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Leaf traits of two Mediterranean perennial tussock grass species in relation to soil nitrogen and phosphorus availability

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Abstract

Studying relationships of plant traits to ecosystem properties is an emerging approach aiming to understand plant's potential effect on ecosystem functioning. In the current study, we explored links between morphological and nutritional leaf traits of two Mediterranean perennial grass species *Stipa tenacissima* and *Lygeum spartum*, widely used to prevent desertification process by stabilizing sand dunes. We evaluated also relationships in terms of nitrogen (N) and phosphorus (P) availability between leaves of the investigated species and the corresponding soil. Our results showed that leaf P was very low in comparison with leaf N for the two investigated species. In fact, chlorophyll content, photosynthesis capacity and water conservation during photosynthesis are mainly linked to leaf nitrogen content. Our findings support previous studies showing that at the species levels, morphological and nutritional leaf traits were not related. On the other hand, significant relationships were obtained between soil N and leaf N for *S. tenacissima* ($P = 0.011$) and *L. spartum* ($P = 0.033$). However, leaf P was not significantly related to soil P availability for both species. We suggest that any decrease in soil N with the predicted increasing aridity may result in reduction in leaf N and thus in worst dysfunction of some biological processes levels.

Key words: leaf traits, *Lygeum spartum*, soil nitrogen, soil phosphorus, *Stipa tenacissima*

Résumé

L'étude des relations entre des caractéristiques d'une plante et les propriétés de l'environnement est une approche émergente visant à comprendre l'effet potentiel de la plante sur le fonctionnement de l'écosystème. Dans cette étude, nous avons exploré les liens entre des caractéristiques morphologiques et nutritionnelles des feuilles de deux espèces de graminées méditerranéennes pérennes, *Stipa tenacissima* et *Lygeum spartum*, largement utilisées pour empêcher les processus de désertification en stabilisant les dunes de sable. Nous avons aussi évalué les relations en termes de disponibilité en azote (N) et en phosphore (P) entre les feuilles des espèces étudiées et le sol correspondant. Nos résultats ont montré que le P des feuilles était très bas par rapport au N des feuilles pour les deux espèces étudiées. En fait, le contenu en chlorophylle, la capacité de photosynthèse et la conservation de l'eau pendant la photosynthèse sont principalement liés au contenu des feuilles en azote. Nos résultats confirment des études antérieures qui montrent qu'au niveau de l'espèce, les caractéristiques morphologiques et nutritionnelles des feuilles ne sont pas liées. D'autre part, nous avons obtenu des relations significatives entre le N du sol et celui des feuilles pour *S. tenacissima* ($P = 0.011$) et *L. spartum* ($P = 0.033$). Cependant, chez les deux espèces, le P des feuilles n'était pas lié à la disponibilité du sol en P. Nous suggérons que toute diminution de l'azote du sol, résultant de l'aridité croissante prédite, pourrait entraîner une réduction de l'azote des feuilles et donc de graves disfonctionnements de certains niveaux de processus biologiques.

Introduction

Studying relationships of plant traits to ecosystem properties is an emerging approach aiming to understand plant's potential effect on ecosystem functioning (Lavorel & Garnier, 2002; Chapin, 2003; De Deyn, Cornelissen & Bardgett, 2008). Nitrogen (N) and phosphorus (P) consti-

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tuting the limiting factors for plant growth have been recognized as the essential nutrient elements for productivity in natural ecosystems (Aidar *et al.*, 2003; Martínez-Sánchez, 2005). It has been reported that soil nutrient availability, mostly N and P, determines the prevalence of certain plant functional traits, such as leaf mass per area (LMA) or its inversed value specific leaf area (SLA) and leaf N content (Grime, 1977; Fyllas *et al.*, 2009; Ordoñez *et al.*, 2009). According to Reich *et al.* (1999) and Wright *et al.* (2004), leaf N content and SLA are tightly linked to plant leaf economic spectrum and potential growth rate. Specific leaf area (SLA) and leaf dry matter content (LDMC) are important traits in plant ecology because they are associated with many critical aspects of plant growth and survival (Shipley & Vu, 2002). According to Vile *et al.* (2005), leaf thickness (LT) that can be estimated by $(SLA \times LDMC)^{-1}$ in laminar leaves plays an important role in leaf and plant functioning and is related to species strategies of resource acquisition and use.

Changes in resource availability have strong influence on traits related to plant resource-use strategies (Craine *et al.*, 2001). Two contrasting plant strategies (resource conservative versus resource acquisitive) have been developed by plant species to strengthen their competitive and responsive abilities under environmental fluctuations (Wright *et al.*, 2004; Tecco *et al.*, 2010). Species with resource conservative strategies dominating dry and nutrient-poor environments (Hobbie, 1992; Aerts, 1995) usually show low SLA, low leaf N content and long leaf lifespan (Reich *et al.*, 1999; Villar & Merino, 2001; Tecco *et al.*, 2010). On the contrary, species with resource acquisitive strategies are more dominant in moist and fertile areas (Grime *et al.*, 1997; Reich *et al.*, 1999) and generally have high SLA, high leaf N content and short leaf lifespan (Reich *et al.*, 1999; Tecco *et al.*, 2010; Laliberte *et al.*, 2012).

Among the most widespread tussock grasses species that occurs in arid and semi-arid environments of the Mediterranean region, we cited *Stipa tenacissima* and *Lygeum spartum. S. tenacissima* is restricted to the Iberian Peninsula and North Africa (Maire, 1968), while *L. spartum* has a wider geographical range and spreads throughout the Mediterranean Basin (Tutin, 1980). These perennial species belonging to the Poaceae family contribute in preventing desertification process by stabilizing sand dunes.

In the current study, we explored links between morphological (LMA and LDMC) and nutritional leaf traits (leaf N and P levels) of *S. tenacissima* and *L. spartum*. Furthermore, we evaluated relationships in terms of nitrogen and phosphorus availability between leaves of the investigated species and the corresponding soil.

Materials and methods

Study site

The current investigation was conducted on May 2013 in a national park located in south-western Tunisia named El Gonna. The study area (34°42′34.14″N, 10°31′20.15″E) is a *S. tenacissima* steppe dominated mainly by the tussock grass *S. tenacissima.* In addition to *S. tenacissima,* some perennial plant species were also present such as *Gymnocarpos decander*, *Helianthemum sessiliflorum*, *Helianthemum kahiricum*, *L. spartum* and *Atractylis serratuloides.*

Soils are alkaline sandy loam, with friable caliches at 10–25 cm depth and gypsum outcrops (Jeddi & Chaieb, 2009).

The climate type is Mediterranean lower arid (Emberger, 1955). The mean annual temperature and the mean annual rainfall were about 18.5°C and 193 mm, respectively.

Sampling protocol

For each species, we sampled eight tussocks for soil and leaf analyses. Each selected tussock must be at least to 50 cm apart from the other tussock. We ran three repetitions per tussock to measure nitrogen and phosphorus contents in the leaves of both investigated species and in the associated soil where tussocks grow in. Obtained values were then averaged.

Leaf morphological measurements

Leaf functional traits measurements were determined following a standardized protocol defined by Cornelissen *et al.* (2003). We selected young, fully expanded and illuminated leaves without herbivore or pathogen damage as recommended by Reich, Walters & Ellsworth (1992), Westoby (1998) and Weiher *et al.* (1999). Six juvenile leaves per tussock were used to measure: LMA defined as the leaf dry mass per leaf area (g cm^{-2}) and LDMC determined as the ratio of leaves dry mass to fresh mass (mg g^{-1}). For the determination of leaf dry mass, leaf samples were oven-dried for 24 h at 105°C.

Soil and leaf tissue analyses of nitrogen and phosphorus

Soil samples were taken underneath each tussock to 10 cm depth, mixed and sieved to 2 mm. Additional young leaves per tussock were collected and rinsed with deionized water to remove dust or soil attached to the leaves. Leaves and soil samples were oven-dried at 105°C for 24 h. The leaves were then crushed. Total N was measured by Kjeldahl procedure. Phosphorus content was determined by the Olsen's bicarbonate extraction (Olsen & Sommers, 1982).

Treatment of data

Determination coefficient (R^2) was used to investigate the relationship between studied parameters. Significance was set at a level of $P < 0.05$.

Results

Summarized leaf traits of the studied tussock grass species *S. tenacissima* and *L. spartum* and the associated soil P and N contents are presented in Table 1. *S. tenacissima* and *L. spartum* have, respectively, a LMA of 0.05 and 0.04 g cm^{-2} and LDMC of 212.7 and 161.27 mg g^{-1}. The concentrations of N and P (mg g^{-1}) in *S. tenacissima* leaves were about 4.9 and 0.48, respectively. These concentrations in *L. spartum* leaves were in the order of 6.49 mg g^{-1} for N and 0.27 mg g^{-1} for P.

Concerning soil N and P values (mg g^{-1}) underneath *S. tenacissima* tussocks, we found that these values were about 0.45 and 0.57, respectively. However, underneath *L. spartum*, soil N was in the order of 0.29 mg g^{-1} and soil P was about 0.36 mg g^{-1}.

Table 1 Leaf traits of the investigated grass species and the associated soil nitrogen and phosphorus availability (mean ± SD)

Leaf traits and the associated soil N and P concentrations	Species	
	Stipa tenacissima	*Lygeum spartum*
Leaf mass per area (LMA) (g cm^{-2})	0.05 ± 0.01	0.04 ± 0.00
Leaf dry matter content (LDMC) (mg g^{-1})	212.7 ± 52.12	161.27 ± 18.49
Leaf nitrogen (mg g^{-1})	4.9 ± 0.46	6.49 ± 0.48
Leaf phosphorus (mg g^{-1})	0.48 ± 0.1	0.27 ± 0.05
Soil nitrogen (mg g^{-1})	0.45 ± 0.06	0.29 ± 0.08
Soil phosphorus (mg g^{-1})	0.57 ± 0.05	0.36 ± 0.07

Our results showed nonsignificant relationships between morphological and nutritional leaf traits (Fig. 1).

Relationships between levels of N and P in the leaves of both studied species and in the associated soil where these tussock grass species grow are presented in Fig. 2. Positive and significant relationships were obtained between soil N and leaf N for *S. tenacissima* ($R^2 = 0.68$, $P = 0.011$) and *L. spartum* ($R^2 = 0.55$, $P = 0.033$). On the contrary, leaf P was not significantly related to soil P for both species (*S. tenacissima*: $P = 0.653$; *L. spartum*: $P = 0.52$).

Discussion

Nitrogen and phosphorous availability in Stipa tenacissima and Lygeum spartum leaves

Our results showed that leaf phosphorous availability was very low in comparison with leaf nitrogen availability for the two investigated species. In fact, nitrogen concentrations are important as they enhance water conservation during photosynthesis in species from low-rainfall areas (Wright & Westoby, 2002), which is the case of our studied species *S. tenacissima* and *L. spartum* dominating driest environments of the Mediterranean basin. Indeed, it has been mentioned that chlorophyll content is linked to leaf nitrogen content (Evans, 1983) and the photosynthetic capacity is principally related to nitrogen content because the proteins of the Calvin cycle and thylakoids represent the majority of leaf nitrogen (Field & Mooney, 1986; Evans, 1989).

Nevertheless, both leaf nitrogen and phosphorus concentrations remained low in the leaves of *S. tenacissima* and *L. spartum.* This can be attributed to the soil type in which the studied species are grown in. In fact, sandy soils support vegetation with low leaf nitrogen and phosphorus (Khan Towhid, 2013).

Relationships between morphological and nutritional leaf traits

The leaf growth of the investigated tussock grass species appeared not related to leaf nitrogen and phosphorus concentrations. In fact, our findings showed that LMA and LDMC were not related to levels of leaf nitrogen and phosphorus. These results corroborate that of Garten (1978) who highlighted that at the species level, LMA or LDMC was not related to plant nutrient concentrations. Furthermore, Domínguez *et al.* (2012) found that LMA

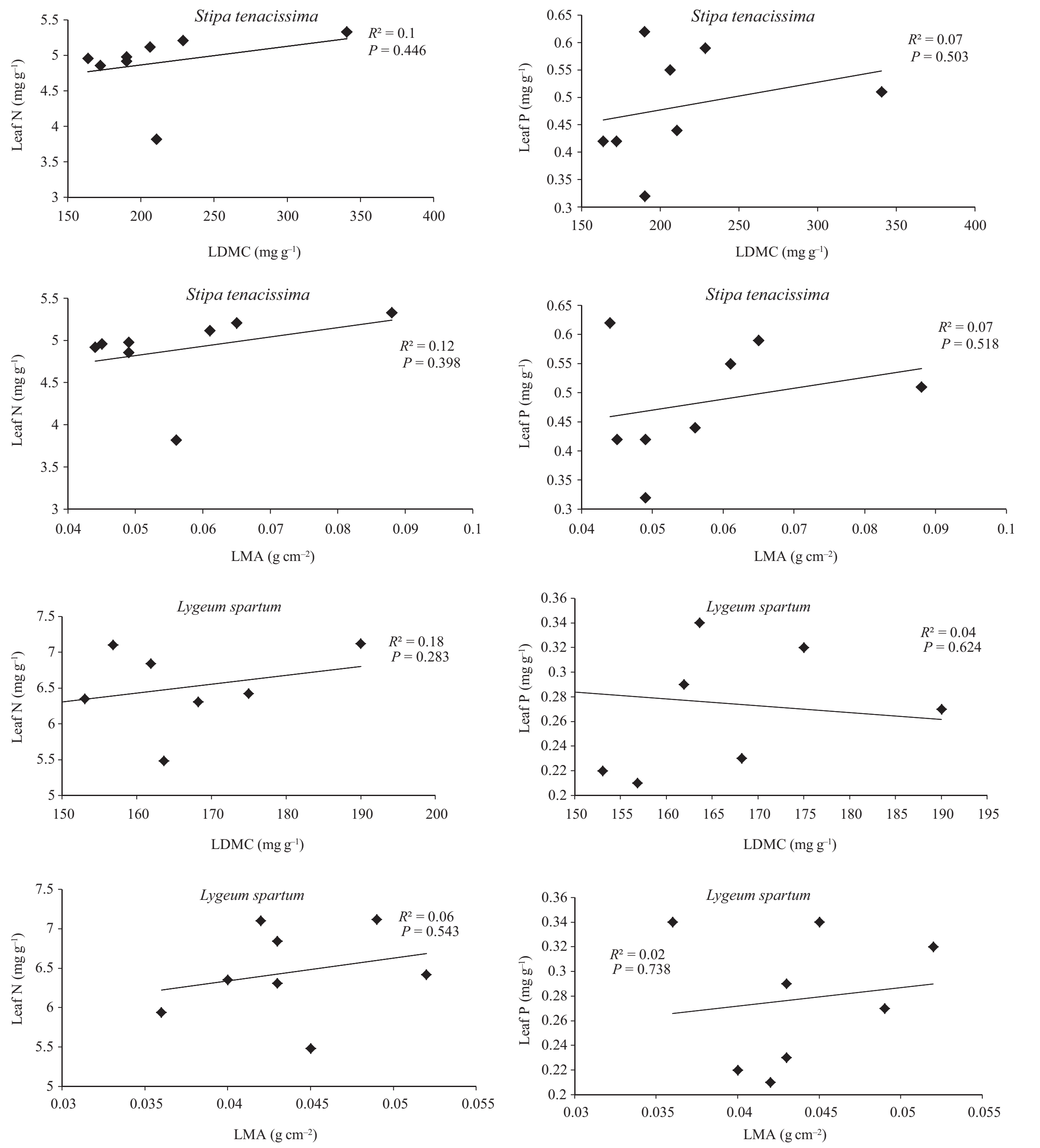

Fig 1 Relationships between morphological and nutritional leaf traits. LDMC, leaf dry matter content; LMA, leaf mass per area; N, nitrogen; P, phosphorus

and LDMC were significantly related to leaf N concentrations at the community level but not at the species level. On the contrary, some previous studies (Reich *et al.*, 1999; Castro-Diéz, Puyravaud & Corelissen, 2000; Shipley & Lechowicz, 2000; Wright *et al.*, 2004; Westoby & Wright, 2006) showed that leaf nitrogen and phosphorus concen-

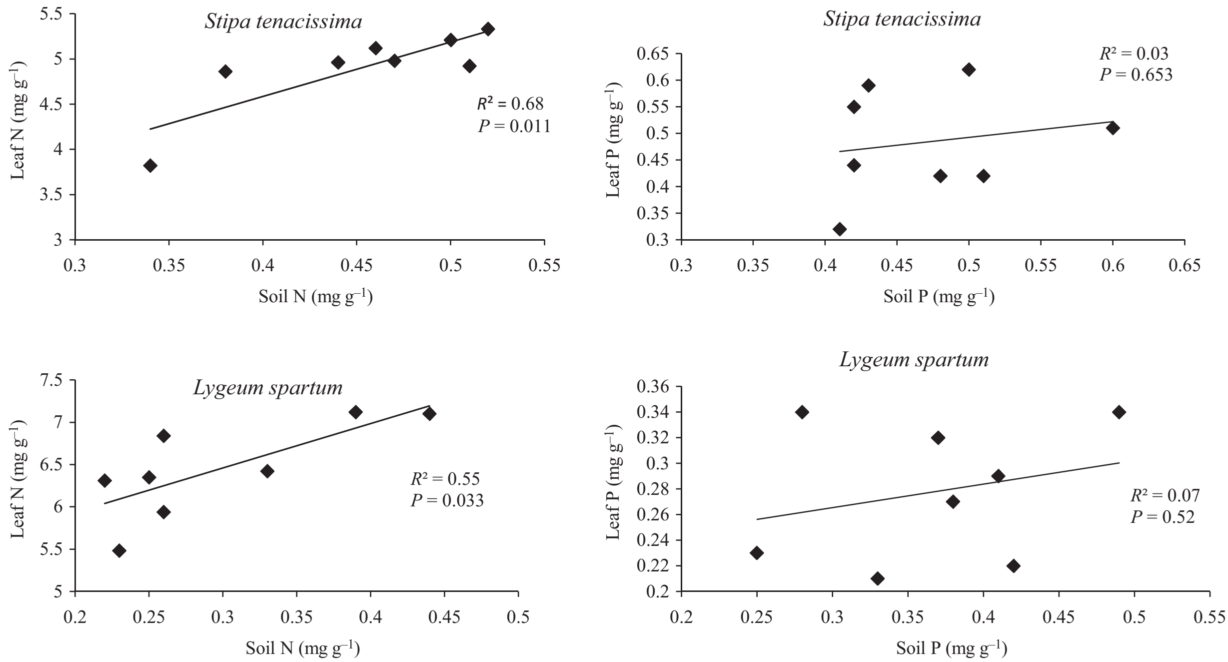

Fig 2 Relationships between leaves of the studied grass species and the associated soil in terms of nitrogen and phosphorus availability. N, nitrogen; P, phosphorus

trations were correlated with leaf mass per unit area across thousands of plant species.

Generally, these conflicting results may be attributed to site- and species-specific differences in nutrient availability which can contribute to significant variability in leaf trait relationships (Santiago & Wright, 2007).

Relationships between leaf N, P levels and the associated soil N and P availability

Leaf nitrogen was positively and significantly related to soil nitrogen for both studied species. So, an increased availability of soil N is associated with higher concentrations of leaf N. These results corroborate with those of Tognetti, Johnson & Michelozzi (1997), Zatylny & St-Pierre (2006), Orwin *et al.* (2010) and He *et al.* (2014) showing that leaf N increased linearly with an increase in soil N. Through this, it can be deduced that the quantity of N in leaves is intimately associated with soil nitrogen levels. Nevertheless, it has been suggested that any predicted increase in aridity will probably reduce the concentrations of soil nitrogen (Finzi *et al.*, 2011; Delgado-Baquerizo *et al.*, 2013). We suggest that any reduction in soil N can decrease leaf nitrogen content, which leads to loss green colour in the leaves and decrease leaf area (Bojović & Marković, 2009). As a result, this can lead to worst dysfunction of some biological processes linked to leaf N levels such as photosynthesis capacity.

On the other hand, leaf P content was not related to soil P availability. Orwin *et al.* (2010) highlighted that relationships of plant traits to soil properties related to P cycling were few and weak compared to those to soil properties related to N cycling. In fact, available phosphorus for plants is derived mainly from mechanical rock weathering (Schlesinger, 1996; Vitousek, 2004).

Anyway, the demand for nutrients among species differs among soils (Buol, 1995). In addition to that, it is likely that the traits expressed by a given plant species will vary depending on the length of time a plant has been growing in a particular patch and differences in soil fertility (Craine & Reich, 2001).

Conclusion

The current study showed that leaf phosphorous availability is very low in comparison with leaf nitrogen availability for the two investigated grass species. In fact, chlorophyll content, photosynthesis capacity and water conservation during photosynthesis are mainly linked to leaf nitrogen content. Our results showed also that there was no link between morphological (LMA, LDMC) and nutritional (N, P) leaf traits of *S. tenacissima* and *L. spartum*. On the other hand, leaf N content appeared to be strongly influenced by soil N availability, but leaf P level was not related to soil P for the both investigated species. We suggest that any decrease in soil N with increasing aridity (Finzi *et al.*, 2011; Delgado-Baquerizo *et al.*, 2013) may results in reduction in leaf nitrogen content and thus in worst dysfunction of some biological processes levels such as photosynthesis.

Further researches are needed to investigate relationships between leaf traits and soil nutrient availability at the community level.

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