

Taxonomic identity, phylogeny, climate and soil fertility as drivers of leaf traits across Chinese grassland biomes

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Abstract Although broad-scale inter-specific patterns of leaf traits are influenced by climate, soil, and taxonomic identity, integrated assessments of these drivers remain rare. Here, we quantify these drivers in a field study of 171 plant species in 174 sites across Chinese grasslands, including the Tibetan Plateau, Inner Mongolia, and Xinjiang. General linear models were used to partition leaf trait variation. Of the total variation in leaf traits, on average 27% is due to taxonomic or phylogenetic differences among species within sites (pure species effect), 29% to variation among sites within species (pure site effect), 38% to joint effects of taxonomic and environmental factors

(shared effect), and 6.2% to within-site and within-species variation. Examining the pure site effect, climate explained 7.8%, soil explained 7.4%, and climate and soil variables together accounted for 11%, leaving 18% of the inter-site variation due to factors other than climate or soil. The results do not support the hypothesis that soil fertility is the “missing link” to explain leaf trait variation unexplained by climatic factors. Climate- and soil-induced leaf adaptations occur mostly among species, and leaf traits vary little within species in Chinese grassland plants, despite strongly varying climate and soil conditions.

Keywords Functional traits · Leaf economics spectrum · Photosynthesis · Soil fertility · Inner Mongolia · Tibetan Plateau

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Introduction

Broad patterns of leaf characteristics are considered to reflect adaptations to variation in the physical environment. Understanding these adaptations is a priority for addressing such fundamental questions as how climate change will influence the world's ecosystems (Garnier et al. 2007; Lavorel et al. 2007). In natural grasslands, cold or dry climate (Walter 1970; Whittaker 1975) together with soil nutrient status (Hooper and Johnson 1999) determines biome boundaries, therefore we would expect leaf traits and trait combinations to vary among the native grassland taxa as temperature, aridity, and soil fertility vary, due to local adaptation to environmental conditions. However, little evidence for these hypothesized relationships has been found.

A number of studies have documented large-scale patterns of, and potential driving forces for plant functional

traits (Castro-Díez et al. 2000; Garnier et al. 1999; He et al. 2009; Niinemets 2001; Reich and Oleksyn 2004; Reich et al. 1997; Shipley and Lechowicz 2000; Wright et al. 2004). These efforts have revealed that (1) regardless of biome, life form, or phylogenetic history, the scaling of bivariate and multiple trait relations are generally similar and predictable (He et al. 2009; Reich and Oleksyn 2004; Reich et al. 1997; Wright et al. 2004); (2) across biomes, species, and research sites, the modulation of leaf traits by climate is surprisingly modest, although some significant patterns can be detected; and (3) differences in key attributes, including life-form, phenology, phylogenetic history, and ecological strategies, are related to variation in leaf traits (Reich et al. 2003).

The potential environmental drivers of leaf trait variation investigated in these broad-scale studies included long-term average temperature, precipitation (Reich and Oleksyn 2004; Reich et al. 1997; Wright et al. 2004), irradiance (Niinemets 2001; Niinemets et al. 1999; Wright et al. 2006), life form variation and species identity (He et al. 2006a, 2008). In some cases, the impact of drought (Niinemets and Valladares 2006), grazing (see review by Díaz et al. 2007), and land-use change (Garnier et al. 2007) on plant traits have also been investigated. A recent study by collecting published studies has quantitatively assessed the relationships between leaf traits and soil nutrient fertility (Ordoñez et al. 2009). However, integrated assessments of these drivers remain rare.

Previous reviews of changes in species traits along soil fertility gradients documented that species from nutrient-rich habitats tend to be fast in resource capture and nutrient turnover, while the reverse is true for species from nutrient-poor habitats (Chapin 1980; Grime 1979). Corresponding to the differences in resource use, species from nutrient-rich habitats have been hypothesized to have a combination of low leaf mass per unit area (LMA), high tissue nutrient concentration [in particular leaf nitrogen (N) and phosphorus (P)], low tissue density, and short leaf lifespan, while species from nutrient-poor habitats have the opposite traits such that the mean residence time of nutrients tends to be maximized through long lifespan or high resorption efficiency of nutrients from senescing organs (Aerts and Chapin 2000; Lavorel et al. 2007; Reich et al. 1991; Westoby et al. 2002). Moreover, examination of species variation within and among sites suggests that variation among species within a site is just as large as the mean differences among sites varying markedly in climate or soils (Wright et al. 2005b). Clearly, separating intra-site and cross-site drivers (biotic and abiotic) of leaf trait variation will be required to understand variation that encompasses both kinds of heterogeneity.

This study includes analyses of the patterns of variation of leaf traits in relation to both broad-scale climatic and

local-scale environmental variation between sites, as well as in relation to inter-specific variation within sites. We further test two contrasting hypotheses: the first hypothesis is that soil fertility can explain a substantial amount of the remaining variation of leaf traits at broad scales after accounting for climatic variables. Consequently, we expected that, with increasing soil fertility as measured by increasing soil organic carbon (SOC), increasing soil total nitrogen (STN) and decreasing bulk density (BD), leaf N, P, photosynthetic rates should increase and LMA decrease among sites. Alternatively, because the formation of soil fertility is strongly associated with climate (Jenny 1941; Schlesinger 1997), a large amount of the explanatory power of soil variables may already be included in climatic variables; thus the second hypothesis is that soil fertility may not explain much of the remaining variation of leaf traits unexplained by climate at the broad scale. These hypotheses were tested using data from a systematic census of 171 species over 174 research sites in the grassland biomes of China.

While comprehensive studies of leaf traits in trees and shrubs have emerged in recent years (McGroddy et al. 2004; Niinemets 2001; Townsend et al. 2007), few synthetic studies of grassland species exist (but see Craine et al. 2005; Tjoelker et al. 2005), particularly in the large natural grassland biomes of Asia. Specifically, we addressed the following questions: (1) How do leaf traits vary along climatic and soil fertility gradients among sites and among taxa? (2) Does soil fertility explain a substantial amount of variation of leaf traits unexplained by climate at broad scale? and (3) How strongly these traits are conserved within species and within phylogenetic lineages?

Materials and methods

Study site, plant species, and measuring procedure

We sampled 171 abundant grassland species at 174 sites in three regions of China: the Tibetan Plateau, the Inner Mongolia Plateau, and Xinjiang (see the three regions in the site map of Fig. S1). The climatic and soil properties of the three regions are presented in Table 1. The leaf trait data from the Tibetan Plateau (He et al. 2006b), data on C:N:P stoichiometry (He et al. 2006a, 2008), and bivariate relationships between leaf productivity (mass-based photosynthetic rate, A_{mass} , N and P concentrations, photosynthetic N-use efficiency, PNUE) and persistence (leaf mass per area, LMA; He et al. 2009) were presented previously.

The 171 species belong to 90 genera and 34 families of vascular plants. We further distinguished three growth forms: grasses (56 species), herbs (78 species) and woody species (37 species). At each site, we selected the dominant

Table 1 Description of the three study regions

	Inner Mongolia	Tibetan Plateau	Xinjiang
No. of sites	42	58	74
No. of sampled species	53	82	49
Latitude (°N)	44.5 (38.4–49.5)	33.5 (28.2–36.3)	43.7 (36.8–48.8)
Longitude (°E)	115.1 (107.3–120.1)	97.5 (86.8–102.9)	84.0 (74.9–93.7)
Altitude (m)	1060 (575–1527)	4019 (2934–5249)	1786 (733–3812)
Climatic variables			
MAP (mm year ⁻¹)	320 (184–427)	468 (239–624)	242 (68–418)
GSP (mm year ⁻¹)	243 (133–316)	314 (184–383)	157 (43–311)
MAT (°C)	2.1 (–2.5–7.9)	–1.2 (–9.7– 7.0)	2.3 (–6.3–12.0)
GST (°C)	17.0 (14.8–20.4)	7.2 (–1.0–13.6)	14.6 (3.8–23.9)
AET (mm)	320 (184–427)	361 (239–436)	241 (68–372)
PET (mm)	546 (478–644)	370 (272–528)	521 (344–804)
VPD (kPa)	0.46 (0.28–0.68)	0.25 (0.12–0.55)	0.46 (0.19–0.94)
Ir (MJ m ⁻² year ⁻¹)	5497 (4998–6202)	6131 (5067–7847)	5522 (5103–6157)
Soil variables			
BD (0–10 cm, g/cm ³)	1.44 (0.90–1.74)	0.82 (0.35–1.44)	0.99 (0.39–1.47)
BD (10–20 cm, g/cm ³)	1.46 (1.09–1.72)	1.02 (0.37–1.43)	0.99 (0.61–1.54)
SOC (0–10 cm, %)	1.13 (0.28–3.33)	7.05 (0.30–17.36)	3.81 (0.09–16.45)
SOC (10–20 cm, %)	0.86 (0.23–2.52)	4.20 (0.43–15.64)	2.7 (0.24–7.76)
STN (0–10 cm, %)	0.13 (0.03–0.31)	0.67 (0.06–1.50)	0.39 (0.02–1.75)
STN (10–20 cm, %)	0.10 (0.04–0.25)	0.42 (0.06–1.24)	0.29 (0.00–0.83)

The ranges of environmental variables across study sites are given in parentheses
MAP Mean annual precipitation, *GSP* growing season precipitation, *MAT* mean annual temperature, *GST* growing season temperature, *AET* actual evapotranspiration, *PET* potential evapotranspiration, *VPD* vapor pressure deficit, *Ir* total irradiance, *BD* bulk density, *SOC* soil organic carbon content, *STN* soil total nitrogen content

species after surveying the entire plant community. As a consequence, the number of species investigated per site varied from 1 (species-poor grasslands with strong dominance structure) to 12 species. Each species could occur at a number of sites, ranging from 1 to 13. Defining the occurrence of a particular species at a particular site as a population, the dataset contained 429 populations (Table S1 for original data table).

The seven leaf traits *LMA*, A_{mass} , A_{area} , N_{mass} , N_{area} , P_{mass} and P_{area} were measured in late July and early August of 2003 and 2004. Descriptions of the sampling protocol and leaf trait measurements were detailed elsewhere (He et al. 2006b, 2008). In brief, in situ photosynthetic rates of current season leaves were measured in the mornings of clear days using red-blue light sources and CO₂ mixers (LI-6400, Li-Cor, Lincoln, NE). The reference CO₂ concentration in the leaf cuvette was maintained at 360 μmol CO₂ mol⁻¹, and leaf cuvette temperature was maintained at 22–25°C, depending on the external temperature. Newly mature leaves of five to ten plants of each species were collected to conduct chemical analyses. Leaf N concentration was assayed using an elemental analyzer (2400 II CHN Elemental Analyzer, Perkin-Elmer, Boston, MA), total P concentration was measured by a molybdate/stannous chloride method (Kuo 1996) after H₂SO₄–H₂O₂–HF digestion (Bowman 1988), and *LMA* was determined by dividing oven-dried (60°C) leaf mass by the corresponding

leaf area measured in the field with a portable leaf-area meter (AM200; ADC Bioscientific, Herts, UK).

We surveyed the soil at 163 of the 174 sites. Sampling procedures and measurement methods for soil bulk density (BD), soil total N (STN) and soil organic carbon (SOC) have been described previously (Yang et al. 2008). BD, STN, and SOC from 0–10 to 10–20 cm depths were used as measurements of soil fertility in the current study. Climatic variables, including mean annual temperature (MAT), mean growing season temperature (from May to August, GST), mean annual precipitation (MAP), and mean growing season precipitation (GSP) were calculated using 50-year temperature and precipitation data (1951–2000) at 680 well-distributed climate stations across China (Climate Database, National Meteorological Bureau of China). Potential and actual evapotranspiration values (PET and AET) were calculated from the monthly climate data according to Thornthwaite (1948), and monthly mean vapor pressure deficit (VPD) was estimated as the difference between the saturation vapor pressure of air and vapor pressure (Campbell and Norman 1998). Solar radiation data were generated from 98 solar radiation observation stations across the country (Piao et al. 2006).

Climate and soil variables were cross-correlated (Table 2). Across 174 sites, AET, PET and VPD were closely associated with GST and GSP. Irradiance, however, was weakly associated with other variables.

Table 2 Correlation matrix for climatic and soil variables

	GST	GSP	AET	PET	VPD	Ir	BD	SOC	STN
GST									
GSP	−0.759								
AET	−0.555	0.867							
PET	0.985	−0.790	−0.607						
VPD	0.887	−0.849	−0.740	0.920					
Ir	−0.517	0.338	0.086	−0.489	−0.255				
BD	0.712	−0.460	−0.323	0.678	0.637	−0.254			
SOC	−0.706	0.552	0.346	−0.689	−0.638	0.309	−0.842		
STN	−0.682	0.544	0.357	−0.668	−0.622	0.298	−0.833	0.981	

Abbreviations as in Table 1. All pair-wise correlations are significant at $P \leq 0.05$ except that between AET and Ir

Data analysis

We used correlation analysis to examine the relationships between leaf traits and climatic and soil variables, and general linear models (GLMs) to summarize the results in analysis of variance (ANOVA) (Schmid et al. 2002). To analyze inter-specific differences in a hierarchical fashion, we used (1) the taxonomic designations to group species into genera and families, and (2) the phylogenetic relationships between species.

The phylogenetic tree was developed with the program Phylomatic (Webb et al. 2008), using a maximally resolved seed plant tree based on the supertree of the Angiosperm Phylogeny Group (Angiosperm Phylogeny Group 2003), with additional detail added for some groups by Stevens (2008). Detailed information was presented in He et al. (2009). The resulting phylogeny, which included all 171 study species, was used to create “phylogenetic groups”, with a first-order group cut at 25 million years before present, and a second-order group cut at 21 million years before present. We chose these ages because they resulted in roughly the same number of groups as the families and genera used in the taxonomic analysis (35 and 88 phylogenetic groups, vs 34 families and 90 genera), allowing us to directly compare the effect of using taxonomic versus phylogenetic groupings to summarize inter-specific differences in our GLM framework. We did not use the phylogenetic distance to the youngest common ancestor as a quantitative variable because, in this case, it is not comparable to the species’ taxonomic identities.

Leaf-trait variation can be partitioned into the following explanatory components: (1) environmental variation among sites within species; (2) inter-specific variation among species within sites; (3) shared variation among sites among species (which cannot be partitioned further because of the correlation between sites and species, i.e., changing species occurrences with changing environmental conditions across sites); and (4) residual variation (for details see He et al. 2009). Variation among sites (1) could

be further partitioned into contrasts for climatic variables, soil variables and remainder, and variation among species (2) could be further partitioned either into taxonomic (family/genus/species) or into phylogenetic (first-/second-order group cut/species) strata. Different fitting sequences were used to test how much variation a term explained if other terms were partialled out (fitted before the term of interest, type-II analysis in SAS terminology, SAS Institute 1999). For example, the fitting sequence “environmental factors → inter-specific factors” allowed us to measure how much variation in leaf traits could be explained by climatic and soil variables among and within species together. With the opposite fitting sequence we could assess how much variation these environmental variables could explain within species only. In sequential analysis, the shared component (3) is combined with either (1) or (2) depending on the sequence of sites and species in the analysis (see analogous procedure for sum of squares in Borcard et al. 1992; Schmid et al. 2002).

By fitting climatic and soil variables in alternative sequences within the site term, we could estimate how much variation they explained in common, much in the same way as shown above for the shared component (3). First, we entered the climatic variables (GST, GSP, AET, and VPD) as a group and then the soil variables as group (BD, SOC, STN of the 0–10 cm soil layer, which was highly correlated with that of the 10–20 cm soil layer) into the model. These variables had been selected after inspection of the correlation matrix between climatic variables, soil variables and leaf traits (Table 2). Then we reversed this sequence and first entered the soil variables as a group and then the climatic variables as a group into the model. Using sequential fitting of terms in GLMs yields for each term a contribution to the total sum of squares. We did not include interactions between environmental and inter-specific factors in our final analysis because they only explained a very small amount of variation. All statistical analyses were calculated with the software product R (R Development Core Team 2007).

Results

Enormous variation in plant traits was observed, with each trait varying across one to two orders of magnitude across all sites and species (Table 3). In line with previous results (He et al. 2006b; Wright et al. 2005a), herbs had lower LMA and higher A_{mass} and P_{mass} than grasses and woody species, and legumes had higher N_{area} and N_{mass} than non-legumes (Table 3).

Leaf mass per area was positively correlated with each of the climate and soil variables GST, PET, VPD and BD, and negatively correlated with GSP, AET, SOC and STN (Table 4). A_{mass} was positively correlated with GSP, AET, SOC, and STN and negatively correlated with VPD and BD (Fig. 1). Among mass-based traits P_{mass} and among area-based traits, A_{area} showed the weakest association with climatic variables. Correlations between N_{mass} , A_{area} and soil variables were not significant. Among the six climatic variables, irradiance showed the weakest relationship with each of the leaf traits.

The climatic variables GST, GSP, AET and VPD as a group had a significant influence on all leaf traits except

P_{mass} (Table 5), explaining 2.0–13.7% of total variation when entered first into the model. The soil variables BD, SOC and STN as a group had a significant influence on all leaf traits except P_{mass} , and A_{area} , explaining 1.3–14.0% of total variation when entered first into the model. Climatic and soil variables together explained 3.4–16.4% of total variation (Table 5), much less than the sum of climatic variables and soil variables analyzed separately, due to the correlation between climatic and soil variables. Remaining site effects (partialling out climate and soil fertility) explained about half of total variation (52.5–60.0%) of leaf traits, by far the largest proportion.

Investigating the influence of inter-specific differences after accounting for all of the environmental factors, N_{mass} , P_{mass} , and P_{area} differed significantly among first-order phylogenetic groups, only A_{mass} differed among second-order groups, and five traits (LMA, N_{mass} , N_{area} , P_{mass} and P_{area}) differed among species within these phylogenetic groups (Table 5). When using taxonomic groupings instead of phylogenetic ones, four leaf traits (A_{area} , N_{mass} , P_{mass} , and P_{area}) varied significantly among families, three (LMA,

Table 3 Leaf traits for two plant functional groupings and regions

		LMA (g m ⁻²)	A_{area} ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	A_{mass} ($\mu\text{mol g}^{-1} \text{s}^{-1}$)	N_{area} (g m ⁻²)	N_{mass} (mg g ⁻¹)	P_{area} (g m ⁻²)	P_{mass} (mg g ⁻¹)
Overall	Mean	93.78 (±4.69)	13.71 (±0.64)	0.17 (±0.01)	2.34 (±0.10)	26.52 (±0.81)	0.16 (±0.01)	1.87 (±0.08)
	Median	82.06	13.20	0.16	2.11	25.16	0.14	1.72
Growth form								
Grass	Mean	101.03 (±7.88) ^a	12.62 (±0.92) ^a	0.16 (±0.01) ^a	2.28 (±0.16) ^a	23.74 (±0.88) ^a	0.15 (±0.01) ^a	1.63 (±0.10) ^a
	Median	85.46	12.12	0.14	1.97	23.06	0.13	1.55
Herb	Mean	79.24 (±5.54) ^b	15.32 (±1.17) ^b	0.21 (±0.02) ^b	2.26 (±0.13) ^a	30.07 (±1.65) ^b	0.17 (±0.01) ^a	2.26 (±0.16) ^b
	Median	69.05	15.24	0.19	2.13	28.37	0.15	2.11
Woody	Mean	99.33 (±9.78) ^a	13.68 (±1.28) ^{ab}	0.16 (±0.02) ^a	2.63 (±0.24) ^b	27.62 (±1.82) ^b	0.17 (±0.02) ^a	1.81 (±0.12) ^a
	Median	86.96	12.90	0.14	2.31	25.34	0.16	1.77
Legume								
No	Mean	94.09 (±5.10) ^a	13.57 (±0.66) ^a	0.17 (±0.01) ^a	2.25 (±0.11) ^a	25.35 (±0.79) ^a	0.16 (±0.01) ^a	1.86 (±0.09) ^a
	Median	81.88	12.86	0.16	2.06	24.10	0.14	1.70
Yes	Mean	91.61 (±11.93) ^a	14.76 (±2.19) ^a	0.19 (±0.03) ^a	2.97 (±0.29) ^b	35.16 (±2.34) ^b	0.16 (±0.02) ^a	1.93 (±0.17) ^a
	Median	84.45	14.42	0.15	2.87	34.83	0.15	1.79
Region								
Inner Mongolia	Mean	96.02 (±6.32) ^a	14.60 (±1.53) ^a	0.17 (±0.02) ^a	2.56 (±0.17) ^a	27.12 (±1.47) ^a	0.17 (±0.01) ^a	1.80 (±0.12) ^a
	Median	88.65	14.73	0.17	2.42	26.12	0.15	1.69
Tibetan Plateau	Mean	81.08 (±4.27) ^b	13.41 (±0.84) ^a	0.17 (±0.01) ^a	2.13 (±0.10) ^b	27.48 (±1.14) ^a	0.15 (±0.01) ^b	1.85 (±0.11) ^a
	Median	74.39	12.49	0.16	2.03	25.85	0.13	1.73
Xinjiang	Mean	112.20 (±14.03) ^a	13.29 (±1.06) ^a	0.18 (±0.03) ^a	2.45 (±0.28) ^{ab}	24.37 (±1.69) ^b	0.19 (±0.02) ^a	1.98 (±0.19) ^a
	Median	85.42	12.57	0.15	1.91	22.40	0.15	1.72

Mean (±95% confidence interval) and medians are shown. Mean with different letters in superscripts are significantly different at the 5% significance level (Tukey’s post hoc test) within the same group

Table 4 Correlations between leaf traits and environmental variables

	LMA	A_{mass}	N_{mass}	P_{mass}	A_{area}	N_{area}	P_{area}
Climatic variable							
GST	0.279****	-0.091*	-0.101**	-0.085*	0.080	0.231****	0.193****
GSP	-0.278****	0.185****	0.215****	0.070	0.037	-0.128***	-0.213****
PET	0.278****	-0.097*	-0.110**	-0.076	0.079	0.223****	0.198****
AET	-0.227****	0.269****	0.186****	0.050	0.165****	-0.098**	-0.187****
Irradiance	-0.098**	-0.035	0.087*	-0.051	-0.111**	-0.035	-0.094*
VPD	0.354****	-0.167***	-0.145***	-0.109**	0.052	0.281****	0.265****
Soil variable							
BD	0.242****	-0.120**	0.000	-0.13***	0.016	0.281****	0.147***
SOC	-0.328****	0.205****	0.003	0.121**	0.018	-0.362****	-0.235****
STN	-0.352****	0.255****	0.065	0.152***	0.047	-0.34****	-0.234****

Abbreviations as in Table 1. Leaf traits, SOC and STN were log-10 transformed prior to analysis

**** $P \leq 0.001$, *** $P \leq 0.01$, ** $P \leq 0.05$, * $P \leq 0.1$

N_{mass} , P_{mass}) among genera within families and four (LMA, N_{mass} , N_{area} , P_{area}) among species within families and genera (Table 5).

Considering all leaf traits together, among-family variation explained 7.3–16.0%, variation among genera within families explained 6.7–11.7% and variation among species within families and genera explained 5.4–10.4%. Similar results were found using phylogenetic groups: first-order phylogenetic groups explained 6.6–15.8% of total variation in individual leaf traits, second-order groups 6.1–10.0% and species-level differences 6.2–10.5% (Table 5). The percent of variance explained by the first- and second-order groupings was essentially identical to that explained by families and genera.

By switching the order of entering site and species factors into the GLMs (see [Materials and methods](#)), we partitioned the leaf trait variation into four components (Fig. 2). The pure effects of site (i.e., environmental variation among sites within species) accounted for 29% (from 24.1 to 35.2%) of variation in leaf traits, and were generally stronger for area- than for mass-based traits (e.g. 35.2% for A_{area} vs 26.6% for A_{mass}). The pure effects of species (i.e., taxonomic or phylogenetic variation among species within sites) accounted for 27% (from 22.7 to 33.8%) of variation, and tended to be stronger for mass-based traits than area-based ones. Except for A_{area} and P_{area} , the shared effect of species and site explained the highest proportion of variation, 38% (from 31.8 to 45.2%), suggesting that the species turnover with environmental factors is a major factor affecting leaf traits. In contrast, the residual variation was small for all leaf traits, 6.2% (from 4.2 to 9.7%), indicating that variation within sites and species had only a weak influence on patterns of variation in leaf traits.

Discussion

Effects of climate and soil on leaf trait variation among sites

Our study revealed that, on average, climatic variables alone explained 7.8%, soil variables alone explained 7.4%, and variation among sites (including variation due to climate and soil and shared variation among sites and among species) explained 67% of the total variation in individual leaf traits. Together, climatic and soil variables explained 11%, indicating that $7.8\% + 7.4\% - 11\% = 4.2\%$ of this variation was shared variation due to climate or soil. Given that in previous studies (and also in this study) much variation among sites was unexplained by broad-scale climatic variations (Wright et al. 2005b), we had hypothesized that soil fertility could be the “missing link” explaining a large part of the unexplained variation. However, despite the large variation in soil fertility among sites (including all natural grassland types of China), the effect of soil was relatively weak, explaining maximally 7.4% of the variation in leaf traits (or minimally $7.4\% - 4.2\% = 3.2\%$, if all shared variation from above were assigned to climate factors). Therefore, our results do not support our first hypothesis, but do support the second hypothesis that soil fertility does not explain much of the remaining variation of leaf traits unexplained by climate at the broad scale.

In general, our results are consistent with previous findings that the influence of climate on broad-scale patterns of variation in leaf traits was quite modest (Wright et al. 2004). However, it must be noted that our dataset focuses on the natural grassland biomes across China and thus covers a much narrower range of climatic variation

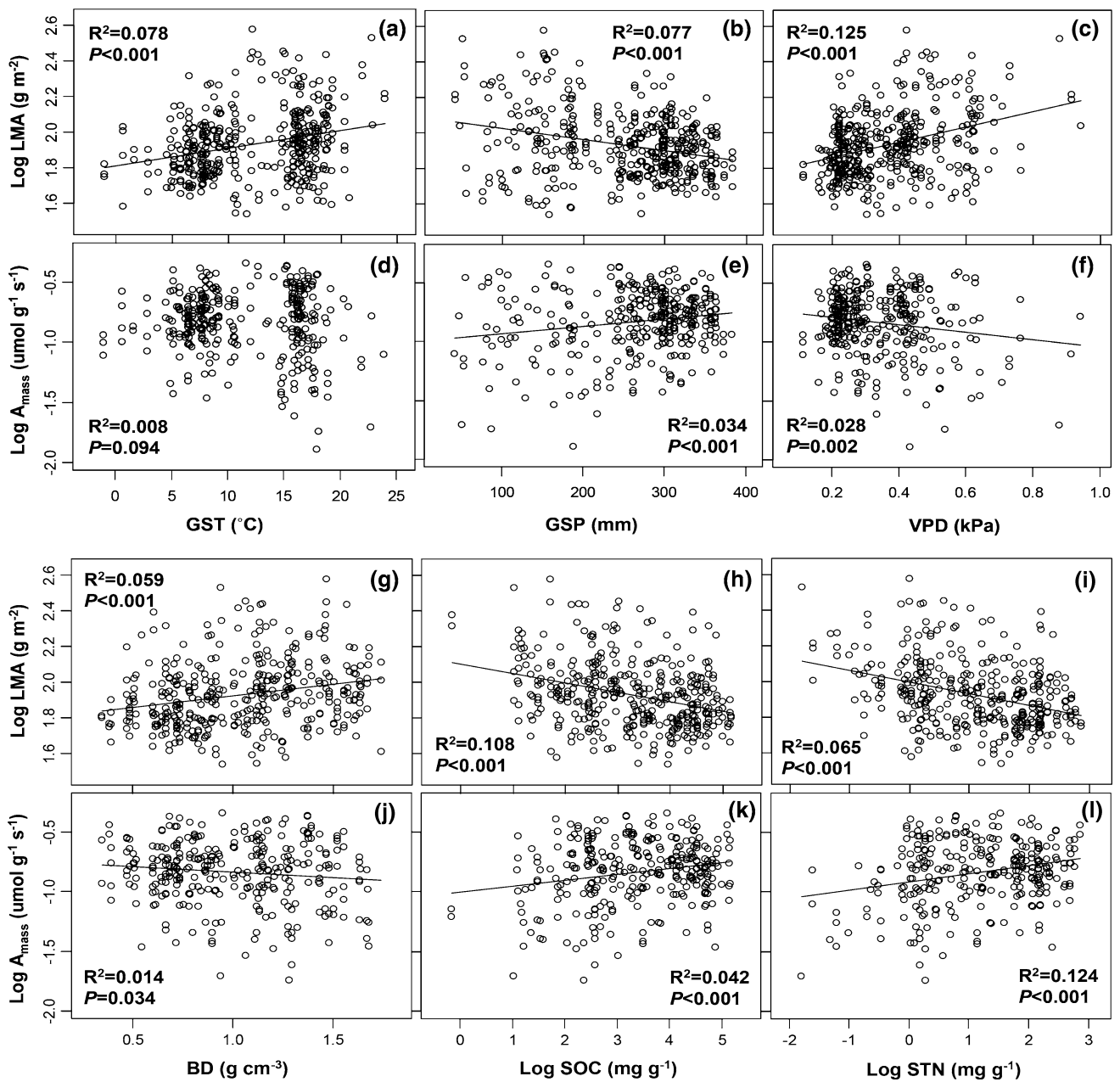


Fig. 1 Relationships between leaf mass per area (LMA) and mass-based photosynthesis (A_{mass}) with each of growing season temperature (GST), growing season precipitation (GSP), vapor pressure deficit (VPD), soil bulk density (BD), soil organic carbon (SOC), and

soil total nitrogen (STN). LMA and A_{mass} are mean for each site (species \times site mean). Regression lines are shown only for relationships that were significant at $P \leq 0.05$

(MAT = -9.7 – 12.0°C ; MAP = 68 – 624 mm year^{-1}) than studies assembling data from several major ecosystem types (e.g., MAT = -16.5 – 27.5°C ; MAP = 133 – $5300 \text{ mm year}^{-1}$ in Wright et al. 2004). In this respect, we consider the modest but highly significant influence of the climatic variables on leaf traits in our study to be biologically relevant. While previous studies had the advantage of large climatic gradients, they had the disadvantage of synthesizing data across widely contrasting biomes and ecosystems, masking potential variation within biomes. Despite

the differences between our study and previous syntheses, the same general pattern emerged here for grasslands, where the large variation among biomes was not a potential confounding factor: LMA increased with increasing temperature and VPD, and decreased with increasing precipitation; while N_{mass} decreased with increasing temperature and VPD. Thus, the present study demonstrates that, even within a single biome, at a regional scale climate leads to the same adaptations in leaf traits as have been found among biomes at a global scale.

Table 5 Summary of general linear models for the effects of environmental variation (climatic variables: GST, GSP, AET and VPD as a group, soil variables: BD, SOC, and STN as a group; site) and taxonomic variation (family; genus; species) or phylogeny (first and second order group; species) on individual leaf traits

	LMA			A_{mass}			A_{area}			N_{mass}		
	<i>df</i>	%SS	<i>P</i>	<i>df</i>	%SS	<i>P</i>	<i>df</i>	%SS	<i>P</i>	<i>df</i>	%SS	<i>P</i>
Climate entered first												
Climate	4	13.69	0.000	4	7.90	0.003	4	4.80	0.049	4	6.22	0.003
Soil	3	2.73	0.059	3	5.81	0.009	3	2.58	0.157	3	3.58	0.025
Soil entered first												
Soil	3	14.04	0.000	3	9.03	0.001	3	1.28	0.457	3	3.31	0.034
Climate	4	2.37	0.164	4	4.68	0.049	4	6.10	0.017	4	6.49	0.002
Site	148	53.09	0.000	122	58.04	0.000	123	60.00	0.000	149	55.54	0.000
Taxonomy												
Family	29	7.38	0.373	25	7.25	0.172	25	7.86	0.041	29	15.94	0.000
Genus	45	10.34	0.048	40	8.33	0.165	42	7.22	0.466	51	9.03	0.005
Species	59	8.54	0.000	46	7.12	0.085	47	7.89	0.509	61	5.37	0.011
Phylogeny												
First order group	30	8.16	0.136	28	6.61	0.543	28	7.41	0.268	30	15.77	0.000
Second order group	44	8.35	0.307	40	9.88	0.043	42	9.05	0.110	47	6.06	0.540
Species	59	9.75	0.000	43	6.21	0.132	44	6.51	0.677	64	8.52	0.000
Residuals	80	4.24		53	5.55		57	9.65		84	4.32	
	N_{area}			P_{mass}			P_{area}					
	<i>df</i>	%SS	<i>P</i>	<i>df</i>	%SS	<i>P</i>	<i>df</i>	%SS	<i>P</i>	<i>df</i>	%SS	<i>P</i>
Climate entered first												
Climate	4	10.15	0.000	4	1.99	0.259	4	9.75	0.000			
Soil	3	6.20	0.001	3	1.37	0.303	3	1.17	0.352			
Soil entered first												
Soil	3	13.76	0.000	3	2.78	0.063	3	7.46	0.000			
Climate	4	2.58	0.146	4	0.58	0.814	4	3.45	0.050			
Site	148	55.26	0.000	150	55.92	0.000	148	52.45	0.000			
Taxonomy												
Family	29	8.02	0.062	29	15.51	0.004	29	11.53	0.002			
Genus	45	7.50	0.222	51	11.68	0.002	45	6.70	0.716			
Species	59	7.96	0.001	63	6.63	0.145	59	10.36	0.010			
Phylogeny												
First order group	30	8.86	0.057	30	14.44	0.002	30	11.44	0.003			
Second order group	44	7.72	0.073	48	9.03	0.262	44	6.69	0.701			
Species	59	6.90	0.004	65	10.34	0.002	59	10.47	0.009			
Residuals	80	4.92		84	6.90		80	8.04				

For the taxonomic variation, “Species” was nested within “Genus”, and “Genus” was nested within “family”, while for the phylogenetic variation, “Species” was nested into “second order group”, and “second order group” was nested within “first order group”. Explanatory terms are listed in the order of their entry into the models. Leaf traits, SOC and STN were log transformed prior to analysis. Abbreviations as in Table 1

Df Degree of freedom, %SS percentage of sum of squares explained

It should be noted, however, that the effects of climate in this study and in previous syntheses were assessed using long-term average climatic data. They might thus not have picked up more subtle non-genetic variation in leaf traits due to the particular climatic conditions of the year in

which leaves were measured. The influence of such small-scale climatic variation on leaf trait variation may partly overlay and obscure the influence of larger-scale variation.

In the present study, we did find one marked difference from previous studies. Irradiance has been found to be a

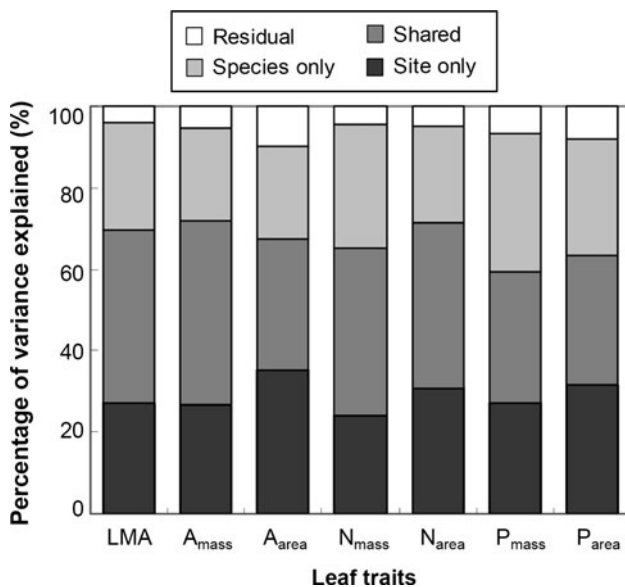


Fig. 2 Effects of species and site on leaf traits, expressed as percentage of variance explained. The variance was partitioned into four components by switching the order of entering site and species factors into the general linear models: *Site only* variance resulting from environmental variation among site within species; *Species only* variance resulting from inter-specific variation within sites; *Shared* shared variance between site and species; *Residual* residual variance within sites and within species

strong climatic driver of leaf trait variation in several recent broad-scale studies (Niinemets 2001; Wright et al. 2004, 2005b, 2006). In this study, however, we found irradiance to be the weakest predictor influencing leaf traits among the six climatic variables. The reason for this difference is probably related to the present focus on grassland vegetation, which in comparison with taller woody vegetation is limited by low precipitation or low temperature while canopy solar radiation is generally high. Nevertheless, the present study is consistent with a recent synthesis in which irradiance had no effect on leaf traits such as LMA (Ordoñez et al. 2009).

Among the average 29% of variation in leaf traits explained by pure site effect (Fig. 2), about 18% (excluding 11% due to climate and soil) is due to inter-site variation other than climate or soil, at least to the extent to which these were comprehensively measured with our climatic and soil variables. This provides an interesting starting point for future research to explore the importance of further among-site differences such as levels of disturbance, type of land-use, degree of herbivory, or intensity of competition—all of which were not quantified in the present study—in explaining leaf trait variation. Synthesis of all these factors would be necessary for a better understanding of broad-scale patterns of plant traits.

Variations in leaf traits among taxonomic groups within site

Even under the same environmental conditions, there are large differences in leaf traits among coexisting taxa (Townsend et al. 2007). The reason for these differences could be (1) phylogenetic differences, i.e., different taxa have evolved in different past environments, (2) related to differences in the “architecture” of different taxa, e.g., differences in plant size (Hirose and Werger 1994), plant canopy architecture (Schmid and Bazzaz 1994), plant phenology (Hikosaka 2004), or (3) “character displacement” (Slatkin 1980). These three forces are not mutually exclusive and likely combine to result in the observed patterns. We tested how such inter-specific differences can explain the variation in the leaf traits studied here using both taxonomic and phylogenetic groupings of species. In all of the analyses, the results from the taxonomic and phylogenetic groupings were similar, indicating that, to a large degree, the taxonomic groupings here represent phylogenetic history quite well.

After accounting for inter-site differences in leaf traits, approximately an additional 11% of the total variation was explained by families, which may reflect a combination of reasons (1)–(3) above. Taxonomic variation among genera within families accounted for 8.7%, and among species within genera accounted for 7.7%. The lower the taxonomic unit, the more likely it will be reason (3), i.e., character displacement from competitive interactions among species sharing the same environment. To our knowledge, this is the first time that differences between taxonomic groups in leaf traits unexplained by environmental factors have been quantified in such detail. It is also interesting to note that the differences among second order group (approximately at genus level), which presumably have evolved within only $25 - 21 = 4$ million years, are so influential in terms of leaf-trait variation.

Shared variation among sites and among species: effect of species shift with sites

Much of our knowledge regarding the patterns of plant traits has been derived from field observations along environmental gradients (see review in Lavorel et al. 2007; Luo et al. 2005), which are often associated with shifts in species composition. In the current study, after taking into account the taxonomic or phylogenetic differences among species within site (27% of the total variation, pure species effect in Fig. 2), including family, genus, and species identities, we find that about 38% of the total variation in leaf traits was jointly explained by taxonomic and environmental factors, i.e., the effect of species shift with sites. This is by far the largest proportion of the variation in leaf traits.

It should be noted that in our and other previous analyses, shared and pure effects of climate (and other inter-site variation) and species were not separated (He et al. 2006a, 2008; Reich and Oleksyn 2004; Wright et al. 2005b). Thus, it was not clear from these analyses how much of the correlation between climatic variables and leaf traits was due to climate-induced species shifts among sites and how much to differences among populations of the same species occurring at different sites. For a better understanding of the drivers, it is of importance to distinguish the different components shaping leaf trait patterns. The ideal analysis would be possible only if all species were transplanted to all sites.

The shared variation contains differences among species occurring at different sites, which can be correlated with climate. In this case it could be that the species occurring at the different sites have evolved under the different climatic conditions. However, in the shared component only a small proportion is related to climatic variation. That is probably due to (1) the current climatic variation has not existed for long enough to lead to “deep” evolution, and (2) migration of species was insufficient to allow them to relocate to those sites that are similar in climate to those in the past when these species evolved. Regarding the latter reason, new evidence was presented recently by Crisp et al. (2009), who found strong support for phylogenetic biome conservatism because of limited biomes shifts. Obviously, even ecologically important leaf traits, for which we might expect rapid evolution due to strong selection pressure, show relatively strong niche conservatism within phylogenetic lineages.

In conclusion, our results indicate that taxonomic factors, including differential selection in past environments, fundamental plant architectural differences, and character displacement [reasons (1)–(3) mentioned above] explain at least as much leaf-trait variation in Chinese grassland biomes as do environmental factors. In addition, the direct effects of climate and soil fertility are of minor importance compared to other inter-site differences in explaining leaf trait variation. Considering the magnitude of phylogenetic and functional differences between coexisting grassland species, it may be not so surprising that leaf adaptations to climate and soil fertility within species are less significant than the forces determining the distribution and abundances of species across sites.

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