

Effects of tussock size and soil water content on whole plant gas exchange in *Stipa tenacissima* L.: Extrapolating from the leaf versus modelling crown architecture

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Abstract

In this study we evaluated daily whole plant transpiration and net photosynthetic rates in *Stipa tenacissima* L. (Poaceae) tussocks of different sizes subjected to three levels of soil moisture. The crown architecture of 12 tussocks was reconstructed with the 3D computer model Yplant taking into account the morphology and physiology of the leaves determined at different soil moisture levels. We also calculated whole plant transpiration by extrapolating leaf transpiration in different senescence conditions measured with a diffusion porometer. This extrapolated transpiration overestimated transpiration, particularly when the soil moisture level was high (>15% of volumetric soil water content). At this high level of soil moisture, large tussocks (>60 cm in diameter), which were sexually mature and had a large leaf surface area, were the most efficient with regard to daily water use efficiency (whole plant net photosynthesis/whole plant transpiration). Whole plant water use efficiency decreased with tussock size primarily because small tussocks exhibited high transpiration rates. Small tussocks were more sensitive to soil drying than large and intermediate ones, presenting a faster rate of leaf senescence as water deficit increased. Leaf acclimation to irradiance, which was significantly influenced by the degree of mutual shading among neighbouring leaves, along with the ontogeny of the tussock and its effect upon leaf senescence were found to be the main mechanisms involved in the different responses to water limitations found in whole plant gas exchange variables. Our results show that the size of each individual plant must be taken into account in processes of scaling-up of carbon gain and transpiration from leaf to stand, as this is a particularly relevant aspect in estimating water use by semiarid vegetation.

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1. Introduction

As [Percy \(1997\)](#) highlighted, little attention has been paid to the processes involved in the behaviour of the whole plant, mainly due to the poor understanding of the development and of the main flows occurring within the individual ([Schulze, 1986](#)). One way of improving our functional understanding of plants at the level of the whole individual is by recognising the variety of processes taking place within the plant and the multiple functions that a given architecture must serve ([Percy and Valladares, 1999](#)). [Levin \(1995\)](#) highlights the fact that scaling-up (e.g.

from leaves to whole plant) involves a gradual process in which knowledge of how information is transferred from one scale to another is crucial with regard to understanding the mechanisms and generating the patterns. This strategy was supported by [Baldocchi et al. \(1991\)](#) who recommend a sequential scaling of ecophysiological variables (focusing mainly on stomatal conductance), scaling only at adjacent scales. From this standpoint, understanding the main processes that take place at the leaf level becomes crucial in the sequential scaling process from the leaf to the stand via the whole plant. Thus for example, the use of architectural models incorporating the main ecophysiological processes and factors affecting plant performance (leaf senescence, micro-climatic conditions, physiological features of the foliage units) have proven to be efficient tools for quantifying net photosynthesis and transpiration ([Ryel et al., 1993](#); [Ryel](#)

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and Beyschlag, 1995; Valladares and Pugnaire, 1999; Zotz et al., 2002; Ramírez et al., 2006) as well as estimating the effect of photoinhibition on the whole plant (Werner et al., 2001a,b; Valladares et al., 2005). The architectural modelling perspective follows a summation-scaling scheme (*sensu* Jarvis, 1995) where physiological variables are calculated in leaf sections with regards to micro-meteorological variables, and, subsequently, the summation of these physiological results calculated in each section is done to estimate results for the whole plant.

Ecophysiological evaluations are conducted mainly at the leaf level, and usually in chambers and under artificial conditions (Kruijt et al., 1997). Extrapolations of these measurements to higher scales usually involve important overestimations of gas exchange processes (e.g. Schulze et al., 1985; Ramírez et al., 2006). There is increasing evidence indicating that evaluations of isolated leaves are not representative of whole plant performance (Beyschlag and Ryel, 1998), and this the main reason why greater attention is being paid to modelling tools that can integrate processes at the level of the whole organism. Two key plant processes such as net photosynthesis and transpiration have been measured at whole plant level with the following aims: (i) to assess the effect of increased atmospheric CO₂ concentration (e.g. Dugas et al., 1997; Morgan et al., 1998; Polley et al., 1999; Roumet et al., 2000; Robredo et al., 2007), (ii) to analyse the consequences of shading among individuals of the same species (Ryel et al., 1994) and that caused by alien species (Matsumoto et al., 2000a), and (iii) to assess extrapolations based on measurements at leaf level (e.g. Schulze et al., 1985; Dugas et al., 1993; Senock and Ham, 1995; Lu et al., 2002; Dragoni et al., 2005; Ramírez et al., 2006).

We carried out a modelling exercise of gas exchange and water use efficiency from the leaf to the whole plant in different-sized specimens of *Stipa tenacissima* L., a clonal, rhizomatous and perennial tussock grass from semiarid areas of the Mediterranean region. The vegetative reproduction of *S. tenacissima* has been considered to be the main spatial colonization strategy of the species (White, 1983; Haase et al., 1995), which can be seen during the tussock aging phase, at approximately 60 years of age onwards (Servicio del Esparto, 1951). At this stage the tussock that has gradually aged from the centre, separates its external parts which form new tussocks (Puigdefábregas and Sánchez, 1996). However, other authors (Gasque, 1999; Gasque and García-Fayos, 2003) have highlighted the importance of sexual reproduction and recruitment in *S. tenacissima* grasslands, proving that this species is capable of forming soil seed banks. Numerous studies have dealt with the responses and high tolerance of *S. tenacissima* to water stress. Pugnaire et al. (1996) and Balaguer et al. (2002) have highlighted a high capacity to respond to rain or temporal water availability pulses during which this species' net photosynthesis rates, water conductance, photosynthetic efficiency of photosystem II and leaf water potential are quickly restored. Whole plant studies have also been carried out in this species using architectural models. Valladares and Pugnaire (1999) have pointed out that the architecture of the tussock, comprising vertical leaves with a high level of self-shading, attenuates the negative effect of high radiation, giving rise to an effective photo-protection mechanism

but reducing whole plant net CO₂ assimilation in this species (Valladares and Pugnaire, 1999). Ramírez et al. (2006) demonstrated that architectural modelling taking leaf senescence into consideration was the best approach for scaling-up transpiration from the leaf to the whole plant in *S. tenacissima*. Given the importance of this species in the redistribution of sediments and water (Puigdefábregas and Sánchez, 1996; Cerdà, 1997), along with its frequent facilitation of other plants and the positive net effect in the restoration of native shrub species (Maestre et al., 2001, 2003), the accurate ecophysiological characterization of the different cohorts of *S. tenacissima* populations becomes highly relevant.

We assessed the whole plant gas exchange behaviour of 12 potted *S. tenacissima* tussocks of different sizes at three contrasting soil moisture contents during a dry-out process mimicking the onset of drought in the field. We compared two whole plant transpiration estimates: the first one was based upon direct extrapolations from porometer measurements, and the second one was based on a 3D crown architectural model that includes relevant physiological information on the foliage units. Self-shading promoted by leaf density within the tussock and leaf acclimation have been shown to play a crucial role in whole plant net photosynthesis (Valladares and Pugnaire, 1999) and transpiration (Ramírez et al., 2006) in this species. For this reason, important whole plant gas exchange differences are expected among tussocks of different sizes due to the fact that as tussocks become larger, a whole suite of morphological characteristics varies in a non-linear way, affecting not only total leaf surface area, leaf density and amount of senescent leaves and dry foliage but also the way leaves are displayed and arranged in a 3D space. On the other hand, as Balaguer et al. (2002) pointed out, there is a reversible senescence processes when *S. tenacissima* leaves were subjected to light and water stresses, so we hypothesized a reduced effect of senescence in large tussocks because the photo-protective effect of self-shading will overcompensate for their reduced photosynthetic capacity, allowing large tussocks a higher overall carbon assimilation and water use efficiency than small tussocks. We argue that the allometric changes experienced in *S. tenacissima* as it grows and the increased accumulation of dead foliage with size are the underlying mechanisms of increased water use efficiency and drought tolerance that can be expected in large plants.

2. Materials and methods

2.1. Experiment specifications

Twelve *S. tenacissima* tussocks were removed from a south-facing hillside in the experimental area Sierra "El Ventós" (Bellot et al., 2004), in the province of Alicante in SE Spain (38°29'N, 0°37'W). All tussocks belonged to three size groups (four tussocks per group) classified by their mean cover diameter (\emptyset), big tussocks: $60 \text{ cm} \leq \emptyset \leq 85 \text{ cm}$, medium tussocks: $30 \leq \emptyset < 60 \text{ cm}$ and small tussocks: $15 \text{ cm} \leq \emptyset < 30 \text{ cm}$. These tussock size groups were established according to the age classification defined by Sánchez (1995) in *S. tenacissima*. We uprooted the tussocks on 18 November 2002 following a rain-

fall event, in order to reduce root damage, and planted each soil block with each individual in three different-sized pots: 5, 25 and 35 L for small, medium and big tussocks, respectively, representing the mean soil volume available for each plant size in the field. Rock outcrops characterize many field sites where *S. tenacissima* is found, and particularly the south-facing hillside in the “El Ventós” catchment, where plant roots are confined to “soil pockets”. We then transferred the pots with their respective plants to an experimental plot at Alicante University, where the plants were watered and kept for approximately 1 year and 8 months before the experiment.

In order to estimate soil moisture (volumetric soil water content), we inserted two pairs of steel rods (length: 15 cm, diameter: 0.5 cm) horizontally into each pot to a depth of 5 cm, and connected them to a TDR system (TDR 100 model, Campbell Scientific Ltd., UK) using calibration equations for the soil in our study area (Ramírez et al., 2007a). In order to expose tussocks to different levels of water stress, we watered the soil in each pot until saturation point was reached, and then initiated a 30-day drying period on 1 July 2004 mimicking natural conditions. In order to reduce soil-drying speed and assess leaf area at each assessed level of soil moisture, we covered the soil surface of the pot with laboratory film (Parafilm M, Pechiney Plastic Packaging, Chicago, USA) immediately after watering. Inspection with the TDR system enabled us to distinguish the moment at which the soil in each pot reached three different soil moisture levels (in volumetric soil water content), 1st: 15–30%, 2nd: 5–15% and 3rd: $\leq 5\%$.

In order to analyse the microclimatic conditions of the transpiration sample dates, we compared daily average wind speed, temperature, relative atmospheric humidity and global radiation on assessed sample dates using the *U* Mann–Whitney non-parametric test (the data did not satisfy the assumptions of the parametric test). A portable meteorological station was used for automatic estimation of: wind direction and speed (Meteo 1 Wind- HOBO, Onset Computer Corporation, USA), air temperature and humidity (HOBO Pro RH/Temp., Onset Computer Corporation, USA), photosynthetic photon flux density (HA-LI HOBO, Onset Computer Corporation, USA) and global radiation (PYR HOBO, Onset Computer Corporation, USA). All data loggers recorded outputs every 5 min. Environmental conditions (1–31 July 2004) were characterized by clear days, with high relative atmospheric humidity (approximately 90%) from 19:00 to 5:00 solar hours (Fig. 1). The mean hourly maximum and minimum temperatures were 37.2 °C (10:00 solar time) and 16.4 °C (4:00 solar time), respectively. Mean maximum water vapour pressure deficit was 2.70 kPa (Fig. 1).

2.2. Morphological characterization

The internal tussock structure of *S. tenacissima* comprises a population of stems formed by connected and ramified nodes. The leaf-growing axis is a tiller located at the apical end of the stem (a detailed morphological description of this species is provided in Sánchez and Puigdefábregas, 1994). In order to calculate tussock leaf area (green and dry leaf area) we sampled the tiller leaf area in the tussock following the approach established

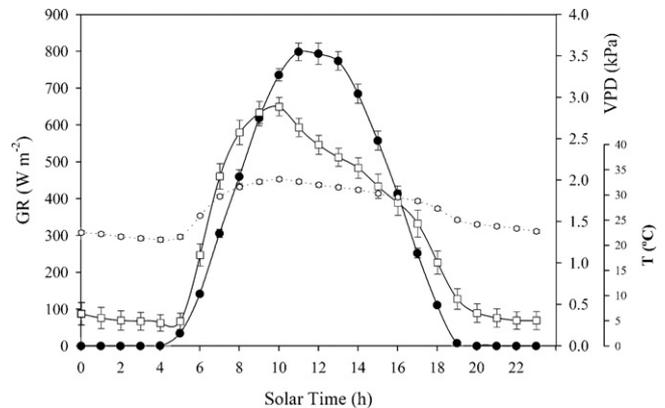


Fig. 1. Mean values (\pm S.E.) of global radiation (black circles, GR), vapour pressure deficit (open squares, VPD) and temperature (open circles, *T*) during the study period (1–31 July 2004).

by Ramírez et al. (2006). This sample perspective facilitates the crown architectural modelling process in this species (Valladares and Pugnaire, 1999). We divided big and medium tussocks into two concentric sectors (internal and external), marking them as: (i) internal sector: 5 and 4 tillers, in big and medium tussocks, respectively, (ii) external sector: 10 and 6 tillers, in big and medium tussocks, respectively. We did not divide the small tussocks into sectors, randomly sampling five tillers for each of these. The leaf area of each tiller was estimated using the relationship between the product of leaf length \times diameter and the area calculated by scanned leaves ($y = 0.982x + 0.063$, $n = 100$, $r^2 = 0.94$, $*P < 0.05$). The area of green foliage was divided into three senescence conditions (1st = optimal, 2nd = intermediate, 3rd = senescent), depending on the degree of dryness from the tip to the base of the limb, which in turn is related to age (Sánchez, 1995; Haase et al., 1999). The chromatic characterization of these senescence conditions was measured in four big tussocks with a portable chlorophyll meter (SPAD-502, Minolta Co. Ltd., Japan), and was simultaneously calibrated using $a + b$ chlorophyll concentration (Chlor_{*a+b*}, mg m⁻²) as determined by Arnon's method (1949) following extraction with 80% acetone (Chlor_{*a+b*} = 20.4 SPAD + 15.1, $r^2 = 0.87$, $P < 0.05$). The mean $a + b$ chlorophyll concentration in each senescence condition was: 1st = 869.3 ± 63.5 mg m⁻², 2nd = 635 ± 49.8 mg m⁻² and 3rd = 312.3 ± 437.5 mg m⁻². This classification was coherent with the senescence process taking place from the wet to the dry season in *S. tenacissima* tussocks in natural conditions (Ramírez et al., 2006). We estimated tussock leaf area in each senescence condition by multiplying average leaf area by sampled tillers by total number of tillers in the tussock. The measurement was repeated when the soil moisture changed to the following soil moisture level. With the aim in mind of comparing tussock size groups in relation to the senescence process, we calculated the relative leaf area of each tiller in each senescence condition. This was calculated by dividing the tiller leaf area (cm²) in each senescence condition-by total green area in the tiller (total green area = 1st + 2nd + 3rd total leaf area senescence condition).

We measured other morphological variables required to model crown architecture using Yplant (see below). To this end,

the big and medium tussock sectors and the base of the small tussocks were divided into four zones according to their orientation: NW, NE, SW and SE. In each zone, we sampled both the internal and external sectors in the big and medium tussocks, 3 and 5 tillers, respectively (36 total tillers by plant), whereas in the small tussocks, in each zone we sampled 5 tillers (20 tillers per plant). At the base of each tiller we measured the following parameters: angle from horizontal, distance from the middle of the tussock, azimuth as the compass direction and lengths. We estimated the angle from horizontal of four sections of all the leaves of each tiller (four to five leaves per tiller) for a total of eight tillers for all big and medium tussocks (four from the northern zone and four from the southern zone) and four tillers for all small tussocks.

2.3. Gas exchange measurements

An infrared gas analyzer (IRGA—LI-COR 6400 model, LI-COR Biosciences Inc., Nebraska, USA) was used to evaluate net photosynthesis versus photosynthetic photon flux density (PPFD) response curves (light response curves) for each leaf senescence condition in the 12 tussocks. A group of between three and four leaf sections was measured in the IRGA chamber. Photosynthetic light response was estimated by the IRGA systems under the following conditions: sequence of programmed PPFD = 2000, 1500, 1000, 500, 200, 100, 50 and 0 $\mu\text{mol m}^{-2} \text{s}^{-1}$, relative humidity in the reference chamber = 35%, CO_2 concentration in the reference chamber = 370 $\mu\text{mol CO}_2 \text{mol}^{-1}$, airflow rate = 500 $\mu\text{mol s}^{-1}$ and vapour pressure deficit in the chamber = 1.9–2.9 kPa. In order to avoid damaging the leaves evaluated, light response curves were measured on a set of different leaves, since the time used for each simulation was approximately 20 min. The temperature programmed for the IRGA chamber was 25 °C for all simulations.

In order to compare the transpiration response to PPFD among leaves belonging to each tussock size group in controlled conditions, we fitted transpiration versus PPFD results (from light response curves measurements) to a linear function

using a regression analysis for each leaf senescence condition, using Sigmaplot for Windows 6.0 software (SPSS, Chicago, IL, USA). At the same time, we used the information obtained from light response curves and supported by the Photosyn Assistant software (Dundee Scientific, Scotland, UK), to calculate: light saturated CO_2 assimilation rate (A_{max} , $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$), dark respiration rate (R , $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$), quantum yield (Φ) and curvature factor (Θ) (Table 1).

Hourly stomatal H_2O conductance (g_s) was measured using a transient porometer (AP4 model, Delta T Devices Ltd., Cambridge, UK). A multi-parameter ventilation meter (Velocalc Plus 8386 model, TSI Incorporated, Minnesota, USA) was used to measure wind speed, air temperature and relative humidity in the area close to the leaf where porometer measurements were taken. Domingo et al. (1996) estimated boundary layer conductance in *S. tenacissima* (g_b) in relation to wind speed (v), obtaining a potential function: $g_b = av^b$, where $a = 0.06$, $b = 0.363$. Total H_2O conductance (g_t , $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$) was calculated assuming that the leaf has stomata on only one side (Field et al., 1989):

$$g_t = \frac{g_b g_s}{g_b + 2g_s} \quad (1)$$

Transpiration rate (E , $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$) was estimated adding a correction factor produced by the mass flow caused by water diffusion passing through intercellular spaces into the stomatal pore (details in Pearcy et al., 1989):

$$E = \frac{g_t(w_i - w_a)}{1 - ((w_i + w_a)/2)} \quad (2)$$

where w_i is the mole fraction derived from saturation vapour pressure at leaf temperature ($\text{H}_2\text{O mol mol}^{-1}$ air) and w_a is the mole fraction derived from vapour pressure at the adjacent atmospheric temperature ($\text{H}_2\text{O mol mol}^{-1}$ air).

The transpiration estimations based on previous detailed methodology shall be referred to as the “porometer method” throughout this work. In order to obtain whole plant transpiration from the porometer method, we extrapolated integrated transpiration weighted for each leaf senescence condition area. For this

Table 1

Mean values (\pm S.E.) of parameters obtained from the light response curves for the three leaf senescence conditions defined and three tussock size groups (big: $\varnothing > 60$ cm, medium: $60 \text{ cm} \geq \varnothing \geq 30$ cm and small: $\varnothing < 30$ cm)

| Senescence condition | Tussock size | A_{max} | R | Φ | Θ |
|----------------------|--------------|------------------|-----------------|------------------|-----------------|
| 1st | Small | 10.32 \pm 2.1 | −1.93 \pm 0.1 | 0.03 \pm 0.004 | 0.82 \pm 0.14 |
| | Medium | 7.29 \pm 1.0 | −1.98 \pm 0.3 | 0.03 \pm 0.006 | 0.82 \pm 0.15 |
| | Big | 11.63 \pm 2.9 | −1.91 \pm 0.2 | 0.03 \pm 0.005 | 0.83 \pm 0.13 |
| 2nd | Small | 2.94 \pm 0.8 | −1.29 \pm 0.4 | 0.02 \pm 0.001 | 0.90 \pm 0.13 |
| | Medium | 3.53 \pm 0.7 | −1.40 \pm 0.1 | 0.02 \pm 0.008 | 0.84 \pm 0.14 |
| | Big | 4.92 \pm 1.3 | −1.01 \pm 0.2 | 0.01 \pm 0.002 | 0.85 \pm 0.12 |
| 3rd | Small | 1.37 \pm 0.4 | −1.24 \pm 0.2 | 0.01 \pm 0.002 | 0.88 \pm 0.13 |
| | Medium | 1.21 \pm 0.3 | −1.02 \pm 0.1 | 0.01 \pm 0.008 | 0.88 \pm 0.14 |
| | Big | 3.11 \pm 0.8 | −0.98 \pm 0.3 | 0.01 \pm 0.004 | 0.89 \pm 0.13 |

A_{max} : light saturated rate of CO_2 assimilation ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$); R : dark respiration rate ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$); Φ : quantum yield; Θ : curvature factor of the light response curve.

purpose, transpiration was estimated in the leaves of each senescence condition, the sample design for big and medium tussocks was: 3 senescence conditions \times 3 leaves \times 4 tussocks = 36 total n , while for small tussocks the sample design was: 2 senescence conditions \times 2 leaves \times 4 tussocks = 16 total n . In the small tussocks we linked the 1st and 2nd senescence conditions, the two assessed senescence conditions being: 1st + 2nd and 3rd. Daily transpiration measurements using the porometer method were taken from 6:00 to 18:00 solar time every 2 h; we attempted to assess the highest number of tussocks possible at each soil moisture level. The sample dates for the porometer method assessment were: July 4th, 13th, 15th, 20th, 25th, 26th and 29th 2004.

2.4. Representativity of the measurements performed

In order to test whether the potted tussocks presented similar physiological behaviour to that of the tussocks in natural field conditions, we measured stomatal conductance (g_s) using a diffusion porometer (AP4, model, Delta T Devices Ltd., Cambridge, UK) at “El Ventós” experimental area on the slope where the tussocks were removed. The g_s measurements in the field were conducted on 8 August 2004, in the same summer season when the potted tussocks were assessed. The g_s was taken from 6:00 to 18:00 solar time every 2 h in 32 samples from 6 *S. tenacissima* tussocks. The sample size in each tussock size group was: 16, 10 and 6 samples for big, medium and small tussocks, respectively.

The range of g_s values obtained in the field in individuals belonging to the three tussock sizes was compared with the range of g_s values of potted tussocks in similar soil moisture. To this end, we randomly inserted 27 pairs of steel rods (0.15 m long and 0.05 m diameter), in order to determine volumetric soil water content by TDR (Campbell Scientific Ltd., Model TDR 100, UK), into the bare soil and the soil under *S. tenacissima* tussocks in the field.

2.5. Whole tussock measurements—Yplant simulations

We assessed the crown architecture of 12 individual tussocks at each soil moisture level using the Yplant model (Percy and Yang, 1996). The reader is referred to Ramírez et al. (2006) for a revision of the advantages and limitations of Yplant modelling compared to other methods of assessing whole plant gas exchange in *S. tenacissima*. We reconstructed tussock architecture following the approach used by Valladares and Pugnaire (1999) and Ramírez et al. (2006). This reconstruction was carried out in three phases (Fig. 2): (i) we counted the total number of base tillers in each defined zone (NW, NE, SW and SE) belonging to two concentric sectors of the tussock. We represented all the base tillers in each sector, randomly assigning: the angle from horizontal, distance from the centre of the tussock, azimuth as the compass direction and lengths obtained from the morphological measurement specified in previous paragraphs (Fig. 2A). For this assignment process we used as a base the values of four variables measured in three and five tillers sampled in each internal and external zone, respectively. (ii) Four represen-

tative types of tiller were produced, for the northern and southern orientation, in each concentric sector of the tussock. Each representative tiller had four to five leaves, and we divided each one in turn into four sections. We placed each leaf section on a senescence condition, determining the area and angle from the horizontal by estimating the average value of all tillers sampled in each sector (Fig. 2B). This procedure was conducted for the 12 tussocks at each soil moisture level based on results of morphological characterization. (iii) The four representative tillers were cloned for each base tiller belonging to the north-south internal and external sectors (Fig. 2C). We built 12 architectural models (4 tussocks \times 3 size groups), modifying these at each soil moisture level.

Physiological parameters obtained from the analysis of light response curves (Table 1), enabled us to use the rectangular hyperbolic response to PFD model in order to calculate the assimilation rate (details in Percy and Yang, 1996). Yplant software uses Leuning’s (1995) model to calculate CO_2 stomatal conductance (g_{CO_2}). The parameters required for this model were taken from studies by Ramírez et al. (2006) based on IRGA measurements. These authors fitted a linear relationship among $A \text{HR}/c_s$ and g_{CO_2} values using a regression analysis ($y = 12.62 + 0.014x$, $n = 84$, $r^2 = 0.69$; $*P < 0.05$) where A is net CO_2 assimilation, HR and c_s are relative humidity and CO_2 concentration on the leaf surface, respectively. Average daily atmospheric temperature, minimum and maximum water vapour pressure deficit, wind speed and direction obtained by the portable meteorological station were included in Yplant software. We ran 36 simulations using Yplant (12 tussocks \times 3 soil moisture levels) on days when porometer assessments were carried out from 6:00 to 18:00 solar time. From these simulations we obtained daily whole plant net photosynthesis and transpiration.

PPFD quantity in all leaf sections was assessed for three tussock size groups using box plot analysis. For this analysis, we used the result of simulation running for midday on July 4th. We created frequency histograms of the proportion of leaf sections receiving 8 levels of PPFD.

2.6. Statistical analysis

Total green and dry leaf area, and relative leaf area by tiller in each senescence condition were compared among each tussock size group and soil moisture level using two- and three-way ANOVAs, respectively. Where required, we transformed these assessed variables using the logarithmic function to correct deviation from normal and heterogeneity of variance.

The daily whole plant transpiration rate (DWT_{ext} , $\text{mol H}_2\text{O m}^{-2} \text{day}^{-1}$) extrapolated for each tussock using porometer method was calculated as follows:

$$\text{DWT}_{\text{total}} = \text{DWT}_1 A_1 + \text{DWT}_2 A_2 + \text{DWT}_3 A_3 \quad (3)$$

$$A_{\text{total}} = A_1 + A_2 + A_3 \quad (4)$$

$$\text{DWT}_{\text{ext}} = \frac{\text{DWT}_{\text{total}}}{A_{\text{total}}} \quad (5)$$

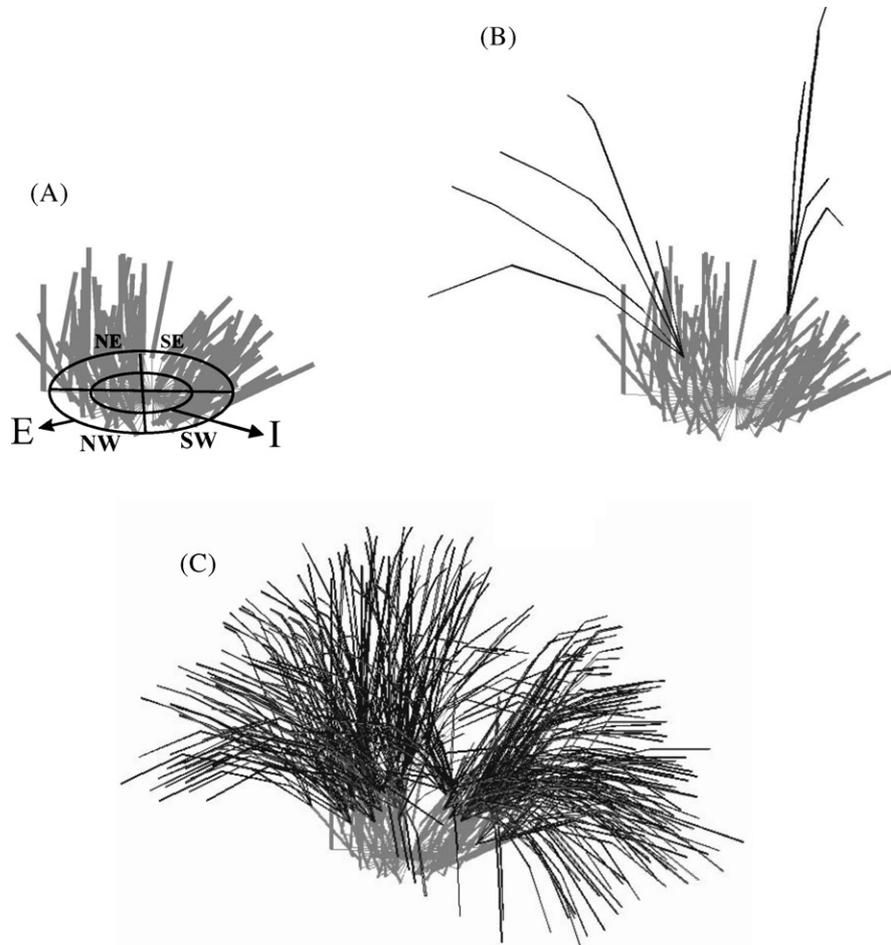


Fig. 2. Sequential diagram showing the construction process of the *Stipa tenacissima* crown architecture modelling using Yplant. (A) Base of the tussock: internal (I) and external (E) concentric sectors divided into four zones: NW, NE, SW and SE. The tiller bases are shown in grey. (B) Leaves (in black) of the two representative tillers belonging to the north and south zones. (C) Leaves of the representative tillers shown in (B) were cloned for each tiller base shown in (A).

where DWT_1 , DWT_2 , and DWT_3 are average values, of transpiration rates integrated over the 6:00 to 18:00 solar time period in leaves belonging to 1st, 2nd and 3rd senescence condition, respectively, and A_1 , A_2 , and A_3 are tussock leaf surface area (m^2) for the 1st, 2nd and 3rd senescence condition, respectively. Statistical analysis of whole plant transpiration was assessed by two-way ANOVA: 3 soil moisture levels \times 3 tussock size groups (big, medium and small).

Ramírez et al. (2006) proved that transpiration scaled-up by Yplant model was a reliable method for estimating whole plant transpiration in *S. tenacissima*. For this reason the transpiration obtained by Yplant simulation in each tussock size group served as a reference of transpiration extrapolated from leaf measurement (using the porometer method). We estimated water use efficiency (WUE, $mmol\ CO_2/mol\ H_2O$) of each individual tussock by dividing daily whole plant net photosynthesis ($mmol\ CO_2\ m^{-2}\ day^{-1}$) by daily whole plant transpiration, both rates as calculated by Yplant. We compared whole plant net photosynthesis and transpiration and WUE among three soil moisture levels and three tussock sizes using the *U* Mann–Whitney non-parametric test (the data did not satisfy the assumptions of the parametric test).

3. Results

3.1. Morphological characterization at each soil moisture level evaluated

One of the main implications of the gradual loss of the soil moisture is an increase in leaf senescence. Many leaves became senescent at low water availability, particularly in the case of small tussocks, which rapidly presented signs of senescence (Table 2). Thus, for the 2nd soil moisture level (from >5 to 15%), the small tussocks showed a lower percentage of their leaves in the 1st senescence condition compared to big and medium tussocks (Table 2). The opposite occurs with big tussocks, due to the fact that, when the tussocks were at the 3rd soil moisture level, the big individuals presented a higher percentage of their leaves in the 2nd senescence condition and a lower percentage in the 3rd senescence condition in comparison with medium and small tussocks (Table 2).

We found significant differences between tussock size groups in the total green (1st + 2nd + 3rd senescence condition) and dry leaf area (Table 2). This result, together with the fact that only the big tussocks produced spikes during the spring of 2004 (Ramírez

Table 2
Average (\pm 1S.E.) of green and dry total leaf area and relative leaf area by tiller under three defined senescence conditions belonging to three tussock size groups (big: $\varnothing > 60$ cm, medium: $60 \text{ cm} \geq \varnothing \geq 30$ cm and small: $\varnothing < 30$ cm) and three volumetric soil water content levels

| Soil moisture levels | Tussock size groups | Total green leaf area (cm ²) | Total dry leaf area (cm ²) | Senescence conditions relative leaf area (%) | | |
|----------------------------|---------------------|--|--|--|----------------|---------------|
| | | | | 1st | 2nd | 3rd |
| >15–30% | Small | 363.0 a (44.0) | 179.9 a (23.6) | 34.2 a (6.0) | 57.3 a (6.2) | 8.6 a (1.5) |
| | Medium | 1036.9 b (59.0) | 525.5 b (39.0) | 42.0 a (5.7) | 47.0 ab (5.17) | 11.0 a (1.4) |
| | Big | 1552.2 c (97.9) | 1288.9 c (90.1) | 47.7 a (3.7) | 36.4 b (3.2) | 15.9 b (1.2) |
| >5–15% | Small | 244.1 a (22.1) | 310.7 a (56.7) | 6.8 a (2.7) | 81.8 a (3.1) | 11.5 a (1.8) |
| | Medium | 991.5 b (67.1) | 586.6 b (45.0) | 27.0 b (4.1) | 57.7 b (3.5) | 15.3 a (1.4) |
| | Big | 1421.0 c (107.8) | 1420.0 c (91.8) | 31.9 b (3.9) | 52.8 b (3.4) | 15.3 a (1.3) |
| <5% | Small | 129.0 a (9.9) | 428.9 a (63.2) | 0.00 | 49.5 a (11.4) | 50.5 a (11.4) |
| | Medium | 460.4 b (30.5) | 1124.7 b (63.2) | 0.00 | 43.4 a (7.6) | 56.6 a (7.6) |
| | Big | 906.5 c (45.1) | 2318.9 c (114.7) | 0.00 | 73.6 b (2.2) | 26.4 b (2.2) |
| <i>F</i> | | | | | | |
| Two-way ANOVA | | | | | | |
| Green leaf area | | | | | | |
| TS | | | | | | |
| SM | | | | | | |
| TS \times SM | | | | | | |
| Dry leaf area | | | | | | |
| TS | | | | | | |
| SM | | | | | | |
| TS \times SM | | | | | | |
| <i>F</i> | | | | | | |
| Three-way ANOVA | | | | | | |
| TS | | | | | | |
| SM | | | | | | |
| SC | | | | | | |
| TS \times SM | | | | | | |
| TS \times SC | | | | | | |
| SM \times SC | | | | | | |
| TS \times SM \times SC | | | | | | |

Different letters next to the values indicate significant differences among tussock size groups at each soil moisture level evaluated (Tukey HDS test, $P < 0.05$). The results of two ANOVAs (two-way for total green and dry leaf area and three-way for relative tiller leaf area) are shown at the bottom (n.s. at $P > 0.05$, * $P < 0.05$, *** $P < 0.001$). TS: tussock size group; SM: soil moisture; SC: senescence conditions.

et al., 2006), justifies and reinforces our size classification, in which the big sizes are sexually mature, with more cover and green and dry leaf area. The medium and small tussocks did not present sexual maturity, showing medium and small green and dry leaf area, respectively.

3.2. Transpiration response and extrapolation from the porometer method

We found no significant differences among the great majority of sample dates in their daily (during the photo-period) average meteorological variables (Table 3). July 25th and 29th were hotter and wetter than 4 and 3 assessed dates, respectively (Table 3). This result enabled us to continue with the individual gas exchange comparison among tussock sizes and relative soil moisture levels.

The relationships between transpiration rate and PPFD, obtained from light curve response measurements, adjusted appropriately to a linear regression model (Fig. 3), and hence

we can see that the medium tussocks show the greatest increase in transpiration rate divided by PPFD (*b*, slope) in comparison with the big and small tussocks. This occurs mainly in the 1st senescence condition, whereas in the 2nd and 3rd it is the small tussocks that show higher slope values than the big and medium ones (Fig. 3).

We found significant differences in the daily extrapolated whole plant transpiration rate among soil moisture levels and tussock size groups, and even the interaction between both factors was significant (Table 4), which would appear to indicate that the differences between each type of tussock size group are determined by each soil moisture level (Fig. 4A).

At the 1st soil moisture level, the medium tussocks showed a higher whole plant transpiration rate ($162.8 \text{ mol H}_2\text{O m}^{-2} \text{ day}^{-1}$), with no significant differences between big and small tussocks (119.8 and $91.7 \text{ mol H}_2\text{O m}^{-2} \text{ day}^{-1}$, respectively, Fig. 4A). This pattern varied at the 2nd soil moisture level, where we found no significant differences between big and medium tussocks (63.9

Table 3

Average meteorological variables registered during photo-period (from 6:00 to 18:00 solar time) on every sample date

| July 2004 | Wind speed (m/s) | Temperature (°C) | Relative atmospheric humidity (%) | Global radiation (W m ⁻²) |
|-----------|------------------|------------------|-----------------------------------|---------------------------------------|
| 4 | 0.19 a (0.05) | 29.7 a (0.6) | 51.4 a (2.8) | 614.9 a (80.6) |
| 13 | 0.25 a (0.06) | 28.5 a (0.7) | 54.9 a (2.9) | 563.5 a (77.1) |
| 15 | 0.27 a (0.05) | 28.5 a (0.6) | 50.5 a (3.1) | 560.3 a (72.0) |
| 20 | 0.18 a (0.04) | 30.2 ab (0.7) | 66.1 ab (3.6) | 477.6 a (67.0) |
| 25 | 0.11 a (0.03) | 33.1 b (0.9) | 65.9 ab (4.7) | 500.6 a (72.4) |
| 26 | 0.28 a (0.06) | 31.5 ab (0.7) | 56.4 ab (3.5) | 513.6 a (75.2) |
| 29 | 0.27 a (0.05) | 29.5 a (0.8) | 71.2 b (4.6) | 413.4 a (82.2) |

Different letters next to the values belonging to each meteorological variable indicate significant differences among sample dates at $P < 0.05$ (Mann–Whitney U -test).

Table 4

Two-way ANOVA summary table assessing daily extrapolated whole plant transpiration from the porometer method among tussock size groups and soil moisture levels

| | d.f. | MS | F |
|------------------------------|------|------|-----------|
| Soil moisture | 2 | 30.3 | 250.02*** |
| Tussock size | 2 | 0.95 | 7.83*** |
| Soil moisture × tussock size | 4 | 0.60 | 4.97*** |
| Error | 94 | 0.12 | |

d.f.: degrees of freedom; MS: mean square.

*** $P < 0.001$.

and 65.9 mol H₂O m⁻² day⁻¹). The small tussocks provided the lowest whole plant transpiration (33.9 mol H₂O m⁻² day⁻¹). It should be kept in mind that at the 2nd soil moisture level, the small tussocks presented the lowest 1st leaf senescence condition area percentage (see Table 2). Finally, at the 3rd soil moisture level (<5%), all tussock sizes showed a reduction in mean transpiration (14.5 mol H₂O m⁻² day⁻¹), no significant differences being detected.

3.3. Representativity of the measurements performed

The average soil moisture (volumetric soil water content) in the field was $7.6 \pm 0.2\%$ ($n = 27$) on 8 August 2004. The stomatal conductance (g_s) values registered in the three size groups of potted tussocks (with soil moisture close to 7.6%) were within the range of g_s measured in field tussocks (Table 5).

Table 5

Comparison of pot experiment measurements with those performed on the site where *Stipa tenacissima* tussocks were removed ("natural conditions")

| | Potted tussocks | | | Field tussocks | |
|--------|-----------------|------|------|----------------|-------|
| | Soil moisture | Min. | Max | Min. | Max. |
| Small | 7.3 | 11.0 | 59.0 | 11.0 | 77.0 |
| Medium | 7.8 | 23.4 | 88.0 | 11.6 | 103.0 |
| Big | 6.6 | 9.2 | 50.0 | 6.2 | 79.0 |

The minimum (min.) and maximum (max.) stomatal conductance values (mmol m⁻² s⁻¹) between 6:00 and 18:00 solar time is shown. The average soil moisture (in % of volumetric soil water content) when the measurements were made in the field (8 August 2004) was $7.6 \pm 0.2\%$ ($n = 27$). The soil moisture (%) in each pot used in the comparison is shown. Three tussock sizes were assessed: big: $\varnothing > 60$ cm, medium: $60 \text{ cm} \geq \varnothing \geq 30$ cm and small: $\varnothing < 30$ cm.

3.4. Transpiration and net photosynthetic rates at whole plant scale

The results of the daily whole plant transpiration rate from Yplant simulation, mainly at the 1st soil moisture level (Fig. 4B), are different from those obtained by means of extrapolation weighted by senescence condition areas using the porometer method (Fig. 4A). Thus, whilst at the 1st, 2nd and 3rd soil moisture levels, calculation of the average extrapolated transpiration were: 124.8, 54.6 and 14.5 mol H₂O m⁻² day⁻¹, respectively (Fig. 4A), the result of the Yplant for the aforementioned three soil moisture levels provides a total average of: 45.8, 37.1 and 18.4 mol H₂O m⁻² day⁻¹, respectively (Fig. 4B). At the 1st and 2nd soil moisture levels the extrapolated transpiration overestimates on average +172.4 and +47.2% the transpiration from Yplant simulation. However, at the 3rd soil moisture level the extrapolated transpiration underestimates on average -21.2% of the transpiration estimated by Yplant. We obtained acceptable linear regression functions between the extrapolated (x) and simulated whole plant transpiration (y); these functions enabled us to correct the overestimation that occurs on estimating the whole plant transpiration using leaf measurements in each tussock size group: big ($y = 12.12 + 0.321x$; $r^2 = 0.53$; $*P < 0.05$), medium ($y = 29.55 + 0.199x$; $r^2 = 0.66$; $*P < 0.05$) and small ($y = 20.42 + 0.408x$; $r^2 = 0.68$; $**P < 0.01$).

The medium and small tussocks presented the highest whole plant transpiration calculated by Yplant at 1st soil moisture level (Fig. 4B), with significant differences between big and small tussocks. At the 2nd soil moisture level the medium tussocks showed higher transpiration, no significant difference being found at 3rd soil moisture level. Big and small tussocks were the ones that showed the highest whole plant net photosynthesis rates, and significant differences between big and medium tussocks were detected (Fig. 4C). The importance of the big tussocks was seen mainly at the 2nd soil moisture level, where the net photosynthesis was significantly higher than medium and small tussocks. When volumetric soil water content was less than 5% (3rd soil moisture level) there was no CO₂ fixation, with the exception of the medium tussocks, which presented a slight net photosynthesis (6 mmol CO₂ m⁻² day⁻¹). Big tussocks presented the highest WUE at the first two soil moisture levels (from >5 to 30%); when the 3rd soil moisture level was reached, however, this efficiency became negative for big and small tussocks, because, as was seen in the previous paragraph, the net photosynthesis

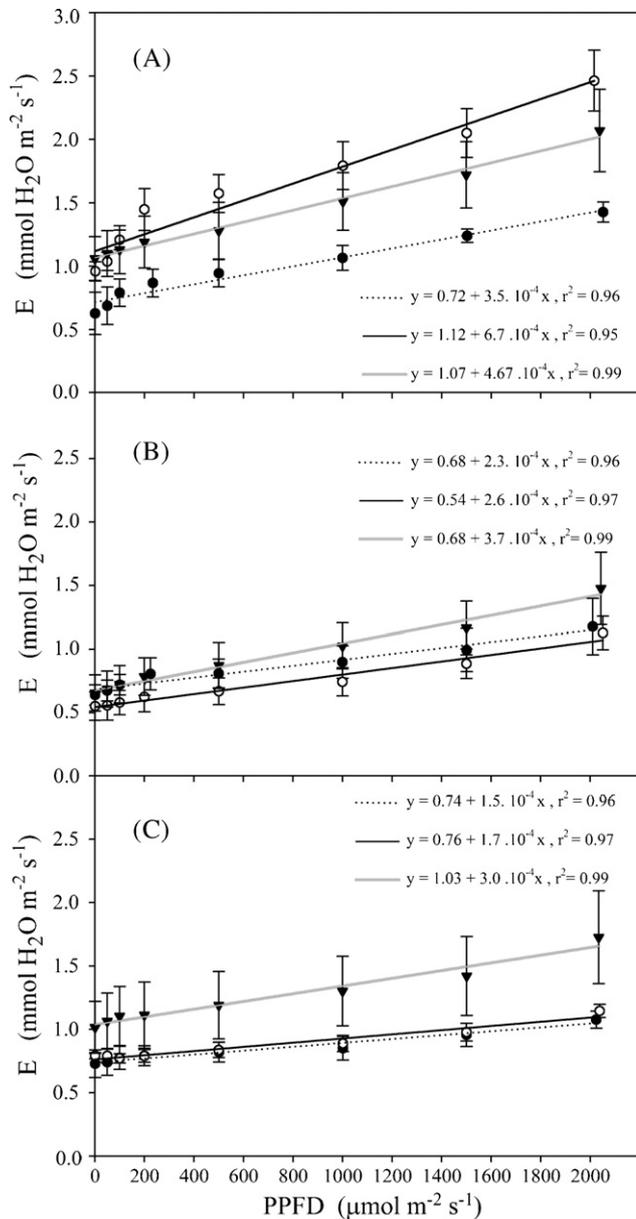


Fig. 3. Scatter plot among average transpiration rate (E , mmol H₂O m⁻² s⁻¹) vs. photosynthetic photon flux density (PPFD, μmol m⁻² s⁻¹) obtained from light response curves for the three tussock size groups (big, black circles: $\varnothing > 60$ cm; medium, open circles: $60 \text{ cm} \geq \varnothing \geq 30$ cm; small, black triangles: $\varnothing < 30$ cm) in each defined leaf senescence condition: (A) 1st, (B) 2nd and (C) 3rd. Fitted straight line from a linear regression analysis for each tussock size group is shown (big = dotted line, medium = black line and small = grey line), all F values of ANOVA were significant (***) $P < 0.001$.

was negative, CO₂ balance being dominated by the respiration process.

As part of the exploratory analysis of the PPFD on the leaf sections in the tussocks, we observed that box size increases from big to small tussocks (Fig. 5), where the upper quartile (75th percentile) occurs at higher PPFD in medium and small tussocks, surpassing 500 μmol m⁻² s⁻¹. This indicates that 75% of the leaf sections evaluated receive a higher range of radiation values as the tussocks get smaller. It should also be pointed out that the upper limit of the boxes is higher in the small tussocks. A more

specific analysis shows that there is a higher percentage (51%) of the leaf sections of the big tussocks within the radiation level between 0 and 200 μmol m⁻² s⁻¹ in comparison to medium and small tussocks (Fig. 5), whereas the latter ones showed a higher frequency of leaf area at radiations of over 800 μmol m⁻² s⁻¹. This analysis quantitatively shows the higher degree of self-shading in the big tussocks, which presented more green and dry leaf area (Table 2) with a lower PPFD, compared to medium and small tussocks.

4. Discussion

A wide range of processes determines photosynthesis and transpiration at whole plant level (Percy and Valladares, 1999) and the analysis of these processes can provide a mechanistic explanation of the influence of plant size on performance (Lusk, 2004; Schmidt and Zott, 2001). The higher radiation levels experienced by individual leaves from tussocks with less leaf area (medium and mainly small plants, see Table 2), is the most plausible explanation for their higher whole plant transpiration. Transpiration cooling is an efficient refrigeration mechanism, preventing the leaf from exposure to lethal temperatures (Grace, 1997). Activating this process, however, means that the plant must compromise efficient water use in order to avoid high temperatures. For Jones (1992) water conservation takes priority over minimising leaf temperature, and this appears to be applicable in the case of semiarid ecosystems in which water shortage can drastically compromise survival. This refrigeration by transpiration cooling, however, is a strategy that has been demonstrated not only in conditions of high water availability, such as in gravelly floodplain habitats (e.g. Matsumoto et al., 2000b), but also in some desert plants (e.g. Schmitt et al., 1993; Laurie et al., 1994). The high transpiration rate of the medium and small-sized *S. tenacissima* tussocks, particularly at high soil moisture levels ($\geq 15\%$ volumetric soil water content), might constitute a mechanism for minimizing overheating of these plants over the summer (“water spender” strategy *sensu* Grace, 1997). The leaves of intermediate and particularly small tussocks of this species presented higher slope and intersection values in their linear relationship between transpiration rate and irradiance than the leaves of the large tussocks (Fig. 3). In accordance with this, Ramírez et al. (2006) found that *S. tenacissima* leaves belonging to tussocks with the smallest leaf area and self-shading exhibited the highest transpiration rates measured by either IRGA or the heat balance method. It is widely known that the leaves exposed to sunlight are more capable of dissipating the excess heat caused by radiation than shaded leaves (Percy, 1997).

Zott et al. (2002), on conducting a morpho-physiological study at whole plant scale in *Vriesea sanguinolenta*, an epiphytic bromeliad, found that big individuals had a higher degree of self-shading, along with higher whole plant daily photosynthetic rates in comparison to small specimens. We also found that the big tussocks of *S. tenacissima* showed a higher degree of self-shading, preventing excessive light from entering, which in turn brought most of the leaves below or very close to the light saturation point. This fact seems to have allowed large tussocks

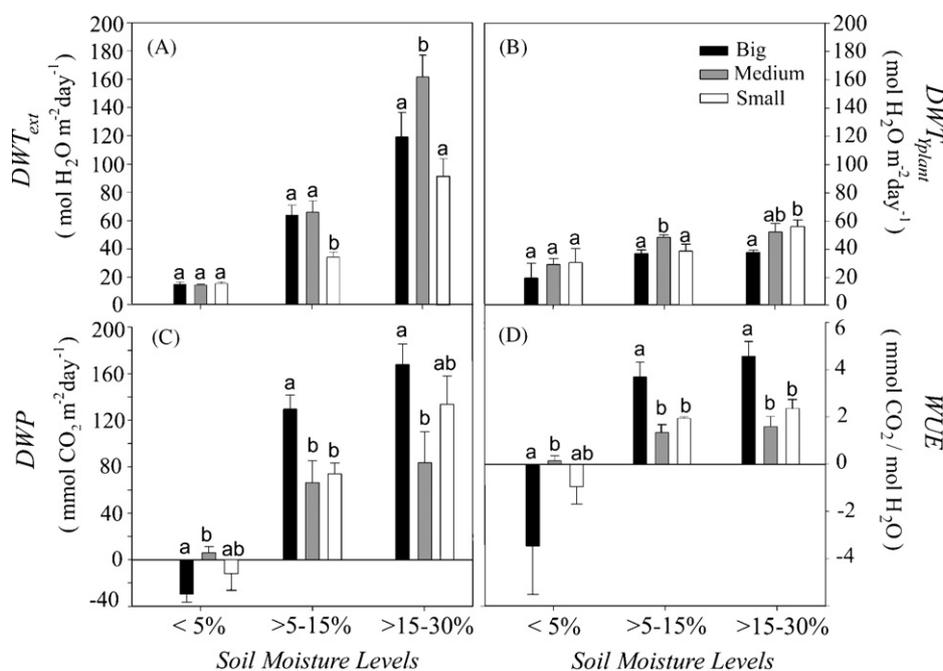


Fig. 4. Comparison of whole plant gas exchange among three tussocks size groups (big: $\varnothing > 60$ cm, medium: $60 \text{ cm} \geq \varnothing \geq 30$ cm and small: $\varnothing < 30$ cm) at each assessed level of volumetric soil water content (1st: $> 15\text{--}30\%$, 2nd: $> 5\text{--}15\%$ and 3rd: 5%). (A) Daily whole plant extrapolated transpiration rate (DWT_{ext}) from the defined porometer method. Yplant simulation: (B) daily whole plant transpiration (DWT_{Yplant}), (C) daily whole plant net photosynthesis (DWP) and (D) whole plant water use efficiency ($WUE = DWT_{Yplant}/DWP$). Different letters in (A) and Yplant simulations (B, C and D) indicate significant differences detected by Tukey HDS test ($*P < 0.05$) and Mann–Whitney U -test ($*P < 0.05$), respectively.

to optimise photosynthesis at whole level plant by minimizing photoinhibition, and to minimize transpiration, which was seen in increased water use efficiency (WUE) in relation to the small tussocks in volumetric soil water contents values $> 5\%$ (Fig. 4C). Large tussocks seem to have reached a size close to an optimum cost-benefit balance in which crown architecture together with leaf acclimation processes maximise carbon gain with the least loss of water (Percy and Valladares, 1999).

Increased water use efficiency at whole plant level has been reported during events of mild water stress in grass species (e.g. Hubick and Farquhar, 1989; Kalapos et al., 1996; Clifton-Brown and Lewandowski, 2000; Cabuslay et al., 2002). However, *S. tenacissima* showed no significant increases in WUE at intermediate levels of soil moisture ($15\text{--}5\%$ of volumetric soil water content, see Fig. 4D), which corresponds to the physiological behaviour of plants more tolerant to water stress (Cabuslay et al., 2002), and which also tallies with a conservative WUE perspective (*sensu* Jones, 1992). Our paper, however, confirms that severe water stress ($< 5\%$, values for dawn leaf water potential of between -5 and < -8 MPa according to studies by Pugnaire and Haase, 1996; Balaguer et al., 2002) significantly and negatively affects WUE at whole plant level in *S. tenacissima*. Photosynthetic performance can be expected to be better in small plants, since these are generally more vigorous and exhibit higher light capture efficiency than large plants (Lusk, 2004; Vesik, 2006). Thus, WUE could be reduced as plant size increases due to a decreased rate of carbon uptake per unit of leaf surface area. However, and contrary to expectations, water use efficiency increased with tussock size in *S. tenacissima*.

An important morphological characteristic in *S. tenacissima* is the accumulation of dead leaves with size and age (Sánchez, 1995). We found significant differences in the total dry leaf area among our three tussock size groups, which was associated with age and not only size since it correlated with the production of flowers (only observed in large plants). On evaluating leaf senescence, we observed that small tussocks were more sensitive to a decrease in soil water, with a faster loss of chlorophylls than in big and medium tussocks. This fast senescence with drought accounted for the abrupt drop in whole plant transpiration in small tussocks. One phenomenon associated with ontogeny is the capacity for gradual acclimation to changing and stressful environments and the development of mechanisms to maximise photosynthetic use of resources such as light and water (Huber and Wiggerman, 1997). It has been shown that a longer period of acclimation to high radiation levels gives rise to improved photosynthetic efficiency in herbaceous species (Noda et al., 2004). The potentially better acclimation to light and water stresses of large plants of *S. tenacissima* was revealed by the fact that large tussocks of this species experiencing drought maintained a higher fraction of photosynthetically active foliage when compared with medium and small tussocks, particularly under severe water deficit (Table 2).

In calculation of gas exchange based on measurements of *S. tenacissima* leaves, Haase et al. (1999) estimated the total daily carbon gain using IRGA measurements and integrating these throughout the day. They obtained values of between 508 and 893 mmol CO₂ m⁻² day⁻¹ during the months with high water availability (September 1993–May 1994) and between

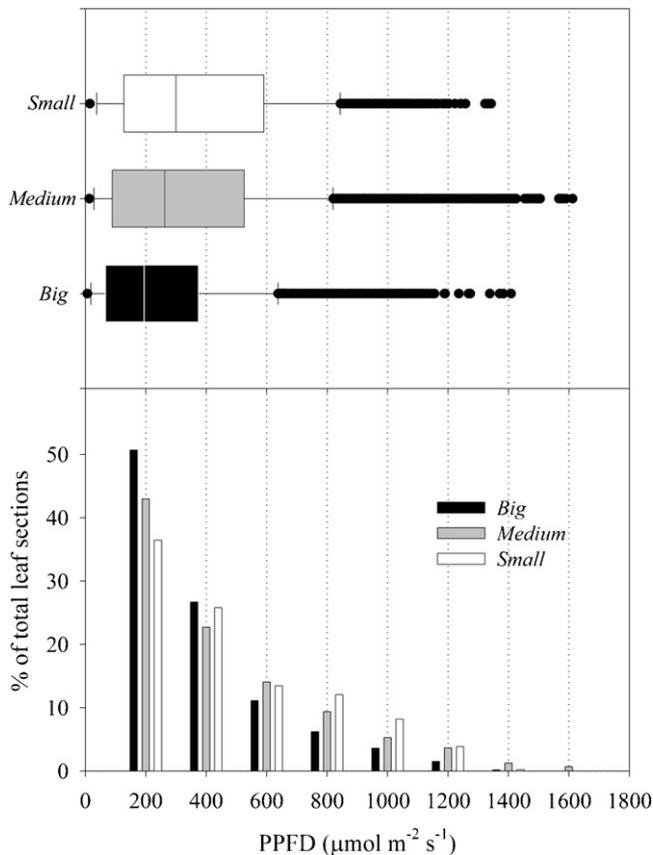


Fig. 5. Box plot analysis and frequency histogram (arranged into eight levels) assessing the photosynthetic photon flux density (PPFD) quantity on the total leaf sections belonging to three tussock size groups (big: $\text{Ø} > 60$ cm, medium: $60 \text{ cm} \geq \text{Ø} \geq 30$ cm and small: $\text{Ø} < 30$ cm). Results of Yplant simulation running for midday on July 4th.

–254 and 292 $\text{mmol CO}_2 \text{ m}^{-2} \text{ day}^{-1}$ during the drier months (June–September 1994). These results are a clear example of the high degree of overestimation that can result from extrapolating leaf level measurements to the whole plant. Valladares and Pugnaire (1999) used the Yplant model to show that a *S. tenacissima* tussock is capable of fixing only 40% of the CO_2 that would have been fixed if all the leaves of the tussock had been horizontal and without overlapping among neighbours. These authors obtained for *S. tenacissima* tussocks a net photosynthesis of between 90 and 250 $\text{mmol CO}_2 \text{ m}^{-2} \text{ day}^{-1}$ approximately, figures which correspond well with our results at a volumetric soil water content of between 5 and 25% (Fig. 4C). With regard to transpiration, Ramírez et al. (2006) showed that modelling tussock architecture considering all aspects of crown architecture including self-shading and leaf senescence was the best method for scaling-up from the leaf to the whole plant in *S. tenacissima* since it tallied well with direct determinations of plant transpiration. As with net photosynthesis, we showed that extrapolations of transpiration using leaf level measurements with IRGA or with a porometer overestimate daily whole plant transpiration by more than 100%, a result similar to what was found by Ramírez et al. (2006) under high soil water moisture. This overestimation, however, is reduced as water deficit increases, since below 5% of

volumetric soil water content, whole plant transpiration approximately matched extrapolations based upon leaf gas exchange measurements (Fig. 4A and B).

An important aspect when comparing gas exchange at the whole plant level among tussocks of different sizes and under different relative soil moisture levels is to avail of similar microclimatic conditions across sampling times. In our case, although wind speed and global radiation were similar among all sampling dates, air temperature and relative humidity exhibited some oscillations, and 2 days in particular (July 25th and 29th, see Table 3) were somewhat different from the rest. However, taking into account that the microclimatic conditions within the crown of *S. tenacissima* are not synchronized with the adjacent atmosphere due to the “shelter effect” of these dense tussocks (Domingo et al., 1996), only a marginal influence on plant performance of the slight temperature increases (July 25th) and relative atmospheric humidity (July 29th) can be expected for these sampling dates. Ramírez et al. (2006) made a comparison of gas exchange measurements between potted tussocks and tussocks naturally growing on the slopes from where the experimental tussocks were taken. They obtained a clear correspondence between these measurements under high soil water content during the spring of 2004. Similar to Ramírez et al. (2006), we have found here a clear correspondence in gas exchange (stomatal conductance) values among field and potted tussocks in soil water deficit conditions (Table 5). All this considered, we can conclude that our experimental setting was realistic with regard to mimicking the natural habitat conditions experienced by nearby populations of *S. tenacissima*.

5. Conclusions

Self-shading within the crown of *S. tenacissima* was a crucial process determining different whole plant gas exchange features in tussocks of different sizes. The higher radiation received by medium and small tussocks associated with a reduced self-shading (Fig. 5), caused an increased transpiration at high water availability levels. Big tussocks with higher photo-protective effects due to increased self-shading showed higher whole plant photosynthetic rates and WUE than small and medium tussocks. Self-shading has also been shown to reduce photo-inhibition (Valladares and Pugnaire, 1999) so all these results collectively suggest that this photo-protective mechanism allow large tussocks to withstand intense water stress minimizing foliar senescence when the soil dries out under high light and heat. Our results highlight the importance of accounting for plant size in scaling-up processes of leaf transpiration and photosynthesis from the leaf to the individual and from this to the stand, as it has been shown by Ramírez et al. (2007b) in *S. tenacissima* stands. This finding together with the differential responsiveness and sensitivity to drought of plants of different size must be taken into account in any consideration of water use efficiency and responses to water limitations in semiarid Mediterranean vegetation. In the very likely scenario of increasing water deficit in the Mediterranean region (e.g. Schröter et al., 2005), we can expect the capacity of the species of reversing senescence (as observed by Balaguer et al., 2002) to be greatly reduced. We have found

here that small tussocks, representing sexual recruits of the population, were more sensitive to severe water deficit and exhibited faster leaf senescence in response to drought than large tussocks. Thus, we can expect an increased risk of establishment failure of *S. tenacissima* by means of sexual reproduction, which, in agreement with Haase et al. (1999), can dramatically reduce the genetic diversity of the populations of this species as water becomes scarcer.

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References

- Arnon, D.L., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.* 24, 1–15.
- Balaguer, L., Pugnaire, F.I., Martínez-Ferri, E., Armas, C., Valladares, F., Manrique, E., 2002. Ecophysiological significance of chlorophyll loss and reduced photochemical efficiency under extreme aridity in *Stipa tenacissima* L. *Plant Soil* 240, 343–352.
- Baldocchi, D.D., Luxmoore, R.J., Hatfield, J.L., 1991. Discerning the forest from the trees: an essay on scaling canopy stomatal conductance. *Agric. For. Meteorol.* 54, 197–226.
- Bellot, J., Maestre, F.T., Hernández, N., 2004. Spatio-temporal dynamics of chlorophyll fluorescence in a semi-arid Mediterranean shrubland. *J. Arid Environ.* 58 (3), 295–308.
- Beyschlag, W.R., Rye, R.J., 1998. Modelling leaf/canopy photosynthesis. In: Raghavendra, A.S. (Ed.), *Photosynthesis: A Comprehensive Treatise*. Cambridge University Press, Cambridge, pp. 305–317.
- Cabuslay, G.S., Ito, O., Alejar, A.A., 2002. Physiological evaluation of responses of rice (*Oryza sativa* L.) to water deficit. *Plant Sci.* 163, 815–827.
- Cerdà, A., 1997. The effect of patchy distribution of *Stipa tenacissima* L. on runoff and erosion. *J. Arid Environ.* 36, 37–51.
- Clifton-Brown, J.C., Lewandowski, I., 2000. Water use efficiency and biomass partitioning of three different *Miscanthus* genotypes with limited and unlimited water supply. *Ann. Bot.* 86, 191–200.
- Domingo, F., Van Gardingen, P.R., Brenner, A.J., 1996. Boundary layer conductance of two native species in southeast Spain. *Agric. For. Meteorol.* 81, 179–199.
- Dragoni, D., Lakso, A.N., Piccioni, R.M., 2005. Transpiration of apple trees in a humid climate using heat pulse sap flow gauges calibrated with whole-canopy gas exchange chambers. *Agric. For. Manage.* 130, 85–94.
- Dugas, W.A., Wallace, J.S., Allen, S.J., Roberts, J.M., 1993. Heat balance, porometer, and deuterium estimates of transpiration from potted trees. *Agric. For. Meteorol.* 64, 47–62.
- Dugas, W.A., Prior, S.A., Rogers, H.H., 1997. Transpiration from sorghum and soybean growing under ambient and elevated CO₂ concentrations. *Agric. For. Meteorol.* 83, 37–48.
- Field, C.B., Ball, J.T., Berry, J.A., 1989. Photosynthesis: principles and field techniques. In: Pearcy, R.W., Ehleringer, J., Mooney, H.A., Rundel, P.W. (Eds.), *Plant Physiological Ecology, Field Methods and Instrumentation*. Chapman & Hall, London, pp. 209–253.
- Gasque, M., 1999. Colonización del esparto (*Stipa tenacissima* L.) en sectores degradados del clima semiárido. Ph.D. Thesis. Politécnica de Valencia University, Spain.
- Gasque, M., García-Fayos, P., 2003. Seed dormancy and longevity in *Stipa tenacissima* L. (Poaceae). *Plant Ecol.* 168, 279–290.
- Grace, J., 1997. Plant water relations. In: Crawley, M.J. (Ed.), *Plant Ecology*, 2nd ed. Blackwell Science Ltd, London, pp. 28–50.
- Haase, P., Pugnaire, F.I., Incoll, L.D., 1995. Seed production and dispersal in the semi-arid tussock grass *Stipa tenacissima* L. during masting. *J. Arid Environ.* 31, 55–65.
- Haase, P., Pugnaire, F.I., Clark, S.C., Incoll, L., 1999. Environmental control of canopy dynamics and photosynthetic rate in the evergreen tussock grass *Stipa tenacissima*. *Plant Ecol.* 145, 327–339.
- Huber, H., Wiggerman, L., 1997. Shade avoidance in the clonal herbs *Trifolium fragiferum*: a field study with experimentally manipulated vegetation height. *Plant Ecol.* 130, 53–62.
- Hubick, K., Farquhar, G.D., 1989. Carbon isotope discrimination and the ratio of carbon gained to water lost in barley cultivars. *Plant Cell Environ.* 12, 795–804.
- Jarvis, P.G., 1995. Scaling processes and problems. *Plant Cell Environ.* 18, 1079–1089.
- Jones, H.G., 1992. *Plants and Microclimate: A Quantitative Approach to Environmental Plant Physiology*, 2nd ed. Cambridge University Press, Cambridge.
- Kalapos, T., Van-Den-Boogaard, R., Lambers, H., 1996. Effect of soil drying on growth, biomass allocation and leaf gas exchange of two annual grass species. *Plant Soil* 185 (1), 137–149.
- Kruijt, B., Ongeri, S., Jarvis, P.G., 1997. Scaling of PAR absorption, photosynthesis and transpiration from leaves to canopy. In: Van Gardinen, P.R., Foody, G.M., Curran, P.J. (Eds.), *Scaling Up: From the Cell to Landscape*. Society for Experimental Biology Seminar Series 63. Cambridge University Press, Cambridge.
- Laurie, S., Bradbury, M., Stewart, G.R., 1994. Relationships between leaf temperature, compatible solutes and antitranspirant treatment in some desert plants. *Plant Sci.* 100, 147–156.
- Leuning, R., 1995. A critical appraisal of a combined stomatal-photosynthesis model for C3 plants. *Plant Cell Environ.* 18, 339–355.
- Levin, S., 1995. The problem of pattern and scale in ecology. In: Powell, T.M., Steele, J.H. (Eds.), *Ecological Time Series*. Chapman & Hall, New York.
- Lu, P., Woo, K.-C., Liu, Z.-T., 2002. Estimation of whole-plant transpiration of bananas using sap flow measurements. *J. Exp. Bot.* 53 (375), 1771–1779.
- Lusk, C.H., 2004. Leaf area and growth of juvenile temperate evergreens in low light: species of contrasting shade tolerance change rank during ontogeny. *Funct. Ecol.* 18 (6), 820–828.
- Maestre, F., Bautista, S., Cortina, J., Bellot, J., 2001. Potential for using facilitation shrubs on a semiarid degraded steppe. *Ecol. Appl.* 11 (6), 1641–1655.
- Maestre, F., Bautista, S., Cortina, J., 2003. Positive, negative, and net effects in grass–shrub interactions in Mediterranean semiarid grasslands. *Ecology* 84 (12), 3186–3197.
- Matsumoto, J., Muraoka, H., Washitani, I., 2000a. Whole plant carbon gain of an endangered herbaceous species *Aster kantoensis* and the influence of shading by an alien grass *Eragrostis curvula* in its gravelly floodplain habitat. *Ann. Bot.* 86, 787–797.
- Matsumoto, J., Muraoka, H., Washitani, I., 2000b. Ecophysiological mechanisms used by *Aster kantoensis*, an endangered species, to withstand high light and heat stresses of its gravelly floodplain habitat. *Ann. Bot.* 86, 777–785.
- Morgan, J.A., LeCain, D.R., Read, J.J., Hunt, H.W., Knight, W.G., 1998. Photosynthetic pathway and ontogeny affect water relations and the impact of CO₂ on *Bouteloua gracilis* (C₄) and *Pascopyrum smithii* (C₃). *Oecologia* 114, 483–493.
- Noda, H., Muraoka, H., Washitani, I., 2004. Morphological and physiological acclimation responses to contrasting light and water regimes in *Primula sieboldii*. *Ecol. Res.* 19, 331–340.
- Pearcy, R.W., Schulze, E.-D., Zimmermann, R., 1989. Measurement of transpiration and leaf conductance. In: Pearcy, R.W., Ehleringer, J., Mooney, H.A., Rundel, P.W. (Eds.), *Plant Physiological Ecology, Field Methods and Instrumentation*. Chapman & Hall, London, pp. 209–253.

- H.A., Rundel, P.W. (Eds.), *Plant Physiological Ecology, Field Methods and Instrumentation*. Chapman & Hall, London, pp. 137–159.
- Pearcy, R.W., Yang, W., 1996. A three-dimensional crown architecture model for assessment of light capture and carbon gain by understory plants. *Oecologia* 108, 1–12.
- Pearcy, R.W., 1997. Acclimation to sun and shade. In: Raghavendra, A.S. (Ed.), *Photosynthesis: A Comprehensive Treatise*. Cambridge University Press, Cambridge, pp. 250–263.
- Pearcy, R.W., Valladares, F., 1999. Resource acquisition by plants: the role of crown architecture. In: Press, M.C., Scholes, J.D., Barker, M.G. (Eds.), *Physiological Plant Ecology, the 39th Symposium of the British Ecological Society held at the University of New York, September 7–9, 1998*, Blackwell Science Ltd., London, pp. 45–66.
- Polley, H.W., Johnson, H.B., Tischler, C.R., Torbert, H.A., 1999. Links between transpiration and plant nitrogen: variation with atmospheric CO₂ concentration and nitrogen availability. *Int. J. Plant Sci.* 160 (3), 535–542.
- Puigdefábregas, J., Sánchez, G., 1996. Geomorphological implications of vegetation patchiness on semi-arid slopes. In: Anderson, M.G., Brooks, S.M. (Eds.), *Advances in Hillslopes Processes*, vol. 2. John Wiley and Sons Ltd, New York, pp. 1027–1060.
- Pugnaire, F.I., Haase, P., 1996. Comparative physiology and growth of two perennial tussock grass species in a semi-arid environment. *Ann. Bot.* 77, 81–86.
- Pugnaire, F.I., Haase, P., Incoll, L., Clark, S.C., 1996. Response of tussock grass *Stipa tenacissima* L. to watering in a semi-arid environment. *Funct. Ecol.* 10, 265–274.
- Ramírez, D.A., Valladares, F., Blasco, A., Bellot, J., 2006. Assessing transpiration in the tussock grass *Stipa tenacissima* L.: the crucial role of the interplay between morphology and physiology. *Acta Oecol.* 30, 386–398.
- Ramírez, D.A., Bellot, J., Domingo, F., Blasco, A., 2007a. Can water responses in *Stipa tenacissima* L. during the summer season be promoted by non-rainfall water gains in soil? *Plant Soil* 291, 67–79.
- Ramírez, D.A., Bellot, J., Domingo, F., Blasco, A., 2007b. Stand transpiration of *Stipa tenacissima* grassland by sequential scaling and multi-source evapotranspiration modelling. *J. Hydrol.* 342, 124–133.
- Robredo, A., Pérez-López, U., Sainz de la Maza, H., González-Moro, B., Lacuesta, M., Mena-Petite, A., Muñoz-Rueda, A., 2007. Elevated CO₂ alleviates the impact of drought on barley improving water status by lowering stomatal conductance and delaying its effects on photosynthesis. *Environ. Exp. Bot.* 59 (3), 252–263.
- Roumet, C., Garnier, E., Suzor, H., Salager, J., Roy, J., 2000. Short and long-term responses of whole-plant gas exchange to elevated CO₂ in four herbaceous species. *Environ. Exp. Bot.* 43, 155–169.
- Ryel, R.J., Beyschlag, W.R., Caldwell, M.M., 1993. Foliage orientation and carbon gain in two tussock grasses as assessed with a new whole-plant gas-exchange model. *Funct. Ecol.* 7, 115–124.
- Ryel, R.J., Beyschlag, W.R., Caldwell, M.M., 1994. Light field heterogeneity among tussock grasses: theoretical considerations of light harvesting and seedling establishment in tussock and uniform tiller distributions. *Oecologia* 98, 241–246.
- Ryel, R.J., Beyschlag, W.R., 1995. Benefits associated with steep foliage orientation in two tussock grasses of the American Intermountain West. A look at water-use-efficiency and photoinhibition. *Flora Jena* 190 (3), 251–260.
- Sánchez, G., Puigdefábregas, J., 1994. Interactions of plant growth and sediment movement on slopes in a semi-arid environment. *Geomorphology* 9, 243–260.
- Sánchez, G., 1995. *Arquitectura y dinámica de las matas de esparto (Stipa tenacissima L.)*, efectos en el medio e interacciones con la erosión. Ph.D. Thesis. Autónoma de Madrid University, Spain.
- Schmitt, A.K., Martin, C.E., Loesch, V.S., Schmitt, A., 1993. Mid-summer gas exchange and water relations of seven C-3 species in a desert wash in Baja California, Mexico. *J. Arid Environ.* 24, 155–164.
- Schmidt, G., Zotz, G., 2001. Ecophysiological consequences of differences in plant size: in situ carbon gain and water relations of the epiphytic bromeliad, *Vriesea sanguinolenta*. *Plant Cell Environ.* 24 (1), 101–111.
- Schröter, D., Cramer, W., Leemans, R., Prentice, I.C., Araújo, M.B., Arnell, N.W., Bondeau, A., Bugmann, H., Carter, T.R., Gracia, C.A., Vega-Leinert, A.C.D.L., Erhard, M., Ewert, F., Glendinning, M., House, J.I., Kankaanpää, S., Klein, R.J.T., Lavorel, S., Lindner, M., Metzger, M.J., Meyer, J., Mitchell, T.D., Reginster, I., Sabaté, M.R.S., Sitch, S., Smith, B., Smith, J., Smith, P., Sykes, M.T., Thonicke, K., Thuiller, W., Tuck, G., Zaehle, S., Zierl, B., 2005. Ecosystem service supply and vulnerability to global change in Europe. *Science* 310 (5752), 1333–1337.
- Schulze, E.-D., Čermák, J., Matyssek, R., Penka, M., Zimmermann, R., Vasíček, F., 1985. Canopy transpiration and water fluxes in the xylem of the trunk of *Larix* and *Picea* trees—a comparison of xylem flow, porometer and cuvette measurements. *Oecologia* 66, 475–483.
- Schulze, E.-D., 1986. Whole-plant responses to drought. *Aust. J. Plant Physiol.* 13, 127–141.
- Senock, R.S., Ham, J.M., 1995. Measurement of water use by prairie grasses with heat balance sap flow gauges. *J. Range Manage.* 48, 150–158.
- Servicio del Esparto, 1951. *Estudios y experiencias sobre el esparto*. Ministerio de Industria y Comercio de Agricultura, Madrid, Spain.
- Valladares, F., Pugnaire, F.I., 1999. Tradeoffs between irradiance capture and avoidance in semi-arid environments assessed with a crown architecture model. *Ann. Bot.* 83, 459–469.
- Valladares, F., Dobarro, I., Sánchez-Gómez, D., Pearcy, R.W., 2005. Photoinhibition and drought in Mediterranean woody saplings: scaling effects and interactions in sun and shade phenotypes. *J. Exp. Bot.* 56, 483–494.
- Vesk, P.A., 2006. Plant size and resprouting ability: trading tolerance and avoidance of damage? *J. Ecol.* 94, 1027–1034.
- Werner, C., Ryel, R.J., Correia, O., Beyschlag, W.R., 2001a. Effects of photoinhibition on whole-plant carbon gain assessed with photosynthesis model. *Plant Cell Environ.* 24, 27–40.
- Werner, C., Ryel, R.J., Correia, O., Beyschlag, W.R., 2001b. Structural and functional variability within the canopy and its relevance for carbon gain and stress avoidance. *Acta Oecol.* 22, 129–138.
- White, F., 1983. *The vegetation of Africa*. Natural Resources Research XX. UNESCO, Paris.
- Zotz, G., Richling, P., Valladares, F., 2002. A simulation study on the importance of size-related change in leaf morphology and physiology for carbon gain in an epiphytic bromeliad. *Ann. Bot.* 90, 437–443.