Response of alfalfa (*Medicago sativa* L.) to diurnal and nocturnal saline sprinkler irrigations.

I: total dry matter and hay quality

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Abstract

Little information is available on the quantitative effects on crops of saline sprinkler irrigations and the presumable beneficial effects of nocturnal versus diurnal irrigations. We measured crude protein content, carbon isotope discrimination and total dry matter of alfalfa (*Medicago sativa* L.) subject to diurnal and nocturnal saline sprinkler irrigations. The work was carried out along the 2004 to 2006 growing seasons with a Triple Line Source sprinkler system using synthetic saline waters dominated by NaCl with an EC ranging from 0.5 to 5.6 dS m^{-1}. The quality of alfalfa hay assessed through its crude protein concentration was not significantly affected by salinity. Carbon isotope discrimination, an indicator of the effect of osmotic stress on plant water status, tended to decrease with increases in salinity. Based on a piecewise linear response model, alfalfa grown under high-frequency saline sprinkler irrigation was shown to be more tolerant (threshold ECe = 3.5 dS m^{-1}) than under low-frequency surface irrigation (threshold ECe = 2.0 dS m^{-1}) at relatively low salinity values, but became more sensitive (slopes of -13.4 % and -7.3 % for sprinkler and surface irrigation) at higher salinity values. No significant differences in total dry matter were found between diurnal and nocturnal saline sprinkler irrigations. The recommended practice of irrigating at night in saline sprinkler irrigation is therefore not supported by our results in alfalfa grown under semiarid conditions.

Keywords: alfalfa, soil salinity, sprinkling irrigation, saline water, carbon isotope discrimination, protein content

Introduction

Irrigated agriculture is required in most arid and semi-arid areas to satisfy the food and fibre needs of an expanding world population. Nevertheless, decreased water resources and increased competition for good quality waters among different users is forcing to irrigate with waters of marginal quality. This situation will be exacerbated with the increasing reuse for irrigation of low-quality drainage and waste waters (Tanji and Kielen 2002).

The development of new irrigated areas and the modernization of old irrigation schemes is taking place in the Middle Ebro River Basin (Spain) and other areas around the world through pressurized systems, in particular above-canopy sprinkler systems. This development will continue in the future because these systems have inherent high irrigation efficiencies, are easily automated and are cost-effective in terms of labour.

However, the use of low-quality waters in above-canopy sprinkler systems poses the potential problems of leaf salt absorption, specific ion toxicity and decreased yields (Bernstein and Francois 1975). The deleterious effects of saline sprinkling irrigations on crop yield are not well documented, and few field works in a limited number of crops have been carried out (Bernstein and Francois 1975; Hoffman et al. 1983; Isla et al. 1997). Most reports are based on studies that demonstrate the higher accumulation of Na^+ and Cl^- in plant tissues exposed to sprinkling saline waters (Francois and Clark 1979; Grattan et al. 1994; Grieve et al. 2003; Maas et al. 1982ab), but only some studies report the corresponding losses in crop yields (Busch and Turner, 1967; Benes et al. 1996). Maas (1985) showed the lack of correlation in different crop species between foliar injury due to toxic ion accumulation and yield losses. Thus, the FAO guidelines (Ayers and Westcot 1985) report the irrigation water Na^+ or Cl^- concentrations causing foliar injury in various crops, but no information is given on corresponding yield decrements.

Several irrigation practices may potentially alleviate the detrimental effects of sprinkling with saline waters. Avoiding hot, dry and windy periods, irrigating at night, controlling sprinkler drift, increasing the sprinkler rotation speeds and the rate of application, and applying short pre- and post-irrigations with fresh water have been recommended to reduce foliar absorption and leaf damage (Ayers and Westcot 1985; Benes et al., 1996; Maas 1985). In particular, irrigating at night has been advocated as a beneficial practice because of lower temperatures, solar radiations and wind speeds, and higher relative humidity’s than at daytime. The corresponding lower evaporation rates at night would reduce the concentrations of the absorbed ions by the wetted leaves, potentially decreasing its deleterious effects on crop yield. However, this sensible approach has not been extensively documented, and only the work of

Busch and Turner (1967) in cotton demonstrated that night-sprinkled yields were significantly better than day-sprinkled yields.

Surface-irrigated alfalfa (*Medicago sativa* L.), one of the most important crops grown in the Middle Ebro River Basin (Spain), has been classified as moderately sensitive to salinity (threshold ECe = 2 dS m^{-1}) (Maas and Hoffman 1977), although recent studies indicate that it may produce higher than expected yields above this threshold value (Grattan et al. 2004). These discrepancies between different studies are not unusual since the specific conditions of the trials can greatly affect crop response. When sprinkler-irrigated using saline waters, Helalia et al (1996) found significant alfalfa yield decrements above those in surface-irrigated systems. However, the salinity tolerance of alfalfa under sprinkler irrigation and the advocated beneficial effects of the above mentioned irrigation practices have not been properly quantified.

The objectives of this study are (1) to determine under controlled field conditions the effects of sprinkling irrigations with saline waters on the quality and yield of alfalfa, and (2) to assess the potential yield benefits of nocturnal *versus* diurnal saline sprinkler irrigations. In a companying publication, leaf ion accumulation and its effect on yield is reported.

Materials and Methods

Experimental design and cultural conditions

The field trials were carried out during the 2004 to 2006 alfalfa growing seasons at the CITA experimental station located in the Middle Ebro River Basin (0º49’W, 41º44’N). The soil of the site is *Typic Xerofluvent* with a silty-clay-loam texture. The experiment was conducted using a triple line source sprinkler system (TLS) (Aragüés et al. 1992) consisting in three parallel sprinkler lines spaced 15 m apart, a distance equivalent to the wetted radius of the sprinkler. In our modified TLS, the two laterals divert saline water (ECiw = 4.5 to 5.6 dS m^{-1}, depending on years) while the central line diverts fresh water (ECiw = 0.4 dS m^{-1}). The central line consist of two parallel lines with half circle sprinklers that irrigate independently the left and right areas for the diurnal and nocturnal irrigation treatments, respectively (Fig. 1). The overlapping of the two laterals and the central sprinkler lines provides an even distribution in the discharge of irrigation water while creating an ECiw gradient at both sides of the central lines.

In 2004, the saline solution was made up with a mixture of sodium and calcium chloride with a final SAR of around 4. In 2005 and 2006 the sodium chloride was increased to provide a SAR of around 16-17. This increase in Na^+ was intended to better ascertain the potential toxic effect of Na^+ in alfalfa. Table 1 summarizes the general characteristics of the irrigation events. In the analysis of the effect of salinity on alfalfa yield, the seasonal-average EC of the applied water (ECaw; Table 1) is used rather than the seasonal-average ECiw to take into account the dilution effect of the seasonal rainfall. Based on the volumes of irrigation (I), rainfall (R) and alfalfa evapotranspiration (ET) (Table 1), the estimated leaching fractions [LF = (I + R – ET)/(I + R)] in the T1 treatments were 2% (2004), 20% (2005) and 26% (2006). These LF´s will increase as yield and ET decrease in the higher saline treatments.

Alfalfa (*Medicago sativa* L., cv. Aragón) was sown with a conventional driller at a seed rate of 35 kg ha^{-1} in March 23, 2004, before the start of the saline irrigations. Fourteen strips (1.55 m wide by 30 m long) were delineated parallel to the sprinkler lines, seven in the diurnal and seven in the nocturnal irrigated areas. These strips correspond to the seven irrigation water salinity treatments (T1 to T7) designated in this trial (Fig. 1). The alfalfa field was fertilized in the winter of each year with application rates of 85 (P_2O_5) and 400 (K_2O) kg ha^{-1}.

Irrigation scheduling

One to three 1.5 h irrigations were given per week to maintain soil water contents close to field capacity and to simulate the typical irrigation frequencies given to alfalfa in the Middle Ebro River Basin. The weekly estimations of alfalfa water needs (ETc = ETo · Kc) were calculated from the reference evapotranspiration (ETo) and the alfalfa coefficients (Kc) using the FAO methodology. The volume of irrigation water applied in each irrigation was measured in 14 pluviometers installed in the center of each strip or salinity treatment (Fig. 1). The volume and EC of rainfall was also measured to calculate the volume and ECaw of the total applied water.

The diurnal irrigations were given between 7:00 and 15:00 while the nocturnal irrigations were given between 1:00 and 5:00 (GMT time) using an irrigation programmer. The meteorological conditions during these time periods were significantly different (Table 2). The diurnal irrigations were given at higher average temperature, wind speed and solar radiation, and lower relative humidity than the nocturnal irrigations.

Water and soil analysis

After each irrigation event, the ECiw of the water collected in each of the 14 pluviometers was measured with a portable EC-meter. The diurnal ECiw were somewhat lower than the nocturnal ECiw due to the prevailing wind direction (Fig. 1) and the corresponding wind drift effect.

The apparent soil electrical conductivity was periodically measured (6, 8, and 10 times in 2004, 2005, and 2006, respectively) during the alfalfa growing period in each salinity treatment with an EM-38 electromagnetic sensor (Geonics Ltd., Ontario, Canada) placed on the ground in its horizontal dipole position. A total of six readings separated 3 m apart were taken in the middle of each salinity treatment. During each growing season, a variable number of points covering the entire range of the EM-38 readings were selected in two dates for calibration purposes. After reading of the EM-38 at each point, soil samples were taken at two depths (0-0.3 and 0.3-0.6 m) and the electrical conductivity of the soil saturation extract (ECe) was measured in the laboratory. The following calibration equations were obtained in each year:

2004: ECe = 4.52 EMh + 0.02, $R^2 = 0.53$, n = 19

2005: ECe = 5.02 EMh - 0.92, $R^2 = 0.71$, n = 60

2006: ECe = 3.56 EMh - 0.07, $R^2 = 0.74$, n = 37

where ECe is the mean of the 0-30 and 30-60 soil depths (dS m^{-1} at 25ºC), EMh is the apparent soil electrical conductivity (dS m^{-1} at 25ºC) measured by the EM-38, and n is the number of points.

Using these calibration equations, the seasonal-average EMh values for each T1-T7 salinity treatment in each year were converted into the corresponding ECe estimates. This estimated 0-60 cm soil depth seasonal-average ECe is the soil salinity index used in each treatment and year, and will be simply referred as ECe.

Figure 2 shows the relationships between ECaw and ECe in each experimental year. As expected, the slopes and intercepts of the regression equations tended to increase from 2004 to 2006 because of the progressive salinization of the soil. The three intercepts were not significantly different ($P > 0.05$), but the 2004 and 2006 slopes were significantly different ($P < 0.01$).

Crop measurements at harvest

Alfalfa was cut five times in 2004 and six times in 2005 and 2006, when about 30% of the plants were flowering. Three areas of 0.8 m^2 were randomly selected as replicates in the central part of each salinity treatment of the diurnal and nocturnal irrigation treatments (Fig. 1), and the total aboveground biomass was collected using a manual cutting machine that simulates a conventional alfalfa harvester. All the harvested material was rinsed three times in deionized water to remove salts and soil deposited in the plants, oven dried at 65ºC until constant weight and weighted. The yield of alfalfa was expressed in Mg ha^{-1} of total dry matter (TDM). Relative yield (%) was obtained dividing the actual yield in each saline treatment by the average yield of the two highest yields.

Plant analyses

A portion of the dry hay was finely ground using a 0.5-mm sieving miller. Total nitrogen in the alfalfa hay was analysed by the dry combustion method. A factor of 6.25 was used to convert total nitrogen to crude protein.

Samples for carbon isotope discrimination ($\delta^{13}C$, relative to Pee Dee Belemnite) were analyzed by an inductively coupled plasma mass spectrometry (ICP-MS) and converted into carbon isotope discrimination (Δ, expressed as $^0/_{00}$) assuming that the $\delta^{13}C$ for atmospheric CO_2 is $-7.85^0/_{00}$ relative to PDB.

Statistical analysis

The statistical analyses were performed using the SAS 9.1 software. Comparison of regression lines was made using an F-test, taking the root mean error (RME) of the overall regression as the error for the pairwise comparisons. The proc NLIN was used to estimate the non-linear models. The significance of the regression analyses was indicated as **, *, and NS for probability levels (P) of < 0.01, < 0.05, and > 0.05, respectively.

Results and Discussion

Effect of salinity on alfalfa total dry matter (TDM)

Applied water (ECaw) and soil (ECe) salinity tended to decrease alfalfa TDM in the diurnal (D) and nocturnal (N) treatments of the 2004-2006 experimental years (Fig. 3). As expected, TDM decreased more in 2005 and 2006 than in 2004 because of the higher ECaw (Table 1) and the build-up of ECe (Fig. 2) in the last two years. In 2004, the regressions of the diurnal and nocturnal treatments were not significant (P > 0.05). In 2005 and 2006, TDM in the nocturnal treatments significantly decreased with salinity, whereas it did not decreased in the diurnal treatments. Sprinkling with saline waters was therefore more detrimental at night than at day time, probably because of the higher soil salinity (ECe) developed in the nocturnal treatment for saline treatments with similar ECaw. Thus, ECe in the highest salinity treatments tended to be 14, 25, and 12 % higher in the nocturnal than in the diurnal treatments, whilst the EC of the applied water was very similar. These results are opposite to those found by Busch and Turner (1967) in cotton sprinkler irrigated with waters of EC = 4.4 dS m^{-1} and SAR = 17.8, where night-sprinkled yields were about 15-39% higher (depending on cultivar) than day-sprinkled yields. However, it should be noted that, contrary to our work, soil salinity at night was 12% lower than at day. Thus, both soil salinity and irrigation water salinity should be taken into account when crops are subject to both stresses.

In order to ascertain the accumulated response of alfalfa to salinity for the three years examined, the relative alfalfa TDM of the D and N treatments for the pooled 2004-2006 experimental years were regressed against ECaw and ECe (Fig. 4). These regressions were not significantly different ($P > 0.05$), indicating that irrigating at night was not beneficial against irrigating at daytime.

Since the D and N treatments were not different, the overall salinity tolerance of alfalfa for the 2004-2006 years was further determined by regressing the accumulated relative TDM of the pooled D and N treatments against the 2004-2006 average ECaw and ECe (Fig. 5). An outlier with an extremely high TDM value was deleted from these regressions. The fitting of relative TDM to ECaw was better using a linear model ($P < 0.05$) than a piecewise linear model ($P < 0.1$). Thus, a threshold ECaw could not be estimated from these observations. In contrast, relative TDM and ECe were significantly correlated ($P < 0.001$) using a piecewise model, with a threshold ECe of 3.5 dS m^{-1} (SE = 0.15 dS m^{-1}) and a 13.4 % yield decrement per unit increase in ECe.

These salinity tolerance values show that alfalfa is initially more tolerant to soil salinity when grown in high-frequency sprinkler systems (threshold ECe = 3.5 dS m^{-1}) than in low-frequency surface systems (threshold ECe = 2.0 dS m^{-1}; Maas and Hoffman, 1977). However, above the threshold ECe the yield decline per unit increase in salinity almost doubles under sprinkler (slope = -13.4%) than under surface irrigation (slope = -7.3%). Hence, under low to moderate saline waters the beneficial effect of high-frequency sprinkler irrigations seems to be more significant than the detrimental effect of leaf salt absorption (i.e., the threshold ECe is higher for sprinkler than for surface irrigation), whereas the opposite occurs for higher saline waters (i.e., above the threshold ECe, the yield decline per unit increase in salinity is higher for sprinkler than for surface irrigation systems.

Effect of salinity on the quality of alfalfa hay

Zhou et al. (1992) have described the detrimental effects of soil salinity on nodulation of *Rhizobium* in alfalfa. Other works (Serraj et al. 1998; Cordovilla et al. 1999) have shown the inter-specific variability in the salinity tolerance of the N-fixation process and the higher tolerance of alfalfa compared to other legumes. Crude protein is a frequently used quality parameter in alfalfa hay because of its significance in animal nutrition. The effect of applied water salinity (ECaw) on crude protein content of the pooled alfalfa cuts was not significant ($P > 0.05$), except in the 2006 nocturnal treatment which showed a significant ($P < 0.01$) increase in crude protein with increasing ECaw (Fig. 6). Similar results were obtained with ECe (data not given). The corresponding analysis for the yearly individual cuttings showed that only three out of the eighteen regressions (i.e., nine cuts x two irrigation treatments) were significant, corroborating that crude protein is independent of ECaw. The small increase in crude protein in 2006 could be explained by an increase in the leaf/steam ratio, as described by Al-Khatib et al. (1993) in alfalfa grown in pots under NaCl treatments.

Using the average plant N concentration and the aboveground TDM, the total N exported by alfalfa ranged from 520 to 550 kg N ha^{-1} $year^{-1}$. Since no N fertilizer was applied to alfalfa and the soil of the experimental plot was relatively low in organic matter (1.8% in the upper 0-35 cm of the soil profile), these high N exports indicate that alfalfa N-fixation was not affected by the salinity values imposed in this trial.

Effect of salinity on alfalfa hay carbon isotope discrimination (Δ)

Carbon isotope discrimination (Δ) in C_3 plants is linearly related to the (p_i/p_a) ratio, where p_i and p_a are, respectively, the intercellular and the atmospheric partial pressures of CO_2. This ratio depends on the leaf stomatal conductance and photosynthetic capacity of a given plant and, therefore, on genetic and environmental factors. Farquhar et al. (1989) concluded that low Δ values are generally associated with low stomatal conductance values. Brugnoli and Lauteri (1991) with cotton and bean, Johnson (1991) with Agropyron, and Isla et al. (1998) with barley found a decrease in Δ of plants subject to salt stress compared to plants grown in non-saline conditions. These results are consistent with the well known detrimental effects of osmotic stress on plant water status.

The average Δ values of alfalfa subject to low (ECe < 3.5 dS m^{-1}) and high (ECe > 3.5 dS m^{-1}) soil salinity values are shown in Fig. 7 for seven cuts performed along 2004 to 2006. Both sampling time and soil salinity had a significant effect ($P < 0.01$) on Δ. Differences among sampling dates may be associated to differences in meteorological conditions and its effect on vapour pressure deficit and, therefore, on stomatal conductance.

Δ was consistently lower in the high than in the low salinity plots, although only in four sampling dates these differences were significant ($P < 0.05$). The observed differences in Δ between the low and high saline plots ranged between 0.09 and 0.85 $^0/_{00}$, with an average value of 0.32 $^0/_{00}$ which is quantitatively small considering that the mean absolute deviation within one randomly selected sample was 0.14 $^0/_{00}$. Finally, alfalfa hay Δ and ECe were significantly correlated ($P < 0.01$) only in 2006, when soil salinity increased due to the saline sprinklings given in 2004 to 2006. These results indicate that soil salinity values up to about 5 dS m^{-1} had a minor effect on Δ and on the water status in alfalfa, probably due to the beneficial effect derived from the high irrigation frequency in this experiment.

Conclusions

The recommended practice of irrigating at night instead than at daytime in saline sprinkler irrigation has been advocated on the basis that the lower nightly evaporation rates will reduce the concentration of the absorbed ions (mainly Na^+ and Cl^-) by the wetted leaves (Ayers and Westcot, 1985; Busch and Turner, 1967). However, the accumulated total dry matter response of alfalfa to saline sprinkling irrigation for the three years examined in our trial was not significantly different in the diurnal and nocturnal treatments. Therefore, irrigating at night was not beneficial in alfalfa grown under our semiarid climatic conditions.

Alfalfa grown under low-frequency surface irrigation has been classified as a moderately sensitive crop to salinity (threshold ECe = 2 dS m^{-1}, slope = -7.3 %) (Maas and Hoffman, 1977). Our results for high-frequency saline sprinkler irrigation show that alfalfa is more tolerant to soil salinity (threshold ECe = 3.5 dS m^{-1}) at relatively low salinity values, but the yield decline per unit increase in ECe above the threshold (slope = -13.4 %) almost doubles that under surface irrigation. Hence, under low to moderate saline waters, high-frequency sprinkler irrigation will increase the tolerance of alfalfa because of the lower water stress and lower evapo-concentration than in low-frequency surface irrigation (Ayers and Westcot 1985). In contrast, for higher saline waters, the detrimental effects of leaf salt absorption and the subsequent accumulation of toxic ions (i.e., Na^+ and Cl^-) by the wetted leaves in saline sprinkling irrigations (Maas 1985) will decrease the tolerance of alfalfa as compared to surface irrigation systems. This conclusion is substantiated in a companying paper.

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Table 1 General characteristics of the irrigation events given in the 2004 to 2006 experimental years of the alfalfa trial.

Experimental year	2004	2005	2006
Number of irrigations	43	39	38
First saline irrigation	May 28	May 10	May 11
Last saline irrigation	October 6	October 5	October 10
Seasonal irrigation (mm)	571	851	821
Seasonal rainfall (mm)	103	133	223
Seasonal alfalfa evapotranspiration (mm)	663	787	778
T1 – T7 ECaw interval [a] (dS m^{-1})	0.4 – 4.4	0.4 – 4.7	0.4 – 4.3

[a] Electrical conductivity of applied water = volume-weighted average of irrigation EC plus rainfall EC

Table 2 Mean temperature (T), relative humidity (RH), wind speed (WS) and solar radiation (SR) measured in the diurnal and nocturnal sprinkler irrigations in the 2004 to 2006 experimental years of the alfalfa trial. The standard deviations of the means are given in parenthesis.

Year	Sprinkler Irrigation	T (ºC)	RH (%)	WS (m s^{-1})	SR (w m^{-2})
2004	Diurnal	27.2 (4.6)	45.9 (14.1)	2.4 (1.3)	616 (196)
	Nocturnal	17.9 (4.4)	77.1 (12.2)	1.5 (1.5)	154 (172)
2005	Diurnal	24.8 (4.0)	48.7 (12.0)	2.3 (1.2)	690 (167)
	Nocturnal	16.3 (3.2)	77.9 (9.4)	1.2 (0.9)	20 (72)
2006	Diurnal	21 (4.7)	57.3 (14.8)	2.4 (1.7)	446 (235)
	Nocturnal	16.8 (4.6)	72.1 (14.7)	1.7 (1.2)	102 (229)

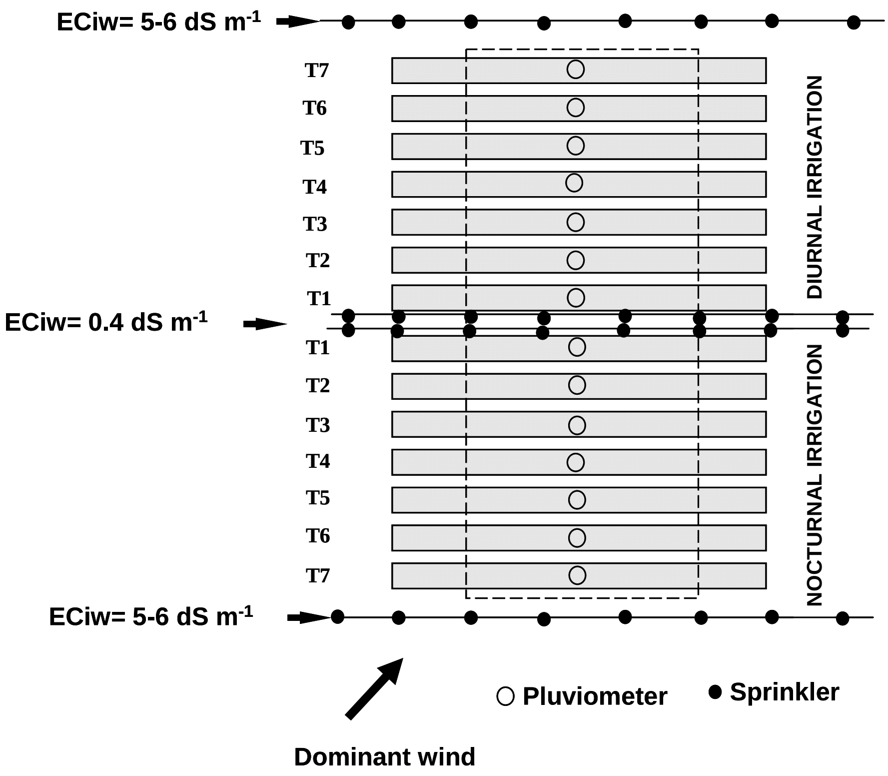

Fig. 1 Diagram of the triple line source sprinkler system: salinity of irrigation waters (ECiw), diurnal and nocturnal irrigation areas, and imposed T1 (low ECiw) to T7 (high ECiw) salinity treatments. The dotted lines delineate the area where the alfalfa was harvested

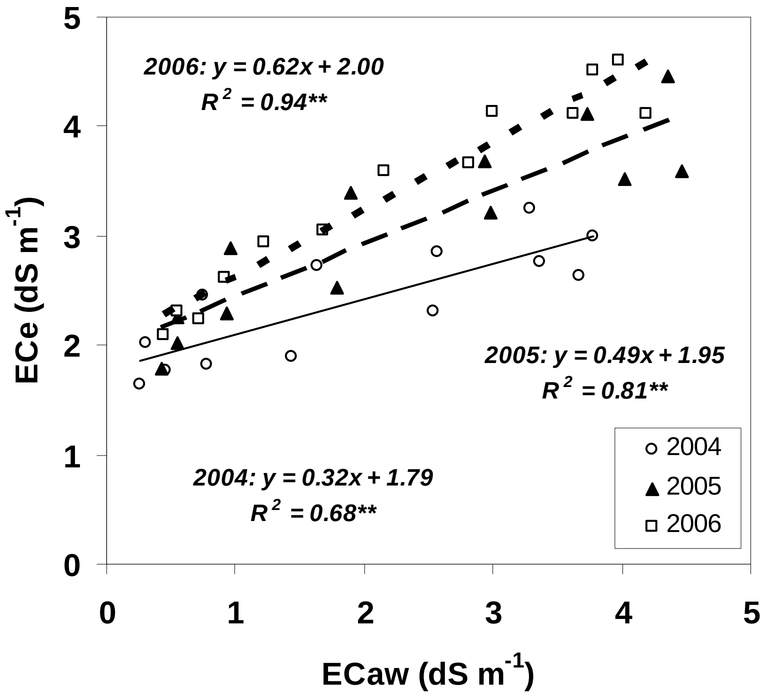

Fig. 2 Relationships between the seasonal-average salinity of the applied water (ECaw) and the estimated seasonal-average soil salinity (ECe) in the 2004 to 2006 experimental years

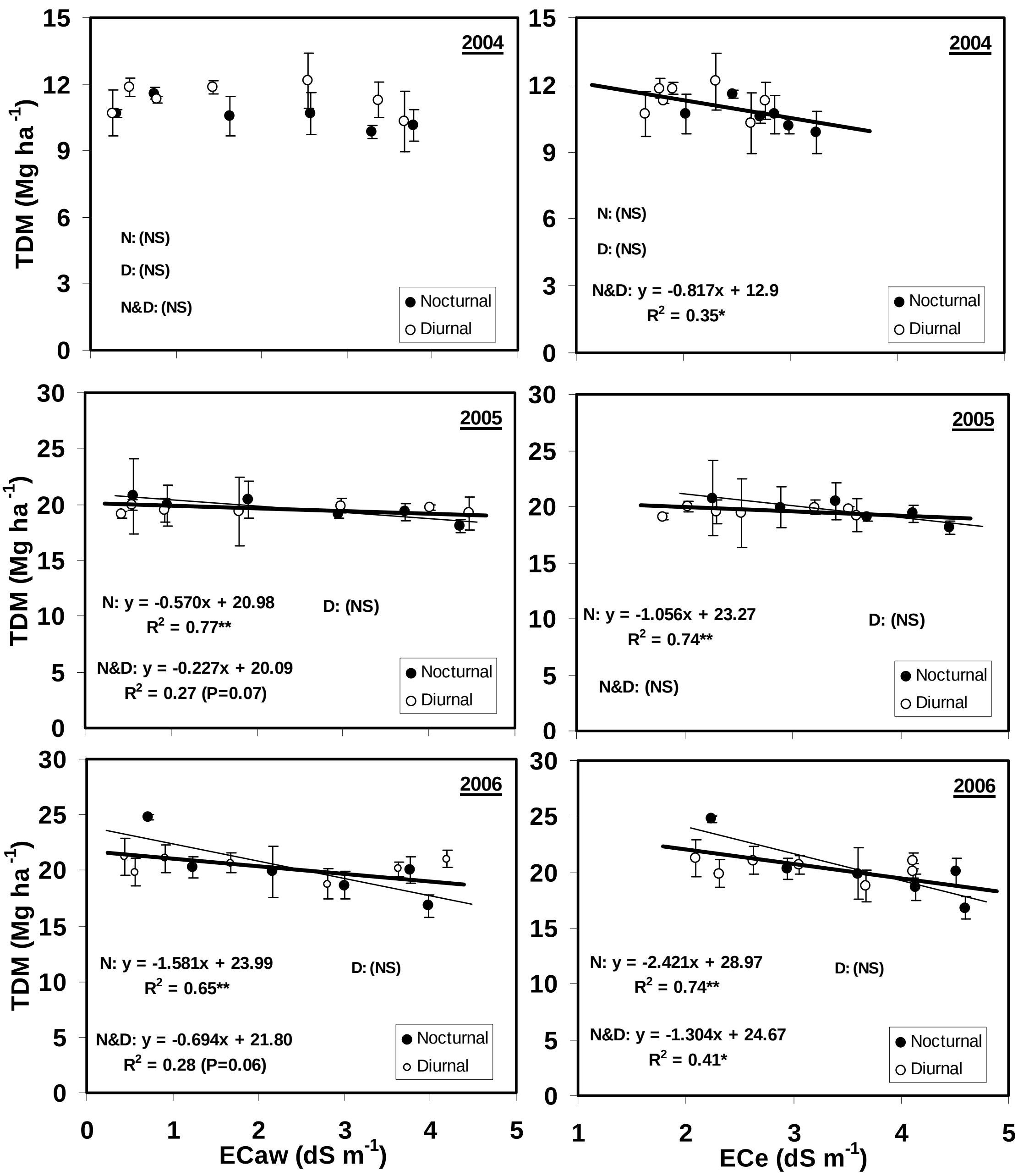

Fig. 3 Effect of applied water (ECaw) and soil (ECe) salinity on alfalfa total dry matter (TDM) harvested in the diurnal (D) and nocturnal (N) irrigation treatments of the 2004 to 2006 experimental years. Vertical bars represent the standard deviation of the mean. When significant ($P < 0.05$), the linear regressions of the D (dotted line), N (thin line) and N&D (thick line) treatments are presented

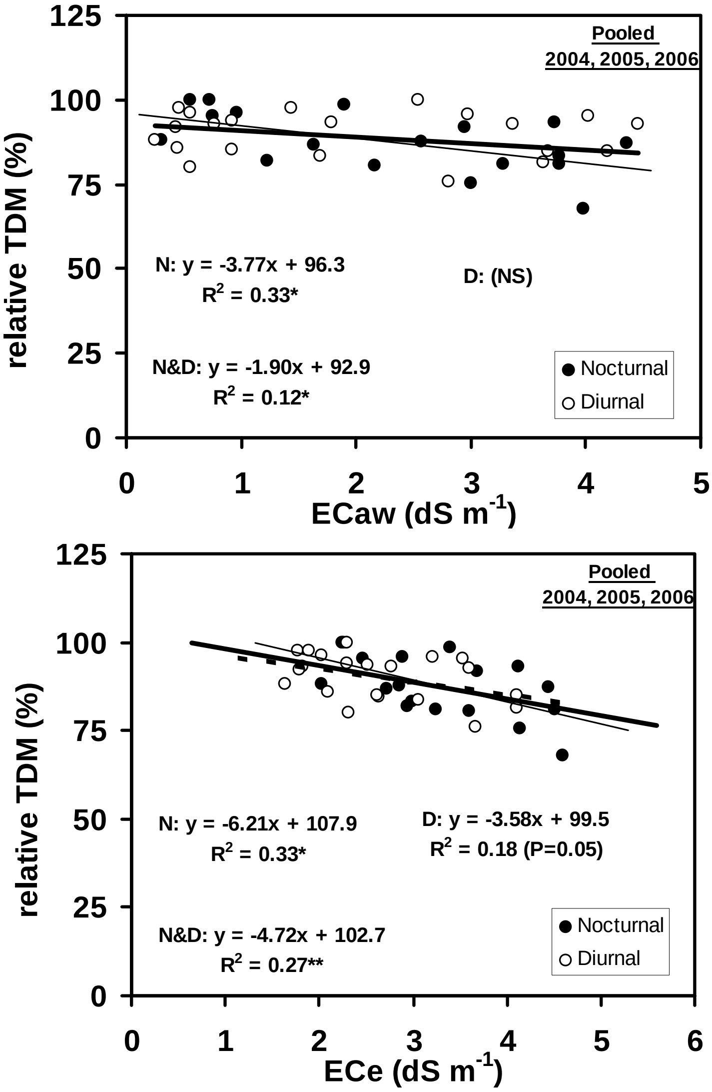

Fig. 4 Effect of applied water (ECaw) and soil (ECe) salinity on relative alfalfa total dry matter (TDM) of the diurnal (D) and nocturnal (N) treatments for the pooled 2004 to 2006 experimental years. When significant ($P \leq 0.05$), the linear regressions of the diurnal (D, dotted line), nocturnal (N, thin line) and N&D (thick line) treatments are presented

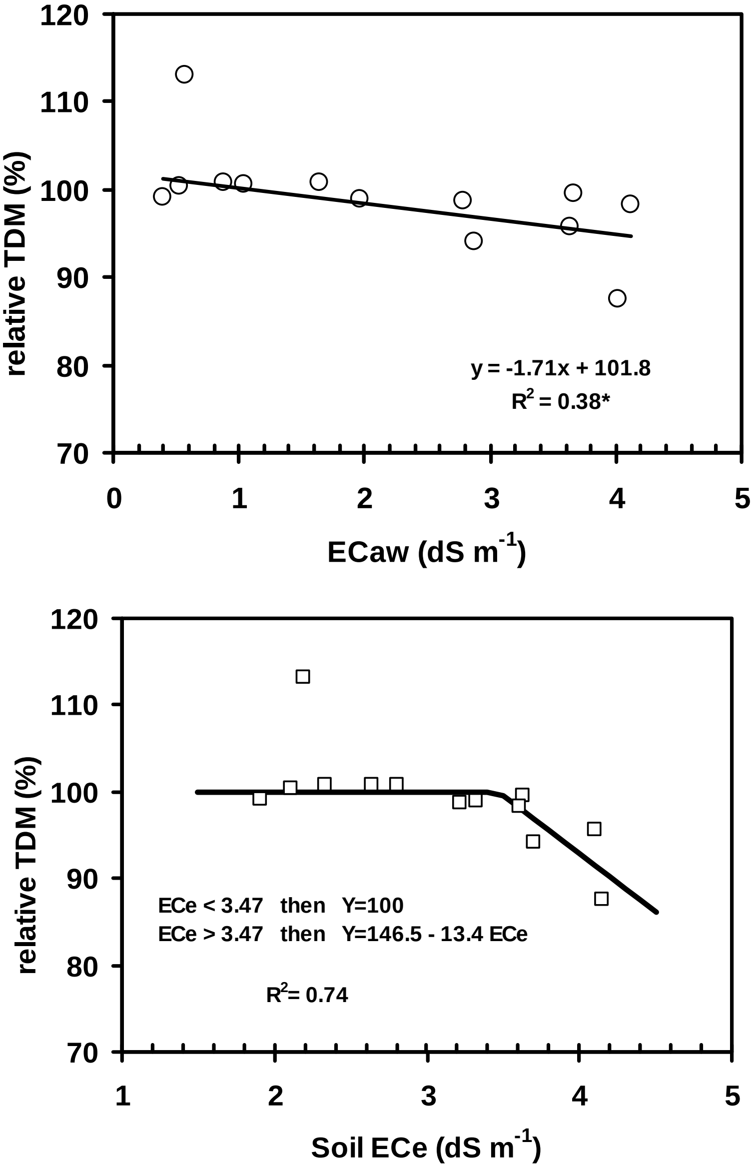

Fig. 5 Relationships between the 2004 to 2006 relative accumulated alfalfa total dry matter (TDM) and the 2004 to 2006 average applied water (ECaw) and soil (ECe) salinity. Relative TDM and ECaw were regressed through a linear model and relative TDM and ECe through a piecewise linear model

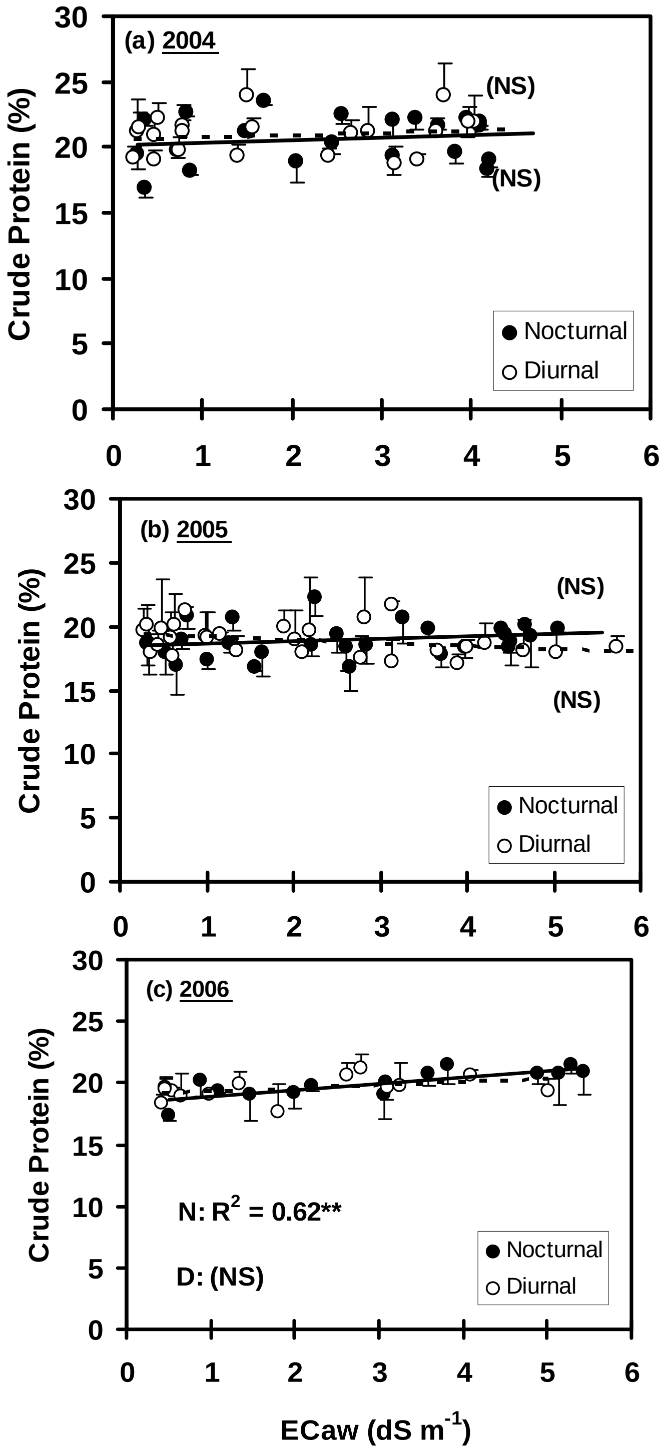

Fig. 6 Effect of applied water salinity (ECaw) on crude protein of alfalfa hay harvested in the diurnal (D) and nocturnal (N) irrigation treatments of the 2004 to 2006 experimental years. The yearly cuts were pooled together. When significant ($P < 0.05$), the linear regressions of the diurnal (dotted line) and nocturnal (thin line) treatments are presented. Vertical bars represent the standard deviation of the mean

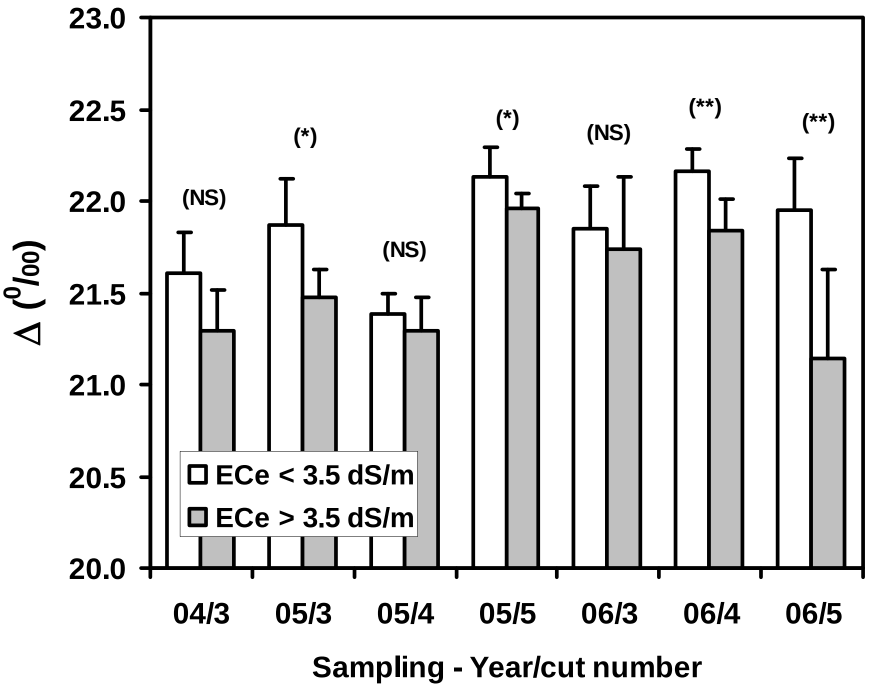

Fig. 7 Average values of alfalfa hay carbon isotope discrimination (Δ, $^{0}/_{00}$) measured at seven sampling times. A pairwise comparison between low (ECe < 3.5 dS m^{-1}) and high (ECe > 3.5 dS m^{-1}) soil salinity was performed for each sampling date. Vertical bars represent the standard deviation of the mean