3.8 ± 0.3	4.4 ± 0.6	5.3 ± 1.0
5.0	44.9 ± 1.6	4.2 ± 0.3	27.8 ± 2.3	12.4 ± 1.2	4.1 ± 0.4	4.2 ± 0.9	7.6 ± 0.9
7.5	44.1 ± 1.3	4.2 ± 0.3	22.3 ± 7.3	11.0 ± 1.7	4.6 ± 0.5	4.5 ± 1.1	9.4 ± 1.1
10.0	39.7 ± 6.6	4.1 ± 0.5	20.4 ± 7.7	10.1 ± 1.4	5.3 ± 0.5	5.4 ± 1.3	11.2 ± 1.9
3rd Period							
0.4	44.0 ± 1.6	4.4 ± 0.4	36.8 ± 3.2	19.4 ± 2.4	4.0 ± 0.4	4.6 ± 1.0	1.6 ± 0.5
2.5	45.5 ± 6.7	4.4 ± 0.3	31.3 ± 1.7	14.8 ± 1.6	4.4 ± 0.6	4.9 ± 0.5	6.8 ± 1.5
5.0	48.6 ± 1.4	4.8 ± 0.3	28.8 ± 2.6	11.9 ± 1.7	4.4 ± 0.3	4.3 ± 1.3	9.0 ± 2.1
7.5	48.0 ± 1.0	5.0 ± 0.3	27.3 ± 1.9	9.6 ± 1.1	4.7 ± 0.2	4.9 ± 0.4	10.7 ± 1.8
10.0	46.1 ± 1.4	4.7 ± 0.5	25.1 ± 3.4	8.5 ± 1.0	5.2 ± 0.3	5.7 ± 0.6	13.1 ± 2.2
Variety	ns	ns	ns	ns	ns	ns	ns
Salinity	*	ns	*	*	*	*	*
Time	*	*	*	*	*	*	*

4.8 g kg^{-1} in periods 1 and 3, respectively). Plant tissue Na increased by a factor of 7.0 between the control treatment and the most saline treatment (10 dS m^{-1}) and by a factor of 2.4 between the first and last study periods.

The mean micronutrient and Cl concentrations in the collected plant material under the different irrigation water qualities and for the three periods are presented in Table 4. Statistical analysis of the data indicates that, with the exception of Zn, the concentrations of all micronutrients were affected by the salinity treatment and by the degree of plant maturity, but no significant differences were found among the varieties tested.

Levels of Mn and Cu in the forage increased with the increases in irrigation water salinity, whereas the Fe, B and Cl content decreased (Table 4). Mn increased by a factor of 1.3 between the control and most saline treatments, with Cu increasing by a factor of 1.1. Levels of Fe fell from 101.5 mg kg^{-1} DM in the control treatment to 92.4 in the 10 dS m^{-1} treatment. In the case of B, the greatest differences in forage concentration were found between the control treatment (mean ∼158.4 mg kg^{-1}) and the treatment with 7.5 dS m^{-1} salinity (mean ∼99.7 mg kg^{-1}). Forage Cl concentration was interesting in that all samples, regardless of treatment or time of sampling contained high levels (i.e. 1.4–2.7% dw). However, the only significant differences in forage concentration that were observed was between plants from the 2.5 dS m^{-1} treatment (mean of 22.2 g Cl kg^{-1}) and those from the 7.5 and 10 dS m^{-1} treatments (mean of 19.0 g Cl kg^{-1}). With regard to the study period, the concentration of all micronutrients, except B, decreased significantly with plant age. For example, average Fe concentration in period 1 was 108.2 mg kg^{-1}, compared to 88.6 mg kg^{-1} in the last period. For the most part, average B levels were lowest in period 1 (mean ∼96.7 mg kg^{-1}) and increased to 135.1 and 126 mg kg^{-1} in the later periods.

4. Discussion

4.1. Impact of irrigation on soil chemical properties

Leaching fractions obtained in this experiment coincide with the concept of "evapotranspiration – soil salinity – leaching fraction" interactions developed by others (Letey et al., 1985). That is, for the same quantity of water applied, when the irrigation water salinity increases, growth decreases, evapotranspiration decreases and the leaching fraction increases, leading to a reduction in soil salinity. Larger accumulation of extractable B in the soils under the least saline treatments was possibly a result of the greater use of water by the plants under these treatments, which translated to lower leaching fractions (Díaz and Grattan, 2009). The observed increase in exchangeable Na levels were likely a result of the saline-sodic nature of the irrigation water. An increase in some nutrients (i.e. Ca, Mg, P, N, Zn) may have been due to the addition of manure and fertiliser at the beginning of the experiment. Uptake and leaching of Zn could occur to a lesser extent than with other micronutrients likely due to fixation/sorption on calcium carbonate (Alloway, 2008).

4.2. Biomass production

The production rate for the alfalfa varieties in our experiment (70–137 kg DM ha^{-1} d^{-1}) were similar or slightly higher than those reported by others in similar studies conducted with saline tolerant varieties of alfalfa. For variety SW8421S, Cornacchione and Suarez (2015) obtained 99 and 69 kg DM ha^{-1} d^{-1} with irrigation water EC of 3 and 12 dS m^{-1}, respectively. Other authors record rates of 74 kg DM ha^{-1} d^{-1} for var. Salado and 86 kg DM ha^{-1} d^{-1} for var. SW9720, with irrigation water EC of 15 dS m^{-1} (Grattan et al., 2004b). These differences in productivity may be due to differences in the environmental conditions (temperature, humidity) and to different soil conditions (physical characteristics, availability of nutrients and water, etc.).

Cornacchione and Suarez (2015) found no significant effect of salinity on the reduction in biomass production up to 12 dS m^{-1} EC in the irrigation water (reduction of ∼25%), while in this experiment reduction is significant from 5.0 dS m^{-1}. These differences are due to the different types of substrate used in each case. Cornacchione and Suarez (2015) used a sand tank system with high irrigation frequency with leaching fractions approaching 100% such that the irrigation water salinity was comparable to the soil water salinity. Since the salinity of the soil solution is about twice that of the saturated soil extract, their 12 dS m^{-1} irrigation treatment is equivalent to an EC_e of about 6 dS m^{-1}. In our study, the high-water hold-

Table 4
Tissue micronutrient concentration at different stages of the experiment: 1st period ~ 1–124 days after initial treatment application, 2nd period ~ 125–269 days after initial treatment application, and 3rd period ~ 270–430 days after initial treatment application; mean ± standard deviation; n = 12; * asterisk denotes significant effects of variety, salinity treatment and time according to a three-way ANOVA and post hoc Tukey test ($p < 0.05$); ns denotes non-significant effects.

EC$_{iw}$ target dS m^{-1}	Fe mg kg^{-1}	Mn	Cu	Zn	B	Cl g kg^{-1}
1st Period						
0.4	113.9 ± 9.2	98.1 ± 20.3	6.2 ± 1.6	27.0 ± 10.7	110.5 ± 29.8	22.7 ± 2.0
2.5	108.5 ± 8.4	101.8 ± 12.4	5.8 ± 1.6	26.6 ± 11.3	93.0 ± 21.9	25.9 ± 3.5
5.0	110.3 ± 8.2	103.8 ± 17.4	6.3 ± 0.9	26.2 ± 8.0	91.9 ± 14.7	27.1 ± 5.8
7.5	107.4 ± 6.6	116.8 ± 14.4	6.7 ± 0.8	26.1 ± 5.0	94.8 ± 11.4	27.3 ± 6.7
10.0	101.2 ± 3.3	110.1 ± 27.1	6.5 ± 0.6	26.0 ± 4.8	93.3 ± 11.3	26.1 ± 5.1
2nd Period						
0.4	104.3 ± 16.3	63.3 ± 5.7	6.9 ± 1.1	24.8 ± 5.0	192.4 ± 34.7	18.5 ± 6.1
2.5	100.9 ± 9.9	76.4 ± 10.3	6.1 ± 1.4	25.2 ± 6.9	136.5 ± 19.2	19.8 ± 6.1
5.0	98.9 ± 5.7	84.1 ± 13.6	6.1 ± 1.6	25.8 ± 4.6	117.9 ± 31.3	16.8 ± 1.8
7.5	95.1 ± 5.1	95.5 ± 19.6	5.9 ± 1.0	27.1 ± 3.0	99.1 ± 28.3	14.3 ± 0.8
10.0	90.2 ± 8.5	107.2 ± 30.5	6.8 ± 1.3	28.6 ± 6.0	129.7 ± 33.2	14.2 ± 1.2
3rd Period						
0.4	86.5 ± 7.6	45.0 ± 4.3	4.6 ± 0.8	19.5 ± 2.0	172.5 ± 41.0	19.8 ± 2.9
2.5	90.9 ± 6.7	48.9 ± 5.9	4.4 ± 0.4	20.9 ± 1.9	135.0 ± 27.6	19.7 ± 1.6
5.0	92.0 ± 5.8	50.9 ± 5.1	5.3 ± 0.7	22.8 ± 3.6	102.8 ± 19.9	17.3 ± 3.5
7.5	88.1 ± 6.3	57.6 ± 7.2	5.3 ± 0.8	23.0 ± 1.9	105.3 ± 14.1	15.5 ± 1.1
10.0	85.7 ± 5.9	58.8 ± 12.6	5.6 ± 1.2	22.7 ± 3.3	115.8 ± 11.8	16.1 ± 1.4
Variety	ns	ns	ns	ns	ns	ns
Salinity	*	*	*	ns	*	*
Time	*	*	*	*	*	*

ing capacity of the fine-textured soil facilitates salt accumulation, including where high leaching fractions are applied.

Alfalfa – traditionally classified as "moderately sensitive" to salinity – is reported to tolerate up to 2 dS m^{-1} EC$_e$ but suffers a productivity decline of approximately 7.3% for each unit increase in EC$_e$ above this threshold value (Maas and Grattan, 1999). Using the current guidelines the theoretical reduction in production would be 77.4% for the lowest salinity (12.6 dS m^{-1}) and 100% for EC$_e$ above 16 dS m^{-1}, significantly higher than those obtained in our experiment. These results confirm that some varieties of alfalfa can grow at much higher salinity than the guidelines indicate without exhibiting significant yield declines. For example, the EC$_e$50 value using the guidelines is 8.8 dS m^{-1} while the EC$_e$50 based on this study is 23.5 dS m^{-1}. Similar results have been reported by other authors, including Putnam et al. (2017) who, following a 3-year field study in a clay loam soil with applications of 5–7 dS m^{-1} EC$_{iw}$, obtained alfalfa production levels similar to those obtained with non-saline water. In the same type of soil, irrigation using water with 8–10 dS m^{-1} EC$_{iw}$ caused yield declines of 10–15% compared to the control treatment, although economically acceptable production was still achieved.

The observed increase in biomass production with plant age was likely a result of the progressive reduction in soil salinity (due to the very high initial ECe) along the experimental period. Results also confirm a lower salt tolerance of variety WL656HQ in forage production with respect to the other two assessed cultivars.

4.3. *Forage quality*

Generally-speaking, forage quality increases as CP, RFV and ME increase and NDF, ADF and ash content decrease (Suyama et al., 2007a). The levels of CP found coincide largely with those reported by other authors for varieties of alfalfa grown in saline conditions (Suyama et al., 2007a; Ferreira et al., 2015). However, some studies report an increase in CP when irrigation water salinity increases (Ferreira et al., 2015). This rise is attributed to a salinity-induced increase in the leaf/stem ratio (Al-Khatib et al., 1992). Leaves are richer in photosynthetic components with high N content, whereas the stems present more structural components with low N content. In our case, no uniform behaviour was observed, the increase in CP content at greater salinity was observed only in the first sampling period (Fig. 4). During subsequent periods, a significant decrease in CP content at the highest level of salinity (10.0 dS m^{-1}) was found. Isotopic N content (δ15N) analyses carried out as part of this study, but addressed separately in a companion paper currently in preparation, indicate that salinity negatively affected atmospheric nitrogen fixation, which may account for the decrease in CP observed under the most saline treatments.

The percentages of fibre found in the present study (19.9–32.3% NDF and 17.1–23.6% ADF) were slightly lower than those reported for variety SW8421S under treatments of 12.7–24.0 dS m^{-1} (Ferreira et al., 2015) but similar to those reported by Robinson et al. (2004) for Salado and SW9720 using waters with 15 and 25 dS m^{-1} (~22–34% NDF) to irrigate alfalfa in sand tanks equivalent to those used later by Cornacchione and Suarez (2015). In both cases, the authors observed a reduction in fibre content with increased irrigation water salinity. Such a reduction, observed by others in other types of forage (Ben-Ghedalia et al., 2001) and which can reach 12.2% in NDF compared to the control treatments, is also attributed to an increase in the leaf-stem ratio with higher irrigation water salinity (Ferreira et al., 2015). In our case, a gradual reduction in ADF content at higher salinity was observed only in the first period (Fig. 4), with a difference of approximately 2% between the control plants and those in the most saline treatments. However, this trend disappeared in subsequent periods and, in general, a slight decrease (~1%) was noted only up to salinity of 7.5 dS m^{-1}, with an increase once again at 10 dS m^{-1} (Fig. 4). In the present experiment, the most saline treatments could have negatively affected leaf production; for example, through a low atmospheric nitrogen fixation and lower water use efficiency, thus decreasing the leaf/stem ratio. Textural differences between the substrates used (sand tanks *vs* clay soil) and differences in the experimental conditions (i.e. amount and frequency of irrigation) are key factors that account for discrepancies between the two studies. Plant age is one of the most important factors influencing fibre content and a significant increase was observed over time, as reported also by other authors for the same varieties (Stefanon et al., 1996; Robinson et al., 2004; Ferreira et al., 2015). Robinson et al. (2004), for instance, found an increase in NDF from 18.6 to 42.6% between the first and fifth cut of alfalfa irrigated with saline water.

A reduction in ash content with increased irrigation water salinity has been reported by others for different forages under greenhouse conditions (Ben-Ghedalia et al., 2001; Robinson et al., 2004; Suyama et al., 2007a). For example, Suyama et al. (2007a) found a reduction of 2% between alfalfa irrigated with non-saline ($0.8\,dS\,m^{-1}$) and highly saline water ($18.0\,dS\,m^{-1}$). However, the reduction in ash content over time observed in this experiment is not consistent with data obtained by Cao et al. (2012), who noted an increase in ash content over time in irrigated alfalfa in saline soils. In our case, the high initial salinity of the soils ($\sim 54\,dS\,m^{-1}$), which was reduced substantially by the irrigations applied before treatments were imposed, may have led to a greater accumulation of salt in the young plants. And indeed this was found. Tissue Cl^-, for example, was 2.3–2.7% dw, regardless of treatment in plants composited from the first series of cuts. The concentrations decreased below 2.0% for all treatments in subsequent sampling periods. It is likely that excessive salt was absorbed by plants in this saline soil at early times while soil saline below the soil surface was still very high. As plants were later stressed, growth and consequently ET was reduced, allowing for high leaching of this highly saline soil. This may explain why tissue Cl^- decreased with increased salinity treatment.

Plant age appears to be the main factor influencing RFV, indicating that the plants' nutritional value declines slightly as they become older. Old plants are mainly associated with increased fibre content as much of the biomass is comprised of woody stems. RFV levels also suggest that variety WL656HQ is slightly lower in quality than the other two analysed. Values obtained here were slightly higher than those reported for alfalfas irrigated with saline water by Ferreira et al. (2015), who observed a significant increase in RFV (~5.2%) when the irrigation water salinity increased from 12.7 to $18.4\,dS\,m^{-1}$ EC_{iw}. Similarly, Suyama et al. (2007a) found ME values of 9.2, 10.0 and $10.3\,MJ\,kg^{-1}$ DM in young alfalfa irrigated using water with 0.8, 11 and $18\,dS\,m^{-1}$ EC_{iw}, respectively, and attributed the increase in ME with salinity to the fact that the plants under saline treatments were less mature than their control treatment counterparts at the time of cutting. In contrast to those studies, no clear positive association was observed here between nutritional quality and the level of salinity in the irrigation water. However, an increase in quality was seen up to 5 and $7.5\,dS\,m^{-1}$ EC_{iw} compared to the control treatments, but not at higher salinities. These dissimilarities could be explained by differences in the substrates used. In our case, we used heavy-textured soils that could potentially reduce soil physical conditions which would impose other abiotic or biotic stresses that limit crop development under high salinity-sodicity conditions.

Based on the quality parameters analysed and regardless of the irrigation water quality, all the varieties can be classed as "supreme" quality (Putnam et al., 2008), adequate for dairy livestock and for producing fast-growing calves (Suyama et al., 2007b), a reflection of their very high nutritional content.

4.4. *Forage mineral composition*

The mineral composition of crops can be affected by salinity, which causes nutritional imbalances due to its effects on nutrient availability, competitive absorption, transport and compartmentation in the tissues (Grattan and Grieve, 1999). Accordingly, the mineral composition of plant tissue in the aerial part of forage plants grown under saline conditions needs to be monitored over time. Here, the concentrations of the different elements analysed were compared with the established maximum tolerable levels (MTLs) in order to evaluate the potential impact on mineral nutrition in ruminants (NRC, 2005).

Of the elements studied in the forages obtained, only K, S and B showed concentrations above the values considered adequate (NRC, 2005; Putnam et al., 2008). Potential problems associated with consumption of these forages may include: i) hypomagnesemia, a metabolic disorder in ruminants affecting the bioavailability of magnesium in the rumen. High concentrations of K in the forage reduce Mg absorption and can trigger this condition (McDowell and Valle, 2000). The maximum tolerable level for K has been set at $20{,}000\,mg\,K\,kg^{-1}$ DM (NRC, 2005). This might be considered conservative given that some research suggests that ruminants can tolerate levels of between $30{,}000–50{,}000\,mg\,K\,kg^{-1}$ DM in their diet without manifesting adverse effects (NRC, 2005). The alfalfa varieties studied here exceeded this value, averaging between 20,000 and $44{,}000\,mg\,K\,kg^{-1}$ DM (Table 3). The fact that K levels generally decrease with increases in irrigation water salinity makes K toxicity caused by alfalfa grown in saline conditions unlikely; ii) polioencephalomalacia (PEM): a high concentration of S in forage can prove toxic to ruminants, manifesting as failures in the central nervous system and brain lesions (Gould, 2000). A further problem associated with high S concentrations is Cu deficiency (Spears, 2003). Cu availability decreases as concentrations of S and Mo increase in the rumen due to the formation of complexes such as thiomolybdates that are not readily absorbed by the animal (Spears, 2003). An increase from 0.2 to 0.4% of S in the diet can cause Cu absorption to fall by 50%. To avoid these problems, the MTL has been set at 0.30 and 0.50% S DM for diets with 85 and 40% forage, respectively (NRC, 2005). Concentrations of this element in the alfalfa varieties studied here were near or above the MTL (mean ~0.30–0.61% DM; Table 3) and were generally higher with higher irrigation water salinity. Accordingly, S accumulation could represent a constraint on the use of these forages; iii) B toxicity: B is considered relatively non-toxic and most animals tolerate it well, even if some research suggests that toxicity may occur in animals that consume high amounts of B (NRC, 2005); findings which have led the MTL to be set at $135\,mg\,B\,kg^{-1}$ DM for livestock (NRC, 2005).

In our study, B concentrations generally decreased with increased irrigation water salinity, an effect already observed by various authors in different crops (Yermiyahu et al., 2008; Díaz and Grattan, 2009). The MTL was exceeded only slightly in the least saline treatments (0.4 and $2.5\,dS\,m^{-1}$). These results suggest the existence of interactions that increase B tolerance under saline conditions. One possible reason for this could be the reduction in plant water uptake under higher salinity levels, which reduces tissue accumulation of B (Yermiyahu et al., 2008). Other authors attribute the reduction in B to interactions with $SO_4{}^{2-}$ and Cl^- (Grattan et al., 2004c). High levels of K, S and B, close to or above MTLs, have been reported for alfalfa by other authors who used saline water for irrigation (Grattan et al., 2004a; Suyama et al., 2007b).

5. Conclusions

The results obtained in this experiment confirm some recent findings concerning the salt tolerance of certain varieties of alfalfa, which suggest that the tolerance guidelines set in the literature for this crop should be reassessed. Our study confirms that alfalfa, traditionally considered moderately sensitive to salinity, can grow in conditions of high soil and irrigation water salinity (e.g. $EC_e \sim 10\,dS\,m^{-1}$; $EC_{iw} \sim 5\,dS\,m^{-1}$) without suffering large reductions in biomass compared to non-saline conditions. These results are particularly important for arid regions, including the easternmost Canary Islands which lack quality water, but do have an abundance of salt-affected water and soils, as well as a high demand for livestock feed, one of the main economic activities in these areas. The forages obtained were of good quality for dairy goat diets, falling into the high category from the standpoint of nutritional quality, based on USDA quality guidelines. In terms of mineral nutrition for ruminants, concentrations of K, B and S, in particular, would require monitoring to determine if forages are approaching maximum tolerable concentrations, above which animals exclusively consuming this forage over extended periods could face potential toxicity. Should this occur, the forages could still be used as a dietary supplement, in conjunction with other feeds with lower mineral content.

Acknowledgements

The present study was funded by the Spanish Ministry for the Economy and Competitiveness (AGL2013-46054-R) and the CajaCanarias Foundation. The authors gratefully acknowledge the assistance provided by staff at the Canarian Institute for Agricultural Research during the experiment. The valuable suggestions and contributions by Dr Daniel Putnam concerning alfalfa seeding and planting density are also gratefully acknowledged.

References

AOAC (Association of Official Analytical Chemists), 2000. Official Methods of Analysis, 17th ed. AOAC, Washington, DC, USA.

AOAC (Association of Official Analytical Chemists), 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed. APHA Publication Office, Washington, DC.

ARC, 1984. The Nutrient Requirements of Ruminant Livestock. Supplement No1. Commonwealth Agric., Bureaux, Slough, U.K.

Al-Khatib, M., McNeilly, T., Collins, J.C., 1992. The potential of selection and breeding for improved salt tolerance in lucerne (*Medicago sativa* L.). Euphytica 65, 43–51.

Alloway, B.J., 2008. Zinc in Soils and Crop Nutrition, 2nd ed. International Zinc Association; Paris : International Fertilizer Industry Association, Brussels.

Ayers, R.S., Westcot, D.W., 1985. Water Quality for Agriculture. FAO Irrig. Drain. Paper 29. FAO, Rome.

Beltran, J.M., Koo-Oshima, S., 2006. FAO Expert Consultation on Water Desalination for Agricultural Applications. FAO Land and Water Discussion Paper, No 5, Rome (Italy) (60 pp).

Ben-Gal, A., Yermiyahu, U., Cohen, S., 2009. Fertilization and blending alternatives for irrigation with desalinated water. J. Environ. Qual. 38, 529–536.

Ben-Ghedalia, D., Solomon, R., Miron, J., Yosef, E., Zomberg, Z., Zukerman, E., Greenberg, A., Kipnis, T., 2001. Effect of water salinity on the composition and in vitro digestibility of winter-annual ryegrass grown in the Arava desert. Anim. Feed Sci. Technolo. 91, 139–147.

Burt, C.M., Clemmens, A.J., Strelkoff, T.S., Solomon, K.H., Bleisner, R.D., Hardy, L.A., Howell, T.A., Eisenhauer, D.E., 1997. Irrigation performance measures efficiency and uniformity. J. Irrig. Drain Eng. ASCE 123 (6), 423–442.

Cao, J., Li, X., Kong, X., Zed, R., Dong, L., 2012. Using alfalfa (*Medicago sativa*) to ameliorate salt-affected soils in Yingda irrigation district in Northwest China. Acta Ecol. Sin. 32, 68–73.

Cornacchione, M.V., Suarez, D.L., 2015. Emergence, forage production, and ion relations of alfalfa in response to saline waters. Crop Sci. 55, 444–457.

Díaz, F.J., Grattan, S.R., 2009. Performance of tall wheatgrass (Thinopyrum ponticum, cv. 'Jose') irrigated with saline-high boron drainage water: implications on ruminant mineral nutrition. Agric. Ecosyst. Environ. 131, 128–136.

Díaz, F.J., Benes, S.E., Grattan, S.R., 2013a. Field performance of halophytic species under irrigation with saline drainage water in the San Joaquin Valley of California. Agric. Water Manage. 118, 59–69.

Díaz, F.J., Tejedor, M., Jiménez, C., Grattan, S.R., Dorta, M., Hernández, J.M., 2013b. The imprint of desalinated seawater on recycled wastewater: consequences for irrigation in Lanzarote Island, Spain. Agric. Water Manage. 116, 62–72.

Djilianov, D., Prinsen, E., Oden, S., Onckelen, H., Müller, J., 2003. Nodulation under salt stress of alfalfa lines obtained after in vitro selection for osmotic tolerance. Plant Sci. 165, 887–894.

Ferreira, J.F.S., Cornacchione, M.V., Liu, X., Suarez, D.L., 2015. Nutrient composition, forage parameters, and antioxidant capacity of alfalfa (*Medicago sativa*, L.) in response to saline irrigation water. Agriculture 5, 577–597.

Gould, D.H., 2000. Update on sulfur-related polioencephalomalacia. Vet. Clin. North Am.: Food Anim. Pract. 16, 481–496.

Grattan, S.R., Grieve, C.M., 1999. Salinity mineral nutrient relations in horticultural crops. Sci. Hortic. 78, 127–157.

Grattan, S.R., Grieve, C.M., Poss, J.A., Robinson, P.H., Suarez, D.L., Benes, S.E., 2004a. Evaluation of salt tolerant forages for sequential water reuse systems. III. Potential implications for ruminant mineral nutrition. Agric. Water Manage. 70, 137–150.

Grattan, S.R., Grieve, C.M., Poss, J.A., Robinson, P.H., Suarez, D.L., Benes, S.E., 2004b. Evaluation of salt-tolerant forages for sequential water reuse systems. I. Biomass production. Agric. Water Manage. 70, 109–120.

Grattan, S.R., Grieve, C.M., Poss, J.A., Suarez, D.L., Smith, T., 2004c. Does Saline Drainage Water Affect Crop Tolerance to Boron? 2003–04 Technical Progress Report: UC Salinity/Drainage Research Program. DANR. University of California, pp. 19–32.

Grattan, S.R., Benes, S.E., Peters, D.W., Diaz, F., 2008. Feasibility of irrigating Pickleweed (*Salicornia bigelovii:* Torr) with hyper-saline drainage water. J. Environ. Qual. 37, S149–S156.

Grattan, S.R., Díaz, F.J., Pedrero, F., Vivaldi, G.A., 2015. Assessing the suitability of saline wastewaters for irrigation of *Citrus* spp.: emphasis on boron and specific-ion interactions. Agric. Water Manage. 157, 48–58.

ISO/IEC 17025, 2005. General Requirements for the Competence of Testing and Calibration Laboratories.

Letey, J., Dinar, A., Knapp, K.C., 1985. Crop-water production function model forsaline irrigation waters. Soil Sci. Soc. Am. J. 49, 1005–1009.

Maas, E.V., Grattan, S.R., 1999. Crop yields as affected by salinity. In: Skaggs, R.W., van Schilfgaarde, J. (Eds.), Agricultural Drainage. Agron. Monograph 38. ASA, CSSA, SSSA, Madison, WI, pp. 55–108.

Martínez-Alvarez, V., González-Ortega, M.J., Martín-Gorriz, B., Soto-García, M., Maestre-Valero, J.F., 2017. The use of desalinated seawater for crop irrigation in the Segura River Basin (south-eastern Spain). Desalination 422, 153–164.

Masters, D.G., Benes, S.E., Norman, H.C., 2007. Biosaline agriculture for forage and livestock production. Agric. Ecosyst. Environ. 119, 234–248.

McDowell, L.R., Valle, G., 2000. Major minerals in forages. In: Givens, D.I., Owens, E., Axford, R.F.E., Omed, H.M. (Eds.), Forage Evaluation in Ruminant Nutrition. CABI Publishing, Oxon, UK, pp. 373–397.

NAFA, 2016. Alfalfa Variety Ratings. Winter Survival, Fall Dormancy and Pest Resistance Ratings for Alfalfa Varieties. National Alfalfa & Forage Alliance.

National Research Council (NRC), 2005. Mineral Tolerance of Animals. National Academy Press Washington, DC, USA.

Putnam, D.H., Robinson, P., DePeters, E., 2008. Forage quality and testing. In: Summers, C.G., Putnam, D.H. (Eds.), Irrigated Alfalfa Management for Mediterranean and Desert Zones. Chapter 16. University of California/Agricultural and Natural Resources (Publication 8302).

Putnam, D.H., Benes, S., Galdi, G., Hutmacher, B., Grattan, S., 2017. Alfalfa (Medicago sativa, L.) is tolerant to higher levels of salinity than previous guidelines indicated: implications of field and greenhouse studies. Geophysical Research Abstracts, Vol. 19. EGU2017-18266-1.

Riera, R., Tuya, F., Ramos, E., Rodríguez, M., Monterroso, O., 2012. Variability of macrofaunal assemblages on the surroundings of a brine disposal. Desalination 291, 94–100.

Robinson, P.H., Grattan, S.R., Getachew, G., Grieve, C.M., Poss, J.A., Suarez, D.L., Benes, S.E., 2004. Biomass accumulation and potential nutritive value of some forages irrigated with saline-sodic drainage water. Anim. Feed Sci. Technol. 111, 175–189.

de la Roza, B., Argamentería, A., 1992. Organic matter digestibility of forages for ruminants. Prediction by sodium hydroxide or sodium chlorite pretreatment in conjunction with enzymatic treatment. A. Biotech. Forum Europe 9 (5), 294–297.

Rozema, J., Flowers, T., 2008. Crops for a salinized world. Science 322, 1478–1480.

Smedema, L.K., Shiati, K., 2002. Irrigation and salinity: a perspective review of the salinity hazards of irrigation development in the arid zone. Irrig. Drain. Syst. 16, 161–174.

Soil Survey Staff, 1996. Soil Survey Laboratory Methods Manual. Soil Survey Invest. Rep. 42. USDA-NRCS, Natl. Soil Survey Center, Lincoln, NE.

Soil Survey Staff, 2006. Keys to Soil Taxonomy, 10th ed. USDA-NRCS. U.S. Gov. Print. Office, Washington, DC.

Spears, J.W., 2003. Trace mineral bioavailability in ruminants. J. Nutr. 133, 1506S–1509S.

Stefanon, B., Pell, A.N., Schofield, P., 1996. Effect of maturity on digestion kinetics of water-soluble and water-insoluble fractions of alfalfa and brome hay. J. Anim. Sci. 74, 1104–1115.

Suyama, H., Benes, S.E., Robinson, P.H., Grattan, S.R., Grieve, C.M., Getachew, G., 2007a. Forage yield and quality under irrigation with saline-sodic drainage water: greenhouse evaluation. Agric. Water Manage. 88, 159–172.

Suyama, H., Benes, S.E., Robinson, P.H., Getachew, G., Grattan, S.R., Grieve, C.M., 2007b. Biomass yield and nutritional quality of forage species under long-term irrigation with saline-sodic drainage water: field evaluation. Anim. Feed Sci. Technol. 135, 329–345.

USEPA, 1994. Method 3015-microwave assisted acid digestion of aqueous samples. In: Test Methods for Evaluating Solid Waste, 3rd ed. USEPA (p. 26).

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods of dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74, 3583–3597.

Yermiyahu, U., Tal, A., Ben-Gal, A., Bar-Tal, A., Tarchitzky, J., Lahav, O., 2007. Rethinking desalinated water quality and agriculture. Science 318, 920–921.

Yermiyahu, U., Ben-Gal, A., Keren, R., Reid, R.J., 2008. Combined effect of salinity and excess boron on plant growth and yield. Plant Soil 304, 73–87.