# Leaf mass per area (LMA) as a possible predictor of adaptive strategies in two species of *Sesleria* (Poaceae): analysis of morphological, anatomical and physiological leaf traits

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Leaf mass per area (LMA) is an important variable in a set of coordinated leaf traits to determine plant species' ecological strategies. We hypothesized that LMA was the main predictor of the adaptive strategies of *Sesleria juncifolia* and *S. nitida* (Poaceae), growing on Mount Terminillo (Central Apennines) and cultivated *ex situ*. We broke LMA down into leaf anatomical components (i.e. leaf tissue density and thickness) and then related them to physiological traits. We found that the different habitats of the two species determine a different control of the anatomical components of the LMA variation. These differences are related to photosynthetic capacity, scaling linearly with leaf biomass investment per unit leaf area. On the whole, our study, taking into account the changes in LMA and its anatomical components, provides a trait framework which could be used to analyze adaptive strategies of other species in dry, mountain grasslands.

## Introduction

Leaf trait variation among plants occurs in a coordinated way (Villar *et al.* 2013) resulting in groups of co-varying traits (Maire *et al.* 2013). Thus, it is important to analyze correlations among traits, investigate the mechanisms highlighting the coordination of them within and among species, and relate the traits to plant strategies (Westoby *et al.* 2002, Maire *et al.* 2013). Leaf mass per area (LMA, ratio of leaf dry mass to leaf area) plays a central role in plant strategies (Westoby 1998, Castro-Diez *et al.* 2000, Gratani *et al.* 2009, Villar *et al.* 2013, Cheng *et al.* 2014). Photosynthetic capacity generally scales linearly with leaf biomass investment per unit leaf area (Gratani & Varone 2006), rendering anatomy the main driver of light-saturated photosynthetic rate (Poorter *et al.* 2009). LMA has been used as an indicator of species position along a gradient from resource-

rich to resource-poor environments (Reich *et al.* 1992, Cornelissen *et al.* 1996, Westoby 1998, Castro–Díez *et al.* 2000). High LMA is advantageous under adverse growing conditions (Poorter *et al.* 2009), where slow tissue turnover is favored (Grime 1977, Chapin *et al.* 1993, Turner 1994). Moreover, high LMA has been interpreted as an adaptation to drought (Salleo & Lo Gullo 1990, Gratani & Varone 2004) generally associated with a long leaf life span (Mediavilla *et al.* 2001, Westoby *et al.* 2002, Catoni & Gratani 2014). Differences in LMA among species can arise from changes in leaf thickness or density within the leaf, or both (Witkowski & Lamont 1991, Niinemets 1999, Poorter *et al.* 2009). Therefore, LMA is an important variable in a set of coordinated traits to determine the species' ecological strategies (Villar *et al.* 2013).

The grass genus *Sesleria* is mainly distributed in Europe (Tutin *et al.* 1980, Di Pietro *et al.* 2004). *Sesleria juncifolia* colonizes calcareous, dry grasslands of the subalpine plain. It is an Amphi-Adriatic species distributed throughout the Balkan Peninsula, and in Italy from 300 to more than 2500 m a.s.l. (Di Pietro *et al.* 2004). *Sesleria nitida* colonizes dry grasslands of the subalpine plain (Wellstein *et al.* 2013, 2014). In Italy, *S. nitida* is encountered on the main carbonatic massifs of the Central and Southern Apennines from 600 to 2000 m a.s.l. (Tutin *et al.* 1980, Pignatti 1982, Brullo & Giusso Del Galdo 2006, Gratani *et al.* 2014, Puglielli *et al.* 2014).

The necessity to achieve more insight into the performance of plants under changing climate requires understanding the traits that cause LMA variation and its physiological consequences (Garnier *et al.* 1999, Niinemets 1999, Niinemets 2001, Poorter *et al.* 2009, Villar *et al.* 2013). Since plant strategies are expressed in correlations of traits, a small set of traits should serve as a surrogate of them (Craine *et al.* 2001). The main objective of this study was to analyze the main determinants of LMA in *S. juncifolia* and *S. nitida* growing at the same altitude on Mount Terminillo (Central Apennines, Italy), but in different habitats. In particular, we tested the hypothesis that LMA variation could be driven by the same leaf traits in these congenerics. Thus, differences in LMA could be used to determine the species' adaptive strategies. To answer these

questions, we broke LMA down into its anatomical and morphological components, and then related them to the physiological traits of the study species.

# Material and methods

## Study area and climate

The study was carried out in the Botanical Garden of the Sapienza University of Rome (41°54′08′′N, 12°31′03′′E), in January–July 2013, on 20 representative transplanted specimens of both *S. nitida* and *S. juncifolia*, collected in June 2012 from Mount Terminillo (Sella di Leonessa, 42°28′28′′N, 13°00′24′′E, 1895 m a.s.l.; *S. nitida* 42°28′24′′N, 13°00′24′′E, 1895 m a.s.l.; *S. juncifolia* 42°28′27′′N, 13°00′21′′E, 1904 m a.s.l.). *Sesleria nitida* thrived on steep screes characterized by discontinuous swards with a steep inclination and erosion resulting from the cryoclastic origin of the soils (D'Ottavio *et al.* 2005), and exposed to a limited cryoturbation due to the prolonged and abundant snow cover in winter. *Sesleria juncifolia* grew along crests on the mountain summit areas exposed to cold winds blowing mainly from the northeast and with a high debris component in the soil as well as large rock outcrops (Costanzo *et al.* 2009), which are snow-free for long periods. The plants are thus exposed to the severity of the climate (Lancioni *et al.* 2011), providing a strong selection pressure.

The climate of Mount Terminillo is characterized by a mean minimum air temperature ($T_{min}$) of the coldest month (February) of –4.3 °C, a mean maximum air temperature ($T_{max}$) of the hottest month (August) of 20.9 °C, and a mean annual air temperature ($T_m$) of 6.6 °C. The average total annual rainfall is 1249 mm and snow occurs from November to May. However, there was no snow on the crests of the summit areas from March onwards (data from the Meteorological Station of Colle Scampetti, Centro Appenninico Carlo Jucci, for the years 1997–2012).

The plants were cultivated in pots (32 cm diameter, and 29 cm deep) containing peat and soil (1:3). The soil comprised silt (8%), clay (32%) and sand (60%). Soil pH was 7.2–7.5. Plants were cultivated outdoors (PPFD > 1000

$\mu$mol photon m$^{-2}$ s$^{-1}$) and watered regularly to field capacity. During the study period, $T_m$ was 15.4 °C, $T_{max}$ (July) was 31.7 °C and $T_{min}$ (February) was 3.5 °C.

## Morphological leaf traits

Measurements of morphological traits were carried out on fully expanded leaves ($n = 20$ for each species) collected at the beginning of June 2013 from the selected plants. The following parameters were measured: leaf area (LA, m$^2$) excluding sheath, by an Image Analysis System (Delta-T Devices, UK) and dry mass (DM, g) after drying at 80 °C to constant mass. The leaf mass per unit of leaf area (LMA, g m$^{-2}$) was calculated as the ratio of DM to LA (Reich *et al*. 1992) and leaf tissue density (LTD, g cm$^{-3}$) as the ratio of LMA to total leaf thickness (LT) (Wright *et al*. 2004).

## Anatomical leaf traits

Measurements of anatomical leaf traits were conducted on fully expanded leaves ($n = 20$ for each species) collected at the beginning of June 2013 from the selected plants and analyzed by light microscopy using an image analysis system (Axiovision AC software) at the midpoint of the leaf, according to Allard *et al*. (1991). Leaf cross-section width was on an average $3 \pm 1$ mm. The following parameters were measured: total leaf thickness (LT, cm); abaxial and adaxial epidermis thickness (ET$_{ab}$ and ET$_{ad}$, cm), thickness of the upper sclerenchyma layers (UST, cm), height and width of the major lateral vascular bundle (HLB and WLB, respectively, cm) and of the central vascular bundle (HCB and WCB, respectively, cm), according to Kuzmanović *et al*. (2009) and Gratani *et al*. (2014). The volume of leaf tissues (t) per unit of leaf area (VA$_t$) was calculated as VA$_t$ = CD$_t$ × CS$_t$ (Villar *et al*. 2013), where CD$_t$ is the total cell number of the tissue on the leaf cross-sectional area, and CS$_t$ is the mean cell size of the tissue. In particular, the volumes of the mesophyll (VA$_m$), epidermis (VA$_e$), vascular plus sclerenchymatous tissues (VA$_{v+s}$) and air spaces (VA$_a$) were calculated.

## Gas exchange measurements

Gas exchange measurements were carried out monthly on fully expanded leaves ($n = 5$ leaves per plant, per species) from January to July 2013. Net photosynthesis per unit leaf area ($A_a$, $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$), stomatal conductance ($g_s$, mol H$_2$O m$^{-2}$ s$^{-1}$), transpiration ($E$, mmol H$_2$O m$^{-2}$ s$^{-1}$), leaf temperature ($T_l$, °C), photosynthetically active radiation (PPFD, $\mu$mol photon m$^{-2}$ s$^{-1}$) and intercellular CO$_2$ concentration ($C_i$, $\mu$mol CO$_2$ mol air$^{-1}$) were measured with an open, infrared CO$_2$ gas analyzer (ADC LCA4, UK), equipped with a leaf chamber (PLC, ADC, UK). Measurements were carried out on cloud-free days (PPFD > 1300 $\mu$mol photon m$^{-2}$ s$^{-1}$) in the morning (from 9:30 to 12:30) to ensure that the maximum daily photosynthetic rate was measured (Reich *et al*. 1995, Varone & Gratani 2007). Net photosynthesis per unit leaf mass ($A_m$, nmol CO$_2$ g$^{-1}$ s$^{-1}$) was also determined.

On each sampling occasion, respiration ($R_D$, $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$) rate measurements were carried out on the same leaves after $A_a$ measurements, as CO$_2$ efflux, darkening leaf chamber by a black paper for 30 min prior to measurements to avoid transient post-illumination bursts of CO$_2$ releasing (Atkin *et al*. 1998). $R_D$ and $A_a$ were recorded as the mean measured rate for three following days per month characterized by the same weather conditions. The ratio between $R_D$ and $A_a$ ($R_D/A_a$) was calculated, according to Galmés *et al*. (2007). The water use efficiency (i.e. the rate of carbon gain per unit of increased stomatal conductance) for both species was evaluated from the relationships between $A_a$ and $g_s$ according to Hetherington and Woodward (2003).

## Statistical analyses

Differences in morphological and anatomical leaf traits between *Sesleria nitida* and *S. juncifolia* leaves were analyzed using MANOVA. Linear regressions were calculated to evaluate the relationships between LMA and LTD, LMA and LT, and LTD and LT. The same variables were plotted against $A_a$ and $A_m$. Regression analysis was also carried out to evaluate the relationships

between LMA and the other considered anatomical leaf traits, between $A_a$ and $g_s$, and between $E$ and $g_s$. Two-way ANOVA was performed on the physiological variables in order to evaluate the effect of the main factors (month and species) and their interaction (month × species) on the response variables. Multiple comparisons were done with Tukey's HSD *post-hoc* test.

## Results and discussion

The assessment of plant response to environmental factors contributes to our understanding of ecological differences among species (Bazzaz 1996). A fundamental question in a global climate change context is how plants respond to new environmental conditions and what mechanisms are involved in the process (Parmesan 2006).

Our results show significant differences in leaf traits (Table 1) and in volumes of the selected leaf tissues per unit area (Table 2) between *S. nitida* and *S. juncifolia*. LMA of both species (medians of 84 and 153 g m$^{-2}$ for *S. nitida* and *S. juncifolia*, respectively) was higher as compared with that of other graminoids (median = 72 g m$^{-2}$; Poorter *et al.* 2009).

In *S. nitida*, LMA correlated positively with LTD ($r^2 = 0.69$, $n = 20$, $p < 0.0001$), and LTD correlated negatively with LT ($r^2 = 0.35$, $n = 20$, $p < 0.001$), while there was no correlation between LMA and LT.

In *S. juncifolia*, LMA was considerably higher than in *S. nitida* (Table 1), LMA correlated positively with both LTD and LT ($r^2 = 0.97$, $n = 20$, $p < 0.0001$; and $r^2 = 0.70$, $n = 20$, $p < 0.0001$; respectively), and LTD correlated positively with LT ($r^2 = 0.52$, $n = 20$, $p < 0.0001$).

The lowest $A_a$ for *S. nitida* and *S. juncifolia* ($6.1 \pm 0.4$ and $5.4 \pm 0.4$ $\mu$mol CO$_2$ m$^{-2}$ s$^{-1}$ for, respectively) was measured in February (air temperature = 7.5 °C); it increased by 87% and 93%, respectively, in April (air temperature = 16.1 °C), and then decreasing by 51% and 67%, respectively, in July (air temperature = 25.6 °C) (Fig. 1a).

**Table 1.** Morphological and anatomical leaf traits of *Sesleria nitida* and *S. juncifolia*. LA = leaf area, DM = dry mass, LMA = leaf mass per area, LTD = leaf tissue density, LT = total leaf thickness, ET$_{ab}$ = abaxial epidermis thickness, ET$_{ad}$ = adaxial epidermis thickness, CD$_m$ = cellular density per total leaf cross-sectional area for mesophyll, CD$_e$ = cellular density per total leaf cross-sectional area for epidermis, UST = thickness of the upper sclerenchyma layers HLB = height of the major lateral vascular bundle, WLB = width of the major lateral vascular bundle, HCB = height of the central vascular bundle, WCB = width of the central vascular bundle, CD$_{v+s}$ = cellular density per total leaf cross-sectional area for vascular plus sclerenchymatous tissues. Mean values (± SE) are shown ($n = 20$). Different letters indicate significant differences between *S. nitida* and *S. juncifolia* leaves (MANOVA followed by Tukey's HSD; $p < 0.05$).

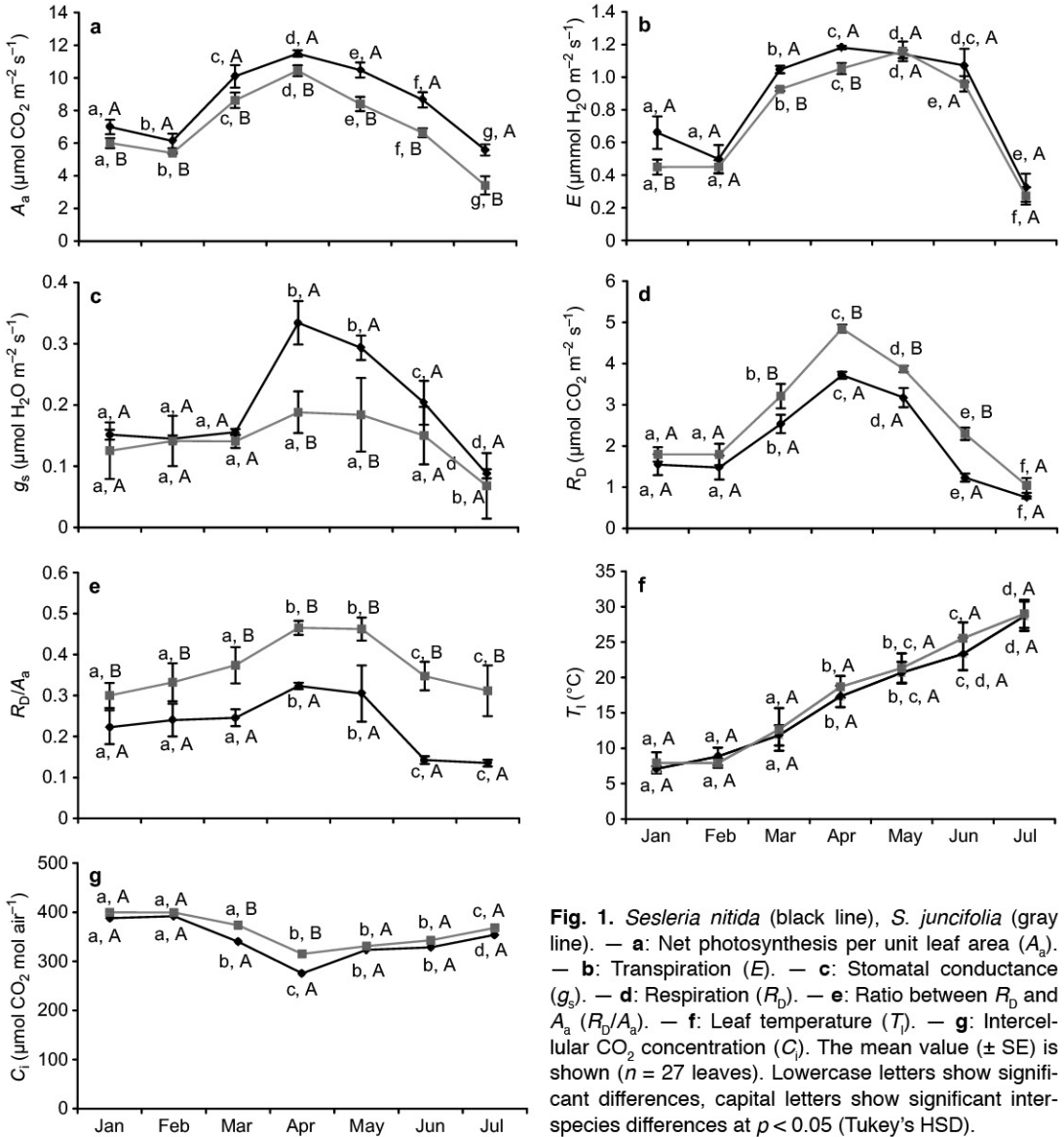| | *S. nitida* | *S. juncifolia* |
|---|---|---|
| **Morphological traits** | | |
| LA (m$^2$) | $0.000492 \pm 0.0002$[a] | $0.000088 \pm 0.000029$[b] |
| DM (g) | $0.04 \pm 0.01$[b] | $0.01 \pm 0.003$[b] |
| LMA (g m$^{-2}$) | $82.7 \pm 8.1$[a] | $148.1 \pm 31.1$[b] |
| LTD (g cm$^{-3}$) | $2.5 \pm 0.3$[a] | $5.8 \pm 0.9$[b] |
| **Anatomical traits** | | |
| Non vascular traits | | |
| LT (cm) | $0.0033 \pm 0.00020$[a] | $0.0026 \pm 0.00014$[b] |
| ET$_{ad}$ (cm) | $0.00027 \pm 0.000017$[a] | $0.00016 \pm 0.000014$[b] |
| ET$_{ab}$ (cm) | $0.00015 \pm 0.000012$[a] | $0.00012 \pm 0.000012$[b] |
| CD$_m$ (number m$^{-2}$) | $9.3 \times 10^4 \pm 7401$[a] | $1 \times 10^5 \pm 2920$[b] |
| CD$_e$ (number m$^{-2}$) | $5.5 \times 10^4 \pm 2773$[a] | $6.2 \times 10^4 \pm 2753$[a] |
| UST (cm) | $0.00099 \pm 0.000050$[a] | $0.000573 \pm 0.000026$[b] |
| Vascular traits | | |
| HLB (cm) | $0.00148 \pm 0.000024$[a] | $0.000815 \pm 0.000086$[b] |
| WLB (cm) | $0.00091 \pm 0.000046$[a] | $0.00055 \pm 0.000035$[b] |
| HCB (cm) | $0.00124 \pm 0.000028$[a] | $0.00071 \pm 0.000049$[b] |
| WCB (cm) | $0.00094 \pm 0.000037$[a] | $0.00061 \pm 0.000037$[b] |
| CD$_{v+s}$ (number m$^{-2}$) | $2.9 \times 10^5 \pm 50873$[a] | $3.9 \times 10^5 \pm 29938$[b] |

**Fig. 1.** *Sesleria nitida* (black line), *S. juncifolia* (gray line). — **a**: Net photosynthesis per unit leaf area ($A_a$). — **b**: Transpiration ($E$). — **c**: Stomatal conductance ($g_s$). — **d**: Respiration ($R_D$). — **e**: Ratio between $R_D$ and $A_a$ ($R_D/A_a$). — **f**: Leaf temperature ($T_l$). — **g**: Intercellular $CO_2$ concentration ($C_i$). The mean value (± SE) is shown ($n$ = 27 leaves). Lowercase letters show significant differences, capital letters show significant interspecies differences at $p < 0.05$ (Tukey's HSD).

**Table 2.** Volumes of the selected leaf tissues per unit area (l m$^{-2}$) of *Sesleria nitida* and *S. juncifolia*. $VA_m$ = volume of the mesophyll per unit area, $VA_e$ = volume of the epidermis per unit area, $VA_{v+s}$ = volume of the vascular plus sclerenchymatous tissues per unit area, $VA_a$ = volume of the air spaces per unit area. Mean values (± SE) are shown ($n$ = 20 leaves). Different letters indicate significant differences between *S. nitida* and *S. juncifolia* leaves (MANOVA followed by Tukey's HSD; $p < 0.05$).

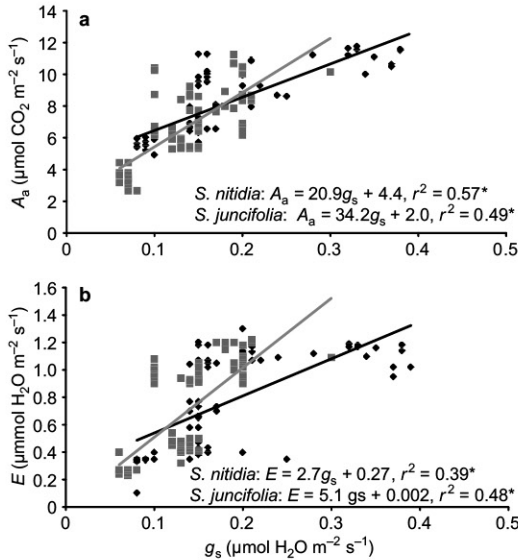| | $VA_m$ | $VA_e$ | $VA_{v+s}$ | $VA_a$ |
|---|---|---|---|---|
| *S. nitida* | $3.6 \times 10^{-7} \pm 3 \times 10^{-8}$ [a] | $2 \times 10^{-7} \pm 2.6 \times 10^{-8}$ [a] | $2.9 \times 10^{-7} \pm 2.4 \times 10^{-8}$ [a] | $1.4 \times 10^{-7} \pm 3 \times 10^{-8}$ [a] |
| *S. juncifolia* | $4.2 \times 10^{-7} \pm 2.9 \times 10^{-8}$ [b] | $2 \times 10^{-7} \pm 2.4 \times 10^{-8}$ [a] | $2.6 \times 10^{-7} \pm 1.4 \times 10^{-8}$ [a] | $1.2 \times 10^{-7} \pm 3 \times 10^{-8}$ [a] |

**Fig. 2.** Regression analysis results. *Sesleria nitida* (black line and symbols), *S. juncifolia* (gray line and symbols). — **a**: Net photosynthesis per unit leaf area ($A_a$) and stomatal conductance ($g_s$). — **b**: Transpiration ($E$) and stomatal conductance ($g_s$). Individual daily measurements carried out in the period January–July 2013 for *S. nitida* and *S. juncifolia*, respectively, were used as experimental units ($n = 189$, * $p \leq 0.01$).

$A_a$ and $g_s$ were significantly (two-way ANOVA followed by Tukey's HSD) higher in *S. nitida* than in *S. juncifolia* (Fig. 1a and c), while the latter had the highest $R_D$ (Fig. 1d). The respective $R_D/A_a$ ratios were $0.24 \pm 0.04$ and $0.33 \pm 0.05$ in February, increasing by 33% and 42%, respectively, in April, and decreasing by 56% and 34%, respectively, in July (Fig. 1e). This was due to a greater decrease in $R_D$ than in $A_a$ (Fig. 1d and a). During the whole study period, $R_D/A_a$ was, on an average, 61% higher in *S. juncifolia* than in *S. nitida*.

According to two-way ANOVA, the effects of *species* ($F_{1,168} = 315.03$, $p < 0.0001$, $n = 182$), *month* ($F_{6,168} = 412.5$, $p = < 0.0001$, $n = 182$) and their interaction ($F_{5,168} = 11.60$, $p = 0.00498$, $n = 182$) on the physiological variables were significant. In particular, most of the variability in $A_a$, $E$, and $R_D$ was explained by *month* while *species* affected mainly $g_s$ and $R_D/A_a$. The $R_D/A_a$ ratio can be considered a simplified characterization of the leaf carbon balance (Galmés *et al.* 2007). During the study period, $R_D/A_a$ was positive for both *S.*

*nitida* and *S. juncifolia* ($0.23 \pm 0.07$ and $0.37 \pm 0.07$, respectively), and it was mainly affected by *species*, despite $A_a$ and $R_D$ being mainly affected by *month*. This means that $A_a$ and $R_D$ changed throughout the year in a way preserving a positive carbon balance in both species; same was found by Liang *et al.* (2013) and Gratani *et al.* (2014) for other species. On the whole, *month* affected the variability more than *species* or their interaction.

$A_a$ and $E$ correlated with $g_s$ (Fig. 2a and b) with the regression slopes being steeper for *S. juncifolia* than *S. nitida*.

In *S. nitida*, $A_a$ was positively correlated with LMA ($r^2 = 0.61$, $n = 20$, $p < 0.001$) and LTD ($r^2 = 0.32$, $n = 20$, $p < 0.001$) but did not correlate with LT. Although correlations between $A_m$ and both LMA and LTD were statistically significant, they were both very weak ($r^2 = 0.19$, $n = 20$, $p < 0.01$; $r^2 = 0.24$, $n = 20$, $p < 0.001$; respectively), which means that LMA and LTD had practically no effect on $A_m$. In addition, $A_m$ was not correlated with LT.

In *S. juncifolia*, $A_a$ was positively correlated with LMA ($r^2 = 0.91$, $n = 20$, $p < 0.0001$), LTD ($r^2 = 0.86$, $p < 0.0001$) and LT ($r^2 = 0.63$, $n = 20$, $p < 0.0001$), while $A_m$ was negatively correlated with LMA ($r^2 = 0.83$, $n = 20$, $p < 0.0001$), LTD ($r^2 = 0.82$, $n = 20$, $p < 0.0001$) and LT ($r^2 = 0.64$, $n = 20$, $p < 0.0001$).

The relationship between LTD and LMA, and LT and LMA, were different. Moreover, LTD was negatively correlated with LT in *S. nitida*. Since an increase in LTD and LT means higher resistance to $CO_2$ diffusion between the leaf intercellular air spaces and carboxylation sites (Niinemets 1999), the negative correlation between LTD and LT results in 17% higher $VA_a$ which increases the $CO_2$ partial pressure at the carboxylation sites (Niinemets 1999) in *S. nitida*. This result is also confirmed by the stronger correlation between LTD and $A_a$ than LTD and $A_m$ pointing the importance of LTD in the photosynthetic process on a leaf area basis in *S. nitida*. In *S. juncifolia*, $A_a$ was positively correlated LTD and LT. A possible explanation of the different trade-off between LTD and LT in *S. juncifolia* may be derived from the control of the photosynthetic process through variations in LTD and LT. In particular, by being positively cor-

related with $A_a$ and negatively with $A_m$, increase in LTD and LT increase transpiration of carbon and water through the leaf surface, and decrease assimilation rates. This could be explained by the stronger control of water loss in *S. juncifolia* through reduction of $CO_2$ diffusion in both the gaseous and liquid phases due to the increase in LT and LTD confirmed by lower $C_i$ and $g_s$ (on an average, 6% and 30% during the study period). In fact, higher LTD — which is due to higher $CD_{v+s}$ and $CD_m$ — correlated positively with LT, resulting in a reduced volume per leaf area of air spaces and in lower photosynthetic rates, associated with an increased water use efficiency, indicated by the greater slope of the correlation between $A_a$ and $g_s$ (*see* Fig. 2a and Hetherington & Woodward 2003). This is confirmed by on average 17% lower $A_a$ in *S. juncifolia* than in *S. nitida*, associated with a greater water use efficiency in the former species. The greater water use efficiency in *S. juncifolia* is also due to an increased stomatal control of transpiration, indicated by the steeper slope of the regression between $E$ and $g_s$ (*see* Fig. 2b). Differences in water use between *S. nitida* and *S. juncifolia* were also confirmed by the greater effect of *species* than *month* on $g_s$. A lower $g_s$ (by 30%) and, thus, a lower $E$ (by 12%) during the study period could explain the 5% higher $T_l$ which might in turn explain the higher $R_D$ (by on average 31%) in *S. juncifolia* than in *S. nitida*. Moreover, the higher respiration rates in *S. juncifolia* could be due to more effective allocation of resources in leaf construction often observed in species whose LTDs are high (Niinemets 2001).

## Conclusions

The results of this research confirm to the importance of leaf mass per area (LMA) in the adaptive strategies of *S. juncifolia* and *S. nitida*. The highlighted differences in leaf traits are adaptations of the two species to their different habitats. Under the conditions on the crests of the summit area, *S. juncifolia* is able to grow due to a smaller leaf area (LA), a higher LMA and a better control of leaf transpiration relative to those of *S. nitida*. On the other hand, the more favorable conditions on the screes explain

the higher $A_a$ in *S. nitida*, which is related to a lower leaf tissue density (LTD) determining a higher $CO_2$ partial pressure at the carboxylation sites. Moreover, the species differ in the way the structural components control variation in LMA and thus the photosynthetic process. In fact, considering that LMA itself may in many cases be the target of selection (Niinemets 1999), our results demonstrate that high LMA is favored in the dry, mountain grasslands colonized by *S. juncifolia* and *S. nitida*. Analysis of the components of LMA (i.e. leaf tissue density and thickness) can provide better insight into the relationship between physiological processes, leaf anatomy and environmental conditions, as pointed out by Witkowski and Lamont (1991), Niinemets (1999) and Poorter *et al*. (2009).

Taking into account changes in LMA and its anatomical components in relationship to physiological parameters, our research reveals differences in the adaptive strategies of *S. nitida* and *S. juncifolia*, providing a complete trait framework which could be used to analyze adaptive strategies of other species in dry, mountain grasslands.

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