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SPECIAL TOPIC ON FUNCTIONAL TRAITS

Phenological variation of leaf functional traits within species

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Abstract A basic assumption of the trait-based approach in plant ecology is that differences in functional trait values are greater between species than within species. We questioned this assumption by assessing (1) the relative extent of inter- and intraspecific leaf trait variation throughout a complete growing season (phenological variation) in a group of deciduous and evergreen woody species, and (2) whether species rankings based on leaf traits were maintained across the growing season. We analysed leaf mass per area (LMA) and leaf nutrient concentrations (C, N, P), including the C:N and N:P ratios. Intraspecific trait variation (ITV) due to phenology was significantly greater than interspecific variation for leaf N concentration on a mass basis (N_m; 68.90 %) and for the leaf C:N ratio (60.60 %), whereas interspecific variation was significantly higher than ITV for LMA (62.30 %) and for leaf C concentration on a mass (C_m) and area (C_a) basis (C_m 70.40 %; C_a 65.30 %). ITV was particularly low for LMA (<20 %).

Species rankings were highly modified by phenology for a number of leaf traits (P_m, N:P ratio) but were relatively well conserved throughout the growing season for others (LMA, N_m). Patterns of ITV across the growing season differed significantly between deciduous and evergreen species for all traits except leaf P but did not vary between native and exotic species. Overall, our results show that intraspecific phenological variation in leaf traits may be similar to or greater than interspecific variation and that temporal patterns of ITV vary considerably among traits and species, especially for leaf nutrient concentrations, factors which can potentially affect quantitative interspecific relationships.

Keywords Deciduous · Evergreen · LMA · N and P concentrations · Species ranking

Introduction

Plant functional traits reflect how plants adapt to variation in their physical environment and biotic interactions. As such, including functional traits in our mechanistic understanding of plant community assembly and ecosystem functioning should improve our ability to predict the effects of environmental changes—including disturbances and climate change—on biodiversity, species distributions and ecosystem processes (Adler et al. 2013; McGill et al. 2006). Many studies have applied the trait-based approach in plant ecology via the description of trait dispersion patterns across environmental gradients (e.g. Shipley et al. 2006; Wright et al. 2004). In this effort, investigations have mostly focused on assessing mean trait values for species or populations, following a basic tenet of trait-based community ecology, namely, that differences in functional trait

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values among species are greater than those within species (Keddy 1992; McGill et al. 2006; Shipley et al. 2016).

Despite the many valuable insights provided by this approach, it has been shown that the use of mean trait values to describe community assembly hides much functional variation at different scales, both within (Bolnick et al. 2011; Violle et al. 2012) and among populations, particularly for species distributed widely across environmental gradients (e.g. Fajardo and Piper 2011). Plants can display considerable intraspecific trait variation (ITV) in response to abiotic filters and even to biotic interactions (Gross et al. 2009; Violle et al. 2012). Notably, ITV is thought to directly influence species interactions, especially in communities dominated by one or a few species (Crutsinger et al. 2006; Niinemets 2015), a commonly found pattern in temperate forests. Several studies have found that consideration of ITV improves the ability of trait-based analyses to detect community assembly processes (e.g. Jung et al. 2010; Kraft et al. 2014; Siefert 2012). Thus, a variance-based approach for the study of community ecology that focuses on the individual would improve our understanding of how communities are ultimately structured (Violle et al. 2012).

Here, we investigated the phenological variation of leaf functional traits within species, a mostly forgotten feature in the trait-based approach. In principle, phenological variation affects both plant functional traits and trait–trait covariation (Wolkovich and Cleland 2014), but its magnitude has rarely been documented in the context of the trait-based approach (but see McKown et al. 2013). Indeed, existing trait sampling protocols (e.g. Cornelissen et al. 2003) recommend measuring traits at the peak of the growing season to minimize phenological variation and facilitate comparison among species. A conspicuous example relates to foliar habit, where deciduous species display noticeable phenological variation during their spring leaf budburst and their colourful autumn leaf shedding; these visual changes in leaves should inevitably be accompanied by changes in functional traits, including leaf nitrogen (N) concentrations. Species with long-lived leaves (i.e. evergreens) may have a different phenological strategy, where leaf functionality may show relatively little seasonal change (Méndez-Alonzo et al. 2012). It is known that seasonality alters, among other things, species' photosynthetic capacity (Grassi et al. 2005; Wilson et al. 2001). Thus, it is possible that results of trait-based studies may be misleading when phenological variation is not taken into account and when traits are compared among plant communities from different locations, sampled at different dates throughout the summer period or measured repeatedly throughout the years (McKown et al. 2013; Niinemets 2015). Although evergreen species by definition always have at least one photosynthetically active leaf cohort to provide the necessary carbon (C) supply, it is not clear whether they differ from deciduous species in

terms of functioning, nutrient relations (e.g. allocation) and relations to herbivores during the growing season (Chabot and Hicks 1982). For example, compared with evergreens, deciduous tree species produce less robust leaves [low leaf mass per area (LMA)], which makes them more prone to mechanical damage, including herbivory, and eventual C limitations (Piper and Fajardo 2014).

In the study reported here, we first determined the magnitude of intraspecific variation of leaf mass per area (LMA) and leaf nutrient concentrations, both leaf functional traits, throughout the entire growing season for a group of 12 Patagonian woody species. We then compared the values obtained to the amount of among-species variation. Decomposing the relative contribution of species-level differences and ITV can be essential in understanding how communities are structured and how they react to environmental changes (de Bello et al. 2011). Thus, we first asked: (1) What is the relative extent of interspecific variation and ITV throughout the growing season? In trait-based studies, it has commonly been assumed that even if ITV is non-negligible, the ranking of species based on their trait values should still be consistent through space and time (Garnier et al. 2001; Rose et al. 2013; Shipley et al. 2016). Our second question was therefore: (2) Are species' rankings based on leaf traits maintained across the growing season? As the practice of phenology relies on documenting visible developmental changes through time, it is much easier to observe phenological changes in deciduous species than in evergreen species given that the latter implicitly show no changes. However, evergreen species produce new leaves every year, and it is not clear whether they follow phenological patterns similar to those of deciduous species. Hence, we compared phenological variation in leaf traits between deciduous and evergreen species, asking: (3) Are patterns of intraspecific variation across the growing season similar between deciduous and evergreen species? Finally, in addition to native woody species, we considered in this study seven exotic species commonly used in reforestation in Patagonia. We justify their inclusion because exotic and native species share the same site conditions but have different overall strategies and different evolutionary history, and differences in leaf trait phenology may therefore reflect these differences. Thus, our last question was: (4) Are patterns of intraspecific variation across the growing season similar between native and exotic species?

Materials and methods

Description of research sites and species

This study was conducted in various locations around the city of Coyhaique (45°57′S, 72°03′W; 380 m a.s.l.), Aysén

Region, Patagonia, Chile. The annual mean temperature in this area is 8.6 °C, and the mean annual precipitation is 890 mm (Coyhaique National Reserve weather station, Dirección General de Aguas, 2004–2013; 400 m a.s.l.). The mean temperature of the warmest month is 14.2 °C, with a potential evapotranspiration of 584 mm (Luebert and Pliscoff 2006). In addition to native deciduous and evergreen tree and shrub species, the woody vegetation of the region includes fast-growing, non-native conifers (e.g. *Pinus contorta*, *P. sylvestris*, *P. ponderosa*) which were planted as part of reforestation projects following fires in the 1950 and 1960s, as well as other exotic woody species introduced for plantation trials or ornamental purposes. We selected three sites around the city of Coyhaique for trait sampling, with each site containing at least three woody species: (1) Baguales (45°31′S, 72°04′W); (2) Reserva Coyhaique (45°32′S, 72°02′W); (3) La Cruz (45°34′S, 72°02′W). In total, we sampled 12 species, including five deciduous and seven evergreen species [Electronic Supplementary Material (ESM) Table S1]. All populations were relatively young (around 50 years old) with good access to light and water.

Sampling and tissue collection and processing

The sampling was conducted between November 2012 and June 2013. Each population was visited on the first week of each month, and at least eight individuals were randomly selected for tissue collection. Sampling was constrained to unshaded adult trees which showed no clear evidence of herbivory or other damage. From each individual tree, we selected one terminal, fully expanded, sun-exposed branch from the current growing season. Branches were cut using a 5.6-m telescoping pole (ARS Corp., Senboku, Japan), labelled and then placed in a cooler for transportation to the laboratory. In the laboratory, the branches were placed in water to minimize leaf dehydration, and two to 20 leaves (depending on the species) were selected from each branch for determination of LMA. These leaves were separately laid flat and photographed with a reference square of 4 cm^2 using a Nikon Coolpix 5000 digital camera (Nikon Corp., Tokyo, Japan), and the total projected leaf area was calculated using the image-processing software SIGMAPROC (Systat Software Inc., Richmond, CA). Leaves were then placed to dry in a forced-air oven (Memmert GmbH, Schwabach, Germany) at 70 °C for 72 h and subsequently weighed with a scale calibrated to 0.0001 g of precision to determine LMA. LMA was computed as the oven-dried weight of leaves divided by their total foliar surface (g m^{-2}) and represents leaf biomass investment (C and nutrients) per light interception area. Finally, the dried leaves were ground to a fine powder using a coffee mill and stored at 4 °C until the chemical analyses. An important consideration when collecting leaves, especially towards the end of the growing season, was to avoid the collection of neoformed leaves, which occur in species with neoformed shoot growth (Hallé et al. 1978), such as *Embothrium coccineum*, *Populus deltoides*, and *Betula pendula*. In such cases, we collected leaves belonging to the proximal section of the current year's shoot, thus standardizing leaf age across species.

Foliar phosphorus (P) was extracted from the tissue by combining 20 mg of dry leaf material in 1 mL of 2 % v/v acetic acid and digesting this mixture for 30 min before centrifugating it at 6000 rpm (Fredeen et al. 1989). Leaf P concentrations were then determined following the method of Murphy and Riley (1962) which, in brief, consists of the formation of an antimony–phosphomolybdate complex and its subsequent reduction to phosphomolybdenum blue, a complex with a bluish-purple colour. The absorbance of this complex is read at 880 nm and converted into a phosphate concentration using a calibration curve of potassium dihydrogen phosphate. Leaf N and C concentrations were determined by a CHN combustion analyser (TruSpec® Micro Analyzer; LECO Corp., St. Joseph, MI) at the Centro de Investigación en Ecosistemas de la Patagonia, Coyhaique, Chile. P, C and N concentrations were expressed on both a leaf dry mass (mg g^{-1}; P_m, C_m and N_m, respectively) and area basis (g m^{-2}; P_a, C_a and N_a, respectively), with the latter calculated by multiplying the mass-based concentrations by LMA. Finally, we calculated the C:N and N:P ratios to assess plant N and P limitations.

Statistical analysis

All values of leaf traits were transformed to their natural logarithms before analyses to meet assumptions of normality. To quantify interspecific and intraspecific trait variation throughout the growing season (question 1), we fitted a linear model to the variance of each trait, with species and month nested within species as random effects (Messier et al. 2010). The resulting variance estimate for species represents interspecific variation, the variance estimate for month within species represents temporal (among-month) intraspecific variation, and the residual variance represents intraspecific variation within months plus measurement error. Using these variance estimates, we partitioned the total variation in each trait into interspecific, intraspecific among-month, and intraspecific within-month components and calculated 95 % bootstrap confidence intervals.

To determine whether species trait rankings were consistent through the growing season (question 2), we calculated the Spearman rank correlation of species mean trait values (Garnier et al. 2001) between each pair of months in which traits were measured. A significant positive correlation between a pair of months indicates consistency in species trait rankings (i.e. species that had relatively high

trait values in 1 month also had high values in the second month, and vice versa). A lack of correlation, or a negative correlation, indicates species trait rankings are inconsistent or reversed between months, respectively.

To assess patterns of intraspecific variation through the growing season and test whether these patterns varied depending on species' foliar habit (deciduous vs. evergreen; question 3) and origin (native vs. exotic; question 4), we fitted linear mixed models to temporal trends for each trait. We modelled temporal trends throughout the growing season using fixed linear and quadratic effects for time (month). We then determined whether species varied in their mean trait values and temporal trends by testing for random intercepts, linear coefficients and quadratic coefficients among species using likelihood ratio tests on nested models fitted using restricted maximum likelihood (Bolker et al. 2009). Retaining the

significant random effects, we next tested the fixed effects of foliar habit, origin and their interactions with time (linear and quadratic terms) using Wald χ^2-tests on nested models fitted using maximum likelihood (Bolker et al. 2009). In this analysis, a significant foliar habit $\times$ time effect would indicate that deciduous and evergreen species differ in their patterns of ITV across the growing season. A significant origin $\times$ time effect would indicate that temporal patterns differ between native and exotic species. All analyses were conducted in R statistical software® (R Development Core Team 2013).

Results

As expected, trait values varied significantly among species and months across the growing season (Fig. 1; ESM Table
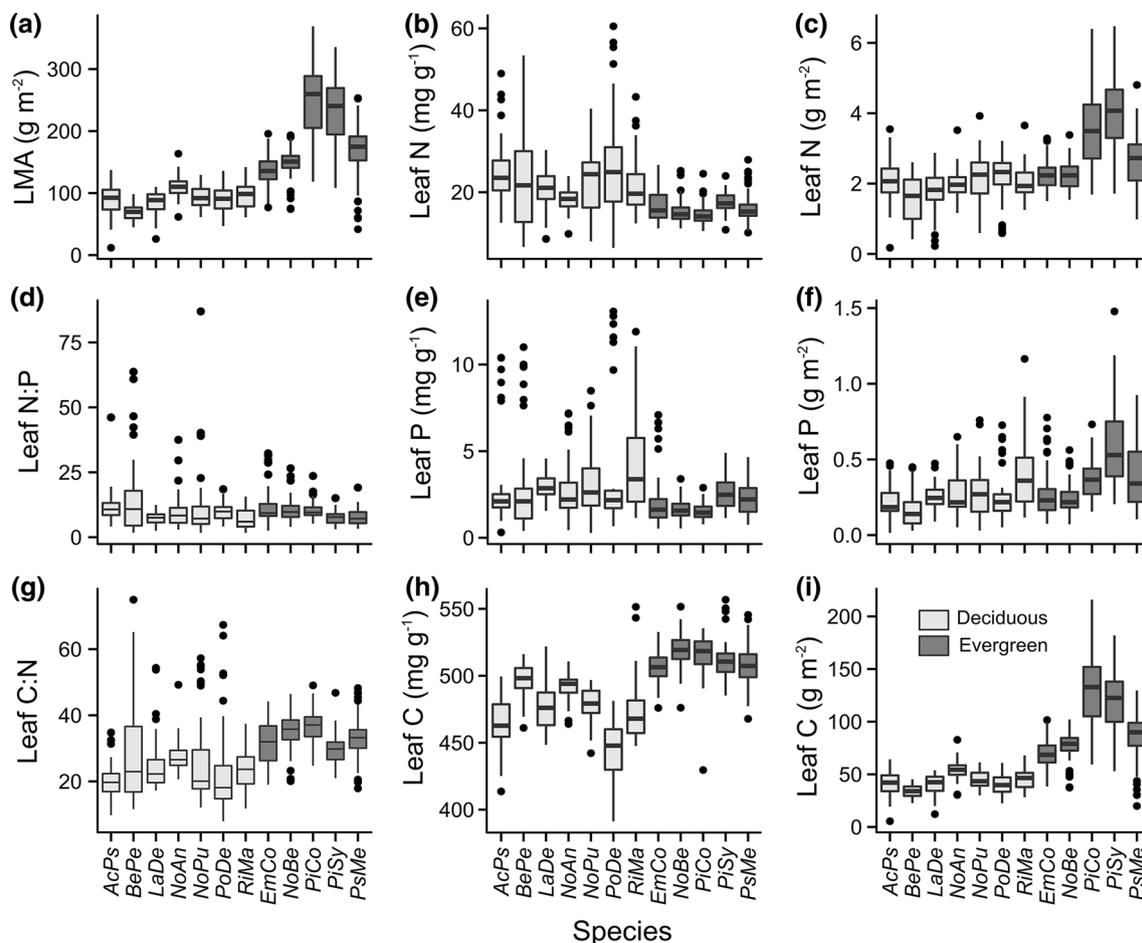
Fig. 1 Variation in leaf mass per area (*LMA*; **a**), in concentrations of leaf nitrogen (*N*; **b**), phosphorus (*P*; **f**) and carbon (*C*; **I**) on a leaf-dry mass basis (N_m, P_m, C_m, respectively), in concentrations of N (**c**), P (**f**) and C (**I**) on a leaf-area basis (N_a, P_a, C_a, respectively) and in the N:P (**d**) and C:N (**g**) nutrient ratios, across a complete growing season (November 2012 throughout May 2013) for deciduous and evergreen tree species growing in the vicinity of Coyhaique (45°34 S, 72°04 W, 384 m a.s.l.), Chile. Tree species abbreviations: *AcPs Acer pseudoplatanus*, *BePe Betula pendula*, *LaDe Larix decidua*, *NoAn Nothofagus antarctica*, *NoPu Nothofagus pumilio*, *PoDe Populus deltoides*, *RiMa Ribes magellanicum*, *EmCo Embothrium coccineum*, *NoBe Nothofagus betuloides*, *PiCo Pinus contorta*, *PiSy Pinus sylvestris*, *PsMe Pseudotsuga menziesii*
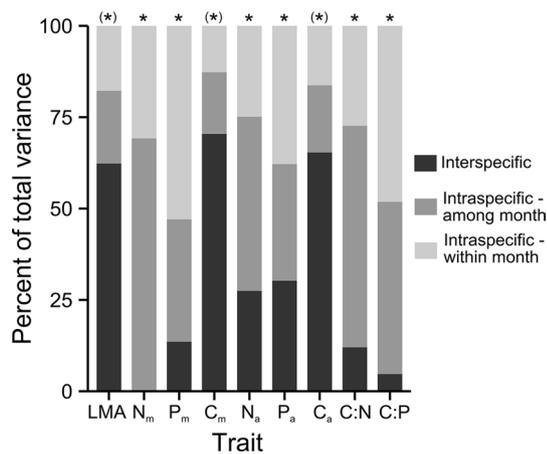
Fig. 2 Decomposition of variation in leaf functional traits across a complete growing season for 12 woody species growing in Patagonia (see Fig. 1 caption for breakdown of species). Variance was decomposed into components representing interspecific variation, intraspecific variation among months (i.e. phenological variation) and intraspecific variation within months. *Asterisks* indicate significant difference between interspecific and intraspecific (among-month + within-month) variance, with *1 asterisk* (*) indicating intraspecific variation > interspecific variation and *asterisk in parenthesis* [(*)] indicating intraspecific variation < interspecific variation. See text and Fig. 1 caption for definition of traits

S2). The relative extent of inter- and intraspecific trait variation throughout the growing season differed among traits (Fig. 2). For some leaf traits, such as LMA (62.30 %), C_m (70.40 %) and C_a (65.30 %), interspecific variation was significantly higher than intraspecific variation. Conversely, for traits such as N_m (0.30 %), P_m (13.60 %) and the C:N (12.00 %), and N:P (4.70 %) ratios, interspecific variation was extremely low when compared to intraspecific variation. Intraspecific variation due to phenology was especially high for N_m (68.90 %) and the C:N (60.60 %) ratio, whereas most intraspecific variation in P_m (52.90 %) was due to within-month differences among individuals.

Species trait rankings were highly modified by phenology for a number of leaf traits (e.g. P_m, N:P), while for others (e.g. LMA, N_m) rankings were relatively well conserved during the growing season (Table 1; ESM Table S3). In particular, species rankings for LMA were highly consistent across the growing season (high month-to-month correlations in species rankings), with the exception of the first month (December). We found similar results for C_m, C_a and, to a lesser extent, C:N and N_a (ESM Table S3). However, other leaf traits, such as P_m, P_a and N:P, had weak month-to-month correlations in species rankings, indicating that species rankings varied across the growing season. N_m showed a unique pattern, with consistency in species rankings early in the growing season (December–March), but a reversal in rankings between the early and late part of the growing season, as indicated by significant negative

correlations between rankings in May and January–March (Table 1).

Results of mixed models fit to trait variation across the growing season showed that there were significant shifts in trait values over time for most traits (significant linear fixed effect of time, t; Fig. 3), with most traits showing nonlinear trends (significant quadratic term for time, t^2; Fig. 3; ESM Fig. S1, Tables S4, S5). Temporal patterns of intraspecific variation differed significantly among species for all traits (significant time-by-species random effects; Fig. 3; ESM Fig. S1). Temporal patterns also differed significantly between deciduous and evergreen species for all traits except P_m and P_a (Fig. 3; ESM Fig. S1). Differences in trends between deciduous and evergreen were especially evident for LMA, N_m and the C:N ratio (Fig. 3). The time–foliar habit interaction term had a significant effect on the variation of LMA, N_m, C_m and the C:N and N:P ratios ($P < 0.05$; Fig. 3). In evergreen species, LMA increased throughout the growing season, while in deciduous species LMA remained relatively constant, with a slight peak in the middle of the season (Fig. 3). There was a strong decrease in N_m (increase in C:N ratio) of deciduous species through the growing season, while in evergreen species N_m was relatively constant, with a small decline (and corresponding peak in C:N ratio) in the middle of the season (Fig. 3). Temporal patterns of intraspecific variation did not differ significantly between native and exotic species for any trait (ESM Tables S4, S5).

Discussion

Examining leaf traits of woody species across an entire growing season, we found that ITV was significantly larger than among-species trait variation for some leaf traits (e.g. N_m) but not for others (e.g. LMA). A number of leaf traits, such as N_m, C:N and P_m, were particularly variable within species (>60 % of total variation), reflecting a combination of phenological (among-month) and individual-level (within-month) variation. Other traits, such as LMA, C_m and C_a, varied more among species than within species. Our results are consistent with those reported by Kazakou et al. (2014) and Siefert et al. (2015), both of whom similarly found that leaf chemical traits (including nutrient concentrations) tended to have greater ITV within and among communities than leaf morphological traits, such as LMA. Most previous studies investigating the importance of ITV have focused on spatial gradients. In general, these studies have found that the relative extent of ITV compared to among-species variation depends on the trait and spatial scale in question (Albert et al. 2010; Fajardo and Piper 2011; Hulshof and Swenson 2010; Messier et al. 2010; Siefert 2012) but that it is often large enough to be

Table 1 Spearman ranking coefficients for the leaf traits of 12 woody species compared month by month during the growing season (December 2012–May 2013)

log-transformed leaf traits[a]	December	January	February	March	April	Log-transformed leaf traits[a]	December	January	February	March	April
ln(LMA)						ln(N_m)					
Jan	0.38						**0.74**				
Feb	0.50	**0.97**					**0.64**	**0.92**			
Mar	0.46	**0.90**	**0.92**				**0.71**	**0.90**	**0.89**		
Apr	0.52	**0.97**	**0.97**	**0.90**			−0.07	−0.03	0.09	0.25	
May	0.43	**0.97**	**0.97**	**0.94**	**0.94**		−0.51	**−0.66**	**−0.67**	**−0.66**	0.17
ln(P_m)						ln(C_m)					
Jan	0.17						**0.81**				
Feb	0.06	0.31					**0.80**	**0.95**			
Mar	0.06	0.47	**0.90**				0.54	**0.85**	**0.91**		
Apr	**0.59**	0.48	0.24	0.22			**0.79**	**0.92**	**0.97**	**0.88**	
May	0.54	0.15	0.17	0.28	0.49		**0.60**	**0.89**	**0.93**	**0.92**	**0.95**
ln(N:P)						ln(C:N)					
Jan	0.31						**0.75**				
Feb	0.11	**0.65**					**0.73**	**0.97**			
Mar	0.22	**0.66**	**0.83**				**0.76**	**0.92**	**0.90**		
Apr	−0.05	0.31	0.17	0.21			0.12	0.26	0.28	0.42	
May	−0.10	0.29	−0.09	0.10	0.59		−0.31	−0.45	−0.36	−0.41	0.25

A significant positive correlation (given in bold font) indicates maintenance of species' trait rankings between months. No correlation indicates a shift in rankings through time. A significant negative correlation indicates a reversal of rankings

All woody species (see Fig. 1 caption for detailed listing of tree species) were growing in the surroundings of Coyhaique city (45°34S, 72°04W, 380 m a.s.l.), Patagonia, Chile

[a] All values of leaf traits were transformed to their natural logarithms before analyses to meet assumptions of normality. Leaf traits analysed were: leaf mass per area (LMA; g m^{-2}); leaf nitrogen (N_m), phosphorus (P_m), and carbon (C_m) concentrations on a leaf-mass basis (mg g^{-1}); N:P and C:N ratios

ecologically relevant. Similarly, we found that the relative extent of ITV along a temporal gradient was higher than among-species variation for some leaf traits, which can affect the quantitative interspecific relationships between traits. Our results suggest that ITV in general, and phenological variation in particular, should be considered for traits such as leaf N and P concentrations, which are important components of the leaf economics spectrum (LES). Perhaps trait measurement protocols should be modified to require sampling at least twice during the growing season in order to capture the necessary variation in these traits. For other leaf traits, such as LMA, using mean values appears to be sufficient to properly characterize species-level variation.

Leaf trait variation across time reflects changes in leaf physiology associated with leaf age and predominant environmental conditions (Pallardy 2008). Although conventional wisdom expects traits to change throughout the growing season (in particular, leaf traits for deciduous species), there have been few previous attempts to explicitly quantify these changes. Some studies, however, have considered the importance of phenology as a source of within-species variation, with mixed results. For example, McKown et al. (2013) found that most of the annual leaf trait variation of *Populus trichocarpa* occurred just following bud set, with relatively little variation during the rest of the growing season. In contrast, Albert (2009) found strong within-species variation across the growing season for several alpine herb and woody species in the French Alps. Mason et al. (2013), working with three species of sunflower, also found that seasonal variation in leaf N concentration and LMA was as high as two-thirds of the total cross-species variation. As stated earlier, this variation is due to some traits appearing to be more plastic than others, particularly with respect to phenology.

It has been recognized that even if ITV is high, interspecific trait rankings may still be maintained across space and time if species display similar spatial and temporal patterns of ITV (Garnier et al. 2001; Shipley et al. 2016). Although we found that species rankings for some traits were conserved along most of the sampling season (e.g. LMA), there were considerable shifts in the rankings of other traits

Fig. 3 Trends of temporal variation for 12 tree species grouped by foliar habit for several foliar traits: **a** log of LMA, **b** log of N concentration on a mass basis (N_m), **c** log of P concentration on a mass basis (P_m), **d** log of C concentration on a mass basis (C_m), **e** C:N ratio, **f** N:P ratio. Inference is indicated for each trait based on the best-fitted model (see "Results"), including random and fixed effects, where time (*t*, in months), foliar habit (*h*, deciduous vs. evergreen) and species (*sp*) are the factors involved; see Fig. 1 caption for details on species. t^2 Significant quadratic term for time. Inference is significant at: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$ (*ns* non-significant effect)
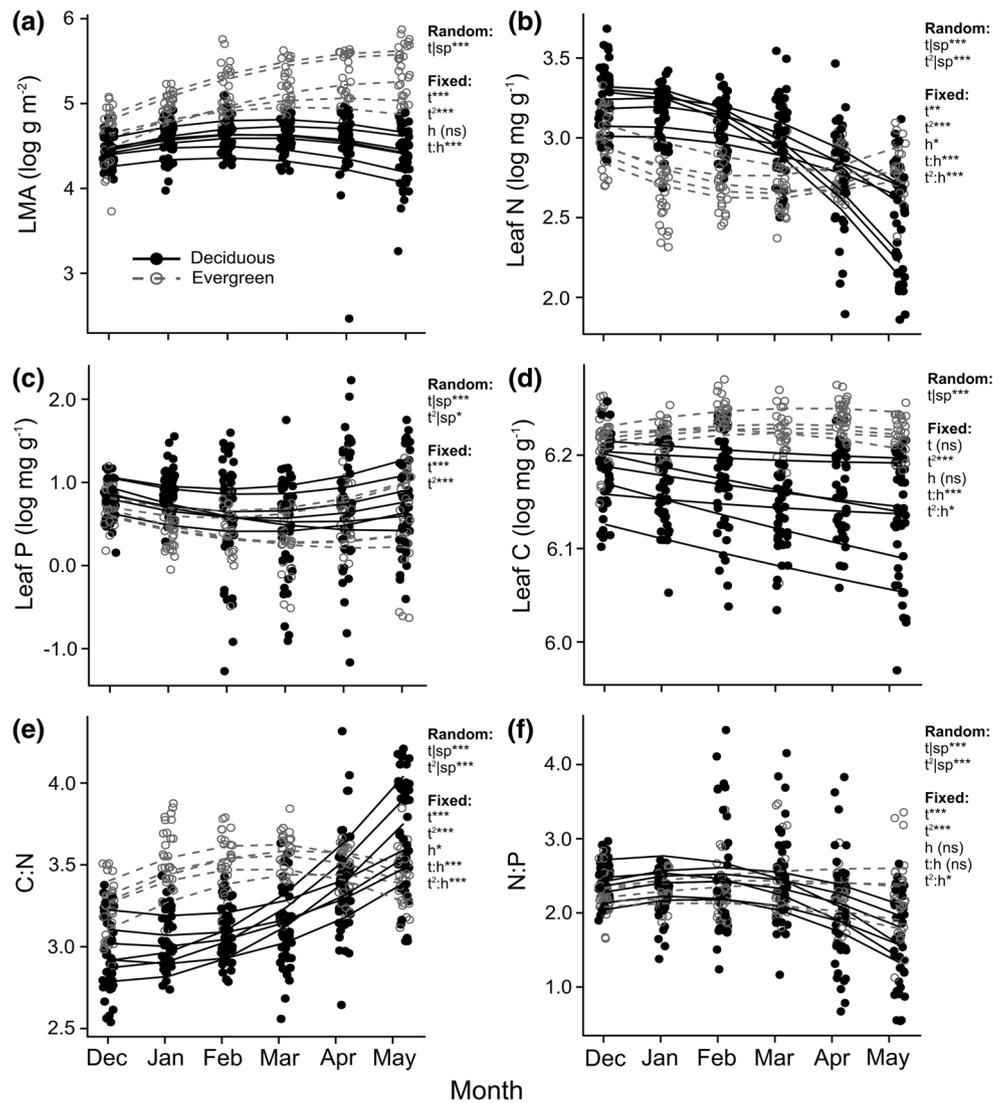
(e.g. P_m). From these patterns, it is clear that there is a relationship between within-species trait variation and maintenance of species rankings, namely, the lower the ITV (e.g. LMA), the less chance that species trait rankings will shift across the growing season. These findings caution strongly against the use of species mean trait values measured at one time point to quantify interspecific differences in traits with high ITV. They also highlight the need to understand how trait values at different phenological stages influence species' responses to the abiotic and biotic environment. For example, it is known that species with high leaf nutrient content tend to be favoured in areas of high resource availability. However, our results show that different species have relatively high nutrient concentrations at different points in the growing season. Testing the degree to which species' trait values at different phenological stages predict their performance or relative abundance along environmental gradients should provide new insights into the trait-based

processes controlling species distributions and community assembly.

One possible explanation for a significant shift in species' ranking for leaf traits is that tree species leaf out (i.e. produce a new cohort of leaves) at different times during the onset of the growing season. In our study, evergreen species generally appeared to leaf out at least 1 month later in the growing season than deciduous species, which is in accordance with observations made in previous studies conducted on broader spatial scales (Davi et al. 2011; Panchen et al. 2014). It is clear that even before deciduous species start to leaf out, evergreen species already have at least one photosynthetically active cohort of leaves; therefore, it is not imperative for evergreens to leaf out earlier in the season.

We found significant differences in temporal patterns between deciduous and evergreen species for all traits except P. LMA increased with leaf age in both deciduous

and evergreen species, probably due to the accumulation of C-rich compounds; however, LMA peaked in the middle of the season in deciduous species and decreased toward the end of the growing season. Removal of C compounds from leaves prior to abscission most likely explains this decrease (Reich et al. 1991). For leaf N, deciduous species had significantly higher values than evergreens, a relatively well-known pattern (e.g. Piper and Fajardo 2014; Walters and Reich 1999). What is less known, however, is how foliar habit and phenology (time) interact with each other. We found a strong decrease in leaf N through the growing season in deciduous but not in evergreen species, indicating that N is acquired and used quite differently during the growing season by woody species depending on their foliar habit. Indeed, deciduous and evergreen species differ markedly in which organ they store N; regardless of the magnitude of concentration, deciduous species mostly store N and C reserves in woody tissues (stems and roots), whereas evergreen species store them in leaves (Piper and Fajardo 2014). We found that leaf C also decreased with leaf age in deciduous species, but at a slower rate than N, resulting in an increasing C:N ratio. We found no difference in phenology of leaf traits between native and exotic species.

Conclusions

In this study, we investigated intraspecific phenological variation in leaf traits, an underappreciated source of trait variation in trait-based plant ecology studies. We found that, for leaf nutrient concentration traits, ITV across a single growing season was significantly greater than interspecific variation across 12 woody species. In contrast, LMA, one of the most useful and commonly measured traits in plant functional ecology studies, varied significantly more among species than within species. Based on our results, we recommend that for highly plastic leaf nutrient concentration traits researchers should sample at least three times during the growing season (early, middle, and late) to be able to capture sufficient variation to properly characterize species' functional properties. Our results and those of other global-scale studies (e.g. Siefert et al. 2015) indicate clearly that the relative extent of ITV varies strongly among traits. Consequently, we propose that trait collection protocols should be updated to explicitly identify traits— and spatial and temporal scales—for which ITV should be considered or, alternatively, can be safely ignored. More importantly, given the large intraspecific phenological variation observed in key functional traits, future research should seek to better understand the consequences of this variation for ecological processes, including species interactions, environmental filtering and ecosystem functioning. Understanding these consequences is especially important in light of the dramatic on-going and predicted shifts in plant phenology brought on by global climate change.

Author contribution statement AF conceived the study, conducted the sampling and processing of samples, helped in the analysis of the data and wrote the manuscript. AS conducted the analysis of the data and helped to write the manuscript.

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