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Specific leaf area and leaf nitrogen concentration in annual and perennial grass species growing in Mediterranean old-fields

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Abstract Specific leaf area (the ratio of leaf area to leaf dry mass) and leaf nitrogen concentration were measured on ten annual and nine perennial grass species growing in two old-fields of southern France, under a sub-humid Mediterranean climate. Specific leaf area (SLA) was found to be significantly higher in annuals than in perennials, but leaf nitrogen concentration expressed on a dry mass basis (LNC_m) was similar in both life-forms; expressed on an area basis, leaf nitrogen concentration (LNC_a) was significantly higher in perennials. The correlation between SLA and LNC_m was negative in annuals and positive in perennials, while that between the inverse of specific leaf area (1/SLA) and LNC_a was positive in annuals and not significant in perennials. It is hypothesized that these contrasting patterns depend on whether the two components of SLA – leaf thickness and density – vary in opposite directions. For nine of the species studied (six annuals and three perennials), relative growth rate data obtained in the laboratory under non-limiting nutrient supply were available; positive correlations were found between these values and both SLA and LNC_m obtained in the field, suggesting that the interspecific differences in structural and chemical characteristics of leaves are maintained under a wide range of growing conditions.

Key words Annuals · Leaf density · Leaf nitrogen concentration · Leaf thickness · Perennials

Introduction

Specific leaf area (SLA, the ratio of leaf area to leaf mass) and leaf nitrogen concentration (LNC) are traits which have been extensively studied, because of their presumed or demonstrated links with leaf gas exchange (e.g. Björkman 1981; Gutschick 1988, for SLA; Field and Mooney 1986; Evans 1989, for LNC). Recently, screening experiments under controlled conditions have also demonstrated the pivotal role of these two characters in the determination of whole-plant relative growth rate (Poorter and Remkes 1990; Garnier 1992; Marañon and Grubb 1993; Meerts and Garnier 1996, for SLA; Poorter et al. 1990; Garnier and Vancaeyzeele 1994 for LNC). SLA has also been shown to be inversely related to leaf longevity (e.g. Reich 1993), and synthesizing results from several sub-disciplines, Shipley (1995) has argued that SLA was a trait connected to a large array of properties affecting the ecology of plant species.

Several studies conducted under controlled conditions have shown that SLA and LNC per unit leaf mass were significantly higher in annuals than in wild herbaceous perennials (Smith et al. 1987; Muller and Garnier 1990; Garnier 1992; Roumet et al. 1996, for SLA; Gavnier and Vancaeyzeele 1994; van Arendonk and Poorter for two *Poa* species; Roumet et al. 1996, for LNC); results are less clear-cut when LNC is expressed on a leaf area basis. However, to our knowledge, no systematic comparison for these traits between the two life-forms has been conducted on plants growing in natural habitats. In the present study, SLA and LNC were measured in annual and perennial grass species growing in two old-fields of southern France, to test whether differences between the two life-forms observed under controlled conditions were also found for plants growing in the field.

Examination of data from the literature produces a confusing picture of the relationship between SLA and LNC expressed on a leaf mass basis. When comparisons are made over a broad taxonomical range or across biomes, this relationship is generally positive (Garnier and Freijsen 1994; Reich and Walters 1994; Schulze et al. 1994), but within a species or group of species of comparable leaf structure, it is either negative or non-existent (e.g. Körner 1989; Reich and Walters 1994). We will argue that these different patterns depend on whether

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the two components of SLA – leaf thickness and density – vary in oppposite directions.

Finally, because data on leaf traits in the field were collected on several species that were also studied under controlled laboratory conditions, an attempt will be made to compare the conclusions obtained in the two situations.

Material and methods

Study sites

The study was conducted during the spring of 1989, in two sites consisting of abandoned old-fields located near Montpellier (France), in a sub-humid Mediterranean climate (di Castri 1981). The first one (Fabrègues: 43°32′N, 3°47′E) is located 10 km south-west of Montpellier, 8 km from the Mediterranean Sea. This was an olive tree orchard abandoned approximately 40 years ago. The second one (Saint-Martin-de-Londres: 43°48′N, 3°44′E; Saint-Martin hereafter) is 25 km north north-west of Montpellier, further inland (32 km from the sea and 28 km north of Fabrègues), and was a vineyard abandoned approximately 30 years ago. Both sites are on calcareous soil, and are typical of the French garrigue (e.g. di Castri 1981). For the period 1981–1990, annual average rainfall was lower (623 vs. 975 mm) while temperature was higher (14.1 vs. 13.2°C) in Fabrègues than in Saint-Martin; at both sites, there is a marked summer drought, and low temperatures in winter.

Sampling and measurements

In Fabrègues, measurements were carried out on six annual and eight perennial grass species at various dates between 28 April and 19 May (Table 1), towards the end of the springtime rainy season; in Saint-Martin, measurements were done on five annual and three perennial grass species, all on 12 May (Table 1). At each site, sampling was conducted within an area of approximately 0.5 ha, and only in open areas (i.e. not under tree cover).

Twenty replicate leaves were taken for each species and tiller type (vegetative or reproductive, see below). In many annual species, there were at most one or two adult leaves present on each tiller at any one time. Therefore, only the youngest fully expanded leaf (i.e. whose ligule was apparent) of a tiller was selected for measurement in both life-forms. For annuals, all the tillers belonged to different individuals, and they were all in the reproductive stage at all dates and sites (Table 1). For perennials, two leaves belonging to different tillers of the same individual were generally selected for measurement; in Fabrègues, heading of tillers had already begun in late April for some species, while in Saint-Martin, all tillers were still vegetative at the time measurements were taken (Table 1).

Leaves were cut and immediately put into a cool chest and brought to the lab where their area was measured with a photoelectric area meter (Metraplan, Chaix, France). Leaves were then oven-dried for 2 days at 70°C, and their dry mass was measured. This was done individually for the 20 replicate leaves. Within each species and tiller type, leaves were then pooled according to their mass and ground. Nitrogen concentration was determined on this ground material with an elemental analyzer (Carlo Erba Instruments, Model EA 1108, Milan, Italy); the number of replicates varied among species (higher for species with larger leaves) and is given in Table 1.

Treatment of data

Specific leaf area was calculated as the ratio of leaf area to leaf dry mass of individual leaves (20 replicates). Leaf nitrogen concentration was expressed on a dry mass basis (LNC_m) and on a leaf area basis (LNC_a), by dividing LNC_m by SLA. Differences in specific leaf area and leaf nitrogen concentrations between sites of sampling and types of tillers were tested with two-way analyses of variance (ANOVA), with species and site or tiller as main effects; differences between life-forms were tested with one-way ANOVAs. Logarithmic transformation was applied to the data when appropriate. Correlations among traits were estimated as Pearson's correlation coefficients among species' means of characters.

The effect of site of sampling (Fabrègues vs. Saint-Martin) was assessed on the three species present at the two sites (*Brachypodium phoenicoides*, *Bromus erectus* and *B. madritensis*) and for comparable tillers (i.e. vegetative for *Brachypodium phoenicoides* and *Bromus erectus*, and reproductive for *B. madritensis*). Because the phenology of perennials lags behind that of annuals, measurements on several perennial species had to be done on vegetative tillers, while they were all taken on reproductive tillers in annuals. Whether leaf traits vary with type of tiller (i.e., either vegetative or reproductive) has thus to be determined as well. This was assessed in Fabrègues, from measurements made on four perennial species (*Brachypodium retusum*, *Bromus erectus*, *Dactylis glomerata* and *Melica ciliata*) which had both vegetative and reproductive tillers on the same individuals (Table 1).

A growth analysis had been conducted in the laboratory under non-limiting conditions (Garnier 1992) on nine of the species studied here (the six annuals *Avena barbata*, *Brachypodium distachyon*, *Bromus hordeaceus*, *B. madritensis*, *Hordeum murinum*, *Lolium rigidum*, and the three perennials *Brachypodium phoenicoides*, *Bromus erectus* and *Dactylis glomerata*). Relative growth rate (the increase in plant biomass per unit time and biomass in the plant) values were taken from this study in an attempt to relate characters of species measured in the laboratory and in the field.

Results

Overview of data and effects of site of sampling and type of tiller

The SLA, LNC_m and LNC_a of each species are given in Table 1. For all three variables there was a 2- to 2.5-fold difference between the highest and the lowest values recorded for the 19 grass species: SLA varied between 15.7 (for the perennial *Brachypodium phoenicoides* in Fabrègues) and 37.7 $m^2\ kg^{-1}$ (for the annual *Vulpia ciliata* in Saint-Martin), LNC_m was between 1.10 (for the perennial *B. phoenicoides*) and 2.50 mmol g^{-1} (for the perennial *Agropyron* sp.), both in Saint-Martin, and LNC_a was between 38.2 (for the annual *Brachypodium distachyon*) and 97.2 mmol m^{-2} (for the reproductive tillers of the perennial *Dactylis glomerata*), both in Fabrègues.

Results of the ANOVAs showed that there was a significant difference between leaves sampled in Fabrègues and in Saint-Martin for SLA ($F = 6.04$, $P < 0.05$), but neither for LNC_m nor LNC_a (the two F values were between 1 and 2, with corresponding P values higher than 0.2). There was no significant effect of type of tiller (vegetative vs. reproductive) on either SLA or LNC_a ($F = 2.53$ and 3.37, respectively for the two variables, with corresponding P values of 0.11 and 0.08), but this effect was significant for LNC_m ($F = 11.3$, $P < 0.001$). Therefore: (1) SLA comparisons between life-forms should be carried out for each site separately, but can be done for the pooled data obtained for vege-

tative and reproductive tillers, and (2) LNC comparisons should be done for leaves taken on similar tillers in annuals and perennials (i.e. reproductive tillers); this is possible only in Fabrègues, where five perennial species were in the reproductive phase at the time of measurements (Table 1).

Table 1 List of species on which leaf mass (*mass*), leaf area (*area*) specific leaf area (*SLA*), leaf nitrogen concentration on a leaf mass (LNC_m) and leaf area (LNC_a) bases were measured. *Tiller* refers to the type of tiller on which the leaf was sampled, either vegetative (*VG*) or reproductive (*RP*). Mass, area and SLA were measured and calculated on 20 replicate leaves. Date of sampling, and number of replicates for the nitrogen measurements (*n*) are also indicated. Numbers between brackets are standard errors. Nomenclature follows Tutin et al. (1980)

Site and species	Tiller	Date (day/month)	Mass (mg)	Area (cm^2)	*SLA* ($m^2\ kg^{-1}$)	LNC_m (mmol g^{-1})	LNC_a (mmol m^{-2})	*n*
FABREGUES								
Annuals								
Aegilops ovata	RP	09/05	4.46 (0.34)	1.04 (0.10)	23.1 (0.5)	2.02 (0.07)	87.9 (4.7)	5
Avena barbata	RP	28/04	4.82 (0.78)	1.19 (0.18)	25.4 (0.7)	1.89 (0.09)	76.6 (4.8)	5
Bromus lanceolatus	RP	09/05	2.32 (0.32)	0.65 (0.07)	28.8 (0.8)	1.34 (0.02)	48.5 (1.6)	4
Bromus madritensis	RP	09/05	1.56 (0.10)	0.57 (0.03)	37.0 (1.1)	1.51 (0.05)	40.8 (1.3)	3
Brachypodium distachyon	RP	28/04	0.69 (0.08)	0.22 (0.02)	32.8 (1.4)	1.23 (–)	38.2 (–)	1
Desmazeria rigida	RP	28/04	1.14 (0.10)	0.26 (0.02)	23.3 (0.7)	1.82 (0.05)	83.8 (8.1)	2
Perennials								
Avenula bromoides	RP	28/04	5.53 (1.11)	0.91 (0.15)	18.4 (0.8)	1.44 (0.09)	87.2 (4.8)	5
Bromus erectus	VG	09/05	29.4 (3.0)	4.78 (0.38)	17.0 (0.5)	1.31 (0.05)	77.6 (3.7)	10
	RP	09/05	9.86 (0.91)	1.93 (0.14)	20.2 (0.5)	1.54 (0.05)	78.6 (3.4)	10
Brachypodium phoenicoides	VG	19/05	47.2 (4.7)	6.91 (0.57)	15.7 (0.7)	1.16 (0.04)	78.3 (2.2)	10
Brachypodium retusum	VG	19/05	3.73 (0.16)	0.66 (0.03)	17.7 (0.4)	1.45 (0.04)	82.7 (3.4)	5
	RP	19/05	4.19 (0.46)	0.75 (0.08)	18.4 (0.5)	1.28 (0.04)	71.7 (4.2)	5
Dactylis glomerata	VG	09/05	11.5 (0.6)	2.35 (0.14)	20.4 (0.6)	1.53 (0.08)	75.3 (5.6)	10
(ssp. *hispanica*)	RP	09/05	4.64 (0.47)	0.99 (0.09)	21.8 (0.7)	2.09 (0.10)	97.2 (3.6)	5
Dichantium ischaemum	VG	28/04	8.15 (0.62)	2.34 (0.17)	29.0 (0.5)	1.44 (0.04)	50.0 (1.2)	5
Melica ciliata	VG	19/05	8.80 (0.41)	2.18 (0.09)	24.9 (0.6)	1.91 (0.06)	82.0 (4.8)	10
	RP	19/05	11.9 (1.1)	2.51 (0.20)	21.5 (0.4)	1.96 (0.05)	92.2 (3.7)	10
Phleum pratense	VG	28/04	6.17 (0.43)	1.62 (0.08)	27.1 (0.8)	1.91 (0.13)	74.0 (6.4)	4
SAINT-MARTIN DE LONDRES								
Annuals								
Bromus hordeaceus	RP	12/05	2.11 (0.22)	0.59 (0.06)	28.2 (0.5)	1.91 (0.05)	68.8 (2.4)	3
Bromus madritensis	RP	12/05	5.71 (0.47)	2.00 (0.14)	35.8 (1.0)	1.55 (0.03)	44.4 (1.4)	5
Hordeum murinum	RP	12/05	4.92 (0.63)	1.44 (0.18)	29.4 (0.6)	2.18 (0.12)	75.1 (2.4)	5
Lolium rigidum	RP	12/05	8.05 (0.74)	2.01 (0.16)	25.3 (0.4)	1.77 (0.07)	71.1 (3.4)	7
Vulpia ciliata	RP	12/05	0.79 (0.07)	0.28 (0.02)	37.7 (1.8)	1.49 (–)	41.6 (–)	1
Perennials								
Agropyron sp.	VG	12/05	16.3 (2.1)	4.23 (0.43)	27.5 (0.8)	2.50 (0.09)	92.4 (5.8)	8
Brachypodium phoenicoides	VG	12/05	36.1 (6.6)	4.91 (0.70)	16.6 (1.2)	1.10 (0.07)	75.9 (6.7)	10
Bromus erectus	VG	12/05	15.8 (2.2)	3.23 (0.32)	22.0 (0.7)	1.50 (0.05)	69.8 (2.6)	10

Table 2 Mean values with standard error between brackets of leaf mass (*mass*), leaf area (*area*), specific leaf area (*SLA*), leaf nitrogen concentration on a leaf mass (LNC_m) and leaf area (LNC_a) bases for annual and perennial grasses growing in two Mediterranean old-fields. Within each site, differences between life-forms were assessed for leaves taken from both vegetative and reproductive tillers for SLA, and only for leaves from reproductive tillers for LNC (see text). The significance levels of analyses of variance testing for differences between life-forms for each leaf trait are also given

Site and life-form	Mass (mg)	Area (cm^2)	SLA ($m^2\ kg^{-1}$)	LNC_m (mmol g^{-1})	LNC_a (mmol m^{-2})
FABREGUES					
Annuals	2.47 (1.08)	0.65 (0.15)	28.5 (0.5)	1.72 (0.07)	67.2 (3.7)
	***	***	***	n.s.	***
Perennials	12.6 (0.8)	2.33 (0.11)	21.0 (0.4)	1.69 (0.05)	85.4 (2.8)
SAINT-MARTIN DE LONDRES					
Annuals	4.31 (1.30)	1.26 (0.16)	31.3 (0.6)	–	–
	***	***	***		
Perennials	22.7 (1.7)	4.13 (0.21)	22.0 (0.8)	–	–

*** $P < 0.001$, n.s. not significant ($P > 0.05$)

Comparisons between annuals and perennials

In both sites, leaves of annuals were substantially smaller than those of perennials (Table 2). Specific leaf area spanned from 23.1 to 37.7 $m^2\ kg^{-1}$ in annuals and from 15.7 to 27.5 $m^2\ kg^{-1}$ in perennials (Table 1), and was significantly higher in annuals, both in Fabrègues and in Saint-Martin (Table 2). When comparisons were restricted to congeneric species, the SLA of annuals was always higher than that of perennials [compare data given in Table 1 for: *Avena barbata* (annual) and *Avenula bromoides* (perennial whose genus has recently been split from *Avena*); *Bromus hordeaceus*, *B. lanceolatus*, *B. madritensis*, (annuals) and *B. erectus* (perennial); *Brachypodium distachyon* (annual) and *B. phoenicoides* and *B. retusum* (perennials)].

Values for LNC_m ranged from 1.23 to 2.18 mmol g^{-1} in annuals and from 1.10 to 2.50 mmol g^{-1} in perennials (Table 1), and no significant difference between the two life-forms was detected for this variable (Table 2). This lack of a difference persists when comparisons were restricted to congeneric species. As a consequence, because SLA was higher in annuals than in perennials, when LNC was expressed on an area basis, values were lower in annuals than in perennials (Table 2). The range covered was between 38.2 and 87.9 mmol m^{-2} in annuals, and between 50.0 and 92.4 mmol m^{-2} in perennials. This difference between life-forms was also observed when congeners were compared (Table 1).

Relationships between SLA and LNC

To examine interdependences between these variables, one must first stress that SLA, LNC_m and LNC_a have different bases of expression. SLA is an area of leaf per unit biomass ($m^2\ kg^{-1}$), LNC_m is an amount of nitrogen per unit leaf biomass (mmol g^{-1}), and LNC_a is an amount of nitrogen per unit leaf area (mmol m^{-2}). The primary relationships should be analyzed with quantities that are homogeneous, i.e. for variables where the mass or the area term appears either as numerator or denominator in both. This is possible if we relate (1) SLA (which is an area/unit mass) to LNC_m (amount of nitrogen/unit mass), and (2) the inverse of SLA (1/SLA, which is a biomass/unit area) to LNC_a (amount of nitrogen/unit area).

These relationships have been drawn using the average values of these different variables for each species (i.e. calculated from data pooled for the different sites and types of tillers). Striking differences appeared between the two life-forms: in annuals, there was a negative correlation between SLA and LNC_m, while in perennials, the association between the two variables was positive (Fig. 1A). The correlations between 1/SLA and LNC_a were quite contrasted between the two life-forms as well, being significantly positive in annuals and positive but not significant in perennials (Fig. 1B). This means that in annuals, at a fixed leaf area, a given increase in leaf biomass (increase in 1/SLA) was accompanied by a sharp increase in the amount of nitrogen in the leaf, while in perennials this latter increase was much less pronounced (except for one species which falls close to annuals at comparable low values of both variables: Fig. 1B).

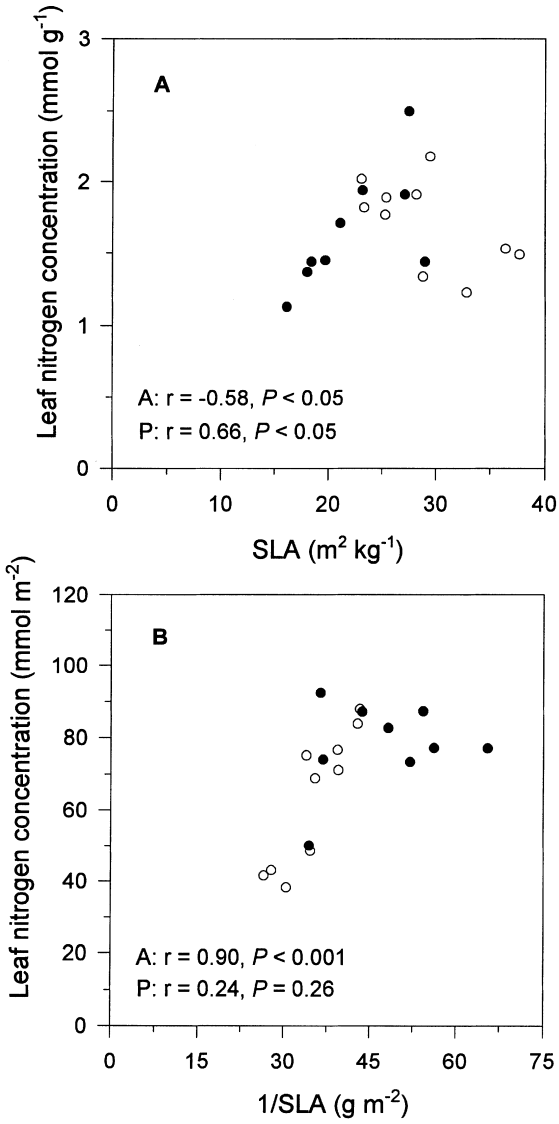

Fig. 1 Correlations between **A** specific leaf area (*SLA*) and leaf nitrogen concentration expressed on a dry mass basis, and **B** the inverse of specific leaf area (*1/SLA*) and leaf nitrogen concentration expressed on an area basis, for annual (○) and perennial (●) grass species growing in Mediterranean old-fields. Pearson's correlation coefficients (*r*) are given on the figures for annuals (*A*) and perennials (*P*), with the level of significance of the correlations

Connection with laboratory data

The correlations between the values of the three variables measured on plants in the field and the relative growth rate of plants measured in the laboratory under non-limiting conditions are presented in Fig. 2. There were significant positive correlations between laboratory

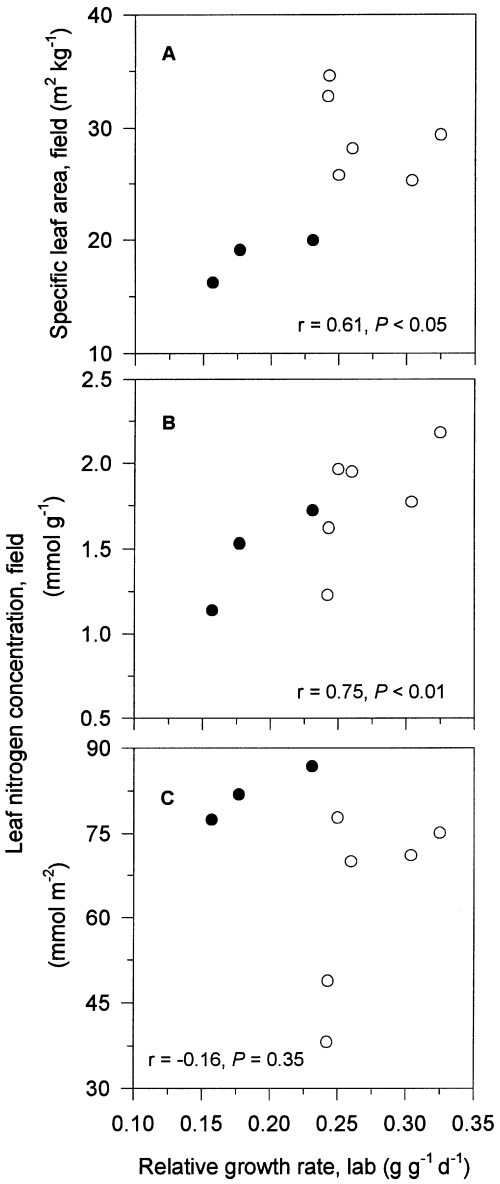

Fig. 2A–C Correlations between relative growth rate measured in the laboratory (Garnier 1992) and leaf traits measured in the field for 6 annual (○) and 3 perennial (●) grass species (see Material and methods section for names of species). **A** Specific leaf area, **B** leaf nitrogen concentration expressed on a dry mass basis, and **C** leaf nitrogen concentration expressed on an area basis. Pearson's correlation coefficients (r) are given on the figures, with the level of significance (P) of the correlations

RGR and specific leaf area on the one hand (Fig. 2A) and laboratory RGR and leaf nitrogen concentration expressed on a dry mass basis (Fig. 2B) on the other hand. There was no detectable correlation between laboratory RGR and LNC_a (Fig. 2C).

Discussion

Values of SLA and LNC found in the present study are in the low to middle range as compared to those found in herbaceous plants growing in the wild (e.g. Mooney et al. 1981; Pammenter et al. 1986; Körner 1989, for both variables; Shipley 1995 for SLA; Gebauer et al. 1988 for nitrogen). As expected, these values are somewhat lower than those found in grasses under productive conditions in the laboratory (Garnier and Vancaeyzeele 1994; van Arendonk and Poorter 1994), a point discussed in some detail by Garnier and Freijsen (1994).

Comparisons between annuals and perennials

The significantly higher SLA found in annuals as compared to herbaceous perennials in the field situation described here (Table 2) corroborates earlier laboratory findings (Smith et al. 1987; Muller and Garnier 1990; Garnier 1992; van Arendonk and Poorter 1994 for two species of *Poa*; Roumet et al. 1996) and common garden experiments (Pitelka 1977). The lack of a difference in leaf nitrogen concentration expressed on a dry mass basis (Table 2) is at variance with data obtained under controlled conditions showing higher LNC_m (and organic nitrogen concentration) in annuals than in perennials under non-limiting nutrient supply (Benech Arnold et al. 1992; Garnier and Vancaeyzeele 1994; van Arendonk and Poorter 1994 for *Poa*; Roumet et al. 1996), but is similar to what has been found in grasslands of southern Spain (Joffre 1990). If we admit that annuals generally have a higher SLA than perennials (see above), data from this latter study – where no SLA data were provided – , suggest that LNC expressed per unit leaf area was probably lower in annuals, in agreement with what was found in the present study (Table 2). This was also found under controlled conditions by Roumet et al. (1996), but is at variance with other data from the laboratory where LNC_a was found to be similar in annuals and perennials (Garnier and Vancaeyzeele 1994) and even higher in the annual *Poa annua* than in the perennial *P. pratensis* (van Arendonk and Poorter 1994).

The discrepancy in LNC_m between laboratory and field data may be interpreted in relation to the difference in nutrient availability between the two types of environments. In the laboratory studies discussed above, plants were grown under non-limiting nutrient supply, a situation where annuals, and fast-growing plants in general have a higher internal nitrogen concentration on a dry mass basis than perennials and slow-growing plants (Poorter and Bergkotte 1992; van der Werf et al. 1993; Garnier and Vancaeyzeele 1994). Under low nutrient supply in the laboratory, such differences in LNC_m between species tend to disappear (van der Werf et al. 1993), and because SLA is relatively insensitive to nitrogen supply, at least in grasses (e.g. Chapin et al. 1988; Muller and Garnier 1990; van de Vijver et al. 1993), LNC_a becomes higher in slow-growing species. Identical LNC_m in both life-forms and higher LNC_a in perennials (slow-growers with a low SLA) are precisely the two findings reported in the present study (Table 2).

What can be the causes of the differences in SLA between annuals and perennials? Witkowski and Lamont (1991) have stressed that specific leaf area could depend on leaf thickness and/or density (amount of biomass per unit leaf volume). A comparison of seven congeneric annual-perennial pairs of grasses (of which nine species are common to those of the present investigation) grown under non-limiting nutrient conditions in the laboratory showed that differences in SLA between life-forms were related to differences in leaf density, but not in leaf thickness (Garnier and Laurent 1994). This was also found for *Poa annua* and *P. pratensis* (van Arendonk and Poorter 1994). However, transverse sections performed on leaves collected in the field for four of the species studied here (*Aegilops ovata*, *Bromus erectus*, *B. madritensis* and *Dactylis glomerata*) showed a negative relationship between SLA and leaf thickness, mainly driven by the high SLA-thin leaves of *B. madritensis* (P. Cordonnier and E. Garnier, unpublished work).

Differences in anatomical features accounting for differences in leaf density between annuals and perennials have been discussed by Garnier and Laurent (1994). The main conclusions were that the higher leaf density of perennials was related to a higher proportion of sclerenchyma and vascular tissues in the leaf volume. These conclusions are likely to be valid under field conditions as well, since a lower proportion of sclerenchyma has also been observed in the annuals *A. ovata* and *B. madritensis* than in the perennials *D. glomerata* and *B. erectus* in the anatomical study mentioned above (P. Cordonnier and E. Garnier, unpublished work; no data on vascular tissues).

Differences in SLA between life-forms may also be related to differences in leaf size: in the present set of data, leaf dry mass tends to increase more rapidly than leaf area (not shown), which implies that SLA is a decreasing function of leaf mass, a point already made by Shipley (1995) in a comparison of 34 herbaceous species growing in the wild. Therefore, the lower SLA of perennials may partly be a consequence of their leaves being larger (Table 2). However, an analysis of variance conducted on species whose mean leaf dry mass was comprised between 1 and 9 mg (see Table 1 for species) still showed a significantly lower SLA in perennials (not shown).

Are differences in leaf traits between life-forms reported here stable over time? Measurements conducted during the first fortnight of April (2–3 weeks before data presented in the present paper were taken) on three annuals (*Aegilops ovata*, *Avena barbata*, *Bromus madritensis*) and three perennials (*Bromus erectus*, *Brachypodium retusum* and *Dactylis glomerata*) show the same tendencies as those presented in Table 2, with significantly higher SLA in annuals than in perennials, slightly (but not significantly) higher LNC_m in annuals, and significantly higher LNC_a in perennials. However, whether similar patterns are maintained over the whole growing season would require further testing.

Relationships between SLA and LNC

A number of studies have reported a positive association between SLA and leaf nitrogen concentration expressed on a dry mass basis, as for perennials in the present study (Fig. 1A; e.g. Sobrado and Medina 1981; Brown and Wilson 1983; Kull and Niinemets 1993 for two out of three tree species; Reich and Walters 1994). This is also true and on a broader scale, when both variables are compared over a wide range of (herbaceous) species (Garnier and Freijsen 1994) or even biomes (Schulze et al. 1994). However, there are a number of reports where no significant (Werk et al. 1983; Körner and Diemer 1987; Reich and Walters 1994) or even a negative relationship (Körner 1989; Reich et al. 1991 for pooled data over a year on three species of trees) were found. Interestingly, extraction of data on annual species from a desert and an old-field community from the extensive survey of Field and Mooney (1986) also yields a negative association ($r = -0.56$, $P < 0.001$, $n = 41$), as found for annuals in the present study (Fig. 1A). Below, an attempt at explaining these contrasting patterns is proposed.

Firstly, as already mentioned, an increase in SLA may be produced by a decrease in leaf thickness and/or a decrease in leaf density (Witkowski and Lamont 1991); secondly, data obtained in the laboratory on grasses (both annuals and perennials) show that LNC_m decreases with increasing density (probably because dense tissues such as sclerenchyma and/or vascular tissues are rich in cell wall material, composed of nitrogen-free or -poor substances), but that it is unaffected by leaf thickness (van Arendonk and Poorter 1994; combination of data from Garnier and Vancaeyzeele 1994 and Garnier and Laurent 1994). Therefore, a concomitant increase in SLA and LNC_m as observed in perennials (Fig. 1A), could be accounted for by a decrease in leaf density; changes in leaf thickness would have only a minor role in this relationship. Whether this actually holds cannot be properly assessed with data on leaf thickness and density available for the two perennials *Bromus erectus* and *Dactylis glomerata*, because differences in both SLA (17.0 and 20.4 $m^2\ kg^{-1}$ for *B. erectus* and *D. glomerata* respectively) and LNC_m (1.31 and 1.53 $mmol\ g^{-1}$ respectively) between the two species are not large enough. The positive correlation found between LNC_m and SLA for taxonomically remote species or across biomes (Garnier and Freijsen 1994; Schulze et al. 1994) suggests that this explanation may be valid when broad comparisons are conducted.

The explanation is less straightforward for annuals: according to what was stated above, a decrease in LNC_m goes with an increase in leaf density. Therefore, to produce the negative trend observed between LNC_m and SLA (on Fig. 2A), SLA has to increase in spite of this increase in leaf density. This is possible only if there is a decrease in leaf thickness, large enough to override the negative effect of leaf density on SLA. Data from leaf transverse sections performed on the annuals *Aegilops*

ovata and *Bromus madritensis* would be in favour of this hypothesis: the lower SLA and higher LNC_m of *A. ovata* (23.1 $m^2\ kg^{-1}$ and 2.02 mmol g^{-1}, respectively) as compared to that of *B. madritensis* (37.0 $m^2\ kg^{-1}$ and 1.51 mmol g^{-1}, respectively) goes with an 80% thicker leaf (149 vs. 64 μm), but a 36% lower leaf density [0.291 vs. 0.419 mg mm^{-3}, calculated as 1/(SLA × thickness)] in the former species.

The same set of hypotheses can be used to explain the area-based relationships presented on Fig. 1B: in perennials, if the increase in 1/SLA is chiefly due to an increase in leaf density, a higher biomass per unit leaf area is obtained with compounds poorer in nitrogen (e.g. proportionally richer in cell wall material); this results in little change in amount of nitrogen per unit area, leading to the lack of a relationship between 1/SLA and LNC_a. In annuals, the increase in 1/SLA is assumed to be the consequence of an increase in leaf thickness, accompanied by a decrease in leaf density. Therefore, a higher 1/SLA is obtained with material progressively richer in nitrogen compounds. This results in a larger amount of nitrogen per unit leaf area, and to a positive correlation between 1/SLA and LNC_a.

Correlations discussed so far have been drawn using mean values of variables for each species, and present an average trend which may differ from that occuring within a species. The positive association between SLA and LNC_m observed at the interspecific level for perennials actually occurs against a complex background of negative, positive or nil relationships at the intraspecific level (Fig. 3A; drawn for two perennial species only for clarity, and representing negative and nil relationships). Similarly, the non-significant association between 1/SLA and LNC_a observed at the interspecific level actually occurs against a background of positive correlations between the two variables at the intraspecific level (Fig. 3B). In annuals, apart from one exception (*Hordeum murinum*), inter- and intra-specific trends are similar (Fig. 3A, B). Taken together, this implies that, at the intraspecific level, the variation in SLA is mainly produced by a change in leaf thickness, accompanied by a change in leaf density in the cases where the relationships between SLA and LNC_m are negative.

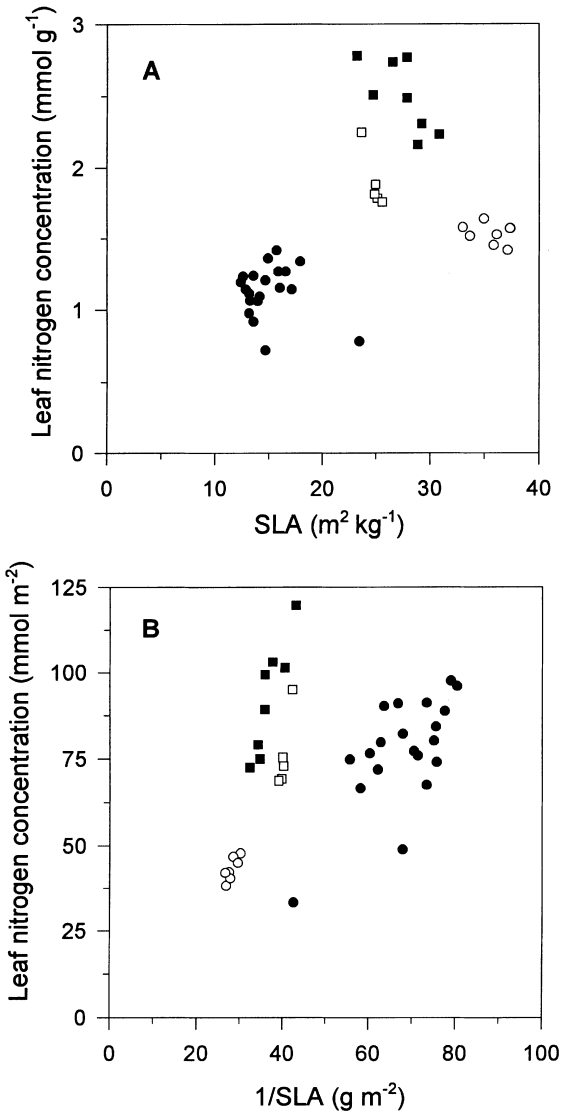

Fig. 3 Within-species relationships between **A** specific leaf area (*SLA*) and leaf nitrogen concentration expressed on a dry mass basis, and **B** the inverse of specific leaf area (*1/SLA*) and leaf nitrogen concentration expressed on an area basis. This figure has been drawn for the 2 annuals *Avena barbata* (□) and *Bromus madritensis* (○), and for the two perennials *Agropyron* sp. (●) and *Brachypodium phoenicoides* (■)

Physiological implications and connection with laboratory data

Some differences in leaf functioning between annuals and perennials are expected on the basis of the structural and chemical differences found here, but these are not straightforward to predict. In particular, the relationship between SLA and photosynthetic rate has not always been found to be consistent among studies (e.g. Werk et al. 1983; Wilhelm and Nelson 1985 and references therein; Pammenter et al. 1986; Körner and Diemer 1987). That between LNC_a and photosynthesis per unit leaf area appears more general though (Field and Mooney 1986; Evans 1989), and since LNC_a tends to be higher in perennials than in annuals (Table 2), we can expect a higher rate of instantaneous photosynthesis per unit leaf area in the former. This conclusion probably does not hold when photosynthetic rates are expressed on a leaf dry mass basis (no difference in LNC_m between life-forms: Table 2).

SLA has also been shown to be inversely related to leaf life-span (e.g. Reich 1993), and we can thus expect a longer leaf longevity in perennials than in annuals. This was indeed observed in a glasshouse study where leaf life-span was found to be 25% longer in the perennial *Bromus erectus* than in the annual *B. madritensis* (E. Garnier, C. Roumet and J. Roy, unpublished work). As nutrients are not completely reabsorbed from senescing leaves, the mean residence time of nutrients (Aerts

1990) is thus likely to be higher in perennials than in annuals.

For plants grown in the laboratory, it was found that the relative growth rate (RGR) of annuals was higher than that of perennials (Garnier 1992), and that this was partly related to differences in SLA (Garnier 1992) and leaf nitrogen concentration expressed on a dry mass basis (Garnier and Vancaeyzeele 1994). Figure 2 shows that when the same leaf parameters (SLA and LNC_m) were measured in the field, they remained related to the RGR measured in the lab. There were some differences in the absolute values of the parameters between the two situations: for the nine species common to the two studies, the average SLA was 24.6 ± 1.6 and 29.4 ± 1.9 m^2 kg^{-1} in field and lab conditions respectively; for LNC_m, these values were 1.66 ± 0.08 and 3.44 ± 0.14 mmol g^{-1} respectively, and for LNC_a, these were 72.5 ± 5.8 and 119 ± 6 mmol m^{-2}, respectively. The causes of these differences are discussed in Garnier and Freijsen (1994), but the main point is that some of the structural differences between annuals and perennials – and more generally between fast- and slow-growing species – in the laboratory are maintained in the field and, surprisingly, with a similar ranking. As discussed above, whether this translates into differences in functioning in the field requires further checking. This result is similar in nature to the finding that SLA correlates positively with the fertility of the habitat of a species, whether it is measured on plants growing in their natural habitat or in the laboratory (Garnier and Freijsen 1994).

Conclusions

Confirming results obtained in the laboratory, specific leaf area was found to be significantly higher in annual than in perennial grasses naturally occuring in old-fields; leaf nitrogen concentration was similar in both life-forms when expressed on a leaf mass basis, but it was higher in perennials when expressed on a leaf area basis. The relationship between these variables differs between the two life-forms; it is hypothesized that this is because in perennials, changes in SLA are driven chiefly by changes in leaf density, while in annuals they are produced by changes in leaf thickness accompanied by changes in leaf density in the opposite direction. In spite of differences in absolute values of specific leaf area and leaf nitrogen concentration between plants growing in the field and in the laboratory, the ranking of species for these two parameters remained very similar in the two situations.

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