Seasonal water-use efficiency and chlorophyll fluorescence response in alpha grass (*Stipa tenacissima* L.) is affected by tussock size

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Abstract

Twelve randomly chosen *Stipa tenacissima* L. individuals were grouped into three tussock size classes, small (ST), medium (MT), and large (LT) with 5.6±0.8, 34.1±4.2, and 631.9±85.8 g of dry green foliar matter, respectively, in three plots with different *S. tenacissima* cover. Instantaneous (WUE_i) and long-term (WUE_l) water-use efficiencies were measured in two seasons of contrasting volumetric soil water content (early winter 21.0±0.8 % and summer 5.8±0.3 %). Maximum photochemical efficiency of photosystem 2 and stomatal conductance in summer assessed the extent of water and irradiance stress in tussocks of different size. WUE_i was lower in MT and ST "water spender" strategies than in LT during the high water-availability season. In summer net photosynthetic rate and WUE_i were higher and photoinhibition was lower in LT than in MT and ST. Significant spatial variability was found in WUE_i. Water uptake was competitive in stands with denser alpha grass and more water availability in summer, reducing their WUE_i. However, WUE_l showed a rising tendency when water became scarce. Thus it is important to explicitly account for plant size in ecophysiological studies, which must be combined with demographic information when estimating functional processes at stand level in sequential scaling procedures.

Additional key words: leaf area index; net photosynthetic rate; photoinhibition; photosystem 2; plant size; semiaridity; stomatal conductance; transpiration rate; water stress.

Introduction

Arid and semiarid Mediterranean vegetation in Northwest Africa and the Iberian Peninsula is dominated by alpha grass (*Stipa tenacissima* L.) steppes covering an estimated surface area of 60 000–80 000 (Le Houérou 1986) and 6 000 km^2 (Servicio del Esparto 1950), respectively, where this species can be more than 90 % of total vegetation cover in some stands (Maestre *et al.* 2007). *S. tenacissima* is a rhizomatous perennial tussock grass capable of quick gas exchange, chlorophyll (Chl) concentration and fluorescence, and xanthophyll-cycle pigment responses to rainfall (Pugnaire and Haase 1996, Pugnaire *et al.* 1996, Haase *et al.* 1999, Balaguer *et al.* 2002) and non-rainfall soil water gains (Ramírez *et al.* 2007a). This opportunistic response to erratic water pulses, along with a photo-protective crown architecture based on vertical green leaves and the accumulation

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Received 23 October 2007, *accepted* 19 December 2007.

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Acknowledgments: We thank Adela Blasco for her assistance with ecophysiological assessments. Financial support was provided by the "Efecto de la cubierta vegetal en el balance hídrico y en la disponibilidad y calidad del agua, propuesta para aumentar la recarga de acuíferos" Project, funded by the CICYT (REN2000-0529HID, CGL2004-03627) and by "AQUADAPT: Energy, Environment and Sustainable Development" Project founded by the European Community (EVK1-2001-00149) and the PROBASE (CGL2006-11619/HID) and CANOA (CGL2004-04919-C02- 01/HID) projects funded by the Spanish Ministry of Education and Science. D.A.R. was at the EEZA on a visiting researcher grant from the *Junta de Andalucía* regional government. The authors thank Deborah Fuldauer for correcting and improving the English of the text.

of dry biomass in the outer crown (Valladares and Pugnaire 1999) allow this species to flourish in harsh, arid environments.

Asexual reproduction was previously considered the main *S. tenacissima* space occupation strategy (White 1983, Le Houérou 1986). However, other studies have since shown that population recruitment by sexual reproduction is also an important mechanism of space colonization in this species (Gasque 1999), which is able to form transient seed banks (Gasque and García-Fayos 2003). Sexual reproduction gives rise to structured populations of alpha grass, in which the stand biomass is distributed among tussocks of different cohorts in non-overgrazed stands (Gauquelin *et al.* 1996). Recent studies analysing stand-scale structural and functional properties in *S. tenacissima* steppes have found that variables such as tussock size and mean distance from each other, as well as richness and spatial pattern of perennial shrubs are significantly related to ecosystem functional attributes (Maestre and Cortina 2004, 2006). Scale-up of ecophysiological variables to stand requires the explicit consideration of population structure, because physiology varies with plant size and ontogenetic stage (*e.g.* Zotz 1997, Schmidt *et al.* 2001, Matzner *et al.* 2003, Vesk 2006). Ramírez *et al.* (2007b) scaled *S. tenacissima* transpiration from the individual to the stand, taking into account different tussock-size classes, and found good correspondence between scaled stand transpiration and stand transpiration calculated with a multi-source evaporation model. Further studies under natural conditions are required to assess other crucial ecophysiological variables related to carbon sequestration and photoinhibition in these steppes. These studies should take into account recent advances in scaling from leaf to individual in both potted and 3-D reconstructed *S. tenacissima* tussocks (Valladares and Pugnaire 1999, Ramírez *et al.* 2006, 2008).

In this study, a semiarid *S. tenacissima* steppe with different tussock densities and water availabilities was studied to find variation ranges and sources of factors determining alpha grass water use and photochemical efficiencies. We hypothesize that differences in tussock ecophysiological response are induced by plant size and are mediated by seasonal development and spatial variability in water availability. Differences according to plant size are expected because tussock aging involves leaf litter accumulation in the crown, where micrometeorological conditions create a "shelter effect" (*sensu* Domingo *et al.* 1996) in the large tussocks (old), decoupling them from the surroundings more than small tussocks (seedling). It is also hypothesised that small plants are more vulnerable to water stress due to their small shallow root system. The results of this study could be used in a near future to carry out sequential scaling of carbon assimilation and photoinhibiton in semiarid steppes dominated by alpha grass.

Materials and methods

Field site: Our study area is a south-facing sub-catchment (19 ha) located in the Ventós-Castelar aquifer recharge area (38º28'N, 0º37'W) in the "El Ventós" catchment near Agost, Alicante, Spain. The minimum and maximum altitudes are 479 and 800 m a.s.l., respectively, and the slope, with SE and SW aspects, ranges from 37 to 73 %. The soil is calcareous regosol (FAO-UNESCO 1988) with a silt loam texture. *S. tenacissima* is the predominant vegetation with 42.97±2.10 % of total plant cover (Ramírez 2006). The climate is "Mediterranean" and "semiarid" (Köppen and Thornthwaite classification, Guijarro 1981) with scant, highly variable precipitation, and higher temperature and water deficit in the summer months (July and August, see Fig. 1). The average yearly precipitation is 269.6 mm and temperature 17.4 ºC (see Ramírez *et al.* 2007a for a detailed description of the soil and meteorological features).

Experimental design: Five 100-m^2 plots in three *S. tenacissima* cover groups in the sub-catchment (Table 1) were sampled. The individuals in the plots were divided in three tussock-size classes defined by their mean cover diameter (Ø) and called large (LT, 0.60 m≤Ø), medium (MT, 0.30 m≤Ø<0.60), and small (ST, Ø≤0.30 m). Distribution of individuals into tussock-size classes was different in each cover group CG, whereby distribution of individuals was more regular in plots with denser *S. tenacissima* cover (CG I), while CG III had a large number of small individuals (Table 1).

Our research was carried out in two periods with different water availability. The wet season corresponded to early winter 2004 (21–23 January), which was preceded by a rainy autumn 2003 (145.3 mm rainfall in October through December 2003, 48.5 % of that year's total precipitation, Fig. 1). The plots used in this early winter season were: 1, 3, and 5 (Table 1). The second period, summer 2004 (10–12, 17–19 August), was

Table 1. Total *Stipa tenacissima* cover in the plots assessed and frequency of total individuals in each tussock-size class (LT 0.60 m≤Ø, MT 0.30 m≤Ø<0.60 m, and ST Ø<0.30 m). CV = variability coefficient [%] of the number of individuals in each tussock-size class.

Group		Total cover	Frequency [%] LT	MT	ST	CV
I	Plot 1	38.6	39.5	23.3	37.2	26.3
	Plot 2	41.4	24.3	25.4	50.3	44.1
II	Plot 3	19.0	30.0	16.7	53.3	55.6
	Plot 4	18.1	47.6	9.5	42.9	62.3
III	Plot 5	8.9	21.7	4.3	73.9	108.8

Table 2. Hourly average of the meteorological variables measured from 06:00 to 18:00 h solar time (±S.E.) on the days when the IRGA was measured in each cover group (CG). T° = temperature [°C], RH = relative atmospheric humidity [%], PPFD = photosynthetic photon flux density [µmol m^{-2} s^{-1}]. The letters in each variable mean significant differences among assessed days as detected by Mann-Withney U test (at $p<0.05$). + Sensor was damaged.

		CG I (21 Jan. 2004)	CG II (23 Jan. 2004)	CG III (22 Jan. 2004)
Early Winter	T°	11.7 a (0.5)	+	12.2 a (0.4)
	RH	55.9 a (0.1)	57.0 b (0.1)	55.7 a (0.1)
	PPFD	658.3 a (70.7)	543.2 a (61.8)	657.5 a (70.4)
		CG I (12 Aug. 2004)	CG II (10 Aug. 2004)	CG III (11 Aug. 2004)
Summer	T°	30.8 a (0.4)	28.6 b (0.4)	29.4 ab (0.7)
	RH	+	+	+
	PPFD	937.4 a (66.3)	1058.2 a (74.9)	1118.8 a (90.6)

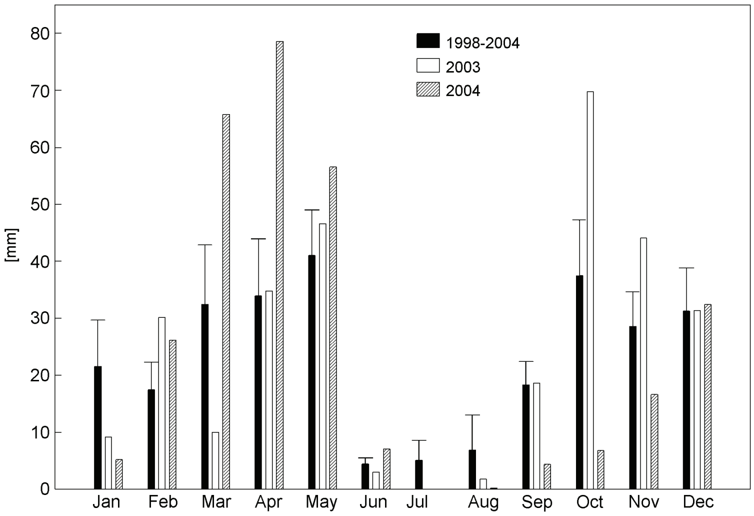

Fig. 1. Total monthly rainfall in 2003 and 2004. *Black bars* are the average monthly precipitation calculated from 7 years' records (1998–2004) from the "Ventós 2" meteorological station (38°27'N, 0°37'W) in our study area.

characterized by high water stress (0.2 mm per month, Fig. 1), and the plots assessed were: 2, 4, and 5 (Table 1).

Gas exchange measurements: We chose 4 individuals at random from each tussock-size class and plot. Gas exchange (net photosynthesis rate, P_N; transpiration rate, E; and total water vapour conductance, g_t) was measured by an infrared gas analyser (*LI-COR 6400*; *LI-COR Biosciences*, USA). Instantaneous water-use efficiency (WUE_i) was estimated by dividing P_N by E. The stomatal conductance (g_s) from IRGA measurements was calculated taking the characteristic hypostomatous *S. tenacissima* leaf into consideration (Field *et al.* 1989):

$$g_s = 1/[(1/g_t) - (2/g_b)] \qquad (1)$$

where g_b is boundary layer conductance calculated by IRGA software.

We marked 3–4 leaf sections taped together to be able to measure the gas exchange in the same leaf-section area at three times: 08:00, 12:00, and 16:00 solar time. The sampling design for the total number of leaf sections (samples) measured in each tussock-size class was: LT 4 cardinal points (NE, NW, SE, and SW) × 2 tussock sectors (in the middle and on the periphery) × 4 individuals = 32 *n*; MT 5 samples (4 cardinal points + 1 in the middle of the tussock) × 4 individuals = 20 *n*; ST 3 samples (1 in the North + 1 in the South + 1 in the middle of the tussock) × 4 individuals = 12 *n*.

In order to keep micro-environmental conditions the same in all the leaf sections assessed in each plot, we set the same photosynthetic photon flux density (PPFD) in the IRGA chamber as the average PPFD at 08:00, 12:00, and 16:00 h solar time in the month the study took place, that is 425, 890, 100 µmol m^{-2} s^{-1} and 940, 1 999, 998 µmol m^{-2} s^{-1} in the study area in early winter and summer, respectively. Air flow rate (500 µmol s^{-1}), reference CO_2 concentration [370 µmol(CO_2) mol^{-1}], and relative humidity (30 %) were also set in the IRGA chamber.

Micrometeorological and soil moisture measurements: The abovementioned IRGA measurements in each plot were taken on continuous days to minimize daily variability in the weather. To analyse this, one PPFD (*HA-LI HOBO*, *Onset Computer Corporation*, USA) and one air temperature and atmospheric humidity sensor (*HOBO*

Pro RH/Temp. model, *Onset Computer Corporation*, USA) were installed in each plot. Internal sensor data loggers recorded output every 5 min. The days evaluated in each cover group (CG) in the early winter season showed no significant differences in the average hourly PPFD, however, a few cloudy periods and more atmospheric relative humidity (57.0±0.1 %) were detected on 23 January (when Plot 3 in CG II was assessed) than the other days (Table 2). In summer, the average hourly PPFD on evaluated days was similar (no significant differences were detected, Table 2), but the 12 August (30.8±0.4 ºC) was hotter than the 10 August (28.6±0.4 ºC).

For measuring the PPFD in the individuals in the three tussock-size classes assessed, 4 PPFD sensors (*HA-LI HOBO*, *Onset Computer Corporation*, USA) were installed, 1 sensor in each of the 3 tussock-size classes and the fourth outside the tussocks. The PPFD sensors were located in the middle of the tussock half-way down from the maximum height of each tussock assessed. The internal data loggers recorded output every 2 min. The measurements were taken on one clear day and one cloudy day (14 and 15 March 2003, respectively).

Volumetric soil water content (θ) in the plots was measured by Time Domain Reflectometry (TDR) (model *TDR 100*, *Campbell Scientific*, UK) in 10 pairs of steel rods (0.1 m long) randomly inserted in the soil. The following equation fitted by Chirino (2003) was used to calculate θ in our study area: $\theta = -5.41 + 3.06\ K - 0.10\ K^2 + 0.02\ K^3$; $r^2 = 0.99$; $p<0.01$, where K is the dielectric constant recorded by the TDR. In the wet and dry periods (early winter and summer) the total average θ (±SE) was 21.0±0.8 and 5.8±0.3 %, respectively.

Chl fluorescence measurements: As in Maestre and Cortina's (2006) work in assessing the physiological performance of *S. tenacissima* stands, we consider summer the best time to compare ecophysiological responses by tussock size, because the individuals are subjected to maximum water and irradiance stresses. We therefore widened our ecophysiological examination by adding two variables measured on three continuous days in each plot (17–19 August). A diffusion porometer (model *AP4*, *Delta T Devices*, Cambridge, UK) was used to measure g_s at 09:00, 13:00, and 17:00 h solar time in a total of 32 samples taken using the following sampling design: LT and MT 3 samples (1 in the North + 1 in the South and + 1 in the middle of the tussock) × 4 individuals = 12 *n*; ST 2 samples (1 in the North + 1 in the South of the tussock) × 4 individuals = 8 *n*.

In vivo Chl fluorescence was measured by a portable pulse-amplitude modulation fluorometer (model *PAM-2000*, *Walz*, Effeltrich, Germany) at 05:00 and 12:00 solar time. For this purpose (as for IRGA measurements), and to avoid a leaf aging effect in the water stress assessment in Chl fluorescence analysis (Nesterenko *et al.* 2006), when comparing tussock-size classes, 3–4 leaf sections (taped together) taken from the middle of both young and mature leaves were assessed (following the age leaf classification of Haase *et al.* 1999 in *S. tenacissima*). These are the most photosynthetically active sections of the foliar tissue (in this species, leaves age from the tip of the lamina to the base). Ramírez *et al.* (2008) detected no significant differences in photosynthesis-irradiance curve for leaf sections from the three tussock sizes under optimal water conditions. 24 samples from each tussock-size class were distributed according to the following sampling design: LT and MT 2 samples (one in the North + one in the South) × 4 individuals = 8 *n*; ST 2 random samples × 4 individuals = 8 *n*.

We estimated the maximum photochemical efficiency of photosystem 2, PS2 (F_v/F_m) at dawn and at midday by: $F_v/F_m = (F_m - F_0)/F_m$, where F_0 = minimal fluorescence yield as measured at weak irradiance after adaptation to the dark, F_m = maximal fluorescence yield after adaptation to the dark. To assess F_v/F_m at midday, the sampled leaves were pre-adapted to the dark one hour before measurement.

Leaf area index of green foliage (LAI_{green}): Target plant LAI_{green} was measured by non-destructive methods using different protocols depending on tussock-size classes. Eight metal parallelepipeds (0.01 m^2 and 0.3 m high) were inserted randomly into LT (32 *n*). The leaf area in the parallelepipeds was calculated by an allometric function fitted by Ramírez *et al.* (2006): $y = 0.982\ x + 0.063$, ($n = 100$, $r^2 = 0.94$, $p<0.05$), where x is the leaf area calculated by multiplying length by width and y is the scanned leaf area. For MT and ST, 10 tillers were chosen at random for leaf area measurement. We counted all the tillers in the MT and ST and measured the basal tussock cover of each individual. The LAI_{green} in MT and ST was estimated by multiplying the average leaf area per tiller ($n = 10$) by the total number and dividing this result by the basal tussock cover area. 30 tillers from random individuals were sampled to estimate the green biomass per individual in the tussocks (12). Sample tiller leaf area was measured and then dried in an oven for 3 d at 60 ºC. A linear function was fitted between leaf area (x) and dry tiller biomass (y) by: $y = 0.048\ x - 0.173$ ($n = 30$, $r^2 = 0.98$, $p<0.05$). Green biomass per individual was calculated by converting the LAI_{green} in each sample unit (the parallelepiped in LT and the sample tillers in MT and ST) into biomass per surface. This was multiplied by the basal surface cover of each individual to find the green biomass per individual.

Statistics: The effect of tussock size in all the ecophysiological variables assessed (gas exchange measured in early winter and summer by IRGA, g_s measured by diffusion porometer, and F_v/F_m) was evaluated by 3-way (Tussock Size, Individual, Time) nested ANOVA with repeated measures in Time factor and Individual nested within Tussock Size factor. The ANOVAs were assessed for each plot in early winter and summer using *SPSS v.14* software (*SPSS*, Chicago, IL, USA). The Tukey HSD post hoc test of 2-way (Tussock Size, Individual) nested ANOVA with Individual nested within Tussock Size factor was used to compare the daily average of each type of gas exchange (measured by IRGA and diffusion porometer) and F_v/F_m at dawn and midday for tussock-size classes. Gas exchange and fluorescence measurements were transformed by a logarithmic function and the arc sine function $[x' = \text{arc sine } (F_v/F_m^{1/2})]$, respectively (Zar 1999) to correct for deviation from the norm and heterogeneity of variance.

Following the procedure of Schulze and Hall (1982) for estimating long-term WUE by the slope of the linear relationship between maximum hourly P_{Nmax} *vs.* g_s, linear regression analysis was performed using *Sigma Plot 2000* software (*Spss Science*, Chicago, IL, USA), to compare the slopes between IRGA measurements for the two seasons.

Hourly temperature, atmospheric relative humidity, and PPFD on continuous days of IRGA assessment were compared using the Mann-Whitney U test. A Spearman correlation matrix between $\text{LAI}_{\text{green}}$ and average F_v/F_m was calculated to assess the photo-protective effect of $\text{LAI}_{\text{green}}$ in individuals in the same tussock-size class.

Results

Ecophysiological comparison of tussock sizes: The 3-way nested ANOVA with repeated measures revealed significant differences for most of the ecophysiological variables assessed in the two seasons. Significant effects were found for both tussock size and time (Table 3). The summer E showed no significant variations among tussock-size classes (except in the plot with the least *S. tenacissima* cover – CG III). Differences in g_s among tussocks of different sizes were significant only in the plot with the highest *S. tenacissima* cover (CG I) (Table 3). In early winter, no significant differences in E were detected for different size tussocks in the plot with intermediate *S. tenacissima* cover (CG II) or for WUE_i in Plot CG III (Table 3).

Table 3. Results of nested and repeated measures of ANOVA (F values) for five ecophysiological variables in three cover groups. The Tussock Size and Time factors and the effects of interaction were analysed by the ANOVA F test at $^{*}p<0.05$, $^{**}p<0.01$, n.s. $p>0.05$. E = transpiration rate, P_N = net photosynthetic rate, WUE_i = instantaneous water-use efficiency, g_s = stomatal conductance, F_v/F_m = maximum photochemical efficiency of photosystem 2. d.f. was always 2 for tussock size and tussock size×time, but 1 for time.

		Tussock Size	Time	Tussock Size×Time
Cover group I	E_{winter}	24.49**	216.10**	17.00***
	$P_{\text{N winter}}$	5.18**	432.30**	2.21 n.s.
	WUE$_{\text{winter}}$	6.20**	327.32**	1.19 n.s.
	E_{summer}	0.56 n.s.	36.13**	4.54 n.s.
	$P_{\text{N summer}}$	10.12**	8.77**	2.50 n.s.
	WUE$_{\text{summer}}$	22.68**	1.61 n.s.	8.84**
	g_s	3.91*	27.12**	0.77 n.s.
	F_v/F_m	5.68**	311.31**	1.10 n.s
Cover group II	E_{winter}	1.81 n.s.	96.58**	4.60 n.s.
	$P_{\text{N winter}}$	3.57*	332.48**	4.01*
	WUE$_{\text{winter}}$	15.95**	187.00**	6.65**
	E_{summer}	0.66 n.s.	65.00**	2.66 n.s.
	$P_{\text{N summer}}$	3.90*	62.65**	19.95**
	WUE$_{\text{summer}}$	4.90**	22.77**	21.11 n.s.
	g_s	2.39 n.s.	86.30**	2.17 n.s.
	F_v/F_m	16.81**	71.01**	1.66 n.s.
Cover group III	E_{winter}	10.30**	103.89**	0.34 n.s.
	$P_{\text{N winter}}$	3.96*	221.12**	3.02 n.s.
	WUE$_{\text{winter}}$	1.19 n.s	182.32**	0.26 n.s.
	E_{summer}	28.98**	5.07*	1.71 n.s.
	$P_{\text{N summer}}$	31.55*	93.98**	1.62 n.s.
	WUE$_{\text{summer}}$	18.30**	92.78**	0.35 n.s.
	g_s	0.33 n.s.	93.55**	0.33 n.s.
	F_v/F_m	8.03**	295.50**	1.62 n.s.

In early winter, MT and ST tussocks had higher E than LT particularly in plots CG I and III (Fig. 2). P_N was highly variable in this season with a complex pattern in each CG plot. Significant differences in P_N were detected among tussock-size classes in plots CG I, II, and III, respectively (Fig. 2). WUE$_i$ consistently increased with tussock size in all CG plots, with significant differences between tussocks in LT and ST. The overall averages of *E*, P_N, and WUE$_i$ in the early winter season were 1.57±0.04 mmol(H_2O) m^{-2} s^{-1}, 5.13±0.16 µmol(CO_2) m^{-2} s^{-1}, and 3.28±0.08 µmol(CO_2) $mmol^{-1}$(H_2O), respectively.

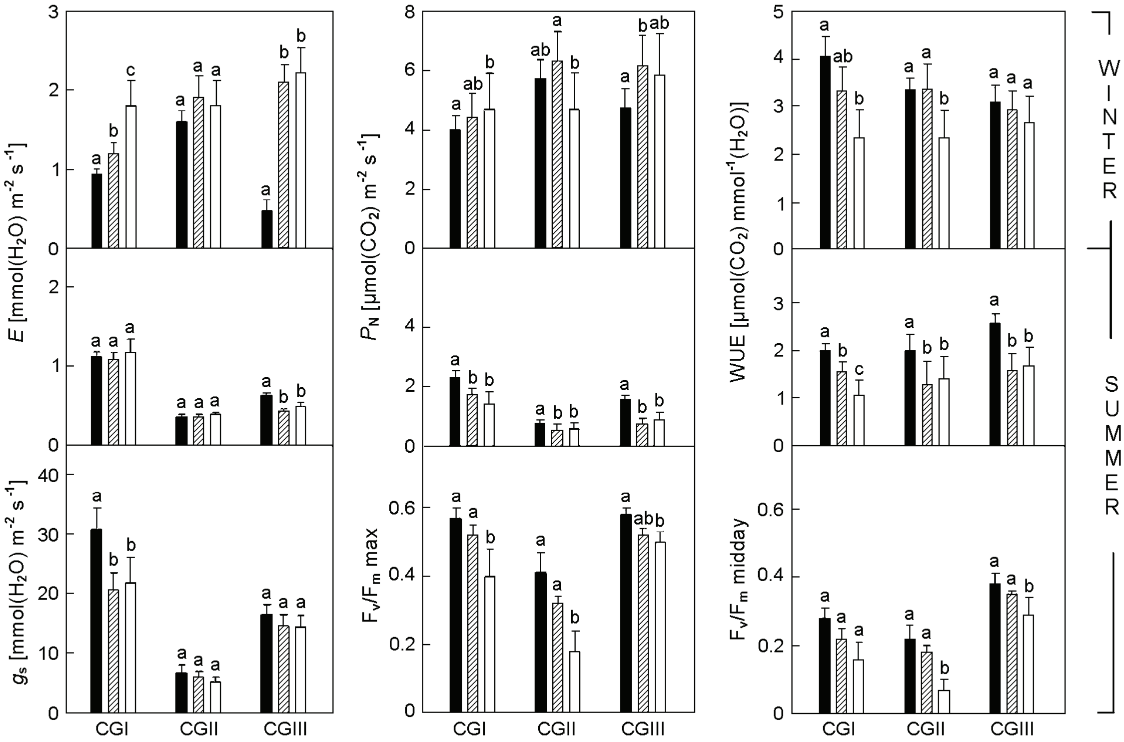

Fig. 2. Daily averages of the ecophysiological variables assessed in early winter and summer 2004. *The letters* mean significant differences detected by Tukey HDS post hoc test of the 2-way nested ANOVA at $p<0.05$. P_N = net photosynthetic rate, *E* = transpiration rate, g_s = stomatal conductance, F_v/F_m max = maximum photochemical efficiency of photosystem 2 (PS2) at dawn, F_v/F_m midday = maximum photochemical efficiency of PS2 at midday. *Black, shaded,* and *white bars* are large, medium, and small individuals, respectively. CG = cover group.

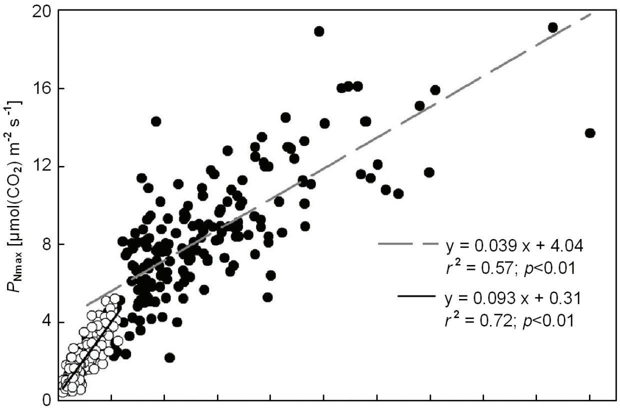

Fig. 3. Scatter plot of maximum net photosynthetic rate (P_{Nmax}) *versus* stomatal conductance (g_s) in the early winter (*black circles*) and summer (*open circles*) from IRGA measurements. *Straight line* fitted by a linear regression analysis is shown for each season (early winter = *grey dashed line*, summer = *black line*; all ANOVA F values were significant at $p<0.01$).

In the water-stress season (summer), no significant differences in *E* were detected among tussock-size classes, except in the plot with the least *S. tenacissima* cover (CG III) where LT had higher *E* than MT and ST (Fig. 2). LT had higher P_N and WUE$_i$ (2.33±0.24, 0.74±0.12) than MT (1.60±0.13, 2.0±0.15) and ST (2.0±0.33 and 2.57±0.20) in CG I, II, and III, respectively. This result was found in g_s in CG I, but no significant differences were detected among different size tussocks in plots CG II and CG III (Fig. 2). Maximum photochemical efficiency of PS2 at dawn (F_v/F_m max) and at midday (F_v/F_m midday) decreased with tussock size (Fig. 2). The overall averages of *E*, P_N, WUE$_i$, g_s, F_v/F_m max, and F_v/F_m midday in summer were 0.67±0.02, 1.29±0.05, 1.81±0.06, 15.49±0.67, 0.46±0.02, and 0.25±0.01, respectively.

The slope of P_{Nmax} over g_s, which was higher with water stress, was 0.039 and 0.093 in early winter and summer, respectively (Fig. 3). The range and variability of P_{Nmax} and g_s were higher in the early winter (2.19–19.10, 21.5–400.1) than in summer (0.42–5.20, 3.4–45.0).

PPFD in the tussocks and photo-protective effect of the foliage: Large individuals were characterized by large green biomass per individual (631.9±85.8 g of dry matter) and lower PPFD inside the tussock than in MT and ST, particularly on clear days (Fig. 4). Similar PPFD was also found in MT and ST (Fig. 4), which had less average green biomass per individual than LT [34.1±4.2 and 5.6±0.8 g(DM), respectively]. We found a marginally significant (at $p \leq 0.1$), positive relationship between LAI_{green} and F_v/F_m (at both dawn and midday) for individuals in each tussock-size class (Table 4) revealing a photo-protective effect of the foliage.

Discussion

With high water availability, *S. tenacissima* leaves subjected to strong irradiance showed a more intense *E* response to PPFD. Ramírez *et al.* (2006) compared *E* values measured with different methods (IRGA, diffusion porometer, stem heat balance) and observed that leaves from tussocks with less leaf biomass (and exposed to strong irradiance) transpired more than leaves from large tussocks. More self-shading in LT than MT or ST was demonstrated in simulated tussocks measured by a 3-D crown architecture model (Ramírez *et al.* 2008). Stronger irradiance on the tussocks may promote possible cooling by transpiration in MT and ST potted plants growing under optimum water availability. Here higher PPFD was found in MT and ST (Fig. 4) where *E* was also higher in response to PPFD. This "water spender" strategy (*sensu* Grace 1997) caused reduced WUE_i in MT and ST with high water availability during early winter. This relatively low WUE_i is within the range found by Chen *et al.* (2005) in *Stipa grandis* [3–4 μmol(CO_2) $mmol^{-1}$(H_2O)] in a semiarid steppe of the Inner Mongolia Plateau.

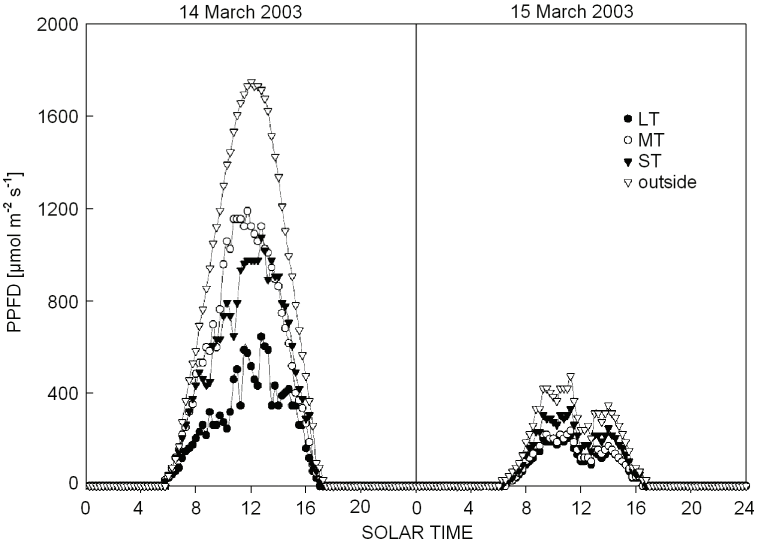

Fig. 4. Photosynthetic photon flux density (PPFD) measured in large (BT), medium (MT), and small (ST) tussocks and outside of the tussocks on clear (14 March 2003) and cloudy (15 March 2003) days. Each sensor was located half-way down from the maximum height of each tussock.

Table 4. Spearman correlation matrix of leaf area index (LAI) *vs.* maximum photochemical efficiency of photosystem 2 at dawn (F_v/F_m max) and midday (F_v/F_m midday). Calculated for three tussock size classes (see legend to Table 1). *n* was always 12.

	Size	$r_{Spearman}$	*p*
LAI *vs.* F_v/F_m max	LT	0.48	0.10
.	MT	0.64	0.02*
	ST	0.50	0.09
LAI *vs.* F_v/F_m midday	LT	0.64	0.02*
	MT	0.48	0.10
	ST	0.50	0.10

The structural photo-protective effect caused by the vertical leaf arrangement and the accumulation of both senescent and green foliage in *S. tenacissima* tussocks is a key mechanism allowing this species to cope with high irradiance and water stresses in semiarid environments (Valladares and Pugnaire 1999). The high photoinhibitory risk experienced by these plants in summer is also counteracted by the photo-protective effect of the de-epoxidation of xanthophylls and by highly dynamic Chl synthesis and degradation that avoids harvesting of excessive photon energy when photosynthesis is impaired by drought and heat (Balaguer *et al.* 2002). The relatively high photochemical efficiency of PS2 at both dawn and midday and also the high P_N observed in large tussocks in summer (Fig. 2) resulted from increased structural photoprotection induced by a large leaf surface area. WUE was positively influenced by these relatively high P_N values in LT. Our findings confirm under natural conditions the results found by Ramírez *et al.* (2008) under controlled conditions using potted tussocks. The agreement of the two studies that LT use water more efficiently than MT and ST has important implications for natural regeneration of the species by sexual reproduction and also for the interpretation of plant-plant interaction under stress.

In grass species, both WUE_i (*e.g.* Toft *et al.* 1989, Kalapos *et al.* 1996) and WUE_l, *i.e.* DM production per water transpired, show a tendency to increase (*e.g.* Clifton-Brown and Lewandowski 2000, Cabuslay *et al.* 2002) with growing water stress in arid and semiarid environments. However, other studies report no changes in WUE with changing water regimes in these species (*e.g.* Zhang *et al.* 1998, Clifton-Brown and Lewandowski 2000). We found a reduction of WUE_i with increased water stress [from 3.28±0.08 to 1.81±0.06 μmol(CO_2) mmol^{-1}(H_2O) in early winter and summer, respectively], an unusual pattern, since the reverse is frequently found for WUE studied during drought, but in agreement with results of Valladares and Sánchez-Gómez (2006). As discussed in the latter study, most studies reporting an increase in WUE with water stress were carried out under mild stress, not the very low water content experienced by many plants under Mediterranean semiarid conditions. However, there was significant spatial variability in WUE_i response in places with more *S. tenacissima* cover (see Table 2) where WUE was lower from early winter to summer, –2.02 μmol(CO_2) mmol^{-1}(H_2O), significantly higher than in places with intermediate and less alpha grass cover [–1.49 and –0.88 μmol(CO_2) mmol^{-1}(H_2O), respectively]. Even though WUE is expected to increase physiologically during drought, the reverse can be expected from an ecological point of view. High WUE is a poor strategy where there is strong competition for water because the water not used by the most efficient individuals remains available for competing neighbours (Jones 1992). Ramírez *et al.* (2007a) demonstrated non-rainfall water gains by direct adsorption of atmospheric water vapour in places with high *S. tenacissima* cover (CG I). These water gains represent an important water resource compensating for the high evapotranspiration demands during the dry summer (Ramírez *et al.* 2007a). The reduction of the instantaneous WUE driven by rising E (Fig. 2), mainly in the denser alpha grass stands (CG I, sector with highest E in summer, Fig. 2) seems to reflect high competition for water resources in summer.

Schulze and Hall (1982) considered the slope of the relationship between P_{Nmax} and g_s as an indicator of WUE_l. Supporting this argument, Jones (1992) emphasised that the main factor influencing WUE_l is the timing and duration of events when the stomata are fully open. Because the slope of P_{Nmax} *vs.* g_s was higher with low water content (Fig. 3), we suggest increased WUE_l with water stress in contrast to the results for WUE_i. Another estimation of WUE_l is the stable carbon composition ratio ($\delta^{13}C$), which is associated with WUE integrated over the lifetime of the tissue (Dawson *et al.* 2002). In our study area, Ramírez (unpublished data) detected a slight reduction in foliar $\delta^{13}C$ from the middle of summer 2006 (–23.9±0.18 ‰, 10 August) to the end of spring 2007 (–24.2±0.11 ‰, 13 June). This evidence further supports the hypothesis that *S. tenacissima* WUE_l increases during the summer water stress period despite WUE_i reduced with drought.

The morphological and physiological leaf features of some plant species improve with ontogenetic development, generally providing better conditions for a high P_N (*e.g.* James and Bell 2001). Similar to our results, Ishida *et al.* (2005) found that the WUE, P_N, and F_v/F_m of individual *Macaranga gigantea* seedlings were lower than in larger individuals (sapling, sucker, and adult trees). The authors suggest that increasing foliar nitrogen content and hydraulic system efficiency during ontogenetic development are the main factors explaining this pattern. At higher soil water availability, gas exchange was higher in the smallest individuals (seedlings) of *Quercus douglasii*, and similar to our findings, this kind of individual (with more negative predawn xylem water potential) had a lower WUE with limited water than larger individuals in California (Matzner *et al.* 2003). An ontogenetic increment of foliar biomass production in cultivated grass species improves photosynthetic capacity during the late reproductive period, allowing these species to translocate resources to increase the grain yield (Calderini *et al.* 1997, Takai *et al.* 2006). Ohsumi *et al.* (2007) found that P_N was higher when rice genotypes were in the developmental stages from panicle initiation to 2 weeks before heading.

Our results reveal that small tussocks (in early development) are more vulnerable to water stress than larger ones, as they are less able to take up water, not only from rainfall but also from non-rainfall soil water in summer due to their small root system. Thus small tussocks are also more vulnerable to photoinhibition. The positive relationship between LAI and F_v/F_m within tussock size groups (Table 4) confirms the importance of self-shading as a structural photoprotective mechanism in this species as suggested by Valladares and Pugnaire (1999).

In conclusion, different ecophysiological responses to water conditions were found in *S. tenacissima* individuals of different size under field conditions, highlighting the importance of a "demographic approach" in physiological ecology (Schmidt *et al.* 2001). Spatial variability was found in WUE_i, emphasizing high resource variability at relatively small spatial scales (19 ha) in semiarid environments. Significant recruitment of seedlings was found in the *Stipa tenacissima* steppes in our study area confirming the importance of sexual reproduction in this species (Gasque and García-Fayos 2003). However, the found greater susceptibility of small tussocks to water deficit suggests that these individuals could be the worst affected in the most likely climate change scenario of increased drought in the Mediterranean region (Christensen *et al.* 2007). This situation could negatively affect sexual reproduction in this species, which is an important way of space colonization in semiarid areas.

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