

# A test of the generality of leaf trait relationships on the Tibetan Plateau

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## Summary

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- Leaf mass per area (LMA), nitrogen concentration (on mass and area bases,  $N_{\text{mass}}$  and  $N_{\text{area}}$  respectively), photosynthetic capacity ( $A_{\text{mass}}$  and  $A_{\text{area}}$ ) and photosynthetic nitrogen use efficiency (PNUE) are key foliar traits, but few data are available from cold, high-altitude environments.
- Here, we systematically measured these leaf traits in 74 species at 49 research sites on the Tibetan Plateau to examine how these traits, measured near the extremes of plant tolerance, compare with global patterns.
- Overall, Tibetan species had higher leaf nitrogen concentrations and photosynthetic capacities compared with a global dataset, but they had a slightly lower  $A_{\text{mass}}$  at a given  $N_{\text{mass}}$ . These leaf trait relationships were consistent with those reported from the global dataset, with slopes of the standardized major axes  $A_{\text{mass}}-LMA$ ,  $N_{\text{mass}}-LMA$  and  $A_{\text{mass}}-N_{\text{mass}}$  identical to those from the global dataset. Climate only weakly modulated leaf traits.
- Our data indicate that covarying sets of leaf traits are consistent across environments and biogeographic regions. Our results demonstrate functional convergence of leaf trait relationships in an extreme environment.

**Key words:** foliar nitrogen, functional traits, grassland, leaf mass per area (LMA), photosynthesis, photosynthetic nitrogen use efficiency (PNUE).

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## Introduction

Leaf mass per area (LMA), nitrogen (N) concentration (on mass and area bases,  $N_{\text{mass}}$  and  $N_{\text{area}}$  respectively), photosynthetic capacity (similarly,  $A_{\text{mass}}$  and  $A_{\text{area}}$ ) and photosynthetic nitrogen use efficiency (PNUE, defined as photosynthetic capacity per unit leaf nitrogen) are fundamental leaf traits, playing key roles in plant functioning (Schulze, 1994; Grime *et al.*, 1997; Mooney *et al.*, 1999; Ackerly, 2004). As a reflection of the dry-mass cost of producing new leaves, LMA correlates positively with leaf lifespan (LL) and negatively with leaf N concentration across species (Reich *et al.*, 1997; Westoby *et al.*, 2002; Wright *et al.*, 2004b). Leaf N concentration

itself is strongly correlated with photosynthetic capacity (Field & Mooney, 1986; Evans, 1989; Reich *et al.*, 1994), as N is essential for the synthesis of Rubisco, the key enzyme of photosynthesis (Field & Mooney, 1986; Taiz & Zeiger, 1998). This correlation provides a useful link between processes on short-term, leaf-level scales and long-term, plant- and stand-level scales, and has been used to estimate maximum CO<sub>2</sub> uptake over a broad range of species (Schulze, 1994; Baldocchi & Harley, 1995; Harley & Baldocchi, 1995; Aber *et al.*, 1996; Williams *et al.*, 1997; Larocque, 2002). Understanding the relationships between these fundamental traits and their large-scale patterns is essential for scaling up ecophysiological processes from the leaf level to the ecosystem level and in

predicting ecosystem functioning in response to environmental change (Ehleringer & Field, 1993; Peterson *et al.*, 1999; Norby & Luo, 2004).

Understanding large-scale patterns of leaf functional traits is a challenging issue of great interest to both plant physiologists and ecologists (Körner, 1989; Yin, 1993; Niinemets, 2001; Reich *et al.*, 2003; Chown *et al.*, 2004; Reich & Oleksyn, 2004; Wright *et al.*, 2005a,b). For example, in an examination of a global dataset, Reich *et al.* (1997) found that leaf traits such as photosynthetic rate and longevity scale predictably with one another, largely irrespective of environment or phylogeny. Wright *et al.* (2005b) similarly found that the effect of climate on the relationships among  $A_{\text{mass}}$ ,  $N_{\text{mass}}$ , LMA, leaf phosphorus (P), dark respiration rate ( $R$ ) and LL was modest, although some patterns appeared. A recent study by Reich & Oleksyn (2004) further pursued the link between climate and leaf traits, finding that leaf N and P decreased with mean annual temperature (MAT) from the 5–10°C range to the warmest MAT. At very low MATs, however, the relatively scarce data available hindered arrival at any definitive conclusions.

The Tibetan Plateau is an ideal place for large-scale ecological studies, because it provides a unique opportunity to examine trends in a high-altitude, cold climate with very low MAT. The plateau represents one of the largest alpine grasslands in the world, yet its vegetation has been underrepresented in global-scale studies (e.g. Reich & Oleksyn, 2004; Wright *et al.*, 2004b). Arctic and alpine plants have adapted to low temperatures, and thus are expected to have developed unique survival mechanisms (Chapin & Körner, 1995), enhancing the value of regional and global studies that include such plants. As the largest geomorphological unit on the Eurasian continent (Sun & Zheng, 1998), the Tibetan Plateau has a mean elevation of > 4000 m, with altitudes ranging from approx. 3000 to 8844 m. The plateau covers 12° of latitude and 28° of longitude, for a total area of approx.  $2.5 \times 10^6$  km<sup>2</sup>, nearly one-quarter of the area of China. As a consequence of uplift in the past several million years (Zheng, 1996; Tapponnier *et al.*, 2001), the Tibetan Plateau has had tremendous impact on the evolution and the development of species and ecosystems (Sun & Zheng, 1998), making it a center of differentiation for new species and a refuge for ancient species (Zhang *et al.*, 1988; Hou & Chang, 1992). In addition, the Plateau is one of the main regions of low-latitude frozen soils in the world (Zhang *et al.*, 1988; Molnar, 1989). Its alpine vegetation remains relatively undisturbed by humans, and thus the Plateau is an ideal region in which to study the responses of natural ecosystems to global climate change.

This study was designed to explore patterns of leaf functional traits in a high-elevation, low-temperature environment. Specifically, our study objectives were (i) to document the leaf functional traits of the flora in an understudied region over broad regional, elevational, and taxonomic ranges, and (ii) to examine how relationships among these traits, measured near the extremes of plant tolerance, compare with global patterns.

## Materials and Methods

### Study sites

Sampling was performed and measurements were taken between late July and early August 2003 along a transect in the Central Tibetan Plateau (Fig. 1). The transect covers latitudes from 28.19 to 36.32°N and longitudes from 86.83 to 100.93°E, and is approx. 2000 km long and 250 km wide (Table 1). Climate variation along the transect is represented by a MAT range of –9.7 to 6.8°C, with mean annual precipitation (MAP) ranging from 239 to 534 mm, and elevation from 2934 to 5249 m (Table 1).

Natural vegetation types along the transect include alpine steppe, alpine meadow, alpine cushion vegetation and scrubland, which are representative of the Tibetan Plateau (Zhang *et al.*, 1988). Alpine meadows, with perennial tussock grasses such as *Kobresia pygmaea* and *Kobresia tibetica*, and alpine steppes, with cold-xerophytic, short, dense tussock grasses such as *Stipa purpurea*, have extensive distributions, and are usually mixed with alpine forbs, including *Polygonum viviparum* and species of *Gentiana* and *Pedicularis* (Zhang *et al.*, 1988). The scrublands are dominated by *Salix oritrepha*, *Potentilla parvifolia*, species of *Rhododendron*, and *Sophora moorcroftiana*.

### Site selection and sampling

We selected 49 more or less evenly spaced sites along the transect by visual inspection of the vegetation, aiming to sample sites subject to minimal grazing and other anthropogenic disturbances (Table 1). Of the 49 sites, 12 were scrub, 12 steppe and 25 meadow. At each site, the dominant species were selected for *in situ* gas exchange measurement and *ex situ* chemical analysis. Nearly all measurements were taken at the flowering stage. In all, we investigated 74 species from 26 families over the 49 sites (Tables 1,2).

### Gas exchange, leaf carbon and nitrogen measurements

*In situ* photosynthetic rates of current-season leaves were measured at saturating light with two open path gas-exchange systems using red-blue light sources and CO<sub>2</sub> mixers (LI-6400; Li-Cor Inc., Lincoln, NE, USA). The on-board pressure and temperature sensors on the LI-6400 corrected for any changes in air density resulting from changes in atmospheric pressure or air temperature, and provided the correct mole fraction of CO<sub>2</sub> (Li-Cor Inc., 2002). Measurements were taken in the morning on clear days, on five to 10 plants of each of the dominant species at each site to account for idiosyncratic measurements. During the measurement at each site, leaf cuvette temperature was maintained at 22–25°C, depending on the external temperature, and relative humidity inside the leaf cuvette was kept at 45–65%. The reference CO<sub>2</sub> concentration in the leaf cuvette was maintained at 360

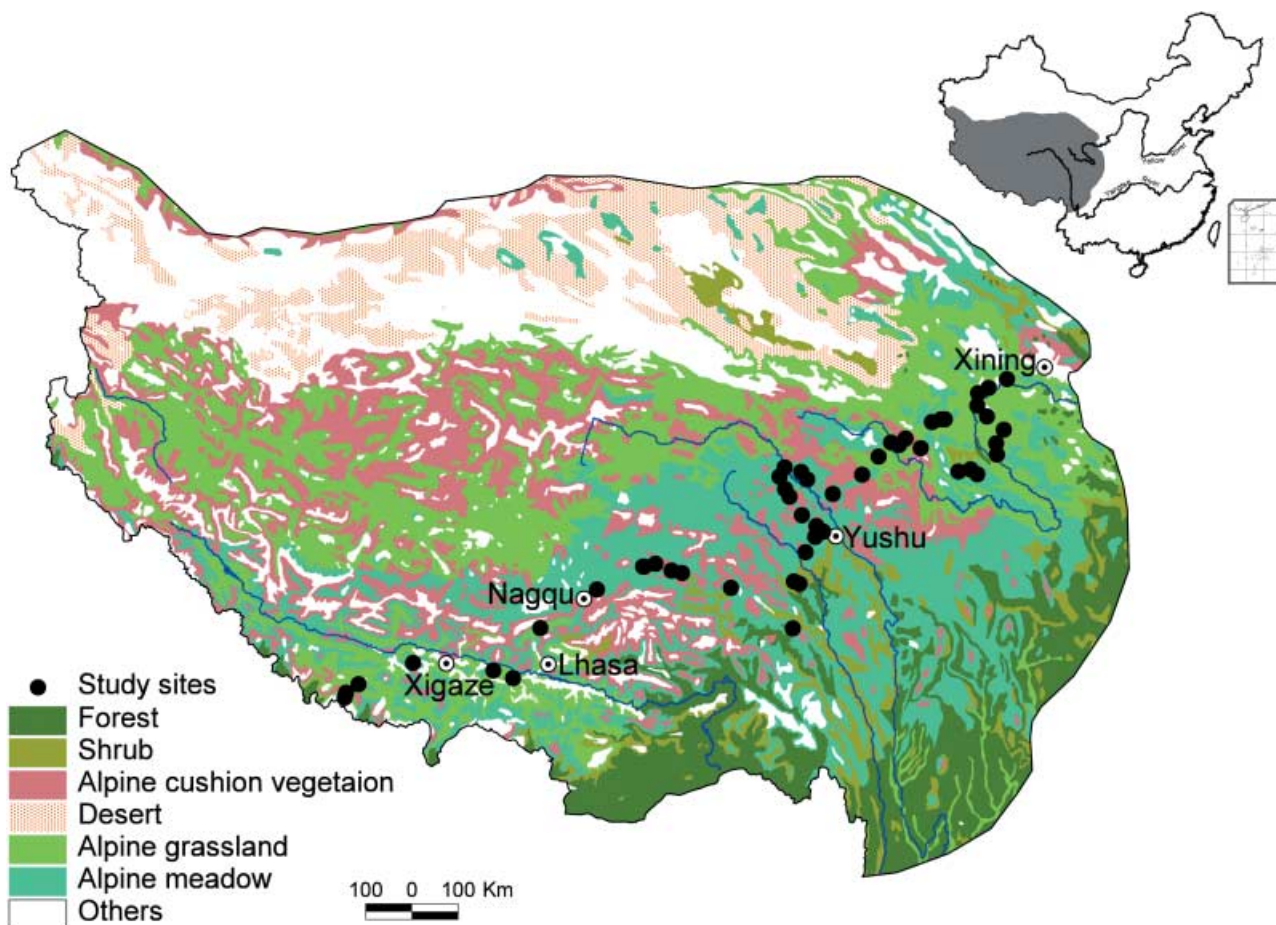


Fig. 1 A vegetation map of the Tibetan Plateau, adapted from the Vegetation Map of China (Hou, 1982), showing the sample sites.

$\mu\text{mol CO}_2 \text{ mol}^{-1}$ , and saturating photosynthetic photon flux density (PPFD, 400–700 nm) was set at  $1500 \mu\text{mol m}^{-2} \text{ s}^{-1}$ . For grasses with needle-like leaves, four to six leaves were placed across the chamber, taking care to avoid self-shading. The leaf area enclosed in the leaf chamber was determined immediately with a portable leaf-area meter (AM200; ADC Bioscientific Limited, Herts, UK). For each gas exchange measurement, a subsample of leaf was taken, its fresh weight was determined with a balance (Acculab Lt-320; Acculab, Measurement Standards Inc., Danvers, MA, USA) and its leaf area was measured. Following photosynthesis measurements, leaves were placed in paper bags and dried in the sun. Leaf samples were oven-dried at  $60^\circ\text{C}$  in the laboratory and their dry masses were measured on a semianalytical balance (Sartorius AG, Goettingen, Germany). The two LMA measurements for the gas-exchange sample and subsample were averaged to yield a combined estimate of LMA.

Dried samples from each plant were ground using a ball mill (NM200; Retsch, Haan, Germany). Total carbon (C) and N concentrations were determined on 5–6 mg of homogenously ground material for each sample using an elemental analyzer (2400 II CHNS/O Elemental Analyzer; Perkin-Elmer,

Boston, MA, USA) with a combustion temperature of  $950^\circ\text{C}$  and a reduction temperature of  $640^\circ\text{C}$ .

#### Climate data and statistical analyses

The climate data used in this study were from 50-year averaged temperature and precipitation records (1951–2000) at 680 well-distributed climate stations across China (Fang *et al.*, 2001; Piao *et al.*, 2003). We calculated MAT, mean growing season temperature (GST) (from May to August), MAP, and mean growing season precipitation (GSP) for each research site from the climate data, based on a linear model using latitude, longitude, and altitude as explanatory variables (Fang *et al.*, 2001; Piao *et al.*, 2003). It should be noted that MAT and GST, and MAP and GSP, were closely correlated ( $R^2 = 0.97$  and  $0.96$ , respectively;  $P < 0.0001$  for both correlations).

In our dataset, some species were frequently sampled. However, at some sites with very few species present, only one species could be sampled. To account for this variation in sample size and imbalance in the number of species per genus, we analyzed the data at three levels: (1) species-by-site level, with individual plant measurements averaged within species

**Table 1** Description of 49 sites where leaf trait measurements were taken

Site	Longitude (°E)	Latitude (°N)	Altitude (m)	MAT (°C)	MAP (mm)	Vegetation type	Species measured <sup>1</sup>
Q01	100.89	36.32	3277	-0.11	405	Steppe	As
Q02	100.46	36.12	2934	2.63	355	Steppe	As, Ci, Ai, Pmu
Q04	100.22	36.00	3078	1.90	362	Steppe	As, Ai
Q06	100.23	35.76	3184	1.53	389	Steppe	Ai, Ic, Sp, Pm, En, Ls
Q07	100.49	35.57	3304	0.76	431	Meadow	Sp, Lv, Kp
Q09	100.93	35.35	3253	1.19	468	Steppe	As, Sp
Q10	100.77	35.08	3565	-0.54	508	Meadow	Lv, Gs, Ssp, Pv
Q11	100.82	34.86	3650	-0.88	534	Meadow	Lv, Gs, Ssp
Q12	100.40	34.45	3938	-2.19	562	Scrub	Oo, So, Ppa, Lr
Q13	100.22	34.53	3727	-0.78	524	Meadow	Lv, Kp, Gs, Pv, Lr, Ag, Pma, Ac, Ap, Pta, Pa, Pd
Q16	99.93	34.47	3930	-1.96	530	Scrub	Ssu, Sa, Gs, Pv, Oo, So, Ppa
Q18	98.97	34.84	4518	-6.01	503	Meadow	Kt, Sgr, Kr, Cm
Q21	99.18	35.36	4158	-4.27	446	Steppe	Ic, Sp
Q22	99.39	35.43	4002	-3.36	440	Steppe	Sp
Q23	99.48	35.44	4089	-4.01	454	Steppe	Sp, Ag
Q24	98.58	34.99	4297	-4.52	446	Steppe	Sgr, Fr
Q25	98.45	34.85	4219	-3.78	438	Steppe	En, Cd, Sgl
Q26	98.25	34.88	4229	-3.80	425	Steppe	Sp, Kk
Q30	97.99	34.58	4278	-3.67	432	Meadow	Kt, Cm
Q31	97.66	34.20	5249	-9.69	534	Meadow	Rr, Sgl, Pn, Sme
Q32	97.02	33.76	4589	-4.05	473	Meadow	Kp, Mi, Kca
Q34	96.37	33.97	4229	-2.10	480	Meadow	Gs, Oo, Kh
Q35	96.20	34.10	4363	-2.99	468	Meadow	Kp, Kc, Oo, Kh, Gf, Rta
Q37	95.80	34.14	4226	-2.20	467	Steppe	Sp, Of
Q38	95.70	33.95	4161	-1.63	470	Meadow	Kp, Sc
Q39	95.88	33.73	4264	-2.06	468	Meadow	Of, Sc
Q40	96.01	33.60	4330	-2.33	467	Scrub	Ppa, Lr, Cj
Q41	96.36	33.28	4292	-1.84	478	Meadow	Kp
Q43	96.74	33.11	4238	-1.40	490	Scrub	Gs, So
Q44	96.91	33.02	3901	0.63	514	Meadow	Lv, Kp, Asi
Q47	96.74	32.90	4286	-1.49	488	Meadow	Gs, Oo, Ppa, Lr
Q48	96.56	32.59	3958	0.72	505	Meadow	Ic, Lv, Sc, Pal, Dc
X01	96.53	31.97	4167	0.05	494	Scrub	Pv, So, Ppa, Ga
X02	96.39	32.00	4191	-0.11	489	Meadow	Rt, Sa, Pg, Ssp, So
X03	96.51	31.10	4631	-1.87	468	Scrub	Rt, Ca, Rsp, Pi, Lg, Kpu, Sso, Ak, Rsp, So
X04	94.96	31.70	4336	-0.58	451	Meadow	Ra, Smo, Bd, Pt, Csp, Lt, Rh, Ppa
X06	93.79	31.84	4014	1.25	445	Meadow	Bd, Lh, Gs
X08	93.54	31.85	4475	-1.43	412	Meadow	Kp, Ppa
X09	93.14	31.93	4478	-1.50	403	Meadow	Kp
X10	92.90	31.84	4307	-0.40	408	Meadow	Ksp, Kh
X12	92.87	31.83	4287	-0.27	409	Meadow	Kp, Kh, Pd
X17	91.69	31.10	4758	-2.73	358	Meadow	Kt
X19	90.81	30.31	4328	1.19	368	Scrub	Ppa
XX1	90.42	29.26	3667	7.01	403	Scrub	Sm
XX2	89.95	29.33	3706	6.80	390	Scrub	Lm
XX3	86.83	28.19	5100	-5.65	244	Scrub	Ht, Ppa
XX4	86.84	28.30	4622	-1.20	272	Scrub	Ppa, Sc
XX5	87.07	28.51	5242	-6.91	239	Meadow	Pp, Sg, Ppa, Rta
XX6	88.15	29.15	4080	3.75	330	Scrub	Sm

Data for latitude, longitude and altitude were obtained with Magellan GPS Field PRO V (Magellan System Corporation, San Dimas, CA, USA). Mean annual temperature (MAT) and mean annual precipitation (MAP) were calculated from 50-year averaged temperature and precipitation records (1951–2000) at 680 well-distributed climate stations across China based on a linear model using latitude, longitude, and altitude as variables. See Fig. 1 for site locations.

<sup>1</sup>See Table 2 for definitions of species codes.

at each site to produce a species-by-site dataset; (2) species level, with measurements averaged within species to produce a dataset of species means; and (3) genus level, with measurements averaged within genera to produce a dataset of genus means.

We used  $\log_{10}$  transformation to normalize the distributions, a common practice in large-scale ecological studies (Sternier & Elser, 2002; McGroddy *et al.*, 2004; Reich & Oleksyn, 2004; Wright *et al.*, 2004b).

**Table 2** Species included in this study (74 in total), and average values of area-based light-saturated photosynthetic rate ( $A_{\text{area}}$ ), mass-based light-saturated photosynthetic rate ( $A_{\text{mass}}$ ), area-based leaf nitrogen concentration ( $N_{\text{area}}$ ), mass-based leaf nitrogen concentration ( $N_{\text{mass}}$ ), leaf mass per area (LMA) and photosynthetic nitrogen use efficiency (PNUE)

Code	Species <sup>1</sup>	Family	FG <sup>2</sup>	LMA (g m <sup>-2</sup> )	$A_{\text{area}}$ ( $\mu\text{mol m}^{-2} \text{s}^{-1}$ )	$A_{\text{mass}}$ ( $\mu\text{mol g}^{-1} \text{s}^{-1}$ )	$N_{\text{area}}$ (g m <sup>-2</sup> )	$N_{\text{mass}}$ (mg g <sup>-1</sup> )	PNUE ( $\mu\text{mol g}^{-1} \text{N s}^{-1}$ )
Ai	<i>Achnatherum inebrians</i>	Poaceae	G	75.0	11.5	0.14	2.53	33.8	4.6
As	<i>Achnatherum splendens</i>	Poaceae	G	112.6	15.9	0.15	3.50	31.2	4.9
Ag	<i>Aconitum gymnantrum</i>	Ranunculaceae	H	60.0	9.7	0.15	1.85	31.6	6.5
Ac	<i>Anemone cathayensis</i>	Ranunculaceae	H	56.3	13.7	0.24	1.73	30.7	7.9
Ak	<i>Arenaria sieversiana</i>	Caryophyllaceae	H	129.9	6.2	0.05	2.18	16.8	2.8
Asi	<i>Artemisia sieversiana</i>	Asteraceae	H	49.5	7.6	0.15	1.86	37.5	4.1
Ap	<i>Astragalus porphyrocalyx</i>	Fabaceae	H	51.2	13.1	0.25	2.57	50.3	5.1
Bd	<i>Berberis diaphana</i>	Berberidaceae	S	101.2	8.7	0.09	1.74	17.2	5.0
Cj	<i>Caragana jubata</i>	Fabaceae	S	91.5	10.1	0.11	2.49	27.2	4.1
Csp	<i>Caragana</i> sp.	Fabaceae	S	65.0	8.6	0.13	2.26	34.8	3.8
Cm	<i>Carex moorcroftii</i>	Cyperaceae	G	59.9	13.9	0.22	1.96	32.5	7.0
Ca	<i>Cotoneaster adpressus</i>	Rosaceae	S	50.8	8.5	0.17	1.10	21.7	7.7
Cd	<i>Cremanthodium discoideum</i>	Asteraceae	H	139.3	17.5	0.13	2.46	17.7	7.1
Dc	<i>Delphinium caeruleum</i>	Ranunculaceae	H	84.7	24.2	0.28	2.09	24.6	11.6
En	<i>Elymus nutans</i>	Poaceae	G	47.9	13.4	0.27	1.70	36.5	8.8
Fr	<i>Festuca rubra</i>	Poaceae	G	48.3	10.7	0.22	1.87	38.7	5.7
Gf	<i>Gentiana farrerii</i>	Gentianaceae	H	78.9	14.9	0.19	2.22	28.2	6.7
Gs	<i>Gentiana straminea</i>	Gentianaceae	H	96.0	16.2	0.17	2.71	28.5	6.0
Ga	<i>Gnaphalium affine</i>	Asteraceae	H	63.9	21.7	0.33	2.45	38.3	8.9
Ht	<i>Hippophae thibetana</i>	Elaeagnaceae	S	78.8	10.9	0.14	3.44	43.7	3.2
Ic	<i>Iris chinensis</i>	Iridaceae	H	146.0	18.7	0.13	3.15	21.9	5.9
Kca	<i>Kobresia capillifolia</i>	Cyperaceae	G	53.0	5.8	0.11	1.44	27.1	4.1
Kh	<i>Kobresia humilis</i>	Cyperaceae	G	58.6	9.9	0.16	1.43	24.5	7.0
Kk	<i>Kobresia kansuensis</i>	Cyperaceae	G	101.0	3.7	0.04	2.31	22.9	1.6
Kp	<i>Kobresia parva</i>	Cyperaceae	G	59.3	5.9	0.10	1.49	25.2	4.2
Kr	<i>Kobresia royleana</i>	Cyperaceae	G	45.7	11.1	0.23	0.99	21.5	11.3
Kpu	<i>Kobresia pusilla</i>	Cyperaceae	G	61.7	8.7	0.14	1.38	22.4	6.3
Ksp	<i>Kobresia</i> sp.	Cyperaceae	G	83.5	14.0	0.17	1.98	23.7	7.0
Kt	<i>Kobresia tibetica</i>	Cyperaceae	G	75.7	11.0	0.14	2.22	29.4	5.0
Kc	<i>Koeleria cristata</i>	Poaceae	G	62.0	4.7	0.08	1.62	26.1	2.9
Lg	<i>Lagotis glauca</i>	Scrophulariaceae	H	110.7	13.2	0.12	1.69	15.3	7.8
Lr	<i>Lamiophlomis rotata</i>	Lamiaceae	H	98.3	17.8	0.16	2.57	26.8	7.4
Lm	<i>Leptodermis microphylla</i>	Rubiaceae	S	63.9	9.1	0.14	1.60	25.0	5.7
Ls	<i>Leymus secalinus</i>	Poaceae	G	98.8	20.0	0.20	2.55	25.9	7.8
Lv	<i>Ligularia virgaurea</i>	Asteraceae	H	96.2	15.0	0.15	1.96	20.3	8.2
Lh	<i>Lonicera hispida</i>	Caprifoliaceae	S	129.0	9.7	0.07	3.63	28.2	2.7
Lt	<i>Lonicera tibetica</i>	Caprifoliaceae	S	58.9	11.3	0.19	1.46	24.8	7.7
Mi	<i>Meconopsis integrifolia</i>	Papaveraceae	H	80.6	21.3	0.26	2.96	36.7	7.2
Mt	<i>Microula tibetica</i>	Boraginaceae	H	69.1	27.4	0.40	2.78	40.2	9.9
Of	<i>Oxytropis falcata</i>	Fabaceae	H	66.2	15.6	0.23	2.64	39.9	5.9
Oo	<i>Oxytropis ochrocephala</i>	Fabaceae	H	57.0	15.8	0.21	2.36	42.1	7.0
Pal	<i>Pedicularis alaschanica</i>	Scrophulariaceae	H	54.7	11.8	0.21	1.55	28.4	7.6
Pi	<i>Pedicularis integrifolia</i>	Scrophulariaceae	H	65.9	12.1	0.18	1.31	19.8	9.3
Pmu	<i>Peganum multisectum</i>	Zygophyllaceae	H	60.1	17.7	0.29	3.24	54.0	5.4
Pp	<i>Phyllophyton pharicum</i>	Lamiaceae	H	73.3	9.7	0.13	1.38	18.9	7.0
Pma	<i>Polygonum macrophyllum</i>	Polygonaceae	H	56.9	13.4	0.23	2.16	38.0	6.2
Pv	<i>Polygonum viviparum</i>	Polygonaceae	H	65.3	12.4	0.19	2.15	32.8	5.8
Pa	<i>Potentilla anserina</i>	Rosaceae	H	60.2	10.7	0.18	1.58	26.3	6.8
Pg	<i>Potentilla glabra</i>	Rosaceae	S	136.4	14.6	0.10	2.32	17.0	6.3
Pn	<i>Potentilla nivia</i>	Rosaceae	H	57.6	6.9	0.12	1.45	25.2	4.7
Ppa	<i>Potentilla parvifolia</i>	Rosaceae	S	86.6	11.7	0.13	1.84	21.9	6.4
Pt	<i>Primula tangutica</i>	Primulaceae	H	45.6	12.4	0.27	1.04	22.8	11.9
Pta	<i>Przewalskia tangutica</i>	Solanaceae	H	54.3	19.0	0.35	3.08	56.8	6.2
Pd	<i>Ptilagrostis dichotoma</i>	Poaceae	G	65.6	7.3	0.11	1.66	25.5	4.4
Rsp	<i>Rheum spiciforme</i>	Polygonaceae	H	58.9	13.5	0.23	1.54	26.1	8.8
Rta	<i>Rheum tanguticum</i>	Polygonaceae	H	115.0	22.2	0.19	3.55	30.8	6.3
Rr	<i>Rhodiola rotundata</i>	Crassulaceae	H	57.7	5.7	0.10	1.78	30.9	3.2

Table 2 Continued

Code	Species <sup>1</sup>	Family	FG <sup>2</sup>	LMA (g m <sup>-2</sup> )	A <sub>area</sub> (µmol m <sup>-2</sup> s <sup>-1</sup> )	A <sub>mass</sub> (µmol g <sup>-1</sup> s <sup>-1</sup> )	N <sub>area</sub> (g m <sup>-2</sup> )	N <sub>mass</sub> (mg g <sup>-1</sup> )	PNUE (µmol g <sup>-1</sup> N s <sup>-1</sup> )
Rsp	<i>Rhododendron</i> sp.	Ericaceae	S	205.8	10.9	0.05	1.90	12.2	5.8
Rt	<i>Rhododendron thymifolium</i>	Ericaceae	S	113.2	8.8	0.07	2.01	17.8	4.3
Rh	<i>Ribes himalense</i>	Saxifragaceae	S	74.0	11.2	0.15	1.86	25.1	6.0
Ra	<i>Rumex acetosa</i>	Polygonaceae	H	53.8	22.5	0.42	2.33	43.3	9.7
So	<i>Salix oritrepha</i>	Salicaceae	S	83.5	14.8	0.16	2.49	30.2	5.9
Sso	<i>Salix souliei</i>	Salicaceae	S	70.9	9.9	0.14	1.63	23.0	6.1
Ssp	<i>Salix</i> sp.	Salicaceae	S	74.4	16.7	0.22	1.73	23.2	9.6
Sg	<i>Saussurea glanduligera</i>	Asteraceae	H	38.0	10.9	0.26	1.21	31.8	9.0
Sgr	<i>Saussurea graminifolia</i>	Asteraceae	H	70.7	8.9	0.12	1.82	25.8	4.9
Sme	<i>Saussurea medusa</i>	Asteraceae	H	65.6	5.1	0.08	1.63	24.9	3.1
Ssp	<i>Saussurea</i> sp.	Asteraceae	H	80.1	23.1	0.30	2.17	26.6	12.0
Ssu	<i>Saussurea superba</i>	Asteraceae	H	72.5	11.4	0.16	1.55	21.4	7.4
Sm	<i>Sophora moorcroftiana</i>	Fabaceae	S	101.8	13.3	0.13	3.34	32.8	4.1
Sa	<i>Spiraea alpina</i>	Rosaceae	S	65.7	9.8	0.14	1.41	22.4	6.9
Smo	<i>Spiraea mongolica</i>	Rosaceae	S	71.0	14.8	0.21	1.86	26.2	8.0
Sc	<i>Stellera chamaejasme</i>	Thymelaeaceae	H	59.0	14.5	0.24	2.15	36.4	6.8
Sp	<i>Stipa purpurea</i>	Poaceae	G	80.5	8.5	0.11	2.03	24.8	4.8

<sup>1</sup>Nomenclature follows that of Wu (1987). Family names follow current practice: Asteraceae = Compositae, Poaceae = Gramineae; Lamiaceae = Labiatae; Fabaceae = Leguminosae.

<sup>2</sup>FG, functional group: H, herb, including annuals, biennials and perennial forbs; S, shrub, including deciduous shrubs and evergreen shrubs; G, grass, including graminoids and sedges.

LMA, leaf mass per area; N<sub>mass</sub> and N<sub>area</sub>, nitrogen concentration on mass and area bases, respectively; A<sub>mass</sub> and A<sub>area</sub>, photosynthetic capacity on mass and area bases, respectively; PNUE, photosynthetic nitrogen use efficiency.

The influence of climate, plant functional group, and taxonomic identity on leaf traits was analyzed with general linear models (GLMs), using *R* and sequential (type-I) sums of squares (Ihaka & Gentleman, 1996; Schmid *et al.*, 2002). The explanatory terms included MAT and MAP as climatic variables, grasses vs herbs vs shrubs as the functional group (FG) variable, plant family as the taxonomic variable, and interactions between these (MAT × FG, MAT × family, MAP × FG, and MAP × family). We switched the order of entry into the model for MAT and MAP to test the explanatory power of each ignoring the other (see e.g. Schmid *et al.*, 2002). The significance of effects was tested with *F*-ratios between mean squares of explanatory terms and appropriate error terms. We also used GST and GSP to replace MAT and MAP, respectively. As the results were similar, for simplicity and for ease of comparison with other studies, we only present here the results with MAT and MAP.

The bivariate relationships of leaf traits were analyzed by fitting standardized major axis (SMA) lines to log-scaled variables (Wright *et al.*, 2004a). Both correlation coefficients (*r*) and SMA slopes were calculated using a DOS-based computer package, (s)<sub>MATR</sub> (Falster *et al.*, 2003). In this program, heterogeneity between SMA slopes is tested via a permutation test. Where deemed nonheterogeneous, a common SMA slope is estimated using a likelihood-ratio method (Warton & Weber, 2002). Differences in SMA elevation (intercept) can then be

tested with the SMA analog of standard analysis of covariance (ANCOVA).

## Results

### Leaf traits of the Tibetan Plateau compared with global data

For all species, the average values of LMA, N<sub>mass</sub>, N<sub>area</sub>, A<sub>mass</sub>, A<sub>area</sub> and PNUE were 78.7 g m<sup>-2</sup>, 28.0 mg g<sup>-1</sup>, 2.1 g m<sup>-2</sup>, 0.16 µmol g<sup>-1</sup> s<sup>-1</sup>, 12.7 µmol m<sup>-2</sup> s<sup>-1</sup> and 6.2 µmol g<sup>-1</sup> s<sup>-1</sup>, and species varied c. 4-, 6-, 4-, 11-, 7-, and 8-fold, respectively (Tables 2, 3). Part of this variation could be explained by differences among plant functional groups (Table 3). Herbs had higher photosynthetic rate (by both A<sub>area</sub> and A<sub>mass</sub>) than shrubs and grasses, higher leaf N concentration (by N<sub>mass</sub>) than shrubs, and higher PNUE than grasses. Comparison of the Tibetan data with the global dataset of Wright *et al.* (2004b) indicates that the present data are within the global ranges (Fig. 2). Overall, the Tibetan species had higher leaf N concentrations (by both N<sub>area</sub> and N<sub>mass</sub>) and photosynthetic rates (by both A<sub>area</sub> and A<sub>mass</sub>), but lower LMA than the average of the global dataset. For PNUE, mean values of the two datasets were not statistically different (Table 3). When individual functional groups were analyzed separately, the Tibetan grasses and herbs were found to have

**Table 3** Leaf traits of the plants on the Tibetan Plateau in comparison with the global dataset (Wright *et al.*, 2004b)

Leaf trait	Growth form	Tibet			Wright <i>et al.</i>		
		<i>n</i>	Mean	SD	<i>n</i>	Mean	SD
LMA	Overall	156	78.7 <sup>A</sup>	26.72	2370	127.7 <sup>B</sup>	118.35
	Grass	47	70.9 <sup>aA</sup>	20.82	125	95.0 <sup>aA</sup>	102.04
	Herb	71	78.3 <sup>abA</sup>	27.14	508	63.6 <sup>bb</sup>	37.53
	Shrub	38	89.2 <sup>bA</sup>	29.47	733	185.6 <sup>cB</sup>	160.97
$N_{\text{mass}}$	Overall	156	28.0 <sup>A</sup>	7.99	2061	19.3 <sup>B</sup>	9.81
	Grass	47	27.3 <sup>abA</sup>	5.41	95	19.5 <sup>ab</sup>	7.40
	Herb	71	30.3 <sup>aA</sup>	9.25	379	28.1 <sup>bA</sup>	10.84
	Shrub	38	24.6 <sup>bA</sup>	6.82	625	14.8 <sup>cB</sup>	8.69
$N_{\text{area}}$	Overall	156	2.1 <sup>A</sup>	0.66	1975	1.9 <sup>B</sup>	0.93
	Grass	47	1.9 <sup>aA</sup>	0.70	95	1.6 <sup>ab</sup>	0.89
	Herb	71	2.2 <sup>aA</sup>	0.62	378	1.7 <sup>ab</sup>	0.86
	Shrub	38	2.1 <sup>aA</sup>	0.64	621	2.1 <sup>bA</sup>	0.96
$A_{\text{mass}}$	Overall	142	0.16 <sup>A</sup>	0.07	770	0.13 <sup>B</sup>	0.10
	Grass	40	0.14 <sup>aA</sup>	0.06	37	0.20 <sup>ab</sup>	0.10
	Herb	65	0.20 <sup>bA</sup>	0.08	141	0.25 <sup>bb</sup>	0.13
	Shrub	37	0.13 <sup>aA</sup>	0.04	234	0.10 <sup>cB</sup>	0.07
$A_{\text{area}}$	Overall	143	12.7 <sup>A</sup>	5.31	825	11.5 <sup>B</sup>	5.93
	Grass	40	9.6 <sup>aA</sup>	4.64	44	16.6 <sup>ab</sup>	8.28
	Herb	66	15.1 <sup>bb</sup>	5.39	157	15.6 <sup>ab</sup>	7.07
	Shrub	37	11.6 <sup>aA</sup>	3.59	244	10.9 <sup>bA</sup>	4.99
PNUE	Overall	143	6.2 <sup>A</sup>	2.42	712	6.4 <sup>A</sup>	3.53
	Grass	40	5.4 <sup>aA</sup>	2.42	37	11.7 <sup>ab</sup>	6.65
	Herb	66	6.9 <sup>bA</sup>	2.57	139	8.7 <sup>bb</sup>	3.24
	Shrub	37	5.9 <sup>abA</sup>	1.71	228	5.3 <sup>cA</sup>	2.37

In multiple comparison tests, the Games–Howell method was used when variances were assumed to be heterogeneous by Levene’s test, and Tukey’s method was used when variances were homogeneous. Means followed by different lower-case or upper-case letters were statistically different at  $P < 0.05$  among functional groups and between datasets, respectively.

LMA, leaf mass per area;  $N_{\text{mass}}$  and  $N_{\text{area}}$ , nitrogen concentration on mass and area bases, respectively;  $A_{\text{mass}}$  and  $A_{\text{area}}$ , photosynthetic capacity on mass and area bases, respectively; PNUE, photosynthetic nitrogen use efficiency; SD, standard deviation.

**Table 4** Summary of general linear models for the effect of mean annual temperature (MAT), mean annual precipitation (MAP), functional group (FG) and family on leaf traits at three taxonomic levels

Factor	Species by site			Species mean			Genus mean		
	df	MS	%SS	df	MS	%SS	df	MS	%SS
LMA									
MAT	1	0.12***	4.0	1	0.04	2.7	1	0.00	0.1
MAP	1	0.03	0.9	1	0.00	0.3	1	0.01	1.1
FG	2	0.09***	6.2	2	0.05*	7.0			
Family	25	0.04***	38.3	25	0.02	40.3	26	0.02	63.8
MAT × FG	2	0.00	0.1	2	0.02	2.8			
MAP × FG	2	0.03	1.8	2	0.02	2.1			
MAT × family	14	0.02*	9.8	11	0.02	17.1	8	0.02	17.2
MAP × family	10	0.01	2.8	6	0.01	4.3	5	0.02	9.5
Residuals	98	0.01	36.1	25	0.01	23.4	7	0.01	8.2

lower PNUE than in the global dataset, while the PNUE values for shrubs of the two datasets were similar.

### Leaf trait relationships across all species

Across species, all leaf traits were correlated with one another (Fig. 2). These trait relationships were consistent with previous results from the global dataset (Wright *et al.*, 2004b). When the data from the present study were compared with the global dataset of Wright *et al.* (2004b), the SMA slopes for mass-based bivariate relationships, for example  $A_{\text{mass}}-LMA$ ,  $N_{\text{mass}}-LMA$  and  $A_{\text{mass}}-N_{\text{mass}}$ , were found to be the same for the two datasets. However, on an area basis, the SMA slopes for  $LMA-N_{\text{area}}$  and  $N_{\text{area}}-A_{\text{area}}$  differed between these two datasets (Fig. 3, Table 3). Furthermore, elevation shifts of the two datasets for  $N_{\text{mass}}-LMA$  and  $A_{\text{mass}}-N_{\text{mass}}$  were both significant, indicating that Tibetan species tended to have a higher  $N_{\text{mass}}$  at a given LMA, and a lower  $A_{\text{mass}}$  at a given  $N_{\text{mass}}$  (lower PNUE).

### Climate modifications of leaf traits

At the species-by-site level, GLM analysis (Table 4) showed that the effect of MAT was significant for LMA and area-based traits ( $N_{\text{area}}$  and  $A_{\text{area}}$ ), but not for mass-based traits ( $A_{\text{mass}}$  and  $N_{\text{mass}}$ ). The effect of MAT was weak, explaining 4.0–6.5% of the total variation in leaf traits. As shown in Fig. 3, among all species, LMA,  $A_{\text{area}}$  and  $N_{\text{area}}$  slightly increased with MAT (Fig. 3a,c,e), while other leaf traits did not shown any clear trend with MAT. FG and family were dominant factors, together explaining 25.3–60.7% of the total variation in leaf traits, and this result was independent of the sequence of entering the factors into the model. The effect of MAP was not significant for any of the leaf functional traits. Among the interaction terms, MAT × family had a significant influence on LMA and  $N_{\text{area}}$ , whereas MAT × FG had a significant influence on PNUE, demonstrating that the three

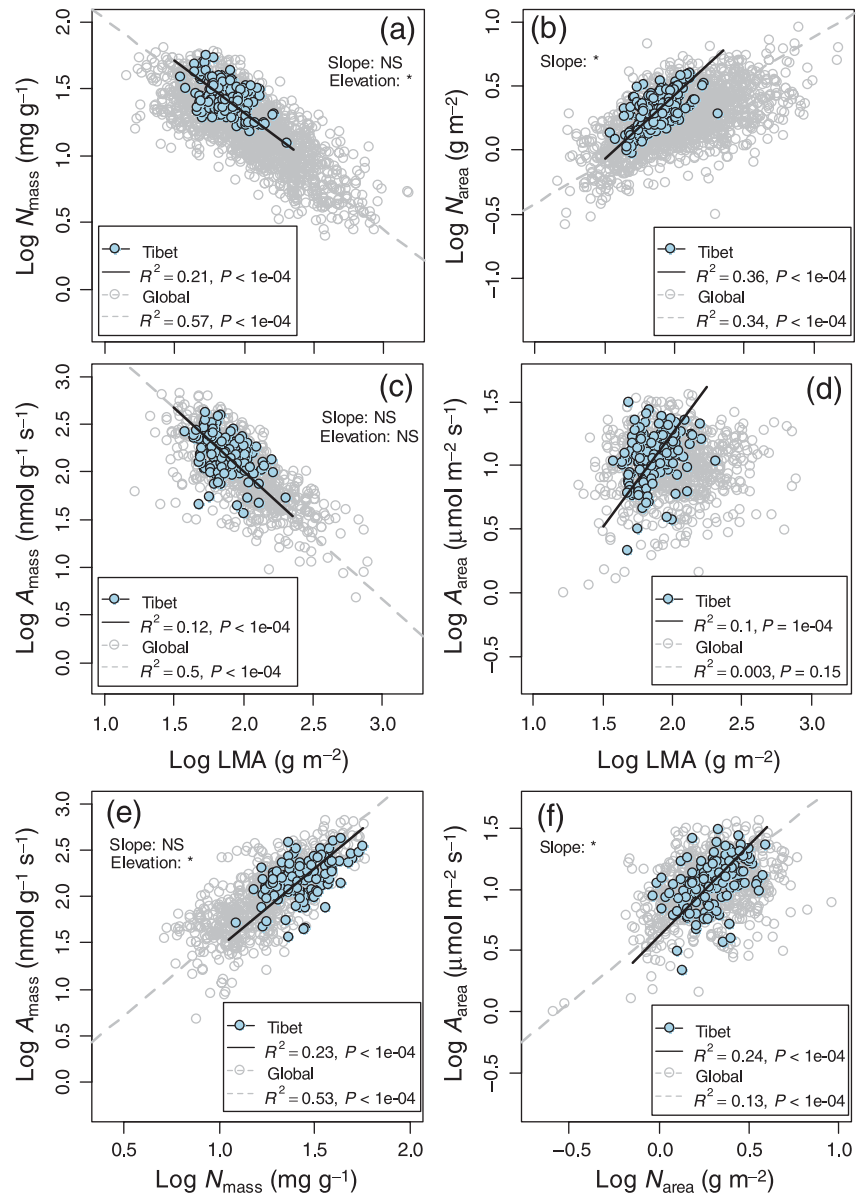
Table 4 Continued

Factor	Species by site			Species mean			Genus mean		
	df	MS	%SS	df	MS	%SS	df	MS	%SS
<i>N<sub>mass</sub></i>									
MAT	1	0.01	0.3	1	0.01	0.7	1	0.00	0.3
MAP	1	0.01	0.5	1	0.01	0.7	1	0.00	0.0
FG	2	0.10***	8.8	2	0.07**	11.6			
Family	25	0.05***	51.9	25	0.03***	61.1	26	0.03	79.1
MAT × FG	2	0.00	0.2	2	0.01	1.0			
MAP × FG	2	0.01	1.4	2	0.01	1.4			
MAT × family	14	0.01	5.9	11	0.01	5.7	8	0.01	5.6
MAP × family	10	0.01	4.7	6	0.00	2.4	5	0.01	7.1
Residuals	98	0.01	26.4	25	0.01	15.3	7	0.01	7.9
<i>N<sub>area</sub></i>									
MAT	1	0.18***	6.5	1	0.08**	6.6	1	0.01	0.9
MAP	1	0.00	0.1	1	0.02	1.9	1	0.01	1.7
FG	2	0.10***	6.8	2	0.02	2.8			
Family	25	0.04***	35.0	25	0.02**	49.1	26	0.02**	77.8
MAT × FG	2	0.00	0.2	2	0.01	1.1			
MAP × FG	2	0.02	1.1	2	0.05**	7.5			
MAT × family	14	0.02*	11.8	11	0.01	12.1	8	0.01	11.6
MAP × family	10	0.01	2.3	6	0.01	4.9	5	0.01	5.0
Residuals	98	0.01	36.2	25	0.01	14.2	7	0.00	3.0
<i>A<sub>mass</sub></i>									
MAT	1	0.04	0.7	1	0.07	2.2	1	0.01	0.8
MAP	1	0.01	0.1	1	0.00	0.0	1	0.01	0.8
FG	2	0.46***	16.8	2	0.31**	20.1			
Family	24	0.06*	24.3	24	0.04	34.7	25	0.05	66.6
MAT × FG	2	0.08	2.9	2	0.08	5.3			
MAP × FG	2	0.08	3.0	2	0.00	0.3			
MAT × family	14	0.02	5.8	11	0.02	6.2	8	0.02	8.2
MAP × family	10	0.01	2.1	6	0.04	8.2	5	0.04	12.0
Residuals	85	0.03	44.3	24	0.03	23.0	7	0.03	11.7
<i>A<sub>area</sub></i>									
MAT	1	0.23**	4.2	1	0.21*	9.2	1	0.03	2.2
MAP	1	0.00	0.0	1	0.00	0.2	1	0.00	0.0
FG	2	0.67***	24.0	2	0.22**	19.7			
Family	24	0.03	11.6	24	0.02	25.4	25	0.03	57.1
MAT × FG	2	0.09	3.1	2	0.02	1.7			
MAP × FG	2	0.03	1.2	2	0.01	1.1			
MAT × family	14	0.03	8.6	11	0.02	9.7	8	0.04	21.3
MAP × family	10	0.01	2.3	6	0.02	5.4	5	0.02	7.6
Residuals	86	0.03	45.1	24	0.03	27.8	7	0.02	11.8
PNUE									
MAT	1	0.01	0.3	1	0.04	1.8	1	0.01	0.6
MAP	1	0.00	0.1	1	0.02	0.8	1	0.02	2.0
FG	2	0.21**	9.6	2	0.10*	10.3			
Family	24	0.03	15.7	24	0.02	30.1	25	0.03	63.8
MAT × FG	2	0.10*	4.5	2	0.05	4.8			
MAP × FG	2	0.03	1.5	2	0.02	2.1			
MAT × family	14	0.03	8.7	11	0.02	9.7	8	0.01	10.3
MAP × family	10	0.03	6.0	6	0.03	8.9	5	0.01	6.0
Residuals	86	0.03	53.7	24	0.03	31.5	7	0.02	17.3

Explanatory variables are listed in the order of their inclusion in the models. Leaf traits were  $\log_{10}$ -transformed before analysis. df, degrees of freedom; MS, mean squares; %SS, percentage of sum of squares explained (%). \*\*\*,  $P < 0.001$ ; \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ .

LMA, leaf mass per area;  $N_{\text{mass}}$  and  $N_{\text{area}}$ , nitrogen concentration on mass and area bases, respectively;  $A_{\text{mass}}$  and  $A_{\text{area}}$ , photosynthetic capacity on mass and area bases, respectively; PNUE, photosynthetic nitrogen use efficiency.





**Fig. 2** Leaf trait relationships for Tibetan species and from the global dataset of Wright *et al.* (2004b). The  $A_{\text{area}}$ –LMA (photosynthetic capacity on an area basis–leaf mass per area) relationship for the global dataset was not significant ( $P > 0.05$ ) and thus the regression line is not shown. ‘Slope’, difference in standardized major axis (SMA) slopes; ‘Elevation’, difference in SMA elevations; NS, not significantly different; \*, significantly different ( $P < 0.05$ ). LMA, leaf mass per area;  $N_{\text{mass}}$  and  $N_{\text{area}}$ , nitrogen concentration on mass and area bases, respectively;  $A_{\text{mass}}$  and  $A_{\text{area}}$ , photosynthetic capacity on mass and area bases, respectively.

relationships between the climatic variable MAT and the leaf traits LMA,  $N_{\text{area}}$  and PNUE differed among plant families or among plant functional groups. For example, LMA increased with MAT in Asteraceae, Poaceae, and Salicaceae, but decreased with MAT in Polygonaceae. Similarly,  $N_{\text{area}}$  increased with MAT in Asteraceae, Poaceae, Lamiaceae and Fabaceae, but decreased with MAT in Polygonaceae. When MAT and MAP were replaced with GST and GSP, essentially the same results were obtained (data therefore not shown).

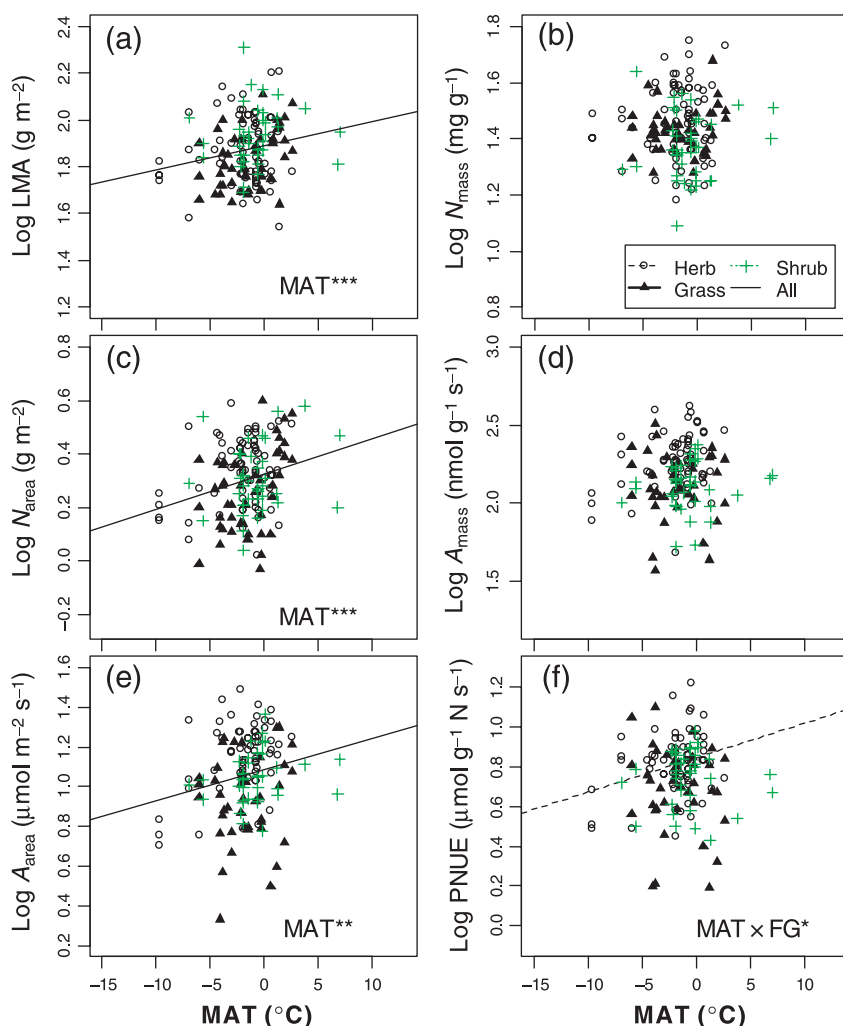
These patterns were generally similar for species means, except that LMA was no longer significantly affected by MAT (now averaged across sites for each species). However, at the genus mean level, the effects of all main factors (excluding the effect of family in  $N_{\text{area}}$ ) and interactions were not significant.

This is not surprising insofar as most genera occurred over a large range of sites and thus explanatory variables were also averaged over these large ranges of sites.

## Discussion

### Overall patterns of leaf traits on the Tibetan Plateau

This work presents, to the best of our knowledge, the first large-scale survey of leaf functional traits on the Tibetan Plateau. Our data indicate that the leaf N concentrations and photosynthetic capacities of Tibetan plants are higher than the global average (Wright *et al.*, 2004b). Furthermore, the leaf trait relationships were in agreement with those reported



**Fig. 3** Leaf traits in relation to mean annual temperature (MAT). Regression lines are shown only for relationships that were significant at  $P < 0.05$ . LMA, leaf mass per area;  $N_{\text{mass}}$  and  $N_{\text{area}}$ , nitrogen concentration on mass and area bases, respectively;  $A_{\text{mass}}$  and  $A_{\text{area}}$ , photosynthetic capacity on mass and area bases, respectively.

previously (Field & Mooney, 1986; Reich *et al.*, 1997; Ackerly, 2004; Wright *et al.*, 2004b).

Why do the Tibetan plants have overall higher leaf N and photosynthetic capacities? Functional group composition is likely a key factor. Most species we surveyed shed their leaves in winter, with only two evergreen species present. Previous studies have shown that evergreen plants usually have lower leaf N and photosynthetic capacities (Field & Mooney, 1986; Westoby *et al.*, 2002; Wright *et al.*, 2005a), so the dominance of deciduous species in our study, and in the flora of the Tibetan Plateau in general, may be one reason for high average leaf N concentrations and photosynthetic capacities. Another potential explanation for this high leaf N is the temperature–plant physiological hypothesis (TPPH) (Reich & Oleksyn, 2004), which predicts that leaf N should rise with decreasing temperature, as high leaf N may compensate for the low efficiency of physiological processes at low temperatures. Possibly because of the narrow MAT range (−9.7 to 6.8°C) in the current study, leaf N did not show any trend with decreasing temperature. However, when the leaf N data from Tibet

were pooled with the dataset of Reich & Oleksyn (2004), the previously observed positive correlation between leaf N and mean annual temperature (MAT) at very low MATs disappeared (He *et al.*, 2006). Thus, the TPPH is one potential explanation for higher leaf N concentrations on the Tibetan Plateau.

In recent years, several reports have documented global-scale variations in leaf functional traits and nutrient status (Reich *et al.*, 1997; Sterner & Elser, 2002; McGroddy *et al.*, 2004; Reich & Oleksyn, 2004; Wright *et al.*, 2004b; Kerkhoff *et al.*, 2005). A similar pattern of trait correlations is observed globally independent of growth form, biome or climate (Reich *et al.*, 1997; Wright *et al.*, 2004b). Despite the high altitude and low MAT of the Tibetan Plateau, which should exert strong evolutionary pressures on plant physiology, we found that interspecific leaf trait relationships on the Tibetan Plateau did not differ substantially from global patterns. Our results thus support the notion of convergent evolution in plant functioning (Reich *et al.*, 1997), with data from near the lower temperature and elevation limits of plant tolerance.

### Photosynthetic nitrogen use efficiency at high altitude

While shrubs on the Tibetan Plateau did not differ significantly in PNUE from shrubs in the global dataset, grasses and herbs had much lower PNUE in Tibet than globally. The lower intercept of the SMA regression line between  $A_{\text{mass}}$  and  $N_{\text{mass}}$  for the Tibet data indicates that the Tibetan species had a lower  $A_{\text{mass}}$  at a given  $N_{\text{mass}}$ , i.e. a lower PNUE (Table 3).

The Tibetan Plateau is characterized by both high altitude and low MAT. These characteristics are not independent, as MAT decreases with altitude. On the one hand, environmental conditions at higher altitudes are typically characterized by low MAT, low air pressure, high wind speed and high UV-B radiation (Friend & Woodward, 1990; Körner, 1999), all of which are considered to lower photosynthetic rates (Chapin *et al.*, 1993). On the other hand, some studies have found that photosynthetic capacity at high altitude is comparable to that at low altitude (Körner, 1999). In addition, studies on alpine plants have revealed that leaf N concentration usually increases with increasing elevation (Körner & Diemer, 1987; Friend *et al.*, 1989; Körner *et al.*, 1989; Friend & Woodward, 1990; Westbeek *et al.*, 1999). As a result, the PNUE of plants at high altitudes is predicted to be lower than that of plants at low altitudes.

The few studies investigating changes in PNUE along altitudinal gradients have supported this deduction (Körner & Diemer, 1987; Vitousek *et al.*, 1990; Hikosaka *et al.*, 2002). For example, Körner & Diemer (1987) found that *in situ* PNUE was 20–30% lower in many herbaceous species at an altitude of 2600 m than at 600 m in the Austrian Alps. Vitousek *et al.* (1990) also found that *in situ* PNUE of a Hawaiian tree species, *Metrosideros polymorpha*, decreased by half as altitude rose from 700 to 2500 m. In contrast, Terashima *et al.* (1993) showed that *in situ* PNUE of several herbaceous species at 4300 m in the Eastern Himalayas was comparable to that observed in lowland herbs. Based on a biochemical model, Terashima *et al.* (1995) argued that the biochemical suppression of photosynthesis should not be as large as has been supposed, because, with lowering of atmospheric pressure, the partial pressure of  $O_2$  decreases as well as that of  $CO_2$ , which results in a reduction of photorespiration, partly compensating for the reduction in  $CO_2$  assimilation. Therefore, the effect of MAT at high altitudes may only partially contribute to this trend in PNUE.

In the present study, we observed a lower PNUE for the herbs and grasses on the Tibetan Plateau compared with the global average. N partitioning between photosynthetic and nonphotosynthetic structures (Loomis, 1997; Hikosaka, 2004) and N allocation within the photosynthetic apparatus (Hikosaka, 2004) may explain this decrease. Such partitioning differences could arise via alterations of leaf anatomy resulting from the falling temperature. Leaf thickness, palisade and parenchyma cell sizes, and the proportion of cell wall to cell volume may influence N partitioning, because these anatomical traits affect the ratio of cell wall mass to whole cell mass and

thus the percentage of protein in each cell (Loomis, 1997). However, little information is available regarding the links between these anatomical traits and leaf N partitioning. These links will be the subject of future studies.

It is worth noting that our measurements were taken at local low air pressure. The Li-Cor 6400 photosynthetic system is designed to correct for any changes in air density resulting from changes in atmospheric pressure or air temperature, and provide the correct mole fraction of  $CO_2$  (Li-Cor Inc., 2002). In addition, the flow meter is a mass flow meter (not a volume flow meter). Thus the difference in gas exchange measurements between high and low altitudes is air pressure. We do not know how different the PNUE would be if we accounted for air pressure. This issue should be addressed in future studies.

### Effects of climate on leaf traits in cold, extremely high-altitude environments

Whereas effects of climate on leaf traits were relatively small in our study, differences among plant functional groups and families were large, together explaining 25.3–60.7% of the total variation in the various leaf traits measured. If mean values for genera were used, the climate- and functional-group-related variations in leaf traits disappeared, indicating that different genera are not very specialized with regard to climatic preferences or functional group. In other words, these climate- and functional-group-related variations most likely reflect evolutionary processes occurring within genera at the intra- and interspecific levels.

Plant functional traits are considered to reflect adaptations to variation in the physical environment and ecophysiological as well as evolutionary trade-offs among different functions within a plant (Cornelissen, 1999; Ackerly *et al.*, 2000; Westoby *et al.*, 2002; Lavore *et al.*, 2006). Thus, the responses of plant functional traits to climate, including responses to extreme low or high temperature, and to gradients of moisture availability, are associated with variations in life form and shifts in species composition (Chapin *et al.*, 1993; Körner, 1999; Wright & Westoby, 2002; Cavender-Bares *et al.*, 2004). Our results suggest that broad comparisons, at least in nontree plant species, should focus on intra- and interspecific variations, because data aggregation at the genus level may sacrifice too much information and thus not allow detection of macroecological patterns among climate, whole-plant functional type and leaf functional traits.

In the past 50 years, there have been numerous integrated surveys of forest and grassland resources in Tibet, with most of the work focusing on vegetation ecology (Chang & Gauch, 1986; Wang, 1988; Zhang *et al.*, 1988). During the 1990s, long-term research on ecosystem structure and functioning began in the major vegetation types of the Tibetan Plateau (Li & Zhou, 1998), and recent studies have examined the productivity of its natural vegetation (Luo *et al.*, 2002). In a

new study on large-scale patterns of leaf N and P stoichiometry by Han *et al.* (2005), 14 out of 753 terrestrial plant species from across China were from Tibet. In spite of these efforts, the functional ecology of alpine plants on the Tibetan Plateau has been underrepresented in recent large-scale comparative studies, such as those of Reich & Oleksyn (2004) and Wright *et al.* (2004b).

Our study fills part of this information gap (Reich, 2005). The global uniqueness of the Tibetan Plateau with regard to its extremely high altitude and large size makes any global compilation incomplete without the inclusion of Tibetan data. More work on the ecology and evolution of plant traits in this region is therefore much needed to improve our understanding of global patterns of, in particular, plant C and N balance and allocation. Considering that global change may contribute to an upwards shift of climatic environments (Parmesan & Yohe, 2003), the Tibetan Plateau may be an important region for future research on plant acclimation and adaptation.

In conclusion, the general pattern of leaf trait relationships on the Tibetan Plateau is consistent with those reported previously on the global scale, providing additional support for convergent evolution in plant functioning. However, some patterns are unique to Tibet. First, overall leaf N concentrations and photosynthetic capacities were higher than the global average. This likely resulted from the dominance of deciduous species in our study, but low-temperature-associated chemical composition and physiological processes may also contribute to this pattern. Secondly, Tibetan species had a slightly lower PNUE, probably as a result of different N partitioning. Thirdly, even in a cold, extreme, high-altitude environment, the modulation of leaf traits by climate was weak, and the variations in leaf traits mainly occurred at the intra- and inter-specific levels, not at the genus level.

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## References

- Aber JD, Reich PB, Goulden ML. 1996. Extrapolating leaf CO<sub>2</sub> exchange to the canopy: a generalized model of forest photosynthesis compared with measurements by eddy correlation. *Oecologia* 106: 257–265.
- Ackerly DD. 2004. Functional strategies of chaparral shrubs in relation to seasonal water deficit and disturbance. *Ecological Monographs* 74: 25–44.
- Ackerly DD, Dudley SA, Sultan SE, Schmitt J, Coleman JS, Linder CR, Sandquist DR, Geber MA, Evans AS, Dawson TE, Lechowicz MJ. 2000. The evolution of plant ecophysiological traits: recent advances and future directions. *Bioscience* 50: 979–995.
- Baldocchi DD, Harley PC. 1995. Scaling carbon-dioxide and water-vapor exchange from leaf to canopy in a deciduous forest. 2. Model testing and application. *Plant, Cell & Environment* 18: 1157–1173.
- Cavender-Bares J, Kitajima K, Bazzaz FA. 2004. Multiple trait associations in relation to habitat differentiation among 17 floridian oak species. *Ecological Monographs* 74: 635–662.
- Chang DHS, Gauch HG. 1986. Multivariate analysis of plant communities and environmental factors in Ngari, Tibet. *Ecology* 67: 1568–1575.
- Chapin FS III, Autumn K, Pugnaire F. 1993. Evolution of suites of traits in response to environmental stress. *American Naturalist* 142: S78–S92.
- Chapin FS III, Körner C. 1995. *Arctic and alpine biodiversity: patterns, causes and ecosystem consequences*. Heidelberg, Germany: Springer.
- Chown SL, Gaston KJ, Robinson D. 2004. Macroecology: large-scale patterns in physiological traits and their ecological implications. *Functional Ecology* 18: 159–167.
- Cornelissen JHC. 1999. A triangular relationship between leaf size and seed size among woody species: allometry, ontogeny, ecology and taxonomy. *Oecologia* 118: 248–255.
- Ehleringer JR, Field CB (eds). 1993. *Scaling physiological processes: leaf to globe*. San Diego, CA, USA: Academic Press.
- Evans JR. 1989. Photosynthesis and nitrogen relationships in leaves of C<sub>3</sub> plants. *Oecologia* 78: 9–19.
- Falster DS, Warton DI, Wright IJ. 2003. (S) MATR: Standardised major axis tests and routines. <http://www.bio.mq.edu.au/ecology/SMATR/>
- Fang J, Piao S, Tang Z, Peng C, Ji W. 2001. Interannual variability in net primary production and precipitation. *Science* 293: 1723a.
- Field C, Mooney HA. 1986. The photosynthesis–nitrogen relationship in wild plants. In: Givnish TJ, ed. *On the economy of plant form and function*. Cambridge, UK: Cambridge University Press, 25–55.
- Friend AD, Woodward FI. 1990. Evolutionary and ecophysiological responses of mountain plants to the growing environment. *Advances in Ecological Research* 20: 59–124.
- Friend AD, Woodward FI, Switsuer VR. 1989. Field measurements of photosynthesis, stomatal conductance, leaf nitrogen and δ<sup>13</sup>C along altitudinal gradients in Scotland. *Functional Ecology* 3: 117–122.
- Grime JP, Thompson K, Hunt R, Hodgson JG, Cornelissen JHC, Rorison IH, Hendry GAF, Ashenden TW, Askew AP, Band SR, Booth RE, Bossard CC, Campbell BD, Cooper JEL, Davison AW, Gupta PL, Hall W, Hand DW, Hannah MA, Hillier SH, Hodgkinson DJ, Jalili A, Liu Z, Mackey JML, Matthews N, Mowforth MA, Neal AM, Reader RJ, Reiling K, RossFraser W, Spencer RE, Sutton F, Tasker DE, Thorpe PC, Whitehouse J. 1997. Integrated screening validates primary axes of specialization in plants. *Oikos* 79: 259–281.
- Han W, Fang JY, Guo D, Zhang Y. 2005. Leaf nitrogen and phosphorus stoichiometry across 753 terrestrial plant species in China. *New Phytologist* 168: 377–385.
- Harley PC, Baldocchi DD. 1995. Scaling carbon-dioxide and water-vapor exchange from leaf to canopy in a deciduous forest. 1. Leaf model parameterization. *Plant, Cell & Environment* 18: 1146–1156.
- He J-S, Fang JY, Wang ZH, Guo D, Flynn DFB, Geng Z. 2006. Stoichiometry and large-scale patterns of leaf carbon and nitrogen in the grasslands of China. *Oecologia*, in press.
- Hikosaka K. 2004. Interspecific difference in the photosynthesis–nitrogen relationship: patterns, physiological causes, and ecological importance. *Journal of Plant Research* 117: 481–494.
- Hikosaka K, Nagamatsu D, Ishii HS, Hirose T. 2002. Photosynthesis–nitrogen relationships in species at different altitudes on Mount Kinabalu, Malaysia. *Ecological Research* 17: 305–313.
- Hou H-Y. 1982. *Vegetation map of the People's Republic of China (1 : 4M)*. Beijing, China: Chinese Map Publisher.

- Hou H-Y, Chang H-S. 1992. The principal types of montane vegetation belts in China and eco-geographical characteristics. *Braun-Blanquetia* 8: 11–17.
- Ihaka R, Gentleman R. 1996. R: a language for data analysis and graphics. *Journal of Computational and Graphical Statistics* 5: 299–314.
- Kerkhoff AJ, Enquist BJ, Elser JJ, Fagan WF. 2005. Plant allometry, stoichiometry and the temperature-dependence of primary productivity. *Global Ecology and Biogeography* 14: 485–598.
- Körner C. 1989. The nutrient status of plant from high altitudes: a worldwide comparison. *Oecologia* 81: 379–391.
- Körner C. 1999. *Alpine plant life: functional plant ecology of high mountain ecosystems*. Heidelberg, Germany: Springer-Verlag.
- Körner C, Diemer M. 1987. In situ photosynthetic responses to light, temperature and carbon dioxide in herbaceous plants from low and high altitude. *Functional Ecology* 1: 179–194.
- Körner C, Neumayer M, Pelaez Menendez-Riedl S, Smeets-Scheel A. 1989. Functional morphology of mountain plants. *Flora* 182: 353–383.
- Larocque GR. 2002. Coupling a detailed photosynthetic model with foliage distribution and light attenuation functions to compute daily gross photosynthesis in sugar maple (*Acer saccharum* Marsh.) stands. *Ecological Modelling* 148: 213–232.
- Lavore S, Díaz S, Cornelissen JHC, Garnier E, Harrison SP, McIntyre S, Pausas JG, Pérez-Harguindeguy N, Roumet C, Urcelay C. 2006. Plant functional types: are we getting any closer to the Holy Grail? In: Canadell J, Pitelka LF, Pataki D, eds. *Terrestrial ecosystems in a changing world. IGBP Book Series*. Heidelberg: Springer-Verlag, (in press.)
- Li WH, Zhou XM (eds). 1998. [*Ecosystems of the Qinghai-Xizang (Tibetan) Plateau and approaches for their sustainable management.*] Guangzhou, China: Guangdong Science and Technology Press (in Chinese).
- Li-Cor Inc. 2002. *Using the LI-6400 portable photosynthesis system*. Publication Number 9806-122. Lincoln, NB, USA: Li-Cor Biosciences, Inc.
- Loomis RS. 1997. On the utility of nitrogen in leaves. *Proceedings of the National Academy of Sciences, USA* 94: 13378–13379.
- Luo T, Li W, Zhu H. 2002. Estimated biomass and productivity of natural vegetation on the Tibetan Plateau. *Ecological Applications* 12: 980–997.
- McGroddy ME, Daufresne T, Hedin LO. 2004. Scaling of C: N: P stoichiometry in forests worldwide: implications of terrestrial Redfield-type ratios. *Ecology* 85: 2390–2401.
- Molnar P. 1989. The geographic evolution of the Tibetan Plateau. *American Scientist* 77: 350–360.
- Mooney HA, Canadell J, Chapin FSI, Ehleringer JR, Körner C, McMurtrie RE, Parton WJ, Pitelka LF, Schulze E-D. 1999. Ecosystem physiology responses to global change. In: Walker B, Steffen W, Canadell J, Ingram J, eds. *The terrestrial biosphere and global change*. Cambridge, UK: Cambridge University Press, 141–189.
- Niinemets Ü. 2001. Global-scale climatic controls of leaf dry mass per area, density, and thickness in trees and shrubs. *Ecology* 82: 453–469.
- Norby RJ, Luo Y. 2004. Evaluating ecosystem responses to rising atmospheric CO<sub>2</sub> and global warming in a multi-factor world. *New Phytologist* 162: 281–293.
- Parmesan C, Yohe G. 2003. A globally coherent fingerprint of climate change impacts across natural systems. *Nature* 421: 37–42.
- Peterson AG, Ball JT, Luo YQ, Field CB, Reich PB, Curtis PS, Griffin KL, Gunderson CA, Norby RJ, Tissue DT, Forstreuter M, Rey A, Vogel CS. 1999. The photosynthesis–leaf nitrogen relationship at ambient and elevated atmospheric carbon dioxide: a meta-analysis. *Global Change Biology* 5: 331–346.
- Piao SL, Fang JY, Zhou LM, Guo QH, Henderson M, Ji W, Li Y, Tao S. 2003. Interannual variations of monthly and seasonal normalized difference vegetation index (NDVI) in China from 1982 to 1999. *Journal of Geophysical Research* 108(D14): 4401. doi:10.1029/2002JD002848.
- Reich P. 2005. Global biogeography of plant chemistry: filling in the blanks. *New Phytologist* 168: 263–266.
- Reich PB, Oleksyn J. 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. *Proceedings of the National Academy of Sciences, USA* 101: 11001–11006.
- Reich PB, Walters MB, Ellsworth DS. 1997. From tropics to tundra: global convergence in plant functioning. *Proceedings of the National Academy of Sciences, USA* 94: 13730–13734.
- Reich PB, Walters MB, Ellsworth DS, Uhl C. 1994. Photosynthesis–nitrogen relations in Amazonian tree species. 1. Patterns among species and communities. *Oecologia* 97: 72–62.
- Reich PB, Wright IJ, Cavender-Bares J, Craine JM, Oleksyn J, Westoby M, Walters MB. 2003. The evolution of plant functional variation: traits, spectra, and strategies. *International Journal of Plant Sciences* 164: S143–S164.
- Schmid B, Hector A, Huston MA, Inchausti P, Nijs I, Leadley PW, Tilman D. 2002. The design and analysis of biodiversity experiments. In: Loreau M, Naeem S, Inchausti P, eds. *Biodiversity and ecosystem functioning: synthesis and perspectives*. Oxford, UK: Oxford University Press, 61–75.
- Schulze E-D. 1994. Relationships among maximum stomatal conductance, ecosystem surface conductance, carbon assimilation rate and plant nitrogen nutrition: a global ecology scaling exercise. *Annual Review of Ecology and Systematics* 25: 629–660.
- Sterner RW, Elser JJ. 2002. *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton, NJ, USA: Princeton University Press.
- Sun HL, Zheng D (eds). 1998. [*Formation, evolution and development of the Qinghai-Xizang (Tibetan) Plateau.*] Guangzhou, China: Guangdong Science and Technology Press (in Chinese).
- Taiz L, Zeiger E. 1998. *Plant physiology*. Sunderland, MA, USA: Sinauer Associates, Inc.
- Taponnier P, Xu Z, Roger F, Meyer B, Arnaud N, Wittlinger G, Yang J. 2001. Oblique stepwise rise and growth of the Tibet Plateau. *Science* 294: 1671–1677.
- Terashima I, Masuzawa T, Ohba H. 1993. Photosynthetic characteristics of a giant alpine plant, *Rheum nobile* Hook. f. et Thoms. and of some other alpine species measured at 4300 m, in the eastern Himalaya, Nepal. *Oecologia* 95: 194–201.
- Terashima I, Masuzawa T, Ohba H, Yokoi Y. 1995. Is photosynthesis suppressed at higher elevations due to low CO<sub>2</sub> pressure? *Ecology* 76: 2663–2668.
- Vitousek PM, Field CB, Matson PA. 1990. Variation in foliar δ<sup>13</sup>C in Hawaiian *Metrosideros polymorpha*: a case of internal resistance? *Oecologia* 84: 362–370.
- Wang JT. 1988. The steppes and deserts of the Xizang plateau (Tibet). *Vegetatio* 75: 135–142.
- Warton DI, Weber NC. 2002. Common slope tests for bivariate error-in-variables models. *Biometrical Journal* 44: 161–174.
- Westbeek MHM, Pons TL, Cambridge ML, Atkin OK. 1999. Analysis of differences in photosynthetic nitrogen use efficiency of alpine and lowland *Poa* species. *Oecologia* 120: 19–26.
- Westoby M, Falster DS, Moles AT, Vesk PA, Wright IJ. 2002. Plant ecological strategies: some leading dimensions of variation between species. *Annual Review of Ecology and Systematics* 33: 125–159.
- Williams M, Rastetter EB, Fernandes DN, Goulden ML, Shaver GR, Johnson LC. 1997. Predicting gross primary productivity in terrestrial ecosystems. *Ecological Applications* 7: 882–894.
- Wright IJ, Groom PK, Lamont BB, Poot P, Prior LD, Reich PB, Schulze ED, Veneklaas EJ, Westoby M. 2004a. Leaf trait relationships in Australian plant species. *Functional Plant Biology* 31: 551–558.
- Wright IJ, Reich PB, Cornelissen JHC, Falster DS, Garnier E, Hikosaka K, Lamont BB, Lee W, Oleksyn J, Osada N, Poorter H, Villar R, Warton DI, Westoby M. 2005a. Assessing the generality of global leaf trait relationships. *New Phytologist* 166: 485–496.
- Wright IJ, Reich PB, Cornelissen JHC, Falster DS, Groom PK, Hikosaka K, Lee W, Lusk CH, Niinemets Ü, Oleksyn J, Osada N, Poorter H, Warton DI, Westoby M. 2005b. Modulation of leaf economic traits and trait relationships by climate. *Global Ecology and Biogeography* 14: 411–421.
- Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JHC, Diemer M, Flexas J,

- Garnier E, Groom PK, Gulias J, Hikosaka K, Lamont BB, Lee T, Lee WJ, Lusk C, Midgley JJ, Navas M-L, Niinemets U, Oleksyn J, Osada N, Poorter H, Poot P, Prior L, Pyankov VI, Roumet C, Thomas SC, Tjoelker MG, Veneklaas EJ, Villar R. 2004b. The worldwide leaf economics spectrum. *Nature* 428: 821–827.
- Wright IJ, Westoby M. 2002. Leaves at low versus high rainfall: coordination of structure, lifespan and physiology. *New Phytologist* 155: 403–416.
- Wu ZY, ed. 1987. *Flora Xizangica*, Vols 1–5. Beijing, China: Science Press.
- Yin X. 1993. Variation in foliage nitrogen concentration by forest type and chinatic gradients in North America. *Canadian Journal of Forest Research* 23: 1587–1602.
- Zhang J, Wang JT, Chen W, Li B, Zhao K. 1988. *Vegetation of Xizang (Tibet)*. Beijing, China: Science Press.
- Zheng D. 1996. The system of physico-geographical regions of the Qinghai-Xizang (Tibetan) Plateau. *Science in China* 39: 410–417.

## Supplementary Material

The following supplementary material is available for this article online.

**Table S1** Dataset for photosynthetic rate, leaf nitrogen concentration, leaf mass per area and photosynthetic nitrogen use efficiency for each species at the 49 sites on the Tibetan Plateau, measured by JSH, WYZ, MZ and CYZ in 2003 (Department of Ecology, College of Environmental Sciences, Peking University, Beijing, China)

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