

## The acquisitive–conservative axis of leaf trait variation emerges even in homogeneous environments

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- **Background and Aims** The acquisitive–conservative axis of plant ecological strategies results in a pattern of leaf trait covariation that captures the balance between leaf construction costs and plant growth potential. Studies evaluating trait covariation within species are scarcer, and have mostly dealt with variation in response to environmental gradients. Little work has been published on intraspecific patterns of leaf trait covariation in the absence of strong environmental variation.
- **Methods** We analysed covariation of four leaf functional traits [specific leaf area (SLA) leaf dry matter content (LDMC), force to tear ( $F_t$ ) and leaf nitrogen content ( $N_m$ )] in six Poaceae and four Fabaceae species common in the dry Chaco forest of Central Argentina, growing in the field and in a common garden. We compared intraspecific covariation patterns (slopes, correlation and effect size) of leaf functional traits with global interspecific covariation patterns. Additionally, we checked for possible climatic and edaphic factors that could affect the intraspecific covariation pattern.
- **Key Results** We found negative correlations for the LDMC–SLA,  $F_t$ –SLA, LDMC– $N_m$  and  $F_t$ – $N_m$  trait pairs. This intraspecific covariation pattern found both in the field and in the common garden and not explained by climatic or edaphic variation in the field follows the expected acquisitive–conservative axis. At the same time, we found quantitative differences in slopes among different species, and between these intraspecific patterns and the interspecific ones. Many of these differences seem to be idiosyncratic, but some appear consistent among species (e.g. all the intraspecific LDMC–SLA and LDMC– $N_m$  slopes tend to be shallower than the global pattern).
- **Conclusions** Our study indicates that the acquisitive–conservative leaf functional trait covariation pattern occurs at the intraspecific level even in the absence of relevant environmental variation in the field. This suggests a high degree of variation–covariation in leaf functional traits not driven by environmental variables.

**Key words:** Leaf functional traits, acquisitive syndrome, conservative syndrome, leaf economics spectrum, intraspecific trait variation, common garden experiment, Fabaceae, Poaceae.

### INTRODUCTION

Leaf functional traits mediate plant responses to environmental conditions and, in turn, influence plant effects on ecosystem properties (Reich *et al.*, 1997; Cornelissen *et al.*, 1999; Díaz *et al.*, 2004; Wright *et al.*, 2004; Shipley *et al.*, 2006b; Violle *et al.*, 2007; Cornwell *et al.*, 2008). Leaf traits related to resource use strategy have been found to consistently correlate with each other. Most of the variation in such traits can be characterized by a single acquisitive–conservative axis. The position of any plant species on this axis is related to its resource

use strategy (Wright *et al.*, 2004) and describes a gradient from ‘fast’ or ‘acquisitive’ species – species with a fast recovery of foliar investment and fast turnover of matter and energy – to ‘slow’ or ‘conservative’ species with traits associated with slow return on investment. This gradient is manifested as a negative correlation between traits related to high net assimilation rate per unit of leaf mass on the one hand, and traits related to long leaf life span on the other. Specific leaf area (SLA =  $1/LMA$ , where LMA is the leaf dry mass per unit leaf area) and leaf nitrogen content per leaf mass ( $N_m$ ) are intimately (and

positively) related to the net assimilation rate both empirically (Wright *et al.*, 2004, 2005a) and theoretically (Shipley *et al.*, 2006a; Onoda *et al.*, 2017). On the other hand, leaf mechanical resistance (to herbivores, wind and other sources of physical damage, often measured as the force to tear;  $F_t$ ) and leaf dry matter content (LDMC) are positively related to leaf life span. Leaf dry matter content is a proxy for the cell cytoplasm ( $V_c$ ) to cell wall volume ( $V_w$ ) ratio ( $V_c/V_w \sim W_m$ ;  $LDMC^{-1} = 1 - W_m$ , where  $W_m$  is the mass of water per dry leaf mass), which could be a key variable explaining covariation patterns among leaf functional traits (Shipley *et al.*, 2006a). A low cytoplasm to cell wall volume ratio (i.e. high LDMC) is associated with well-defended, long-lived leaves, whereas a high ratio (i.e. low LDMC) enables the high metabolic activity in ‘fast’ species. Mechanical resistance is considered one of the main causes of leaf life span variability (Wright *et al.*, 2004). Leaf thickness, fibre content and density affect leaf mechanical resistance, which enhances leaf life span in the face of herbivory and physical damage (Onoda *et al.*, 2011; Kitajima *et al.*, 2012). Leaf mechanical resistance depends on leaf thickness, density and the specific toughness per unit of density. Therefore leaf mechanical resistance is expected to be correlated with SLA (negatively) and LDMC (positively). However, because most of the variability in leaf mechanical resistance (55–74 %) depends on specific toughness per unit of density (Onoda *et al.*, 2011), an important degree of independence remains between leaf mechanical resistance and SLA or LDMC (see also Grubb, 2016 for a discussion of departures from these general trends among leaf functional traits).

All the proposed causal hypotheses for the acquisitive–conservative axis are at the level of the leaf and are based on biophysical and physiological principles that determine relationships among traits (Wright *et al.*, 2004; Shipley *et al.*, 2006a; Blonder *et al.*, 2011, 2013, 2015; Onoda *et al.*, 2017, but see Grubb, 2016). As a consequence, covariation patterns should be independent of scale or level of organization. In other words, the covariation pattern among leaf functional traits at the intraspecific level is expected to mirror the global interspecific pattern.

The acquisitive–conservative axis was originally observed within global databases of diverse taxa collected across broad environmental gradients (Reich *et al.*, 1997; Wright *et al.*, 2004; Díaz *et al.*, 2004, 2016). Also, leaves from different populations of the same species distributed across broad geographical scales (e.g. its entire range of distribution), or under contrasting growing conditions, usually reproduce the interspecific global patterns (Albert *et al.*, 2010a; Jackson *et al.*, 2013; Richardson *et al.*, 2013; Hu *et al.*, 2015; Niinemets, 2015; Martin *et al.*, 2017; Fajardo and Siefert, 2018; Xiong and Flexas, 2018; Hayes *et al.*, 2019; Sartori *et al.*, 2019). At these broad scales, climatic and soil properties have been shown to drive the leaf functional trait variation (Reich and Oleksyn, 2004; Wright *et al.*, 2005b; Ordoñez *et al.*, 2009; Hidaka and Kitayama, 2011; Moles *et al.*, 2014).

This strong, consistent leaf functional trait covariation pattern at both the interspecific and intraspecific level appears to weaken or even change direction as the taxonomic (Anderegg *et al.*, 2018) or organization level (Fajardo and Siefert, 2018) of analysis gets finer. At the intraspecific level and local scale (i.e.

from metres to a few kilometres but certainly far smaller than the geographic distribution range of a given species), where environmental conditions are less variable, leaves from individuals of the same population do not always show a covariation pattern similar to the global interspecific pattern (Blonder *et al.*, 2013; Hu *et al.*, 2015). These observations, together with some patterns of genetic correlations (i.e. the proportion of variance that two traits share due to genetic causes), have led to the suggestion that natural selection has played a bigger role than genetic constraints in the evolution of the acquisitive–conservative covariation pattern (e.g. Donovan *et al.*, 2011). Also, it has been proposed that the acquisitive–conservative covariation pattern in leaf traits might not consistently hold at local scales and, at the population level, this pattern should be present among ecotypes within species only when these ecotypes span bioclimatic zones (Messier *et al.*, 2017). However, none of the previous studies has explicitly addressed the leaf functional trait covariation pattern under constant environmental conditions. If selection is the main driver in the evolution of the acquisitive–conservative covariation pattern, then homogeneous environmental conditions should lead to stabilizing selection on each trait, and the intraspecific leaf functional trait covariation pattern should become weaker and more prone to deviation from the global interspecific pattern. Exploring variation in the absence of environmental variation (at lower levels of organization) thus helps elucidate whether biophysical constraints on leaf properties are a product of, or independent from, the environment.

Whether or not the leaf functional trait covariation pattern at the intraspecific level reproduces the global interspecific pattern is also a relevant question beyond plant functional biology. The global interspecific acquisitive–conservative axis is a powerful concept to link vegetation and ecosystem processes (Grime, 2001; Díaz *et al.*, 2004; Garnier *et al.*, 2004; Suding *et al.*, 2008; Funk *et al.*, 2017) and predicts plant community changes (Shipley *et al.*, 2006b; Shipley, 2015; Warton *et al.*, 2015). If the leaf functional trait covariation pattern underlying the considerable intraspecific trait variability (now documented by many authors, such as Albert *et al.*, 2010a, b; Messier *et al.*, 2010; Siefert *et al.*, 2015) does not mirror the global interspecific covariation pattern, it follows that the acquisitive–conservative framework could not integrate plant ecology across all organization levels.

In the present study, we ask the following question. At the intraspecific level and when the leaf functional trait variation is not mainly driven by environmental conditions, is the covariation pattern of leaf functional traits consistent with the global interspecific acquisitive–conservative covariation pattern? To address this question, we studied the leaf functional trait covariation patterns in six grasses and four woody legumes. We complemented the study of plants from a field area with small environmental (climatic and edaphic) variation with their descendants grown under common conditions. Growing plants in a common garden removes environmentally induced phenotypic plasticity and its effects on covariation patterns, but not local adaptation (i.e. population genetic change due to environmental conditions) that may still be present among genotypes. Therefore, to rule out climatic and edaphic variables as explanatory factors for genetic

trait variation and covariation patterns, we selected a narrow study area with little environmental variability. Additionally, we analysed the intraspecific leaf functional trait variation as a function of these environmental variables to test if the remaining environmental variability is relevant to the leaf functional trait variation, and could affect the leaf functional trait covariation pattern, in our study. We analysed the intraspecific covariation of leaf functional traits and compared them with the global interspecific covariation pattern. We focused on two speciose, locally abundant and well represented plant families within the study area: Fabaceae and Poaceae. These families differ in their patterns of trait covariation (Adams *et al.*, 2016), which further motivated a comparison of the trait patterns observed at local intraspecific scales with those at the global interspecific scale, for each family.

We measured SLA,  $N_m$ , LDMC and leaf mechanical resistance under two conditions: in the field in order to account for natural local intraspecific variability (which is partially shaped by phenotypic plasticity), and in a common garden, where the effects of phenotypic plasticity are controlled and phenotypic differences should be mostly the expression of genetic variability (maternal effects cannot be ruled out).

## MATERIALS AND METHODS

### Study area and species

The field study area is located in central Argentina, at the southern extreme of the South American Gran Chaco (approx. 31°18′–31°32′S and 65°23′–65°32′W). The sampling area is approx. 25 km long north to south and 10 km wide east to west. The climate is sub-tropical and semi-arid (Cabido *et al.*, 1994) with a mean annual precipitation of 514.8 mm concentrated in spring–late summer (October–March) and a mean annual temperature of 19.6 °C (Supplementary data Table S1). Soils vary from Torriorthents (Entisols order) to Camborthids ustolics (Aridisols order) (Cabido *et al.*, 1994). Vegetation corresponds to an open-canopy xerophytic forest with the trees *Aspidosperma quebracho-blanco* and *Prosopis flexuosa* as canopy and sub-canopy dominants, respectively. The shrub layer is often dense and dominated by *Mimozyanthus carinatus*, *Senegalia gilliesii* and *Larrea divaricata*. Land use, logging and livestock grazing result in a mosaic of communities with different relative abundances of these species and varying proportions of bare soil (Cabido *et al.*, 1994).

We measured the leaf functional traits of six perennial  $C_4$  grasses (*Aristida mendocina*, *Gouinia paraguayensis*, *Neobouteloua lophostachya*, *Sporobolus pyramidatus*, *Leptochloa crinita* – formerly *Trichloris crinita* –, *Leptochloa pluriflora* – formerly *Trichloris pluriflora*) and four woody legume species (*Vachellia aroma* – formerly *Acacia aroma* –, *Senegalia gilliesii* – formerly *Acacia gilliesii* –, *Prosopis flexuosa* and *Prosopis torquata*) (see Supplementary data Table S2 for the species list including authorities). All these species are common and often abundant in the Chaco ecosystem (Cabido *et al.*, 1993, 1994).

### Sampling protocol

In the study area, 39–49 sampling points were selected for each species. For each species, sampling points were separated by at least 100 m to avoid cross-pollination both for grasses (Bateman, 1947; Griffiths, 1950; Jain and Bradshaw, 1966; Caisse and Antonovics, 1978) and for woody legumes (Vilardi *et al.*, 1988; Saidman and Vilardi, 1993; Butcher *et al.*, 1998; Bessega *et al.*, 2000, 2005; Casiva *et al.*, 2004). For grasses, each sampling point corresponded to a group of three individuals of a given species with <20 m distance from each other. For grasses, at each sampling point, we collected a leaf sample from each of these three individuals. For woody legumes, each sampling point corresponded to a single individual of a given species. For woody legumes, at each sampling point, we collected two leaf samples from the sun-exposed outer canopy of one reproductively mature plant, one sample from the lowest branch and one sample from the highest branch reachable (approx. 2 m). Each leaf sample (for both grasses and woody legumes) consisted of three or more fully developed leaves. A total of 1172 leaf samples were analysed from plants in the field (850 leaf samples from 850 grass individual plants and 322 leaf samples from 161 woody individual plants).

### Leaf functional trait measurements

All leaf samples were processed independently and used to measure SLA ( $\text{mm}^2 \text{mg}^{-1}$ ), LDMC (proportion),  $F_l$  ( $\text{N mm}^{-1}$ ) and  $N_m$  (%), following the protocols of Pérez-Harguindeguy *et al.* (2013). In woody legumes, all measurements were performed on leaflets (excluding rachis), therefore  $F_l$  could not be measured because leaflets were too small to be handled into our measuring device. Leaf nitrogen content per leaf mass was measured using an Elementary Analyzer Perkin Elmer 2400 Series II (USA). Due to financial limitations, we only were able to measure  $N_m$  in two species per family. We selected the most contrasting ones in terms of growth form and habit: the grasses *L. pluriflora* and *N. lophostachya* and the woody legumes *P. flexuosa* and *P. torquata*.

### Soil properties and climatic variables

At each sampling point, we measured surface compaction (pocket penetrometer), compaction at 0–5 cm and 5–10 cm (dynamic cone penetrometer INTA-Villegas) and volumetric soil water content at 3 inches (Field Scout TDR 100 Soil Moisture Meter, Spectrum Technologies, Inc.). Additionally, we collected a pooled soil sample (four samples 0–10 cm deep) from each sampling point to determine pH (PHS-3E pH Meter, Arcano), conductivity (PHS-3E pH Meter, Arcano), organic matter content (Walkley and Black technique, Sparks *et al.*, 1996), total nitrogen content (Elementary Analyzer Perkin Elmer 2400 Series II), extractable phosphates (Olsen technique, Sparks *et al.*, 1996), silicates (Wei-min *et al.*, 2005) and cation ( $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$  and  $\text{K}^+$ ) concentration (atomic absorption spectroscopy, Laboratorio de Edafología del Departamento de



Recursos Naturales de la Facultad de Ciencias Agropecuarias, Universidad Nacional de Córdoba, Argentina). Soil samples were pooled by the closeness of the sampling points, grouping all samples into 22 composite samples. The other variables were averaged following the same grouping factor before statistical analysis. Also, we extracted 19 standard WorldClim bioclimatic variables (Supplementary data Table S1) from WorldClim 2.1 at 30 s resolution (approx. 1 km<sup>2</sup>) (Fick and Hijmans, 2017).

#### *Common garden experiment*

From all sampling points, we collected seeds and produced seedlings that were grown in pots in a common garden at Córdoba National University, Argentina. For woody legumes, seeds from a given sampling point were all from a single mother plant, the same plant whose leaves were measured, so they constituted a maternal family (full or half-siblings). In the case of grasses, seeds from a sampling point were collected from several random plants (within a 20 m radius circular plot) so the resulting seedlings are not necessarily maternal siblings. Nevertheless, we treated the plants produced with seeds from the same sampling point as ‘genetic families’ for both woody legumes and grasses. For all the pots, we used a commercial potting soil similar to field soil (Supplementary data Table S1). For grasses, we used 0.79 L (10 cm diameter, 10 cm height) pots. For woody legumes, we used 2.4 L (10 cm diameter, 30 cm height) pot. All pots were weeded weekly during the growing season and monthly in winter. Herbivory by ants and aphids was controlled by applying insecticide to all plants when herbivores were detected. All plants were watered in short pulses (1–3 min) with automatic sprinklers, evenly spaced and homogeneously (Christiansen’s uniformity coefficient: CUC = 87 %) covering the whole surface where the pots were placed. Each watering day, plants received four pulses. Irrigation (frequency and pulse duration) was adjusted weekly or more frequently to ensure enough moisture and avoid overwatering. We successfully obtained seedlings from the seeds from 28–40 sampling points per species. A year after germination, we measured the leaf functional traits of these plants, 2–4 individual plants per sampling point. We took one leaf sample per plant, each sample consisting of three or more fully developed leaves. A total of 1195 individual plants (and samples) were analysed in the common garden experiment. At that time, grasses had reached maturity and reproduced sexually. In contrast, woody legumes were still juvenile.

#### *Data analysis*

If two leaf functional traits are affected by the same environmental variable, this environmental effect will produce a correlation between the two traits. Therefore, in the case of plants growing in the field, for each species separately, we tested for correlations (Pearson correlation test) between each leaf functional trait (log<sub>10</sub>-transformed) and each environmental variable. We then tested for significant correlation (*P*-values < 0.05) among each environmental variable and two or more leaf functional traits.

We next analysed the correlation (Pearson correlation test) between pairs of leaf functional traits for each species and growing condition, both in the field and in the common garden separately, as well as correlations for the global interspecific dataset as a whole, and for the Fabaceae and Poaceae families within it (see below). To compare each of these correlations, we computed Fisher’s *z* effect size (Fisher, 1925). Additionally, we computed the overall effect size for the intraspecific correlation in our Fabaceae and Poaceae species in the field and in the common garden by using the ‘rma’ function from the R package ‘metafor’ (Viechtbauer, 2010).

Finally, we computed the slopes of the relationships between pairs of leaf functional traits in each one of our species, in the global interspecific, in the Fabaceae interspecific and in the Poaceae interspecific datasets. To do that, we performed standardized major axis (SMA) regressions (Warton *et al.*, 2006) where both elevation (i.e. intercept) and slope varied freely. We used the R-package ‘smatr 3’ (Warton *et al.*, 2012) and the pair-wise comparisons among the resulting slopes. *P*-values were adjusted using the Sidak correction (Šidák, 1967) to reduce false positives among multiple comparisons. All leaf functional traits were log<sub>10</sub>-transformed to achieve normality and homoscedasticity across the whole dataset (in analyses of both correlation and slopes). For multiple comparison tests among slopes, only groups with significant correlation (*P*-value ≤ 0.05) between leaf functional traits were included.

In common garden conditions, the whole covariation pattern (phenotypic covariation) can depend on the covariation between families (genetic correlation, i.e. an estimate of the additive genetic effect that is shared between our pair of traits) and/or covariation within families. So, in the case of leaf functional traits for which several measurements were taken per genetic family (SLA, LDMC and F<sub>l</sub>), we performed the same analyses (correlation and slopes comparisons) for genetic families, to assess the genetic correlation between leaf functional traits. All analyses were performed within R version 3.6.1 (R Core Team, 2019).

#### *Interspecific leaf functional trait data*

The global interspecific, Fabaceae and Poaceae covariation patterns were obtained from the publicly available data in the TRY global communal database ([www.try-db.org](http://www.try-db.org); Fitter and Peat, 1994; Shipley, 1995, 2002; Cornelissen, 1996; Cornelissen *et al.*, 1996, 1999, 2003, 2004; Atkin *et al.*, 1997, 1999, 2015; Medlyn *et al.*, 1999; Meziane and Shipley, 1999; Pyankov *et al.*, 1999; Castro-Diez *et al.*, 2000; Shipley and Lechowicz, 2000; White *et al.*, 2000; Wilson *et al.*, 2000; Meir *et al.*, 2002; Shipley and Vu, 2002; Loveys *et al.*, 2003; Quested *et al.*, 2003; Xu and Baldocchi, 2003; Adler *et al.*, 2004, 2014; Díaz *et al.*, 2004; Givnish *et al.*, 2004; Wright *et al.*, 2004; Craine *et al.*, 2005, 2009, 2011, 2012, 2013; Louault *et al.*, 2005; Sheremetev, 2005; Vile, 2005; Cavender-Bares *et al.*, 2006; Kazakou *et al.*, 2006; Kerkhoff *et al.*, 2006; Michaletz and Johnson, 2006; Preston *et al.*, 2006; Campbell *et al.*, 2007; Craven *et al.*, 2007; Meir and Levy, 2007; Price and Enquist, 2007; Scherer-Lorenzen *et al.*, 2007; Swaine, 2007; Kleyer *et al.*, 2008;

Kraft *et al.*, 2008; Shiodera *et al.*, 2008; Kattge *et al.*, 2009, 2011; van de Weg *et al.*, 2009, 2011; Wirth and Lichstein, 2009; Baraloto *et al.*, 2010; Freschet *et al.*, 2010; Laughlin *et al.*, 2010, 2011; Messier *et al.*, 2010; Ordonez *et al.*, 2010; Blonder *et al.*, 2011, 2012, 2013, 2015, 2016; Butterfield and Briggs, 2011; Campetella *et al.*, 2011; Chen *et al.*, 2011; de Araujo *et al.*, 2011; Milla and Reich, 2011; Onoda *et al.*, 2011; Prentice *et al.*, 2011; Tucker *et al.*, 2011; Sandel *et al.*, 2011; Yguel *et al.*, 2011; Yu *et al.*, 2011; Adriaenssens, 2012; Beckmann *et al.*, 2012; Choat *et al.*, 2012; Frenette-Dussault *et al.*, 2012; Gutiérrez and Huth, 2012; Han *et al.*, 2012; Minden *et al.*, 2012; Powers and Tiffin, 2012; Rolo *et al.*, 2012; Spasojevic and Suding, 2012; Vergutz *et al.*, 2012; Williams *et al.*, 2012; Wright and Sutton-Grier, 2012; Auger and Shipley, 2013; Boucher *et al.*, 2013; Demey *et al.*, 2013; Dahlin *et al.*, 2013; Guy *et al.*, 2013; Kichenin *et al.*, 2013; Lukeš *et al.*, 2013; Martinez-Garza *et al.*, 2013; Joseph *et al.*, 2014; Minden and Kleyer, 2014; Muir *et al.*, 2014; Seymour *et al.*, 2014; Siefert *et al.*, 2014; Slot *et al.*, 2014; Smith *et al.*, 2014; Takkis, 2014; van der Plas and Olff, 2014; Walker, 2014; Ciccarelli, 2015; La Pierre and Smith, 2015; Li *et al.*, 2015; Maire *et al.*, 2015; Minden and Kleyer, 2015; Tribouillois *et al.*, 2015; De Vries and Bardgett, 2016; Gos *et al.*, 2016; Lhotsky *et al.*, 2016; Schroeder-Georgi *et al.*, 2016; J. M. Sharpe and N. Solano, unpubl. res.; E. Chacón-Madrigal *et al.*, 2018). Once entries with ErrorRisk (indication for outliers, distance to mean in standard deviations) >4 were excluded, the dataset contained information on 10 369 species from 309 taxonomic families. From these, 5403 species from 249 taxonomic families including ferns, gymnosperms and angiosperms contain information for at least two leaf functional traits. The observations are widely distributed over the world (Supplementary data Fig. S1).

## RESULTS

### Overall leaf functional trait variation and environmental effects

In general, the ten Chaco species measured in this study were clustered in a relatively narrow section of the global interspecific variability for each leaf functional trait (Supplementary data Fig. S2). Bioclimatic variables showed little variability among sampling points [coefficient of variation (CV) <4 %], whereas some soil properties were quite variable (e.g. soil Mg<sup>2+</sup> content and conductivity show a CV of 48.3 % and 43.3 %, respectively) (Supplementary data Table S1). However, only one correlation (between SLA and F<sub>t</sub>) for two species (*S. pyramidatus* and *L. pluriflora*) could be partially explained by the effects of environmental variables. In *S. pyramidatus*, soil water content correlated positively with SLA ( $r = 0.363$ ,  $P$ -value <0.0001) and negatively with F<sub>t</sub> ( $r = -0.446$ ,  $P$ -value <0.0001) (Fig. 1). In *L. pluriflora*, soil conductivity correlated negatively with SLA ( $r = -0.517$ ,  $P$ -value <0.0001) and positively with F<sub>t</sub> ( $r = 0.523$ ,  $P$ -value <0.0001) (Fig. 1). No other pair of leaf functional traits from any species correlated significantly with any other environmental variable. Therefore, environmental variation within the study area could not explain most of the covariation patterns observed (presented below).

### Trait–trait covariation patterns

The intraspecific covariation of the leaf functional traits generally followed the expected pattern. We observed a negative correlation for the LDMC–SLA, F<sub>t</sub>–SLA, LDMC–N<sub>m</sub> and F<sub>t</sub>–N<sub>m</sub> trait pairs, but a positive correlation for the SLA–N<sub>m</sub> and F<sub>t</sub>–LDMC trait pairs (Supplementary data Table S3; Fig. S3). Only some species in some conditions showed non-significant correlations, but the effect size was always in the expected direction (e.g. *L. crinita* in common garden for the F<sub>t</sub>–SLA correlation) and the overall effect sizes for intraspecific correlations (Fabaceae intraspecific field, Fabaceae intraspecific common garden, Poaceae intraspecific field and Poaceae intraspecific common garden) followed the expected pattern in all cases (Supplementary data Fig. S3). The intraspecific covariation pattern was similar to the global, the Fabaceae and the Poaceae interspecific patterns for most of the pairs of leaf functional traits, but was different for the F<sub>t</sub>–N<sub>m</sub> and F<sub>t</sub>–LDMC pairs of traits. For these, the interspecific patterns showed no correlation.

### Trait–trait slopes

The intraspecific slopes always followed the expected qualitative trend, i.e. a negative slope for the pairs LDMC–SLA (Fig. 2A), F<sub>t</sub>–SLA (Fig. 2C), LDMC–N<sub>m</sub> (Fig. 3A) and F<sub>t</sub>–N<sub>m</sub> (Fig. 3C), and a positive slope for the pairs LDMC–F<sub>t</sub> (Supplementary data Fig. S4A) and SLA–N<sub>m</sub> (Supplementary data Fig. S4C). This pattern was observed in leaf functional traits of individuals both collected in the field and grown in the common garden (Figs 2B, D and 3B, D; Supplementary data Fig. S4B, D). Even family means showed similar patterns (Supplementary data Fig. S5; Tables S4 and S5).

While the intraspecific patterns of leaf functional trait covariation in this study matched those expected on a global scale, many of the intraspecific slopes significantly differed from the global interspecific slope as well as from the slopes of its respective taxonomic family (Figs 2B and 3B; Supplementary data Fig. S4D). Specifically, for the LDMC vs. SLA relationship, ten out of 20 cases (species × growing condition) and three out of ten species have slopes shallower than the global interspecific slope (Fig. 2B; Supplementary data Table S6). Within Fabaceae, three species (*P. flexuosa* field, *P. flexuosa* common garden, *P. torquata* field and *V. aroma* field) showed shallower slopes than the interspecific Fabaceae slope (Fig. 2B; Supplementary data Table S6). Within Poaceae, two species (*N. lophostachya* field, *N. lophostachya* common garden and *S. pyramidatus* field) showed slopes steeper than the interspecific Poaceae slope (Fig. 2B, Supplementary data Table S6). For the F<sub>t</sub> vs. SLA relationship, three out of 12 cases and one out of six grasses have slopes steeper than the global interspecific slope (Fig. 2D; Supplementary data Table S7). However, none of the grasses (F<sub>t</sub> was not measured for woody legumes) was different from the interspecific Poaceae slope. For the LDMC vs. N<sub>m</sub> relationship, three out of eight cases had slopes shallower than the global interspecific pattern (Fig. 3B; Supplementary data Table S8). Within Fabaceae, one species (*P. flexuosa* field, *P. flexuosa* common garden) showed shallower slopes

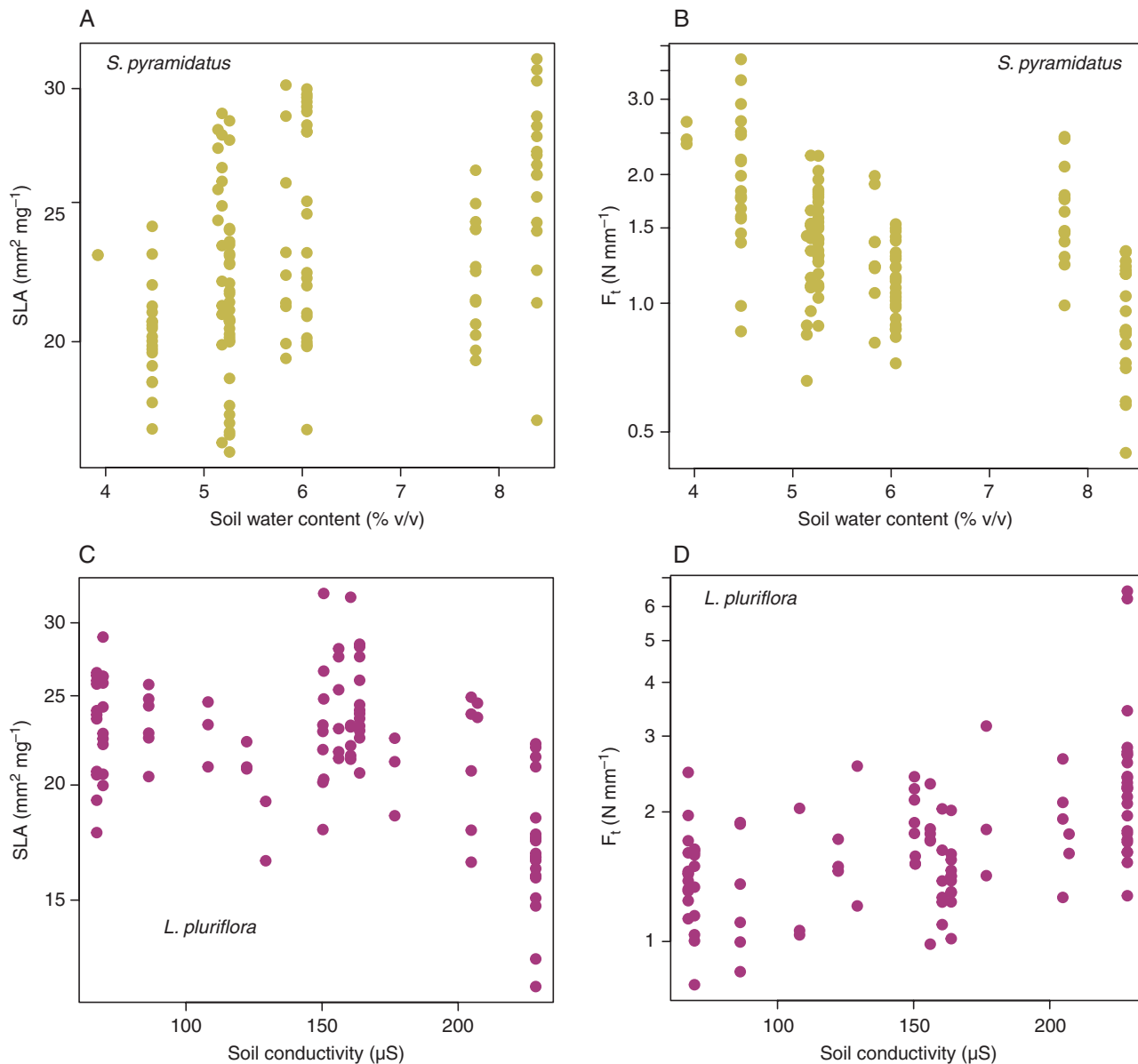


FIG. 1. Relationship between leaf functional traits and environmental variables. Here we show the only two cases where an environmental variable (Supplementary data Table S1) affects simultaneously two leaf functional traits within a species. SLA, specific leaf area.  $F_t$ , force to tear.

than the interspecific Fabaceae slope, which is in turn steeper than the global interspecific (Fig. 3B; Supplementary data Table S8). Within Poaceae, just one case (*L. pluriflora* field) showed a slope shallower than the interspecific Poaceae slope (Fig. 3B; Supplementary data Table S8). For the  $N_m$  vs. SLA relationship, three out of eight cases and one out of four species (all grasses) have steeper slopes than the global and Poaceae interspecific slopes (Supplementary data Fig. S4; Table S9). For the  $F_t$  vs.  $N_m$  and the  $F_t$  vs. LDMC relationships, at the intraspecific level, there were strong correlations for most cases (Supplementary data Table S3; Fig. S3). Moreover, the intraspecific slopes of all cases with significant correlation were similar (Fig. 3D; Supplementary data Fig. S4A).

#### Field vs. common growing conditions

For a given species, the relationship between a pair of leaf functional traits sometimes differed depending on whether the plants were grown in the field or in the garden. These differences appeared more common for the LDMC vs. SLA pair of traits (Fig. 2B) than others (e.g.  $F_t$  vs. SLA; Fig. 2D). The slopes of the relationship between leaf functional traits never changed sign under different growth conditions (even in genetic family analysis); however, in some cases, the correlation became non-significant. This was more common in pairs of traits involving  $N_m$ , in the common garden and in woody legumes (Supplementary data Table S3). For the rest, only  $F_t$  vs. SLA for *L. crinita* in the common garden showed a non-significant

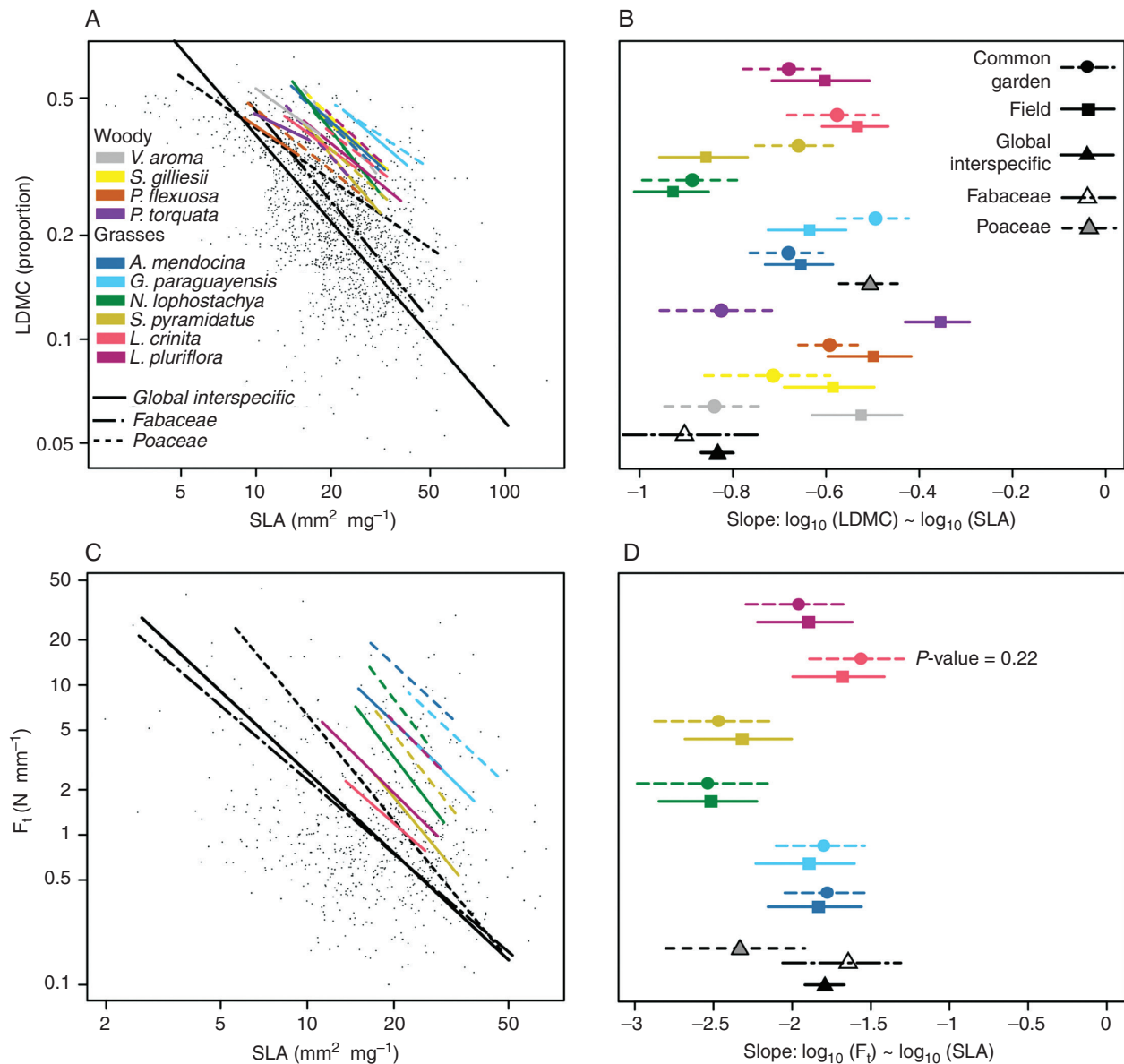


FIG. 2. Relationship between leaf dry matter content (LDMC), force to tear ( $F_t$ ) and specific leaf area (SLA). Different colours represent different species, continuous lines and squares represent the field condition, and dashed lines and circles represent the common garden condition. Black lines and dots show the same analysis and data for the global, Fabaceae and Poaceae interspecific dataset obtained from the TRY datasets. Left panels (A and C) show the SMA lines for each species at each condition, the global interspecific data and the Fabaceae and Poaceae interspecific data. Each dot represents one species; intraspecific data (individual plants) are not shown. Right panels (B and D) show the estimated slope and 95 % confidence interval for each group. When an estimated slope comes from a non-significant correlation, it is indicated in the right panel by reporting the corresponding  $P$ -value and it is not shown in the left panel. Results from multiple comparisons of the slopes are shown in [Supplementary data Tables S6 and S7](#).

correlation ([Supplementary data Table S3](#)). The correlation between a pair of leaf functional traits was significant in the common garden but not in the field only in the case of SLA and  $N_m$  in *P. flexuosa* ([Supplementary data Table S3](#)).

## DISCUSSION

If the acquisitive–conservative axis arises from biophysical constraints on leaf functional traits, it should be present across levels of organization and be independent from the magnitude of environmental variation. Yet patterns at the intraspecific level

have been reported to deviate from the global interspecific pattern ([Donovan et al., 2011](#); [Niinemets, 2015](#); [Martin et al., 2017](#); [Anderegg et al., 2018](#); [Xiong and Flexas, 2018](#); [Hayes et al., 2019](#)). We sought to test whether phenotypic plasticity or local (genetic) adaptation might explain such deviation. We found that the intraspecific leaf functional trait covariation pattern within each of the ten selected species, belonging to two contrasting taxonomic families and growth forms, are consistent with the theoretically expected acquisitive–conservative axis. These covariation patterns are also similar to the interspecific trait covariation pattern, although the strengths of the correlations are variable.



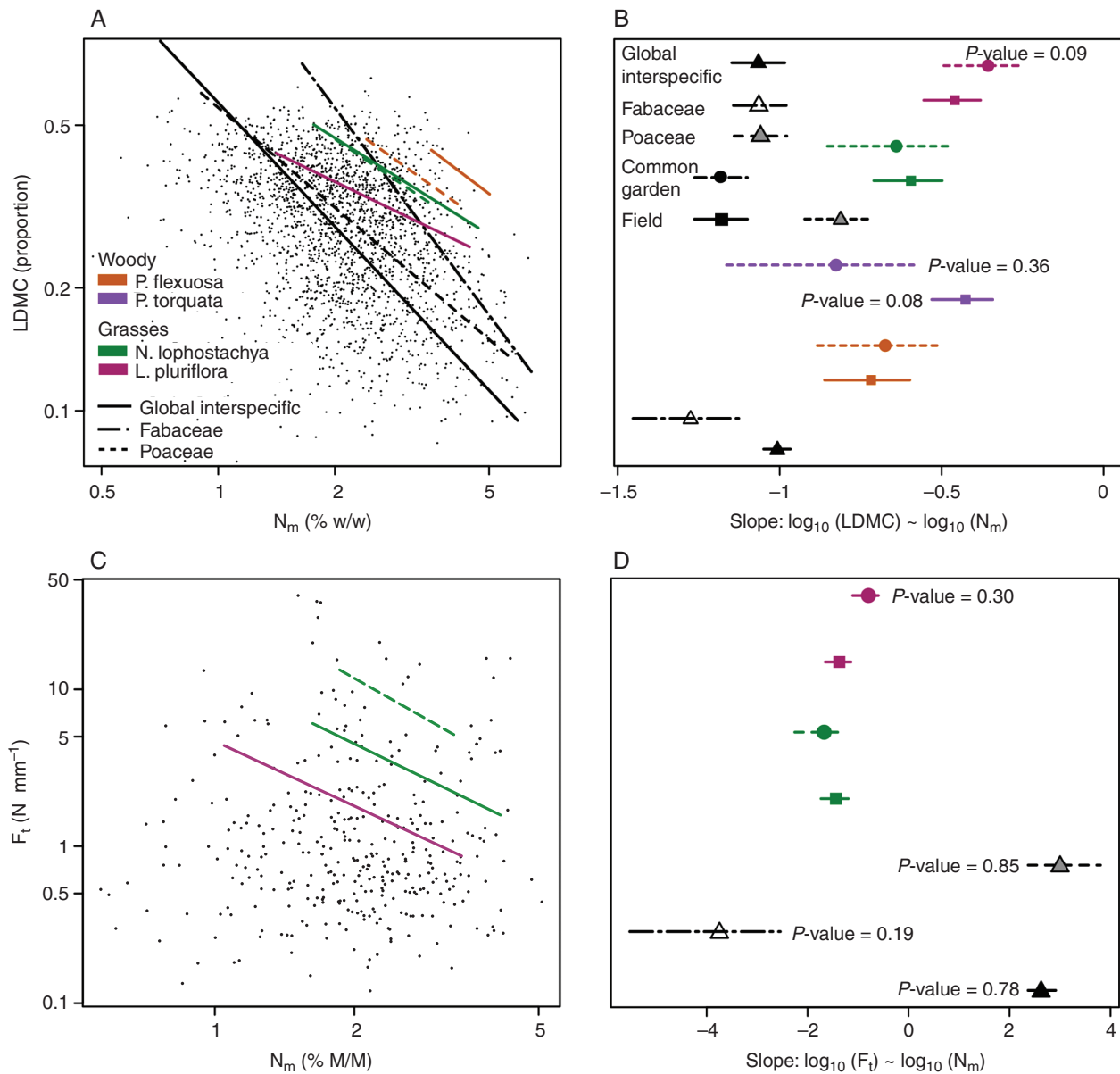


FIG. 3. Relationship between leaf dry matter content (LDMC), force to tear ( $F_t$ ) and leaf nitrogen content ( $N_m$ ). Different colours represent different species, continuous lines and squares represent the field condition, and dashed lines and circles represent the common garden condition. Black lines and dots show the same analysis and data for the global, Fabaceae and Poaceae interspecific dataset obtained from the TRY datasets. Left panels (A and C) show the SMA lines for each species at each condition, the global interspecific data and the Fabaceae and Poaceae interspecific data. Each dot represents one species; intraspecific data (individual plants) are not shown. Right panels (B and D) show the estimated slope and 95 % confidence interval for each group. When an estimated slope comes from a non-significant correlation, it is indicated in the right panel by reporting the corresponding  $P$ -value and it is not shown in the left panel. Results from multiple comparisons of the slopes are shown in [Supplementary data Table S8](#). In the  $F_t$  vs.  $N_m$  relationship, there were no significant differences in slopes among those with correlation different from zero. There are only two species per taxonomic family in the intraspecific analyses.

This suggests that the trade-off between resource acquisition and conservation, which has been extensively documented among species, also operates within species. This is in accordance with previous studies at the intraspecific level (Albert *et al.*, 2010a; Vasseur *et al.*, 2012; Blonder *et al.*, 2013; Jackson *et al.*, 2013; Richardson *et al.*, 2013; Niinemets, 2015; Hu *et al.*, 2015; Martin *et al.*, 2017; Anderegg *et al.*, 2018; Fajardo and Siefert, 2018; Xiong and Flexas, 2018; Hayes *et al.*, 2019; Sartori *et al.*, 2019). However, those studies focused on trait covariation along environmental gradients, and/or under contrasting growing conditions

(Supplementary data Table S3). We gained further insight by focusing on intraspecific covariation patterns under less variable conditions in the field to avoid the effect of major environmental gradients such as rainfall and temperature (i.e. local adaptation), as well as in a common garden experiment to capture the genetic rather than environmental underpinning (phenotypic plasticity) of leaf functional trait correlations. Although we detected some environmentally driven variation in SLA and  $F_t$  for two species, this was an exception rather than a commonality, and does not influence the study conclusions.



We found that the pattern of covariation related to the acquisitive–conservative axis persists at the intraspecific level, both in the field and in a common garden experiment, even when environmental variation among sampling points (in the field) cannot explain such covariation. This suggests that, in our dataset, plastic responses or local adaptation (in response to selection) are not the primary determinants of the intraspecific acquisitive–conservative covariation pattern, as previously proposed (Donovan *et al.*, 2011). On the contrary, it seems that the same trade-offs shaping the global interspecific acquisitive–conservative axis operate at the intraspecific level.

Donovan *et al.* (2011) showed that the genetic correlation between leaf functional traits may be variable among species and even between populations of the same species. They showed that such genetic correlations may be opposite to what is expected on the basis of the global acquisitive–conservative interspecific axis, leading them to conclude that natural selection should be the main force shaping the acquisitive–conservative covariation pattern. However, covariance among leaf functional traits is modulated by plasticity (Sherrard *et al.*, 2009). In our work, field conditions are different from common garden conditions in many ways, e.g. water availability. These differences induced variable effects in the slopes according to the leaf functional trait pair considered, i.e. there is some degree of plasticity in such covariance structure, and some trait–trait correlations may be more plastic than others. So, even when the main trends in the acquisitive–conservative pattern were independent of environmental variation, this heterogeneity could modulate the strength and slope of the covariation pattern.

The relationship between SLA and LDMC is mediated by leaf thickness and leaf density (Vile *et al.*, 2005). Moreover, there is a link between leaf morphology and chemical composition such that the thicker the leaf is, the greater is the LDMC (Roderick *et al.*, 1999). It follows that the steeper the negative LDMC–SLA slope, the more steeply positive the LDMC–thickness slope would be. With respect to the LDMC– $N_m$  slopes, if leaf nitrogen is assumed to be located mostly in the cytosol (the liquid phase) and not in the extracellular matrix (Roderick *et al.*, 1999),  $N_m$  is inversely proportional to LDMC (Shipley *et al.*, 2006a), and the log–log relationship should have a slope equal to  $-1$ . While, the less steep the negative slope, the less nitrogen there should be (proportionally) in the extracellular matrix (the solid phase). Leaf N allocation to cell walls ranges from 2.8 % to 25 % (90 % quantile range) (Onoda *et al.*, 2017). This N allocation trade-off may have important functional consequences because of its effect on the photosynthetic nitrogen use efficiency. Among our studied cases, grasses tend to show a shallower slope than legume species in the LDMC– $N_m$  relationship. This indicates that the N content of the cytoplasm per unit of nitrogen content of the cell wall ( $N_{\text{cyt}}:N_{\text{wall}}$ ) is greater in grasses than in legumes.

The acquisitive–conservative axis (and its variants, such as the leaf economics spectrum) has proven to be a useful concept to understand vegetation dynamics and ecosystem processes, as well as being relevant to functional and comparative plant ecology (McGill *et al.*, 2006; Reich, 2014). The evidence presented here confirms that, in general, the acquisitive–conservative covariation pattern is valid from the global level to the local population level, which has already been demonstrated in

a number of other studies (Blonder *et al.*, 2013; Hu *et al.*, 2015; Fajardo and Siefert, 2018). Our study goes further by showing that the pattern occurs at the intraspecific level even when environmental conditions do not explain it, and it also holds in the absence of environmental variation. This strongly suggests that natural selection, while clearly modulating this pattern (e.g. Wright *et al.*, 2005b), is not indispensable for its emergence. Our findings also reinforce the idea of one underlying cause for the pattern across scales and levels of organization. However, all the different causal hypotheses for the leaf economics spectrum (Wright *et al.*, 2004; Shipley *et al.*, 2006a; Blonder *et al.*, 2011, 2013, 2015; Onoda *et al.*, 2017) have found empirical support at some levels of organization, but failed when tested out of their domains (Blonder *et al.*, 2015). Among all the new studies that could be carried out to further elucidate the causes of these patterns, operating across levels of organization, the combination of phenotypic integration, i.e. the study of complex patterns of covariation among functionally related traits in a given organism, and pleiotropy, i.e. the phenomenon of a single gene affecting multiple traits, frameworks (Pigliucci, 2003; Paaby and Rockman, 2013; Geiler-Samerotte *et al.*, 2020), is arguably the most promising. For example, the fact that the leaf functional trait covariation pattern holds in the absence of environmental variation could be explained by the fact that highly integrated phenotypes (stronger covariation among traits) have higher adaptive value (Damián *et al.*, 2020) and therefore are selected for. However, different types of pleiotropy (Paaby and Rockman, 2013; Geiler-Samerotte *et al.*, 2020) could also explain the covariation pattern even if no selective advantage arises from integrated phenotypes. An experimental design combining both theoretical frameworks could shed light on the mechanisms driving the acquisitive–conservative axis at different levels.

### Conclusions

Our study indicates that the acquisitive–conservative leaf functional trait covariation pattern occurs at the intraspecific level, in a similar way to the well-known global interspecific pattern, even in the absence of relevant environmental variation in the field. This pattern remains mostly consistent even when the possible plastic responses were removed by common growing conditions. This suggests a high degree of variation–covariation in leaf functional traits not driven by environmental variables, i.e. not shaped by selection or plasticity. Our study supports the idea that genetic (or developmental/biophysical) constraints are the main determinants in the evolution of the acquisitive–conservative axis in the leaf functional traits; natural selection then operates, modulating it.

### SUPPLEMENTARY DATA

Supplementary data are available online at <https://academic.oup.com/aob> and consist of the following. Figure S1: geolocation of the entries in the TRY public dataset. Figure S2: distribution of the four traits considered in our analysis across the interspecific global dataset, the

interspecific Fabaceae and Poaceae and the intraspecific variability. Figure S3: Fisher's  $z$  effect size for all correlations among leaf functional traits. Figure S4: relationship between force to tear and leaf dry matter content as well as specific leaf area and nitrogen content per leaf mass. Figure S5: SMA slopes of the relationship between force to tear, leaf dry matter content and specific leaf area, from plants in the field and from genetic families in the common garden. Table S1: edaphic and climatic description of the sampling points, the common garden potting soil from our study, as well as the available data from previous studies about intraspecific covariation patterns of leaf functional traits. Table S2: full botanical names of the studied species. Table S3: correlation coefficient for each pair of leaf functional traits in each species and condition. Table S4: slopes of  $\log_{10}(\text{LDMC}) \sim \log_{10}(\text{SLA})$  for each species and condition and groups resulting from multiple comparison test. Table S5: slopes of  $\log_{10}(F_t) \sim \log_{10}(\text{SLA})$  for each species and condition and groups resulting from multiple comparison test. Table S6: slopes of  $\log_{10}(\text{LDMC}) \sim \log_{10}(\text{SLA})$  for each species and condition and groups resulting from multiple comparison test. Table S7: slopes of  $\log_{10}(F_t) \sim \log_{10}(\text{SLA})$  for each species and condition and groups resulting from multiple comparison test. Table S8: slopes of  $\log_{10}(\text{LDMC}) \sim \log_{10}(N_m)$  for each species and condition and groups resulting from multiple comparison test. Table S9: slopes of  $\log_{10}(N_m) \sim \log_{10}(\text{SLA})$  for each species and condition and groups resulting from multiple comparison test.

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#### DATA ACCESSIBILITY

The original datasets of leaf functional traits from grasses and woody legumes, both in the field and in the common garden are available at <http://hdl.handle.net/11086/17167>.

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