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Original article

Assessing transpiration in the tussock grass *Stipa tenacissima* L.: the crucial role of the interplay between morphology and physiology [☆]

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ABSTRACT

Plant transpiration has a key role on both plant performance and ecosystem functioning in arid zones, but realistic estimates at appropriate spatial-temporal scales are scarce. Leaf and tiller morphology and crown architecture were studied together with leaf physiology and whole plant water balance in four individual plants of *Stipa tenacissima* of different sizes to determine the relative influence of processes taking place at different spatial and temporal scales on whole plant transpiration. Transpiration was estimated in potted plants by leaf-level gas exchange techniques (infrared gas analyzer and porometer), by sap flow measurements, and by integrating leaf physiology and crown architecture with the 3-D computer model Yplant. Daily transpiration of each individual plant was monitored using a gravimetric method, which rendered the reference values. Leaves on each individual plant significantly varied in their physiological status. Young and green parts of the leaves showed five times higher chlorophyll concentration and greater photosynthetic capacity than the senescent parts of the foliage. Instantaneous leaf-level transpiration measurements should not be used to estimate plant transpiration, owing to the fact that extrapolations overestimated individual transpiration by more than 100%. Considering leaf age effects and scaling the estimates according to the relative amount of each foliage category reduced this difference to 46% though it was still significantly higher than gravimetric measurements. Sap flow calculations also overestimated tussock transpiration. However, 3-D reconstruction of plants with Yplant and transpiration estimates, considering both the physiological status and the daily pattern of radiation experienced by each individual leaf section within the crown, matched the gravimetric measurements (differences were only 4.4%). The complex interplay of leaf physiology and crown structure must be taken into account in scaling up plant transpiration from instantaneous, leaf-level measurements, and our study indicates that transpiration of complex crowns is easily overestimated.

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1. Introduction

A dry summer season and high precipitation variability are some typical characteristics of Mediterranean semiarid regions. The fact that evapotranspiration (ET) demand is not matched via precipitation most of the year becomes a serious problem in these regions because it exerts a limitation in the water supply for setting up vegetation, which particularly affects farming activities (Piñol et al., 1995; Thornes, 1999). ET has paramount importance in water resource management, environmental studies and plant production (Pérez Cueva, 1994; Bellot et al., 1999; Gracia et al., 1999). However, its estimate has been considered very difficult to infer, requiring a special focus on landscapes formed by disperse patchy vegetation, as in semiarid environments (Domingo et al., 1999).

Transpiration, a fundamental part of ET, is regulated via stomatal opening in the leaf. The Penman–Monteith equation (Monteith, 1965) had been the main method used for scaling up transpiration from leaf to canopy. This method has been applied in some works considering the stand of vegetation as a mono or multilayer “big leaf” (Leuning et al., 1995; Valentini et al., 1995; Infante et al., 1997). Scaling up is a gradual process and to know how information is shifted from one scale to another is crucial for understanding the mechanisms of pattern generation, which in turn are extremely important for resource management (Levin, 1995). This transfer of information must be done in a sequential manner, and with the use of contiguous scales. Thus, Baldocchi et al. (1991) recommend three adjacent scales to reach this objective: sub, operational and macroscale. In this sense, the exploration and understanding of the individual level may be the key to predict canopy level responses from a leaf—individual—canopy scaling perspective.

To understand and describe transpiration at the level of whole plant requires recognizing the heterogeneity of processes that occur inside the plants (Pearcy and Valladares, 1999). Once the main mechanisms are detected we shall be able to estimate variables such as photosynthesis or transpiration in the whole individual. Following Pearcy (1997), architecture, senescence and acclimatation of leaves are the main processes that can help in studying the whole individual. The comparison of different methods (cuvette, porometer, heat balance, deuterium) for evaluating the step from leaf transpiration to the whole individual has been performed on potted trees (e.g. Schulze et al., 1985; Dugas et al., 1993) and herbaceous species (Baker and Van Bavel, 1987; Senock and Ham, 1995). In these latter species, three-dimensional architectural models have allowed the study of transpiration in tussock grasses, these proved to be very useful tools for the study of processes at an individual scale (Ryel et al., 1993; Ryel and Beyschlag, 1995).

Stipa tenacissima L. is a perennial tussock grass, endemic in the Western Mediterranean region (Djebaili, 1988) being most abundant in the Iberian Peninsula. This species was used as raw material for manufacturing paper, thread, baskets and jute, which were commercialized in the semiarid zones of Spain where *S. tenacissima* grows. The socioeconomic value of this species favored its management:

- pruning to increase productivity;
- reclamation and reforestation of *Stipa* areas including planting and farming (Servicio del Esparto, 1951).

This species covers semiarid areas with patchy spatial distribution, covering more than 600,000 ha of natural and semi-natural areas in the Southeast of Spain (Servicio del Esparto, 1950). The mosaic structure of *S. tenacissima* grassland has great importance in the distribution of water and sediments, as the tussock microsite (patch) favors greater water infiltration, soil moisture, litter and sediment accumulation (Sánchez, 1995; Puigdefábregas and Sánchez, 1996; Cerdà, 1997), whereby these characteristics generally create a potential that facilitates restoration (Maestre et al., 2001).

Scaling up processes in this species is not an easy task. Litter accumulation on the tussock causes a “shelter effect”, that results in a poor ‘coupling’ between the inside of the tussock and the surrounding atmosphere (Domingo et al., 1996), and has been the main cause of the poor results obtained in scaling up aerodynamic conductance from leaf conductance to tussock level in *S. tenacissima* (Domingo et al., 1997). The great importance of the aforementioned species in semiarid environments highlights the need of improving studies on its water relations. We hypothesized that individual or tussock level measurements in *S. tenacissima* following standard procedures overestimate transpiration because these have not taken into consideration crown architecture and leaf aging processes. The influence of these two factors will be reflected in that some active parts of the tussock may have been shaded and, together with senescent leaves, could be the cause of a significant reduction in tussock transpiration.

Our objective was to explore the relative importance of the different processes involved in the transpiration of this tussock species by comparing different methods, and to assess the importance of considering leaf physiological status and crown architecture in the scaling from the leaf to the whole plant.

2. Materials and methods

2.1. Implementation of the experiment

We used 12 tussocks of *S. tenacissima* belonging to three different size groups (hereinafter G, M and S tussocks), G: 60–85 cm, M: 40–60 cm and S: < 30 cm diameter of average ground cover. The blocks of soil holding each tussock were removed from a micro-basin facing south of the “El Ventós” experimental area (Bellot et al., 2004), located in the province of Alicante, in SE Spain (38°29'N, 0°37'W). The average density of *S. tenacissima* in this area was 0.66 ± 0.099 tussocks per m², where the group sizes G + M and S accounted for 49.8% and 17.3% of the total *Stipa* cover, respectively. We unearthed the tussocks on 18/11/2002, after a rainfall event, to reduce root damage. Subsequently, the blocks of soil containing each plant were put into pots and the remaining volume was filled up with sieved soil. The plants were maintained approximately during 1 year and a half before conducting

the experiment on an experimental plot located at the University of Alicante.

Four tussocks belonging to size groups G and M (two plants of each group) were used for this experiment. Plants were watered up to field capacity, and the soil of the pot was covered with laboratory film (Parafilm M, Pechiney Plastic Packaging, Chicago, USA) to avoid water loss through soil evaporation.

2.2. Morphology and classification of leaves and tillers

The tussocks were divided into two concentric sectors (internal and external) as had been worked out by Valladares and Pugnaire (1999), these were marked as:

- internal sector: five and four tillers, in G and M tussocks, respectively;
- external sector: ten and six tillers, in G and M tussock, respectively.

Tiller leaf area was estimated using the relationship between the product of leaf length \times width and the surface, calculated by scanned leaves ($y = 0.982x + 0.063$, $N = 100$, $R^2 = 0.94$, $P < 0.05$). The surface of green foliage was divided into three leaf physiological stages (LPS 1 = optimal, 2 = intermediate, 3 = senescent), depending on the degree of dryness from the tip to the base of the lamina, which in turn is related to age (Sánchez, 1995; Haase et al., 1999). The dead parts of the leaf were considered as the last LPS stage (LPS 4 = dead). The chromatic characteristics of the LPS 1–3 was measured in four G tussocks with a portable chlorophyll meter (SPAD-502, Minolta Camera Co. Ltd, Japan), and was simultaneously calibrated using chlorophyll concentration as determined by Arnon's method (Arnon, 1949) after extraction with 80% acetone.

The sectors were divided into four zones according to their orientation: NW, NE, SW and SE. We sampled in each zone both the internal and external sectors, three and five tillers, respectively (36 total tillers by 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, and we estimated the angle from horizontal of four sections of all the

leaves of each tiller (four to five leaves for tiller) in a total of eight tillers (four from the north and four from the south zone).

2.3. Continuous measurements: gravimetric and sap flow monitoring

Each pot with the tussocks was placed on load cells (M-40 and M-30 models, TILCA, S.L. Zaragoza, Spain; bearing 60 and 30 kg of capacity, respectively). The weight loss of the pot containing the tussock (gravimetric method), estimated between 06:00 and 18:00 solar time, was due to tussock transpiration. This value served us as a reference or standard method. We installed six stem-flow gauges (2 mm diameter, SAG2-WS Dynagage model, Dynamax Inc., Houston, USA): two in each of the G tussocks and one in each of the M tussocks. Gauges were set at the base of the tillers, and connected to a data logger (CR10 Campbell Scientific Model, Ltd, UK), that registered sap flow velocity measurements every 5 min, and averaged every 15 min. The heat balance method was applied to calculate sap flow (theoretical basis: Sakuratani, 1981; Baker and van Bavel, 1987), where the thermal conductance constant (k_{sh}) of the radial heat flux was estimated as the minimal apparent k_{sh} between 00:00 and 04:00 hours, assuming no sap flow during this period. When finishing the evaluation and removing the sensors we estimated the leaf area of each tiller measured with the purpose of relating sap flow velocity to leaf area, obtaining in this manner the so called "leaf-related sap flow" (Q_l , sensu Edwards et al., 1996). Tussock transpiration was calculated multiplying Q_l by the total surface of the leaves. We will refer to the heat balance method as "sap flow method" in the following text.

A meteorological portable station (Onset Computer Corporation, USA) was used for automatic estimation of: wind direction and speed (Meteo 1 Wind-HOBO), air temperature and humidity (HOBO Pro RH/Temp.), photosynthetic photon flux density (PPFD, HA-LI HOBO) and global radiation (PYR HOBO). All data loggers recorded outputs every 5 min. The environmental conditions (3–7 April 2004) was characterized by clear days, except for 5 April, as that day the sky were partially covered between 11:00 and 15:00 solar time (Fig. 1). The vapor pressure deficit (VPD) showed a pattern similar to that

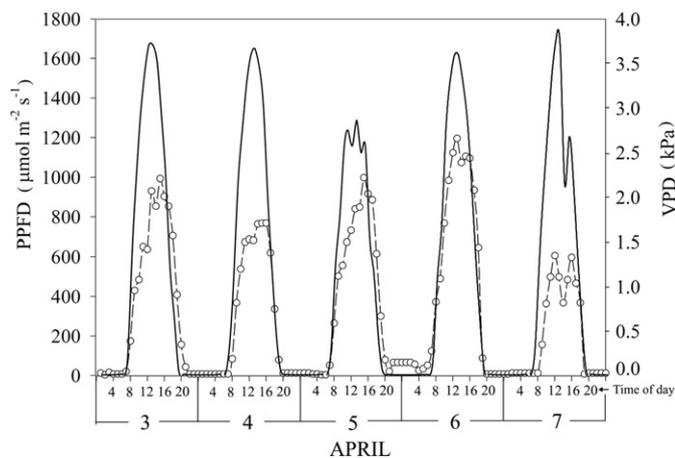


Fig. 1 – PPFD (solid line) and VPD (open circle and dashed line) during the study period (3–7 April 2004).

of the PPFD, with moderate values (1.52–2.66 kPa) between 12:00 and 15:00 solar time during the period 3–6 April 2004 (Fig. 1). However, the last day of evaluation was characterized by a greater atmospheric humidity during the photoperiod (average relative humidity = 69.6%) whereby in comparison to the other days, VPD showed lower values during 09:00 and 16:00 solar time (Fig. 1).

2.4. Discontinuous measurements

An infrared gas analyzer (IRGA - LI-COR 6400 model, LI-COR Biosciences Inc., Nebraska, USA) was used to estimate direct transpiration rates in eight and six replicates for each G and M tussocks, respectively. Each replica consisted of three to four leaves taped together, that were placed in the IRGA's chamber fixing the PPFD so that all samples would be under the same conditions. The fixed PPFD values were: 885, 1940 and 920 $\mu\text{mol m}^{-2} \text{s}^{-1}$ according to environmental PPFD at the solar time when the measurements were taken (08:00, 12:00 and 16:00 solar time). The conditions of the IRGA's reference chamber set for the three evaluations were: boundary layer conductance (g_b) = 2.30 $\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$, CO_2 concentration in the reference cell (CO_2) = 370 $\mu\text{mol CO}_2 \text{mol}^{-1}$, airflow rate = 500 $\mu\text{mol s}^{-1}$. Relative humidity in the reference cell (RH_c) and VPD based on air temperature during the three times of the day evaluated at the IRGA chamber took on the following values: RH_c 08:00 and 16:00 = 40%, RH_c 12:00 = 35%, $\text{VPD}_{8:00}$ = 2.2–2.4 kPa, $\text{VPD}_{12:00}$ = 2.9–3.0 kPa and $\text{VPD}_{16:00}$ = 2.3–2.4 kPa.

PPFD versus net photosynthesis (light response), and intercellular carbon dioxide concentration and net photosynthesis (A/ci response) responses curves were simulated using IRGA for LPS 1–3 in the four tussocks. The relationship between transpiration and PPFD was fitted with a linear regression analysis for each of the 1–3 LPS. At the same time, using the information inferred from curves and supported by the Photosyn Assistant software (Dundee Scientific, Scotland, UK), we calculated: light saturated CO_2 assimilation rate (A_{max} , $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$), dark respiration rate (R , $\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$), absorbed PPFD quantum yield (Φ), maximum rate of carboxylation ($V_{c\text{max}}$, $\mu\text{mol m}^{-2} \text{s}^{-1}$) and maximum rate of electron transport (J_{max} , $\mu\text{mol m}^{-2} \text{s}^{-1}$). Photosynthetic light response was estimated by the IRGA systems under the following conditions: sequence of programmed PPFD = 0, 50, 100, 200, 500, 1000, 1500 and 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$, RH_c = 35%, CO_2 = 370 $\mu\text{mol CO}_2 \text{mol}^{-1}$. The measurements of A/ci response curves were performed under the following conditions: CO_2 sequence = 400, 300, 200, 100, 50, 400, 400, 600 and 800 $\mu\text{mol CO}_2 \text{mol}^{-1}$, RH_c = 40% and PPFD = 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The temperature programmed for the IRGA chamber was 25 °C for all simulations.

Hourly stomatal H_2O conductance (g_s) was measured using a transient porometer (AP4 model, Delta T Devices Ltd., Cambridge, UK) in a total of five leaves from each tussock. Measurements were taken from 07:00 to 18:00 solar time. A multi-parameter ventilation meter (Velocicalc Plus 8386 model, TSI Incorporated, Minnesota, USA) was used to measure wind speed, air temperature and relative humidity in the area near to the leaf where porometer measurements were taken. Domingo et al. (1996) estimated boundary layer

conductance in *S. tenacissima* (g_b) in relation to with wind speed (v). It was described with a power function: $g_b = a v^b$, where: $a = 0.06$, $b = 0.363$. Total H_2O conductance (g_t) was calculated taking into consideration that this species has hypostomatous leaves (Field et al., 1989):

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

$\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$.

Transpiration rate (E) 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 Percy et al., 1989):

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

$\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$.

Where:

w_i = mole fraction derived from saturation vapor pressure at leaf temperature ($\text{mol H}_2\text{O mol}^{-1}$ air);

w_a = mole fraction derived from vapor pressure at the adjacent atmospheric temperature ($\text{mol H}_2\text{O mol}^{-1}$ air).

All E estimates based on: stomatal conductance (measured by porometer), boundary layer and VPD extrapolated to total green area shall be referred to as "porometer method" throughout this work.

2.5. Representativity of the measurements performed

With the purpose of verifying the representativity of E obtained from the leaves of *S. tenacissima*, measurements were also taken from field tussock ("natural conditions"). The operations were conducted at Sierra "El Ventós", on the slope where the removal of tussocks took place and where soil water conditions were similar to those of the experiments done in pots (volumetric water content in the soil $\approx 0.25 \text{ m}^3 \text{ m}^{-3}$). On 21 and 23 April 2004, respectively, evaluations of E using the porometer and IRGA methods were conducted. The methodology and experimental design used was the same as for pots, but in this case we evaluated the range of rate values obtained in the total of samples ($n_{\text{IRGA}} = 28$ and $n_{\text{porometer}} = 18$) corresponding to four tussocks (two of each G and M size). In the case of measurements performed with the porometer, E was thereafter integrated throughout the whole day between 06:00 and 18:00 solar time and between 08:00 and 16:00 solar time for the evaluations done using IRGA. The range of E values obtained with pots during the operation was compared to those in the field.

2.5.1. Scaling up transpiration to the whole plant

The morphology and classification of leaves and tillers, photosynthetic parameters and micro-meteorological information described in previous paragraphs enabled us to use the Yplant software (Percy and Yang, 1996). This 3-D architectural model is used to determine the influence of crown geometry on physiological processes such as photosynthetic light utilization or transpiration and on the energy balance of both the crown and the individual foliage units.

We built the architecture of the four *S. tenacissima* tussocks following the approach used by Valladares and Pugnaire (1999). We counted the total number of base tillers in

Table 1 – Characteristics of the four tussocks evaluated: total number of tillers, total leaf area under four physiological status (LPS 1–4), and green leaf area index (LAI) for the four tussocks assessed (1 standard error)

	Tillers	Total area of LPS (m ²)				Green LAI
		1	2	3	4	
G1	78	0.150 (0.013) a	0.038 (0.006) n.s.	0.013 (0.003) n.s.	0.211 (0.015) a	1.85 (0.13) n.s.
G2	61	0.094 (0.010) b	0.029 (0.004) n.s.	0.018 (0.003) n.s.	0.167 (0.012) b	1.60 (0.13) n.s.
M1	73	0.050 (0.011) c	0.031 (0.006) n.s.	0.008 (0.002) n.s.	0.059 (0.009) c	1.60 (0.18) n.s.
M2	74	0.081 (0.014) bc	0.033 (0.006) n.s.	0.012 (0.002) n.s.	0.076 (0.008) c	2.32 (0.28) n.s.

Different letters next to the values belonging to each variable indicate significant differences among the four tussocks at $P < 0.05$ (Mann-Whitney U -test), n.s. means no significant differences among the four tussocks tested by Kruskal-Wallis ANOVA by Ranks (at $P > 0.05$).

each defined zone (NW, NE, SW and SE) belonging to two concentric sectors of the tussock. We represented total of base tillers in each sector assigning randomly: 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. Four representative tiller types were produced, for the northern and southern orientation, in each concentric sector of the tussock. Each representative tiller had four to five leaves, dividing each in turn into four sections. Each leaf section was located on a LPS, determining the surface and angle from the horizontal by estimating the average value of all tillers sampled in each sector. The four representative tillers were cloned for each base tiller belonging to north-south internal and external sectors.

The Farquhar et al. (1980) photosynthesis model was used in the ecophysiological model of Yplant, where the parameters required for the model were obtained from light and A/c_i responses (Table 2). Leuning's (Leuning, 1995) model was used to calculate CO_2 stomatal conductance (g_c) required by Yplant. The parameters needed for this model were calculated based on punctual IRGA measurements in four tussocks performed during three periods of the day, fitting a linear function between CO_2 assimilation and g_c using a regression analysis. Transpiration in each leaf section was calculated using the linear regression between E and the PPF of the light response. To this aim we worked using total daily output of the Yplant model (between 06:00 and 18:00 solar time), taking from the referred model the PPF received by each section of the leaf (belonging to a given LPS) in such a manner that when applying the equation $E = f(\text{PPFD})$, we obtained an E for each of the leaf sections of the whole tussock.

Hourly transpiration values calculated with each method (IRGA, porometer and sap flow methods) were integrated in the period from 06:00 to 18:00 solar time and then extrapolated to the total green area. The linear relationship between transpiration and PPF (obtained by light response curves) allowed us to calculate the tussock's hourly transpiration by weighing total leaf area in the LPS 1–3 and using incident PPF data (obtained by the micro-meteorological station). The hourly PPF registered by the meteorological portable station and the linear relationship fitted (PPFD versus E) for each LPS, allowed us to extrapolate tussock transpiration weighted by LPS total area. This methodology was named IRGA-LC.

The tussock transpiration estimates determined with IRGA, IRGA-LC, sap flow, porometer extrapolations and Yplant simulation integrated from 06:00 to 18:00 solar time, were compared to the gravimetric method.

3. Results

3.1. Morphological characteristics

Both tussock size (G and M) showed similar morphological characteristics, however, G1 individuals exhibited the largest LPS 1 surface. The LPS 4 (dead biomass) was the best variable to discriminate G and M size classification (Table 1). Regarding tiller level, we observed no significant differences between LPS (1–3) relative surface in the four tussocks. Average values of 65.6%, 25.13% and 9.27% of total green area/tiller were represented by LPS 1–3, respectively.

Most of the tillers had a Southward orientation, except for tillers of G tussock in the NW zone and of M tussock in the

Table 2 – Mean values (± 1 standard error) of indirect (SPAD units, $N = 50$), and direct estimations ($Chlor_{a+b}$, $N = 16$) of total chlorophyll concentration and parameters obtained from the photosynthesis-irradiance and photosynthesis-internal CO_2 concentration curves ($N = 12$) for the three LPS

LPS	Chlorophyll estimations			Light response				A/ci response	
	SPAD	$Chlor_{a+b}$ ($mg\ g^{-1}$)	A_{max}	R	L.C.P.	Φ	Γ	$V_{c_{max}}$	J_{max}
1	41.09 (1.21) a	1.041 (0.03) a	11.14 (1.55) a	-1.45 (0.27) a	50.03 (5.39) a	0.029 (0.004) a	81.96 (7.68) a	35.0 (3.8) a	143.23 (18.15) a
2	28.31 (0.58) b	0.618 (0.02) b	6.36 (1.17) b	-0.81 (0.10) b	74.35 (19.31) a	0.014 (0.003) b	191.47 (21.7) b	15.37 (2.9) b	69.54 (13.06) b
3	16.58 (0.51) c	0.276 (0.01) c	2.98 (0.92) c	-0.67 (0.22) b	287.54 (102.7) b	0.003 (0.002) c	324.86 ^a (24.1) c	13.52 ^a (2.01) b	61.38 ^a (12.01) b

A_{max} = light saturated rate of CO_2 assimilation ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$), R = dark respiration rate ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$), Φ = quantum yield, L.C.P. = light compensation point ($\mu mol\ m^{-2}\ s^{-1}$), Γ = CO_2 compensation point ($\mu mol\ CO_2\ mol^{-1}$), $V_{c_{max}}$ = maximum rate of carboxylation ($\mu mol\ m^{-2}\ s^{-1}$), J_{max} = maximum rate of electron transport ($\mu mol\ m^{-2}\ s^{-1}$). Different letters next to the values belonging to each parameter indicate significant differences among the three LPS at $P < 0.05$ (Mann-Whitney U -test).

^a Two erroneous curves were taken out of the analysis whereby this average has been calculated with two samples less ($N = 10$).

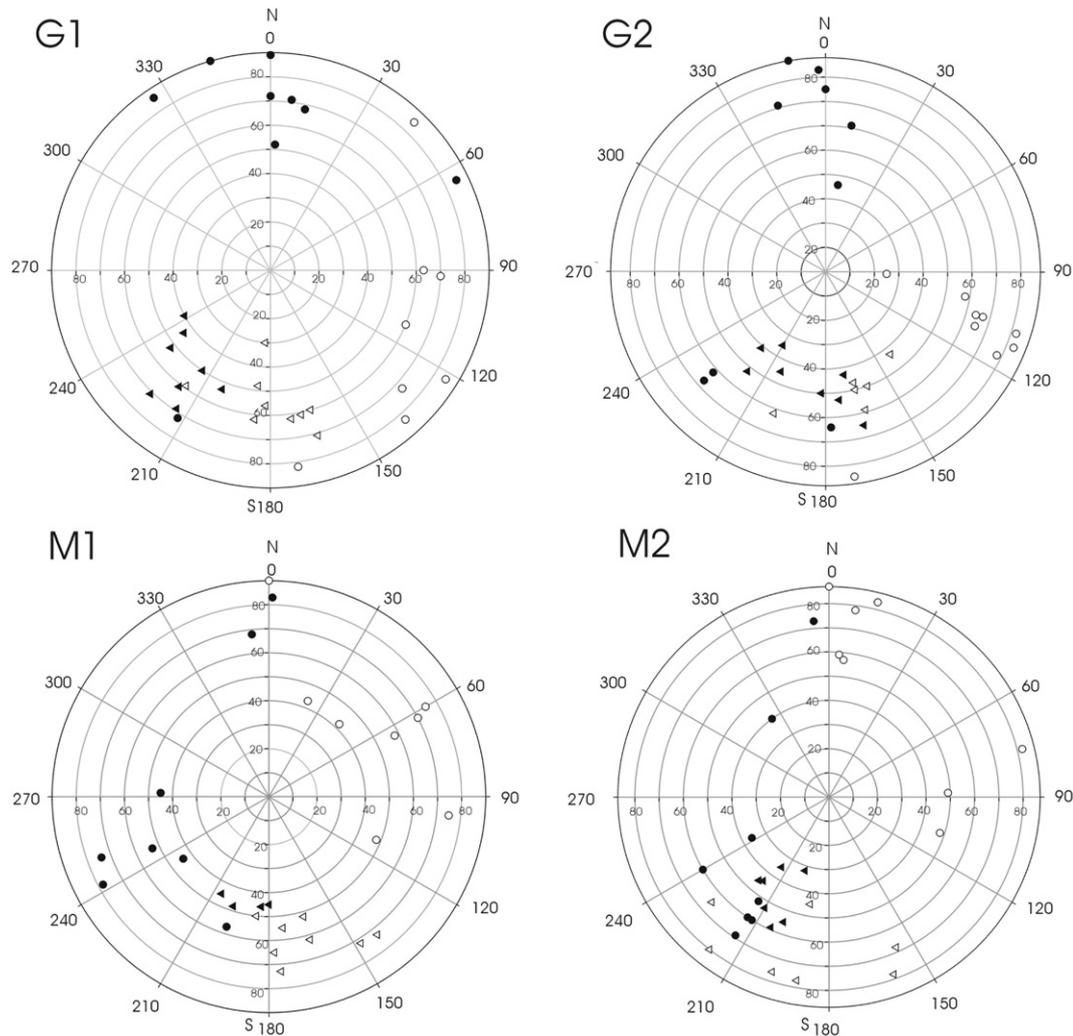


Fig. 2 – Polar scatter plot showing the azimuth as the compass direction and the angle from horizontal (cross internal scale 0–90°) of sampled tillers in four sectors of the tussock: N–W = blade circles, NE = open circles, SW = black triangles, SE = open triangles.

NE zone, that presented a Northward orientation (Fig. 2). Maximum horizontal inclination was found in tillers at SW and SE zones, this characteristic had an influence in the leaning appearance of tussocks with a south aspect.

Leaves of representative tillers were characterized by a progressive loss of green biomass from the internal (shoot) to the external leaf (dry leaf) (Fig. 3). G tussocks showed the highest LPS 4 value and a greater average number of leaves in comparison to tussocks M (5.20 ± 0.15 , 4.73 ± 0.18 , 4.30 ± 0.21 and 4.20 ± 0.13 leaves in G1, G2, M1 and M2, respectively).

3.1.1. Physiological measurements

A good fit of the calibration function was achieved when adjusting a linear function between SPAD (x) and chlorophyll $a + b$ concentration (y , Chlor_{a+b}): $y = 0.028x - 0.1694$, $R^2 = 0.917$, $P < 0.05$. The LPS (1–3) evaluated with SPAD (Table 2) showed significant differences in Chlor_{a+b} .

The LPS 1 (class with the highest chlorophyll concentration) achieved the highest A_{max} and Φ values (Table 2). L.C.P.

was significantly higher in the oldest leaves, indicating less efficient use of light. The parameters obtained from the A/c_i measurements also detect differences between the LPS. The leaves in LPS 1 exhibited a lower CO_2 compensation point, though greater maximum rates of carboxylation ($V_{c_{\text{max}}}$) and electron transport (J_{max}), in comparison with leaves in LPS 2 and 3 (Table 2).

The linear regression analysis between E and PPFD obtained from the simulation in the IRGA chamber, explained over 84% of variability in all cases (Table 3), where the slope of the functions presented the pattern: LPS 1 > LPS 2 > LPS 3, except for tussock G1 where no significant differences were found between the slopes of LPS 2 and 3.

3.2. Sap flow

In general, the daily pattern of sap flow was characterized by an important early morning sap recharge (Fig. 4). Subsequently, this flow continued more or less intensively depending on the orientation or the degree of radiation on the

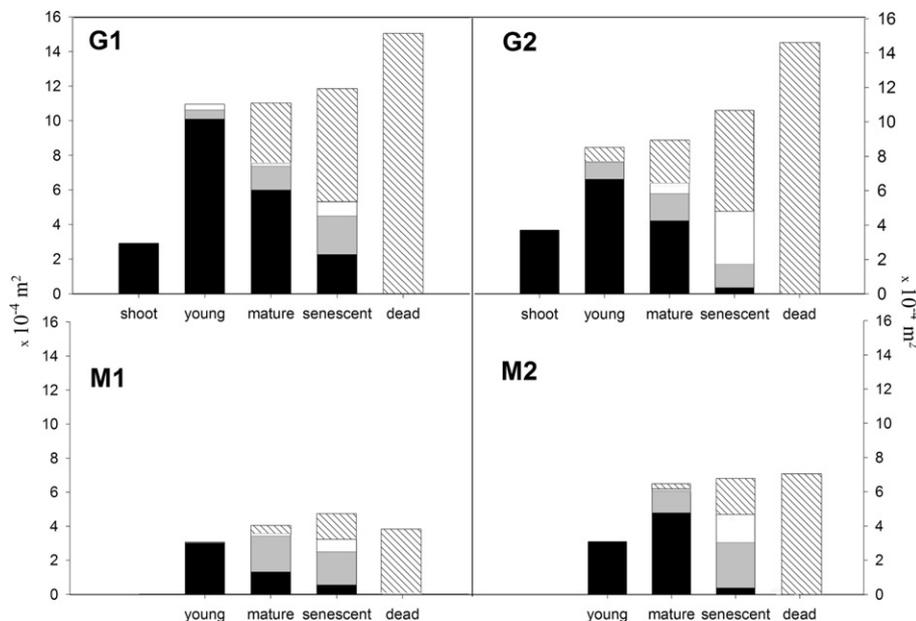


Fig. 3 – Mean area ($\times 10^{-4} \text{ m}^2$) of each leaf cohort belonging to a representative tiller. Different colors represent the LPS: 1 = black bar, 2 = gray bar, 3 = white bar and 4 = striped bar.

Table 3 – Linear regression of transpiration rate (E , $\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$) versus PPFD ($\mu\text{mol m}^{-2} \text{ s}^{-1}$) for LPS 1–3 obtained from four different tussocks

	LPS	a	$b \cdot 10^{-4}$	R^2	F
G1	1	1.019	$5.41 \pm 0.96 \text{ a}$	0.84	32.0
	2	0.319	$1.49 \pm 0.25 \text{ b}$	0.86	36.2
	3	0.684	$2.02 \pm 0.23 \text{ b}$	0.93	74.7
G2	1	0.836	$8.98 \pm 0.55 \text{ a}$	0.98	264.7
	2	0.686	$5.45 \pm 0.24 \text{ b}$	0.99	510.0
	3	0.419	$1.20 \pm 0.10 \text{ c}$	0.96	158.6
M1	1	1.223	$8.13 \pm 0.56 \text{ a}$	0.97	209.6
	2	1.360	$5.01 \pm 0.27 \text{ b}$	0.98	352.1
	3	0.439	$2.04 \pm 0.11 \text{ c}$	0.98	372.9
M2	1	0.689	$7.72 \pm 1.06 \text{ a}$	0.90	52.9
	2	0.431	$3.51 \pm 0.15 \text{ b}$	0.99	579.7
	3	0.568	$1.33 \pm 0.12 \text{ c}$	0.96	132.8

Values of a and b parameters for the relationship: $y = a + bx$, are given. All F values of ANOVA were significant ($P < 0.001$). The comparison of slope values (b) with the LPS of each tussock is also shown, here different letters point out significant differences between LPS when evaluating the confidence interval of the t -test ($P < 0.05$).

gauge. As an example, the M1 individual showing the smallest total green surface ($0.088 \pm 0.01 \text{ m}^2$), and in consequence, the lowest self-shading, had the highest leaf-related sap flow (Q_i) value ($166.7 \text{ mol H}_2\text{O m}^{-2} \text{ day}^{-1}$). In contrast, the G1 individual with a greater total green surface ($0.209 \pm 0.014 \text{ m}^2$) achieved the lowest daily Q_i in the gauge placed on sunny tillers ($38.9 \text{ mol H}_2\text{O m}^{-2} \text{ day}^{-1}$).

One of two gauges on G1 and G2 individuals was placed on tillers having a southern orientation (180° azimuth) and on tillers at the periphery of the tussock where self-shading was greater because the tillers were leaning in this area (Fig. 2). In consequence, these gauges showed a lower daily Q_i value (7.78 and $6.67 \text{ mol H}_2\text{O m}^{-2} \text{ day}^{-1}$ for G1 and G2,

respectively). Likewise, radiation intensity influenced the hourly Q_i pattern. Gauges on the tussocks oriented north registered early morning, midday and evening recharges. Conversely, gauges on the tillers oriented south (with greater inclination) only reflected early morning sap flow recharge.

3.2.1. Gas exchange at the leaf-level

Transpiration (Fig. 5) showed a similar pattern as daily PPFD and VPD, with an increase towards midday and a decrease in the evening (Fig. 1). When conducting a linear regression analysis between average E calculated using the porometer method in four tussocks ($N = 20$) and hourly PPFD, we obtain as a result: $y = 8.01 \times 10^{-4} x + 0.895$ ($R^2 = 0.67$, $P < 0.01$). This function is very similar to that obtained by simulation in the IRGA chamber, especially in the LPS 1 (Table 3).

In general, average IRGA transpiration values were close to the extreme values of the box plot of porometer transpiration. The same was true for sap flow analysis; M1 reached significantly higher transpiration estimates (ANOVA of repeated measurements, Tukey's honest significant differences test).

Transpiration rates obtained in the experiment using pots, integrated throughout the daytime evaluation period, are found to be within the same range as those measured on individuals under natural conditions (Table 4). This indicates the high representativity of our results.

3.2.2. Scaling up: different results with different protocols

The simulation of architecture of the tussock using Yplant was done for tussocks leaning southwards (Fig. 6). The daily integration of transpiration calculated by Yplant showed the best estimates of water transpired per day by the whole plant (WT, Fig. 7), with values similar to the standard method with a difference of only $5.2 \pm 2.8 \text{ g H}_2\text{O day}^{-1}$ (+4.4%) and with the

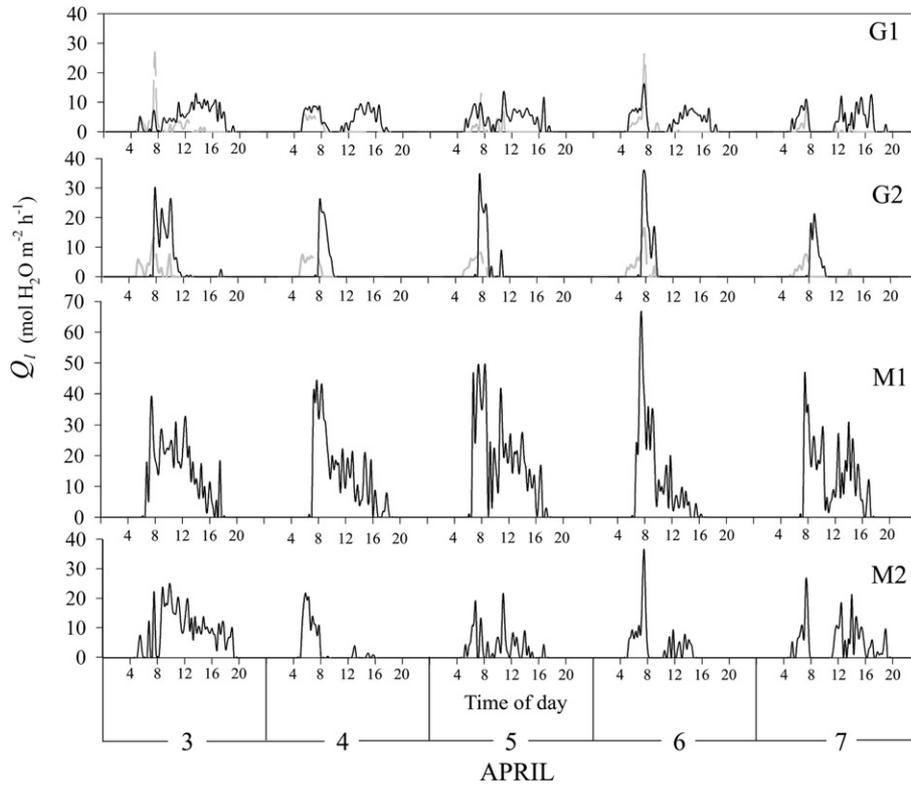


Fig. 4 – Leaf-related sap flow (Q_l) values estimated through the heat balance method, period 3–7 April 2004. In G1 and G2 tussocks, black and gray lines represent estimates from the gauges on sunny and shaded tillers, respectively.

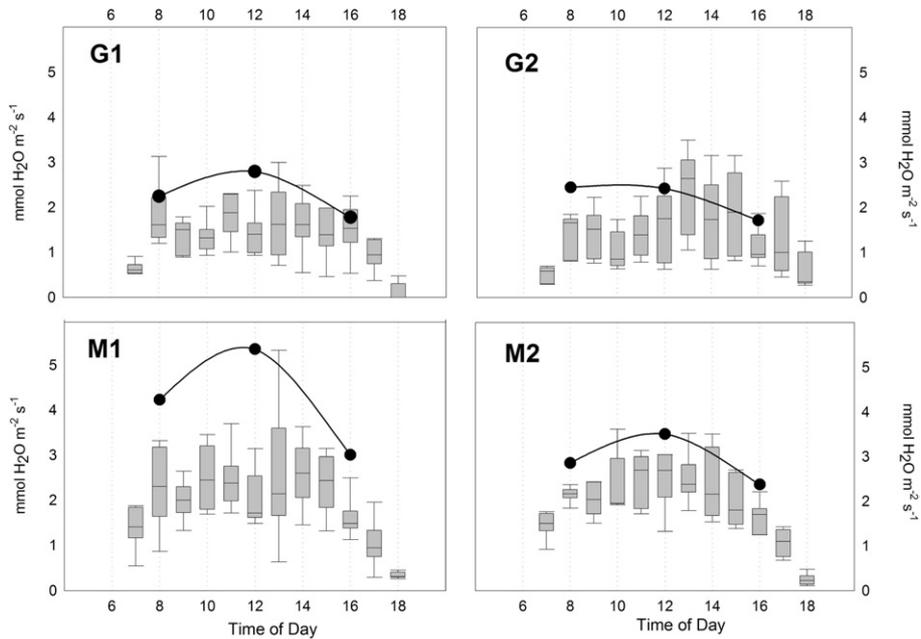


Fig. 5 – Hourly transpiration box plots based on porometer, boundary layer and VPD estimates. Black circles represent mean transpiration obtained by the IRGA method.

Table 4 – Comparison of pot experiment measurements with those performed on the site where *S. tenacissima* tussocks were removed

Method	POTS (experiment)	FIELD (natural conditions)
IRGA	44.4–166.7	22.2–166.7
Porometer	27.8–116.7	38.9–166.7

The range of transpiration rate values integrated along the evaluation period is shown. Units in $\text{mol H}_2\text{O m}^{-2}$ per period. The evaluation period was: between 08:00 and 16:00 and between 06:00 and 18:00 solar time for the IRGA and porometer methods, respectively.

best fit to the linear regression (Table 5). In spite of the great effort and complexity involved in modeling *S. tenacissima* tussock architecture by Yplant, this method is more rapid than others for calculating whole transpiration.

The extrapolation of E measured by IRGA gave the highest overestimation of WT with a difference of $111.9 \pm 11.35 \text{ g H}_2\text{O day}^{-1}$ (+125.8%) in comparison to the gravimetric method.

Effort was relatively low when performing measurements using IRGA. Thus, the advantage of this method is that a great replication capacity.

Sap flow extrapolation results depended on the position of the gauges (Fig. 7). North gauges extrapolation (mainly in G tussocks) was better than south gauges, in comparison with the gravimetric method. However, gauges exposed to sunlight in M1 tussock presented the worst transpiration estimate. Sap flow was much faster and less complex than with the other methods; however it underestimated +92.6% of WT. As with the Sap flow method, porometer measurements reflected a great underestimation for WT with a mean difference of 72.6 ± 10.5 (+77.4%) $\text{g H}_2\text{O day}^{-1}$ when using the gravimetric method (Table 5).

After the Yplant simulation, scaling up using IRGA-LC showed acceptable results, with values close to those of the gravimetric estimates (a mean difference of $41.1 \pm 6.62 \text{ g H}_2\text{O day}^{-1}$ or +46.9% of overestimation). This methodology equally showed a good regression line when compared with

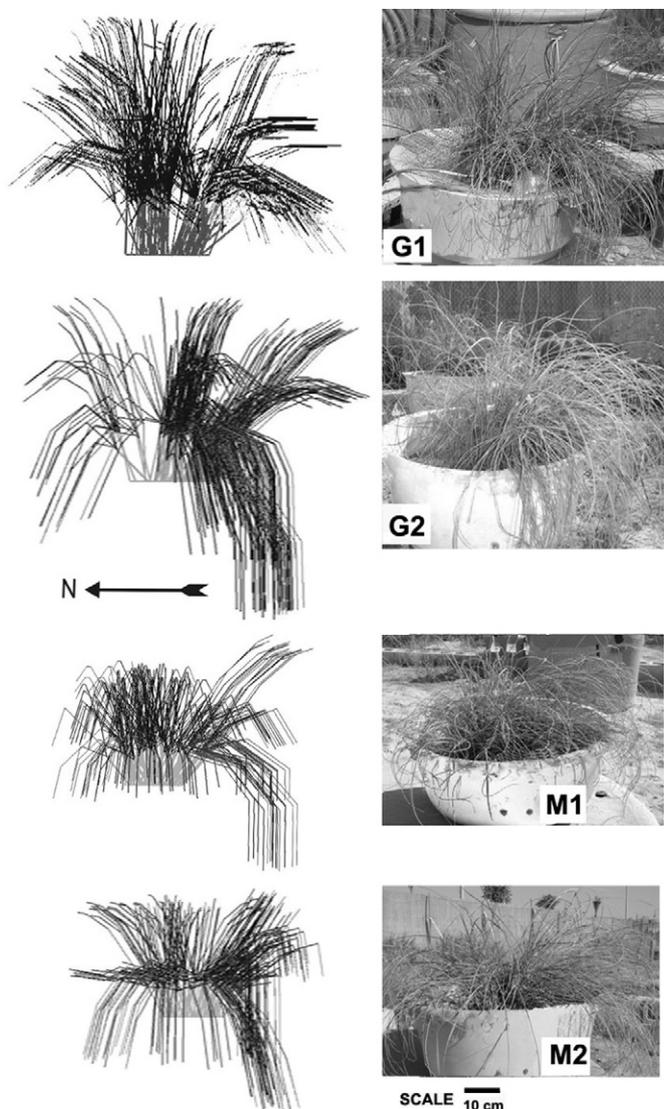


Fig. 6 – On the left, architectural structure as a result of the Yplant simulation in the four tussocks assessed. On the right, a picture of the four tussocks.

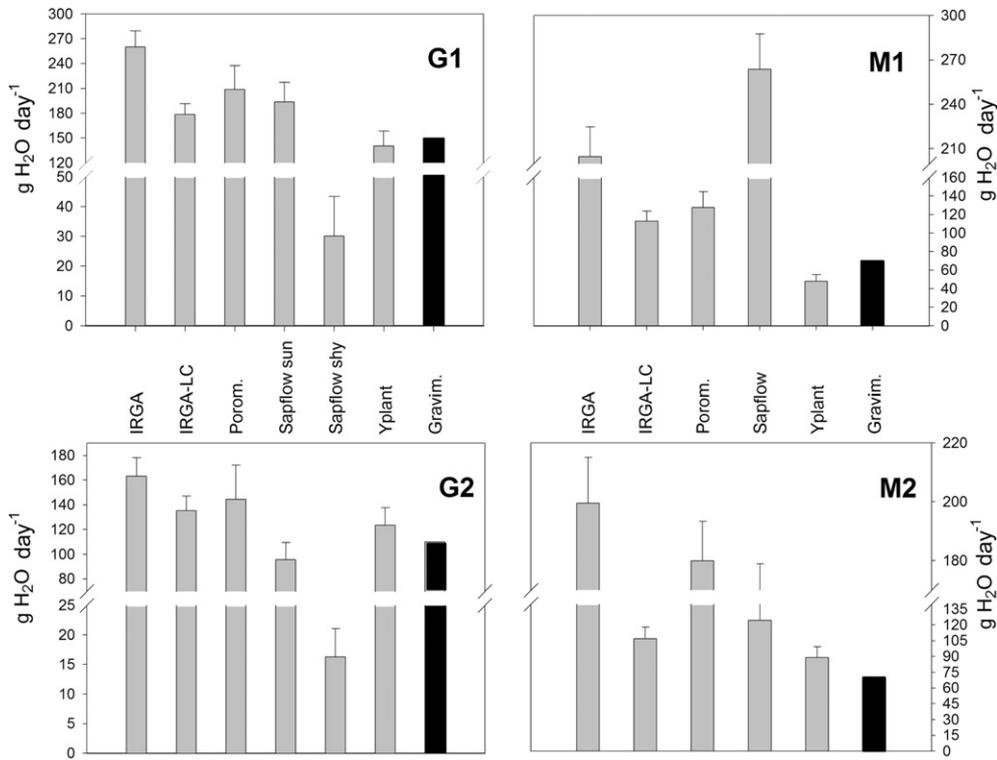


Fig. 7 – Estimation of water transpired per day by the whole plant measured (g H₂O day⁻¹) by five methodologies: infrared gas analyzer and total green surface (IRGA), light curve transpiration response discriminated by LPS area (IRGA-LC), total transpiration based on porometer, boundary layer and VPD estimates extrapolated to total green surface (Porom.), sap flow calculated in sunny (Sap flow sun.) and shaded (Sap flow shy.) tillers and total green surface, Yplant complemented by light curve transpiration response information. The gravimetric method (black bar) was used as standard.

Table 5 – Assessment and comparison of water transpired per day by the whole plant (WT) estimated with five methodologies using the gravimetric method as reference

Assessment criteria	IRGA	IRGA-LC	Porometer	Sap flow	Yplant
WT difference g H ₂ O day ⁻¹ (1 standard error)	111.9 (11.35)	41.1 (6.62)	72.6 (10.5)	76.9 (25.3)	5.2 (2.8)
Percentage MO	+125.8	+46.9	+77.4	+92.6	+4.4
One sample t-test	t _{cal} = 9.85 N = 28**	t _{cal} = 6.21 N = 50**	t _{cal} = 6.89 N = 24 **	t _{cal} = 3.04 N = 20**	t _{cal} = 0.03 N = 16 n.s.
Linear regression	a = 149.8, b = 0.57 R ² = 0.55 n.s.	a = 29.2, b = 1.12 R ² = 0.83*	R ² < 0.5	R ² < 0.5	a = -5.86, b = 1.10 R ² = 0.99*
Complexity/effort	Medium	Low	High	Very low	Very high
Speed of measurement	High	Medium	High	Very high	Very high

Infrared gas analyzer and total green area (IRGA), light curve transpiration response discriminated (IRGA-LC) by three LPS, total transpiration based on porometer, boundary layer and VPD estimates extrapolated onto total green area (porometer), heat balance method (Sap flow) and Yplant simulation. % MO = magnitude of overestimation in percentage terms. Differences between estimated TT (using five methodologies) and the gravimetric method were evaluated by one sample t-test (** significant differences between E mean at P < 0.01). Linear regression analysis between each methodology versus the gravimetric method (a = coefficient, b = slope, * indicate significant differences in the F value of ANOVA regression at P < 0.05).

the gravimetric method and a low complexity and low effort for data management.

4. Discussion

By comparing to the gravimetric method as the standard, a great overestimation in tussock transpiration was found when leaf transpiration measurements were extrapolated. IRGA, porometer and sap flow methodologies rendered dif-

ferences of +125.8%, +77.4% and +92.6%, respectively, relative to transpiration measured with the gravimetric method (Table 5). This confirms that the scaling up process in *S. tenacissima* is not a simple leaf to tussock extrapolation (Domingo et al., 1996; 1997). However, when considering LPS as a consequence of the aging process in leaves, scaling up of leaf-level gas exchange measurements was improved to +46.9% in comparison with the gravimetric method (IRGA-LC method). Crown architecture simulated by Yplant appeared to be the best procedure to estimate tussock tran-

piration in *S. tenacissima* (only a 4.4% difference with gravimetric method). According to Pearcy and Valladares (1999), the identification of the main gradients or heterogeneity of processes that affect leaf populations may lead to a very useful approach for studies at the level of the individual plant. In our case, aging and crown architecture were the most important processes to consider when scaling up transpiration, resulting in a complex interplay between morphology and physiology, confirming our initial hypothesis.

With reference to architecture, leaf orientation of tussock grass in dry and high light environments has two main implications:

- those leaves presenting a more vertical orientation cause greater self-shading, preventing photoinhibition and increasing water use efficiency in comparison to horizontal leaves (Ryel and Beyschlag, 1995);
- avoidance of excessive radiation on the tussocks due to its morphology reduces simultaneously CO₂ assimilation at an individual level (Valladares and Pugnaire, 1999).

This tradeoff between CO₂ capture and radiation avoidance in *S. tenacissima*, however, depends on environmental conditions. This species has been considered “opportunistic” regarding water pulses (Pugnaire et al., 1996). In particular, under conditions of sufficient water availability, there is a quick increase of the water vapor conductance, photosynthesis and photosystem II efficiency (Pugnaire and Haase, 1996; Haase et al., 1999; Balaguer et al., 2002). Similarly, our results confirm the morphological response of *S. tenacissima* to abundant water availability conditions, being leaves and tillers prone to horizontal leaning (Fig. 2).

The amount of leaf area in the tussock had an important impact on transpiration rates (*E*). The tussock with smallest leaf area (M1) presented the highest *E* measured using the IRGA and heat balance methods. A lower self-shading between the leaves of this tussock would have allowed a greater radiation input, and therefore a greater increase in temperature. Under these circumstances, this high transpiration could have acted as a refrigerating mechanism (Larcher, 1995; Nilsen and Orcutt, 1996; Grace, 1997; Matsumoto et al., 2000). However, with respect to the heat balance method, the high *E* measured could have also been due to the bias that this method exhibits for grass species under high temperature conditions (Senock and Ham, 1995).

Dry biomass accumulation in *S. tenacissima* is a typical morphological characteristic in this species. This retention of dead leaves and litter fall creates a shelter effect where wind speed and air turbulence into the tussock are different to the surrounding atmospheric conditions (Domingo et al., 1996). The Yplant simulation in *S. tenacissima* had previously confirmed that the increase of dry biomass has only little influence on light interception and carbon gain (Valladares and Pugnaire, 1999). The quantity of dry biomass in tillers of this species is proportional to its aging (Sánchez, 1995). Dry biomass and sexual maturity (spike presence) in the tussocks were the characteristics that discriminated our classification into G and M.

With reference to the aging process in *S. tenacissima*, it develops from the tip to the base of the limb, increasing leaf

drying when tissues get older (Sánchez, 1995). In this regard, the LPS approach used in this work reflected the aging processes (Fig. 3). Chlorophyll *a* + *b* concentration values obtained in the LPS (from 1 to 3) were similar to the range of chlorophyll *a* + *b* concentrations in wet to dry seasons obtained by Balaguer et al. (2002). This proves that the degree of aging in the cohorts of leaves is coherent or equivalent to seasonal water responses. Age and phenology effects have been tested in the scaling up of net CO₂ assimilation and H₂O vapor conductance from leaf to tussock (Valladares and Pugnaire, 1999) and canopy level (Haase et al., 1999) in *S. tenacissima*. In both cases, the effect of tissue aging was not significant in comparison to the seasonal effect of water availability. Our study was performed under high water availability conditions, in this situation; age together with light interception improved the scaling up of transpiration from leaf to individual. Elimination of the age factor when using IRGA-LC and Yplant methodologies entails a significant increase of the mean difference (+71.4% and +15.08%, respectively) in comparison to the gravimetric method (Table 6).

Ryel and Beyschlag (1995) found an independent response of transpiration to leaf orientation when both leaf and air temperature were similar. In other conditions, transpiration was lower in tussocks having a steeper leaf orientation, in avoiding excessive radiation (Ryel et al., 1993). A reduction of leaf surface exposure in *S. tenacissima* tussocks, clipping 20% of live tillers, produced a significant availability of soil moisture in the tussock as a result of the reduction of transpiring surface, what suggested an important competition among tillers (Puigdefábregas and Sánchez, 1996). Our results emphasize the important dependence of transpiration on PPFD in soils with high water content and under controlled environmental conditions (VPD, CO₂ concentration and air-flow rate), this relationship being more intense when the tissue is younger (Table 3).

When studying seasonal transpiration estimated according to the heat balance method, Chirino (2003) described *S. tenacissima* as a water wasting species, upon comparing the daily transpiration values with those obtained in other dominating species (*Quercus coccifera*, *Pinus halepensis* and *Pistacia lentiscus*). However, our results question this hypothesis because we found an overestimation when applying this methodology to *S. tenacissima*. This could also explain the differences found by Chirino (2003) when comparing ET estimates for *S. tenacissima* grassland with those for other species (*Q. coccifera*, *P. halepensis* and *P. lentiscus*) from a model

Table 6 – Tussock transpiration differences in percentage terms estimated by four methods compared with the gravimetric method

	IRGA-LC (%)	IRGA-LC Without age effect (%)	Yplant (%)	Yplant without age effect (%)
G1	+ 48.8	+ 83.3	+ 8.0	+ 23.9
G2	+ 23.1	+ 42.7	+ 5.6	+ 17.4
M1	+ 61.1	+ 76.3	- 2.4	+ 2.6
M2	+ 52.7	+ 83.4	+ 6.3	+ 16.4
Mean	+ 46.4	+ 71.4	+ 4.4	+ 15.08

The four methodologies evaluated were: Light curve transpiration response (IRGA-LC) discriminated by three LPS, Light curve transpiration response without three LPS, Yplant simulation and Yplant without age effect.

based on potential evaporation and soil moisture, and with estimates from sap flow extrapolations (–52.4%).

In conclusion, taking into consideration aging processes as well as crown architecture in a tussock species like *S. tenacissima*, scaling of transpiration from leaf to individual is significantly affected following a summation-scaling scheme (*sensu* Jarvis, 1995). However, this process can be very complicated and laborious, particularly when using models or techniques such as Yplant that require detailed morphological measurements in each individual plant. In fact, calculating canopy transpiration at the stand level with this kind of approach is not feasible unless a number of simplifications can be made. Our study has shown extent of the overestimation when using instantaneous methodologies in transpiration measurements (IRGA, porometer, sap flow), which are common ecophysiological approaches, and the need for taking into account the fraction of senescent foliage, which reduces transpiration in active foliage, as well as the geometric arrangement of all the foliage units in addition to their corresponding physiological activity. The high degree of correspondence among transpiration rates indicated by our results in potted tussocks and in tussocks under natural field conditions justifies the use of the current methodology in future studies. In this context, future efforts should consider the way in which scaling of transpiration from the leaf to the whole plant is affected by plant size and soil moisture. These two variables can significantly affect the present findings, particularly in plants with complex crowns growing in arid sites.

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REFERENCES

- Amon, D.L., 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology* 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 and 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. *Agricultural and Forest Meteorology* 54, 67–84.
- Baker, J.M., Van Bavel, C.H.M., 1987. Measurement of mass flow of water in the stems of herbaceous plants. *Plant Cell and Environment* 10, 777–782.
- Bellot, J., Sánchez, J.R., Chirino, E., Hernández, N., Abdelli, F., Martínez, J.M., 1999. Effect of different vegetation type on the soil water balance in semi-arid areas of South Eastern Spain. *Phys. Chem. Earth (B)* 24, 353–357.
- Bellot, J., Maestre, F.T., Chirino, E., Hernández, N., de Urbina, J. O., 2004. Afforestation with *Pinus halepensis* reduces native shrub performance in a Mediterranean semiarid area. *Acta Oecologica* 25, 7–15.
- Cerdà, A., 1997. The effect of patchy distribution of *Stipa tenacissima* L. on runoff and erosion. *Journal of Arid Environments* 36, 37–51.
- Chirino, E., 2003. Influencia de las precipitaciones y de la cubierta vegetal en el balance hídrico superficial y en la recarga de acuíferos en clima semiárido. Ph.D. thesis. Alicante Univ., Spain.
- Djebaili, S., 1988. Connaissances actuelles sur l'alfa (*Stipa tenacissima* L.): autoécologie, phénologie, productivité et valeur nutritive. *Biocénoses* 3 (1–2), 43–53.
- Domingo, F., Moro, M.J., Sanchez, G., Brenner, A., Van Gardingen, P.R., 1997. Leaf and canopy boundary layer conductances of two semiarid species (*Retama shaerocarpa* L. Boiss. and *Stipa tenacissima* L.). *Mediterránea Serie de Estudios Biológicos* 16, 37–41.
- Domingo, F., Van Gardingen, P.R., Brenner, A.J., 1996. Leaf boundary layer conductance of two native species in southeast Spain. *Agricultural and Forest Meteorology* 81, 179–199.
- Domingo, F., Villagarcía, L., Brenner, A., Puigdefábregas, J., 1999. Evapotranspiration model for semi-arid shrublands tested against data from SE Spain. *Agricultural and Forest Meteorology* 95, 67–84.
- Dugas, W.A., Wallace, J.S., Allen, S.J., Roberts, J.M., 1993. Heat balance, porometer, and deuterium estimates of transpiration from potted trees. *Agricultural and Forest Meteorology* 64, 47–62.
- Edwards, W.R.N., Becker, P., Čermák, J., 1996. A unified nomenclature for sap flow measurements. *Tree Physiology* 17, 65–67.
- Farquhar, G.D., von Caemmerer, S., Berry, J.A., 1980. A biochemical model of photosynthetic CO₂ assimilation in leaves of C3 species. *Planta* 149, 78–90.
- Field, C.B., Ball, J.T., Berry, J.A., 1989. In: Pearcy, R.W., Ehleringer, J., Mooney, H.A., Rundel, P.W. (Eds.), *Photosynthesis: Principles and Field Techniques*. In: *Plant Physiological Ecology, Field Methods and Instrumentation*. Chapman and Hall, London, pp. 137–160.
- Grace, J., 1997. Plant water relations. In: Crawley, M.J. (Ed.), *Plant Ecology*. Second ed. Blackwell Science Ltd, London (pp. 28–50).
- Gracia, C.A., Tello, E., Sabaté, S., Bellot, J., 1999. An integrated model of water dynamics and forest growth. In: Rodà, F., Retana, J., Gracia, C., Bellot, J. (Eds.), *Ecology of Mediterranean Evergreen Oak Forest*, Serie Ecological Studies Vol. 137. Springer-Verlag, Berlin, pp. 163–180.
- Haase, P., Pugnaire, F., Clark, S.C., Incoll, L., 1999. Environmental control of canopy dynamics and photosynthetic rate in the evergreen tussock grass *Stipa tenacissima*. *Plant Ecology* 145, 327–339.
- Infante, J.M., Rambla, S., Joffre, R., 1997. Modelling transpiration in holm-oak savannah: scaling up from the leaf to the tree scale. *Agricultural and Forest Meteorology* 87, 273–289.
- Jarvis, P.G., 1995. Scaling processes and problems. *Plant Cell and Environment* 18, 1079–1089.
- Larcher, W., 1995. *Physiological Plant Ecology*. Third ed. Springer-Verlag, Berlin (506 pp).

- Leuning, R., 1995. A critical appraisal of a combined stomatal-photosynthesis model for C3 plants. *Plant, Cell and Environment* 18, 339–355.
- Leuning, R., Kelliher, F.M., De Pury, D.G.G., Schulze, E.-D., 1995. Leaf nitrogen, photosynthesis, conductance and transpiration: scaling from leaves to canopies. *Plant, Cell and Environment* 18, 1183–1200.
- Levin, S., 1995. The problem of pattern and scale in Ecology. In: Powell, T.M., Steele, J.H. (Eds.), *Ecological Time Series*. Chapman and Hall, New York, pp. 278–326.
- Maestre, F., Bautista, S., Cortina, J., Bellot, J., 2001. Potential for using facilitation shrubs on a semiarid degraded steppe. *Ecological Applications* 11 (6), 1641–1655.
- Matsumoto, J., Muraoka, H., Washitani, I., 2000. Ecophysiological mechanisms used by *Aster kantoensis*, and endangered species, to withstand high light and heat stresses of its gravely floodplain habitat. *Annals of Botany* 86, 777–785.
- Monteith, J.L., 1965. Evaporation and environment. *Symp. Soc. Exp. Biol.* 19, 205–234.
- Nilsen, E.T., Orcutt, D.M., 1996. *The Physiology of Plant Under Stress—Abiotic Factors*. John Wiley and Sons, Inc, New York (588 pp).
- 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 and Hall, London, pp. 137–160.
- 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. 264–272.
- 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, 7–9 September 1998*. Blackwell Science Ltd Oxford, pp. 45–66.
- Pérez Cueva, A.J., 1994. *Atlas Climàtic de la Comunitat Valenciana (1961–1990)*. Sèrie: Publicacions de Divulgació Tècnica Col.lecció: “Territori” N° 4. Conselleria d’Obres Públiques, Urbanisme i Transports, Generalitat Valenciana, Valencia - Spain.
- Piñol, J., Terradas, J., Àvila, A., Rodà, F., 1995. Using catchments of contrasting hydrological conditions to explore climate change effects on water and nutrient flows in Mediterranean forest. In: Moreno, J.M., Oechel, W.C. (Eds.), *Global Change and Mediterranean-type Ecosystems*. Springer-Verlag, Berlin, pp. 251–264.
- Puigdefàbregas, J., Sánchez, G., 1996. Geomorphological implications of vegetation patchiness on semi-arid slopes. In: Anderson, M.G., Brooks, S.M. (Eds.), *Advances in Hill-slope Processes*, vol. 2. John Wiley & Sons, Ltd, New York, pp. 1027–1060.
- Pugnaire, F.I., Haase, P., Incoll, L., Clark, S.C., 1996. Response of tussock grass *Stipa tenacissima* to watering in a semi-arid environment. *Functional Ecology* 10, 265–274.
- Pugnaire, F.I., Haase, P., 1996. Comparative physiology and growth of two perennial tussock grass species in a semi-arid environment. *Annals of Botany* 77, 81–86.
- Ryel, R.J., Beyschlag, W., Caldwell, M.M., 1993. Foliage orientation and carbon gain in two tussock grasses as assessed with a new whole-plant gas-exchange model. *Functional Ecology* 7, 115–124.
- Ryel, R.J., Beyschlag, W., 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.
- Sakuratani, T., 1981. A heat balance for measuring water flux in the stem of intact plants. *Journal of Agricultural Meteorology* 37, 9–17.
- 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 Univ., Spain.
- Schulze, E.-D., Cermák, J., Matyssek, R., Penka, M., Zimmermann, R., Vasíček, F., Gries, W., Kucera, J., 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.
- 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, 1950. *El esparto y su economía*. Ministerio de Industria y Comercio de Agricultura, Madrid, Spain.
- Servicio del Esparto, 1951. *Estudios y experiencias sobre el esparto*. Ministerio de Industria y Comercio de Agricultura, Madrid, Spain.
- Thornes, J., 1999. Mediterranean desertification: the issues. In: Balabanis, P., Peter, D., Ghazi, A., Tsogas, M. (Eds.), *Mediterranean Desertification Research Results and Policy Implications*. Proceeding of the International Conference 29 October to 1 November 1996, Crete, Greece, vol. 1. Office for Official Publications of the European Communities, Luxembourg.
- Valladares, F., Pugnaire, F.I., 1999. Tradeoffs between irradiance capture and avoidance in semi-arid environments assessed with a crown architecture model. *Annals of Botany* 83, 459–469.
- Valentini, R., Gamon, J.A., Field, C.B., 1995. Ecosystem gas exchange in a Californian grasslands: seasonal patterns and implications for scaling. *Ecology* 76 (6), 1940–1952.