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Abstract

1. The leaf economic spectrum (LES) describes consistent and strong correlations among plant functional traits in a multidimensional trait space, which reflects fast or slow rate of return on carbon investment in leaves. These relationships between LES functional traits are strong at global scales and consistent across species and biomes. However, there is evidence that the LES relationships may be weak or absent at smaller scales due to different factors affecting traits locally.

2. Here we quantify: (1) the intraspecific functional trait variation within a model clonal species, *Populus tremuloides*; (2) how important is microclimate in determining intraspecific trait variation; (3) whether and how much trait-trait, trait-environment relationships in this species are consistent with global interspecific LES patterns; (4) how trait – trait and trait – environment relationships change with ploidy level. We collected leaf functional traits from 15 aspen clones using a hierarchical sampling design along a large environmental gradient in Colorado, we analyzed trait - trait and trait – environment relationships within and across *Populus tremuloides* genotypes and how these relationships changes with ploidy level.

3. We found: (1) the highest variation within clones, indicating high plasticity; (2) opposite of what we would expect, microclimate was a weak predictor of functional traits (3) trait – trait relationships at the intraspecific scale were not consistent with the LES; (4) we found significant differences in physiology and shifts in resource-use trade-offs in trait – trait and trait – environment relationships for diploids and triploids.

4. *Synthesis.* We conclude that the LES does not hold at the intraspecific scale for Quaking aspen, meaning that at finer scales there are not strong constraints determining the strategies plants can use. These findings also show that the ploidy level can affect and shift the LES trait – environment relationships and that microenvironment which is thought to be direct driver of trait variation at finer scales, does not predict trait variation at the intraspecific scale for Quaking aspen functional traits.

Contents

Introduction

The leaf economic spectrum (LES) describes consistent and strong correlations among plant functional traits in a multidimensional trait space (Reich et al., 1997; Reich and Oleksyn, 2004; Wright et al., 2004; Wright et al., 2005). The LES reflects the ecological trade-offs and constraints of leaves around the resource use. Particularly, the LES reflects fast or slow rate of return on carbon investment in leaves (Reich et al., 1997; Wright et al., 2004). Key traits associated with the LES are: specific leaf area (SLA), leaf dry matter content (LDMC), photosynthetic capacity (A_{max}), leaf nitrogen content (LNC), leaf phosphorous (LNP), dark respiration (R_d) and leaf lifespan (LL) (Reich et al., 1997; Reich et al., 1999; Wright et al., 2004; Wright et al., 2005). On the one side of the spectrum there are leaves with high photosynthetic rates, high SLA, high leaf N concentrations and short leaf lifespans. These leaves are associated with fast turnover of carbon investment in leaves and express a resource-acquisitive or "live fast, die young" strategy. On the other end of the spectrum are leaves with low photosynthetic rates, low SLA and N concentrations and long leaf lifespans and thus associated with a resource-conservative or "live long and prosper" ecological strategy (Reich et al., 1997; Reich et al., 1998; Reich et al., 1999; Wright et al., 2004). These strategies are based on the assumption that more expensive leaves per unit mass requires more time to pay back its construction cost than do leaves with smaller construction cost (Chabot and Hicks, 1982; Kikuzawa and Lechowicz, 2011). These relationships or correlations between the LES functional traits are strong at global scales and consistent across species and biomes (Reich et al., 1997; Wright et al., 2004). The main reason for the strong LES trait correlations at the global scale is assumed to rely on leaf level biophysical constraints that lead to mandatory relationships between traits (Reich et al., 1997). For example, A_{max} often increases linearly with nitrogen per unit area, since the proteins of photosynthetic machinery are nitrogen dependent, especially Rubisco protein (Lambers et al., 2008). At the same time, with increasing photosynthesis, the use of photosynthate increases due to maintenance costs, which leads to high respiration (Reich et al., 1998).

However, there is evidence that LES relationships may be weak or absent at smaller scales because abiotic and biotic factors affecting trait variation locally might be different at larger scales, which in turn can blur the LES patterns (Armbruster et al., 2007; Funk and Cornwell, 2013; Messier et al., 2017). Besides that, the LES is usually applied to compare trait relationships across species and thus assuming fixed traits at the species level. Therefore,

there is still a need for evidence to strengthen this hypothesis and understand why contrasting LES patterns are observed at smaller scales. This study is interested in the LES variation at the intraspecific level i.e. within one clonal species Accordingly, there are four important questions to understand.

Question 1: How is trait variation partitioned among different ecological scales for a single clonal species (leaf, branch, tree, clone, site)? To understand why the LES patterns may not hold at smaller scales, there is a need to understand what is causing trait variation at different spatial scales and where most of the variation is found. Many studies in trait-based ecology emphasize intraspecific functional trait variation and how it is partitioned across scales, because it allows us to understand where most of the trait variation is found and what factors are causing it (Albert et al., 2010; Messier et al., 2010; Violle et al., 2012; Niinemets, 2015; Messier et al., 2017; Fajardo and Siefert, 2018). The variation at smaller scales is affected both by genotype and environment, and clonal species can give us insight into understanding how important plasticity might be relative to local adaptation in intraspecific trait variation.

Question 2: How important is microclimate in determining intraspecific trait variation? This question is particularly interesting in the LES context, because the LES assumes modest effect of environment on leaf trait variation globally (Wright et al., 2004). However, we know that some of the trait variation we observe at smaller scales is in direct response to various local factors rather than responses to regional means (Armbruster et al., 2007; Stark et al., 2017). For example, the variation we observe at the leaf level can directly reflects the plastic responses to varying light intensity in the tree canopy (Kikuzawa and Lechowicz, 2011). Individual variation in the same species can reflect genetic differences between individuals, developmental stochasticity, age differences between individuals (Messier et al., 2017). Individual variation can also reflect plastic responses to the microclimatic variation due small-scale topography and structure, which creates a fine-scale mosaic of microclimates (Armbruster et al., 2007).

Question 3: Are trait - trait, trait - environment relationships at each ecological scale consistent with the global interspecific LES patterns? We know that both plasticity and selective pressures can shape the physiology of traits and functionality of organisms occupying these local environments, that could in turn shape the expected trait - trait and trait - environment relationships (Sultan, 1995; Armbruster et al., 2007; Blonder et al., 2013; Opedal et al., 2015; Stark et al., 2017; Anderegg et al., 2018). For example, plasticity can reverse trait – trait relationships at the leaf and individual level (Blonder et al., 2013; Anderegg

et al., 2018). It could also be that natural selection acting on individual clones, leading to genotype specific responses to microenvironment (Sultan, 1995). On the other hand, if strong underlying genetic mechanisms control traits, that can lead to coordinated responses of traits at multiple scales (Shipley et al., 2006; Vasseur et al., 2012). Several possible scenarios make it important to assess whether the LES patterns hold also at the intraspecific scale.

Question 4: How do trait - trait and trait - environment relationships change with ploidy level and genotype? Polyploidy is interesting in the LES context as well, because polyploidy can alter gene expression, which in turn can affect the plant morphological and functional traits, which in turn drive plant functional trait – environment relationships. Therefore, it is very important to incorporate ploidy level in studies that address questions of trait - trait and trait - environment relationships (Greer et al., 2017).

To answer these questions, we need a study organism where separation of variation caused by environment and genotype is possible. The latter is possible with a broad species range encompassing substantial microclimatic variation and organism where polyploidy is common. Clonal species allow separation of the variation caused by environment and genotype. Quaking aspen (*Populus tremuloides* [Salicaceae]) occurs over large geographical (from central Mexico to northern Alaska), environmental (from valleys up to steep talus slopes) and altitudinal (0-3700 m.a.s.l.) gradients and therefore is an ideal study system to answer questions 1-3 (Morgan, 1969; Keddy, 1992; Mock et al., 2008; Mock et al., 2012; Meier et al., 2015; Greer et al., 2017) (Fig.1, Fig.2).

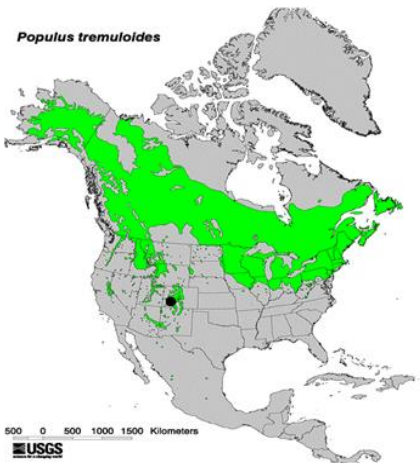

Figure 1 Range of Populus Tremuloides and the location of study area marked in black. Obtained from U.S. Geological Survey – Digital representation of "Atlas of United States Trees" by Elbert L. Little Jr.

Quaking aspen is a dominant species throughout the landscape of the Rocky Mountains in North America (Morgan, 1969; Barnes, 1975; Clair et al., 2010; Meier et al., 2015). The species is capable of reproducing both sexually and asexually by root suckers, also called clonal growth (Mitton and Grant, 1996). Clonal growth is considered as an adaptation to high altitudes and xeric conditions and it 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). Aspen is also known for its variation in ploidy level, with diploids and triploids being the most common chromosomal sets, giving us the possibility to answer Question 4 (Mock et al., 2012; Greer et al., 2017). Polyploidy is common in plant species, however, most of the species do not have a mix of different ploidy level individuals occupying same geographical area (Soltis and Soltis, 1995). Polyploidy in aspen is particularly interesting in the LES context for two reasons. First, it presents the opportunity to study diploids and triploids simultaneously along altitudinal gradient of the same geographic area, which is not common for most of the plants. Second, diploids and triploids occupy different environmental spaces: triploids occupy lower altitudes with higher temperatures, as opposed to diploids, which are found at high altitudes, which is associated with lower temperatures (Mock et al., 2012).

By collecting leaf functional traits from 15 aspen clones using a hierarchical sampling design along a large environmental gradient in Colorado, we analyzed trait - trait and trait – environment relationships within and across *Populus tremuloides* genotypes and how these relationships changed with ploidy level.

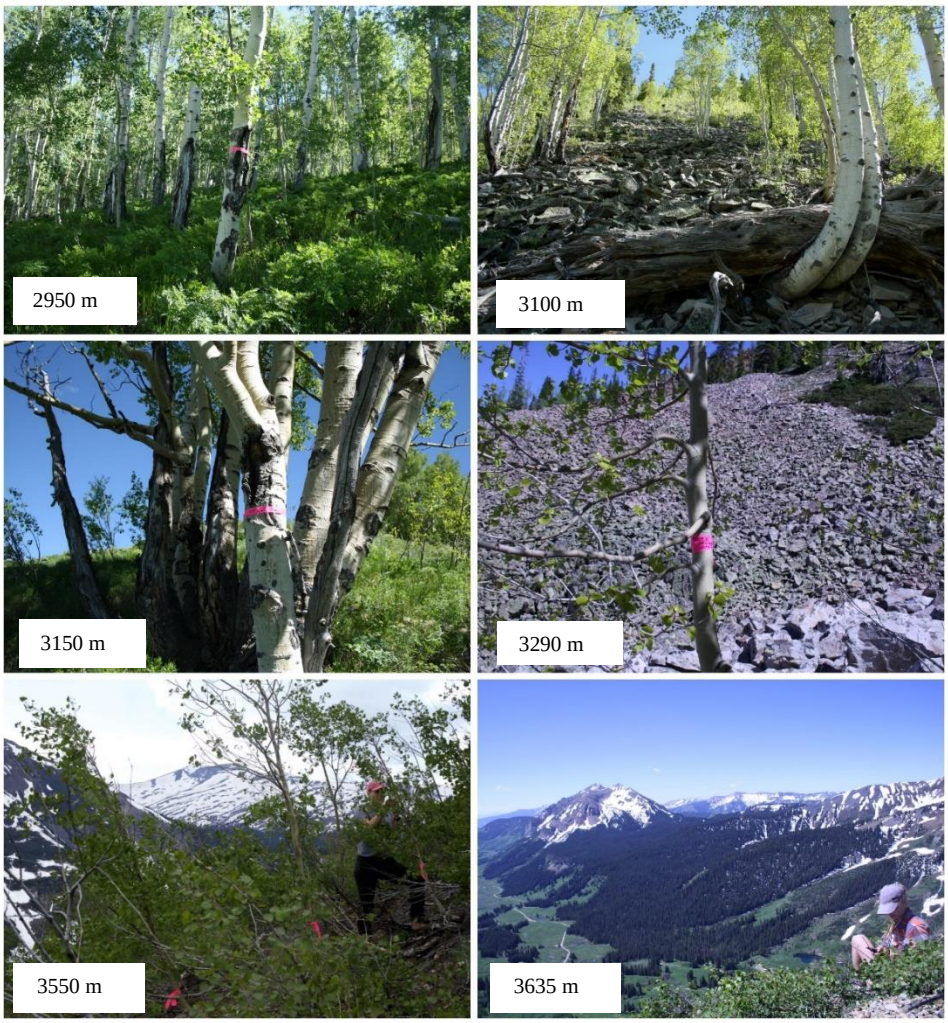

Figure 2 The diversity of aspen clones spanning the altitudinal gradient and experiencing various microclimates in the Gothic area, Rocky Mountains, Colorado.

Materials and methods

Study area and field sites

The study was carried out in the Gunnison National Forest, Gothic, the Rocky Mountain Biological Laboratory (RMBL), Colorado (latitude N 38.5°, 106° W, elevation: 2900 – 3635 m above sea level) (Fig.3). Monthly mean temperatures in the study area range from -9.5℃ to -4.7℃ and from 10.5 ℃ to 12.5℃ for the coldest and warmest months, respectively (W.R.C., 2018). The area is characterized as a heterogeneous mosaic of Engelmann spruce-subalpine fir forests, aspen forests and subalpine meadows (Morgan, 1969). Large, continuous, high-density aspen stands are located on foothill and mountain slopes from river bottom at ca. 2900 m up to tree line ca. 3600 m (Meier et al., 2015). Ca. 90% of the area in Gunnison National forest is on sedimentary rock and ca. 10 % on igneous rock (Langenheim, 1962).

Four sites on S to SW facing slopes with continuous aspen stands along an altitudinal gradient were selected: representing low (Gothic 2900 – 3150 m), middle (Deer Creek 3000 – 3250 m), high (Avery 3100 – 3300m) and alpine (Bellview 3270 – 3635 m) elevations (Fig.3). (Fig.2, Table 1). The distance between sites ranges from ca. 1.5 km to 5 km.

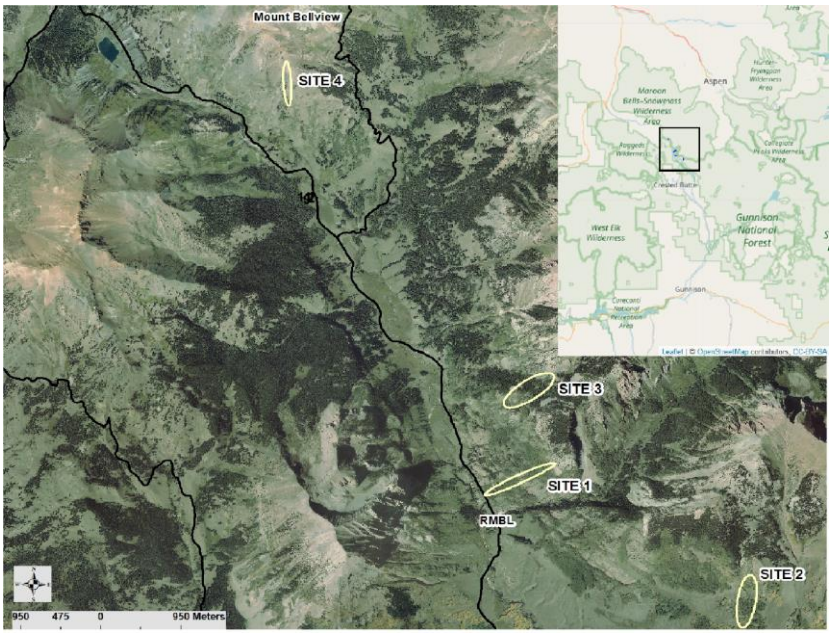

Figure 3 Map of location of 4 study sites in the Gunnison National Forest, Gothic, the Rocky Mountain Biological Laboratory (RMBL), representing low elevation (Site 1), middle (Site 2), high (Site 3), alpine (Site 4).

Table 1 Latitudes, longitudes, and elevations of the study sites

Site name	Site nr.	Plot nr.	Elevation range for site (m.a.s.l.)	Coordinates at the center of the plot (Lat, Long)	Elevation at center of the plot (m.a.s.l.)
Gothic (low)	1	1	2924-3127	38.96105, -106.98800	2952
	1	2		38.96200, -106.98559	2991
	1	3		38.96256, -106.98390	3030
	1	4		38.96294, -106.98250	3072
	1	5		38.96351, -106.98160	3107
Deer Creek (middle)	2	1	3013-3208	38.94659, -106.95370	3037
	2	2		38.94792, -106.95400	3071
	2	3		38.94844, -106.95409	3091
	2	4		38.94925, -106.95440	3132
	2	5		38.95092, -106.95380	3196
Avery (high)	3	1	3116-3295	38.97050, -106.98649	3142
	3	2		38.97061, -106.98579	3172
	3	3		38.97184, -106.98430	3217
	3	4		38.97325, -106.98220	3260
	3	5		38.97384, -106.9818	3290
Bellview (alpine)	4	1	3271-3635	39.00464, -107.01792	3272
	4	2		39.00889, -107.01839	3550
	4	3		39.01050, -107.01900	3635

Plot design and sampling strategy

A hierarchical sampling design was used, where five plots per site, ten trees per plot, two branches per tree, and four leaves per branch were selected. An exception was made for the alpine Bellview site due limited elevational range of aspen cover. Here only three plots were selected: two "low" elevation plots with 10 trees and one high elevation plot with two dwarf aspen trees. In total 172 trees were selected, yielding sampling of 344 branches and 1376 leaves.

Each plot consists of a randomly chosen tree along the altitudinal gradient with a previously determined altitude (40 m altitudinal distance between plot centers) from which 10 trees for measurements were selected stratified randomly, using the point polar method with the closest distance from the central tree.

Trait selection

Seven functional leaf traits were measured: **(i)** Photosynthetic capacity (A_{max}), the maximum photosynthetic rate at which leaves fix carbon measured at ambient temperature and fixed CO_2 level (390 ppm) expressed as μmol m^{-2} s^{-1}. **(ii)** Dark respiration (R_d) which reflects the use of photosynthate, expressed as μmol m^{-2} s^{-1}. **(iii)** Leaf nitrogen content (LNC) which is the total amount of nitrogen in unit of oven dry leaf mass and is expressed in mg g^{-1}. (**vi**) Specific leaf area (SLA), which is the one-sided area of a fresh leaf, divided by its oven-

dry mass expressed as cm^2 g^{-1} (Perez-Harguindeguy et al., 2013). **(v)** Leaf dry-matter content (LDMC), which is the oven dry leaf mass divided by its water-saturated fresh mass, expressed in mg g^{-1} (Perez-Harguindeguy et al., 2013). LDMC reflects the tradeoff in investing resources in structural tissues versus liquid-phase processes. **(vi)** Leaf lifespan (LL) in months which is defined as the period from emergence to the fall of the leaf (Kikuzawa and Lechowicz, 2011). In this study the focus is on the period when the leaf can carry out its photosynthetic function, therefore the leaf lifespan here is considered as the time from the end of the leaf expansion until leaf abscission. **(vii)** Ploidy level (diploid or triploid) refers to the number of sets of homologous chromosomes in the genome of a cell (Campbell et al., 2008). Trait means and standard deviations for each clone can be seen in Table S1.

In addition, tree diameter at 50 cm from ground (diameter at breast height, DBH) was used as a proxy for stem age, which is known to affect leaf traits (Smith et al., 2011).

Gas exchange (A_{obs}, R_d)

Five trees at each plot were selected for maximum photosynthetic and dark respiration measurements. Two healthy mature leaves from one branch were selected (n=172). Lower branches were collected using hand pruners, whereas higher branches were reached using a slingshot and aerial saw (Bigshot Launcher, Sherrill Inc., NC, USA) (Fig.4). Each branch was cut and kept under water to avoid embolisms. Leaves were retained to the branch during A_{obs} and R_d measurements. A portable photosynthesis system (CIRAS-3 with PLC3 18x25 mm standard leaf cuvette, PP Systems, Amesbury, MA, USA) was used to measure A_{max} and R_d. Two environmental conditions were controlled: CO_2 reference (390 ppm) and PAR (photosynthetic active radiation) was kept near irradiance levels (1200 W/m^2) with temperature near or above ambient. After waiting two to three minutes for value to stabilize, six measurements at 10 second intervals were collected for A_{max} and R_d and later averaged. Measurements were recorded once a stable value was obtained for both A_{max} and R_d. The IRGAs were zeroed and matched after each leaf. After measurement, leaves were cut from the branch, placed in a plastic bag with moist tissue paper and stored in a cooler. Due to varying field conditions, it was not possible to obtain maximum photosynthetic rates, therefore we further refer to observed photosynthesis (A_{obs}), rather than maximum photosynthesis.

Figure 4 Data collection of branches from highest aspen branches using a slingshot.

LNC

Twenty 5 mm diameter large leaf disks from twenty accessory leaves from both branches per tree were oven dried and later pooled at tree level (n=172). Approximately 3.0 mg of the dried leaf discs were loaded into 5 mm x 9 mm tin capsules and analyzed for %N by mass spectrometry at the Stable Isotope Facility at the University of California at Davis.

Spectral measurements

Previous studies have shown that partial least-squares regression on spectroscopic reflectance measurements provide reliable estimates of leaf biochemical, nutritional, and morphological properties (Wold et al., 2001; Serbin et al., 2014; Chavana-Bryant et al., 2017; Wu et al., 2017). We therefore used this method for increasing sample sizes for expensive (LNC) and time consuming (R_d, A_{obs}) functional traits and for gap-filling of missing trait data.

Measurements were collected using a hand-held spectroradiometer (ASD FieldSpec HandHeld 2; ASD inc, CO, USA) with a 325 – 1075 nm spectral range and sampling interval of 1.5 nm for the region 325 - 1075 nm. The spectrometer was fitted with a plant probe with a built in light source. The spectrometer was optimized every 15 minutes to ensure measurement quality (Chavana-Bryant et al., 2016). After every optimization a 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 30 sec. long reflectance measurements per leaf were collected and averaged for eight leaves per tree (n=1376).

To estimate LNC, A_{obs} and R_d for leaves where measurements were not available, a partial least square regression (PLSR) modeling approach was used using the 'plsr' function

from the *pls* package within R statistical framework, version 3.3.2 (Wehrens and Mevik, 2007; R Core Team, 2017). 10-fold cross-validation using 90% of the calibration data set 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}. Predicted values for A_{obs} were calculated using 65 components and RMSE = 3.58 μmol m^{-2} s^{-1}, whereas for R_d – 30 components were used and RMSE = 0.95 μmol m^{-2} s^{-1}. It is important to note that obtained LNC values at leaf level are under assumption, that the pooled data of LNC per tree applies to the leaf level.

SLA/LDMC

Eight healthy adult leaves from two branches per tree (four leaves per branch) (n=1376) were collected for SLA and LDMC. Leaf collection followed the same procedure as for gas exchange measurements. On trees where photosynthetic measurements were taken, six leaves per branch for SLA and LDMC measurements were collected in addition to the two leaves already collected. In the lab, leaves were cut from the petiole, scanned at 300 dpi using a CanoScan LiDE220 Color Image Scanner (Canon U.S.A., Inc.). Leaf area was obtained using "Leaf size and shape analysis code" in MATLAB (The MathWorks, Inc.) available at http://www.benjaminblonder.org/leafarea. Then water-saturated fresh mass was obtained, followed by oven drying each leaf sample at 70°C for 72 h and determining dry mass (Perez-Harguindeguy, 2013).

LL

In this study, leaf lifespan is considered as the period from fully matured photosynthetically active leaf until leaf senescence (leaf lifespan = date of leaf senescence - date of fully expanded leaf). Repeated measurements of the length and the width of ten leaves (n=180) on one easily accessible understory tree in each study plot were used to obtain the time when the leaves stopped expanding. Leaf length provided a better estimate of leaf growth than leaf width, therefore it was used as a measure of leaf growth. Because leaf growth was in progress in eight of eighteen plots by the start of the measurements (16.06.2017.), the first day of measurement was considered as the end of leaf growth; such measurements are unlikely to be biased by more than 7 days. For the remaining ten plots, leaf growth was assessed using nonlinear least squares regression (NLS), where parameter estimation is based on minimizing the sum of square residuals and the end of the growth was obtained from the model with 95% of the asymptotic value. We used the 'nls*'* function from the *nls* package within R (Bates et al., 2007; R Core Team, 2017). A sigmoidal function $length=a-be^{-cx}$ was fitted in R.

Similarly, repeated measurements of the leaf senescence were conducted on the same ten leaves by measuring the percentage of leaves that turned color (0%, 25%, 50%, 75%, 100%) and the number of leaves lost from the observed branch. Due to plot accessibility limitations during leaf senescence measurements, many plots have missing data for senescence, therefore to be able to compare leaf lifespan across different plots, the end of growing season was considered when at least 20% of measured leaves on the branch were 100 % discolored.

Microsatellite analysis

Ploidy level and clone identity was determined with microsatellite analysis (Mock et al., 2008; Mock et al., 2012). Two to three leaves were collected from each focal tree (n=172) and dried using silica gel. DNA was subsequently extracted from each dried leaf sample using the E.Z.N.A HP plant DNA mini kit (Omega Bio-tek Inc., GA, USA). For this study we used twelve unlinked microsatellites, three developed by Smulders et al. (2001) (WPMS 014-016), three developed by Tuskan et al. (2004) (ORPM 028, 059 & 206) and six sourced from http://www.ornl.gov (PMGC 433, 510, 575, 667, 2571 & 2658). DNA amplifications were carried out in two multiplexes of six microsatellites in 10 µl reactions containing 2.4 µl of one of the multiplexed primer combinations (0.1-0.4 µM primer concentrations), 1 µl template DNA, 5 µl Qiagen Multiplex PCR Master Mix and 1.6 µl RNAse-free water. We used a 'touchdown' PCR protocol adapted from Cole (2005), with an initial denaturation at 92 °C for 5 min, followed by 9 cycles of 45 sec at 92 °C, 45 sec at 59 °C (dropping by 1 °C each cycle to 50 °C) and 60 sec at 72 °C. This was followed by 21 cycles of 45 sec at 92 °C, 45 sec at 50 °C and 60 sec at 72 °C, with a final extension step of 5 min at 72 °C. After PCR, 1 µl of the reaction was added to a solution of 9.35 µl formamide and 0.15 µl of the Applied Biosystems' GeneScan 500 LIZ size standard. Fragments were subsequently sized on a 3130xl Genetic Analyzer (Applied Biosystems, CA, USA) and scored with GeneMapper Software v4.0 (Applied Biosystems, CA, USA). 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 clone if all alleles for the ten markers were identical. Individual plants were furthermore defined as triploid if three alleles were observed 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. Clone and ploidy level distribution across study area can be seen in Table S1.

Microclimatic variables

Four environmental variables were selected to represent microclimate: **(i)** Average midday (10:00 - 14:00) temperature in July, ^{o}C (JulydayT), as data collection was conducted during July. **(ii)** Average midday light intensity in July, lux (JulydayL). Data for temperature and light intensity were recorded using waterproof Pendant Temperature/Light 64K Data Loggers (UA-002-64) (Onset Computer Corporation, MA, USA) from June 25 through September 22, 2017. 172 data loggers (one for each tree) with recording interval of 5 minutes, were placed in the tree canopies using a Bigshot Launcher (Sherrill Inc., NC, USA). The sensors were orientated upwards and no radiation shielding was applied to capture natural light levels (Fig.5). **(iii)** Gravimetric water content (GWC) is the mass of water per mass of dry soil, calculated as (*Wet soil(g) – Dry soil(g)) /Dry soil(g)* (Black et al., 1965). One soil core (ca. 2 cm x 15 cm) per tree (N=172) was taken in a ~1m radius of the base of the tree. Where soils were too rocky to collect soil cores, a similar volume of soil from 0 – 15cm 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 #10 mesh (2mm) sieve, weighted, dried at 60^{o}C for 48 hours and weighed again. **(iv)** Soil pH was obtained with a hand-held pH meter (Handheld pH/mV/°C meter, pH 1100 H, VWR International, PA, United States) by using 10g of dried soil. In cases where the soil sample was less than 10 g, the whole soil sample was used. Soil was weighed into a test tube, then 50 ml of distilled water was added, then the sample was shaken until homogeneous. The pH meter was turned on after the soil pH probe tip was inserted into the soil. The value was recorded when the reading displayed on the soil pH meter stabilized to a constant value. Microclimate variable means and standard deviations for each clone can be seen in Table S2.

Figure 5 Data logger place in tree canopy with sensors orientated upwards.

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, 2011; R Core Team, 2017). The MICE package was used in this study

for two reasons: 1) to obtain data of DBH data that was missing for one plot (ten missing observations); 2) PLS cannot be performed with missing data, therefore, the MICE package was used to fill in missing data for the following variables: 13 values for SLA, 1 value for LDMC, 1032 values for A_{obs} and R_d and 1204 values for LNC. Ten imputations for each variable were performed and averaged.

Statistical analysis

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., 2014) within the R statistical framework, version 3.3.2 (R Core Team, 2017). LMM 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 in the following descending (nested) order: site, clone, tree branch and leaf (Johnson, 2014). Conditional and marginal R^2 values were calculated with the 'r.squaredGLMM' function in the *MuMIn* package (Barton and Barton, 2018). A conditional-R^2 described the variation explained by both fixed and random effects, and a marginal R^2 described the variation explained by fixed effects alone.

We performed variance partitioning analysis for each of the measured leaf traits (response variable) to assess the intraspecific functional trait variation, which was structured across different nested organizational levels in our dataset (site, clone, tree, branch and leaf) with 'lmer' function in the *lme4* package and 'varcomp' in the *varComp* package (Qu and Qu, 2017).

To test trait - trait relationships, one functional trait was entered as response variable, and another trait as predictor. Trait data was log-transformed to be able to compare our results with those of Wright et al. (2004) and because log transformation has been shown to linearize these relationships. Response and predictor variables were chosen based on the LES trait patterns presented in Wright et al. (2004). Random factors for all LMM (also trait - environment) were: tree nested in clone, nested in site (1|Site/Clone/Tree). Trait combinations that were tested included: SLA-LDMC, SLA-LNC, SLA-LL, LL-LNC, SLA-A_{obs}, A_{obs}-LNC, A_{obs}-LL, R_d -A_{obs}, R_d- LL, R_d-LNC, R_d-SLA.

In order to see whether trait - trait relationships in aspen are consistent with the global interspecific LES patterns, we used the GLOPNET data set 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 4). The overall slope was

obtained on log-transformed data performing a linear regression model in R, where one trait was entered as the response variable and the other as the predictor variable (Table 4).

To quantify the trait - environment relationships, we made LMM where a trait was entered as response variable and microenvironment variables - temperature, light intensity, pH, gravimetric water content and DBH (as proxy for tree age) were entered as fixed factors. To account for environmental variables being on different scales, both response and fixed factors were z-transformed using the *scale* function in R. Collinearity between predictor variables was checked and a minimum Pearson correlation coefficient between two environmental variables was set at $|r| < 0.5$. All four environmental variables met these requirements and were kept in the models.

To determine whether there was a different slope for each clone, we made likelihood ratio tests for both trait - trait and trait - environment models where we compared a model with only random intercept (1/Site/Clone/Tree/Branch) to a model with both random intercept and random slope (trait|Clone) or (microcl. variable|Clone) depending on model type.

We also determined whether trait - trait and trait - environment relationships changed with ploidy level using separate models. For both trait – trait and trait – environment relationships we performed similar trait – trait and trait – environment models as presented before but with ploidy as an extra fixed factor. A likelihood ratio test was performed, to test whether there were differences in ploidy slopes by testing model with and without interaction.

Because leaf lifespan was only measured for one to two trees per clone, the leaf lifespan values were averaged per clones, and hence a different random structure was used for models on leaf lifespan. As it was not possible to test whether clones have different slopes for LL, we performed one model in which we test the overall relationship and whether relationships change for different ploidy levels by adding ploidy level as fixed factor to the model, to see whether there are different slopes and/or intercepts for diploids and triploids. The likelihood ratio test was used to test for differences in ploidy slopes by testing models with and without interaction.

The statistical significance of trait-trait and trait-environment relationships was tested using a 95% CI approach and considered statistically significant if CI does not overlap 0. 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

Variance partitioning

Using variance partitioning analysis, we found that most of the variation is within clone, where 26-55% is at leaf level (Fig.6). Some variation is found at the tree level (11-28%) and clone level (6-24%). Among all traits LNC and LDMC had highest fraction of variance at the clone level. Differences between sites do account for some variation in LDMC (16%), LNC (10%), and SLA (9%). In R_d and A_{obs}, site does not account for any variation. Very little variation (1-12%) was found at the branch level.

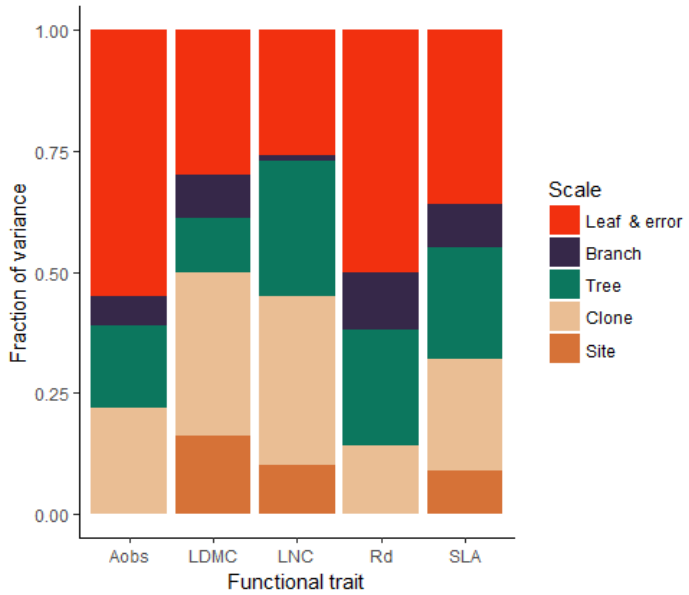

Figure 6. Variance partitioning analysis for leaf functional traits. Variance decomposition at leaf, tree, clone, ploidy and site scale for A_{obs}, LDMC, LNC, R_d SLA presented as fraction of variance.

Trait - trait relationships

In all trait - trait comparisons, where we compared a model of random clone intercept with a model of random clone intercept and slope, the model with random clone intercept and slope showed statistically significant improvement for: SLA-LDMC, A_{obs}-LNC, A_{obs}-SLA, A_{obs}-R_d, R_d-LNC, R_d-SLA (Table S3). The exceptions were for SLA-LNC (X^2=2.78, df=3, P=0.43), R_d-LNC (X^2=2.59, df=3, P=0.436), A_{obs}-LNC (X^2=5.55, df=3, P=0.14), where the model with only random clone intercept was supported, indicating that there were no differences between clones.

Note that for LL only one model was performed, giving us the overall slope of LL-trait relationship and ploidy slope differences. This is because we had one to two trees for observing LL at the clone level, thus it was not possible to obtain individual clone slopes (but see Methods section). Our dataset covered a reasonable range of trait values for A_{obs} and R_d (Table 4). However, our maximum photosynthetic values reached only up to 18 μmol m^{-2} s^{-1} in comparison to the GLOPNET maximum of 30 μmol m^{-2} s^{-1}. Our LNC and LDMC values in the 5%-95% range were considerably high (LNC: 2.32% to 3.41 %, LDMC:0.32 to 0.48 g g^{-1}) in comparison to GLOPNET dataset (LNC: 0.91 to 2.54%, LDMC: 0.10 to 0.42 g g^{-1}). Our obtained SLA values covered ca 40% of the global SLA range and 1.96% of the global LL range (Table 4).

Table 4 5-95% quantile trait range of traits used from GLOPNET and our study dataset

Dataset	SLA cm^2 g^{-1}		LDMC g g^{-1}		LNC %		Aobs/Amax umol m-2 s-1		Rd umol m-2 s-1		LL months	
	5%	95%	5%	95%	5%	95%	5%	95%	5%	95%	5%	95%
Global	55.00	225.62	0.10*	0.42*	0.91	2.54	5.95	18.30	0.62	2.42	1.38	36.00
Our	101.36	174.46	0.32	0.48	2.32	3.41	2.51	14.57	0.47	2.96	2.53	3.20

*Data 5-95% quantile range for LDMC taken from TRY databse presented in (Kattge et al., 2011).

From the eleven leaf functional trait pairs tested in a single clonal species, we found six statistically significant trait pair relationships at the species scale on log-transformed data: A_{obs}-SLA, SLA-R_d, SLA-LDMC, LNC-LL, A_{obs}- LNC, A_{obs} -LL (Table 5, Fig.7,8,10). From these pairs that were found to be significant in this study, four trait pair relationships were opposite of what is expected from the global LES. These trait pairs were A_{obs}-SLA, R_d-SLA and LNC-LL, A_{obs}-LL (Table 5, Fig.7,8,10). This result indicates that all the other trait pairs having nonsignificant weak or no relationships at the species scale (Table 5, Fig.7, Fig.10), which is surprising and in contrast with the global LES.

One trait pair, SLA-LDMC, was consistent at all studied scales – species scale i.e. overall aspen scale and clone scale (Fig7a.). We do not have comparison with GLOPNET, but according to theory we know that SLA scales negatively and strongly with LDMC, therefore we can conclude that SLA-LDMC relationship within and among clones is also consistent with global patterns (Kattge et al., 2011; Perez-Harguindeguy et al., 2013).

Two trait pairs SLA-LNC, Rd-A_{obs} were only consistent at global and overall aspen scales (Fig.7b, Fig.8b). The slope strengths decreased at the species scale and clone scales for SLA-LNC and R_d-A_{obs} in comparison to the global interspecific LES slope (Fig.7b,g, Fig.8b,g). For example, overall SLA-LNC slope in Quaking aspen was 0.05, while the LES slope was 0.95. Similarly, the overall R_d-A_{obs} slope in aspen was 0.11 and LES slope was 0.49 (Table 5).

Comparing trait relationships among individual clones, overall there is no consistency in whether individual clone slopes are becoming weaker than overall clone slope (Fig.7, Tables S4). Some clone slopes are consistent with the overall clone slope and have either similar, weaker, or stronger relationship; some clones have the opposite direction to the relationship from the overall slope (Fig.7, Table S4).

Three trait pairs that were opposite of what is expected from the LES (SLA-R_d, A_{obs}-SLA, LNC-R_d) and were consistent both at the species and clone scale – having reversed relationships, however, we could not see a pattern, whether individual clone slopes are becoming weaker than the overall slope, but we saw that the strength of the slope is clone specific (Table 5, Table S4). Note, that LNC- R_d relationship is not statistically significant (Table 5).

For one trait pair (A_{obs}- LNC) we found a much stronger slope (0.79) at the species scale in comparison to the interspecific LES (0.09), but because we were not able to obtain "true" A_{max} values, slopes of the LES and our data set cannot be compared. But again, at the clone level, there was no consistency in strength and direction of clone slopes.

Due to different data structure, the graphical representation of LL is different (Fig10). We were able to obtain the overall slope of our study sites and the differences in the ploidy levels. The overall slope of all trait pairs with LL (SLA-LL, LNC-LL, Aobs-LL, Rd-LL) had opposite direction of what is expected from the LES (Table 5).

Table 5 Overall aspen slopes of trait-trait relationships and their 95% CI in comparison with global interspecific slope obtaine dfrom GLOPNET dataset. Trait pairs that are marked with bold are considered statistically significant.

Dependent variable	Independent variable	Overall aspen lope	CI	Global slope	CI
SLA	**LDMC**	-0.75	(-0.96 , -0.55)	-	-
SLA	LNC	0.05	(-0.04 , 0.14)	0.95	(0.91,0.98)
Aobs	**SLA**	-0.62	(-1.14 , -0.09)	-0.09	(-0.11, -0.06)
Rd	**SLA**	-1.67	(-1.94 , -1.48)	-0.43	(-0.45,-0.40)
SLA	LL	0.39	(-1.29 , 2.09)	-0.39	(-0.40, -0.37)
Aobs	**LNC**	0.79	(0.41 , 1.18)	0.09	(0.05,0.12)
Rd	LNC	-0.37	(-0.77 , 0.03)	-0.18	(-0.22,-0.14)
LNC	**LL**	0.14	(0.04 , 0.25)	-0.26	(-0.27,0.25)
Rd	Aobs	0.11	(-0.04 , 0.26)	0.49	(0.46,0.51)
Aobs	**LL**	0.87	(0.36 , 1.39)	-0.11	(-0.12, -0.10)
Rd	LL	0.12	(-1.77 , 2.01(	0.05	(0.03,0.06)

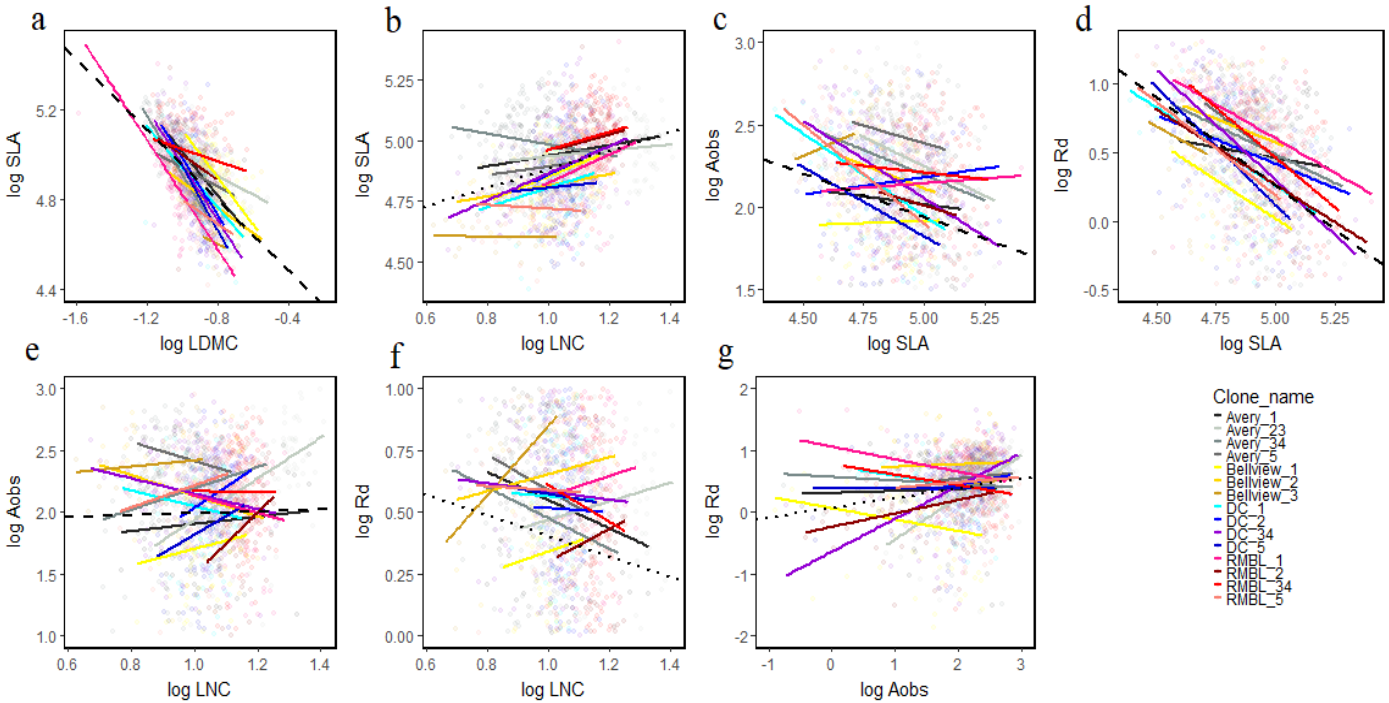

Figure 7 Trait-trait relationships for different functional trait pairs on log-transformed data: **a** SLA and LDMC, **b** SLA and LNC, **c** A_{obs} and SLA, **d** Rd and SLA, **e** A_{obs} and LNC **e** Rd and LNC **f** R_d and A_{obs}. Each graph represents the overall slope (dashed or dotted) and individual clone slopes. Dashed line indicates that relationship is significant, dotted not significant. Numbers at the upper left corner indicate the slope of relationship between two traits from the GLOPNET dataset and the overall aspen slope in our study.

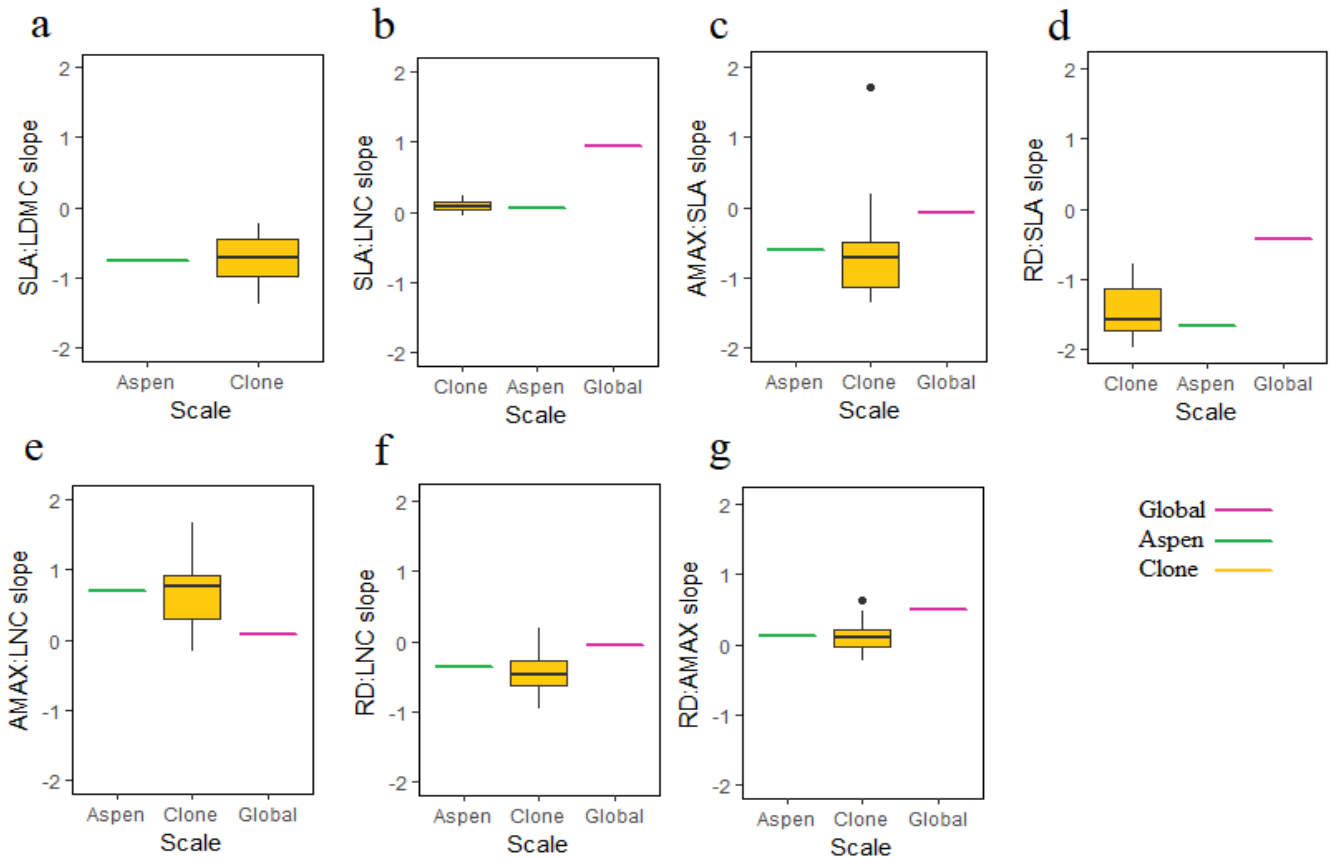

Figure 8 Boxplot graphs representing mean for slope (y axis) for trait - trait relationship at three scales (x axis): individual clone slopes (Clone), overall aspen relationship (Aspen) and global interspecific scale from GLOPENT dataset (Global).

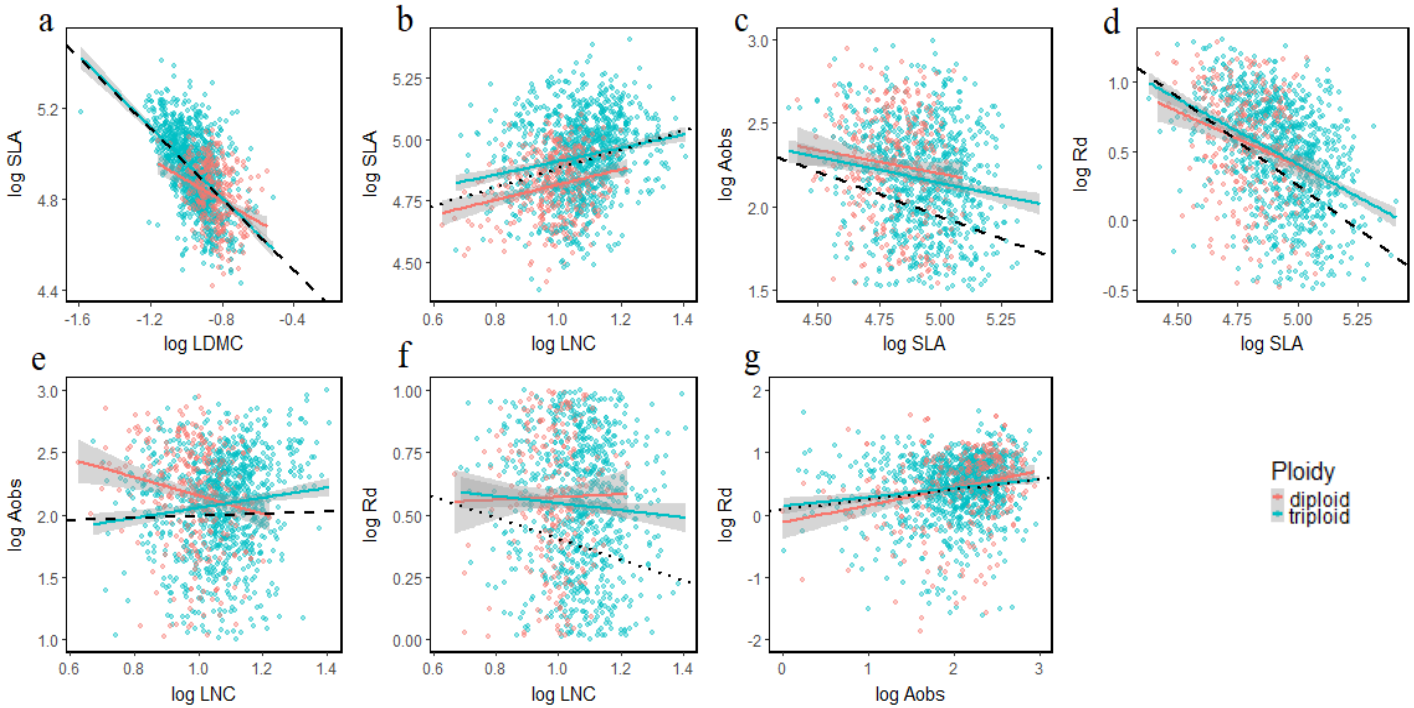

Figure 9 Ploidy level differences for different functional trait pairs on log-tranformed data: **a** SLA and LDMC, **b** SLA and LNC, **c** A_{obs} and SLA, **d** R_d and SLA, **e** A_{obs} and LNC **e** R_d and LNC **f** R_d and A_{obs}. Each graph represents the overall slope (dashed or dotted) and individual ploidy slopes. Dashed line indicates that relationship is significant, dotted – not significant. Blue colored slope -represents triploids (n=1011) and pink colored- diploids (n=292).

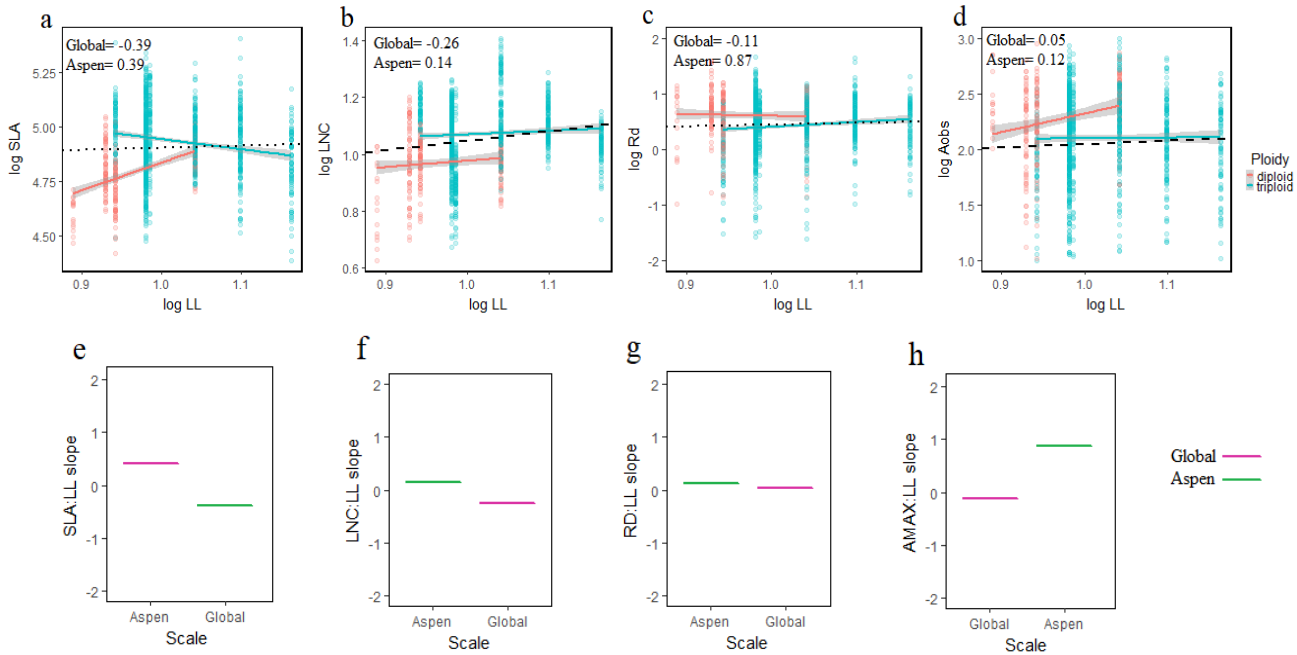

Figure 10 Overall trait - trait relationships with LL and their differences with ploidy level on log-transformed data: **a** SLA and LL, **b** LNC and LL, **c** R_d and LL, **d** A_{obs} and LL. Each graph represents the overall slope (dashed or dotted) and individual ploidy slopes. Dashed line indicates that relationship is significant, dotted – not significant. Blue colored slope -represents triploids (n=1011) and pink colored- diploids (n=292). Boxplot graphs **(e-g)** representing mean for slope (y axis) for trait-trait relationship at two scales (x axis): individual overall aspen relationship (Aspen) and global interspecific scale (Global). Due to structure of data it was not possible to obtain individual clone slopes for LL. Numbers at the upper left corner indicate the slope of relationship between two traits from the GLOPNET dataset and the overall aspen slope in our study.

Trait - environment relationships

In all trait - environment comparisons, where we compared a model of random clone intercept with a model of random clone intercepts and slopes, the models with random clone slope and intercept were supported for all traits, indicating that clones are statistically different from each other (Table S5).

Overall microenvironment is not a very strong predictor of explaining trait variation of aspen functional traits (Fig.11, Table S6). Temperature was a statistically significant predictor for LNC, A_{obs} and R_d trait variation (Fig.11.a,d,e). Light intensity was a significant predictor for SLA and LDMC variation (Fig.8.b, c), however none of these effects were very strong. Tree age had significant effect on LNC and SLA and A_{obs} (Fig.11.a,b). Soil moisture and pH did not explain any variation in leaf functional traits.

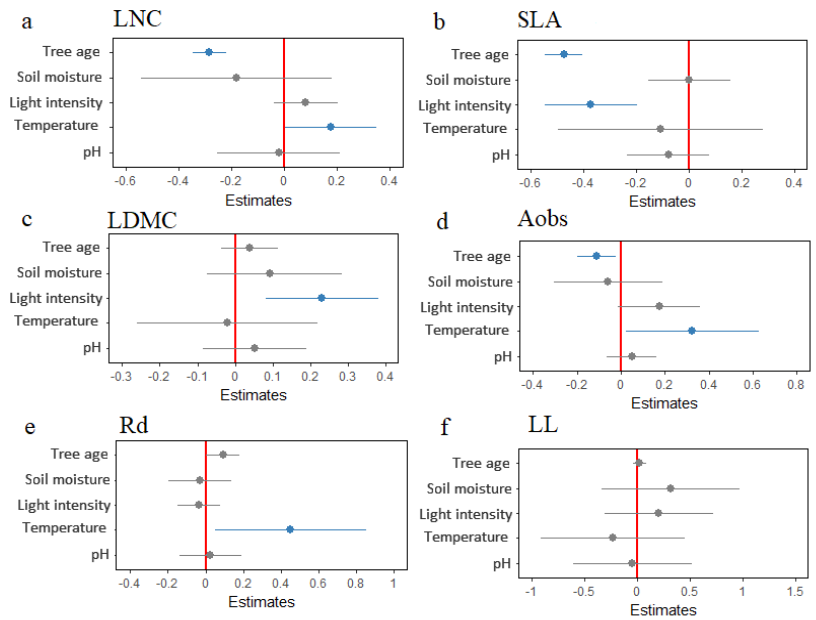

Figure 11 Summary figure of LMM models testing the overall effect of standardized microenvironmental variables (Tree age, Soil moisture, Light intensity, Temperature and pH) on all functional traits: a leaf nitrogen (LNC), **b** specific leaf area (SLA), **c** leaf dry matter content (LDMC), **d** observed photosynthesis (A_{obs}), **e** dark respiration rate (R_d), **f** leaf lifespan (LL). Center points of the bars show parameter estimates and bars - SE. Center points colored in blue with its associated SE that are not overlapping zero and are considered statistically significant.

Between clones the relationships were neither strong or consistent in explaining trait variation and they did not differ remarkedly from overall trait - environment relationships (Tables S7-S11). Few trait-environment pairs were consistent between clones – LNC-temperature (Fig.12a), LNC-light (Fig.12b), LDMC-light (Fig.14b), A_{obs}-pH (Fig.15c), Rd-light (Fig.16b). The other trait-environment pairs had inconsistent relationships, which might indicate that different strategies coexist in the site (Table S7-S11). The strength of relationships also varied from clone to clone, indicating genotype specific responses to environment, except for LNC-temperature, which had the same slope across all clones (0.74) and was stronger than the overall LNC-temperature slope (0.17). (Fig. 12a, Table S7). We can clearly see that different clones are occupying different environmental spaces, possibly indicating local adaptation of a genotype to particular environment. We found that some trait-environment relationships do share the same responses and others do not. For example, LNC is increasing with increasing temperature, thus LNC becoming accessible to plants (Fig. 12a). And in response to that we see that A_{obs} and R_d are increasing with temperature most likely reflecting higher the nitrogen availability in the soil in warmer temperatures and easier with increasing temperature (12 from 14 clones) (Fig. 15a, 16a).

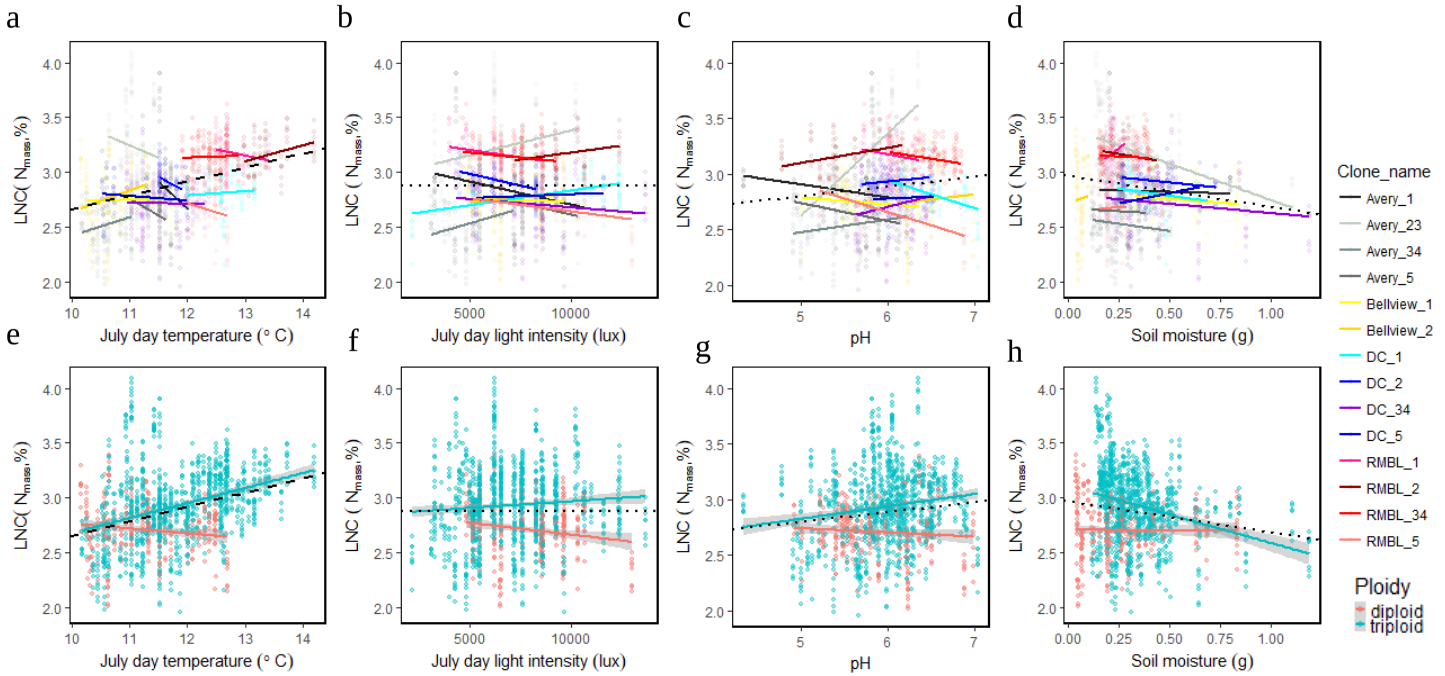

Figure 12 The relationships between LNC and microenvironmental variables. Graphs **a-d** show the overall relationship of LNC and microenvironmental variables (dashed or dotted line) and individual clone slopes (colored lines). Graphs **e-h** show the same relationships as in a-d, but with differences in ploidy levels- blue color indicates triploids and pink - diploids. Graphs **a,e** represents LNC- temperature relationship, **b, f** LNC- light intensity, **c,g** LNC - pH, **d,h** LNC- soil moisture. Dashed line indicates that relationship is significant, dotted – not significant.

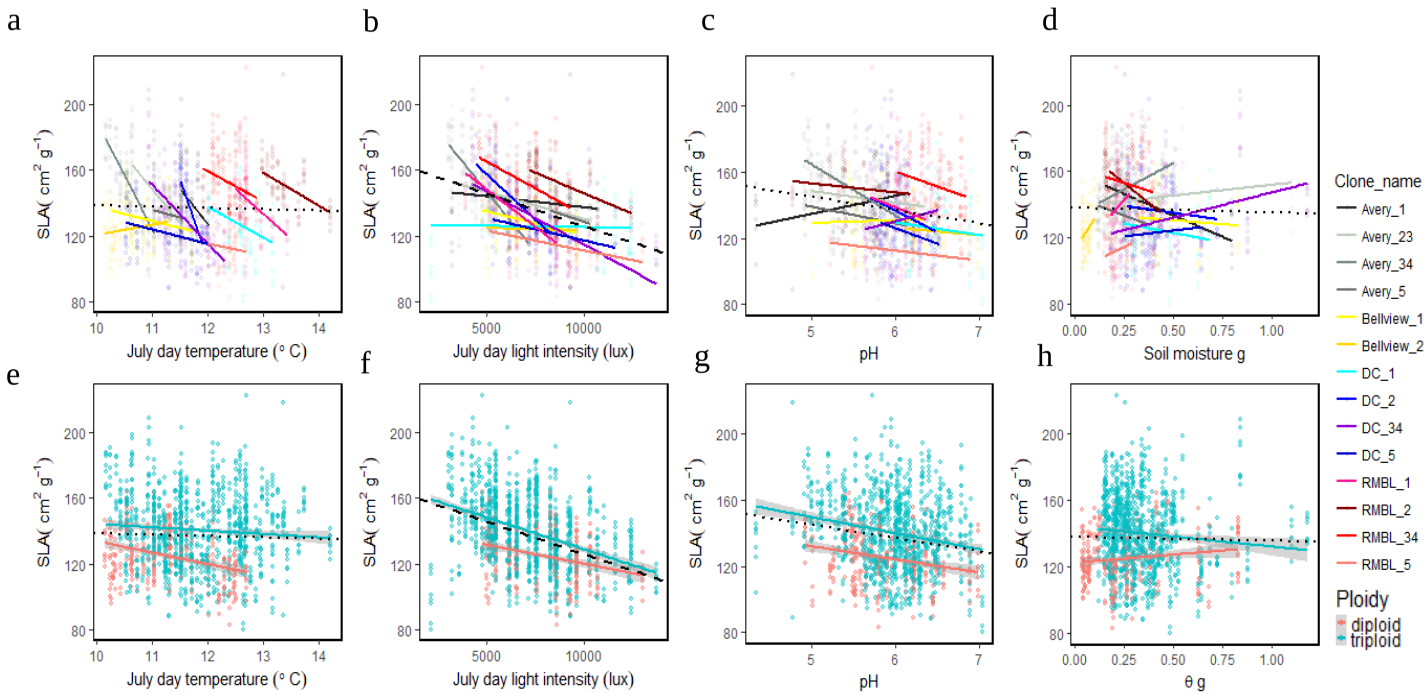

Figure 13 The relationships between SLA and microenvironmental variables. Graphs **a-d** show the overall relationship of SLA and microenvironmental variables (dashed or dotted line) and individual clone clone slopes (colored lines). Graphs **e-h** show the same relationships as in a-d, but with differences in ploidy levels- blue color indicates triploids and pink - diploids. Graphs **a,e** represents SLA- temperature relationship, **b,f** SLA- light intensity, **c,g** SLA - ph, **d,h** SLA- soil moisture. Dashed line indicates that relationship is significant, dotted – not significant.

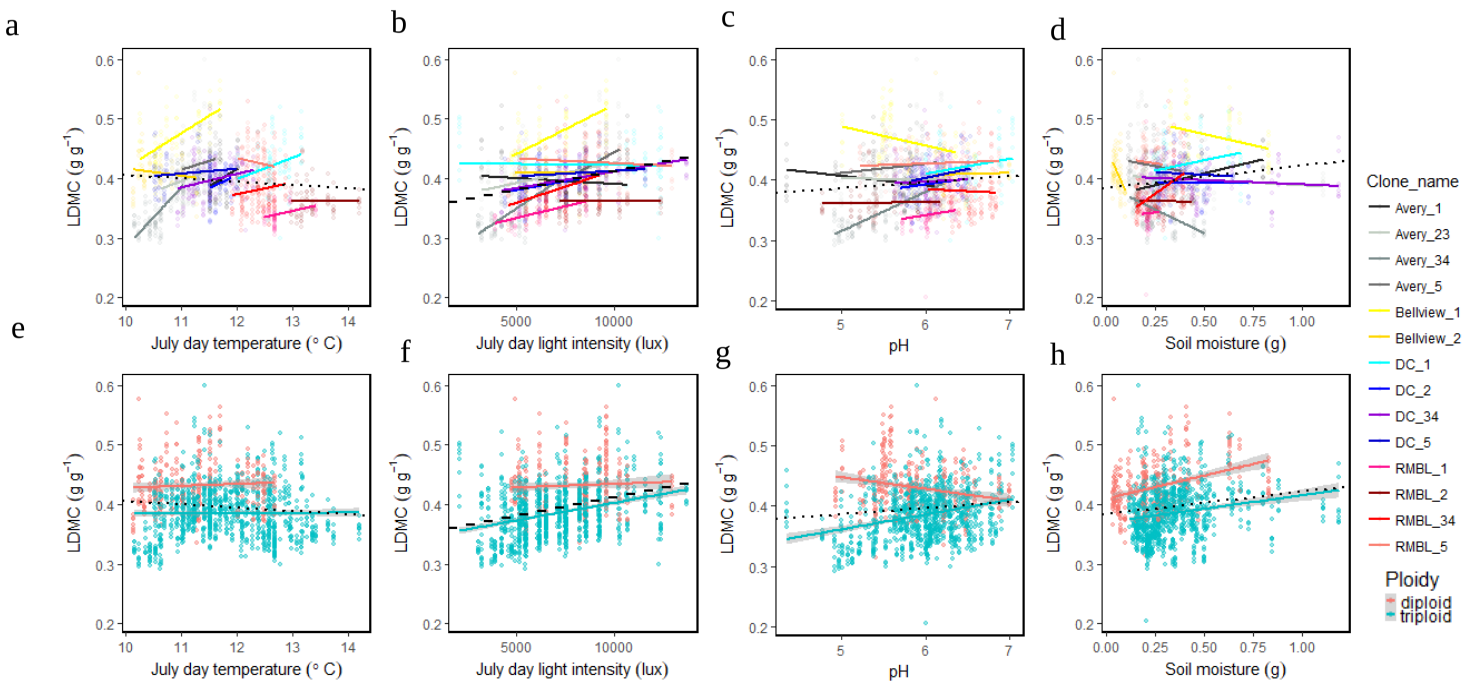

Figure 14 The relationships between LDMC and microenvironmental variables. Graphs **a-d** show the overall relationship of LDMC and microenvironmental variables (dashed or dotted line) and individual clone slopes (colored lines). Graphs **e-h** show the same relationships as in a-d, but with differences in ploidy levels- blue color indicates triploids and pink - diploids. Graphs **a,e** represents LDMC- temperature relationship, **b,f** LDMC- light intensity, **c,g** LDMC - ph, **d,h** LDMC- soil moisture. Dashed line indicates that relationship is significant, dotted – not significant.Dashed line indicates that relationship is significant, dotted – not significant.

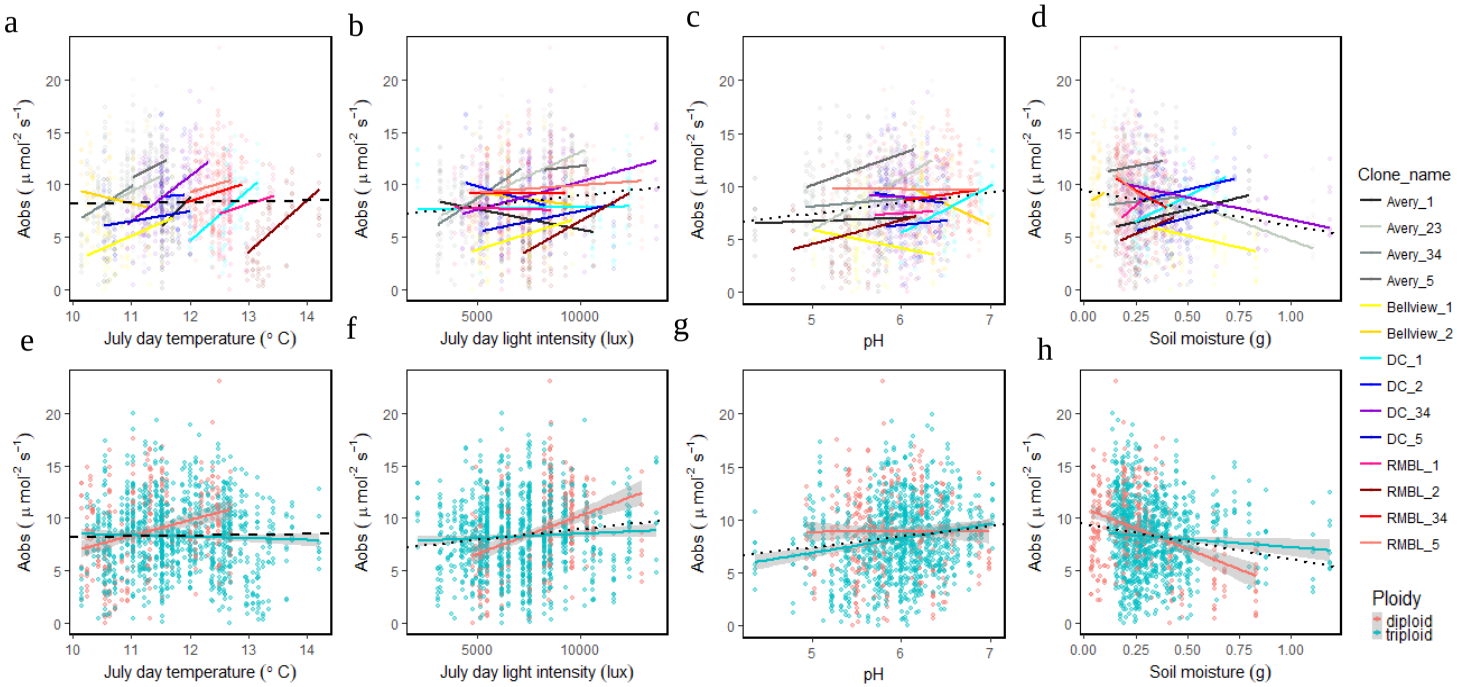

Figure 15 The relationships between A_{obs} and microenvironmental variables. Graphs **a-d** show the overall relationship of A_{obs} and microenvironmental variables (dashed or dotted line) and individual clone clone slopes (colored lines). Graphs **e-h** show the same relationships as in a-d, but with differences in ploidy levels- blue color indicates triploids and pink - diploids. Graphs **a,e** represents A_{obs} - temperature relationship, **b,f** A_{obs} - light intensity, **c,g** A_{obs} - ph, **d,h** A_{obs} - soil moisture. Dashed line indicates that relationship is significant, dotted – not significant. Dashed line indicates that relationship is significant, dotted – not significant.

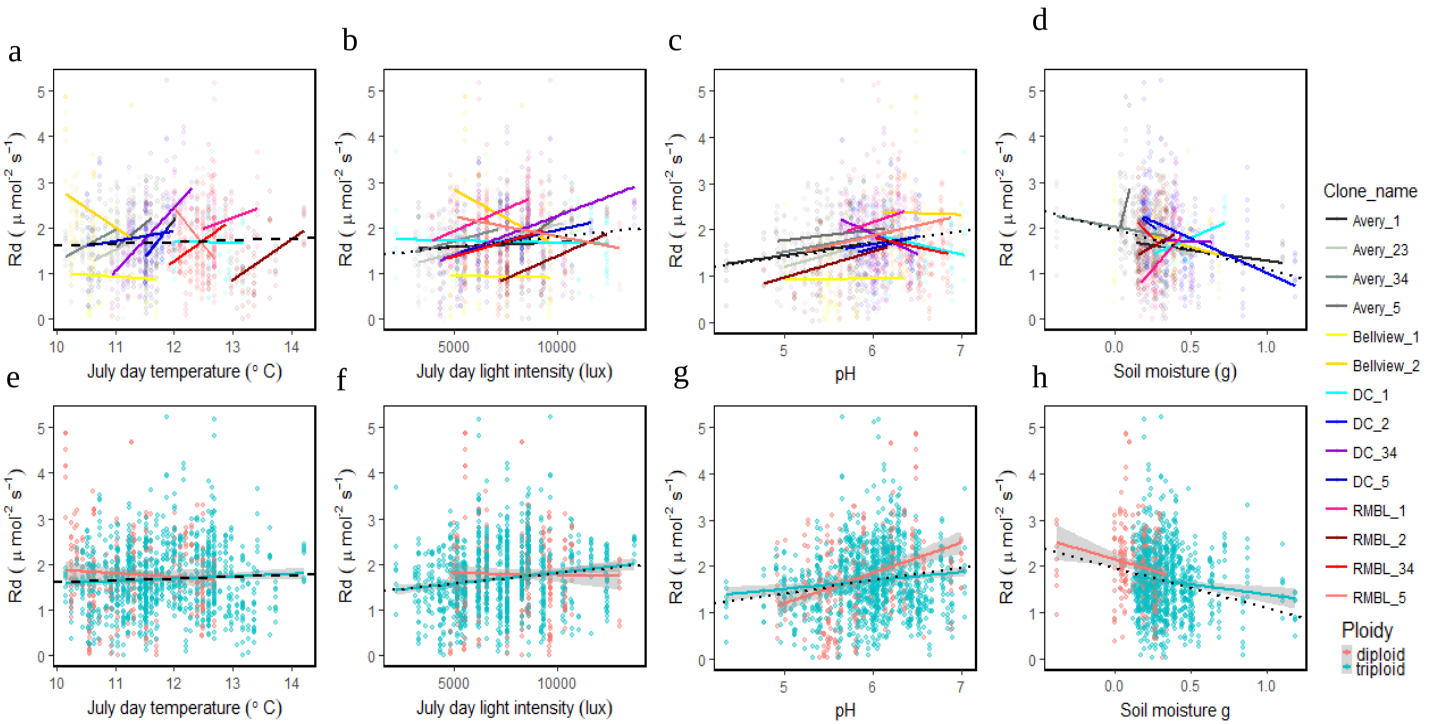

Figure 16 The relationships between R_d and microenvironmental variables. Graphs **a-d** show the overall relationship of R_d and microenvironmental variables (dashed or dotted line) and individual clone clone slopes (colored lines). Graphs **e-h** show the same relationships as in a-d, but with differences in ploidy levels- blue color indicates triploids and pink - diploids. Graphs **a,e** represents R_d - temperature relationship, **b,f** R_d - light intensity, **c,g** R_d - ph, **d,h** R_d - soil moisture. Dashed line indicates that relationship is significant, dotted – not significant. Dashed line indicates that relationship is significant, dotted – not significant.

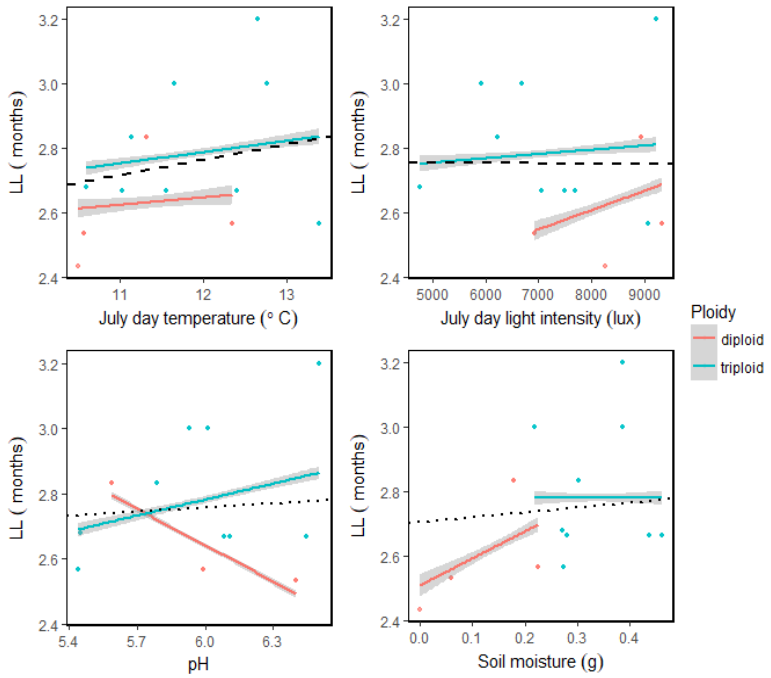

Figure 17 The relationships between LL and microenvironmental variables. Graphs show the overall relationship of LL and microenvironmental variables (dashed or dotted line) and the differences in ploidy levels- blue color indicates triploids and pink - diploids. Graph **a** represents LL - temperature relationship, **b** LL - light intensity, **c** LL - pH, **d** LL - soil moisture.

Ploidy level differences

For trait - trait relationships we found significant differences in intercepts for ploidy level trait pair comparisons (SLA-LDMC, A_{obs}-LNC, A_{obs}-SLA, SLA-LNC, LNC-LL, SLA-LL, R_d-A_{obs}, Rd-LNC, RD-SLA but A_{obs}-LL (Fig.9, Fig.10, Table S12). Three trait pairs had significant differences in ploidy level slopes: A_{obs}-LNC (X^2=9.23, df=1, 0.002), SLA-LL (X^2=5.46, df=1, 0.02), R_d-A_{obs} (X^2=9.80, df=1, 0.001), however, the diploid and triploid slopes were significant only for SLA-LL, for diploids being positive 1.54 (95% confidence interval (CI): 0.07 to 3.01) and for triploids being negative -1.80 (95% CI: -3.38 to -0.23). For A_{obs}-LNC and Rd-A_{obs} only triploid slopes was significant, most likely because triploids had a larger sample size, thus larger trait coverage that enables us to see the pattern (Table S13).

We found that triploid aspen clones occupy somewhat larger trait spaces compared to diploid clones, which occupy a smaller fraction of environmental or trait space. Diploids have smaller SLA, lower LNC concentrations, higher LDMC and shorter lifespans compare to triploid aspen clones (Fig.9, Fig.10, Table S13). We also found that different ploidy levels

occupy different environment spaces (Fig.12-16e-h). Diploids are found at lower temperatures, higher pH, lower soil moisture and higher irradiance, all factors associated with more stressful and harsher environments (Fig. 12-17e-h).

We also found that in trait - environment relationships, four traits had differences in ploidy slopes – LNC, SLA, LL and Rd (Table S14). Some interesting patterns emerged between different ploidy levels for specific trait and microenvironmental variable relationships. Although not significant, for SLA triploids across all microenvironmental variables had consistently steeper slopes that did diploids, again indicating more plasticity in triploids. For LL, diploids and triploids shared the same response to temperature (positive LL - temperature relationship), however, diploids were more sensitive to pH and triploids were more sensitive to light. In other words, this means that only diploids had significant pH-LL relationship and only triploids had significant light-LL relationship (Table S15), indicating diploids and triploids differ in their responses to environment.

However, when it comes to A_{obs} and R_d we do not see that diploids have smaller R_d and A_{obs} values compared to triploids. Instead we see that there is similar variation for these trait values.

Discussion

The hierarchical sampling design, the clonal nature of Quaking aspen, its wide distribution and the mix of ploidy levels gave us opportunity to answer our four key questions about the LES trait patterns at the intraspecific scale: (1) we found the highest variation within clones, indicating high plasticity; however, some traits had high fraction of variance at the clone level, possibly indicating the importance of local adaptation in trait variation; (2) trait – trait relationships at the intraspecific scale were not consistent with the LES indicating that there are not strong set of constraints on the strategies a plant can use at finer scales; (3) opposite of what we would expect, microclimate was a weak predictor of functional traits; (4) we found significant differences in morphological traits and shifts in resource-use trade-offs for diploids and triploids.

Together these findings suggest that the expected LES trait-trait patterns fall apart, and they are not bounded by strong constraints and tradeoffs at the at the intraspecific scale. These findings also suggest the ploidy level can affect and shift the LES trait - trait and trait - environment relationships and microenvironment does not predict trait variation at the intraspecific scale for Quaking aspen functional traits.

Variance partitioning

We found that for all traits the largest part of the total variation was found within clone, indicating high within-clone / plastic variability (Fig. 6.) (Albert et al., 2010; Messier et al., 2010; Messier et al., 2017). However, the amount of variation at each scale was trait dependent. The most variation within clones in A_{obs}, R_d and SLA came from differences between leaves, reflecting the plasticity in leaves within the canopy in response to the varying irradiance, creating variation in sun vs. shade leaves along the tree canopy (Messier et al., 2017). The highest part of the variance in LNC and LDMC was at clone level, most likely indicating that genotypic differences among clones play an important role in determining variation in these traits. This idea is supported by the fact that we found little effect on microenvironment in trait variation, therefore we can conclude that the variation at the clone level most likely reflects genetic differences between clones rather than mix of genetics and environment.

LNC showed somewhat equally distributed variances among leaves (0.26) and trees (0.28) and clones (0.35) in comparison with other traits (Fig.6). This means that LNC is equally sensitive at different scales and that processes at these scales (leaf - leaf position in the canopy, tree - microclimate variation among individuals and clone - genetic differences between clones) have equal importance in determining trait variation (Messier et al., 2010). We found very little variation at the site level for LNC (0.10), which is somewhat in contrast with Messier et.al. (2017) who found that largest fraction of the variation in LNC was at the highest scales (site and plot scale), instead of smaller scales. This contrasting result might be explained by aspen`s clonal nature, where genetic differentiation of clones in adaptive traits have contributed to high variation within sites due to contrasting selective forces and varying environmental conditions (Clair et al., 2010). Similarly, in SLA we found equal variances at the tree and clone level, again indicating that variation among individuals and genotype might play equally important roles in trait variation. Results of trait variation in SLA are consistent with previous studies, showing that SLA is sensitive at multiple scales (Messier et al., 2017). The same study also hypothesized that theoretically highly correlated traits should exhibit similar variance across scales, which was not the case, because different traits had different sensitivities at different scales (Messier et al., 2017). We found similar results in our study for SLA and LDMC, which had the strongest correlation (Fig.7a), but they had differing variances across scales (Fig.6). For example, differences in variance among clones were lower in SLA compare to LDMC (0.23 and 0.34 respectively) and differences at the tree scale were

considerably higher in SLA than LDMC (0.23 and 0.11). This result is also in contrast with Messier et.al. (2010), who found equal variances across all scales for these two traits (SLA and LDMC) (Fig.6).

Together with other studies these findings strengthen the need to account intraspecific trait variation and processes causing them at various scales and go beyond the mean trait values which are currently used in most of the studies (Albert et al., 2010; Messier et al., 2010; Messier et al., 2017). More importantly, in the context of the LES, high plasticity within aspen clones and large fraction of variance at the clone scale means that both plasticity and genetic differences between clones can be important in determining trait variation and should be accounted for. These results also question the LES current approach of assuming fixed trait values at the species scale, especially for species that have high genetic and phenotypic diversity (Anderegg et al., 2018).

Trait - trait relationships

Our analysis does not support the hypothesis for trait - trait relationships being consistent with global interspecific patterns at intraspecific scale in Quaking aspen, as we observed:

1. only one trait pair was consistent with LES at all scales;
2. some of trait pairs have opposite direction of relationship of what is expected from LES;
3. not all trait pair combinations at the clone level had the same direction as the overall slope;
4. many trait pairs that are thought to be significant at global scales were statistically nonsignificant.

It is important to understand why in general we might expect significant trait - trait relationships, and what are possible causes of deviations from the LES observed in this study. There are several possible explanations for consistent negative (such as SLA-LDMC in Fig.7a) or positive relationships at all scales- global, species, among clones and within clones, indicating scale independence for this relationship consistent with the LES. These causes are: genetic constraints, such as pleiotropy and linkage disequilibrium, natural selection and biophysical constraints (Shipley et al., 2006; Vasseur et al., 2012; Messier et al., 2017). First, the unified and coordinated responses in trait relationships at different scales are thought to indicate strong underlying genetic mechanisms in controlling trait relationships, (Shipley et al., 2006; Vasseur et al., 2012). It can be pleiotropy, where one gene affects multiple traits and thus causing genetic correlation between the LES traits or linkage disequilibrium where pairs of genes cause genetic correlation (Donovan et al., 2011). For example, silencing of pleiotropic genes in *Arabidopsis thaliana* leads to coordinated adjustment of plant traits

indicating that several pleiotropic genes drive correlations between functional traits and that these traits are genetically constrained (Vasseur et al., 2012). However, Donovan et.al. (2011) suggest selection as the main explanation for why only certain combinations of traits are observed at the LES. They argue that because they found a reasonable amount of genetic variation in studied traits, unstable leaf trait combinations may occur, but selection acting on these trait combinations will eliminate the individuals who are functioning ineficciently (Donovan et al., 2011). This is reasonble because if the genetic mechanisms are strong and drive the patterns observed in the LES, we would see the same trait -trait relationships at all scales, however, we found nonsignificant, weak or reversed relationships at different scales, therefore suggesting different selective agents acting on traits at different scales rather than strong genetic underlying mechanisms. Another possible explanation for strong relationships between two traits might be due to biophysical constraints, that leads to mandatory relationships between traits (Lambers et al., 2008).

Due to biophysical constraints it is not possible to obtain high rates of photosynthesis with limited nitrogen, but the opposite could happen (low photosynthesis and high nitrogen concentration) –when the resources are reallocated, for example, towards defense against insect herbivores at lower elevations or harsh environmental conditions at higher elevations, instead of promoting higher photosynthesis (Lambers et al., 2008; Moles et al., 2011; Hulshof et al., 2013). For example, invertebrate herbivores affect plant traits both directly by consuming plant species and indirectly by promoting changes in plant traits, such as changes in morphology, phenology, physiology, chemical compound shifts towards defense etc., also called trait mediated indirect interactions (Utsumi et al., 2010, Firn et al., 2017). This means that for example weaker (SLA-LNC) and nonsignificant overall and individual clone slopes (R_d-A_{obs}) relationships in comparison with the interspecific LES slopes could because the nitrogen is invested into defense rather than into photosynthetic processes. If all nitrogen would be devoted to primary metabolism, we would see strong relationships, because photosynthetic processes are regulated by the availability and investment of nitrogen into photosynthetic machinery, which in turn would promote higher photosynthesis and thus higher respiration (Lambers et al., 2008). But does that mean that aspen uses nitrogen-rich compounds into herbivore defense directly?

Short lived species are thought to invest mobile compounds into herbivore defense, such as alkaloids, phenolics and cyanogenic glycosides, that are present at small concentrations and they are mostly carbon - based compounds (Coley et al., 1985). The most common herbivore deterrents in aspen are phenolics, particularly - phenolic glycosides and

condensed tannins, which suggests that nitrogen rich compounds in aspen are not used for defense directly (Millard and Way, 2011). A more realistic explanation would be reduced primary metabolism due to the maintenance of mobile compounds which has high metabolic cost, which in turn leads to reallocation nitrogen towards defense indirectly (Coley et al., 1985). It could also be that aspen is investing less nitrogen in leaves and in that way reducing risks of being more attractive to herbivores if they are present on the landscape. The latter could be true as aspen tends to store nitrogen in trunk and roots, thus it might be a strategy to avoid herbivore attractiveness, reduce the amount of secondary metabolites needed to deter herbivores and thus reduce the metabolic cost associated with maintenance of defense compounds (Millard and Grelet, 2010; Millard and Way, 2011).

Many trait pairs had statistically nonsignificant and weaker relationships at the species scale in comparison to the global interspecific LES slope (Table 5), which is surprising and in contrast with the LES, as many of these are expected to be significant according to the global LES (Reich et al., 1997; Wright et al., 2004; Wright et al., 2005). However, the weaker relationships at species scale in comparison to global LES is not surprising, as it is assumed that strength of relationships decreases with decreasing scales. Smaller scales are associated with smaller environmental heterogeneity and therefore smaller trait coverage, that leads to weaker or absent LES (Messier et al., 2017). Besides that, Funk and Cornwell (2013) argued that the strength between two LES and other traits will be stronger with sufficient variation in LL. It could be true, as our study shows that, we covered 1.96% (2.53 to 3.20 months) of the LL in comparison with global LES (1.38 to 36 months). But within aspen, who is limited by length of growing season, this variation can be considered large. Besides that, we covered considerable range of other leaf trait values (Table 4), thus we argue that explanation of small trait coverage leading to weak or not significant relationships is not the main explanation.

Nonsignificant or weak relationships in trait pairs with R_d and A_{obs} might have arisen due to measurement error in A_{obs} and R_d measurements. First, were unable to get the "true" A_{max} in most cases during data collections. A_{max} is more a fixed trait, while A_{obs} varies quite a bit with environmental and measurement conditions. Second, we had a small sample size for photosynthesis and dark respiration, and in order to increase sample size, we used predicted values from reflectance spectra which had high root mean square errors for predicted data (RMSEP): for photosynthesis it was 3.58 and for dark respiration – 0.95. Both factors might have led to inconsistency in these measurements, and thus not represent the maximum photosynthetic and respiration values that these traits are meant to represent. However, we

believe that the inability to collect maximum rates of photosynthesis might have accounted for most of the error, as RMSEP were quite small proportionally.

Theoretically because of biophysical constraints, some trait relationships related to primary metabolism should at least maintain the direction of the general trait - trait relationship (Vasseur et al., 2012; Anderegg et al., 2018). But this is not what we observed in this study. Instead, we found several trait pairs with opposite slopes from the global LES vs. species or clone scale, such as the trait pairs R_d-SLA an A_{obs}-SLA (Fig.7c,d). We observed negative R_d-SLA and A_{obs}-SLA relationships at the species scale and clone scale in contrast with the LES slopes, where positive relationships between these traits are expected (Wright et al., 2004) (Table 5) . Anderegg et al. (2018) suggested two possible explanations for reverse relationships at smaller scales: genetic differentiation, which is a result of different drivers of variation at different scales and plasticity. As were not able to treat sun and shade leaves separately, this result most likely reflects the plasticity of sun vs shade leaves within the tree canopy, where there are higher photosynthetic rates for sun exposed leaves, which are smaller and thicker than shade leaves, for acclimation to increased irradiance and temperature that are considered as stressors (Kikuzawa and Lechowicz, 2011). Other studies have reported similar patterns of reverse relationships due to plasticity within the canopy (Blonder et al., 2013; Anderegg et al., 2018). For example, Blonder et.al. (2013) found that among species there are positive photosynthesis and leaf mass per area relationships, which can be negative within species. Similarly, Anderegg et.al. (2018) found that with decreasing scales the relationship reversed between leaf lifespan and leaf mass per area, thus becoming positive.

At the global scale, negative SLA, R_d, A_{obs} relationships with LL are associated with conservative ecological strategies, where longer leaf lifespans are needed to pay back their construction cost (Kikuzawa and Lechowicz, 2011). However, 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, in order to maximize the overall carbon gain and avoid respiratory carbon losses and maintenance costs during unfavorable period for photosynthesis (Kikuzawa and Lechowicz, 2011; Caldararu et al., 2014). Therefore, for deciduous species such as aspen, longer growing seasons are associated with less stressful environment, higher temperatures, higher nutrient concentrations, and the ability to construct more expensive leaves and thus longer LL (Meier et al., 2015). The significant positive LNC-LL and A_{obs}-LL relationship supports this interpretation of longer leaf lifespans being more beneficial for aspen.

We found that two significant trait pair slopes with LNC at the clone level did not differ from the overall species slope (SLA-LNC, A_{obs}-LNC) (Fig.7b,e), possibly meaning that nitrogen allocation is species specific not clone specific.

When we compared the strengths of the relationships of overall aspen slopes to individual clone slopes, for most of the trait pairs there is no consistency, whether individual clone slopes are becoming stronger or weaker than overall slope (Table 5, Table S1). It could be due to random variability or measurement error. But we argue that this rather might be due to clone specific responses to the environment they are occupying as a strategy for aspen to cope with divergent microenvironments, thus individual clones responding differently to microenvironment and biotic stressors. That in turn leads to clone specific trait - trait relationships.

To sum up, these findings show that LES do not hold at the intraspecific level of Quaking aspen suggesting that there is scale dependency in the LES trait relationships, which should be considered and possibly tested *a priori* when interpreting trait variation in relation to the LES. The reversed relationships at finer scales might indicate, that at the intraspecific scale the LES patterns are not constrained and plants can have many strategies to cope with the biotic and abiotic factors. Some of the absent and weak trait pair relationships suggests, that biotic selective factors such as herbivory might have an important role. This is intriguing and therefore should be more explored in future.

Trait - environment relationships

Surprisingly, microenvironment did not explain functional trait variation in aspen. This result is opposite of what is expected, that microclimate drives the variation in functional traits locally (Armbruster et al., 2007). Marginal r^2-values ranged from 0.06 to 0.25 for linear mixed effect models. These values are very low in comparison to Stark et.al. (2017), where the values were reasonably high and ranged from 0.23 to 0.47 and supports the low effect of our measured microclimatic variables in aspen traits. This could be the result varying of ecological strategies among clones within a species e.g. both positive and negative clone responses to environmental variables, which act to weaken the overall relationship (Moles et al., 2014). One of such examples is SLA-temperature relationship, where we had overall weak negative relationship (with slope -0.11), and at the clone level there were 7 negative clone slopes and 7 positive slopes (Table S3). The overall negative SLA-temperature relationship might indicate that temperature is a stress factor to which leaves respond with reduced SLA in order to avoid leaf damage, similar to increased irradiance (Lambers et al., 2008). However,

some clones might experience temperature as a limiting factor and thus increase SLA with increasing temperature. But we would need temperature response curves to be entirely sure. It could also be that in clones that responded positively to increasing temperature, we sampled similar leaves (healthy mature), whereas in some clones we had leaves that are experienced more varied light conditions and thus responded to the sun-shade gradient accordingly (Kikuzawa and Lechowicz, 2011).

In some traits only temperature and light intensity had weak statistically significant effects (Fig.11). The absence of plant functional trait and temperature relationships is surprising, because temperature has been considered of being as one of the major determinants of plant traits at a global scale, as it is directly influencing all plant physiological processes, such as metabolic rate, growth rate, energy balance etc. (Reich and Oleksyn, 2004; Moles et al., 2014). However, the effect of environment in trait coordination in the LES was found to be modest (Wright et al., 2004). Our study and Anderegg et al. (2018) found similar results - weak overall trait- environment relationships within species. This is surprising as local factors are supposed to affect traits directly (Armbruster et al., 2007; Stark et al., 2017). The absence of strong trait response to temperature would be surprising if the temperature would affect functional traits directly and/or would be the only limiting factor. In most cases especially at smaller scales, the case is more complex, as many abiotic and biotic factors act on traits simultaneously, therefore plants might not exert very strong trait-environment relationships due to different trade-offs at the smaller scales (Reich and Oleksyn, 2004; Moles et al., 2014). And these various small-scale biotic and abiotic factors allow selection to act on traits giving different genotypes ability to persist in environment in their own optimal way, and our clone specific responses support this idea.

Another surprising result is that soil moisture did not have a strong effect on trait variation (Fig.8), because water availability is considered important to foliar function, and this result might be due to measurement error because we were not able to collect soil samples consistently (such as a certain amount of days without high precipitation), might have led to high error in these measurements (Ordoñez et al., 2009). Besides that, our study has only examined two soil properties – pH and soil moisture. As many traits (SLA, LNC) are known to be affected by soil fertility, the incorporation of soil nitrogen and phosphorous might have explained more variation than did our measured properties (Ordoñez et al., 2009). We also covered spatially large areas, with differing substrate type that might have explained reasonable variation trait- soil properties results.

Some traits had consistent trait - environment relationships at different scales and some traits had varying responses to microclimate at the clone level, indicating that the consistency of trait-environment relationships depends on the trait. The consistent LNC-temperature relationships among the clones reflects the biochemical hypothesis that states that leaf nitrogen reflects the nitrogen availability in the soil, which becomes harder to take up in colder temperatures due to reduced movement of nutrients in colder environments (Reich and Oleksyn, 2004). If we assume that this relationship is driven by biochemical processes, then it might indicate that nitrogen uptake is species specific not clone specific and thus similar to all clones across the environmental gradient. The overall negative and inconsistent SLA-temperature relationship (Table S3) at the clone level indicates that different clones are having different drivers of trait variation. The overall negative SLA-temperature relationship is opposite of what we would expect, because temperatures are associated with higher SLA and temperatures with lower SLA values.

Ploidy level differences

This study was able to show the covariation between traits in relation to different ploidy levels. Our measurements and analysis support previous research of diploid and triploid aspen clones differing in morphology and function (Greer et al., 2017). First, we found that diploids occupy the part of environmental gradient that is associated with lower temperatures, lower soil moistures, higher pH and higher light intensity than triploids (Fig. 12-16e-h). All these factors are consistent with the fact that diploids occupy more stressful environments, as in Greer et.al. (2017) who explored individual trait relationships.

We found that diploids have lower SLA and LNC and higher LDMC compared to triploid aspen clones, which is consistent with Greer et.al. (2017). We also found that A_{obs} and R_d do not differ between ploidy levels (Table S10, Table S11, Fig.9c, d. Fig. 10). We could assume that photosynthetic and respiration rates did not differ between diploids and triploids, but because we believe there was substantial error in these measurements the patterns observed may have happened by chance, and we conclude that these results do not have biological significance.

Overall the direction and slope of ploidy levels for trait-trait pairs was somewhat similar for diploid and triploid aspen clones, except for SLA – LL trait pair, where we found a significant negative relationship for triploid aspen clones and a significant positive relationship for diploid aspen clones (Fig.10a, Table S10).

Although not significant, we found opposite directions for ploidy slopes for several trait-environment pairs (LNC-temperature, LNC-moisture, SLA-moisture, LDMC-pH and LL-pH (Fig.12a, Fig.12d, Fig.13d, Fig.14c, Fig.17c, Table S12). This result shows that different ploidy levels not only occupy different trait-environment spaces, but also that they have different strategies, where diploids are associated with slower return on carbon investment in leaves and thus on being on one side of the LES and triploids being on the other side of the LES – with faster return on carbon investment in leaves.

The morphological differences in traits and their differing environmental preferences and differences in ploidy slopes for some trait-trait and trait-environment pairs indicate that different ploidy levels have different trade-offs related to resource acquisition, diploids being more resource-conservative and triploids mostly resource-acquisitive which falls on the LES major axis. Even though diploids and triploids occupying one end of the global LES, they are occupying slightly different positions within the "aspen space". Besides that, we also see that triploid aspen clones are occupying somewhat whole trait and environment spaces with a higher density of trait values on the one side of spectrum. This might indicate more plasticity in triploid aspen clones than diploid clones, which occupy a smaller fraction of trait space and are always at the one or another end of the spectrum. The plasticity for triploids being higher can be seen in the SLA-LDMC example, where we found a statistically significant steeper slope for triploid than diploid aspen clones (Fig.9a).

Within aspen these can be considered as important and significant differences changes in physiology and shifts in resource-use trade-offs. These results partially support our hypothesis for Question 3 of trait-trait and trait environment relationships changing with ploidy level depending on the trait pair considered and should give awareness for further studies that incorporates LES dimension and use study species with different ploidy levels.

Conclusion

Evidence from this study suggests that high intraspecific variability within a single clonal species and different trait sensitivities at various spatial scales, strengthens the need to account for intraspecific trait variation and processes causing them at various scales. These findings should encourage to go beyond the mean trait values, if one is willing to properly understand the processes causing trait variation (Albert et al., 2010; Messier et al., 2010; Messier et al., 2017).These findings also strengthen the need to account for species-specific characteristics, life histories and biogeographies in functional trait approach and go beyond treating functional traits as fixed entities at the species scale.

The results of the contrasting LES trait pair relationships in one species along an altitudinal gradient and the clone and ploidy specific trait - trait and trait - environment relationships are striking and strengthens the hypothesis that LES might not hold at smaller scales due to various factors affecting traits locally. This means that at the smaller scales there are not necessarily strong set of constraints on the strategies plants can use. This is very important for community assembly and ecosystem functioning studies, therefore there is a need to test *a priori* whether an LES trait dimension is present at study scale and species of interest (Messier et al., 2017). These findings also suggest that if clonal and ploidy structure is not accounted in ecological studies, then conclusions are drawn only for a particular fraction of a species and not the species in general (Mock et al., 2008). This is important, as the ploidy is common in plants, but its importance in the ecological studies have been neglected (Soltis and Soltis, 1995; Mock et al., 2008; Greer et al., 2017). These findings should also be considered when predicting climate change, vegetation-climate, and land use changes from models that use the LES traits.

We also present evidence that microclimate which is known to be a direct driver of trait variation locally might be weak. In this study, multiple environmental factors are acting on traits simultaneously in determining final set of traits. Particularly in aspen the genotype and the clone age were stronger drivers in determining traits, indicating that genetic component and developmental factors should be more incorporated in trait-based studies. This study was limited to abiotic drivers as possible drivers of trait variation and thus suggest, the biotic drivers such as herbivory might have shaped the trait-trait and trait-environment relationships by forcing leaves to reallocate the resources towards defense, which might have led to weak or nonsignificant patterns. The incorporation of biotic drivers and possibly

disentangling absent trait-trait and trait - environment relationships is intriguing, which should be more explored in future research.

Trait - based approach and the LES are powerful tools that gives ability to explain and generalize the functioning of organisms and understand trait variation in terms of ecological trade-offs and constraints, which is crucial for predictive ecology and ongoing climate change. However, findings from this study show that the LES does not have a power to explain trait variation at the intraspecific scale, as plants at finer scales are not strongly constrained to follow certain strategies that enables them to persist in a given environment. Besides that, the shifts in strategies in a single species depending on a ploidy level or genotype, questions the ability for trait - based approach to explain plant functioning without accounting for species identity and many ongoing processes within species and should give awareness for future studies.

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References

Albert, C.H., Thuiller, W., Yoccoz, N.G., Soudant, A., Boucher, F., Saccone, P., Lavorel, S., 2010. Intraspecific functional variability: extent, structure and sources of variation. Journal of Ecology 98, 604-613.

Anderegg, L.D., Berner, L.T., Badgley, G., Sethi, M.L., Law, B.E., HilleRisLambers, J., 2018. Within-species patterns challenge our understanding of the leaf economics spectrum. Ecology letters.

Armbruster, W.S., Rae, D.A., Edwards, M.E., 2007. Topographic complexity and terrestrial biotic response to high-latitude climate change: variance is as important as the mean, Arctic alpine ecosystems and people in a changing environment, Springer, pp. 105-121.

Barnes, B.V., 1975. Phenotypic variation of trembling aspen in western North America. Forest Science 21, 319-328.

Barton, K., Barton, M.K., 2018. Package 'MuMIn'.

Bates, D., DebRoy, S., Gay, D., 2007. nls: Nonlinear Least Squares. R Core Team.

Bates, D., Mächler, M., Bolker, B., Walker, S., 2014. Fitting linear mixed-effects models using lme4. arXiv preprint arXiv:1406.5823.

Black, C.A., Evans, D., Dinauer, R., 1965. Methods of soil analysis. American Society of Agronomy Madison, WI.

Blonder, B., Violle, C., Enquist, B.J., 2013. Assessing the causes and scales of the leaf economics spectrum using venation networks in. Journal of Ecology 101, 981-989.

Buuren, S., Groothuis-Oudshoorn, K., 2011. mice: Multivariate imputation by chained equations in R. Journal of statistical software 45.

Caldararu, S., Purves, D., Palmer, P., 2014. Phenology as a strategy for carbon optimality: a global model. Biogeosciences 11, 763.

Campbell, N., Reece, J., Urry, L., Cain, M., Wasserman, S., Minorsky, P., Jackson, R., 2008. Biology 8th Edition‖, Pearson Education, USA.

Chabot, B.F., Hicks, D.J., 1982. The ecology of leaf life spans. Annual review of ecology and systematics 13, 229-259.

Chavana-Bryant, C., Malhi, Y., Wu, J., Asner, G.P., Anastasiou, A., Enquist, B.J., Caravasi, C., Eric, G., Doughty, C.E., Saleska, S.R., 2017. Leaf aging of Amazonian canopy trees as revealed by spectral and physiochemical measurements. New Phytologist 214, 1049-1063.

Clair, S., Samuel, B., Mock, K.E., LaMalfa, E.M., Campbell, R.B., Ryel, R.J., 2010. Genetic contributions to phenotypic variation in physiology, growth, and vigor of western aspen (Populus tremuloides) clones. Forest Science 56, 222-230.

Cole, C.T., 2005. Allelic and population variation of microsatellite loci in aspen (Populus tremuloides). New Phytologist 167, 155-164.

Coley, P.D., Bryant, J.P., Chapin, F.S., 1985. Resource availability and plant antiherbivore defense. Science 230, 895-899.

Donovan, L.A., Maherali, H., Caruso, C.M., Huber, H., de Kroon, H., 2011. The evolution of the worldwide leaf economics spectrum. Trends in Ecology & Evolution 26, 88-95.

Fajardo, A., Siefert, A., 2018. Intraspecific trait variation and the leaf economics spectrum across resource gradients and levels of organization. Ecology.

Funk, J.L., Cornwell, W.K., 2013. Leaf traits within communities: context may affect the mapping of traits to function. Ecology 94, 1893-1897.

Greer, B.T., Still, C., Cullinan, G.L., Brooks, J.R., Meinzer, F.C., 2017. Polyploidy influences plant–environment interactions in quaking aspen (Populus tremuloides Michx.). Tree physiology, 1-11.

Hulshof, C.M., Violle, C., Spasojevic, M.J., McGill, B., Damschen, E., Harrison, S., Enquist, B.J., 2013. Intra-specific and inter-specific variation in specific leaf area reveal the importance of abiotic and biotic drivers of species diversity across elevation and latitude. Journal of Vegetation Science 24, 921-931.

Johnson, P.C., 2014. Extension of Nakagawa & Schielzeth's R2GLMM to random slopes models. Methods in Ecology and Evolution 5, 944-946.

Kattge, J., Diaz, S., Lavorel, S., Prentice, I.C., Leadley, P., Bönisch, G., Garnier, E., Westoby, M., Reich, P.B., Wright, I.J., 2011. TRY–a global database of plant traits. Global change biology 17, 2905-2935.

Keddy, P.A., 1992. Assembly and response rules: two goals for predictive community ecology. Journal of Vegetation Science 3, 157-164.

Kikuzawa, K., Lechowicz, M.J., 2011. Foliar Habit and Leaf Longevity, Ecology of Leaf Longevity, Springer, pp. 1-6.

Lambers, H., Chapin, F.S., Pons, T.L., 2008. Photosynthesis, Plant physiological ecology, Springer, pp. 11-99.

Langenheim, J.H., 1962. Vegetation and environmental patterns in the Crested Butte area, Gunnison County, Colorado. Ecological Monographs 32, 249-285.

Meier, G.A., Brown, J.F., Evelsizer, R.J., Vogelmann, J.E., 2015. Phenology and climate relationships in aspen (Populus tremuloides Michx.) forest and woodland communities of southwestern Colorado. Ecological Indicators 48, 189-197.

Messier, J., McGill, B.J., Enquist, B.J., Lechowicz, M.J., 2017. Trait variation and integration across scales: is the leaf economic spectrum present at local scales? Ecography 40, 685-697.

Messier, J., McGill, B.J., Lechowicz, M.J., 2010. How do traits vary across ecological scales? A case for trait-based ecology. Ecology letters 13, 838-848.

Millard, P., Grelet, G.-a., 2010. Nitrogen storage and remobilization by trees: ecophysiological relevance in a changing world. Tree Physiology 30, 1083-1095.

Millard, P., Way, D.A., 2011. Tree competition and defense against herbivores: currency matters when counting the cost. Tree physiology 31, 579-581.

Mitton, J.B., Grant, M.C., 1996. Genetic variation and the natural history of quaking aspen. Bioscience 46, 25-31.

Mock, K.E., Callahan, C.M., Islam-Faridi, M.N., Shaw, J.D., Rai, H.S., Sanderson, S.C., Rowe, C.A., Ryel, R.J., Madritch, M.D., Gardner, R.S., Wolf, P.G., 2012. Widespread triploidy in Western North American aspen (Populus tremuloides). PLoS One 7, e48406.

Mock, K.E., Rowe, C.A., Hooten, M.B., Dewoody, J., Hipkins, V.D., 2008. Clonal dynamics in western North American aspen (Populus tremuloides). Mol Ecol 17, 4827-4844.

Moles, A.T., Bonser, S.P., Poore, A.G., Wallis, I.R., Foley, W.J., 2011. Assessing the evidence for latitudinal gradients in plant defence and herbivory. Functional Ecology 25, 380-388.

Moles, A.T., Perkins, S.E., Laffan, S.W., Flores-Moreno, H., Awasthy, M., Tindall, M.L., Sack, L., Pitman, A., Kattge, J., Aarssen, L.W., 2014. Which is a better predictor of plant traits: temperature or precipitation? Journal of Vegetation Science 25, 1167-1180.

Morgan, M., 1969. Ecology of aspen in Gunnison County, Colorado. American Midland Naturalist, 204-228.

Niinemets, Ü., 2015. Is there a species spectrum within the world-wide leaf economics spectrum? Major variations in leaf functional traits in the Mediterranean sclerophyll Quercus ilex. New Phytologist 205, 79-96.

Opedal, Ø.H., Armbruster, W.S., Graae, B.J., 2015. Linking small-scale topography with microclimate, plant species diversity and intra-specific trait variation in an alpine landscape. Plant Ecology & Diversity 8, 305-315.

Ordoñez, J.C., Van Bodegom, P.M., Witte, J.P.M., Wright, I.J., Reich, P.B., Aerts, R., 2009. A global study of relationships between leaf traits, climate and soil measures of nutrient fertility. Global Ecology and Biogeography 18, 137-149.

Perez-Harguindeguy, N., Diaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry, P., Bret-Harte, M., Cornwell, W.K., Craine, J.M., Gurvich, D.E., 2013. New handbook for standardised measurement of plant functional traits worldwide. Australian Journal of botany 61, 167-234.

Qu, L., Qu, M.L., 2017. Package 'varComp'.

R Core Team, 2017. Team, R.C., 2017. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. 2016. Retrieved from https://www.r-project.org/.

Reich, P.B., Ellsworth, D.S., Walters, M.B., Vose, J.M., Gresham, C., Volin, J.C., Bowman, W.D., 1999. Generality of leaf trait relationships: a test across six biomes. Ecology 80, 1955-1969.

Reich, P.B., Oleksyn, J., 2004. Global patterns of plant leaf N and P in relation to temperature and latitude. Proceedings of the National Academy of Sciences of the United States of America 101, 11001-11006.

Reich, P.B., Walters, M.B., Ellsworth, D.S., 1997. From tropics to tundra: global convergence in plant functioning. Proceedings of the National Academy of Sciences 94, 13730-13734.

Reich, P.B., Walters, M.B., Ellsworth, D.S., Vose, J.M., Volin, J.C., Gresham, C., Bowman, W.D., 1998. Relationships of leaf dark respiration to leaf nitrogen, specific leaf area and leaf life-span: a test across biomes and functional groups. Oecologia 114, 471-482.

Serbin, S.P., Singh, A., McNeil, B.E., Kingdon, C.C., Townsend, P.A., 2014. Spectroscopic determination of leaf morphological and biochemical traits for northern temperate and boreal tree species. Ecological Applications 24, 1651-1669.

Shipley, B., Lechowicz, M.J., Wright, I., Reich, P.B., 2006. Fundamental trade-offs generating the worldwide leaf economics spectrum. Ecology 87, 535-541.

Smith, E.A., Collette, S.B., Boynton, T.A., Lillrose, T., Stevens, M.R., Bekker, M.F., Eggett, D., St Clair, S.B., 2011. Developmental contributions to phenotypic variation in functional leaf traits within quaking aspen clones. Tree physiology 31, 68-77.

Smulders, M., Van Der Schoot, J., Arens, P., Vosman, B., 2001. Trinucleotide repeat microsatellite markers for black poplar (Populus nigra L.). Molecular Ecology Resources 1, 188-190.

Soltis, D.E., Soltis, P.S., 1995. The dynamic nature of polyploid genomes. Proceedings of the National Academy of Sciences 92, 8089-8091.

Stark, J., Lehman, R., Crawford, L., Enquist, B.J., Blonder, B., 2017. Does environmental heterogeneity drive functional trait variation? A test in montane and alpine meadows. Oikos 126, 1650-1659.

Stöcklin, J., Kuss, P., Pluess, A.R., 2009. Genetic diversity, phenotypic variation and local adaptation in the alpine landscape: case studies with alpine plant species. Botanica Helvetica 119, 125-133.

Sultan, S., 1995. Phenotypic plasticity and plant adaptation. Acta botanica neerlandica 44, 363-383.

Tuskan, G.A., Gunter, L.E., Yang, Z.K., Yin, T., Sewell, M.M., DiFazio, S.P., 2004. Characterization of microsatellites revealed by genomic sequencing of Populus trichocarpa. Canadian Journal of Forest Research 34, 85-93.

Vasseur, F., Violle, C., Enquist, B.J., Granier, C., Vile, D., 2012. A common genetic basis to the origin of the leaf economics spectrum and metabolic scaling allometry. Ecology Letters 15, 1149-1157.

Violle, C., Enquist, B.J., McGill, B.J., Jiang, L., Albert, C.H., Hulshof, C., Jung, V., Messier, J., 2012. The return of the variance: intraspecific variability in community ecology. Trends Ecol Evol 27, 244-252.

W.R.C., 2018. Western Regional Climate Center, Retrieved from http://wrcc.dri.edu

Wehrens, R., Mevik, B.-H., 2007. The pls package: principal component and partial least squares regression in R.

Wold, S., Sjöström, M., Eriksson, L., 2001. PLS-regression: a basic tool of chemometrics. Chemometrics and intelligent laboratory systems 58, 109-130.

Wright, I.J., Reich, P.B., Cornelissen, J.H., Falster, D.S., Garnier, E., Hikosaka, K., Lamont, B.B., Lee, W., Oleksyn, J., Osada, N., Poorter, H., Villar, R., Warton, D.I., Westoby, M., 2005. Assessing the generality of global leaf trait relationships. New Phytol 166, 485-496.

Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F., Cavender-Bares, J., Chapin, T., Cornelissen, J.H., Diemer, M., 2004. The worldwide leaf economics spectrum. Nature 428, 821-827.

Wu, J., Chavana-Bryant, C., Prohaska, N., Serbin, S.P., Guan, K., Albert, L.P., Yang, X., Leeuwen, W.J., Garnello, A.J., Martins, G., 2017. Convergence in relationships between leaf traits, spectra and age across diverse canopy environments and two contrasting tropical forests. New Phytologist 214, 1033-1048.

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Table S1 Means, standard deviations (SD), sample sizes (n) for leaf traits SLA, LDMC, LNC, A_{obs}, Rd and LL and ploidy level for each clone

Table S1 Means, standard deviations (SD), sample sizes (n) for leaf traits SLA, LDMC, LNC, A_{obs}, R_d and LL and ploidy level for each clone

							SLA ($cm^2\ g^{-1}$)		LDMC ($g\ g^{-1}$)		LNC (%)		Aobs ($\mu mol\ m^{-2}\ s^{-1}$)		Rd ($\mu mol\ m^{-2}\ s^{-1}$)		LL (months)
Site name	**Site nr.**	**Plot nr.**	**Clone name**	**Ploidy level**	**Nr. of trees /clone**	**n**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**
Gothic (low)	1	1	RMBL 1	Triploid	10	79	138.23	22.72	0.34	0.03	3.17	0.19	7.44	3.43	2.16	0.89	3.00
		2	RMBL 2	Triploid	10	78	150.58	17.17	0.36	0.03	3.15	0.17	5.46	3.27	1.2	0.63	2.56
		3	RMBL 34	Triploid	20	159	151.82	18.91	0.38	0.04	3.14	0.15	9.11	3.13	1.61	0.74	2.66
		4															
		5	RMBL 5	Diploid	10	80	112.88	12.51	0.43	0.03	2.68	0.20	9.72	3.36	1.83	0.74	2.56
DeerCreek (middle)	2	1	DC 1	Triploid	10	80	125.39	19.57	0.42	0.04	2.81	0.18	7.84	3.13	1.68	0.64	3.20
		2	DC 2	Triploid	10	80	136.91	23.56	0.39	0.03	2.93	0.13	9.0	2.54	1.68	0.49	3.00
		3	DC 3 4	Triploid	20	160	131.67	23.87	0.40	0.03	2.72	0.27	8.82	3.55	1.82	0.89	2.66
		4															
		5	DC 5	Triploid	10	80	123.41	15.35	0.41	0.03	2.78	0.15	6.26	3.07	1.67	0.76	2.66
Avery (high)	3	1	Avery 1	Triploid	10	80	141.89	20.24	0.40	0.04	2.83	0.38	6.8	3.03	1.6	0.78	NA
		2	Avery 23	Triploid	17	136	142.64	16.65	0.40	0.04	3.20	0.39	9.41	4.1	1.59	0.63	2.83
		3															
		4	Avery 3 4	Triploid	13	103	150.59	23.52	0.34	0.004	2.52	0.29	8.48	3.65	1.67	0.53	2.63
		5	Avery 5	Diploid	10	80	133.08	10.97	0.42	0.03	2.64	0.18	11.78	2.8	1.89	0.7	2.83
Bellview (alpine)	4	1	Bellview 1	Diploid	10	80	130.06	17.44	0.47	0.04	2.75	0.17	4.36	3.06	0.75	0.8	NA
		2	Bellview 2	Diploid	10	68	124.08	13.6	0.41	0.03	2.76	0.35	8.75	3.61	2.36	0.99	2.53
		3	Bellview 3	Diploid	2	16	100.15	7.2	0.45	0.02	2.34	0.32	11.1	2.95	1.88	0.89	NA

Table S2 Means, standard deviations (SD) for microclimatic variables: July day temperature, light intensity, soil moisture and pH for each clone

Table S2 Means, standard deviations (SD) for microclimatic variables: July day temperature, light intensity, soil moisture and pH for each clone

Site name	Site nr.	Site nr.	Plot nr.	Clone name	Nr. of trees /clone	Mean July day temperature	SD	Mean July light intensity	SD	Mean soil moisture	SD	Mean pH	SD
		1	1	RMBL 1	10	12.77	0.26	5891.37	1254.9	0.22	0.02	6.01	0.21
		1	2	RMBL 2	10	13.39	0.35	9114.41	1563.56	0.28	0.08	5.44	0.38
Gothic	1	1	3	RMBL 34	20	12.40							
(low)		1	4				0.24	7047.38	1303.28	0.28	0.09	6.45	0.25
		1	5	RMBL 5	10	12.35	0.20	9323.42	1995.41	0.22	0.04	5.92	0.46
		2	1	DC 1	9	12.65	0.30	9217.4	3537.87	0.39	0.15	6.5	0.33
		2	2	DC 2	10	11.66	0.14	6680.13	1369.92	0.38	0.14	5.98	0.25
DeerCreek	2	2	3	DC 3 4	20	11.56							
(middle)		2	4				0.39	7494.04	2232.33	0.46	0.25	6.08	0.22
		2	5	DC 5	10	11.03	0.50	7694.36	1861.24	0.42	0.11	6.14	0.25
		3	1	Avery 1	10	11.67	0.15	7024.46	2097.90	0.35	0.19	5.63	0.46
		3	2	Avery 23	17	11.14							
Avery	3	3	3				0.22	6235.28	1898.51	0.30	0.23	5.79	0.35
(high)		3	4	Avery 3 4	13	10.60	0.27	4778.53	1154.74	0.27	0.11	5.44	0.43
		3	5	Avery 5	10	11.33	0.20	8935.72	696.59	0.18	0.20	5.57	0.34
		4	1	Bellview 1	10	10.88	0.46	6671.06	1746.56	0.57	0.17	5.62	0.33
Bellview	4	4	2	Bellview 2	10	10.59	0.35	6883.76	1372.17	0.06	0.02	6.39	0.25
(alpine)		4	3	Bellview 3	2	10.50	0.30	8266.75	1067.24	NA	NA	NA	NA

Table S3 Likelihood ratio tests (LRT) for trait-trait relationships testing whether models with only random clone intercepts are better than models with random intercepts and slopes

Table S3 LRT tests for trait-trait relationships testing whether models with only random clone intercepts are better than models with random intercepts and slopes

Trait	X^2	*df*	P-value
SLA-LDMC	80.24	3	$2.2e^{-16}$
SLA-LNC	2.78	3	0.43
SLA -A_{obs}	5.55	3	0.14
SLA -R_d	17.81	3	0.001
A_{obs}-LNC	35.48	3	$9.66\ e^{-08}$
A_{obs} -RD	66.56	3	$2.33\ e^{-14}$
R_d -LNC	2.58	3	0.46
SLA-LL	-	-	-
LNC-LL	-	-	-
A_{obs} -LL	-	-	-
R_d -LL	-	-	-

Table S4 Individual clone slopes for trait pairs

Table S4 Individual clone slopes for trait pairs

Trait pait/ Clone name	SLA LDMC	SLA LNC	SLA A_{obs}	SLA R_d	A_{obs} LNC	A_{obs} RD	R_d LNC
Avery 1	-0.98	0.03	-0.62	-1.62	0.76	0.06	-0.97
Avery 23	-0.39	0.02	-1.14	-1.70	1.58	0.63	0.19
Avery 34	-1.37	-0.04	-1.30	-1.13	0.82	-0.04	-0.84
Avery 5	-0.49	0.07	-0.56	-1.72	-0.08	0.16	-0.47
Bellview 1	-0.94	0.10	0.19	-3.05	1.34	-0.23	-0.85
Bellview 2	-0.43	0.15	-0.44	-0.77	-0.16	0.10	-0.05
Bellview 3	-1.06	0.24	-0.72	-1.54	0.11	0.11	-0.46
DC 1	-0.71	0.14	-1.05	-1.74	0.42	-0.03	-0.24
DC 2	-0.68	0.07	-0.04	-1.10	0.55	0.22	-0.43
DC 34	-0.98	0.09	-1.35	-2.17	0.47	0.49	-0.58
DC 5	-1.18	0.15	-1.13	-1.96	0.96	-0.03	-0.62
RMBL 1	-0.90	0.06	1.72	-1.10	1.68	-0.10	-0.23
RMBL 2	-0.41	-0.03	-1.10	-1.92	0.89	0.23	-0.63
RMBL 34	-0.23	-0.04	-0.53	-1.47	0.82	-0.10	-0.47
RMBL 5	-0.60	0.22	-1.16	-2.08	0.20	0.23	-0.31

Table S5 LRT for trait-environment relationships testing whether models with only random clone intercepts are better than models with random intercepts and slopes

Table S5 LRT tests for trait-trait relationships testing whether models: random clone intercept vs. random clone intercept and slope

Trait	X^2	*df*	**P-value**
LNC	144.47	12	$2.22e^{-16}$
SLA	83.927	12	$7.32\ e^{-13}$
LDMC	56.28	12	$1.07\ e^{-07}$
Aobs	34	12	0.001
Rd	56.32	12	$1.05\ e^{-07}$
LL	-	-	-

Table S6 Estimates and confidence intervals for trait-environment relationships

Table S6 Estimates and confidence intervals for trait-environment relationships.

Env. variable	LNC	SLA	LDMC	Aobs	Rd	LL
Temperature	**0.17(0.001,0.35)**	-0.11(-0.50,0.28)	-0.02(-0.26,0.21)	**0.32(0.02,0.63)**	**0.45(0.04,0.85)**	**0.96(0.88,0.94)**
Light intensity	0.08(-0.04,0.20)	**-0.37(-0.55,-0.20)**	**0.23(0.08,0.37)**	0.17(-0.13,0.36)	-0.04(-0.15,0.07)	-0.33(-0.37,-0.27
Soil moisture	-0.18(-0.54,0.18)	0.00(-0.15,0.15)	0.09(-0.09,0.28)	-0.06(-0.31,0.19)	-0.02(-0.19,0.13)	-0.10(-0.15,-0.05)
pH	-0.02(-0.25,0.21)	-0.08(-0.24,0.08)	0.05(-0.09,0.18)	0.05(-0.06,0.16)	0.03(-0.13,0.19)	**0.23(0.18, 0.28)**
Tree age	**-0.28(-0.34,-0.22)**	**-0.48(-0.55,-0.40)**	0.04(-0.04,0.11)	**-0.11(-0.19,-0.23)**	**0.09(0.001,0.18)**	0.02(-0.02, 0.07)

Table S7 Individual clone slopes for trait-environment relationships for LNC

Table S7 Individual clone slopes fo LNC

Clone name	Temperature	Light	Moisture	pH
Avery 1	0.74	0.05	0.10	-0.10
Avery 23	0.74	0.04	0.41	1.10
Avery 34	0.74	0.07	-1.51	-0.28
Avery 5	0.74	0.01	0.20	-0.23
Bellview 1	0.74	0.03	0.25	0.14
Bellview 2	0.74	0.07	0.29	0.11
DC 1	0.74	0.08	0.15	0.06
DC 2	0.74	0.09	0.34	0.42
DC 34	0.74	0.22	0.31	-0.06
DC 5	0.74	0.06	0.31	0.21
RMBL 1	0.74	0.08	0.02	0.42
RMBL 2	0.74	0.11	-0.02	0.30
RMBL 34	0.74	0.16	-0.11	0.34
RMBL 5	0.74	0.07	0.14	-0.18

Table S8 Individual clone slopes for trait-environment relationships for SLA

Table S8 Individual clone slopes for SLA

Clone name	Temperature	Light	Moisture	pH
Avery 1	0.08	-0.08	-0.09	-0.07
Avery 23	-1.11	-0.40	0.31	-0.40
Avery 34	-0.11	-0.93	0.10	-0.33
Avery 5	0.00	-0.51	-0.14	-0.11
Bellview 1	0.06	-0.57	-0.16	0.12
Bellview 2	0.83	-0.47	0.07	-0.13
DC 1	-0.47	-0.07	-0.01	-0.08
DC 2	0.22	-0.35	0.24	0.17
DC 34	-1.20	-0.21	-0.06	0.05
DC 5	-0.06	-0.31	-0.04	-0.25
RMBL 1	-0.02	-0.37	0.02	-0.04
RMBL 2	0.33	-0.38	-0.09	-0.05
RMBL 34	0.51	-0.46	-0.07	0.14
RMBL 5	-0.57	-0.11	-0.06	-0.13

Table S9 Individual clone slopes for trait-environment relationships for LDMC

Table S9 Individual clone slopes for LDMC

Clone name	Temperature	Light	Moisture	pH
Avery 1	-0.08	0.06	0.29	0.02
Avery 23	-0.09	0.39	0.01	0.01
Avery 34	0.14	0.36	-0.14	0.35
Avery 5	0.02	0.35	-0.10	0.02
Bellview 1	0.28	0.53	0.18	-0.36
Bellview 2	-0.20	0.17	-0.04	0.03
DC 1	0.21	0.02	0.17	0.06
DC 2	-0.13	0.22	-0.01	0.11
DC 34	0.23	0.18	0.01	0.22
DC 5	-0.01	0.18	0.02	0.06
RMBL 1	-0.36	0.29	0.22	0.17
RMBL 2	-0.27	0.14	0.04	0.08
RMBL 34	-0.15	0.27	0.74	-0.01
RMBL 5	0.10	0.08	-0.06	-0.04

Table S10 Individual clone slopes for trait-environment relationships for A_{obs}

Table S10 Individual clone slopes for Aobs

Clone name	Temperature	Light	Moisture	pH
Avery 1	0.10	-0.10	0.22	0.05
Avery 23	0.18	0.39	-0.37	0.08
Avery 34	0.22	0.71	0.10	0.03
Avery 5	0.45	0.34	0.21	0.06
Bellview 1	0.35	0.01	-0.15	0.04
Bellview 2	-0.09	0.12	-0.35	0.04
DC 1	0.25	-0.04	0.31	0.06
DC 2	0.72	0.08	0.18	0.04
DC 34	1.03	-0.02	-0.07	0.04
DC 5	0.17	0.10	0.08	0.05
RMBL 1	0.39	0.06	0.00	0.05
RMBL 2	-0.07	0.27	0.11	0.06
RMBL 34	0.27	0.36	-0.83	0.05
RMBL 5	0.56	0.16	-0.25	0.03

Table S11 Individual clone slopes for trait-environment relationships for R_d

Table S11 Individual clone slopes for Rd

Clone name	Temperature	Light	Moisture	pH
Avery 1	0.78	-0.04	0.00	0.12
Avery 23	0.95	-0.02	-0.27	0.15
Avery 34	0.35	-0.05	-0.09	0.01
Avery 5	0.47	-0.04	-0.24	0.00
Bellview 1	0.55	-0.02	0.14	0.07
Bellview 2	-0.50	-0.05	-0.10	0.03
DC 1	0.15	-0.04	-0.07	-0.10
DC 2	0.56	-0.03	0.09	-0.04
DC 34	1.38	-0.06	-0.13	-0.14
DC 5	0.26	-0.04	-0.10	0.12
RMBL 1	0.38	-0.04	-0.04	0.07
RMBL 2	0.15	-0.03	0.24	0.16
RMBL 34	1.05	-0.03	0.31	-0.25
RMBL 5	-0.20	-0.05	-0.15	0.14

Table S12 LRT tests for trait-trait relationships for ploidy

Table S12 LRT tests for trait-trait relationships for ploidy

Trait	X^2	df	P-value
SLA-LDMC	0.97	1	0.32
SLA-LNC	1.73	1	0.19
SLA -A_{obs}	1.62	1	0.20
SLA -R_d	1.95	1	0.16
A_{obs}-LNC	9.23	1	0.002
A_{obs} -RD	9.80	1	0.001
R_d -LNC	0.28	1	0.59
SLA-LL	5.46	1	0.02
LNC-LL	0.49	1	0.48
A_{obs} -LL	0.04	1	0.83
R_d -LL	1.74	1	0.19

Table S13 Ploidy slopes and intercepts for trait-trait pairs

Table S13 Ploidy slopes and intercepts for trait-trait pairs

Trait pair/		**SLA**	**SLA**	**SLA**	**SLA**	**A_{obs}**	**A_{obs}**
Ploidy level		**LDMC**	**LNC**	**A_{obs}**	**R_d**	**LNC**	**RD**
Diploid	Intercept	4.23 (4.14,4,32)	4.73(6.62,4.85)	5.49(4.34,6.64)	8.63(7.49,9.76)	2.36(1.52,3.20)	0.57(0.21,0.94)
	Slope	-0.65(-0.72,-0.58)	0.05(-0.04,0.13)	-0.71(-0.94,-0.48)	-1.72(-1.95,-1.49)	-0.28(-1.90,0.52)	-0.10(-0.21,0.01)
Triploid	Intercept	4.29(4.20,4.37)	4.88(4.76,5.00)	5.46(4.29,6.62)	8.88(7.74,10.03)	0.69(0.16,1.21)	0.16(-0.05,0.39)
	Slope	-0.65(-0.72,-0.58)	0.05(-0.04,0.13)	-0.71(-0.94,-0.48)	-1.72(-1.95,-1.49)	1.43(0.51,2.35)	0.21(0.08,0.34)

Trait pair/		**SLA**	**LNC**	**A_{obs}**	**R_d**	**R_d**
Ploidy level		**LL**	**LL**	**LL**	**LL**	**LNC**
Diploid	Intercept	3.27(1.85,4.69)	0.64(0.04,1.25)	1.21(-0.54,2.96)	-0.28(-1.64,1.07)	0.73(0.24,1.20)
	Slope	1.54(0.07,3.01)	0.31(-0.31,0.93)	1.08(-0.72,2.90)	0.91(-0.48,2.31)	-0.38(-0.79,0.02)
Triploid	Intercept	5.22(4.63,5.81)	0.74(0.11,1.38)	0.83(-1.04,2.70)	-0.55(-2.00,0.89)	0.79(0.31,1.26)
	Slope	-1.80(-3.38,-0.23)	0.31(-0.31,0.93)	1.08(-0.72,2.90)	0.91(-0.48,2.31)	-0.38(-0.79,0.02)

Table S14 LRT tests for trait-environment relationships for ploidy

Table S14 LRT tests for trait-environment relationships for ploidy

Trait	X^2	*df*	P-value
LNC	10.87	4	0.03
SLA	24.19	4	$7.32e^{-05}$
LDMC	8.32	4	0.08
Aobs	7.20	4	0.13
Rd	32.54	4	$1.48\ e^{-06}$
LL	44.05	4	$6.27\ e^{-09}$

Table S15 Estimates for trait-environment relationships for different ploidy levels

Table S15 Estimates for trait-environment relationships for different ploidy levels

Trait		**Environmental variable**			
LNC		Temperature	Light	pH	Moisture
diploid	intercept	-0.79(-1.63,0.06)			
	slope	0.02(-0.65,0.71)	-0.01(-0.46,0.42)	-0.13(-0.40,0.14)	0.05(-0.27,0.37)
triploid	intercept	1.10(-0.27,0.94)			
	slope	-0.18(-0.89,0.48)	0.22(-0.21,0.68)	-0.12(-0.06,0.63)	0.22(-0.48,0.17)
SLA					
diploid	intercept	-0.76(-1.56,0.03)			
	slope	-0.07(-0.67,0.56)	-0.14(-0.52,0.23)	-0.05(-0.28,0.17)	0.03(-0.24,0.30)
triploid	intercept	1.02(0.16,1.81)			
	slope	-0.52(-1.09,0.22)	-0.33(-0.59,0.20)	-0.36(-0.55,0.06)	0.17(-0.14,0.42)
LDMC					
diploid	intercept	0.84(0.13,1.55)			
	slope	0.27(0.06,0.45)	0.24(0.16,0.34)	0.12(0.04,0.21)	0.09(0.03,0.15)
triploid	intercept	-1.07(-1.76,-0.43)			
	slope	0.27(0.06,0.45)	0.24(0.16,0.34)	0.12(0.04,0.21)	0.09(0.03,0.15)
A_{obs}					
diploid	intercept	0.35(-0.51,1.25)			
	slope	0.54(-.27,0.76)	0.04(-0.06,0.15)	0.21(0.10,0.31)	-0.23(-0.31,0.15)
triploid	intercept	-0.61(-1.46,0.30)			
	slope	0.54(-.27,0.76)	0.04(-0.06,0.15)	0.21(0.10,0.31)	-0.23(-0.31,0.15)
R_d					
diploid	intercept	0.28(-0.93,1.47)			
	slope	-0.10(-0.87,0.69)	-0.32(-0.79,0.15)	0.26(-0.02,0.54)	-0.30(-0.66,0.06)
triploid	intercept	-0.65(-1.85,0.77)			
	slope	1.04(0.26,1.94)	0.01(-0.14,0.82)	-0.19(-0.60,0.01)	-0.04(-0.25,0.48)
LL					
diploid	intercept	-0.08(-1.26,1.09)			
	slope	0.48(0.24,0.71)	-0.20(-0.36,-0.03)	0.06(-0.07,0.19)	0.0004(-0.30,0.31)
triploid	intercept	-0.14(-0.37,0.09)			
	slope	0.97(0.27,0.72)	-0.39(-0.33,0.01)	0.27(0.07,0.35)	-0.12(-0.43,0.19)