

PLANT FUNCTIONAL TRAIT RESPONSES ACROSS AN EXPERIMENTAL NITROGEN GRADIENT

MSc Masters Proposal
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SUMMARY

Trait-based ecology seeks to understand how environmental variation influences plant functional traits, performance, and fitness. The ‘performance paradigm’ proposes that multivariate trait phenotypes integrate into performance traits that drive organismal fitness, but does not explicitly incorporate the plastic responses of traits to environment. To address this knowledge gap, I will investigate how key leaf functional traits — including leaf mass per area (LMA), leaf dry matter content (LDMC), chlorophyll content (CHL), and the quantum yield of photosystem II (PSII) — modulate the relationship between biomass growth rates and soil nitrogen availability. Three plant species were grown across a broad nitrogen gradient, and leaf functional traits and whole-plant biomass growth rates were measured. As a next step, Structural Equation Models will evaluate hypothesized causal relationships linking nitrogen availability, leaf functional traits, and biomass growth rates. By clarifying the pathways that connect environmental variation to plant functional and performance traits, the findings will contribute to our understanding of plant responses to nutrient gradients, with applications in global change ecology.

INTRODUCTION

Since the 1960s, human activity has doubled the amount of nitrogen entering terrestrial ecosystems, primarily driven by the expansion of agriculture and industrial production (Bracken et al., 2015; Vitousek et al., 1997). This surge of nitrogen deposition has profound ecological consequences, notably accelerating the cycling and balance of nitrogen, carbon, and phosphorus in our forests and soils (Templer et al., 2012). Therefore, it is crucial to understand how pollution disrupts key natural resources like wetlands, forests, and riparian zones near polluting areas. In this theme, there is an implicit incentive to understand the consequences of nitrogen pollution on individual plant physiology, particularly focusing on leaf structure and function. Plant physiology, through carbon and nutrient absorption, ultimately drives ecosystem processes by regulating energy flow and resource dynamics across trophic levels.

Eco-physiological research highlights a need to link plant traits and leaf nutritional status to clarify how nutrient enrichment alters ecosystem function (Wilcots et al., 2022; Meyer-Grünefendt et al., 2016; Stevens et al., 2015). Leaf functional traits are a central focus because they govern carbon fixation and energy storage in the biosphere. From a carbon economic perspective, leaf mass per area (LMA) reflects the degree of structural investment in leaf tissues, constrained by a fundamental trade-off between durability and photosynthetic efficiency (Reich et al., 1997). Leaf dry matter content (LDMC) captures leaf tissue density and water retention, providing insights into resource conservation strategies. Chlorophyll content indicates nitrogen allocation to photosynthetic function, directly affecting the efficiency of light capture and carbon assimilation. Finally, the quantum yield of photosystem II (PSII) measures the efficiency of photosynthetic metabolism under saturating light conditions.

To bridge the gaps between environmental change, individual plant physiology, and effects on whole-ecosystem function, researchers often propose an integrated “functional trait” framework (Díaz et al., 2016; Wright et al., 2004). Functional traits are defined as any morphological, physiological, or phenological characteristics that impact individual fitness indirectly through their effects on performance

traits (Figure 1a; Violle et al., 2007). These traits form hierarchical relationships that scale environmental responses from individual to demographic levels and ultimately to ecosystem processes like nutrient cycling and biomass production. Recently introduced as part of a faceted “performance paradigm,” the functional trait approach links commonly studied traits to demographic parameters of interest, such as survival probability, reproduction, and biomass accumulation (Violle et al., 2007). However, a critical gap remains in understanding how environmental variation shapes trait plasticity and its corresponding influences on individual performance.

Building on this framework, my thesis investigates how environmental variation drives variation in key leaf functional traits and biomass growth - a performance trait. I will investigate how soil nitrogen availability drives variation in leaf traits and whole-plant biomass growth rates. I conducted a greenhouse study focusing on four leaf functional traits: LMA, LDMC, chlorophyll content, and the quantum yield of PSII. These traits were chosen for their relevance in linking nitrogen availability to plant resource-use strategies (Pérez-Harguindeguy et al., 2013). For the final part of my thesis, I will use structural equation models (SEMs) to explore how nitrogen availability, directly and indirectly, influences trait phenotypes and links these responses to biomass production (Onoda et al., 2017; Shipley et al., 2006).

I aim to answer the following research questions in my thesis.

- Q1.*** How do LMA, LDMC, chlorophyll content, and the quantum yield of PSII vary across a large nitrogen gradient?
- Q2.*** How does variation in LMA, LDMC, chlorophyll content, and the quantum yield of PSII modulate growth response to nitrogen?

EXPERIMENTAL METHODS

Plant Material 37 individuals of *Borago officinalis* (Boraginaceae; borage), 21 individuals of *Hordeum vulgare* (Poaceae; barley), and 39 individuals of *Raphanus sativus* var. "Rudi" (Brassicaceae; radish) were planted in 5-inch plastic pots in the BioSciences Greenhouse at the University of British Columbia. Seeds were sown in a general-purpose plant growth medium (Pro-Mix BX; Premier Tech, Rivière-du-Loup, QC, Canada). Pots were hand-watered weekly to field capacity and re-watered as needed, depending on weather and light conditions. To minimize effects of spatial variation in environmental conditions, pot positions were randomized weekly. Ammoniacal nitrogen treatments were applied at eight concentrations (0, 5, 10, 15, 20, 25, 30, and 35 mM), prepared through serial dilution, and delivered with a rinsed plastic watering can.

Trait Measurements LMA and LDMC were estimated using standard techniques (Pérez-Harguindeguy et al., 2013). Wet and dry biomass were measured with an analytical balance (Sartorius AG, Göttingen, Germany). Leaf area was measured using a Canoscan LiDE 400 flatbed scanner (Canon Inc., Tokyo, Japan). Leaf area was estimated using ImageJ via the "LeafArea" R package, with additional image pre-processing (clean-up and masking) performed in MATLAB. PSII fluorescence measurements were measured using a LI-600N leaf porometer/fluorometer (LI-COR, inc.; Lincoln, NE, United States).

Spectroscopy Spectral curves were obtained with an SVC 1024i-series spectroradiometer (Spectra Vista Corp., Poughkeepsie, NY, United States). For each plant, three leaf spectroscopy curves were measured for a single leaf and then averaged by barcode ID to produce a single representative curve per individual. Leaf chlorophyll (CHL) content was determined through numerical inversion of the PROSPECT-PRO radiative transfer model (Feret et al., 2021; Jacquemoud & Feret, 1990; Blonder et al., 2020).

Structural Equation Modeling SEMs will evaluate hypothesized links between environment, functional traits, and crop biomass (**Figure 1a**). **Figure 1b** conceptualizes the relationships between nitrogen treatment, LMA, LDMC, CHL, and vegetative biomass growth as a measure of plant performance. Beginning with the base model illustrated in Figure 1b, I will use the "piecewiseSEM" package (Lefcheck, 2016) to evaluate empirical support for hypothesized paths; d-separation will be used to identify any missing links following the analytical recommendations of Michaletz et al., 2018. Multiple models will be constructed based on hypothesized links and compared to each other through AICc (corrected Akaike information criterion) and CFI (comparative fit index).

PRELIMINARY RESULTS

The 6-week greenhouse experiment was completed in May 2024, and primary statistical analyses were done in R version 4.4.2.

Leaf Quality Appearance and morphological characteristics varied considerably across the nitrogen gradient for all model species, as evidenced by leaf image scans (**Figure 2**).

Functional Trait Responses Measured leaf traits from biomass and spectroscopy exhibited saturating effects across the nitrogen gradient, shown with OLS linear regression (**Figure 3**). **Figure 4** presents SMA (standard major axis) correlation plots for log-log pairwise relationships among LMA, LDMC, CHL, and PSII fluorescence. **Figure 5** shows the divergence of multiple traits in principle-component space across leaf samples for low (0 - 15 mM) and high (20 - 35 mM) nitrogen treatments. R² values from regression models used in **Figure 3** and **Figure 4** are listed in **Table 1** and **Table 2**, respectively.

All my data and R code is available on my GitHub page:

<https://github.com/Gromulus-Romulus/Leaf-Nitrogen-Responses-2025>

PROPOSED TIMELINE

September 2023 – January 2024 [Finished]

- Develop study questions and design experiments
- First committee meeting

February – December 2024 [Finished]

- Harvest and grow plants from greenhouse study
- Begin statistical analyses (PCA, SEM, etc.)

January 2025 [Current]

- Defend M.Sc. thesis proposal

February – March 2025

- Draft M.Sc. thesis
- Apply to graduate

April 2025

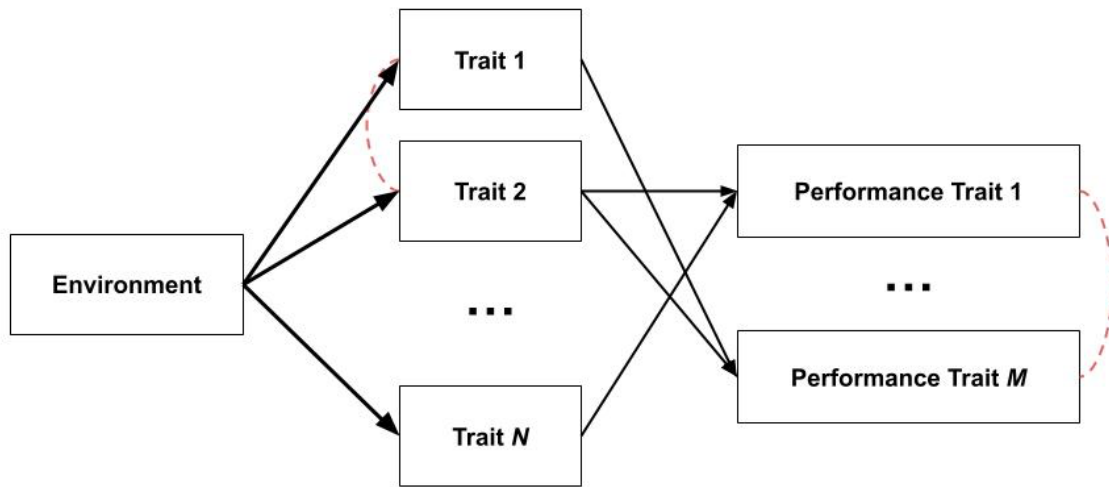
- Defend M.Sc. thesis and graduate with M.Sc.
- Submit thesis as manuscript for peer-review

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a



b

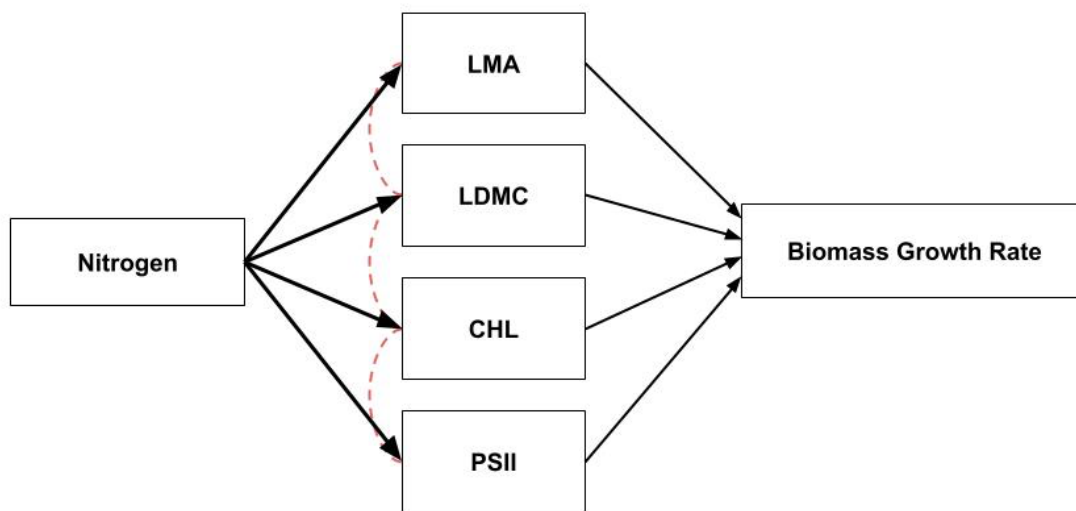


Figure 1. Hypothesized causal relationships linking environmental variation to plant functional traits, performance, and fitness within the performance paradigm. (a) Conceptual illustration of the morphology-performance-fitness (MPF) framework proposed by Arnold (1983) and elaborated by Violle et al. (2007), showing how environmental variables influence functional traits, which subsequently affect organismal performance and fitness. (b) Path diagram applying the MPF framework to plants along a nitrogen availability gradient, depicting hypothesized relationships between nitrogen availability, functional traits (leaf mass per area, LMA; leaf dry matter content, LDMC; chlorophyll content, CHL; fluorescence yield, PSII), and a performance trait (biomass growth rate). Solid directional arrows represent direct effects, while orange dashed paths indicate potential phenotypic covariances among traits.

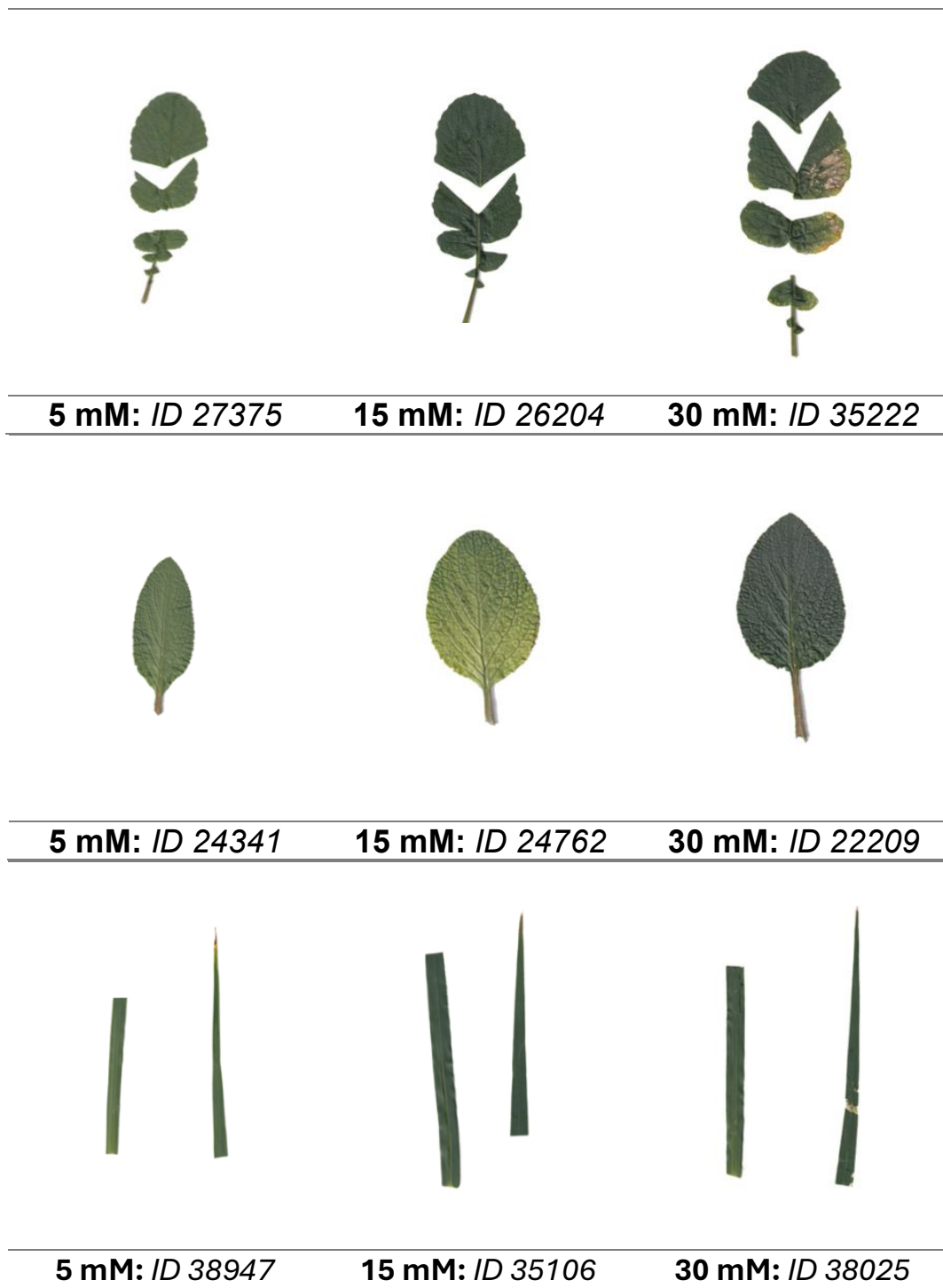


Figure 2. Effect of nitrogen availability on leaf traits in three plant species. Leaf scans show leaf morphology and color variation across three nitrogen treatments (5 mM, 15 mM, and 30 mM). Species from top to bottom are *Raphanus sativus* (radish), *Borago officinalis* (borage), and *Hordeum vulgare* (barley). Rows correspond to a single species, and columns correspond to a single nitrogen treatment.

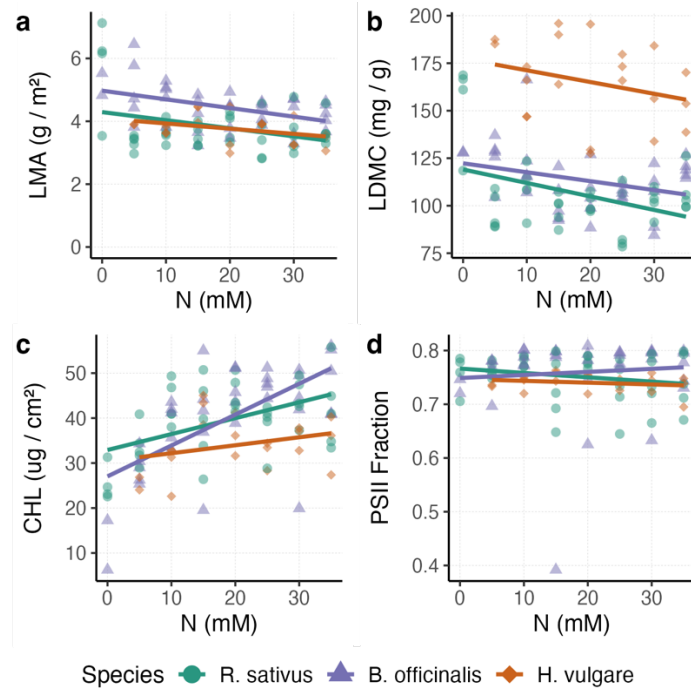


Figure 3. Responses of leaf traits along a nitrogen availability gradient. Panels illustrate variation in (a) leaf dry matter content (LDMC), (b) leaf mass per area (LMA), (c) chlorophyll content (CHL), and PSII fluorescence fraction (d). Horizontal axes for all panels represent the applied mM concentration of ammoniacal nitrogen (N mM). Trendlines were fit using OLS regression of variables with respect to nitrogen treatment.

	LMA	LDMC	CHL	PSII
<i>R. sativus</i>	<u>0.103*</u>	<u>0.157*</u>	<u>0.245*</u>	0.0460
<i>B. officinalis</i>	<u>0.190*</u>	0.104	<u>0.408*</u>	0.00678
<i>H. vulgare</i>	0.140	0.09	0.085	0.0405

Table 1. Rows order each crop species, R2 values from OLS regression are listed for LMA, LDMC, CHL, and PSII fluorescence yield. R2 values with asterisks (*) indicate that the corresponding p-value was significant (less than 0.05).

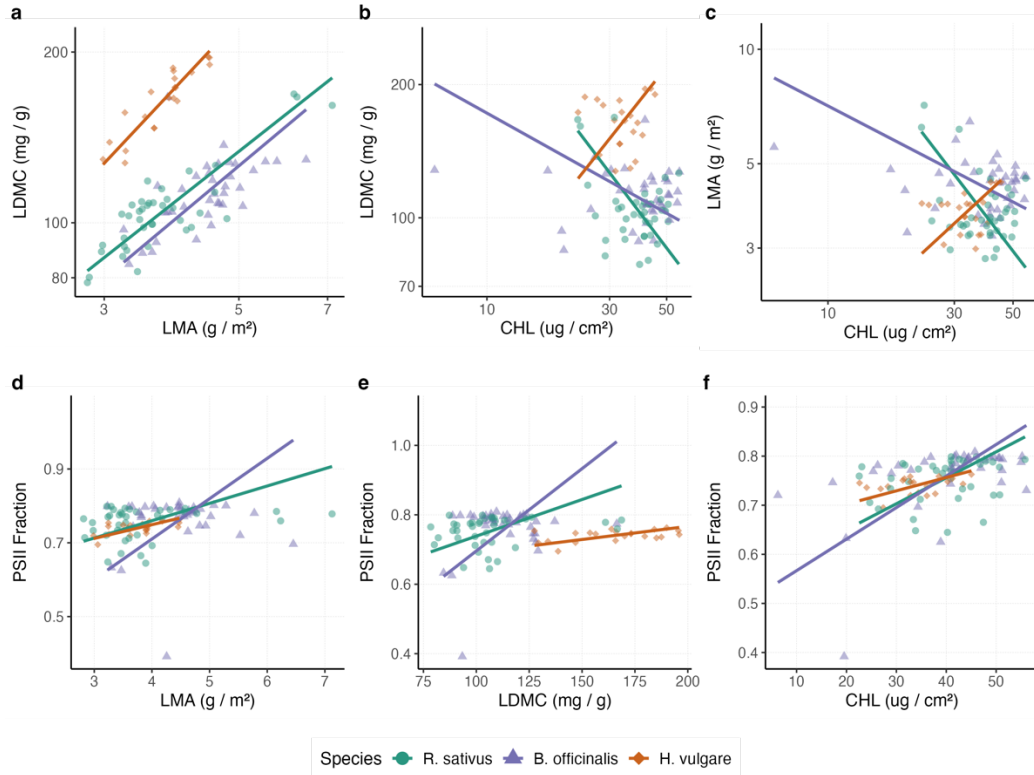


Figure 4. Pairwise scaling relationships between (a) leaf mass per area (LMA) and chlorophyll content (CHL), (b) leaf dry matter content (LDMC) and CHL, and (c) LDMC and LMA. Also included are PSII fluorescence measurements plotted with respect to LMA (d), LDMC (e), and CHL (f). Trendlines represent standard-major-axis (SMA) regression models fitted on log-transformed variables.

	LMA-LDMC	LDMC-CHL	LMA-CHL
<i>R. sativus</i>	<u>0.730*</u>	0.093	<u>0.108*</u>
<i>B. officinalis</i>	<u>0.315*</u>	0.004	0.0259
<i>H. vulgare</i>	<u>0.816*</u>	0.000513	0.03027

	PSII-LMA	PSII-LDMC	PSII-CHL
<i>R. sativus</i>	0.0435	0.0290	0.0425
<i>B. officinalis</i>	0.00859	0.0798	<u>0.229*</u>
<i>H. vulgare</i>	<u>0.362*</u>	<u>0.219*</u>	0.120

Table 2. For each species, ordered by rows, R2 values from SMA log-log regression are listed for all relationships illustrated in **Figure 4**. R2 values with asterisks (*) and underlines indicate that the corresponding *p*-value was significant (less than 0.05).

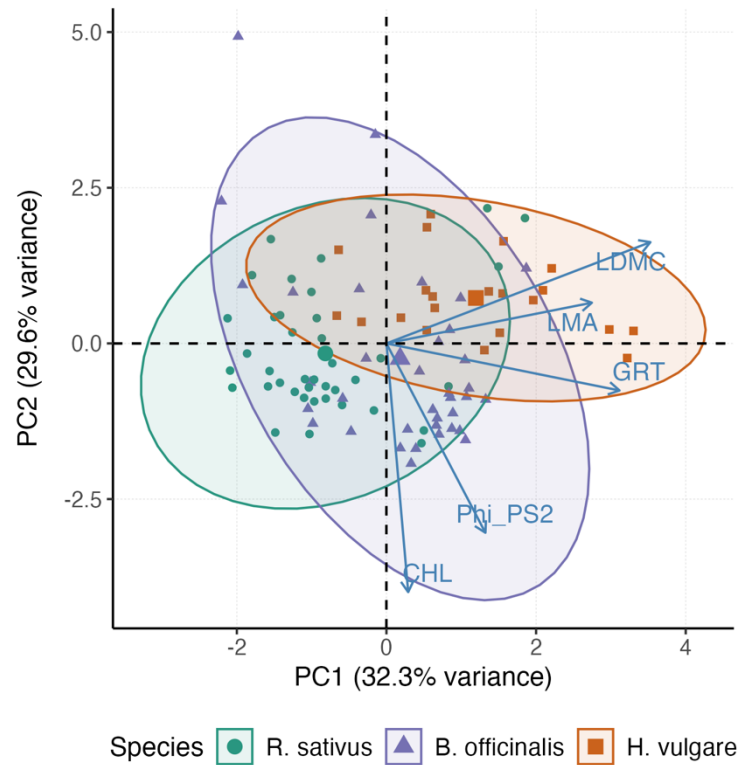


Figure 5. PCA ordination plot of traits of interest for preliminary analysis with ellipsoids grouped by species. GRT represents the normalized rate of growth, dividing measured above-ground vegetative biomass by 42: the number of days in the experiment. Approximately 70% of the variation in trait space was well explained by 2 component axes (with 95% confidence).