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Research article

Relaxation of the leaf economics spectrum within and across quaking aspen *Populus tremuloides* genotypes

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Plant functional traits typically show strong covariation, e.g. as in the worldwide leaf economics spectrum (LES). Covariation is thought to arise from selective forces and physical constraints. However, processes shaping covariation at interspecific scales may differ from those at intraspecific scales. Potential sources of intraspecific trait variation include variation in genetics, abiotic environment, and biotic environment; any of these factors may cause divergence from interspecific patterns. Quaking aspen, *Populus tremuloides* (Salicaceae), is a widespread tree species ideal for assessing these processes' role in the intraspecific LES, due to its clonal growth along environmental gradients, intraspecific variation in cytotype (ploidy level), and variation in biotic interactions (herbivory pressure). Here, we investigate genotype, cytotype, microclimate and herbivory as potential drivers in shaping LES trait–trait and trait–environment relationships at intra-specific scales. We studied 15 quaking aspen genotypes that varied in cytotype (diploid or triploid) along an elevation gradient in southwestern Colorado. We show that LES tradeoffs substantially weaken at the intraspecific scale in this species. Among genotypes, trait–trait slopes ranged from positive, weak, strong, negative, or absent compared to the global LES. We also found that cytotypes varied in resource-use tradeoffs, and that increasing insect herbivory pressure decreased the strength of several trait–trait relationships. Microenvironment was a weak predictor of intraspecific functional trait variation in quaking aspen. *Synthesis.* In quaking aspen, there are relaxed constraints on LES trait co-variation. Variation in genotype, cytotype, and herbivory pressure each contribute to this pattern. Relaxation of the LES may enable more flexible responses to environmental stressors through both genetic and plastic mechanisms.

Keywords: cytotype, functional traits, genotype, intra-specific variation, leaf economics spectrum, polyploidy, *Populus tremuloides*, quaking aspen

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Introduction

The leaf economics spectrum (LES) describes consistent and strong correlations among plant functional traits (Reich et al. 1997, Reich and Oleksyn 2004, Wright et al. 2004, 2005). It has been widely used for understanding general patterns in plant trait variation, how plants adapt to different environments, and how these adaptations influence ecosystem processes, including responses to climate change (Wang et al. 2023). The traditional view of the LES reflects the ecological tradeoffs and constraints of plants around resource use, i.e. fast or slow rate of return on carbon investment in leaves. The LES characterizes variation across diverse plant species, due to the assumption that trait relationships rely on leaf-level biophysical constraints or selective forces which lead to common relationships between traits (Reich et al. 1997, Wright et al. 2004).

While the LES has generally been shown to be consistent across species and biomes, emerging research has increasingly challenged this universality by demonstrating weak or absent LES relationships at more fine-grained biological scales, e.g. at the intraspecific level, resulting in decoupling of LES trait relationships among individuals of the same species or leaves within one individual (Anderegg et al. 2018, Xiong and Flexas 2018, Umaña and Swenson 2019, Ramírez-Valiente et al. 2020). While earlier studies often attributed the LES to individual-level trait tradeoffs (Shipley et al. 2006), more recent evidence suggests that species-level LES patterns are better explained by niche specialization – that is, evolutionary adaptation of species to specific ecological environments, leading to consistent trait combinations or syndromes that reflect long-term selective pressures (Agrawal 2020). This perspective helps explaining why trait correlations may appear robust across species and yet break down within species. Nonetheless, the exact processes driving these divergences in trait relationships across biological scales – particularly at the intraspecific levels – remain incompletely understood.

There are many hypotheses regarding species-level trait covariation which can be attributed to three primary mechanisms: genetic correlations, phenotypic plasticity and population-level local adaptation (Agrawal 2020). Strong genetic constraints have been suggested to govern the tradeoffs between the trait relationships within the LES, at least in annual rosette species like *Arabidopsis thaliana* (Vasseur et al. 2012). Phenotypic plasticity allows the same genotype to express different phenotypes depending on the environmental conditions and is especially thought to be common in environments with predictable variability (Donovan et al. 2011, Vasseur et al. 2012, De Kort et al. 2020). While abiotic factors are often emphasized, biotic interactions such as herbivory may also play a critical role in shaping trait expression and covariation, particularly through their influence on phenotypic plasticity. Unlike abiotic factors, which often regulate consistent directional pressures (e.g. light, temperature or nutrient availability), biotic factors such as herbivory can be more variable and context-dependent, potentially inducing more localized plastic responses.

A key question is whether LES patterns within species arise across environmental gradients because genetic variation is large, so local adaptation is important, or because phenotypic plasticity is large and individual genotypes can express a wide range of trait values. In the first scenario, trait variation is structured genetically, and within genotype, there may be little or no variation. In the second, any genotype could potentially occupy the full trait space if exposed to sufficient environmental variation, reflecting high phenotypic plasticity (Auld et al. 2009, Murren et al. 2015); the particular occupation of trait space could then be driven by the extant genetic correlations and the particular abiotic/biotic conditions. These alternatives reflect the relative importance of genetic variation versus phenotypic plasticity. By examining trait variation within genetically identical individuals (i.e. clones), we can estimate a lower bound for the importance of plasticity in shaping trait covariation of LES patterns, even in the absence of direct estimates of genetic correlations or local adaptation. The broader implication is that multiple processes, typically ignored at interspecific scale, may significantly influence trait–trait and trait–environment relationships within species. Understanding genetic variation in the context of biotic and abiotic environments can help clarify underlying processes that may shift inter- and intra-specific patterns.

Phenotypic plasticity and the LES

Intraspecific variation of traits can reflect plastic responses to small-scale variation in abiotic conditions, such as microclimatic variation (Armbruster et al. 2007), as well as stress-related abiotic drivers which can either increase or decrease intraspecific trait variation (Helsen et al. 2017), and influence competition and community assembly (Bennett et al. 2016, Niu et al. 2020, Kitagawa et al. 2022). Thus, some of the trait variation observed at smaller scales could be in direct response to various local factors and/or stressors rather than responses to regional means (Armbruster et al. 2007, Stark et al. 2017). For example, plasticity can reverse trait–trait relationships among plant individuals or among leaves (Blonder et al. 2013, Anderegg et al. 2018). Plasticity and selective pressures can shape the trait expressions of individual plants occupying these local environments, which can shape the expected trait–trait and trait–environment relationships (Sultan 1995, Armbruster et al. 2007, Opedal et al. 2015, Stark et al. 2017, Anderegg et al. 2018).

Small-scale variation in biotic conditions, such as herbivory pressure, can also shape intraspecific variation in morphological and chemical leaf trait expression associated with herbivory defence (Brown et al. 1991, Ruiz-Guerra et al. 2020). A large portion of the (leaf) carbon can be reallocated towards defence and thus is relevant in the context of the LES. Lind et al. (2012), for example, showed that after browsing, the leaves of spicebush *Lindera benzoin* have higher water content, carbon content, and specific leaf area (SLA), but lower nitrogen content. Furthermore, progressive exclusion of herbivores from grass vegetation over five seasons showed that invertebrate herbivory can influence the plant's resource use efficiency and thus the position of its leaves on the LES (Firn et al. 2017). However, the importance of herbivore defence on LES trait expression remains understudied (Defossez et al. 2018).

Intraspecific genetic variation includes genotypic variation and cytotype variation (i.e. ploidy level – the number of copies of each chromosome), which can also be relevant to the LES. Polyploidy (more than two complete sets of chromosomes) is common in plant species, both in natural populations as well as in crop species (Otto and Whitton 2000, Van de Peer et al. 2017, Li et al. 2022). Although most of these polyploid species occur in just one cytotype, many species have multiple cytotypes sometimes occurring in the same location (Soltis and Soltis 1995).

Polyploidy at interspecific or intraspecific scale may alter genetic, biochemical, physiological, and developmental characteristics of organisms, thus providing/enabling shifts in phenotypes and creating the opportunity to adapt or occupy new or broader niches (Levin 1983, Comai 2005, Van de Peer et al. 2017); but see Wei et al. (2019). Polyploids are often found to have different phenotypes from diploids, such as thicker, broader leaves, increased seed size, but fewer seeds and fewer stems per plant (Levin 1983). Differences in functional leaf and wood traits have also been observed between diploids and polyploids of the same species (Li et al. 1996, Hao et al. 2013, Zhang et al. 2017, Wei et al. 2019). For example, opposite LES tradeoffs related to nutrient, water, and light use have been found in cytotypes of *Dianthus broteri* (López-Jurado et al. 2022).

Traits affected by polyploidy can shift species' position along the LES towards the 'slow' or 'fast' return end of the spectrum, which can affect their ability to cope with environmental change (Van de Peer et al. 2021, 2017). Alternatively, increased number of copies of each chromosome can potentially increase genetic diversity, which can result in a wider range of phenotypes within a population – not necessarily through phenotypic plasticity, but through genotypic variation. Thus, variation in cytotype-related phenotypes and tradeoffs can lead to shifts along the fast–slow continuum in species, though the mechanisms and direction remain unclear.

Clonal species as an ideal model system

Clonal species with large genets covering environmental gradients provide natural experiments that allow differentiation of the impact of plasticity (response to abiotic/biotic drivers) and genetic variation on intraspecific trait variation. In non-clonal species, assessing these effects requires common gardens (Kröber et al. 2014) or other experimental manipulations under controlled conditions (Vasseur et al. 2012, Sartori et al. 2019). Quaking aspen *Populus tremuloides* (Salicaceae) is an ideal model species in this context, allowing simultaneous assessment of genetic variation and phenotypic plasticity of intraspecific LES trait variation in natural populations. Quaking aspen occurs over large geographical (from central Mexico to northern Alaska), environmental (from valleys up to steep talus slopes) and elevational (sea level to treeline) gradients (Morgan 1969, Keddy 1992, Mock et al. 2008, Meier et al. 2015, Greer et al. 2017; Fig. 1). It also is the most widely distributed tree species throughout North America and plays a crucial ecological role (Morgan 1969, Barnes 1975, Clair et al. 2010, Meier et al. 2015). Climate change-induced shifts in precipitation and increased temperatures are expected to increase the frequency and duration of droughts, and have extensive ecological and economic consequences for ecosystems, including aspen (IPCC 2021). Aspen has already experienced widespread drought-induced die-off, where in some areas up to 17% of the population has been affected (Worrall et al. 2010, 2013). Thus, understanding mechanisms that allow aspen to adapt in the face of a changing climate is crucial.

Aspen is capable of reproducing both sexually and asexually (clonally) by root suckers (Mitton and Grant 1996). Clonal growth is potentially an advantage in stressful environments and is thought to be the main mode of reproduction in quaking aspen in the Rocky Mountains, where it forms large multi-ramet clones (Mitton and Grant 1996, Stöcklin et al. 2009). The species also has intraspecific variation in cytotype, occurring as both diploid and triploid (Einspahr et al. 1963).

Together these features mean it is possible to study the effects of environmental variation within and between genotypes, as well as to contrast genotypes of different cytotypes, using natural populations rather than common gardens.

Here we investigate in situ responses of leaf functional traits to variation in genotype, cytotype, microclimate and insect herbivory in natural mature populations of quaking aspen. Our aim is to disentangle the extent to which trait covariation within species is structured by genetic differences such as those between genotypes or cytotypes, or by environmentally induced plasticity. We asked the following questions: 1) are intraspecific (between genotype, within genotype) trait–trait relationships consistent with the interspecific LES, and what do they reveal about the role of phenotypic plasticity? 2) How do genetic factors (cytotype and genotype) affect leaf trait–trait and trait–abiotic environment relationships, and what do they reveal about the role of genetic factors in the LES? 3) Does the biotic environment (insect herbivory) also alter leaf trait–trait relationships in aspen?

Material and methods

Study area and field sites

The study was carried out in the Gunnison National Forest, near the town of Gothic and the Rocky Mountain Biological Laboratory (RMBL), in Colorado (38°3'N, 105°6'W). In the study area the elevation range was 2900–3635 m a.s.l. (Fig. 1). Monthly mean temperatures in the study area ranged from −9.5 to −4.7°C and from 10.5 to 12.5°C for the coldest and warmest months, respectively (Western Regional Climate Center 2018). The area is characterized as a heterogeneous mosaic of Engelmann spruce–subalpine fir forests, aspen forests, and subalpine meadows (Morgan 1969). Large,

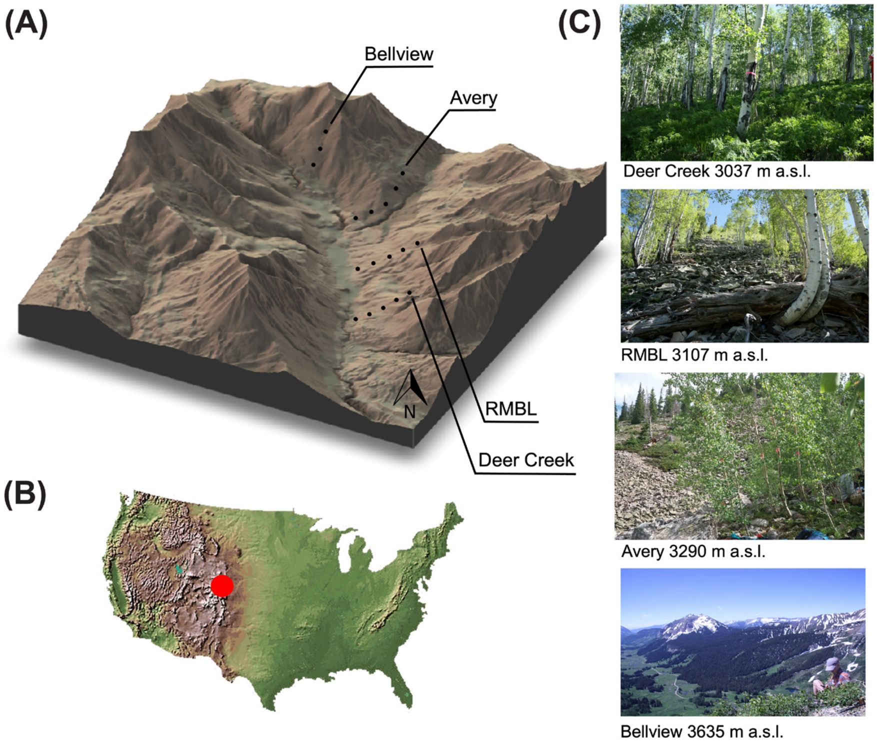

Figure 1. The study sites (A) are located in western Colorado (B) near the Rocky Mountain Biological Laboratory (RMBL). C) Four sites were selected representing low ('RMBL' 2900–3150 m a.s.l.), middle ('Deer Creek' 3000–3250 m a.s.l.), high ('Avery' 3100–3300 m a.s.l.) and alpine ('Bellview' 3270–3635 m a.s.l.) elevations. Dots are representing five sampling plots along a gradient within each site. The distances between sites ranges from ca 1.5 km to 5 km.

continuous, high-density aspen stands are located on foothill and mountain slopes from river bottom at ca 2900 m a.s.l. up to tree line at ca 3600 m a.s.l. (Fig. 1; Meier et al. 2015). Approximately 90% of the region is on sedimentary or metamorphic rock and 10% on igneous rock (Langenheim 1962).

Four sites were selected on south- to southwest-facing slopes with continuous aspen stands along an altitudinal gradient representing low ('RMBL' 2900–3150 m a.s.l.), middle ('Deer Creek' 3000–3250 m a.s.l.), high ('Avery' 3100–3300 m a.s.l.), and very high ('Bellview' 3270–3635 m a.s.l.) elevations (Fig. 1). The distances between sites ranges from ca 1.5 to 5 km.

Plot design and sampling strategy

We used a hierarchical sampling design, where five plots per site, ten trees per plot, two branches per tree, and four leaves per branch were selected for trait sampling. An exception was made for the alpine Bellview site due to limited elevational range of aspen cover. Here only three plots were selected: two plots with 10 trees and one plot with two dwarf aspen trees. In total 172 trees were selected, yielding 344 branches.

Plots were selected along a previously determined altitudinal gradient with the plots at 40 m elevational distances. A central tree was selected for each plot and used for stratified random selection of the surrounding 10 study trees by using the point polar method.

Trait measurements

Six functional leaf traits related to the LES were measured: photosynthetic capacity (A_{max}), dark respiration (R_d), specific leaf area (SLA), leaf dry-matter content (LDMC), leaf nitrogen content (LNC), and leaf lifespan (LL). A_{max}, the maximum photosynthetic rate at which leaves fix carbon measured at ambient temperature and fixed CO_2 level (390 ppm) expressed as $\mu mol\ m^{-2}\ s^{-1}$; R_d reflects the use of photosynthate, expressed as $\mu mol\ m^{-2}\ s^{-1}$. Two leaves from five trees at each plot were selected for in situ A_{max} and R_d measurements ($n = 150$); Specific leaf area (SLA, $cm^2\ g^{-1}$) (Perez-Harguindeguy et al. 2013); leaf dry-matter content (LDMC, $mg\ g^{-1}$) (Perez-Harguindeguy et al. 2013). Eight healthy adult leaves from two branches per tree (four leaves per branch) were collected for SLA and LDMC ($n = 1376$). For LNC, the nitrogen per unit of oven dry leaf mass ($mg\ g^{-1}$), we produced twenty 5 mm diameter leaf discs from additional leaves on the two collected branches per tree. These discs were oven dried and later pooled at tree level before LNC analysis ($n = 172$); LL in months was defined as the period (no. of days/30) between the end of leaf expansion and abscission (c.f. leaf photosynthetic functioning period) (Kikuzawa and Lechowicz 2011). Aspen continuously flushes its leaves throughout the season, but the bulk of the leaves are flushed at the start of the season and these are presumably the ones being measured for target traits. In addition, DBH was used as a proxy for ramet age, which is known to affect leaf traits (Smith et al. 2011). Trait means and standard deviations for each genotype, and detailed methods of functional trait collection are in the Supporting information.

Spectral measurements

Previous studies have shown that partial least-squares regression on leaf-level hyperspectral reflectance measurements provides reliable estimates of several leaf functional traits at the inter- and intraspecific level (Serbin et al. 2019, Helsen et al. 2021a, 2021b). We therefore used this method for increasing sample sizes for resource-intensive traits (LNC, R_d, A_{max}).

Leaf spectra were collected using a hand-held spectroradiometer (ASD FieldSpec HandHeld 2; ASD Inc) with a 325–1075 nm spectral range and 1.5 nm resolution. The spectrometer was fitted with a leaf clip with a built-in light source. The spectrometer was optimized every 15 min to ensure measurement quality (Wehrens and Mevik 2007). After every optimization, dark current and white reference measurements were collected to maintain calibration. Each leaf was blotted dry before measurement and the black reference panel was wiped. Three reflectance measurements per leaf were collected and averaged for eight leaves per tree on the same leaves used for SLA and LDMC measurements ($n = 1376$).

Missing data

We filled a small number of observations for missing data for several datasets using the 'MICE' (multivariate imputation by chained equations) package in R (Buuren and Groothuis-Oudshoorn 2010, www.r-project.org). Missing data were filled for: 13 values for SLA (0.9%), 1 value for LDMC (0.07%), and DBH data that were missing for one plot (10 missing observations, 0.72%). Ten imputations for each variable were performed and averaged.

Microclimatic variables

Four environmental variables were sampled to represent microclimate: average midday (10:00–14:00 h) temperature in July (°C), average midday light intensity in July (lux), gravimetric soil water content (GWC, g), and soil pH (Supporting information). Temperature and light intensity were recorded using waterproof pendant temperature/light 64K data loggers (UA-002-64) (Onset Computer Corporation). Data loggers (one for each tree, $n = 172$) with a recording interval of 5 min were placed in the tree canopies using a Bigshot Launcher (Sherrill Inc.). The sensors were placed in the middle of the aspen canopy orientated upwards and no radiation shielding was applied, in order to capture natural light levels. We used July temperature and light intensity averages to reflect the microenvironment conditions experienced by the leaves at the time of the trait expression. This ensured the environmental data are directly relevant to the traits measured, and in this context, the plant traits themselves can be considered as biotic proxies of the prevailing environmental conditions. However, we acknowledge that early-season leaf development may also play an important role in shaping trait expression. While such early-season data could be informative, they may be correlated with July conditions, and earlier measurements were not feasible due to limited site access caused by snow cover and road closures.

One soil core (2 cm diameter × 15 cm depth) was taken in a ~ 1 m radius of the base of each tree ($n = 172$). Where soils were too rocky to collect soil cores, a similar volume of soil from 0–15 cm depth was collected using a trowel. Soil samples were sealed in plastic bags and stored in insulated bags for transport to the lab, where they were stored in a refrigerator. Soils were then sieved through a 2 mm sieve, weighed, dried at 60°C for 48 h and weighed again. Soil pH was obtained with a hand-held pH meter (Handheld pH/mV/°C meter, pH 1100 H, VWR International) by using 10 g of the dried soil used for GWC. In cases where the soil sample was less than 10 g, we used the whole soil sample (Black et al. 1965).

Insect herbivory

To estimate the background herbivory rates and to be able to link them with leaf functional traits, we collected herbivory data at the end of the growing season (end of July to mid-August 2017). First, we cut two 30–50 cm large branches per tree (north and south facing) and counted the total number of leaves and how many of these leaves were herbivorized (exhibited evidence of herbivory). From the herbivorized batch of the leaves, we randomly selected five leaves, removed the petiole and scanned them at 300 dpi using a CanoScan LiDE220 color image scanner (Canon). Scanned leaves were used for determination of leaf area consumed by insect herbivores, which was estimated by reconstructing the damaged leaf margin using Adobe Photoshop. Then herbivorized leaf area was obtained using MATLAB-based image analysis code (available at https://github.com/bblonder/leafarea). The leaf area consumed (%) was calculated as the difference in area between the herbivorized leaf and the reconstructed leaf divided by the reconstructed leaf area.

Partial least squares regression (PLSR) for spectral data

LNC, A_{max} and R_d were measured on a subset of leaves; for the remaining leaves these trait values were extrapolated from the collected leaf spectra. We used a partial least square regression (PLSR) modelling approach using the *plsr* function from the 'pls' package in R ver. 3.3.2 (Wehrens and Mevik 2007, www.r-project.org), using the full spectral range of the leaves for which LNC, A_{max} and R_d were measured. Ten-fold cross-validation using 90% of the calibration dataset with 10% data as validation was adopted. The PLSR model was evaluated using root mean square error (RMSE). Predicted values for LNC were calculated using 100 components. The PLSR model for LNC had $RMSE = 0.03$ mg g^{-1} ($NRSME = 1.04\%$). Predicted values for A_{max} were calculated using 65 components and $RMSE = 3.58$ µmol m^{-2} s^{-1} ($NRSME = 0.41\%$), whereas for R_d – 30 components were used and $RMSE = 0.95$ µmol

m^{-2} s^{-1} (NRSME = 0.54%). We extrapolated 1226 values for A_{max} and R_d and 1204 values for LNC by running the PLSR model on leaf spectra.

Cytotype and genotype assessment

Cytotype (diploid or triploid) and genotype were determined for 2–3 leaves for each focal tree based on amplification of twelve unlinked microsatellites. See Blonder et al. (2020) for a detailed description of DNA extraction and microsatellite amplification. Cytotype inferred from microsatellite data has shown high confidence in previous studies of quaking aspen (Mock et al. 2008, 2012, Blonder et al. 2020). More specifically, due to high genetic diversity and heterozygosity in aspen, 97% correct classification for cytotype has been obtained using 6–10 microsatellite markers (Mock et al. 2012). Furthermore, no linkage disequilibrium was found indicating independent inheritance and limited sexual reproduction among genotypes (Blonder et al. 2020), thus allowing us to treat each genotype as a distinct genetic individual. Two markers (ORPM 206 and PMGC 2571) failed to amplify reliably, resulting in a total of ten scored microsatellite markers. Individual plants were assigned to the same genotype if all alleles for the ten markers were identical. Individual plants were furthermore defined as triploid if three alleles were observed at at least one of the ten markers. Individual plants were defined as putative diploid when a maximum of only two alleles were observed for each marker. Genotype and cytotype distribution across the study area can be seen in the Supporting information. We have two genotypes (RMBL_1 and RMBL_2) that differ in allele identities at only one locus, which might indicate that the difference is caused by genotyping error rather than by true genetic differences. However, genotypes for these two genotypes occurred over multiple individuals, with consistent allele patterns that were clearly spatially segregated over different plots, strongly suggesting that they reflect true genetic differences (Supporting information). Therefore, we retained the inference that they are separate genotypes. We identified 15 genotypes (five diploids, ten triploids).

Statistical analysis

Trait–trait and trait–environment relationships within and across aspen genotypes

All statistical analyses were performed using linear mixed-effect models (LMM) fitted with maximum likelihood (ML) using the *lmer* function from the 'lme4' package (Bates et al. 2015) in R ver. 3.3.2 (www.r-project.org). LMMs were chosen to account for the non-independence of the data due to hierarchical sampling, which were taken into account in the models as random effects (intercepts) in the following descending (nested) order: site, genotype, tree branch and leaf (residuals) (Johnson 2014).

To assess trait–trait relationships within and across aspen genotypes, one functional trait was entered as response variable, and another trait as predictor. Response and predictor variables were chosen based on the LES trait patterns presented in Wright et al. (2004) and Díaz et al. (2016), and both predictor and response variables were log-transformed. Trait combinations that were considered included: SLA-LDMC, SLA-LNC, SLA-LL, LL-LNC, SLA-A_{max}, A_{max}-LNC, A_{max}-LL, R_d-A_{max}, R_d- LL, R_d-LNC, R_d-SLA.

To assess trait–environment relationships, we performed LMMs with similar random structure as mentioned earlier, where a trait was entered as response variable and all microenvironment variables – temperature, light intensity, pH, gravimetric water content and DBH (as proxy for ramet age) were entered as fixed factors. To account for environmental variables being on different scales, both the response and all fixed factors were z-transformed. Potential collinearity issues between predictors were checked using a maximum Pearson correlation coefficient of $|r| < 0.5$ as a cut-off. All five predictors met these requirements and were kept in the models.

To determine whether there was a different slope in the correlation between the response and predictor for each genotype, we made likelihood ratio tests for both trait–trait and trait–environment relationship LMMs, where we compared a model with only random intercept 1/Site/Genotype/Tree/Branch to a model with both random intercept and random slope trait|Genotype or microclimate variable|Genotype depending on the model. Genotype slopes were extracted using the *ranef'* function from the 'lme4' package (Bates et al. 2015).

Random intercepts for all LMMs testing trait–trait relationships were 1|Site/Genotype/Tree/Branch and 1|Site/Genotype/Tree for trait–environment relationships. The exception was LL, where leaf lifespan was only measured for one to two trees per genotype, hence for which 1|Site was used as random intercept.

Trait–trait relationships

To examine whether intraspecific trait–trait relationships in aspen are consistent with the global interspecific LES pattern, we used the GLOPNET dataset from Wright et al. (2004). We obtained 4792 observations from 203 different species with 279 unique SLA values, 245 LNC values, 187 LL values, 228 A_{max} values and 216 R_d values (Table 1). We obtained the slopes of the relationships between the functional trait pairs of interest at three levels: interspecific LES slope from the GLOPNET dataset (hereafter called 'global') (Wright et al. 2004), aspen (species) level (the slope across all aspen data in our study), and the genotype (intraspecific) level (individual genotype slopes from data in our study). For each scale, we performed a general linear model (global) or an LMM as described above (aspen and genotype levels) on log-transformed data with one functional trait as predictor variable and another functional trait as response variable. We compared the slopes between functional traits to assess the change of the relationships across the three scales.

Cytotype differences and herbivory effects on trait–trait and trait–environment relationships

To test for the effects of cytotype on trait–trait and trait–environment relationships, we performed trait–trait and

Table 1.The 5–95% quantile trait range of functional traits across the scales. Scales: GLOPNET dataset (interspecific LES), functional trait values of quaking aspen *Populus tremuloides* from GLOPNET dataset and our study dataset of quaking aspen. Functional traits (SLA – specific leaf area; LDMC – leaf dry matter content; LNC – leaf nitrogen; A_{max} – photosynthetic capacity; R_d – dark respiration; LL – leaf lifespan). Data 5–95% quantile range for LDMC taken from the TRY database presented in Kattge et al. (2020).

Scale	n	SLA		LDMC		LNC		A_{max}		R_d		LL	
		$cm^2\ g^{-1}$		$g\ g^{-1}$		%		$\mu mol\ m^{-2}\ s^{-1}$		$\mu mol\ m^{-2}\ s^{-1}$		months	
		5%	95%	5%	95%	5%	95%	5%	95%	5%	95%	5%	95%
Interspecific LES (GLOPNET)	4792	55	226	0.10*	0.42*	0.91	2.54	5.95	18.3	0.62	2.42	1.38	36.0
Aspen	150	109	132	NA	NA	1.90	2.60	7.95	11.90	1.84	1.84	3.60	6.0
Genotype	172	101	174	0.32	0.48	2.30	3.41	2.49	14.58	0.48	2.98	2.53	3.2

trait–environment models as mentioned above, except we entered cytotype (diploid, triploid) and its interaction with the trait or microclimate variable as fixed factors. We used the *emtrends* function from the 'emmeans' package (Lenth 2024) to obtain the estimated marginal means and test for differences between cytotype slopes for trait–trait and trait–environment relationships.

To assess if and how trait–trait relationships change with background insect herbivory (leaf area eaten, %), we entered one functional trait as response variable, the other trait, herbivory and its interaction as fixed factors. Because herbivory was measured and averaged at the tree level, the random factors for all trait–trait pairs was 1|Site/Genotype, except 1|Site for LL.

For statistical significance interference, we assessed whether the 95% CI for each parameter included zero. However, due to multiple comparisons for trait–trait relationships, the confidence intervals should be interpreted carefully due to possible type I error, and focus should be on model estimates and biological significance.

Results

Are intraspecific (between-genotype, within-genotype) trait–trait relationships consistent with the interspecific LES, and what do they reveal about the role of phenotypic plasticity?

We examined whether intraspecific trait–trait relationships in aspen align with global patterns described by the LES. Maximum photosynthetic rate in our study reached 14 μmol $m^{-2}\ s^{-1}$ in comparison to the GLOPNET dataset maximum of 18 μmol $m^{-2}\ s^{-1}$. LNC and LDMC values in this study were high (LNC 5–95% quantile: 2.30–3.41%, LDMC: 0.32–0.48 g g^{-1}) when compared to the GLOPNET dataset (LNC: 0.91–2.54%, LDMC: 0.10–0.42 g g^{-1}; Table 1).

From the eleven leaf functional trait pairs tested in a single clonal species, we found that seven trait pairs SLA-LNC, A_{max}-SLA, A_{max}-LNC, Rd-LNC, R_d-A_{max}, R_d-SLA, A_{max}-LL showed the same directional relationships as those observed in the global interspecific LES (Fig. 2A–G). However, trait pair relationships with LL (SLA-LL, LNC-LL; Fig. 2H, I) had an opposite (positive) slope direction compared to the one expected from the interspecific LES (negative). There was no significant relationship for Rd-LL (Supporting information).

Although the direction of the relationships for SLA-LNC and R_d-A_{max} was consistent with the global LES and the aspen scale, the correlation was not statistically significant (Fig. 2). The SLA–LNC slope at the global scale was 0.95 [95% CI: 0.91; 0.98] compared to 0.03 [−0.06; 0.11] at the aspen scale; the R_d-A_{max} slope at the global scale was 0.49 [0.46; 0.51] whereas at the aspen scale it was lower 0.11 [−0.04; 0.25] (Fig. 2A, E).

For three trait pairs (A_{max}-SLA, R_d-SLA and A_{max}-LNC) the slope strengths increased at the aspen scale compared to global interspecific LES. The slope for A_{max}-SLA was −0.09 [−0.11; −0.06] at the global scale compared to −0.62 [−1.16; −0.08] at the aspen scale; for A_{max}-LNC the slope was 0.09 [0.05; 0.12] at the global scale compared to 0.68 [0.07; 1.29] at the aspen scale. Furthermore, there was no consistent relationship between the traits within genotypes (Fig. 2). For example, while there was no SLA–LNC relationship at the aspen level, the within-genotype slopes ranged from −0.15 to 0.10 (Fig. 3A). Similarly, the overall aspen slope between Amax and LNC was 0.68, and the within-genotype slopes ranged from −1.36 to 1.11 (Fig. 3B).

How do genetic factors (cytotype and genotype) affect leaf trait–trait and trait–abiotic environment relationships, and what do they reveal about the role of genetic factors in the LES?

Overall, microenvironment was a weak predictor of functional traits in aspen (Fig. 4) except in the following cases. Temperature was a positive predictor for LL slope = 0.94 [0.71; 1.16], A_{max} slope = 0.58 [0.21; 0.96], R_d slope = 0.46 [0.03; 0.89], and a negative predictor for SLA slope = −0.39 [−0.68; −0.09] (Fig. 4 A–D). Furthermore, SLA was the only functional trait for which other microenvironmental variables – light intensity, pH and proxy for ramet age (DBH) – had a strong negative effect (Fig. 4D). For LNC and LDMC none of the microclimate variables were strong predictors (Fig. 4E, F), except light intensity for LDMC (Fig. 4F). For the individual genotypes, trait–environment relationships were neither strong nor consistent in explaining trait variation (Supporting information).

We found that triploid aspen genotypes occupied larger trait (Table 2) and environment (Table 3) spaces than diploid genotypes. Diploids had a smaller SLA (5–95% quantile: 100–144 $cm^2\ g^{-1}$, n = 5), LNC (2.33–3.02%), and LL range (2.5–2.8 months) than triploids (110–171 $cm^2\ g^{-1}$,

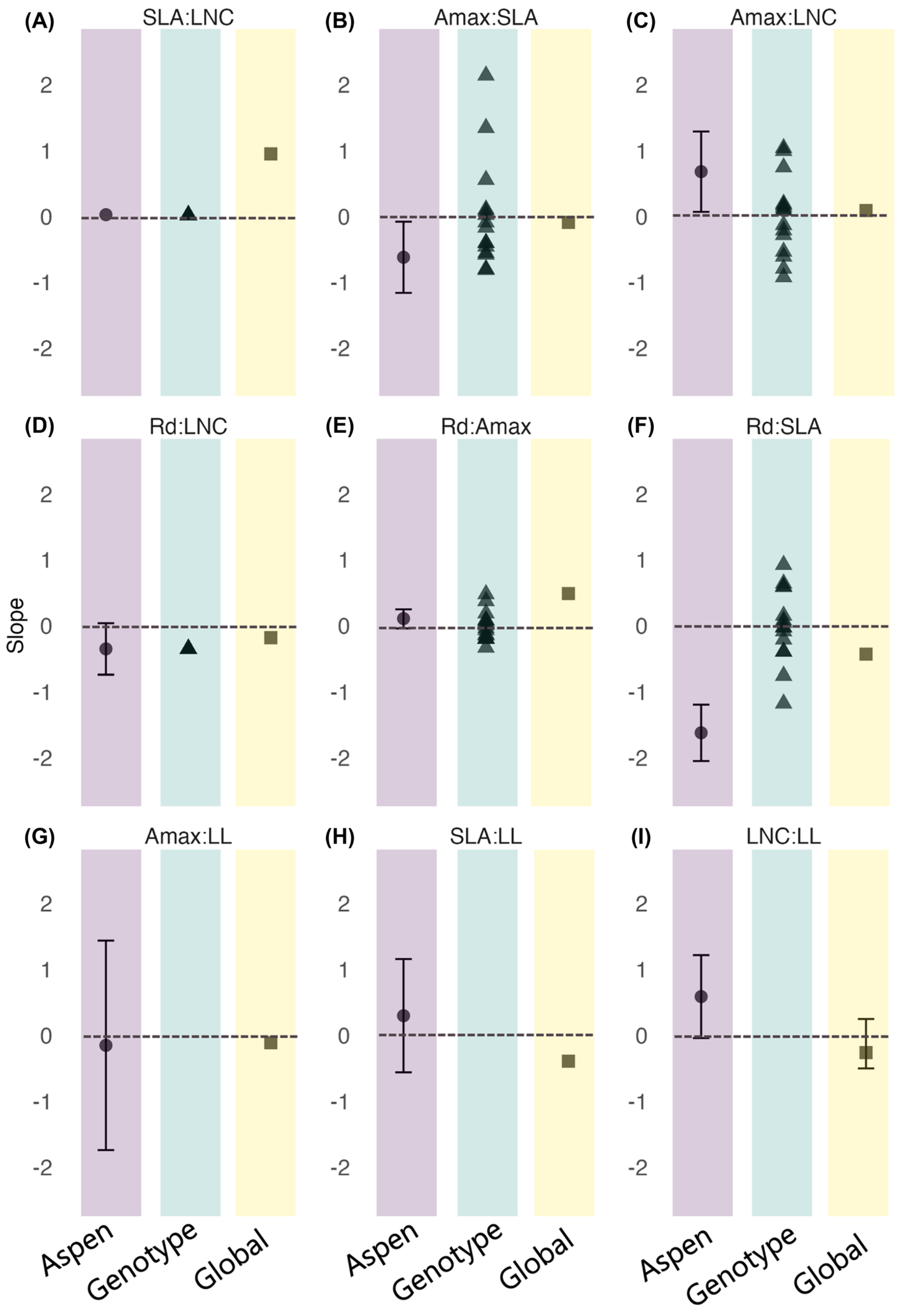

Figure 2. Trait–trait relationships of functional trait pairs across scales tested in this study. The three scales tested were: 'aspen' and 'genotype' scales from data collected this study, and 'global' (data from GLOPNET dataset from Wright et al. 2004). Slope coefficients (± CI) are extracted from the models testing each functional trait pair on log-transformed data. Functional traits: photosynthetic capacity (A_{max}, µmol m^{-2} s^{-1}; dark respiration (R_d, µmol m^{-2} s^{-1}), leaf nitrogen content (LNC, mg g^{-1}), specific leaf area (SLA, cm^2 g^{-1}), leaf dry matter content (LDMC, g g^{-1}), leaf lifespan (LL, months).

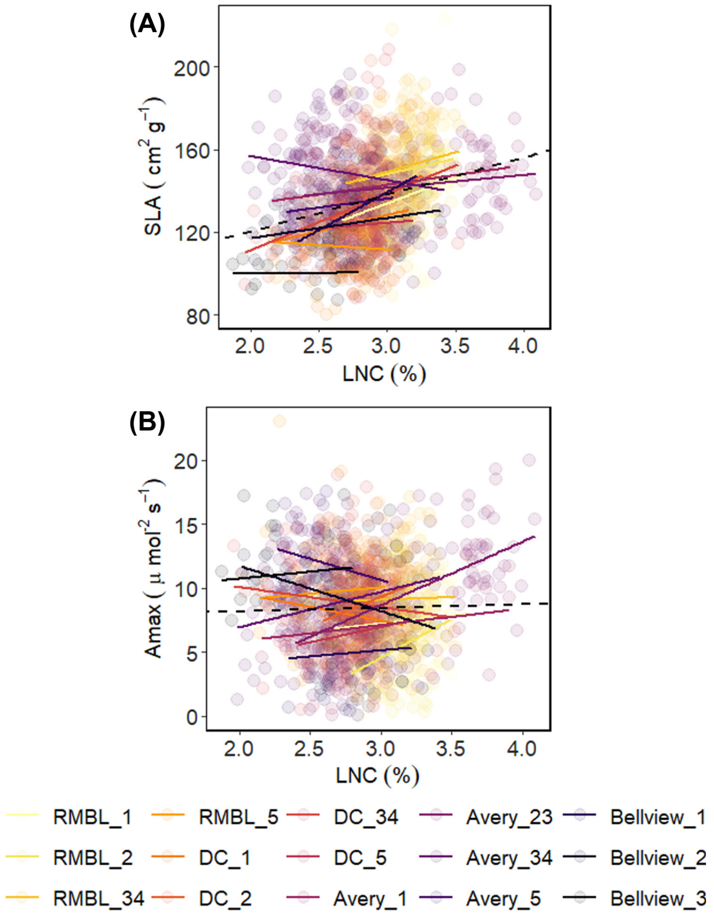

Figure 3. Trait–trait relationships for 15 individual genotypes. (A) The relationships between leaf nitrogen (LNC) and specific leaf area (SLA); (B) the relationships between LNC and photosynthetic capacity (A_{max}). Each solid coloured line defines one aspen genotype; the dashed line indicates the overall aspen slope from this study. The relationship between the functional trait pairs is presented also as raw data.

2.41–3.40%, and 2.5–3.2 months, respectively, n = 10). For environmental variables such as temperature, the 5–95% quantile for temperature in diploids was 10.2–12.5°C, while in triploids it was 10.5–13.2°C.

There were opposite-sign trait–trait relationships between diploids and triploids (Supporting information) for three trait–trait pairs: SLA–LL and Rd–A_{max}, and A_{max}–LNC. For triploids, no relationship between SLA and LL was observed (slope: −0.38 [−1.03; 0.28]), whereas for diploids SLA increased with increasing LL (slope: 1.67 [0.14; 3.20]) (Fig. 5A). For triploids A_{max} increased positively with increasing LNC with the slope value of 1.17 [0.73; 1.61], whereas no consistent correlation occurs for diploids −0.34 [−1.18; 0.50] (Fig. 5B).

Diploids and triploids differed in their responses to microenvironment (Supporting information). For example, for diploids no consistent correlation was observed between LDMC and pH (slope: −0.42 [−1.12; 0.29]) whereas for triploids it was positive (slope: 0.42 [0.06; 0.77]); Supporting information). R_d and pH were positively correlated for diploids (slope: 1.25 [0.38; 2.13]), whereas no relationship was found for triploids (slope: 0.29 [−0.17; 0.76]). Soil moisture had a negative effect on R_d for both diploids and triploids, but the strength of the slope was stronger for diploids (slope diploids: −2.29 [−4.2; −0.4], slope triploids: −0.96 [−1.9; −0.1]; Supporting information).

Does the biotic environment (insect herbivory) also alter leaf trait–trait relationships in aspen?

Herbivory altered several trait–trait relationships (Supporting information). We found a negative effect of herbivory on A_{max}–SLA and R_d–LNC, and a positive effect of herbivory on LDMC-LL trait pairs (Fig. 6A–C) and, although not significant, also on A_{max}–LNC (Fig. 6D). As the % of leaf area eaten increased, the relationship between A_{max} and SLA became significantly more negative (Fig. 6A). On the other hand, the A_{max}–LNC relationship became weaker (Fig. 6D).

Discussion

We assessed how genotype, cytotype, abiotic environment, and biotic environment (insect herbivory) influenced the intraspecific LES in natural mature populations of quaking

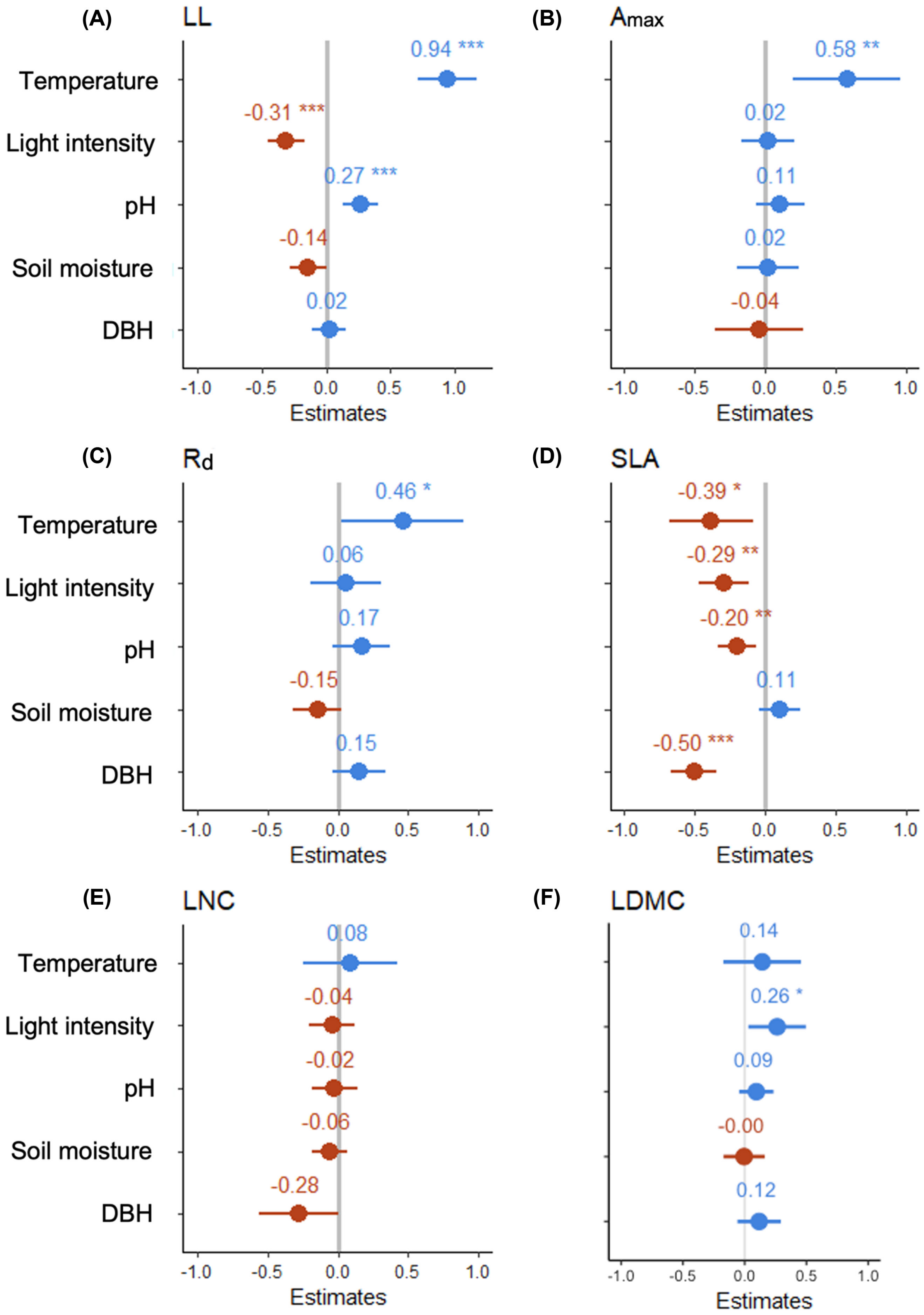

Figure 4. Coefficient plots from multiple regression testing trait–environment relationships for quaking aspen. Five microenvironment variables (y-axis) were tested to explain the functional traits (A) leaf lifespan (LL), (B) A_{max} (photosynthetic capacity); (C) R_d (dark respiration); (D) SLA (specific leaf area); (E) LNC (leaf lifespan); (F) LDMC (leaf dry matter content). Both the response variables and predictor variables (microenvironment variables) were z-transformed during the analysis. Results are shown as mean + 95% confidence intervals from the linear mixed effect models, and the p-value above the mean (colours indicate the positive (blue) or negative (red) effect of microenvironment variables on functional traits).

Table 2.The 5–95% quantile trait range of functional traits for diploids and triploids. Functional traits (SLA – specific leaf area; LDMC – leaf dry matter content; LNC – leaf nitrogen; A_{max} – photosynthetic capacity; R_d – dark respiration; LL – leaf lifespan).

Cytotype	n	SLA		LDMC		LNC		A_{max}		Rd		LL	
		$cm^2\ g^{-1}$		$g\ g^{-1}$		%		$µmol\ m^{-2}\ s^{-1}$		$µmol\ m^{-2}\ s^{-1}$		months	
		5%	95%	5%	95%	5%	95%	5%	95%	5%	95%	5%	95%
Diploid	42	100	144	0.40	0.50	2.33	3.02	3.10	13.7	0.71	2.63	2.5	2.8
Triploid	130	111	171	0.33	0.44	2.41	3.39	4.20	12.8	0.91	2.64	2.5	3.2

aspen. Our in situ approach allowed us to fully represent the complexity of factors driving trait variation in this species.

Trait covariation within species: phenotypic plasticity and genotypic contributions to the relaxation of the LES

For the first research question, we found that within quaking aspen: 1) several functional trait pairs were not consistent with the interspecific LES at all scales; 2) for many functional trait pairs there was no evidence of trait covariation which is found at the interspecific LES; 3) some trait pair relationships were opposite, stronger, or weaker than the same relationships in the interspecific LES; 4) across genotypes there was no consistency in the trait–trait and trait–environment relationships.

The strength of the trait relationships usually decreases at smaller spatial scales, which is often associated with smaller environmental heterogeneity and therefore smaller trait coverage, that can lead to weaker or absent LES relationships (Messier et al. 2017, Anderegg et al. 2018, Xiong and Flexas 2018, Umaña and Swenson 2019, Ramírez-Valiente et al. 2020). While our data range for this study covered a considerable fraction of the global trait variation for aspen, we found that some functional trait pairs had absent or weak relationships. Funk and Cornwell (2013) argued that the strength between two LES traits and other traits will be stronger with sufficient variation in leaf lifespan. We were not able to falsify this hypothesis. Data collected in this study show 2% (2.5–3.2 months) coverage of the leaf lifespan in aspen in comparison with the interspecific global LES (1.38–36 months). Thus, non-LL factors are likely more important at these scales.

Reversed relationships at the intraspecific level are suggested to be a result of genetic differentiation and plasticity (Anderegg et al. 2018). In this study, we were able to work with multiple genotypes to investigate trait relationships both among and within genotypes. Among the aspen genotypes, we did not find any consistency in whether individual genotype slopes were stronger or weaker than the interspecific LES or aspen slopes for almost all functional trait pairs. The contrasting trait–trait responses among genotypes may reflect genotype-specific adaptations to the microenvironment and biotic conditions they occupy (Agrawal 2020). Individual genotypes can exhibit distinct physiological and morphological trait relationships depending on the local conditions they experience, such as light availability, soil moisture, or herbivore pressure. This variation is not necessarily unique to aspen, but in clonal species like aspen, where genetically identical individuals can occupy diverse environments, such plasticity may be particularly pronounced. These genotype-specific trait configurations could enhance population-level resilience by enabling different genotypes to respond optimally to varying stressors – such as drought or insect outbreaks – which are expected to increase in frequency and severity under climate change (Worrall et al. 2013, Chen et al. 2018).

Our study showed that trait–trait relationships in aspen differed from the interspecific LES at the global scale (Wright et al. 2004). At the interspecific level, lower values of SLA, R_d and A_{max} and longer LL are associated with conservative ecological strategies, where longer leaf lifespans are needed to pay back their construction cost (Kikuzawa and Lechowicz 2011). Indeed, for deciduous species, short growing seasons at higher altitudes, nutrient limitation, lower temperatures and, in general, more stressful environments all are disadvantageous for plant growth. The foliar function, and thus the growing season, sets the limit for LL. In response, plants drop their leaves at the end of growing season, to avoid maintenance costs during the unfavourable period for photosynthesis (Kikuzawa and Lechowicz 2011, Caldararu et al. 2014). For deciduous species such as aspen, longer growing seasons are potentially associated with less stressful environments, higher temperatures, higher nutrient concentrations, and the ability to construct more expensive leaves and thus longer LL (Meier et al. 2015). However, recently, climate-manipulation experiments in deciduous tree species showed increased productivity during the growing season that led to earlier leaf abscission (Zani et al. 2020). Other studies, on the other hand, did not find significant relationships between productivity, growing season, and leaf senescence (Lu and Keenan 2022). This observational study was not able to assess

Table 3.The 5–95% quantile trait range of microenvironment variables for diploids and triploids.

Cytotype	n	Temperature		Light intensity		Soil moisture		pH	
		°C		Lux		%		–	
		5%	95%	5%	95%	5%	95%	5%	95%
Diploid	42	10.2	12.5	5136	10 350	0.00	0.67	5.6	5.9
Triploid	130	10.5	13.2	3840	11 441	0.15	0.70	5.7	6.7

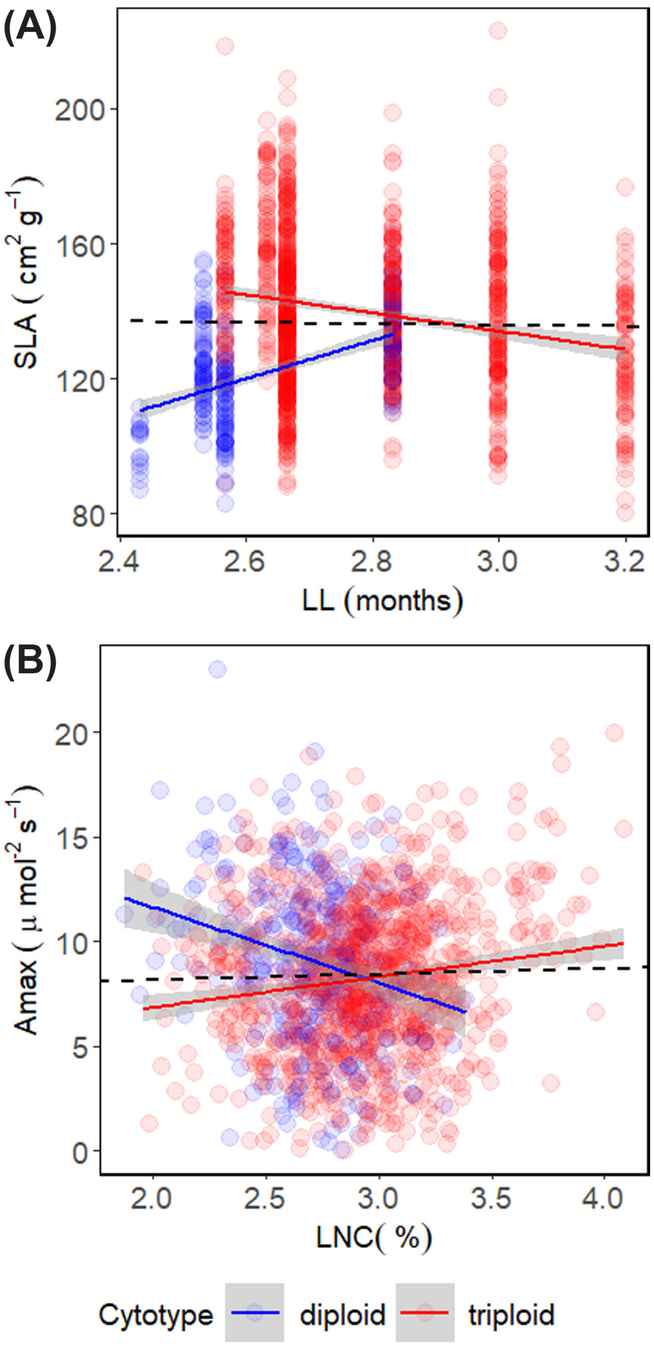

Figure 5. Cytotype level differences for trait–trait relationships for diploids and triploids. (A) The relationship between leaf lifespan (LL) and specific leaf area (SLA); (B) the relationship between LNC and photosynthetic capacity (A_{max}). Each solid coloured defines cytotype; the dashed line indicates the overall aspen slope from this study. The relationship between the functional trait pairs is presented as raw data.

the physiological mechanisms underlying LL variation, but it is clear that coordination of LL with other traits and environmental variables can vary substantially within species.

Given that quaking aspen is the most widely distributed tree species in North America with a continental distribution spanning diverse climates and ecosystems, it is possible that LES patterns may emerge at the broader scales. However, our study focused on a highly localized context, where intraspecific variation – driven by genotype, cytotype, and biotic interactions such as herbivory – which may override broader-scale trait coordination.

While our observational study was limited to a single growing season and did not assess the physiological mechanisms underlying trait variation, it provides a valuable snapshot of how functional traits, including LL, coordinates with other traits and environmental variables across spatial gradients. We acknowledge that temporal dynamics, such as interannual variability in climate and phenology, may also play a significant role in shaping these relationships, and future studies incorporating multi-year data would be essential to fully understand the drivers of variation within species.

Cytotype and genotype effects on trait–environment and trait–trait relationships

Abiotic factors – temperature being among the major drivers of plant traits globally – directly influence plant physiological processes such as metabolic rate, growth rate, and energy balance (Reich and Oleksyn 2004, Moles et al. 2014). However, we did not find that microenvironment explained functional trait variation for most of the functional traits in aspen. This could be the result of varying ecological strategies among genotypes within a species, e.g. both positive and negative genotype responses to the same environmental variable, which act to weaken the overall species–scale relationship (Moles et al. 2014) or a high heritability and low plasticity in functional traits (Geber and Griffen 2003) that might be non-responsive to the microclimatic variables we measured.

Alternatively, we may not have measured the relevant abiotic factors (Maclean et al. 2021) over the relevant spatial, temporal scales or with due respect to life stage responses. For example, the seedlings of aspen are more vulnerable to variations in microclimate variation than adult trees (Landhäusser et al. 2019). It is also possible that the trait responses occur on longer timescales than those captured in our single-season study and thus may not align with the temporal resolution of our environmental data. Detailed edaphic measurements would enable us to better understand the role of microclimate in intraspecific functional trait variation. Furhermore, the temporal and spatial scales of our study, limited to one region and one year, may not have captured the full climatic niche, potentially affecting the detection of trait–microclimate responses.

Cytotypes differ in the environments they occupy and the traits they exhibit (Li et al. 1996, Van de Peer et al. 2021). Polyploidy can be a key trait in the resilience of organisms facing environmental change (Van de Peer et al. 2021, 2017). Trait differences in cytotypes have been shown in many plant species, including aspen (Greer et al. 2017, Wei et al. 2019); however, the trait variation in relation to the LES have not (DeRose et al. 2022). This study was able to show the covariation between traits in relation to differences in cytotype. Our measurements and analysis support previous research of

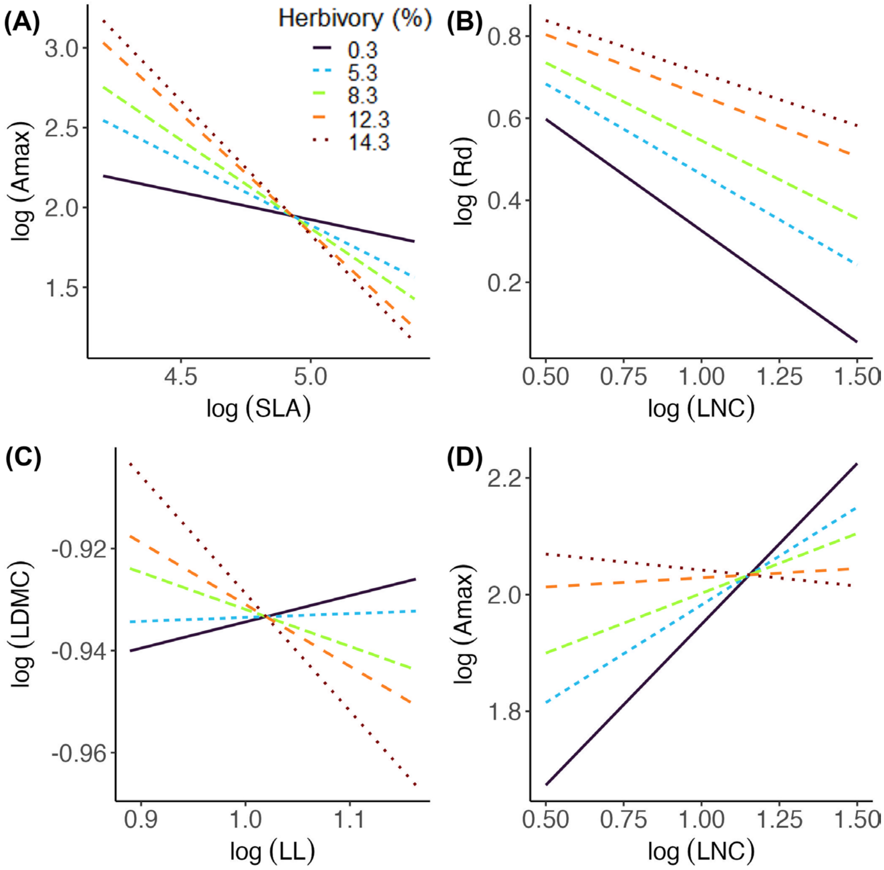

Figure 6. Modelled herbivory effects on trait–trait relationship. Data are presented from linear mixed effect models for four trait pairs, where herbivory altered trait–trait relationship. (A) the relationship between photosynthetic capacity (A_{max}) and specific leaf area (SLA). (B) the relationship between A_{max} and leaf nitrogen (LNC). (C) the relationship between dark respiration (R_d) and LNC. D) the relationship between leaf dry matter content (LDMC) and leaf lifespan (LL). Each slope represents the percentage of the leaf area eaten that varies from 0.30 to 14% of the leaf area removed.

diploid and triploid aspen genotypes differing in morphology and function (Greer et al. 2017, DeRose et al. 2022). First, we found that diploids occupied the part of environmental gradient that is associated with lower temperature, lower soil moisture, higher pH and higher light intensity than triploids, consistent with Mock et al. (2012) and Blonder et al. (2021, 2022). Second, we observed differences in functional traits: diploids had lower SLA and LNC and higher LDMC compared to triploid aspen genotypes, consistent with Greer et al. (2017) and Blonder et al. (2022). DeRose et al. (2022) found that triploids have higher concentrations of phenolic glycosides which protect against herbivory and might give them a growth advantage over the long term.

Cytotype also influenced trait–trait relationships. For example, diploids and triploids had opposite SLA–LL relationships. For triploids, no relationship was found between SLA and leaf lifespan, whereas for diploids SLA increased with increasing leaf lifespan. On the contrary, while for triploids LNC increased with increasing A_{max}, no relationship was found between these traits for diploids. We found opposite directions for cytotype slopes for several trait–environment pairs. These results suggest that cytotypes in aspen have different tradeoffs related to resource acquisition, diploids being more resource-conservative and triploids mostly resource-acquisitive. However, the smaller sample size for diploids (24% of all individuals genotyped versus 76% for triploids) could have resulted in absent, weaker, or fewer trait–trait or trait–environment relationships for diploids. But even with this smaller sample size we were able to depict differences in ecological strategies and trade-offs for diploids and triploids.

Triploid aspen genotypes had a larger range in trait and environment values, likely facilitated by environmental heterogeneity. This finding might indicate more plasticity in triploid aspen genotypes than diploid genotypes. While triploids are thought to have lower fertility than diploids (Jackson 1976, Zielinski and Mittelsten Scheid 2012), their high phenotypic plasticity may enable high long-term survival over temporally variable environmental conditions they experience.

Biotic drivers of trait covariation: altered trait relationships under increasing background insect herbivory levels

Trait relationships are often embedded within a complex network of trade-offs involving multiple, and often unknown, traits, which can influence the observed pairwise correlations

(Agrawal 2020), and which may be sensitive to the environmental drivers, both abiotic and biotic. In this study we found that one biotic environment variable influenced trait–trait relationships. With increasing herbivory, aspen increased photosynthesis and respiration at a lower rate. While we did not directly measure defence compounds or nitrogen allocation, this pattern may be consistent with shift in resource investment away from growth-related processes and toward defence. This interpretation is supported by previous research showing that the cost for defence in aspen generally is high (Kruger et al. 2020). For example, main defence compounds in aspen such as salicinoid phenolic glycosides (SPGs) and condensed tannins can contribute up to 20% of the leaf dry mass, and the contribution for SPGs increases when plants are under herbivory stress (Kruger et al. 2020, Lindroth et al. 2023). Such a substantial investment suggests that shifts in resource allocation under herbivory significantly alter trait–trait relationships.

Our results show that background herbivory can substantially alter or reverse the trait–trait relationships. However, defence against herbivores in aspen varies strongly in response to genotype (Stevens and Lindroth 2005, Rubert-Nason et al. 2015, Lindroth et al. 2023) and nutrient availability and this variation could not be accounted for in our study (Donaldson and Lindroth 2007). Robust herbivory surveys and collection of defence traits, which are investigated together with functional traits and their variation with genotype, are crucial. The potential changes in chemical traits in response to herbivory that might alter trait–trait relationships, indicate complex interactions, and further emphasize the need for linking defence (Defossez et al. 2018) and chemical traits into trait-based ecology (Müller and Junker 2022).

Conclusion

Our study demonstrated that trait relationships central to the LES substantially weakened at the intraspecific scales in quaking aspen. Our findings suggest that interspecific LES patterns are shaped by a complex interplay of genetic factors (genotype, cytotype) and phenotypic plasticity, leading to relaxation of the globally observed LES tradeoffs at local scales. Rather than occupying fixed positions in trait space, aspen genotypes exhibited diverse and context-dependent trait values. The overall species-level pattern is the outcome of a complex interplay between the genetic variation and plastic responses to biotic and abiotic environment. The relaxation of the LES tradeoffs at local scales may enable more flexible responses to environmental stressors, through both genetic and plastic mechanisms.

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Author contributions

Jolanta Rieksta: Conceptualization (lead); Data curation (lead); Formal analysis (lead); Investigation (lead); Methodology (equal); Project administration (lead); Resources (equal); Software (lead); Validation (equal); Visualization (lead); Writing – original draft (lead); Writing – review and editing (lead). **Bente J. Graae**: Data curation (supporting); Funding acquisition (lead); Resources (equal); Supervision (supporting); Writing – review and editing (equal). **Kenny Helsen**: Data curation (equal); Formal analysis (equal); Methodology (supporting); Validation (equal); Writing – review and editing (equal). **Rozália E. Kapás**: Data curation (supporting); Formal analysis (supporting); Writing – review and editing (supporting). **Richard Strimbeck**: Data curation (supporting); Funding acquisition (lead); Methodology (equal); Resources (equal); Supervision (lead); Writing – review and editing (equal). **Benjamin Blonder**: Conceptualization (equal); Data curation (supporting); Funding acquisition (lead); Resources (equal); Supervision (lead); Validation (supporting); Writing – review and editing (equal).

Data availability statement

Data for assessing relationships between traits at the interspecific scale are from GLOPNET (Wright et al. 2004) and were accessed via TRY database (Kattge et al. 2020).

Data are available from the Figshare: https://figshare.com/s/1a56f9f98ba2e8e6af51 (Rieksta et al. 2025).

Supporting information

The Supporting information associated with this article is available with the online version.

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