Plasticity of ecophysiological traits structure how herbaceous plant lineages persist in shaded environments

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Running Head: Ecophysiology of herbaceous plant lineages

# Abstract

Key Words:

# Introduction

The earliest angiosperm ancestors shared ecophysiological traits associated with shady, disturbed, and wet habitats (Feild *et al.*, 2004). Plasticity in these traits, developed through natural selection, then allowed angiosperms to expand into brighter and more evaporative terrestrial habitats characterized as water-limited instead of light limited (Feild & Arens, 2007). The same degree of flexibility has not been widely detected in the modern diversification of spore-bearing plant taxa. As a consequence, the Cretaceous radiation of angiosperms initiated a tremendous shift in of terrestrial plant communities, which included reciprocal trends in diversity of spore-bearing plants (Lupia *et al.*, 1999). Most modern taxa of spore-bearing plants are now believed to have diversified following this angiosperm radiation, so that angiosperm dominant communities now define the ecological niches that spore-bearing plants must inhabit (Schneider *et al.*, 2004).

Our understanding of the ecophysiology of the modern spore-bearing plants, including ferns and lycophytes, is limited. However, studies have shown that these two groups possess unique functional traits and physiological behaviors that likely influence their ecology. Ferns have been shown to be more conservative in stomatal closure, relative to xylem dysfunction, than angiosperms (Brodribb & Holbrook, 2004). Xylem of ferns have been shown to have greater hydraulic efficiency than conifer or angiosperm xylem, while still maintaining comparable resistance to cavitation (Pittermann *et al.*, 2011). Microphylls of lycophytes also likely led to distinct ecophysiology between ferns with megaphylls, even in shared habitats (Campany *et al.*, 2019). Last, ferns and lycophytes have limited flexibility to water stress, compared to angiosperms, as a consequence of a passive stomatal function (McAdam & Brodribb, 2013). These studies reveal several remaining canalized functions in ferns and lycophytes, which undoubtedly affect how spore-bearing taxa persist in a now angiosperm dominated landscape.

Studies that examine that broadly comparate the physiology of ferns and lycophytes to seed plants are rare. Therefore, it is difficult to derive widely applicable hypotheses of the ecophysiological behavior of these groups. From the present studies available, ferns and lycophytes appear to have lower photosynthetic rates than seed plants (Brodribb *et al.*, 2005, 2007; Carriquí *et al.*, 2015; Campany *et al.*, 2019). This would suggest that non-seed plants may be at a competitive disadvantage when growing alongside seed plants. However, only Carriquí *et al.* (2015) has systematically compared the physiology of these groups when growing in similar habitats (a common garden experiment).

Free-sporing plants exhibit trends reciprocal to those of angiosperms of equivalent latitudes

Watkins *et al.* (2007) has demonstrated that the gametophyte generations of many tropical ferns are markedly desiccation-tolerant, with tolerance being closely linked to ecological preference. In seed plants, desiccation tolerance has been essentially lost from vegetative tissues. Additional work on ferns indicates a tight link between carbon and nitrogen relations with species distribution (Watkins 2005). Work on fern hydraulics is in its early stages; however, a study on tropical ferns suggests that ferns have a significantly more resistive vascular system than seed plants. A recent study on temperate species proposes that ferns are more similar to gymnosperms in their hydraulic properties (Watkins).

\*\*add these cites as evidence, not as cites in opening sentence.

A primary controlling factor in fern hydraulics is stomatal function. A series of elegant studies have shown that stomatal control of ferns and lycophytes differs fundamentally from seed plants (Ruszala *et al.*, 2011; McAdam & Brodribb, 2012) . Evidence on fern and lycophyte stomata indicates that these groups have inefficient stomatal systems, likely possessing a hydro-passive mechanism which influences stomatal opening and closing. Specifically, unlike seed plants, fern and lycophyte stomata fail to close in response to abscisic acid (ABA) addition (McAdam & Brodribb, 2012). Additionally, recent evidence also suggests that co-occurring tropical ferns and lycophytes optimize their ecophysiology differently (Campany *et al.*, 2019). Consequently, the evolution or adaptation of physiological traits may impact the differential distribution of ferns,lycophytes and seed plants to each other.

The goal of this study is to examine the comparative ecophysiology of a number of temperate ferns and lycophytes to angiosperms. First, we compared a suite of plant functional traits between co-occurring herbaceous ferns, lycophytes and angiosperm in identical closed canopy habitats. In a pairwise comparison, we then evaluated the potential of fern and angiosperm species to adapt to open-light habitats. Given increasing evidence of canalized physiological function in non-seed plants, we hypothesized that ferns and lycophytes would have lower photosynthetic rates and stomatal conductance values in comparison to the nearby angiosperms. This, in turn, would impact aspects of leaf anatomy, nutrient relations, and water use efficiency. We also predict that more severe canalized physiological function affects the ecology of lycophytes in a manner that prevents them from establishing in full sun environments.

# Methods

## Study site and species

This study was conducted at two sites in upstate New York, USA. Data for most species were gathered at the Edmund Niles Hyuck Preserve (ENHP), characterized by mixed hardwood hemlock forests along the Helderberg escarpment in the foothills of the Catskill mountain range. Data from the second site were gathered at a forested natural area on the campus of Colgate University in Hamilton, New York, situated in the foothills surrounding the Chenango Valley. Forests at the Colgate University site are younger, but consist of a similar species richness and composition as ENHP.

We examined a series of fern, lycophyte, and angiosperm species at both sites (Table 1). To examine ferns relative to light-analogous angiosperms, we first selected a fern species and then located the nearest neighboring angiosperm growing in similar habitat conditions. Surveyed angiosperm taxa were always herbaceous and similar in size and stature to the neighboring fern species. At the ENHP, we sampled across two distinct habitats: understory low-light areas (closed) and open canopy high-light areas (open). At the Colgate University site, additional low-light fern and lycophyte species were surveyed.

**HOW MANY**

## Physiological Measurements

To generate comparative physiological data, we followed two procedures. First, we generated a series of detailed light response curves for a select number of species from each plant group (fern = 5, angiosperm = 5, lycophytes = 3). Light response curves were conducted for five individuals of each species. Second, to increase our comparative sample size, we surveyed light saturated rates of photosynthesis (An) and stomatal conductance (gs) for additional species at the ENHP. All gas exchange parameters were generated using the LiCor 6400 XT Photosynthesis System (LiCor Biosciences, Lincoln, NE USA).

Light response curves were conducted by decreasing the light intensity in the 2x3 cm gas exchange cuvette in small step changes. For each light response curve, The CO2 in the leaf cuvette was set to a flow rate of 300 mol s-1 and at ambient atmospheric [CO2] (400 ppm). Temperature was not explicitly controlled in the leaf cuvette. Cuvette leaf temperatures ranged from 27-30 °C and leaf vapor pressure deficit (VPD) averaged 1.37±0.40 kPa. Initial light measurements were made using the built-in quantum sensor to determine typical ambient light levels. These measurements were used to generate light response curves using appropriate light levels for high-light and low-light species separately. The light levels were not identical for each species, with several points being added or subtracted with each run. Broadly, light response curves followed the same template for high light or low light species. For high light species, light levels started at a PPFD of 1500 mol m-1 s-1 and then consisted of 16 additional steps to O mol m-1 s-1. For low light species, light response curves started at a PPFD of 500 mol m-1 s-1, followed by 13 additional steps to O mol m-1 s-1. Light response data for each curves was fit to a non-rectangular hyperbola model (Equation 6 in Lobo *et al.* (2013)). The light compensation point (LCP) was calculated as the PPFD at which the net photosynthetic rate equaled zero from the linear phase of each light response curve. Quantum yield () was calculated as the initial slope of the assimilation rate and mitochondrial respiration in the light (Rd) at the step change where PPFD was 0 mol m-1 s-1.

From these light response curves, we also developed an understanding of the levels at which low-light and high-light species reached light saturated photosynthetic rates. As a result, additional point measurements of leaf gas exchange were conducted at at a PPFD of 1200 mol m-1 s-1 for high-light species and 600 mol m-1 s-1 for low-light species. All other cuvette parameters were identical to those used for the light response curves. Leaf-level instantaneous water use efficiency (WUE) was calculated as An divided by leaf transpiration from gas exchange measurements.

## Foliar chemistry

Foliar tissue was sampled following gas exchange measurements for all species at ENHP and used for nutrient analyses. Samples were dried to a constant mass and ground using a Wig-L-Bug (Sigma-Aldrich, St. Louis, USA). Foliar nitrogen content (N) was measured using a Costech Analytical Elemental Analyzer (Valencia, USA), with the percentage N in samples calculated by comparison with certified standards. Foliar phosphorus concentrations (P) were determined using an ash digestion process (D’Angelo *et al.*, 2001) preceded by color development and absorbance measurement on an Astoria Paciﬁc colorimetric autoanalyzer (Clackamas, Oregon, USA).

## Stomatal anatomy

Stomatal density (SD) was measured by directly counting stomata on the abaxial leaf surface under 40x magnification with a field of view of 0.622 mm2. Stomatal density was calculated from 8 non-overlapping foliar regions for five individuals of each species.

## Statistial analyses

Principal component analysis, utilizing the ‘vegan’ package (Oksanen *et al.*, 2019), was used to explore how measured functional traits were distributed and co-varied among plant groups and canopy type. Linear mixed-effect models were used to test responses of functional traits to categorical fixed effects of plant group, habitat type and/or reproduction type (spore vs seed). The interaction between plant group and habitat was tested for functional trait comparisions between analagous fern and angiosperm species. To test for broad differences among plant groups, individual plant species were treated as random effects in each model. Tukey’s post-hoc test were performed in conjunction with ANOVA to determine which mean values of functional traits were different among fixed effect treatments with the ‘multcomp’ package (Hothorn *et al.*, 2008). Pairwise comparisons with the ‘emmeans’ package (Lenth, 2019) were calculated to investigate interactions between plant group and habitat, if present. For bivariate trait relationships, responses of dependent variables were analysed with linear mixed-effect models, with species as a random effect and plant group and/or canopy type as categorical fixed effects. Explained variance (R2) of mixed models were computed as in Nakagawa & Schielzeth (2013), in which the marginal R2 represents variance explained by fixed factors and the conditional R2 by both fixed and random factors. All tests of statistical significance were conducted at an level of 0.05. All analyses were performed with R 3.5.1 (R Core Team, 2018).

# Results

## Ecophysiology among plant lineages and habitats

We first explored how each of 10 measured functional traits varied and were distributed among co-occurring ferns, angiosperms and lycophytes in closed habitats. In total, 52.9 % of the trait variation was accounted for in two dimensions, encompassing differences in resource economics, physiology and anatomy of the surveyed taxa. Interestingly, there was minimal overlap among component scores for angiosperms compared to spore-bearing ferns and lycophytes (Figure 1). Specifically, angiosperm species were associated with gas exchange, anatomical and stoichiometric functional traits related to optimizing carboxylation. In contrast, lycophyte and fern species were most associated with physiological traits related to maintaining postie carbon balance.

Next, we explored how the same suite of 10 functional traits were distributed among open and closed habitats, in both angiosperms and ferns. In total, 55.9 % of the trait variation was accounted for in two dimensions. Component scores for angiosperms and fern species broadly diverged across open and closed canopy habitats, however, associations with physiological traits in open habitats were stronger for angiosperms (Figure 2). Specifically, angiosperms appear more plastic in their ability to up-regulate traits related to photosynthetic gas exchange with increased light availability. Overall, these diverging relationships with measured functional traits suggest a highly variable in situ ecophysiology for coexisting angiosperm, fern and lycophyte taxa. This is evident in broad differences in mean values of ecophyioslogical traits across the three plant lineages and among habitat types (Table 2).

## Leaf gas exchange

Ferns and angiosperms growing in low-light habitats had similar An, however, in open habitats angiosperms had 74.0 % higher rates of An than ferns (canopy x plant group, *P* = 0.043, Figure 3A). Rates of An for angiosperms in open habitats were 154.2 % higher than angiosperms in closed habitats (*P* =0.001, t=-4.742), while rates of An for ferns were statistically similar across habitat types. Overall, ferns and angiosperms in open habitats had nearly 3-fold higher gs than comparable species in closed habitats (*P* = 0.003). Rates of gs for angiosperms in open habitats increased nearly two-fold compared to angiosperms in closed habitats (*P* = 0.0341, t=-2.973), while rates of gs for ferns were statistically similar across habitat types (Figure 3B). Neither ferns nor angiosperms differed in WUE across the open and closed habitats, and WUE was statistically similar bewteen the two plant groups.

In closed habitats, variation in gas exchange parameters was largely associated with individual species variation (random effect) instead of functional differences between plant groups. No differences between An were detected between between angiosperms, ferns or lycophytes. Rates of gs were highest in angiosperms (*P* = 0.001, Figure 4A). Overall, the spore-bearing plant species had 50.5 % lower gs than angiosperms (*P* = 0.004, Figure 5A). Water-use efficiency was 88.5 % higher in lycophytes than ferns and angiosperms (*P* = 0.005), while ferns and angiosperms where statistically similar (Figure 4B).

## Light response curve parameters

Overall, ferns and angiosperms had similar across open and closed habitats. Quantum yields were also equivalent across angiosperms and ferns in open and closed habitats. Shifts in Rd between open and closed habitats differed for angiosperms but not for ferns (habitat x plant group, *P* < 0.001, Figure 3C). Specifically, Rd in angiosperms was 4 fold greater in open compared to closed habitats (t=-4.802, *P* = 0.013). Similarly, shifts in LCP between open and closed habitats differed for angiosperms but not for ferns (habitats x plant group, *P* < 0.001, Figure 3D). Specifically, the LCP in angiosperms increased nearly 4-fold in open compared to closed habitats (t=-4.382, *P* = 0.019).

In closed canopy habitats, the LCP of spore-bearing plant species were equivalent and 161.9 % higher than angiosperm species (*P* = 0.0195, Figure 5C). Photosynthetic quantum was statistically similar between each plant group, but lycophytes and ferns were broadly higher than angiosperms. Spore-bearing plant species had equivalent Rd, and Rd was nearly 4-fold lower than in angiosperms (*P* = 0.0178, Figure 5D)

## Stoichiometry

Ferns and angiosperms in open habitats had marginally lower foliar N content compared to similar species growing in closed habitats (-17.2 %, *P* = 0.073), with a large proportion of variation attributed to individual species differences. No differences in foliar P content were detected between open and closed habitats for either ferns or angiosperms. Across all habitats, foliar P content was 22.1 % higher in angiosperms than in ferns (*P* = 0.034). Neither ferns nor angiosperms species differed in their N:P ratios across the two habitat types.

Within closed habitats, lycophytes have 46.3 % lower foliar N content than ferns and angiosperms, which do not statistically differ (*P* < 0.001, Figure 4C). Lycophytes also had 24.0 % lower foliar P content than angiosperms (*P* = .026), while ferns were similar to both groups (Figure 4D). Overall, spore-bearing species had 18.7 % lower foliar P content than angiosperm species (*P* = 0.008, Figure 5B). The N:P ratios were not statistically different across angiosperms, ferns and lycophytes in closed habitats.

The relationship between An and foliar N content depended on both plant group and habitat type (habitat x plant group, *P* = 0.028). Specifically, the positive slopes of the relationship between An and N differed for ferns and angiosperms in open habitats, while no relationship between An and N was found for either group in closed habitat (Figure 6).

## Stomatal anatomy

Overall, differences in stomatal density (SD) were not detected for either ferns or angiosperms between open and closed habitats. Across habitats, however, ferns had a significantly lower SD than analogous angiosperms (-52.0 %, *P* < 0.001). Additionally, no differences in SD were detected among species of ferns, angiosperms and lycophytes in closed habitats. Variation in SD in closed habitats was largely due to individual species variation.

# Discussion

* which way should we tell the story?

1. fundamental differences in shade, then how angiosperms succeed in light
2. or vice versa

# Acknowledgements

We thank

# Tables

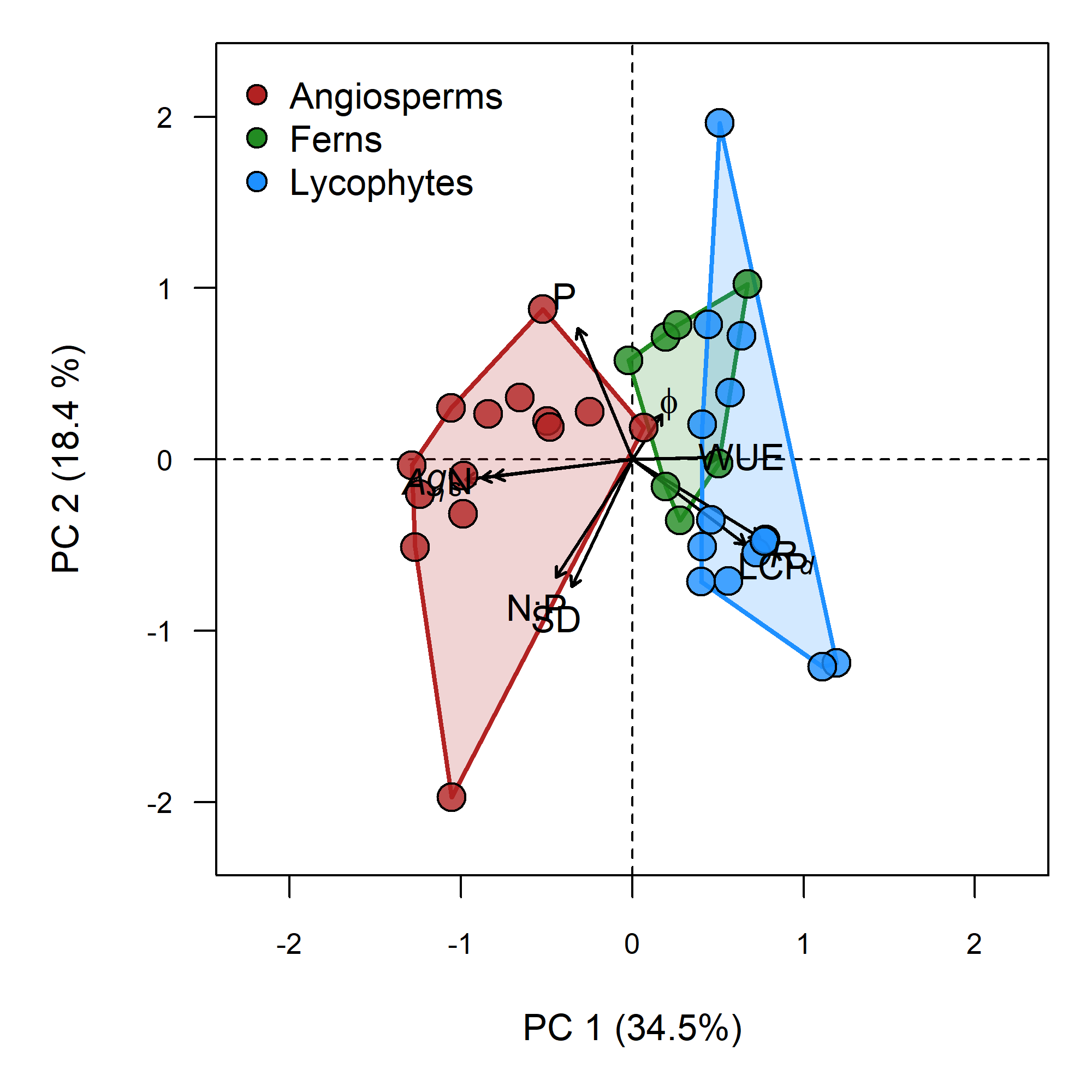
**Table 1**. Surveyed species of angiosperms, ferns and lycophytes across both open canopy high-light and closed canopy low-light habitats. All surveyed taxa were herbaceous and similar in size and stature.

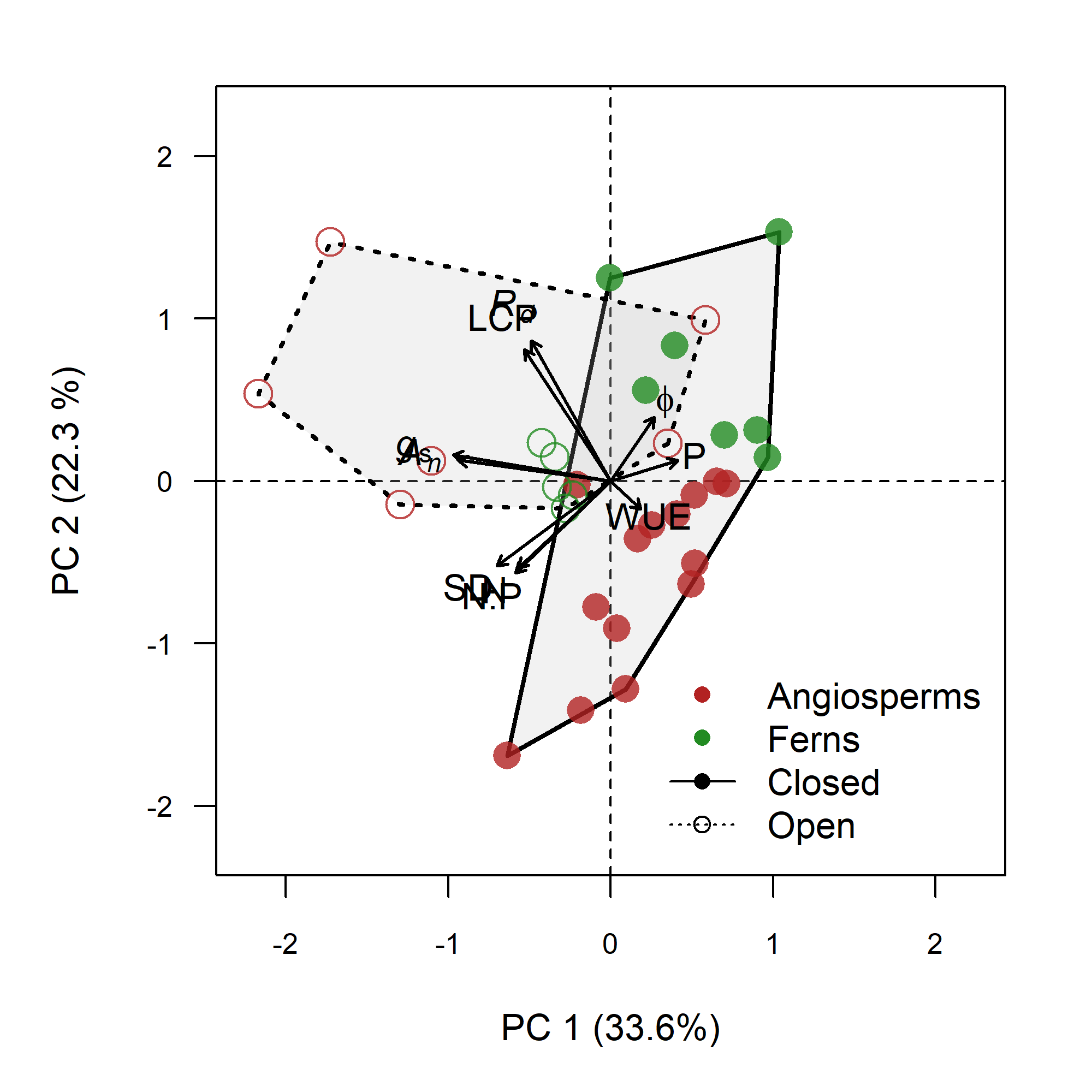
|  |  |  |  |
| --- | --- | --- | --- |
| **Species** | **Plant Lineage** | **Collection Site** | **Canopy** |
| Ageratina altissima (L.) King & H. Rob. | Angiosperm | Huyck\_Preserve | Closed |
| Arisaema triphyllum (L.) Schott | Angiosperm | Huyck\_Preserve | Closed |
| Eupatorium purpureum L. | Angiosperm | Huyck\_Preserve | Open |
| Eurybia divaricata (Aster divaricatus) (L.) G.L. Nesom | Angiosperm | Huyck\_Preserve | Closed |
| Impatiens pallida Nutt. | Angiosperm | Huyck\_Preserve | Closed |
| Lysimachia ciliata L. | Angiosperm | Huyck\_Preserve | Open |
| Rumex acetosella L. | Angiosperm | Huyck\_Preserve | Open |
| Tussilago farfara L. | Angiosperm | Huyck\_Preserve | Open |
| Urtica dioica L. | Angiosperm | Huyck\_Preserve | Closed |
| Viola sp. | Angiosperm | Huyck\_Preserve | Closed |
| Adiantum pedatumL. | Fern | Huyck\_Preserve | Closed |
| Athyrium filix-femina(L.) Mertens | Fern | Huyck\_Preserve | Open |
| Botrychium dissectumSpreng. | Fern | Colgate\_University | Closed |
| Botrychium multifidum(Gmel.) Rupr. | Fern | Colgate\_University | Closed |
| Dennstaedtia punctilobula(Michx.) T. Moore | Fern | Huyck\_Preserve | Open |
| Deparia acrostichoides(Sw.) M. Kato | Fern | Huyck\_Preserve | Closed |
| Dryopteris cristata(L.) Gray | Fern | Huyck\_Preserve | Closed |
| Dryopteris intermedia(Muhl. Ex Willd.) Gray | Fern | Huyck\_Preserve | Closed |
| Dryopteris marginalis(L.) Gray | Fern | Huyck\_Preserve | Closed |
| Onoclea sensibilis L. | Fern | Huyck\_Preserve | Open |
| Osmundastrum cinnamomeumL. | Fern | Huyck\_Preserve | Open |
| Osmunda claytoniana L. | Fern | Huyck\_Preserve | Closed |
| Phegopteris connectilis(Michaux) Watt | Fern | Huyck\_Preserve | Closed |
| Polystichum acrostichoides(Michx.) Schott | Fern | Huyck\_Preserve | Closed |
| Pteridium aquilinum(L.) Kuhn | Fern | Huyck\_Preserve | Closed |
| Thelypteris noveboracensis(L.) Nieuwland | Fern | Huyck\_Preserve | Closed |
| Dendrolycopodium dendroideum(Michx.) A. Haines | Lycophyte | Huyck\_Preserve | Closed |
| Dendrolycopodium obscurum(L.) A. Haines | Lycophyte | Colgate\_University | Closed |
| Diphasiastrum complanatum (L.) Holub | Lycophyte | Colgate\_University | Closed |
| Diphasiastrum digitatum (Dill. Ex A. Braun) Holub | Lycophyte | Huyck\_Preserve | Closed |
| Lycopodium clavatum L. | Lycophyte | Huyck\_Preserve/Colgate\_University | Closed |
| Spinulum annotinum (L.) Haines | Lycophyte | Colgate\_University | Closed |

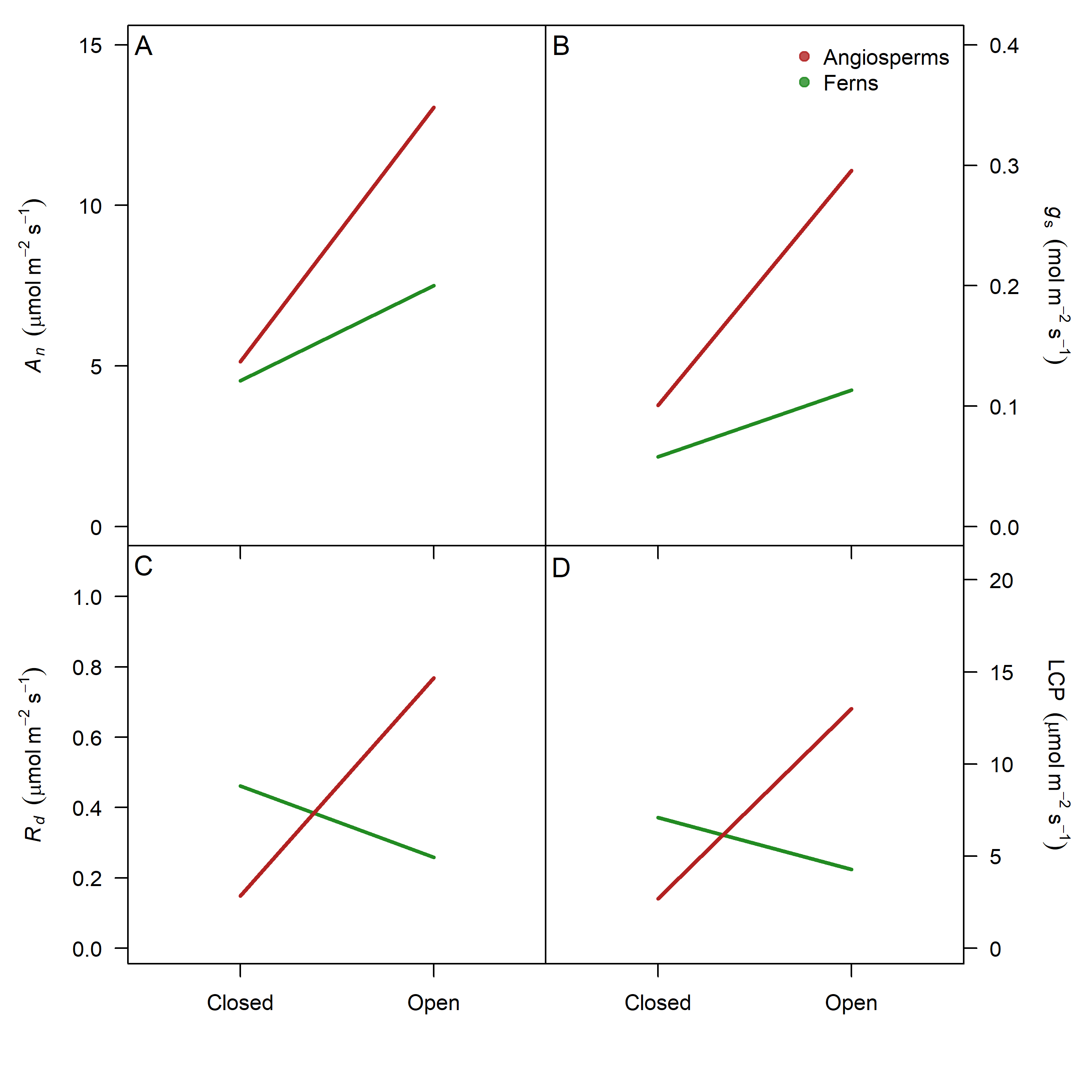
**Table 2**. Pooled ecophysiological traits for different plant lineages in open light and closed habitats. Each value reflects the mean (± 1 standard error) for each treatment.

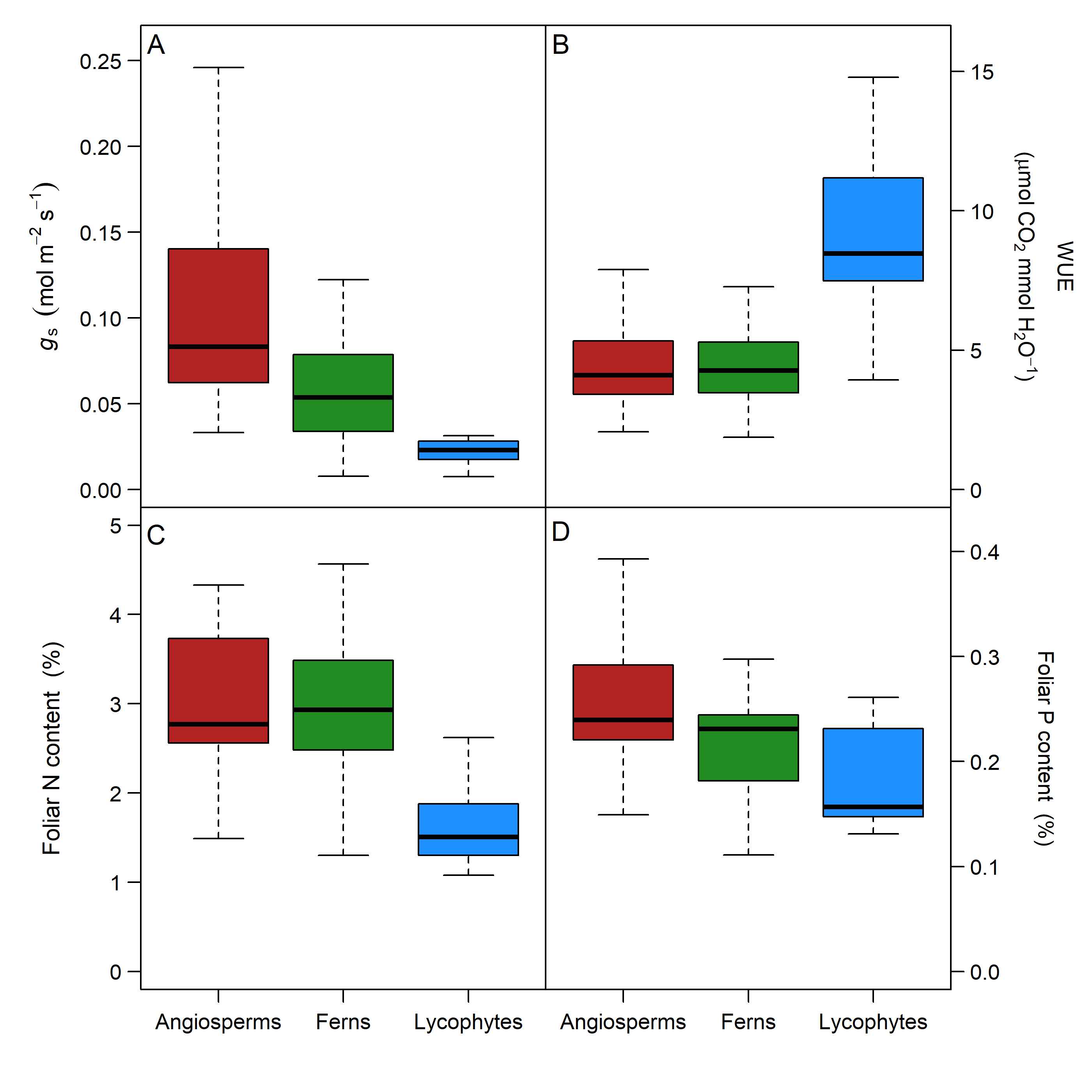
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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Lineage** | **Canopy** | ***A*n (mol m-2 s-1)** | ***g*s (mol m-2 s-1)** | **Foliar Nitrogen(%)** | **Foliar Phosphorus (%)** | **N:P** | **WUE (mol CO2 mmol H2O-1)** | **SD (mm-2)** | **(mol CO2 mol photons-1)** | ***R*d (mol m-2 s-1)** | **LCP (mol~m-2~s-1)** |
| Angiosperm | Closed | 5.1 (0.21) | 0.10 (0.011) | 3.0 (0.14) | 0.26 (0.014) | 13.3 (1.27) | 4.5 (0.34) | 29.2 (1.89) | 0.055 (0.0052) | 0.14 (0.022) | 2.5 (0.41) |
|  | Open | 13.1 (1.12) | 0.30 (0.046) | 2.4 (0.19) | 0.22 (0.007) | 11.6 (1.03) | 4.4 (0.18) | 32.5 (3.17) | 0.067 (0.0034) | 0.77 (0.146) | 13.0 (2.83) |
| Fern | Closed | 4.5 (0.30) | **0.06 (0.004)** | 3.0 (0.10) | 0.21 (0.008) | 13.8 (0.71) | 4.8 (0.28) | 14.3 (1.26) | 87.729 (87.6545) | 0.46 (0.056) | 7.1 (1.11) |
|  | Open | 7.5 (0.58) | 0.11 (0.013) | 2.5 (0.09) | 0.17 (0.012) | 15.0 (0.86) | 5.5 (0.47) | 15.2 (1.96) | 0.062 (0.0015) | 0.26 (0.039) | 4.3 (0.67) |
| Lycophyte | Closed | 3.6 (0.20) | 0.02 (0.002) | 1.6 (0.07) | 0.20 (0.023) | 10.8 (0.89) | 8.8 (0.75) | 24.9 (3.04) | 0.085 (0.0088) | 0.48 (0.085) | 7.1 (1.27) |

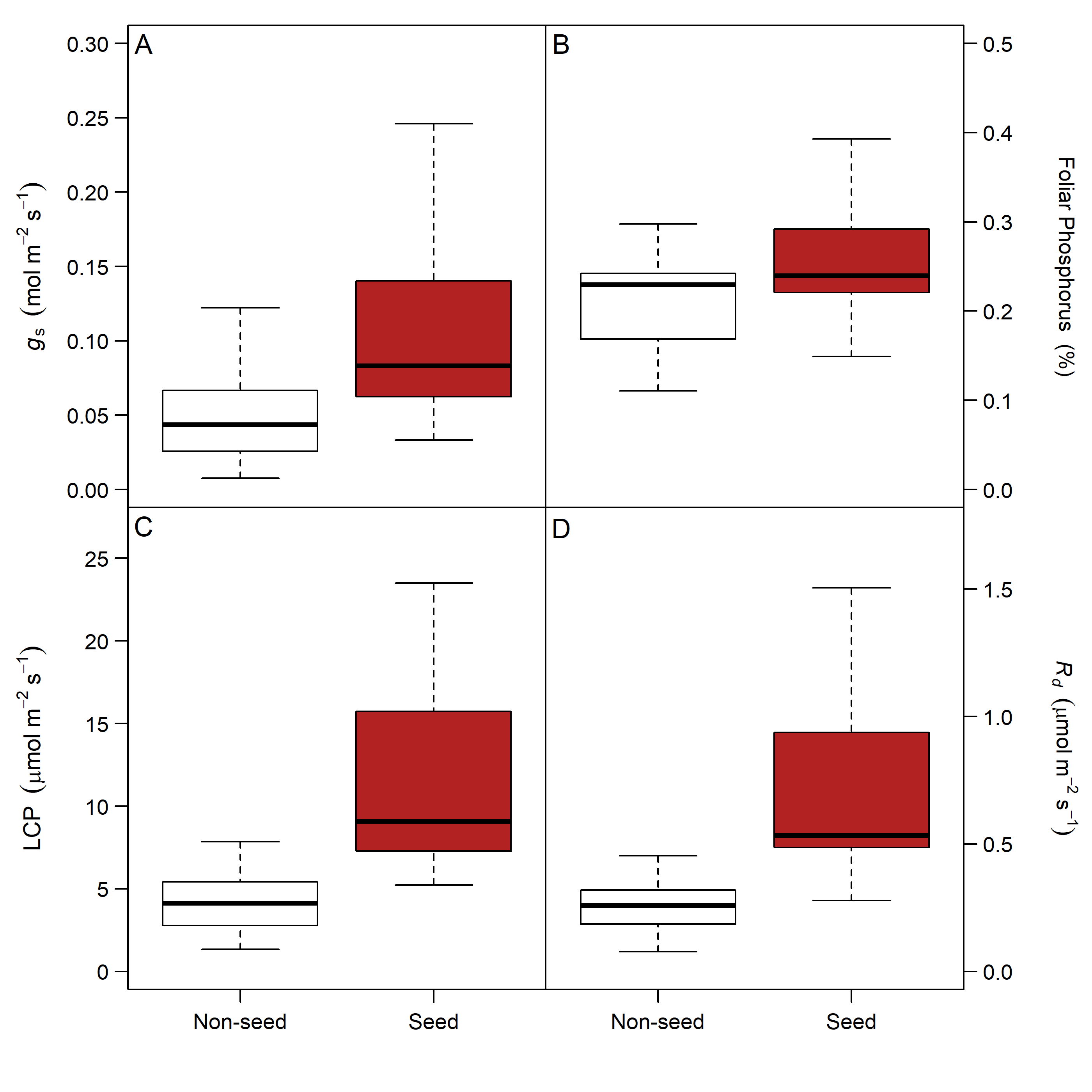
# Figures

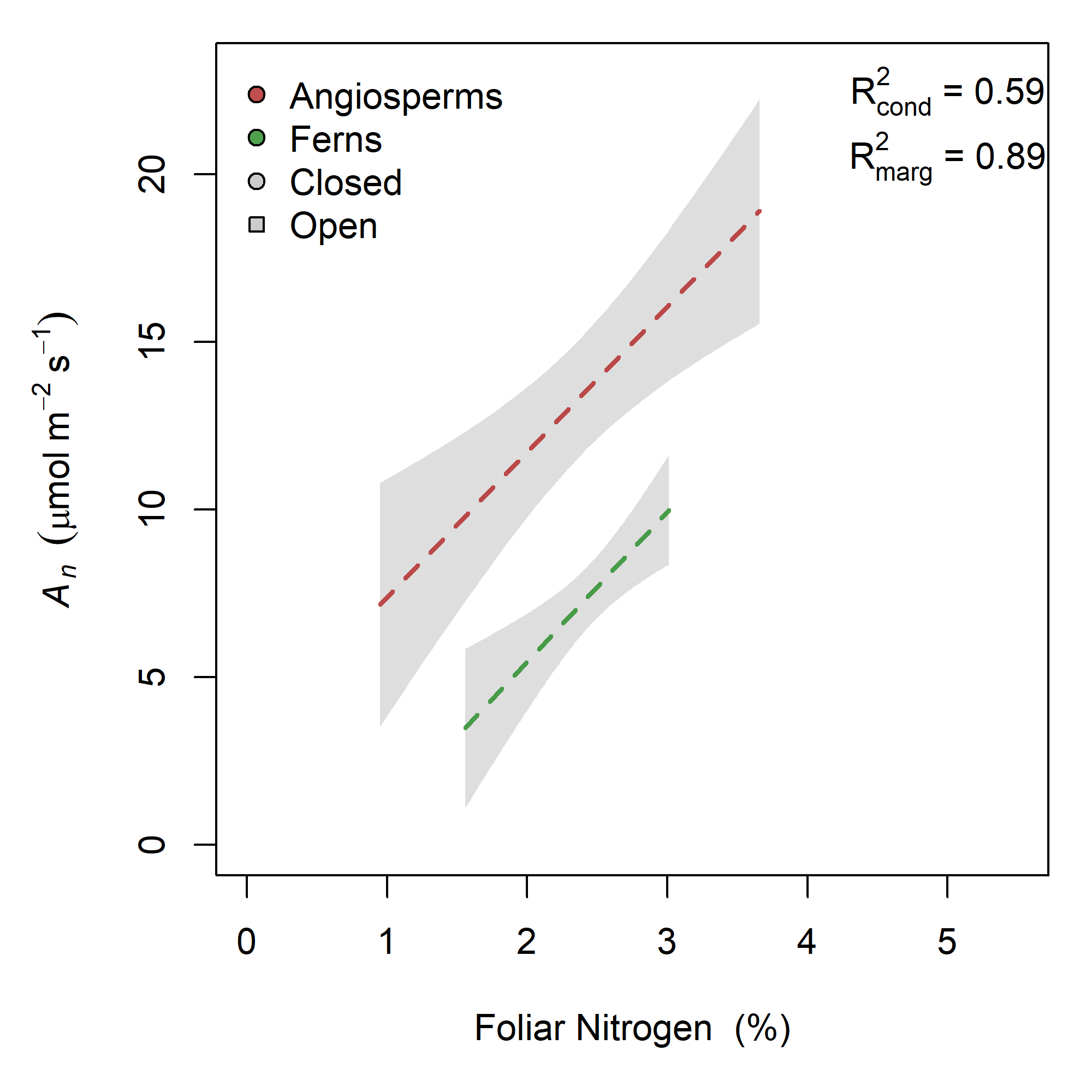
 **Figure 1**. Redundancy analysis of measured functional traits across con-occurring herbaceous angiosperm, fern and lycophyte species in closed habitats. With the 10 measured variables, 52.9 % of the trait variation was accounted for in two dimensions. Variables include net photosynthesis (An), stomatal conductance (gs), instantaneous water use efficiency (WUE), stomatal density (SD), the light compensation point (LCP), quantum yield (), dark respiration (Rd), foliar nitrogen (N) and phosphorus (P) content and the foliar nitrogen to phosphorus ratio (N:P).

 **Figure 2**. Redundancy analysis of measured functional traits across con-occurring herbaceous angiosperms and fern species in open and closed habitats. With the 10 measured variables, 55.9 % of the trait variation was accounted for in two dimensions. Variables include net photosynthesis (An), stomatal conductance (gs), instantaneous water use efficiency (WUE), stomatal density (SD), the light compensation point (LCP), quantum yield (), dark respiration (Rd), foliar nitrogen (N) and phosphorus (P) content and the foliar nitrogen to phosphorus ratio (N:P).

 **Figure 3**. Differences in mean physiological traits between co-occurring herbaceous angiosperms and fern species across open and closed habitats.

 **Figure 4**. Differences in mean physiological traits between con-occurring herbaceous angiosperm, fern and lycophyte species in closed habitats.

 **Figure 5**. Differences in mean physiological traits between co-occurring herbaceous seed plants (angiosperms) and spore-bearing plants (ferns + lycophytes) in closed habitats.

 **Figure 6**. Positive relationships between light saturated photosynthesis (An) and foliar nitrogen content existed for herbaceous angiosperm and fern species in open habitat, but not in closed habitats. For significant linear relationships, dashed lines represent model fits between traits and grey shaded areas are 95 % confidence intervals for the mean. Conditional and marginal R2 are reported for the overall model fit.

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