Coordination of trait responses to above and belowground resource limitation in cultivated sunflower (*Helianthus annuus* L.)

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Summary

- Plant responses to resource limitations can be interpreted within an economic framework of maximizing uptake of the most limiting resource. Here we examine biomass allocation, the effect of allometry, and coordination among allocational, morphological and anatomical traits in response to above and belowground resource limitation in cultivated sunflower.
- We grew seedlings of ten sunflower genotypes for three weeks in a factorial of light (50% shade) and nutrient (10% supply) limitation in the greenhouse and measured leaf, stem, fine root, and taproot traits.
- Under resource limitation, more biomass was allocated to the organ capturing the most limiting resource. Allometric scaling accounted for a substantial portion of trait responses, but key traits showed additional active adjustment. Anatomical trait responses were less plastic and less directionally consistent across organs than allocational and morphological traits. Nutrient and light limitation shifted biomass allocational traits along a common axis, however, in opposite directions.
- Both allometric and active adjustments to resource limitation drove key traits to be more acquisitive. Traits generally shifted along a common axis but alternated greater above or belowground acquisitive allocational traits depending on resource limitation. These results highlight the contrast between adjustments to resource limitation at the ecological versus evolutionary scale.

Keywords: allocation, morphology, anatomy, allometry, plasticity, K_s, SLA, SRL

Introduction

The extent of plant trait adjustment in response to a changed environment is generally considered as the plant's phenotypic plasticity (Valladares *et al.,* 2007, Nicotra *et al.,* 2010). This plasticity, according to the balanced growth hypothesis or functional equilibrium theory (Poorter *et al.,* 2012; Robinson *et al.,* 2010) can serve to maximize the uptake of the most limiting resource (Chapin, 1991, Bloom *et al.,* 1985). For example, increased mass allocation to leaves under shade, or increased allocation to roots under nutrient limitation alleviates some of the stress caused by resource limitation (Shipley & Maziane, 2002; Sugiura & Tateno, 2011). Thus, on the scale of environmentally induced plasticity, plants are expected to independently adjust allocation to alleviate above- or belowground resource limitation. However, how plants adjust a broader range of traits to multiple limiting resources (Freschet *et al.,* 2018), whether above and belowground traits are adjusted comparably (Freschet *et al.,* 2015), and how allometric scaling influences these trait adjustments (Reich 2018), have been a source of debate.

At evolutionary scales, across species or populations with a species, plant functional traits are generally expected to exhibit a coordinated shift due to resource limitation, i.e., the leaf, stem, root and whole-plant economics spectrum (Wright *et al.,* 2004; Fortunel *et al.,* 2012; Reich, 2014; Díaz *et al.,* 2016). On this "whole plant economic spectrum" species or populations adapted to higher resource conditions are generally expected to have resource acquisitive or "faster" traits , to allow for fast growth, while species or populations adapted to poorer resource conditions are generally expected to have "slower" traits to support long tissue lifespan under slow growth. The "slower" traits in one organ and trait due to one limiting resource are expected to be reflected in "slower" traits across all organs in the plant (Reich, 2014). However, much remains unclear about how the expectation of integrated trait responses at the "whole plant economic spectrum" (Reich, 2014) level plays out at the level of environmentally induced plasticity in terms of coordination among a broad set of traits and plant organs.

Ostensibly, this seems to present a contradiction. At the ecological scale, plant above and belowground traits are expected to independently respond to the environmental resource limitations to maximize acquisition of the most limiting resource. Yet at an evolutionary scale, populations and species are generally expected to respond to the environmental resource limitation with more conservative traits across all organs, following the "plant economic spectrum." Whether or not the responses at ecological timescales to resource limitation follow the same trajectory as those at evolutionary timescales can shed light on how these global trait correlations arise. Certainly there exists a body of literature that highlights the discrepancy between intra and interspecific response to resource limitation (e.g Niinements, 2015; Temme *et al.,* 2017, Anderegg *et al.,* 2018). Thus, while the question <u>if</u> ecological and evolutionary trait adjustments align can be a bit of a strawman, the question of <u>how much</u> and at what scale these broad global patterns translate to responses of plants at the ecological scale remains important to address.

Plant anatomical traits are often overlooked in plant trait studies due to time and budget constraints. However, variation in anatomical traits underlie or contribute to morphological and physiological traits that have received much attention (Kong *et al.,* 2014; Scoffoni *et al.,* 2015, John *et al.,* 2017). For example, palisade parenchyma thickness is positively correlated to leaf thickness (Catoni *et al.,* 2015) and photosynthetic rate (Chatelet *et al.,* 2013). A thicker cortex could provide a relative larger site for mycorrhizal infection and higher resource uptake in thicker roots (Kong *et al.,*

2014). Root cortex thickness, due to the size of cortical cells (Eissenstat & Achor, 1999) strongly affects fine root diameter (Gu *et al.,* 2014; Guo *et al.,* 2008). Exploring how responses in anatomical traits align with other responses of other traits will promote our understanding of how plants adjust to changing environmental conditions from tissue, to organ, to architecture.

A factor to consider in understanding plant traits shifts under resource limitation is how allometric scaling affects traits (Shipley & Meziane, 2002; Reich, 2018; Osnas *et al.,* 2013). For example, shoot:root ratio increases with size for herb plants yet decreases for woody species (Poorter *et al.,* 2012). Thus, allometric scaling associated with a smaller plant under limiting resources could be a strategy to acquire the most limiting resource at a given plant size (Coleman *et al.,* 1994; Müller *et al.,* 2000; Reich, 2018). However, there is evidence for active adjustments beyond those accounted for by allometry that affect resource capture. For example greater plant height for a given mass under low light conditions (Rice & Bazzaz, 1989). By explicitly taking biomass into account we can separate trait adjustments to resource limitation into an allometric scaling due to plant biomass and additional active component.

Here we examine trait responses to light and nutrient limitation of traits across different trait categories (biomass allocation, morphology and anatomy) and organs (leaf, stem and root) in cultivated sunflower. Prior research has shown strong plastic responses to resource limitation and other environmental factors in *Helianthus* (Donovan *et al.,* 2014; Bowsher *et al.,* 2017; Pilote, 2017). By borrowing ideas form the plant economic spectrum we seek to place another brick in the wall (*sensu* Reich, 2018) in understanding plants responses to resource limitation at differing scales; focused here on contrasting responses across trait categories. Specifically, we sought to answer the following questions: 1) Are biomass allocation responses to light and nutrient limitations consistent with the balanced growth hypothesis? 2) Does allometry account for most/all of the trait responses to resource limitation? 3) Do different categories of traits (allocational, morphological, anatomical) show a coordinated trait shift due to resource limitation across all organs?

Material and Methods

Experiment design

To address our questions we selected a diverse set of 10 cultivated sunflower genotypes (**Table S1**) from a larger diversity panel used for genomic dissection of traits (Mandel *et al.,* 2011, Nambeesan *et al.,* 2015, Masalia *et al.,* 2018). We grew six replicates of each genotype in a factorial design of two nutrient treatments (rich and poor) and two light treatments (sun and shade), totaling 240 plants, at the Botany greenhouses of The University of Georgia USA in March 2018. Achenes were sown in seedling trays and allowed to grow for seven days after which seedlings were transplanted to 5 liter pots filled with a 3:1 sand:calcinated clay mixture (Turface MVP, Turface Athletics, Buffalo Grove, IL). Pots were arranged in a split plot design of 6 replicate blocks with half of each block under 50% (high density woven polyethylene cloth) shade (**Fig. S1**). Each block subsection consisted of two, randomly distributed, replicates of each genotype supplied with either 40g or 4g fertilizer (Osmocote Plus 15-9-12 with micronutrients, Scotts, Marysville, OH, USA). Greenhouse temperatures were set to maintain 18–24 °C, and supplemental lighting was provided to maintain a 15/9-h photoperiod.

Plant harvest and trait measurements

Plants were harvested 3 weeks after transplanting (4 weeks after germination). At harvest plant

height (from soil surface to top of apical meristem) and stem diameter (midway between cotyledons and first leaf pair) were measured. Plants were separated into root, leaf (including cotyledons), and stem (including bud if present—rarely) for dry biomass measurements. Prior to drying, each organ was subsampled for tissue for anatomical and morphological analysis. Fresh biomass of subsamples was measured, converted to dry biomass based on the ratio of fresh/dry biomass of remaining tissue organs, and added to the whole plant biomass prior to analysis.

For leaf anatomical traits, one recently matured fully expanded leaf was sampled from each replicate, cutting a 1×0.5 cm rectangle out of the leaf center. For stem anatomical traits, a 5 mm length stem segment (centered between the cotyledon and the first leaf pair) was cut from each replicate. For fine root anatomical traits, a single representative lateral root attached to the tap root near to the root/stem junction with an intact root tip was selected, and a 1 cm segment was cut 2 cm from the apex of the fine root. For the tap root anatomical traits, a 1 cm segment was cut 4 cm below the root/stem junction. All tissue subsamples were fixed in FAA (formalin–acetic acid-alcohol solution: 50% ethanol (95% concentration), 5% glacial acetic acid, 10% formaldehyde (37% concentration), and 35% distilled water).

Fixed anatomy subsamples were processed at the University of Georgia Veterinary Histology Laboratory. Each sample was embedded and gradually infiltrated with paraffin, sliced with a sledge microtome, mounted to a slide, and stained with safranin and fast green dye. Slides were imaged with a camera mounted Zeiss light microscope using ZEN software (Carl Zeiss Microscopy, Oberkochen, Germany). Cell and tissue dimensions of leaf, stem, fine and tap roots were traced using Motic Images Advanced 3.2 software (Motic Corporation, Xiamen, China). Theoretical hydraulic conductivity (Ks, $kg \cdot s^{-1} \cdot m^{-1} \cdot MPa^{-1}$) for each sample was calculated, based on the Hagen-Poseuille equation (Tyree & Ewers, 1991): $\mathrm{ks} = (\pi\rho/128\eta\mathrm{Aw})\sum_{i=1}^{n} d_i^4$. where ρ is the density of water (988.3 $kg \cdot m^{-3}$ at 20 °C); η is the viscosity of water (1.002×10^{-9} MPa·s at 20 °C); Aw is the stele (vascular) cross-section area, d is the diameter of the ith vessel and n is the number of conduits in the xylem.

After anatomical trait samples were collected, the rest of the leaf, stem, and fine and tap root of each plant were scanned (Espon, Expression1680, Japan). Total length and volume of fine root, tap root and stem, as well as leaf area was measured using WinRhizo (v. 2002c, Regent Instruments, Quebec, Canada). All tissue was dried at 60°C for 48 h and weighed. Total plant dry mass was calculated as the sum of all plant parts, including the subsamples for anatomical traits. Specific leaf area (SLA, $cm^2 \cdot g^{-1}$) and specific root length (SRL, $m \cdot g^{-1}$) were calculated as the ratios of leaf area to leaf dry mass, and root length to root dry mass, respectively. Tissue density ($g \cdot cm^{-3}$) was calculated as the ratio of dry mass to volume for stem, tap and fine root, respectively. Leaf dry matter content (LDMC, $mg \cdot g^{-1}$), referred to as leaf tissue density, was measured as leaf dry mass divided by leaf fresh mass (Kramer-Walter *et al.,* 2016; Wilson *et al.,* 1999). Mass fractions for each tissue were calculated as proportions of total plant dry mass ($g \cdot g^{-1}$).

Data analysis

The statistical analysis for the phenotypic data was performed using R v3.5.1 (R Core Team). To obtain genotype means from our split plot design, a mixed effects model was fitted using the package *lme4* (Bates *et al.,* 2018) with genotype, light and nutrient level and all interactions as fixed effects and light treatment within block as random factor. Least-square (LS) means of all trait values

without random factor were estimated from this model using the R package *emmeans* (Lenth *et al.,* 2018). To test the effect of genotype and treatment on measured traits we fitted a less expansive mixed effects model with genotype, light and nutrient level as well as the interaction between light and nutrient level as fixed effects (following Freschet *et al.,* 2018) and light treatment within block as random factor. From this model, fixed effects were then tested using a Walds Chi-square test in a type III Anova using the package *car* (Fox *et al.,* 2018). Differences between treatments were tested using a Tukey test corrected for multiple comparisons. We then estimated the influence of plant allometry on the significance of nutrient and light limitation effects on traits by adding (log-transformed) plant biomass as a fixed factor to the models and recalculating means and significance. The relative changes in trait value in each treatment were averaged across genotypes as the value in control (high nutrient & unshaded conditions) minus that in the treatment, and divided by the value at control.

A principal component analysis (PCA) was applied to all the trait data and each trait category of all genotypes, respectively, to determine major sources of variation across multiple traits and identify whether there were concerted trait adjustments to limitation in above or belowground resources. Differences between treatments were tested using, Bonferroni corrected, Hotellings-t test on the first two principal components.

To quantify and compare plastic responses of individual traits to growth conditions, we calculated the relative distance plasticity index (RDPI, Valladares *et al.,* 2006, Scoffoni *et al.,* 2015) for all ten genotypes as ($x_i' - x_i / x_i' + x_i$), where x_i and x_i' are the mean trait values of genotypes grown under control (high-light and high-nutrient) and resource limited condition. In addition, the RDPI after correcting for plant size (total biomass) was also calculated. Differences in RDPI from zero (no plasticity) were determined using T-tests. Data visualizations were made using SigmaPlot 10.0 and *ggplot2* (Wickham *et al.,* 2018).

Results

Plant total biomass and allocation response to resource limitation

Across all genotypes, plant biomass decreased under light, nutrient, and combined limitation (21.43%, 53.25%, and 65.82%, respectively) with additive effects of both stresses on whole plant biomass (**Fig. 1a, Table 1**). The ratio of leaf mass to fine root mass (LFRratio) increased under shade (+82.61%) due to increased mass allocation to leaf mass (LMF, +3.67%) and decreased allocation to fine roots mass (FineRMF, -41.36%) (**Fig. 1b**). In contrast, LFRratio decreased under low nutrients (-45.65%) due to decreased LMF (-12.06%) and an increased FineRMF (+71.42%). While there was no interaction of light and nutrient limitation on whole plant biomass, there was an interaction on allocational traits, as reflected in LMF, stem mass fraction (SMF), and FineRMF (**Table 1**). When biomass was included as a covariate to account for allometric scaling, the effect of nutrient limitation on allocational traits was similar to when biomass was not included, indicating an active adjustment to resource limitation. However, after allometric scaling was taken into account there was no effect of shade on leaf mass fraction, indicating that increased LMF under shade was largely driven by the reduction in plant size and not an active adjustment to resource limitation (**Table 1**).

Morphological traits adjustments

Light and nutrient limitations differed in their effect on plant morphological traits. Shade affected all morphological traits except for fine root diameter (FRD) (**Table 1**). Light limitation led to strong

increases in values for resource acquiring traits such as SLA (+40.53%, **Fig. 2a**), SRL (fine root, +31.81%, **Fig. 2b**; tap root +86.92%), and a moderate increase in plant height (Height, +15.04%). However, strong decreases under shade were also found for other organ dimensions, e.g. leaf thickness (LT, -10.8%), stem diameter (SD, -18.02%), tap root diameter (TRD, -28.84%) tissue density assessed as leaf dry matter content (LDMC, -20.57%); stem tissue density (STD, -22.89%), and fine root tissue density (FineRTD, -18.76%). When taking plant size into account, adjustments to root traits in response to shade were shown to be mainly driven by allometric scaling (**Table 2**). However, for plants were significantly taller for their mass under light limitation (**Table 2)**.

In contrast to the effect of shade, nutrient limitation affected only 4 out of 12 morphological traits (**Table 1**). Nutrient limitation decreased SD (-15.6%) and FRD (-7.25%), and increased the fine root specific root length (FineSRL, +19.82%) (**Fig. 2b)** and TapRTD (+16.56%) (**Table 2, Fig. 2b**). When including biomass into the model adjustments to these traits remained broadly the same.

Anatomical traits adjustment

Overall, shade had a large effect on leaf, stem, and tap root anatomical traits. Shade decreased leaf palisade parenchyma layer thickness (LPT, -12.05%, **Fig. 5a**), stem cortex thickness (SCT, -11.57%, **Fig. 5b**), stem vascular bundle thickness (SVT, -26.2%) and stem xylem thickness (SXT, -24.69%). Belowground, shade reduced tap root cortex thickness (TRCT, -12.93%) and stele diameter (TRSD, -33.64%) (**Table 2**). Vascular tissue adjustment to shade led to decreased leaf hydraulic conductivity (LKs, -19.02%) but increased stem hydraulic conductivity (SKs, +32.36%). Taking plant biomass into account, the effect of shade on SCT, SVT, TRCT and TRSD was revealed to be mainly driven by allometric scaling (**Table 2**). However, plants did have a far greater stem and tap root hydraulic conductivity for their mass under shade (SKs, +95.94; TRKs, +94.08%) (**Table 2**).

In contrast to shade, nutrient limitation predominately affected fine root anatomy. Nutrient limitation decreased fine root cortex thickness (FRCT, -7.72%, **Fig. 5c**) and fine root stele diameter (FRSD, -7.95%) (**Tables 2**). When taking plant biomass into account the negative effects of nutrient limitation on FRCT and FRSD remained (**Table 2**).

Functional traits plasticity and coordinated shift under stress

Across all genotypes, traits categories differed in magnitude of plasticity depending on resource limitation (**Fig. 3**). Biomass allocational traits showed relatively larger magnitude in plasticity, followed by morphology and anatomy (**Fig. 3a**), based on simple means per category in absolute RDPI. On average, light limitation induced a higher relative distance plasticity index (RDPI) values than nutrient limitation (**Fig. 3**). When allometric scaling is factored out, the average magnitude of RDPI values decreased across all trait categories. Notable exceptions were plant height, SKs, and taproot hydraulic conductivity (TRKs), for which RDPI increased after taking plant biomass into account. Interestingly, the magnitude of RDPI in morphology and anatomy switched rank when accounting for trait shifts due to biomass differences (**Fig. 3b**).

When the genotype means for traits in all three traits categories (allocation, morphology, and anatomy) and all treatments were included in a principal component analysis, the first two axes explained 34.4% and 13.4% of the variation, indicating correlated trait responses (**Fig. 4a**). However, light and nutrient limitations did not result in a coordinated shift in all traits along the same axis. The

first principal component provided stronger separation for light treatments and the second principal component provided stronger separation for nutrient treatments (**Fig. 4b-d**). This separation of response to light and nutrient limitation is significant when the effect of plant biomass is taken into account (**Fig. S2a**). The lack of a common shift at the whole plant level is most clear in allocational traits where light and nutrient limitation move traits towards opposite ends of the ordination plane (**Fig. 4b**). As reflected in the effect of light and nutrient stress on biomass, the adjustments to traits due to light limitation were more pronounced then due to nutrient limitation as can be seen by the greater separation between light levels in the PCA biplot. When the effect of plant biomass on trait value is taken into account PCA results are similar. (**Fig. S2**).

Discussion

Here we assessed the direction, magnitude, and coordination of allocation, morphological and anatomical trait responses to light or/and nutrient limitation in cultivated sunflower. Specifically, we sought to answer the following questions: 1) Are trait responses to light and nutrient limitations consistent with the balanced growth hypothesis? 2) Does allometry account for most/all of the trait responses to resource limitation? 3) Do different categories of traits (allocational, morphological, anatomical) show a coordinated trait shift due to resource limitation across all organs? Broadly, we found responses to be consistent with balanced growth; resource limitation was associated with more biomass being allocated to the organs acquiring the limiting resource. Although portions of the plant trait responses were accounted for by allometric scaling (i.e., trait changes due lower biomass of stressed plants) there were additional active adjustments to key traits beyond allometric scaling. We found evidence for coordination of trait adjustments in response to resource stress. However, for allocational traits light and nutrient limitation led to opposite responses that reflect alternating above and belowground traits adjustment becoming more acquisitive depending on resource limitation.

Balanced growth and resource limitation

Consistent with the functional equilibrium or balanced growth hypothesis, plants increase allocation to the plant part acquiring the most limiting resource, both through allometric scaling and additional active responses (Bloom *et al.,* 1985; Poorter *et al.,* 2012). Lower light availability resulted in increased relative investment in aboveground plant organs (higher LMF and SMF, lower FRMF) and the formation of leaves with greater SLA and lower LDMC allowing for light acquisition at a lower carbon cost. Lower nutrient availability resulted in, higher FineRMF and SRL, allowing for a greater exploration of soil volume at reduced carbon and nutrient cost (Hill *et al.,* 2006). Thus, we find results in sunflower to be consistent with those in other growth forms (e.g., grasses, Siebenkäs *et al.,* 2015; shrubs, Valladares *et al.,* 2000a; trees, Reich *et al.,* 1998, Kramer-Walter & Laughlin, 2017). Both above and belowground trait adjustments serve to increase light and nutrient acquisition at reduced absolute biomass invested into the tissue acquiring the limiting resource.

Interestingly, shade had more effect on belowground traits than nutrient limitation on aboveground traits (**Table 1, 2**). Others have found similar results. For example, researchers found higher SRL under shade (Ryser & Eek, 2000), yet unchanged SLA under poor nutrient condition (Siebenkäs *et al.,* 2015). Potentially mechanisms might include hormonal signals transported between source and sink or light signals regulating the root growth and development via light conduction from stem to root (Sun *et al.,* 2005). A thinner fine root and thinner cortex under nutrient limitation would decrease the metabolic cost per unit soil explored (Galindo-Castañeda *et al.,* 2018; Jaramillo *et al.,* 2013). Thus

for a complete picture of plant trait adjustments to resource limitation both above and below ground traits should be taken into account.

Besides a response to resource limitation, many traits showed a strong correlation with plant biomass, with larger plants having a thicker leaf, stem and tap root (**Table 1**). However, even when accounting for plant biomass, treatment effects remained significant for major traits, such as SLA and FineSRL, LPT and FRCT. These adjustments to the major morphological and anatomical traits influencing resource acquisition indicate that light and nutrient limitation had a substantial active effect on plant shoot and root traits beyond allometry. Generally, the allometric adjustments were in a similar direction as the active adjustments (**Fig 3**). Thus, both allometric scaling as well as active trait adjustments served to ameliorate some of the effects of resource limitation.

Hierarchy of trait adjustments

Consistent with other studies, plasticity of mass allocational traits was larger than that of morphological traits (Kramer-Walter & Laughlin, 2017, though opposite Poorter *et al.,* 2012). In addition anatomical traits showed the smallest plasticity, consistent with responses to nutrient limitation in grass (Cai *et al.,* 2017), and light and water limitation in trees (Xu *et al.,* 2015; Catoni *et al.,* 2015). Thus we found trait plasticity to be highest in allocation traits, followed by morphology and anatomy. However, it should be noted that differences in the extent of plasticity for different categories of traits may be species specific. For example, oak (*Quercus robur)* seedlings were more plastic in physiological traits under shade, yet beech (*Fagus sylvatica)* was more plastic in morphological traits (Valladares *et al.,* 2002). Xu *et al.,* (2015) found this sequence of greater to lower plasticity in leaf physiology, morphology, and anatomy in response to limited light or water conditions. Shifts in individual component anatomical traits could add up to larger shifts in morphological traits. For example, leaf thickness is highly correlated with palisade parenchyma (Scoffoni *et al.,* 2015) and root diameter with cortex and/or stele thickness (Kong *et al.,* 2014). Thus, this sequence of trait plasticity might reflect an inherent hierarchy due to the scale at which these traits operate.

In general, pulling out allometric effects decreased the magnitude of adjustment to anatomical traits. However, after accounting for variation associated with allometric scaling, the effects RDPI of stem and taproot Ks increased especially under shade (**Fig 3**). Given that height increased under shade, a higher efficiency of stem and root transport could offset greater resistance due to a longer transport distance (Plavcová & Hacke, 2012). It should be noted, however, that this increased stem and root Ks comes at the cost of increased risk of xylem embolism in shaded plants (Tyree & Zimmermann, 2002). The coordination of stem and root Ks indicates a possible equilibrium between these two components of the plant hydraulic continuum (Maurel *et al.,* 2010; Rodríguez-Gamir *et al.,* 2016), insuring efficient movement of carbon to the roots and nutrients to the leaves. However, the opposite response in leaf Ks provides a puzzling counterpoint that would be worth assessing in more experiments. Additoinally, the anatomical dataset collected in this work provides an excellent resource for state of the art anatomical water flow models (Couvreur *et al.,* 2018), to further shed light on the consequences of these anatomical trait adjustments for plant hydraulics.

Trait syndrome and resource strategy

Contrary to the evolutionarily conservative strategy generally expected under low resource conditions (Reich 2014, Díaz *et al.,* 2016), our results at scale of environmentally induced plasticity

showed that traits shift towards values generally thought to be resource acquisitive under stress (**Fig. 2-4; Table 1,2**). For instance, plants growing under lower resource conditions, with lower biomass, had thinner leaves, stem, fine and tap roots, as well as narrower leaf palisade, stem vascular tissue, fine root cortex and tap root stele, but higher SLA and SRL. These coordinated trait shifts depended on the limiting resource or organ. For example, higher SLA and thinner LPT were only significant under shade, while higher FineSRL and thinner FRCT and FRSD were only significant under poor nutrient condition (**Table 2**). Thus while there was some coordination, shifts were not such that the resource use strategy at the whole-plant level shifted from conservative to acquisitive, even though the individual organ showed more acquisitive traits consistent with increased capture ability for the limiting resource.

Few studies have assessed the phenotypic response of whole plants in terms of biomass allocation, organ morphology and anatomy simultaneously, and even less under multiple resource limitation (Givnish 1988; Pratt *et al.,* 2010). Here, our research demonstrates that major traits from all three categories shift in response to resource limitation, and the extent and direction of the responses is not only associated with changes in plant size (allometric scaling), but includes an additional active adjustments beyond size. Moreover, our results suggest a hierarchy of trait adjustment from anatomical to morphological to allocational. The suite of trait adjustments, across genotypes, broadly results in a more acquisitive suite of traits at limiting resources, albeit with significant differences between light and nutrient limitation. Here, our results highlight the disconnect between the whole plant economic strategy observed at broad global scales and the responses of plants leaves, stems, and roots from anatomy to allocation at the scale of environmentally induced plasticity. In this cultivated crop, arguably released from selection pressures in the wild, plants adjust traits opposite the global pattern and are not coordinated at the whole plant level. A key missing piece in understanding global trait covariance lies in reconciling these ecological scale responses of balanced growth to responses at evolutionary scales.

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Author contributions

YW, LAD, and AAT conceived and designed the experiment. YW carried out the experiment, measurements, and anatomical analyses. YW and AAT performed the statistical analysis. YW wrote the initial draft with substantial revision by AAT and LAD in subsequent drafts. AAT takes responsibility for the integrity of this work from conception to finished whole.

Captions

Table 1 &2. See tables document

Figure 1. Biomass and mass allocation of leaf, stem, tap root, and fine root tissue across a factorial of light and nutrient limitation. Light/Shade, direct sun vs 50% shade. Rich/poor, high nutrients vs 10% nutrient concentration. **(a)** Average whole plant biomass across genotypes (n=10), stacked by

358 average tissue contribution. **(b)** Average mass allocation to tissue types per treatment. Error bars
359 denote standard error across genotypes. Different letters represent significant ($p<0.05$) Tukey post
360 hoc differences between treatments.

361 **Figure 2**. Leaf and fine root morphology across a factorial of light and nutrient limitation.
362 Light/Shade, direct sun vs 50% shade. Rich/poor, high nutrients vs 10% nutrient concentration. **(a)**
363 specific leaf area (SLA, $cm^2\ g^{-1}$), **(b)** specific root length (SRL, $m\ g^{-1}$), **(c)** ratio of leaf area to root
364 length (SLA/SRL, $cm^2\ m^{-1}$). Points indicate genotype (n=10) mean (n=5-6) at a given treatment.
365 Boxplots show distribution of values. Different letters represent significant ($p<0.05$) Tukey post hoc
366 differences between treatments.

367 **Figure 3**. Trait plasticity in response to resource limitation. Points indicate the average (n=10)
368 relative distance plasticity index (RDPI) in response to light limitation (dark blue, Shade-Rich) or
369 nutrient limitation (light green, Light-Poor), or combined (light blue, Shade-Poor). Point size
370 represents a T-test significance ($p<0.05$) of RDPI being different from zero (large points) or not (small
371 points). **(a)** RDPI values taken from base measurements. **(b)** RDPI values when correcting trait values
372 for biomass, allometry. Trait abbreviations as in Table 1.

373 **Figure 4**. Principal component analysis (PCA) of leaf, stem and roots traits in response to resource
374 limation. Light/Shade, direct sun vs 50% shade. Rich/poor, high nutrients vs 10% nutrient
375 concentration. Panels represent **(a)** all traits, **(b)** allocation traits, **(c)** morphological traits, and **(d)**
376 anatomical traits. Different letters indicate significant Hotellings-T test, after Bonferroni correction,
377 between treatments on the first two principal components. Trait abbreviations as in Table 2.

378 **Figure 5**. Leaf, stem, and fine root anatomy across a factorial of light and nutrient limitation.
379 Light/Shade, direct sun vs 50% shade. Rich/poor, high nutrients vs 10% nutrient concentration. **(a)**
380 leaf palisade parenchyma thickness (µm), **(b)** stem vascular bundle width (µm), **(c)** fine root cortex
381 thickness (µm). Points indicate genotype (n=10) mean (n=5-6) at a given treatment. Boxplots show
382 distribution of values. Different letters represent significant ($p<0.05$) Tukey post hoc differences
383 between treatments

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Table 1. Allocational, morphological, and anatomical traits across a factorial of light and nutrient limitation. Light/Shade, direct sun vs 50% shade. Rich/poor, high nutrients vs 10% nutrient concentration. Means±SE of 10 genotypes estimated marginal mean (based on 5-6 replicates) from our split-plot design. Effects of genotype (G), light (L) and nutrient (N) stresses and their interactions (L×N) on plant traits data. With or without plant total biomass (Mass) as a covariate. ***:p<0.001, **:p<0.01, *:p<0.05, ns: not significant.

							Statistics				-including biomass				
Category	**Trait**	**Unit**	**Light-Rich**	**Light-Poor**	**Shade-Rich**	**Shade-Poor**	**G**	**L**	**N**	**L×N**	**Mass**	**G**	**L**	**N**	**L×N**
Allocation	*Total Biomass*	g	1.70±0.14	1.28±0.07	0.77±0.05	0.56±0.05	**	**	**	ns					
	Leaf mass fraction	$g \cdot g^{-1}$	0.68±0.006	0.59±0.001	0.70±0.01	0.66±0.01	**	**	**	**	**	**	ns	**	**
	Stem mass fraction	$g \cdot g^{-1}$	0.16±0.009	0.16±0.009	0.20±0.001	0.21±0.02	**	**	ns	*	*	**	**	ns	**
	Fine root mass fraction	$g \cdot g^{-1}$	0.13±0.007	0.21±0.001	0.074±0.004	0.10±0.007	**	**	**	**	**	**	**	**	**
	Tap root mass fraction	$g \cdot g^{-1}$	0.04±0.002	0.04±0.003	0.027±0.002	0.03±0.002	**	**	ns	ns	ns	**	**	ns	ns
	Ratio of leaf and fine root		5.58±0.33	2.93±0.22	9.96±0.65	6.58±0.40	**	**	**	**	**	**	**	**	**
	Ratio of SLA and FineSRL		1.30±0.07	1.13±0.06	1.50±0.11	1.23±0.04	**	*	*	ns	ns	**	ns	*	ns
Morphology	*Leaf thickness*	µm	257.71±8.38	248.87±7.23	228.86±4.75	227.24±6.37	**	**	ns	ns	ns	**	**	ns	ns
	Specific leaf area	$cm^2 \cdot g^{-1}$	349.53±11.04	362.46±6.47	487.72±8.96	493.42±18.32	**	**	ns	ns	**	**	**	ns	ns
	Leaf dry mass content	$mg \cdot g^{-1}$	102.95±2.66	106.92±2.55	87.33±2.78	90.91±2.69	ns	**	ns	ns	ns	ns	ns	ns	ns
	Stem diameter	mm	5.23±0.18	4.65±0.10	4.30±0.13	3.88±0.11	**	**	**	ns	**	**	*	**	ns
	Stem tissue density	$g \cdot cm^{-3}$	0.039±0.003	0.037±0.002	0.028±0.001	0.031±0.002	*	**	ns	ns	*	*	*	ns	ns
	Fine root diameter	µm	404.95±8.32	374.96±8.15	388.27±1.95	348.22±8.61	**	ns	**	ns	ns	**	ns	**	ns
	Specific fine root length	$m \cdot g^{-1}$	288.10±11.34	342.28±17.57	377.10±21.55	423.75±23.45	**	**	**	ns	**	**	ns	**	ns
	Fine root tissue density	$g \cdot cm^{-3}$	0.033±0.002	0.033±0.001	0.027±0.002	0.028±0.001	**	**	ns	ns	*	**	*	ns	ns
	Tap root diameter	µm	1337.49±62.57	1228.76±59.19	937.87±42.74	900.58±36.41	**	**	ns	ns	**	**	ns	ns	ns
	Specific tap root length	$m \cdot g^{-1}$	4.46±0.43	5.04±0.56	7.35±0.90	10.51±2.06	**	**	ns	ns	**	**	ns	ns	ns
	Tap root tissue density	$g \cdot cm^{-3}$	0.037±0.002	0.043±0.002	0.033±0.002	0.033±0.002	**	**	**	*	**	**	ns	**	ns
	Height	cm	14.62±0.82	14.33±0.93	16.75±1.11	16.43±1.64	**	**	ns	ns	**	**	**	*	ns
Anatomy	*Leaf palisade thickness*	µm	141.35±4.60	136.74±4.61	124.16±3.96	122.22±3.20	**	**	ns	ns	ns	**	*	ns	ns
	Leaf spongy thickness	µm	79.96±4.93	74.11±4.63	70.79±2.85	73.60±4.85	**	**	*	*	ns	**	*	*	*
	Leaf Ks	$kg \cdot s^{-1} \cdot m^{-1} \cdot MPa^{-1}$	4.55±0.45	4.27±0.53	3.66±0.42	3.34±0.33	**	**	ns	ns	**	**	ns	ns	ns
	Stem cortex thickness	µm	262.41±15.68	266.45±12.93	227.65±11.37	225.16±10.32	**	**	ns	ns	**	**	ns	ns	ns
	Stem vascular thickness	µm	651.28±22.70	593.58±21.77	479.75±21.92	436.78±19.99	**	**	**	ns	**	**	ns	ns	ns
	Stem xylem thickness	µm	314.49±11.57	293.74±7.17	234.98±9.27	205.33±8.60	**	**	ns	*	**	**	ns	ns	ns
	Stem Ks	$kg \cdot s^{-1} \cdot m^{-1} \cdot MPa^{-1}$	8.62±0.77	9.86±0.89	11.12±1.11	9.56±1.09	**	**	ns	*	**	**	**	**	*
	Fine root cortex thickness	µm	136.38±2.99	125.46±3.58	127.44±4.13	113.25±2.07	*	ns	*	ns	ns	*	ns	*	ns
	Fine stele diameter	µm	113.38±5.27	103.50±4.28	111.68±5.01	104.40±4.44	**	ns	**	ns	ns	**	ns	*	ns
	Fine root Ks	$kg \cdot s^{-1} \cdot m^{-1} \cdot MPa^{-1}$	0.54±0.07	0.63±0.16	0.66±0.15	0.64±0.13	**	ns	ns	ns	ns	**	ns	ns	ns
	Tap root cortex thickness	µm	209.14±8.67	209.20±8.06	182.66±13.98	186.40±9.60	**	**	ns	ns	**	**	ns	ns	ns
	Tap root stele diameter	µm	888.75±60.60	769.73±44.59	569.64±22.97	517.38±30.58	**	**	**	ns	**	**	ns	ns	ns
	Tap root Ks	$kg \cdot s^{-1} \cdot m^{-1} \cdot MPa^{-1}$	27.16±4.14	28.32±4.08	29.43±3.84	30.49±4.31	**	ns	ns	ns	**	**	**	ns	ns

Table 2. Relative shift in allocational, morphological, and anatomical traits across a factorial of light and nutrient limitation. Light/Shade, direct sun vs 50% shade. Rich/poor, high nutrients vs 10% nutrient concentration. Means±SE of 10 genotypes relative shift in treat value as compared to Light-Rich treatment. Base values as measured and after correcting for the effect of biomass on trait value. Greyed out numbers are not significantly different from zero (T-test p>0.05).

			Light-Poor		Shade-Rich		Shade-Poor	
Category	**Trait**	**Abreviation**	*Base Δ%*	*Mass cor Δ%*	*Base Δ%*	*Mass cor Δ%*	*Base Δ%*	*Mass cor Δ%*
Allocation	*Total Biomass*	TotalBiomass	-21.43±(5.94)		-53.25±(3.14)		-65.82±(2.81)	
	Leaf mass fraction	LMF	-12.06±(1.97)	-12.85±(1.85)	3.67±(1.47)	0.37±(1.39)	-2.94±(1.88)	-7.62±(1.7)
	Stem mass fraction	SMF	-2.07±(4.09)	-1.95±(4.08)	23.97±(3.81)	24.4±(3.8)	32.92±(5.55)	33.57±(5.53)
	Fine root mass fraction	FineRMF	71.42±(10.45)	84.47±(12.91)	-41.36±(3.29)	-27.23±(4.67)	-19.59±(5.56)	4.82±(7.78)
	Tap root mass fraction	TapRMF	6.81±(3.73)	7.92±(3.72)	-21.21±(3.7)	-18.49±(3.87)	-12.85±(5.86)	-8.57±(6.07)
	Ratio of leaf and fine root	LFRratio	-45.65±(5.3)	-47.15±(4.95)	82.61±(13.59)	53.12±(11.61)	20.81±(8.55)	-12.25±(7.04)
	Ratio of SLA and FineSRL	SLAFineSRLratio	-12.1±(4.1)	-12.59±(4.04)	16.35±(6.65)	14.37±(6.73)	-3.39±(4.77)	-6.12±(4.66)
Morphology	*Leaf thickness*	LT	-3.11±(2.33)	-3.16±(2.33)	-10.8±(1.64)	-10.98±(1.63)	-11.47±(2.1)	-11.73±(2.1)
	Specific leaf area	SLA	4.25±(2.36)	0.85±(2.84)	40.53±(4.17)	27.59±(3.34)	42.24±(6.03)	24.2±(4.83)
	Leaf dry mass content	LTD	-0.69±(3.82)	2.47±(4.22)	-20.57±(3.42)	-12.26±(3.92)	-19.82±(4.36)	-7.12±(4.97)
	Stem diameter	SD	-15.6±(3.82)	-11.26±(2.55)	-18.02±(3.42)	0.25±(2.77)	-27.56±(3.96)	-1.15±(2.57)
	Stem tissue density	SDensity	-0.21±(6.89)	3.96±(7.23)	-22.89±(5.73)	-12.55±(6.33)	-17.62±(4.13)	-1.74±(4.94)
	Fine root diameter	FRD	-7.25±(1.85)	-7.1±(1.82)	-3.91±(3.02)	-3.32±(3.01)	-13.82±(2.15)	-12.96±(2.11)
	Specific fine root length	FineSRL	19.82±(6.72)	15.72±(6.44)	31.81±(7.99)	19.58±(6.31)	50.35±(13.43)	32.56±(11.54)
	Fine root tissue density	FineRTD	2.22±(5.63)	5.59±(6.3)	-18.76±(4.39)	-10.31±(4.38)	-13.92±(3.33)	-0.44±(4.35)
	Tap root diameter	TRD	-6.2±(6.59)	1.13±(6.2)	-28.84±(4.18)	-1.62±(6.83)	-31.25±(4.05)	9.52±(6.25)
	Specific tap root length	TapSRL	16.66±(10.54)	-1.91±(11.5)	86.92±(35.39)	-7.72±(13.43)	163.95±(39.39)	25.7±(18.47)
	Tap root tissue density	TapRTD	16.56±(5.96)	22.11±(6.1)	-11.38±(6.17)	0.99±(6.59)	-9.65±(5.94)	9.51±(6.55)
	Height	Height	-0.28±(6.84)	6.26±(4.95)	15.04±(5.91)	41.59±(4.38)	11.95±(9.83)	50.23±(7.5)
Anatomy	*Leaf palisade thickness*	LPT	-2.95±(2.64)	-2.43±(2.63)	-12.05±(1.28)	-10.27±(1.3)	-13.1±(2.23)	-10.49±(2.18)
	Leaf spongy thickness	LST	-7±(3.2)	-6.74±(3.24)	-10.34±(2.82)	-9.42±(2.85)	-7.61±(3.53)	-6.26±(3.56)
	Leaf Ks	LKs	-8.09±(4.91)	0.01±(6.03)	-19.02±(5.46)	9.59±(6.3)	-25.09±(6.07)	18.45±(9.99)
	Stem cortex thickness	SCT	3.82±(6.52)	6.77±(6.05)	-11.57±(4.97)	-1.25±(5.33)	-12.06±(5.51)	3.39±(6.16)
	Stem vascular thickness	SVT	-8.7±(2.17)	-1.97±(2.65)	-26.2±(2.78)	-4.41±(2.89)	-32.68±(2.93)	0.41±(3.59)
	Stem xylem thickness	SXT	-5.83±(3)	0.83±(2.32)	-24.69±(3.24)	-2.39±(3.52)	-34.18±(2.98)	-1±(3.07)
	Stem Ks	SKs	17.4±(8.3)	39.01±(11.45)	32.36±(10.82)	95.94±(17.02)	19.83±(18.39)	107.23±(29.44)
	Fine root cortex thickness	FRCT	-7.72±(2.86)	-7.26±(2.86)	-5.9±(4.28)	-4.18±(4.3)	-16.43±(2.97)	-13.93±(2.94)
	Fine stele diameter	FRSD	-7.95±(3.29)	-7.75±(3.26)	-1.14±(2.58)	-0.35±(2.55)	-7.31±(3.07)	-6.14±(3.07)
	Fine root Ks	FRKs	14.42±(26.24)	14.31±(26.19)	30.55±(24.63)	30.06±(24.56)	17.28±(15.36)	16.67±(15.32)
	Tap root cortex thickness	TRCT	0.77±(3.86)	8.32±(3.17)	-12.93±(5.06)	11.5±(7.25)	-10.59±(3.93)	24.66±(4.63)
	Tap root stele diameter	TRSD	-10.47±(7.24)	-1.01±(9.76)	-33.64±(4.96)	-0.47±(11.43)	-39.98±(4.24)	9.48±(11.54)
	Tap root Ks	TRKs	9.34±(7.58)	34.04±(18.67)	18.56±(11.35)	94.08±(32.24)	21.85±(12.56)	128.2±(40.12)

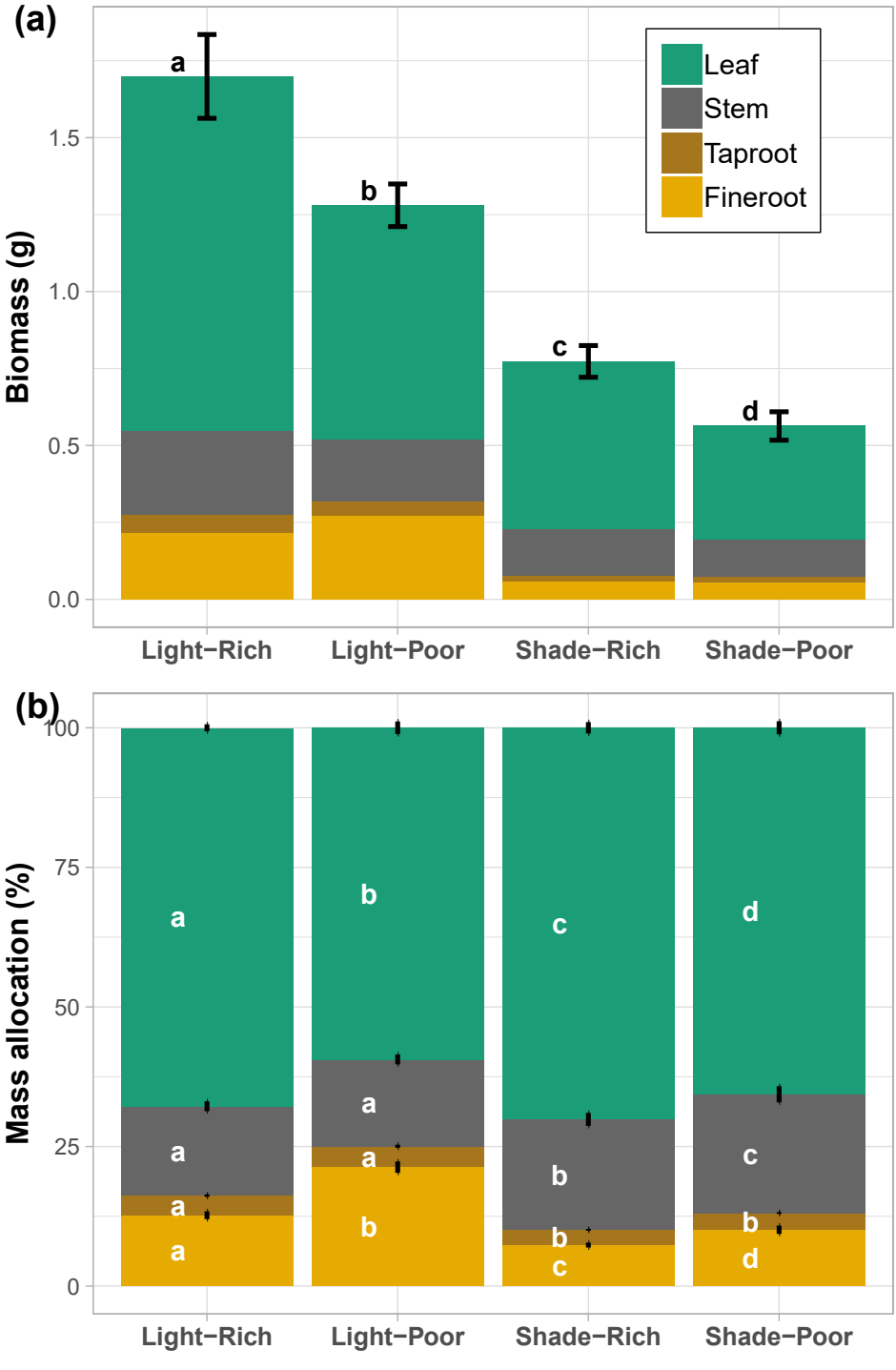

Figure 1. Biomass and mass allocation of leaf, stem, tap root, and fine root tissue across a factorial of light and nutrient limitation. Light/Shade, direct sun vs 50% shade. Rich/poor, high nutrients vs 10% nutrient concentration. (a) Average whole plant biomass across genotypes (n=10), stacked by average tissue contribution. (b) Average mass allocation to tissue types per treatment. Error bars denote standard error across genotypes. Different letters represent significant ($p<0.05$) Tukey post hoc differences between treatments.

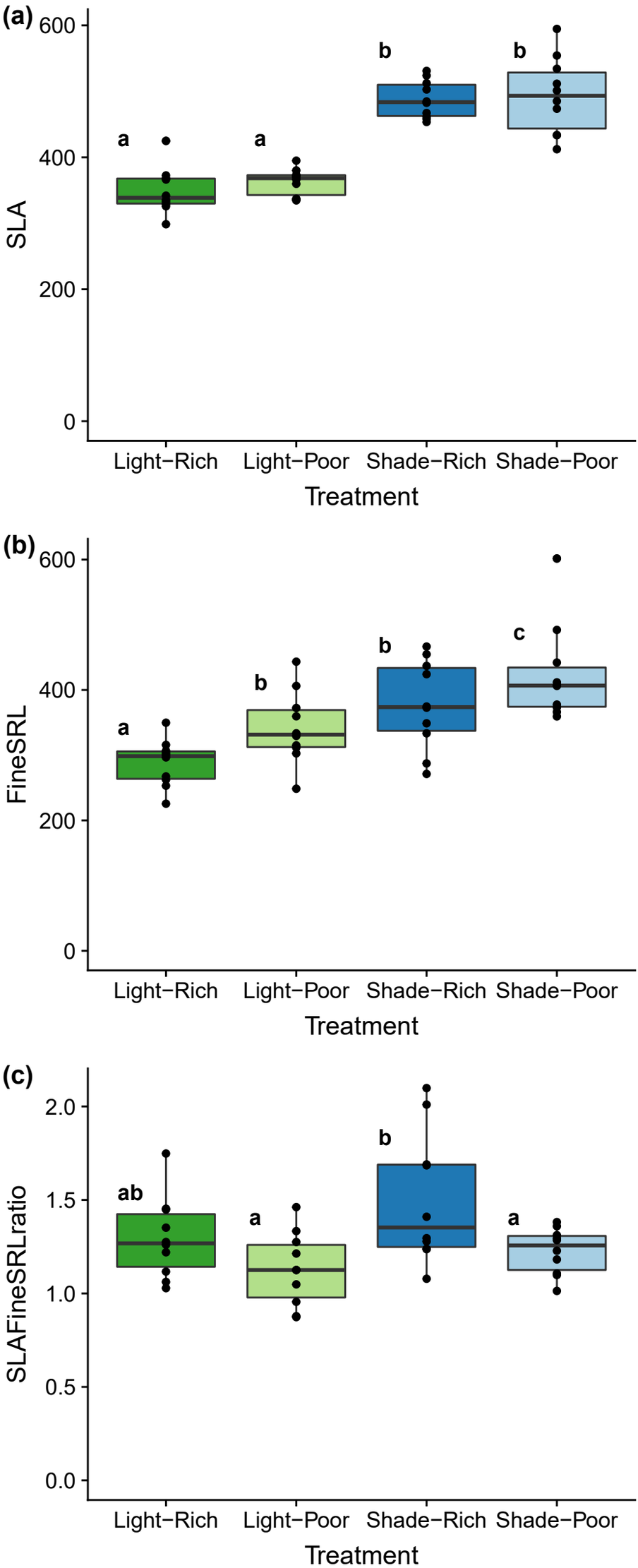

Figure 2. Leaf and fine root morphology across a factorial of light and nutrient limitation. Light/Shade, direct sun vs 50% shade. Rich/poor, high nutrients vs 10% nutrient concentration. (a) specific leaf area (SLA, cm2 g-1), (b) specific root length (SRL, m g-1), (c) ratio of leaf area to root length (SLA/SRL, cm2 m-1). Points indicate genotype (n=10) mean (n=5-6) at a given treatment. Boxplots show distribution of values. Different letters represent significant ($p<0.05$) Tukey post hoc differences between treatments.

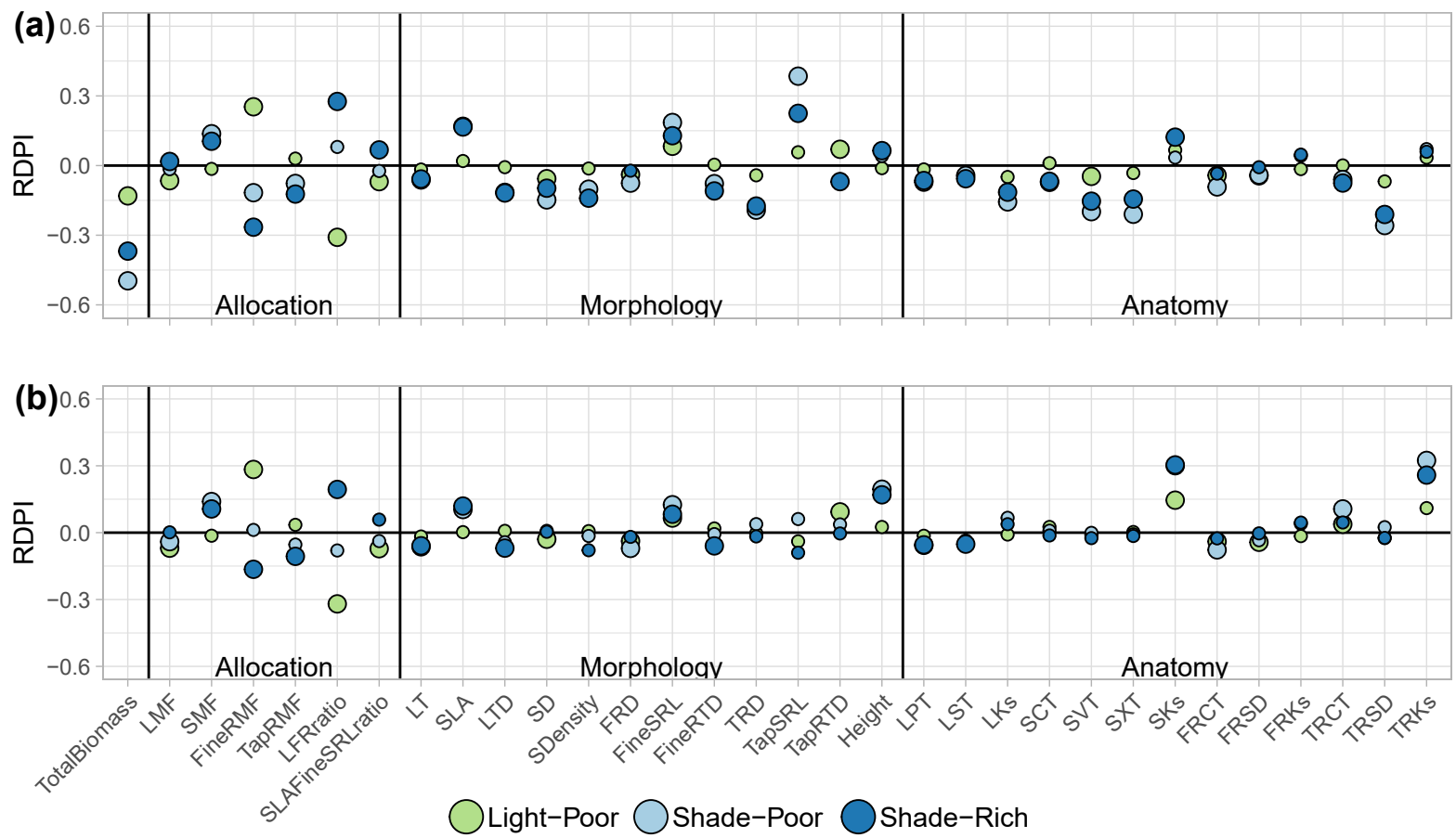

Figure 3. Trait plasticity in response to resource limitation. Points indicate the average (n=10) relative distance plasticity index (RDPI) in response to light limitation (dark blue, Shade-Rich) or nutrient limitation (light green, Light-Poor), or combined (light blue, Shade-Poor). Point size represents a T-test significance ($p<0.05$) of RDPI being different from zero (large points) or not (small points). (a) RDPI values taken from base measurements. (b) RDPI values when correcting trait values for biomass, allometry. Trait abbreviations as in Table 2.

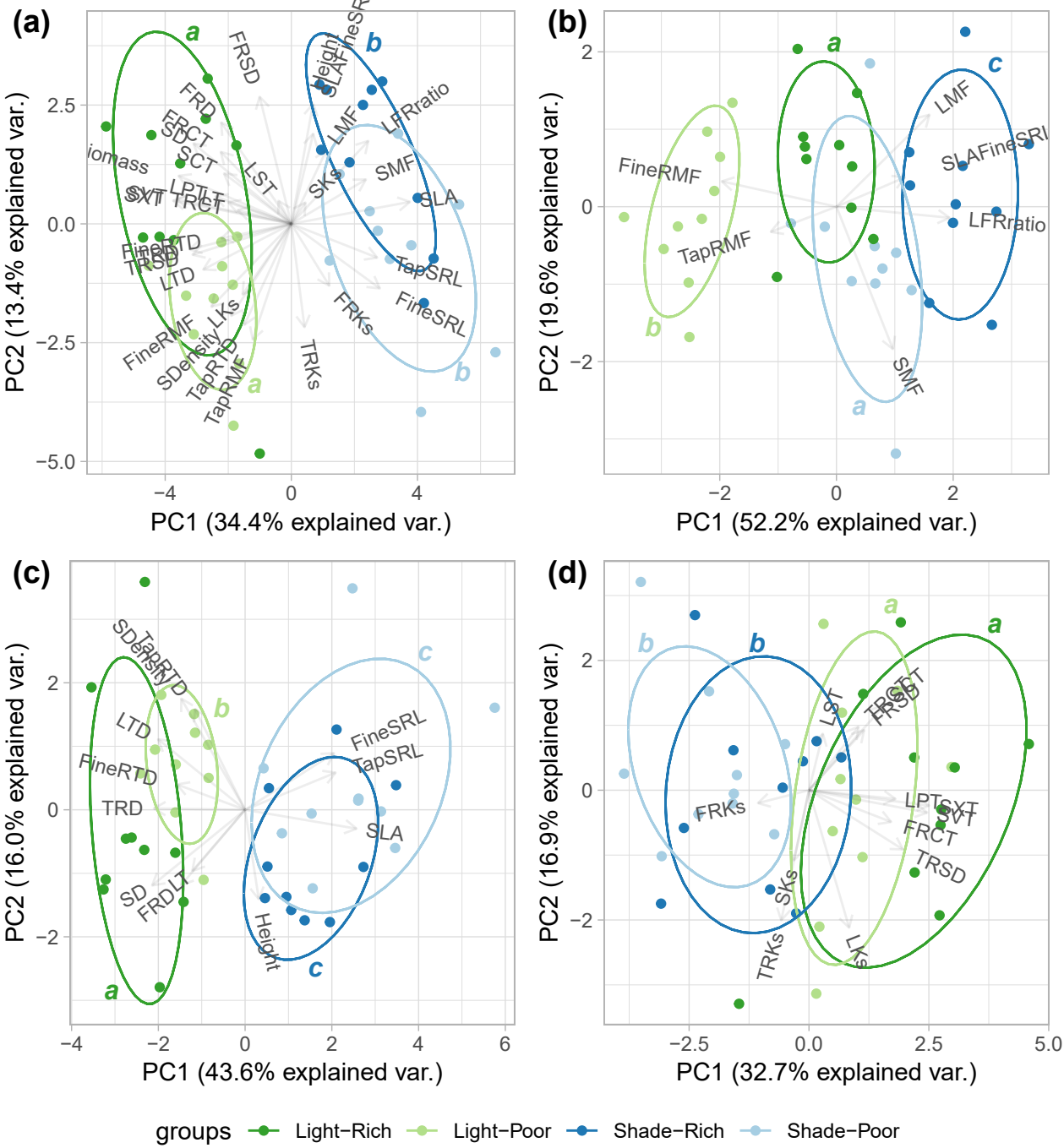

Figure 4. Principal component analysis (PCA) of leaf, stem and roots traits in response to resource limation. Light/Shade, direct sun vs 50% shade. Rich/poor, high nutrients vs 10% nutrient concentration. Panels represent (a) all traits, (b) allocation traits, (c) morphological traits, and (d) anatomical traits. Different letters indicate significant Hotellings-T test, after Bonferroni correction, between treatments on the first two principal components. Trait abbreviations as in Table 2.

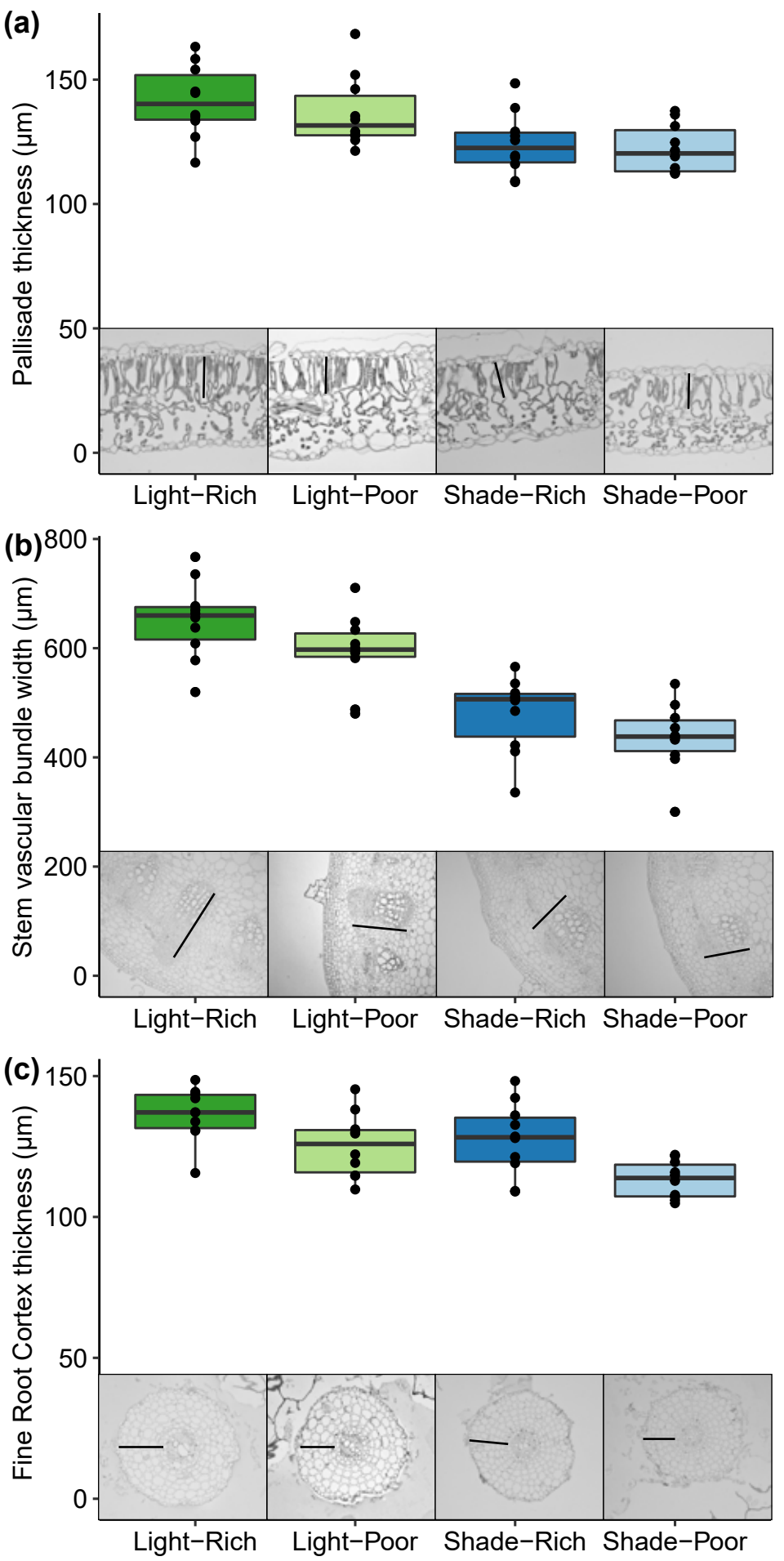

Figure 5. Leaf, stem, and fine root anatomy across a factorial of light and nutrient limitation. Light/Shade, direct sun vs 50% shade. Rich/poor, high nutrients vs 10% nutrient concentration. (a) leaf palisade parenchyma thickness (µm), (b) stem vascular bundle width (µm), (c) fine root cortex thickness (µm). Points indicate genotype (n=10) mean (n=5-6) at a given treatment. Boxplots show distribution of values. Different letters represent significant ($p<0.05$) Tukey post hoc differences between treatments.