Leaf palatability is driven by trait variation within *Quercus robur* crowns

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Abstract

Plant–herbivore interactions vary across spatial and temporal scales in response to environmental gradients. However, despite substantial variation in leaf traits even within individuals and under similar environmental conditions, the role of leaf traits in shaping leaf palatability remains poorly understood. Here, we disentangled the effects of leaf trait variation from those of crown strata (sun vs. shade) and season on leaf palatability within the crowns of *Quercus robur* forest trees. Palatability was measured in no-choice feeding bioassays with *Lymantria dispar* caterpillars as consumed leaf volume per unit caterpillar mass. We related palatability to structural traits, including leaf dry matter content (LDMC) and leaf mass per area (LMA), and proxies for chemical traits including flavonol, anthocyanin, and nitrogen balance index (NBI). We found that more palatable leaves had higher NBI but lower LDMC, LMA, and flavonol content. Shade leaves were generally more palatable than sun leaves, reflecting trait differences between crown strata. Leaf palatability fluctuated among seasons and didn't strictly mirror seasonal shifts in leaf traits. Our findings highlight that leaf palatability arises from spatially structured variation in leaf traits within tree crowns, whereas temporal palatability variation is highly complex and driven by factors beyond structural and chemical leaf traits.

Keywords

palatability; plant–herbivore interactions; leaf traits; oak; spongy moth

Introduction

Insect herbivory constitutes a key ecological process that constrains plant growth, health, and reproduction[1,2]. In an arms race with herbivores, plants have evolved diverse strategies to mitigate herbivore damage[3,4]. These strategies involve an investment in structural and chemical defensive traits that trade off with plant growth[5–9]. Thus, structural traits related to leaf toughness, such as leaf dry matter content and leaf mass per area, can constrain herbivores by increasing the energetic and temporal costs of feeding[10] but plants need to economize on such traits and may not invest equally in all their leaves. The same goes for chemical traits, among which secondary metabolites such as flavonoids can deter herbivore feeding by decreasing the digestibility or increasing the toxicity of plant tissues[11,12], while plants or leaves with high nitrogen content are generally more attractive to herbivores[13,14]. Collectively, variation in these structural and chemical traits can shape plant palatability to herbivores across biological scales, from communities to individuals[15–18].

Leaf traits can vary substantially within individual plants[19,20]. For example, because of microclimatic gradients within tree crowns, sun-exposed leaves are typically tougher and richer in secondary metabolites than shaded leaves in the lower crown[21,22]. While these traits help tolerate UV radiation and thermal stress, they can also reduce palatability and thus decrease herbivory[12,23]. In addition, sun-exposed leaves typically have lower nitrogen per unit dry mass than shaded leaves, rendering them less attractive for herbivores[24,25]. Linking intra-individual variation in leaf traits to palatability therefore provides insight into how herbivores respond to nuanced trait variation and how growth–defense trade-offs are expressed within plant individuals.

Leaf traits can also vary across growing seasons. Early-season leaves are often softer and more nutrient-rich, becoming progressively tougher and less nutritious as they mature[13]. In contrast, seasonal patterns of chemical traits are less uniform and do not follow a single directional trend. Concentrations of some secondary metabolites, such as flavonoids, often peak during mid-season, whereas others, including tannins, tend to accumulate later across the growing season[13,26,27]. These heterogeneous yet important trends in structural and chemical traits render seasonal variation in leaf palatability difficult to predict.

The effects of leaf traits on palatability among and within species have been widely studied[15,17]. However, at the scale most relevant to individual plant fitness—the intra-individual scale, where leaves are exposed to steep microclimatic gradients and undergo seasonal changes in their structural and chemical properties—trait–palatability relationships remain poorly understood. In this study, we aim to disentangle the roles of within-individual variation, crown strata, and temporal variation in shaping pedunculate oak (*Quercus robur)* leaf palatability for the generalist herbivore caterpillar *Lymantria dispar*. Pedunculate oaks are of particular interest because they support diverse herbivorous insects[28], among which *L. dispar*, can cause substantial ecological and economic damage during outbreaks[29–32].

We hypothesize that (i) palatable leaves are associated with greater nitrogen content and low structural and defensive investment. (ii) Shade leaves are more palatable than sun leaves because they are generally softer, richer in nitrogen, and less chemically defended. (iii) We expect leaf palatability to decline over the season as leaf quality decreases, structural investment increases, and secondary metabolites accumulate.

Materials and methods

Study site and leaf sampling

We conducted our study in the Marburg Open Forest, Germany (50°50'26.7"N, 8°41'06.8"E). There, we sampled 1420 fully expanded leaves from eight mature *Quercus robur* trees from June to September in 2023 and 2024. Half of these leaves were from the upper, sun-exposed, south-facing crown and the rest from the lower, shaded, north-facing crown. We sampled leaves regardless of flush or prior herbivore damage to reflect natural conditions, where multiple herbivore species share leaf resources and feeding may occur in successive events.

Leaf trait measurements

In the laboratory, we cut a 2 cm diameter leaf disc for a subsequent palatability bioassay and measured traits on the remaining leaf material. We measured nitrogen balance index (NBI, as a proxy for nitrogen content), anthocyanin, and flavonol content (as proxies for secondary metabolites) at the base, middle, and top of each leaf using a Dualex leaf-clip meter FORCE-A[33] and averaged these measurements for each trait per leaf. We measured fresh leaf mass after disc removal, oven-dried the leaves at 60 °C for 24 hours and measured dry mass using an electronic balance (0.0001 g precision). Thereafter, we calculated leaf dry matter content (LDMC) as the ratio of dry to fresh mass and leaf mass per area (LMA) as dry mass per unit leaf area.

Caterpillar rearing and palatability bioassay

We reared caterpillars of the generalist herbivore *Lymantria dispar*, hatched from egg clutches, under constant laboratory conditions at 21 °C, 65% relative humidity, and a 12:12 hour photoperiod. We fed the caterpillars an artificial diet consisting of 814 ml water, 15 g agar, 120 g wheat germ, 8 g Wesson salt, 25 g casein, 5 g ascorbic acid, 2 g

sorbic acid, 1 g methylparaben, 0.1 g chlortetracycline, 0.1 g iron citrate, and 10 g vitamin mixture[34].

We conducted a no-choice feeding experiment with fourth-instar caterpillars under the same rearing conditions. Prior to feeding, we starved the caterpillars for 24 hours to standardize hunger levels. Then, we weighed the starved caterpillars and transferred them to Petri dishes containing moistened filter paper and a 2 cm leaf disc. We allowed caterpillars to feed for 24 hours in 2023. Because caterpillars completely consumed the leaf discs in some cases, we reduced the feeding duration to 14 hours in 2024 to ensure food availability did not limit consumption. After the feeding period, we scanned the discs, quantified the remaining leaf area, and subtracted it from the initial area to obtain the consumed area. We then calculated consumed leaf volume by multiplying the consumed leaf area by the leaf thickness measured with a digital caliper to 0.01 mm precision. Finally, we expressed palatability as consumed leaf volume per unit caterpillar body mass ($cm^3\ g^{-1}$).

Statistical analysis

We assessed the effect of leaf traits, crown strata, and season on leaf palatability using linear mixed-effects models. Before model fitting, we assessed the correlations among leaf traits using the *cor* function and visualized them with the R package *corrplot*[35]. Because of strong correlations among leaf traits (Spearman's rho > 0.7; Fig. S1), we performed principal component analysis (PCA) using the *prcomp* function to summarize their variation into uncorrelated axes and capture trait gradients along the growth-defense spectrum, which we used in subsequent models. Then, we fitted our models using the *glmmTMB* function of the eponymous R package with a Gaussian distribution[36]. We set consumed leaf volume as the response variable and used PC1 and PC2 scores,

crown strata, and season as explanatory variables to disentangle leaf-trait effects while accounting for variation among crown strata and seasons. Leaves that were not consumed were excluded from the analysis. In a separate model, we included only crown stratum and season as explanatory variables to assess overall differences in palatability between sun and shade leaves and among seasons. All models further included tree identity as a random effect to account for variation among individual trees. We accounted for heteroscedasticity by fitting residual variance as a function of the explanatory variables using the *dispformula* argument in *glmmTMB*[36]. We analyzed the 2023 and 2024 data separately because feeding times differed during the palatability assay. Lastly, we evaluated model assumptions and goodness of fit using simulated residual diagnostics from the R package *DHARMa*[37]. We used *emmeans* and *multcomp* packages to extract predicted effects[38,39]. All statistical analyses were performed using R version 4.6.0[40].

Results

The first two PCA axes explained 76.5% of the total variation in leaf traits, with PC1 and PC2 accounting for 57.6% and 18.9%, respectively (Fig. 1). PC1 captured a growth–defense gradient, ranging from leaves with greater structural and chemical defense investment at negative scores, characterized by higher flavonol content (loading = −0.530), leaf mass per area (−0.500) and leaf dry matter content (−0.443) to leaves with higher nitrogen balance index at positive scores, reflected by the positive loading of the nitrogen balance index (0.450; Fig. 1, Fig. S2). In contrast, PC2 was primarily characterized by a strong positive loading of anthocyanin content (loading = 0.818; Fig. 1, Fig. S2). Sun and shade leaves were distinctly separated in the multivariate trait space, whereas leaves from different seasons largely overlapped (Fig. 1).

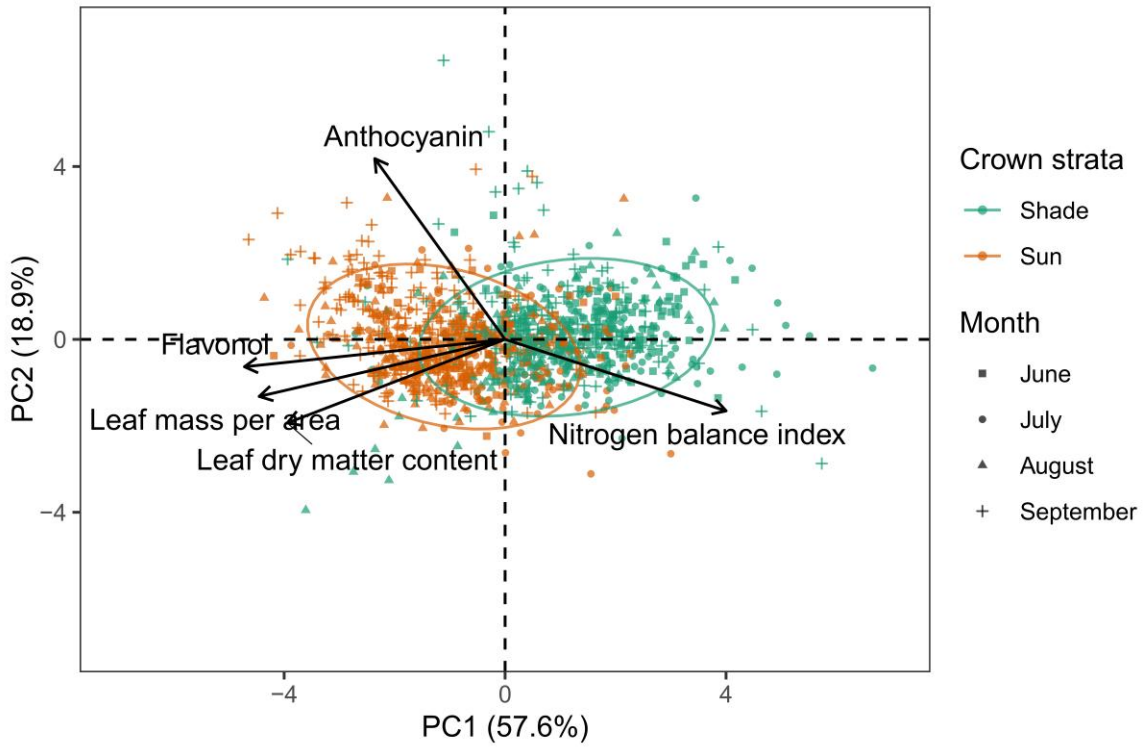


Fig. 1 Principal component analysis of variation in *Quercus robur* leaf traits (n = 1224). Green and orange points represent leaves from shaded and sun-exposed crowns, respectively. Ellipses indicate the 95% confidence intervals for each crown stratum. Point shapes distinguish between the sampling months.

Leaves with higher nitrogen balance index and lower flavonol, leaf mass per area, and leaf dry matter content tended to be more palatable in both years (Fig. 2A, C). However, this relationship was significant in 2024 (p = 0.009; Fig. 2C; Table S2) but not in 2023 (p = 0.111; Fig. 2A; Table S1). Leaf palatability was not significantly associated with anthocyanin in 2023 (p = 0.051; Fig. 2B; Table S1) and 2024 (p = 0.724; Fig. 2D; Table S2).

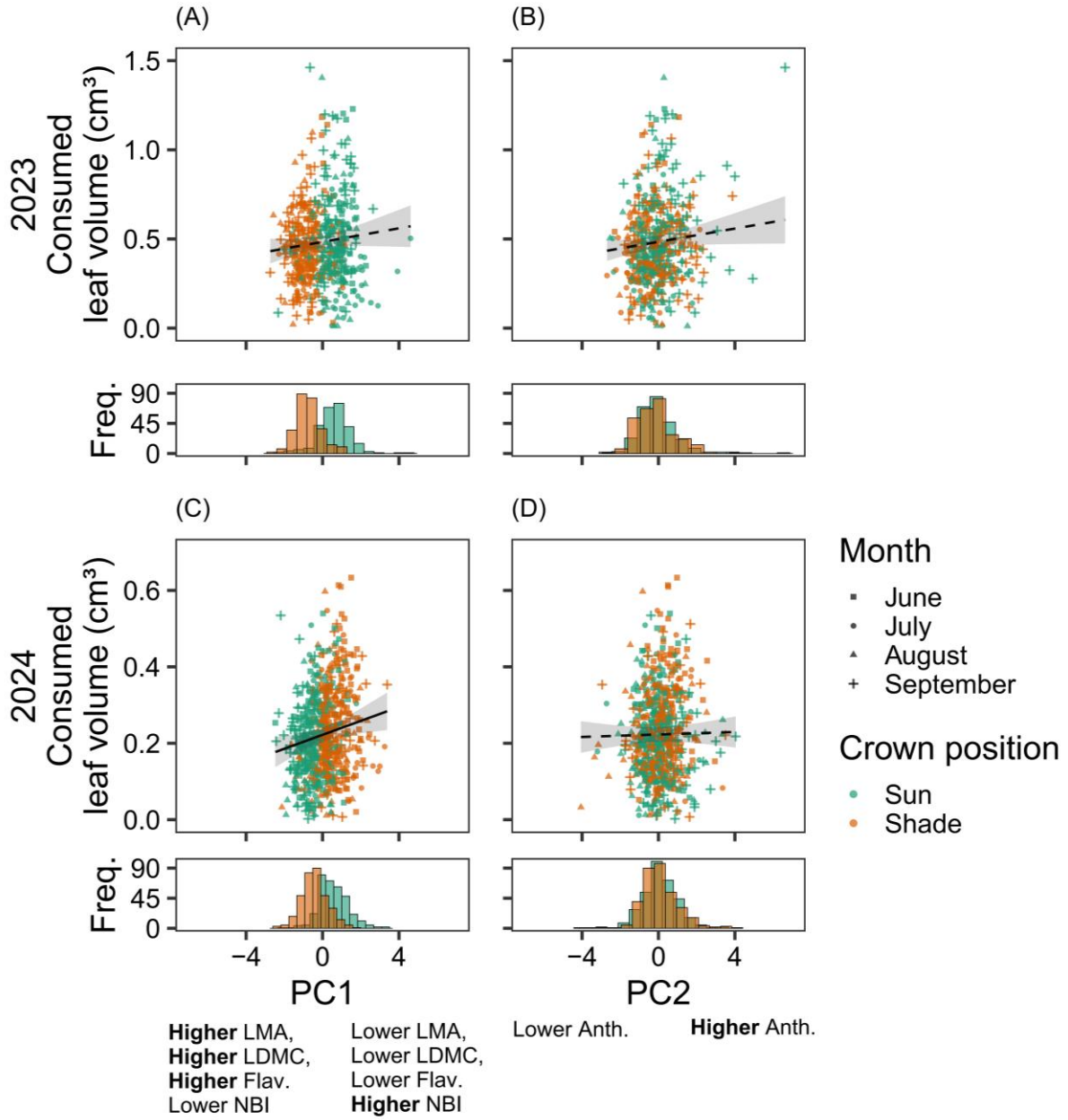


Fig. 2 Relationships between consumed leaf volume (leaf palatability) and the first two principal component axes (PC1 and PC2) in 2023 (A, B) and 2024 (C, D). Positive PC1 scores represent leaves with lower leaf mass per area (LMA), leaf dry matter content (LDMC), and flavonol content, and a greater nitrogen balance index (NBI), whereas negative PC1 scores represent the opposite combination of traits. Positive PC2 scores indicate higher anthocyanin levels, whereas negative PC2 scores indicate lower anthocyanin levels. Points represent individual leaves. Solid regression lines indicate

significant relationships (p <0.05). Dashed lines indicate non-significant relationships. Grey ribbons represent 95% confidence intervals. Histograms below each panel show the distributions of PC scores for sun and shade leaves.

Shade leaves were more palatable than sun leaves in both years (Fig. 3A, C; Table S3-S4), consistent with differences in the summarized trait PC1 and individual traits (LDMC, LMA, flavonol and NBI) between crown strata (Fig. S3, S4). In 2023, leaf palatability was significantly lower in July than in other months (Fig. 3B, Table S3), whereas in 2024, it peaked in July and August (Fig. 3D, Table S4). In 2023, seasonal variation in leaf palatability was inversely aligned with the nitrogen balance index (Fig. S5), whereas in 2024 it was aligned with leaf dry matter content, leaf mass per area, and flavonol (Fig. S6).

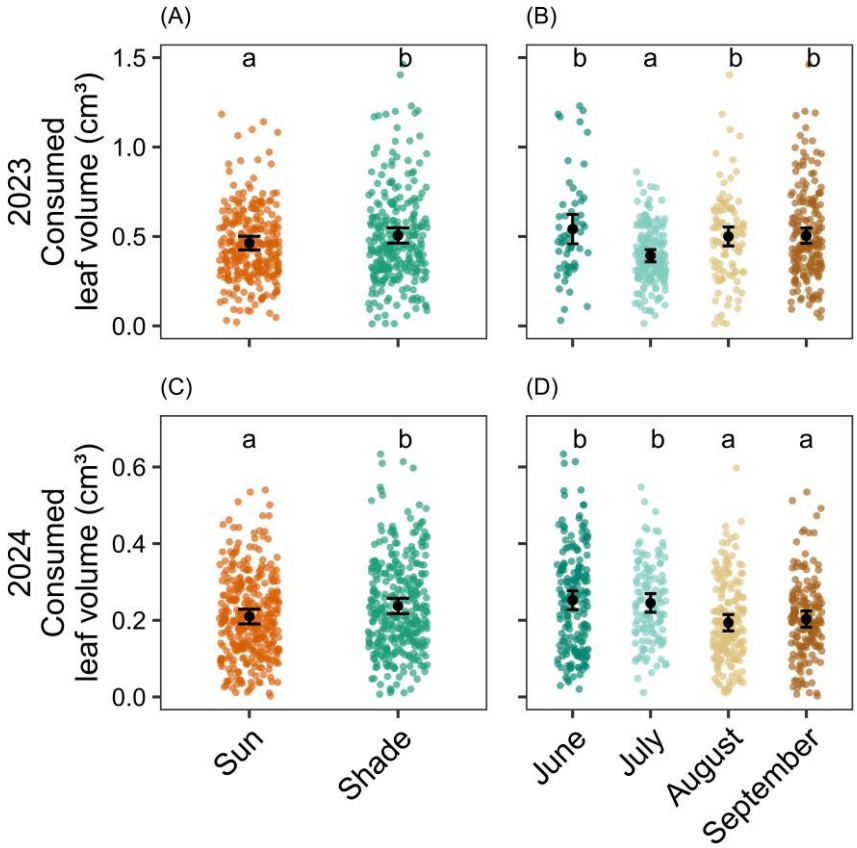


Fig. 3 Leaf palatability between crown strata and among seasons. Colored points represent individual leaves (N_{2023}: Sun = 276, Shade = 267, June = 57, July = 191, August = 104, September = 191; N_{2024}: Sun = 346, Shade = 335, June = 184, July = 126, August = 190, September = 181). Black points represent the estimated marginal means. Bars represent 95% confidence intervals. Different lowercase letters indicate significant differences in leaf palatability among groups based on HSD-Tukey tests ($p < 0.05$).

Discussion

Focusing on intra-individual variation in mature pedunculate oak trees, our study provides detailed insight into how spatial and temporal variation in leaf traits drives leaf palatability. Palatable leaves generally showed lower structural investment, higher nitrogen content, and reduced chemical defenses, consistent with coordinated growth–defense trade-offs within individuals. Overall, shade leaves are more palatable than sun leaves, although the variation was large in both groups. Seasonal variation in palatability fluctuated inconsistently between years and did not necessarily mirror seasonal increases in structural traits and proxies for chemical defense.

Consistent with previous studies, we found that leaves with higher nitrogen and lower leaf dry matter content, leaf mass per area, and flavonol content were more palatable[10,41]. These relationships align with the leaf economics spectrum, in which resource-acquisitive leaves combine high nutrient concentrations with relatively low structural investment and chemical defense[7,9], underscoring that, also within individuals, softer and more digestible leaves are more attractive to herbivores[10]. Palatability also declined with flavonol, highlighting that this secondary compound further deters herbivore feeding[11,12]. These patterns suggest that structural and chemical traits jointly influence leaf palatability. More broadly, our results indicate that growth–

213 defense trade-offs, which underpin this trait coordination, occur even among leaves
214 from the same plant, creating a heterogeneous response-scape of plant–herbivore
215 interactions.

216 However, prolonged feeding may have weakened the relationship between
217 palatability and leaf traits in 2023, as palatable leaf discs were often completely
218 consumed and caterpillars continued feeding on less palatable leaves when no
219 alternative food was available. This may have broader ecological implications, as a
220 similar constraint may arise during *L. dispar* outbreaks, when sustained feeding pressure
221 depletes more palatable foliage and forces herbivores to turn to less attractive feeding
222 alternatives. Thus, structural and chemical defense traits may initially slow
223 consumption, but given sufficient time, herbivores may continue feeding, potentially
224 resulting in extensive or even complete defoliation.

225 Our results further show that leaf palatability was not significantly associated with
226 anthocyanin content. While previous studies have suggested that anthocyanins function
227 as a warning signal of unpalatable leaves due to a higher concentration of chemical
228 defenses[42,43], our findings indicate that anthocyanin itself does not function as a cue for
229 our generalist herbivore model and high exposure (i.e., under no-choice conditions).
230 Nevertheless, the defensive role of anthocyanins may become apparent primarily when
231 herbivores can choose among leaves, rendering it a minor constraint[43,44], especially in
232 herbivores with local mass outbreaks such as *L. dispar*, which can have up to 10,000
233 individuals per tree[45].

234 As expected, shade leaves were generally more palatable than sun leaves,
235 reflecting the overall differences in leaf traits between crown strata. In 2024, leaf
236 palatability more closely followed seasonal changes in leaf traits, with reduced

palatability associated with lower NBI and simultaneously higher LDMC, LMA, and flavonol concentrations. However, contrary to our hypothesis, leaf palatability did not decline over the season in 2023. Instead, higher late-season palatability coincided with a lower nitrogen balance index, despite increases in LDMC, LMA, and flavonols. Thus, whereas specialized herbivores may react more strongly to leaf toughness and chemical defenses, the response of our generalist herbivore model suggests compensatory feeding, in which herbivores increase consumption to offset declining nutritional quality[46,47]. This highlights that herbivore feeding behavior adds further complexity to seasonal plant–herbivore interactions.

Conclusion

Our findings demonstrate that individual tree crowns create heterogeneous feeding environments, where variation in leaf traits drives resource quality for herbivores. However, declining nutritional quality and increasing leaf defenses do not necessarily reduce herbivore feeding, suggesting that these traits may provide limited protection, particularly against defoliation during pest outbreaks, which in turn can compromise forest productivity and alter resource availability for diverse oak-associated insect communities[29,30].

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Author Contributions

AANAR, SP, and NF conceptualized and designed the study. AANAR processed and analyzed the data and led manuscript writing. TT, SW, ARW, LS, CL, and EM contributed to the field and laboratory work. AB, LO, MYB, and RRJ contributed to developing the sampling design. AB, LO, NF, and RRJ provided laboratory facilities. All authors contributed to preparing and revising the manuscript and approved the final version for publication.

Competing interests

The authors declare no competing interests.

Data availability

Data will be made available publicly in DataPLANT upon acceptance of the manuscript

Supporting information

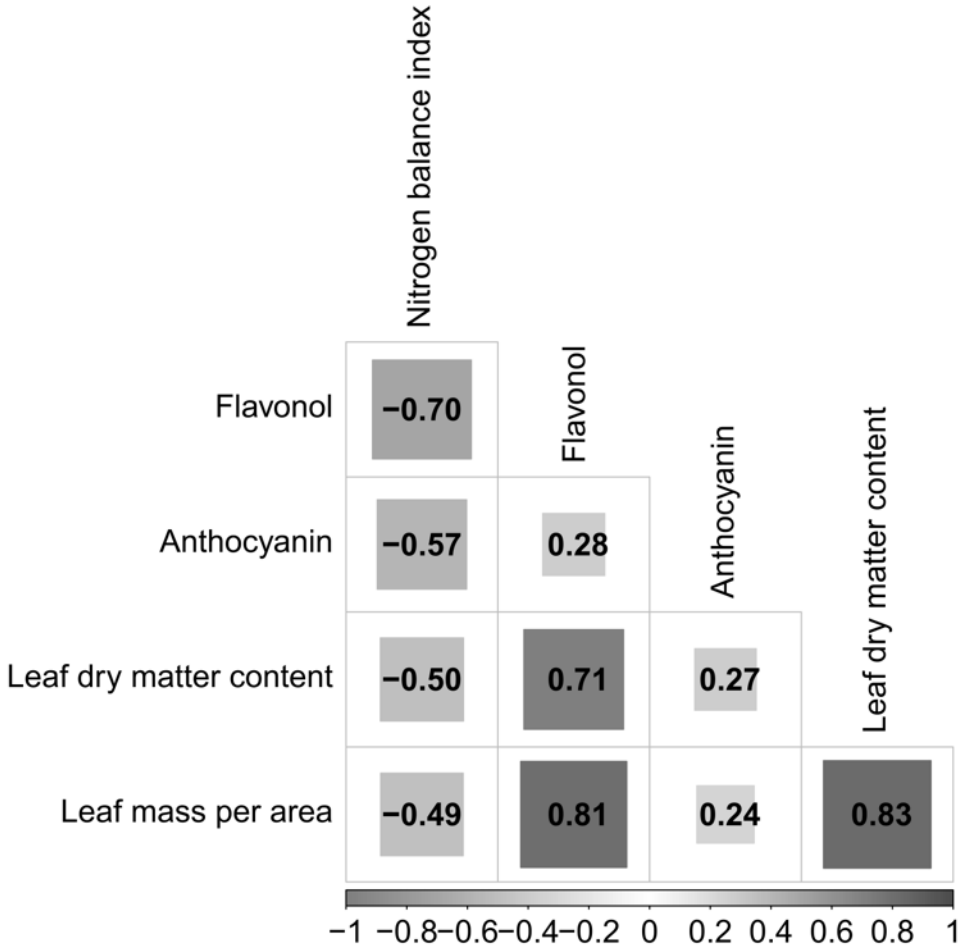
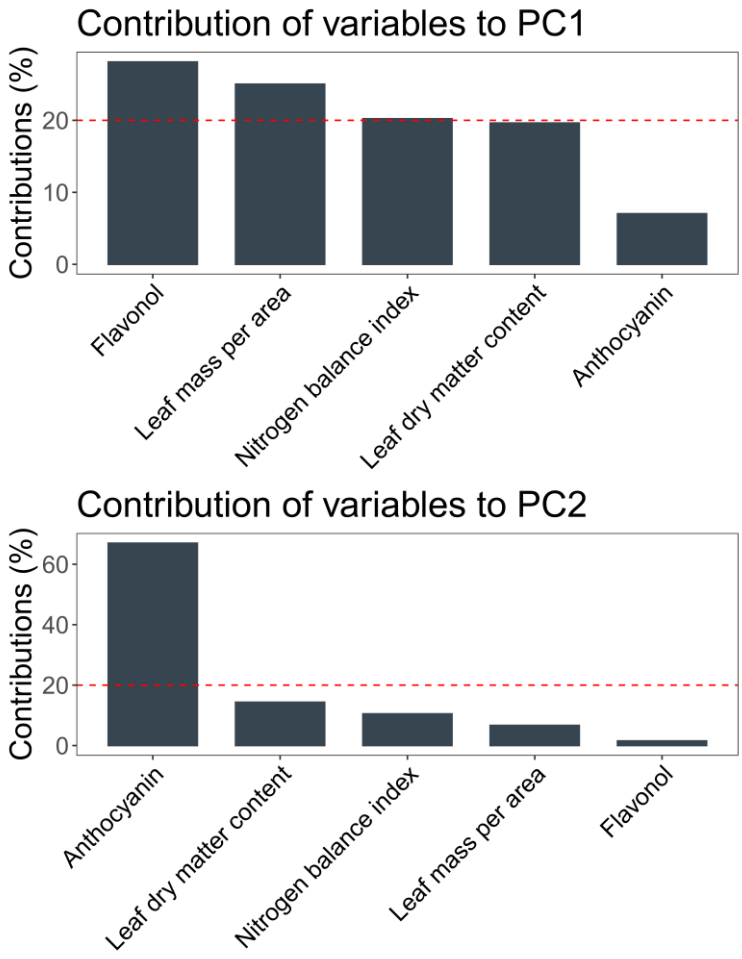
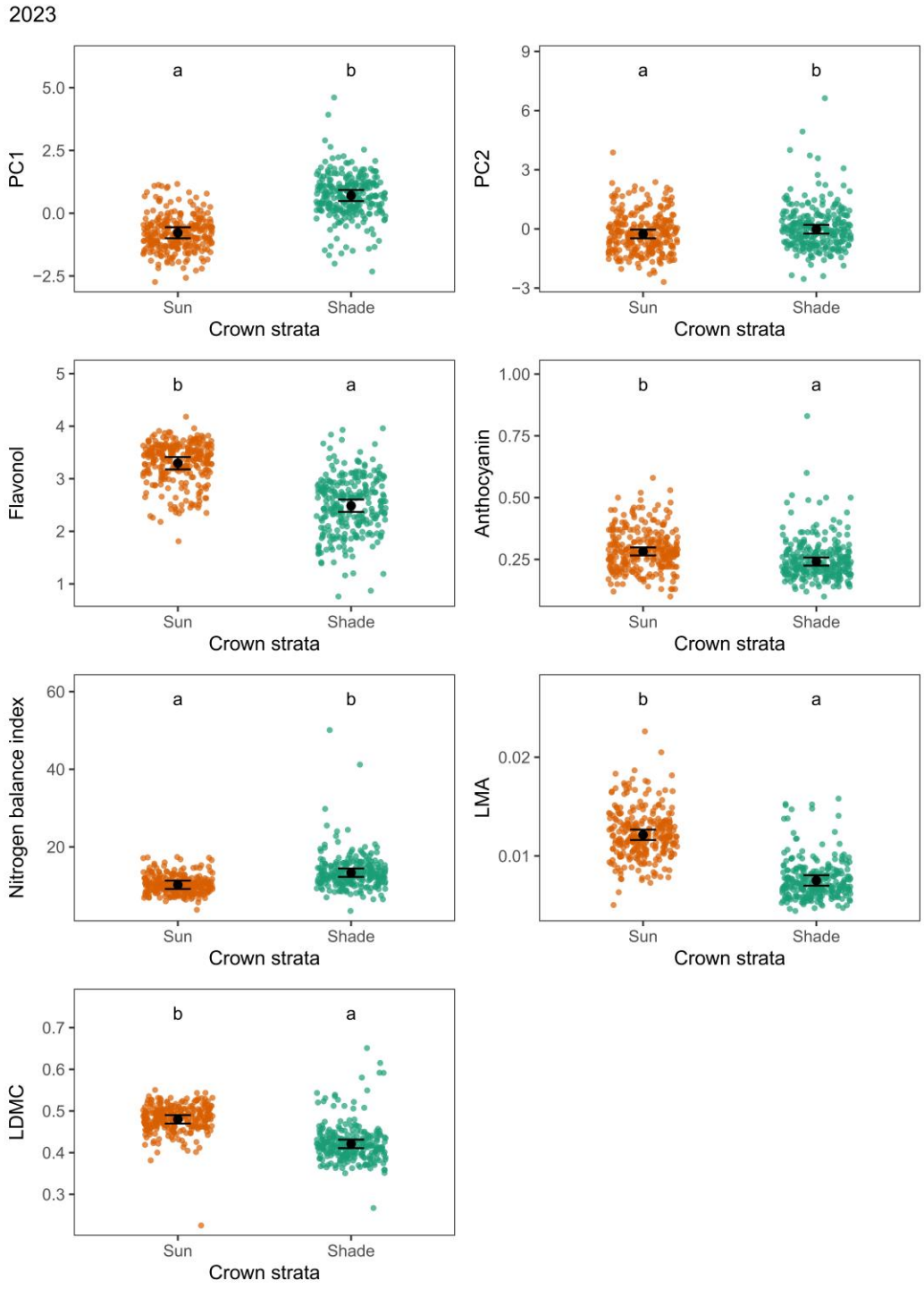


Fig. S1 Spearman's rank correlation coefficients (rho) of pairwise relationships between leaf traits in eight individuals of *Q. robur*



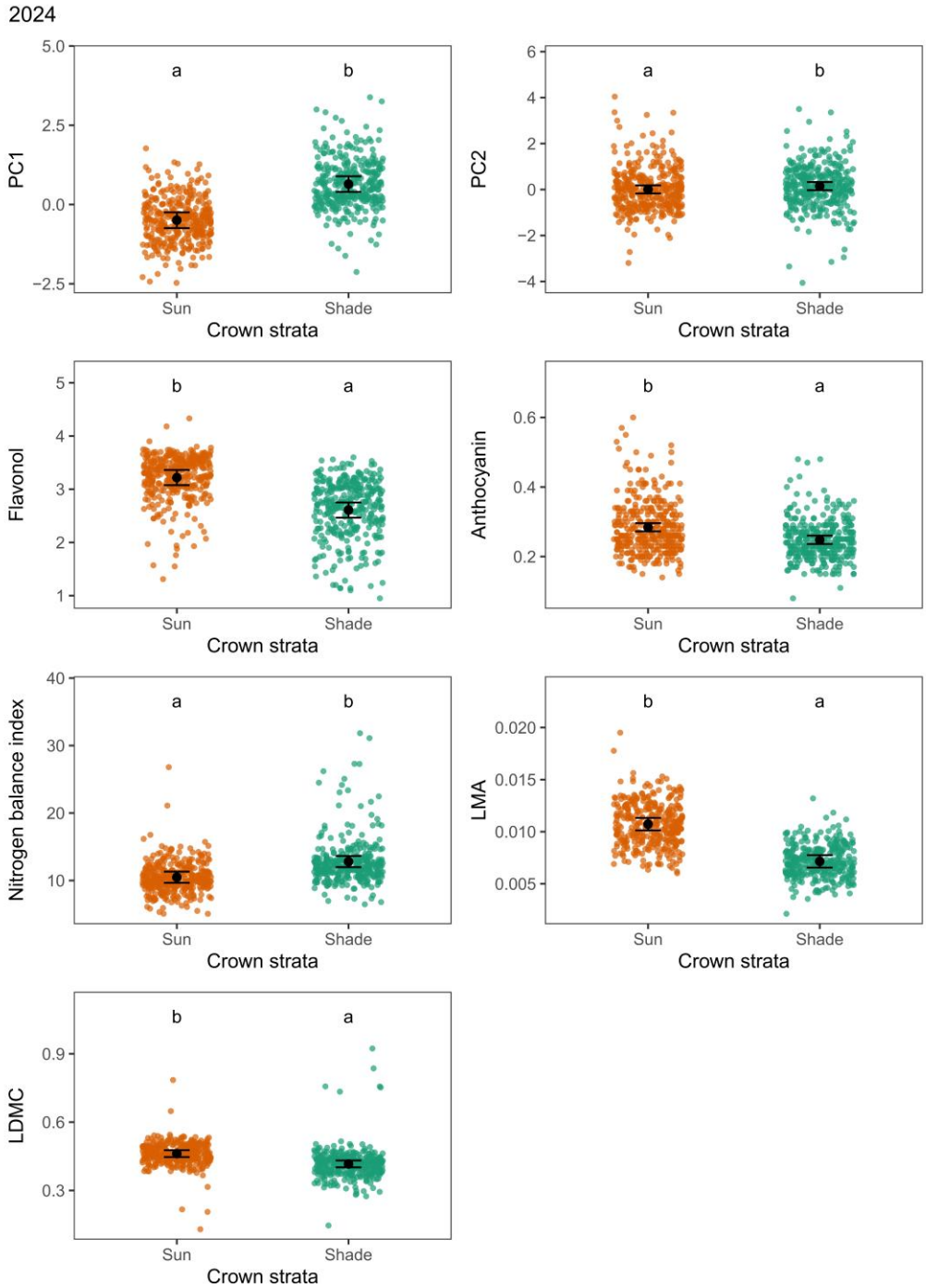
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397 **Fig. S2** The contribution of leaf traits to the different PCA axes. Traits that exceed the
398 expected average contribution (dashed red line) are the most influential within each
399 component.



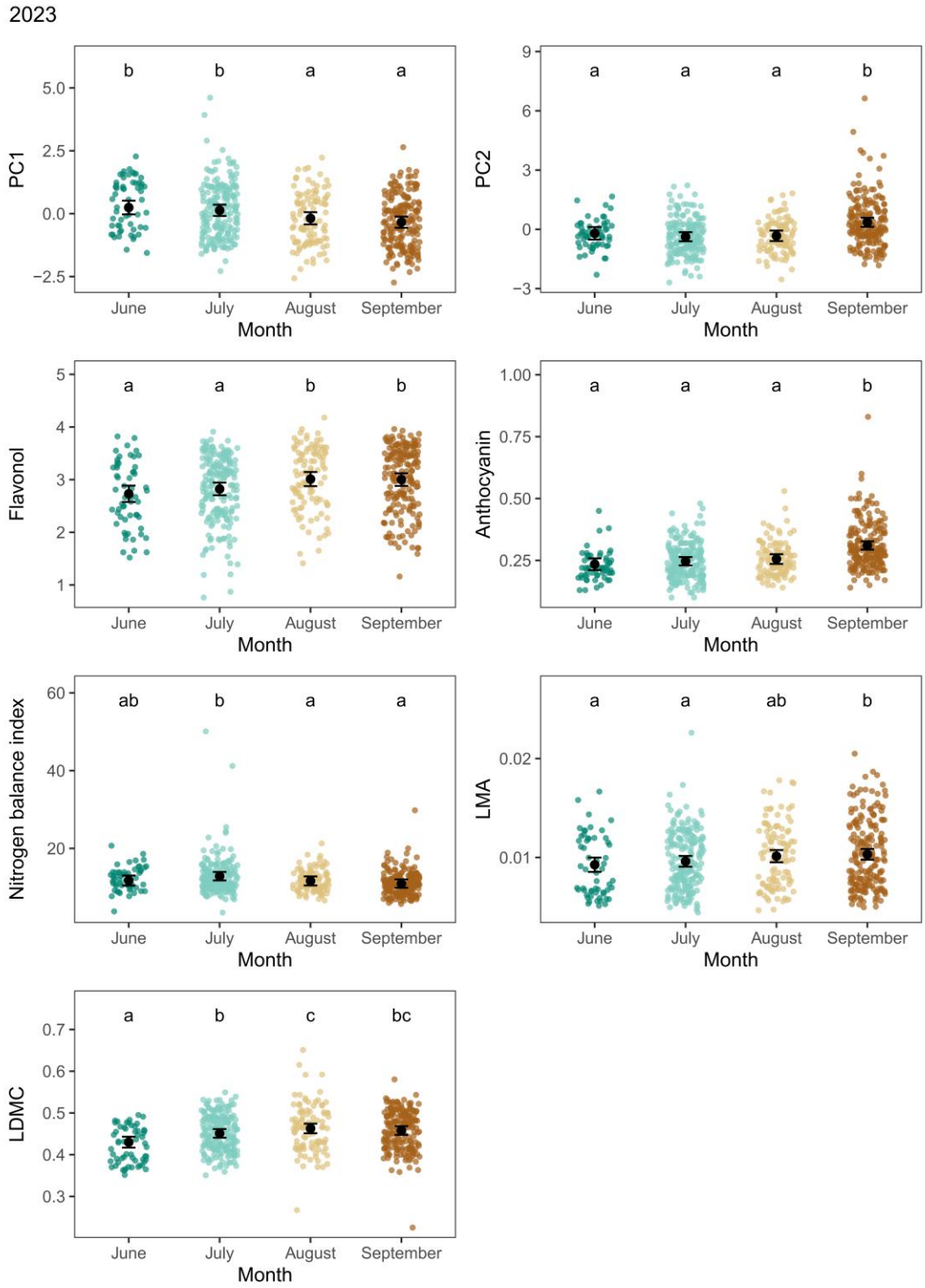
400

401 **Fig. S3** Differences in individual leaf traits between crown strata in 2023. Colored points
402 represent individual leaves (n = 543). Black points represent model-estimated marginal
403 means. Error bars indicate 95% confidence intervals. Lowercase letters indicate Tukey-
404 adjusted differences at $p < 0.05$



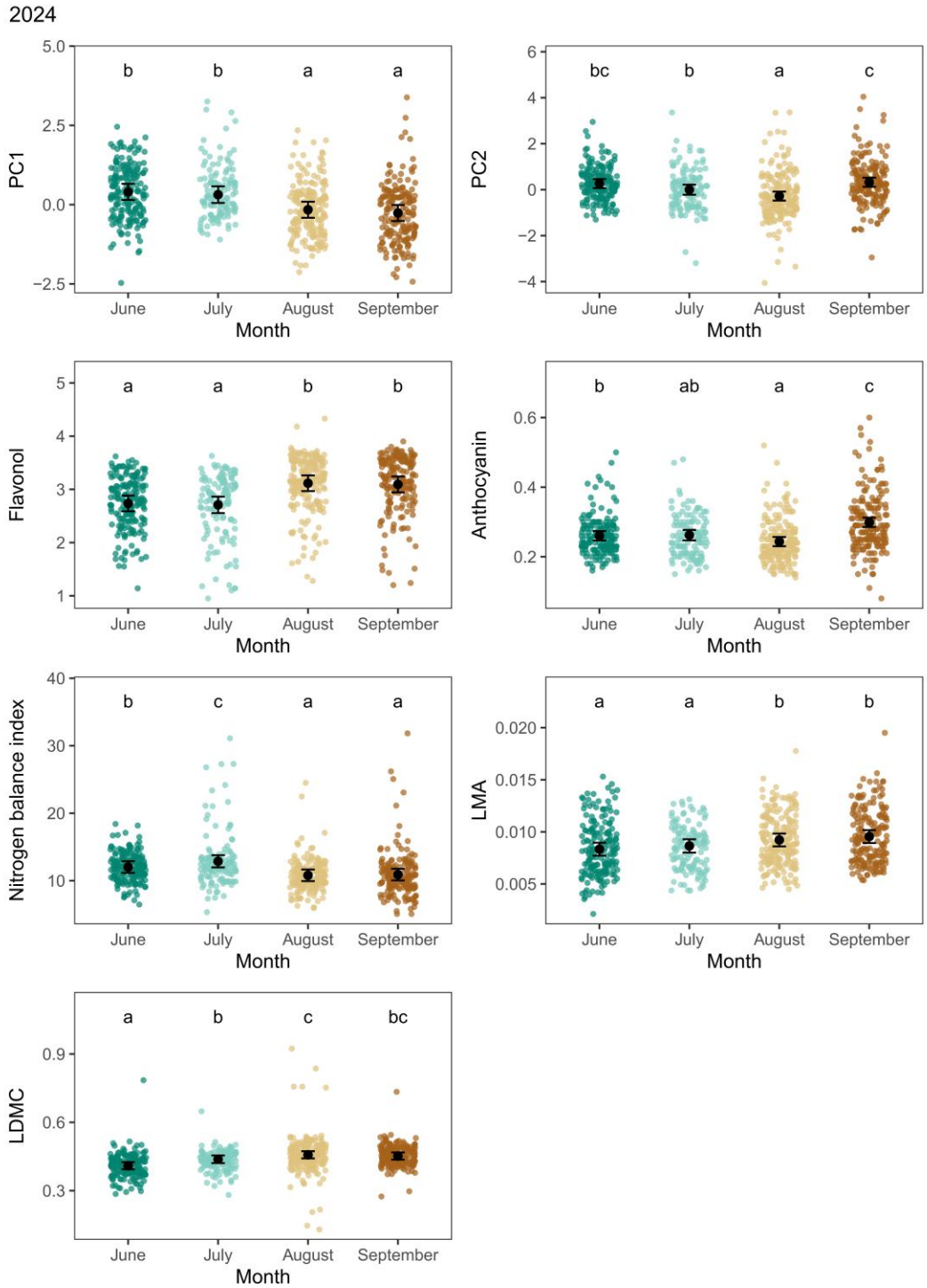
405

406 **Fig. S4** Differences in individual leaf traits between crown strata in 2024. Colored points
407 represent individual leaves (n = 681). Black points represent model-estimated marginal
408 means. Error bars indicate 95% confidence intervals. Lowercase letters indicate Tukey-
409 adjusted differences at $p < 0.05$



410

411 **Fig. S5** Seasonal variation of individual leaf traits in 2023. Colored points represent
412 individual leaves (n = 543). Black points represent model-estimated marginal means.
413 Error bars indicate 95% confidence intervals. Lowercase letters indicate Tukey-adjusted
414 differences at $p < 0.05$



415

416 **Fig. S6** Seasonal variation of individual leaf traits in 2024. Colored points represent
417 individual leaves (n = 681). Black points represent model-estimated marginal means.
418 Error bars indicate 95% confidence intervals. Lowercase letters indicate Tukey-adjusted
419 differences at $p < 0.05$

Table S1 Linear mixed-effects model for the year 2023 with consumed leaf volume as response variables and PC leaf traits, crown strata, and season as explanatory variables. Significant effects ($p < 0.001$) are bold.

Predictors	**Estimate**	**SE**	**z**	***p***
(Intercept)	0.524	0.041	12.812	**<0.001**
PC axis1	0.019	0.012	1.596	0.111
PC axis2	0.019	0.010	1.948	0.051
Crown strata [Shade]	0.017	0.024	0.730	0.465
Month [July]	-0.133	0.039	-3.397	**<0.001**
Month [August]	-0.023	0.044	-0.527	0.598
Month [September]	-0.032	0.042	-0.771	0.441
Random effects				
Variance among trees	0.001323			
Observations	543			
N trees	8			
AIC	-117.7			
BIC	-53.2			
Log-likelihood	73.8			

Table S2 Linear mixed-effects model for the year 2024 with consumed leaf volume as response variables and PC leaf traits, crown strata, and season as explanatory variables. Significant effects ($p < 0.001$) are bold.

Predictors	**Estimate**	**SE**	**z**	***p***
(Intercept)	0.241	0.014	17.841	**<0.001**
PC axis1	0.018	0.007	2.624	**0.009**
PC axis2	0.002	0.005	0.353	0.724
Crown strata [Shade]	0.006	0.011	0.517	0.605
Month [July]	-0.004	0.013	-0.327	0.744
Month [August]	-0.047	0.013	-3.642	**<0.001**
Month [September]	-0.036	0.013	-2.859	**0.004**
Random effects				
Variance among trees	0.000632			
Observations	681			
N trees	8			
AIC	-1031.2			
BIC	-963.3			
Log-likelihood	530.6			

Table S3 Linear mixed-effects model for the year 2023 with consumed leaf volume as response variables and crown strata and season as explanatory variables. Significant effects ($p < 0.001$) are bold.

Predictors	**Estimate**	**SE**	**z**	***p***
(Intercept)	0.520	0.042	12.286	**<0.001**
Crown strata [Shade]	0.043	0.017	2.514	**0.012**
Month [July]	-0.149	0.041	-3.642	**<0.001**
Month [August]	-0.041	0.045	-0.905	0.366
Month [September]	-0.037	0.043	-0.861	0.389
Random effects				
Variance among trees	0.001527			
Observations	543			
N trees	8			
AIC	-124.8			
BIC	-77.5			
Log-likelihood	73.4			

Table S4 Linear mixed-effects model for the year 2024 with consumed leaf volume as response variables and crown strata and season as explanatory variables. Significant effects ($p < 0.001$) are bold.

Predictors	**Estimate**	**SE**	**z**	***p***
(Intercept)	0.239	0.013	18.294	**<0.001**
Crown strata [Shade]	0.028	0.008	3.392	**<0.001**
Month [July]	-0.007	0.013	-0.540	0.589
Month [August]	-0.059	0.012	-4.888	**<0.001**
Month [September]	-0.049	0.012	-4.106	**<0.001**
Random effects				
Variance among trees	0.000527			
Observations	681			
N trees	8			
AIC	-1048.2			
BIC	-998.4			
Log-likelihood	535.1			