LARISSA AREAL DE CARVALHO MÜLLER

COMO A ESPECTROSCOPIA FOLIAR PODE CONTRIBUIR PARA A CARACTERIZAÇÃO FUNCIONAL DA VEGETAÇÃO DE ALTITUDE?

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Botânica, para obtenção do título de *Doctor Scientiae*.

Orientadora: Andreza Viana Neri

Coorientadora: Cibele Hummel do Amaral

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RESUMO

MÜLLER, Larissa Areal de Carvalho, D.Sc., Universidade Federal de Viçosa, setembro de 2022. **Como a espectroscopia foliar pode contribuir para a caracterização funcional da vegetação de altitude?** Orientadora: Andreza Viana Neri. Coorientadora: Cibele Hummel do Amaral.

Esse trabalho teve como objetivo investigar as características funcionais das espécies de vegetação de Altitude neotropicais por meio da relação de traços funcionais e espectroscopia foliar. Para a caracterização funcional coletamos a reflectância foliar, pigmentos foliares (clorofila a, clorofila b, total de clorofila, carotenoide), área foliar (LA), massa foliar por área (LMA), conteúdo de matéria seca foliar (LDMC) e a altura de cada 10 indivíduos de 32 espécies ao longo do pico da Pedra do Pato na Serra do Brigadeiro - MG. Estimamos os traços foliares a partir da reflectância foliar usando regressões parciais do mínimos quadrados (PLSR) e avaliamos a qualidade das estimativas através do coeficiente de determinação (R^2), a raiz quadrada média do erro de predição (RMSEP) e a % RMSEP (RMSEP como porcentagem da característica). Após as estimativas, realizamos análises de agrupamento hierárquico com os valores estimados e com os traços observados e definimos o número de grupos foram formados usando um corte híbrido. Comparamos os dendrogramas e realizamos o teste de Kruskal-Wallis para analisarmos a variação dos traços entre os grupos de cada agrupamento. Posteriormente, analisamos a distribuição das espécies no espaço multidimensional das análises de componentes principais para os traços observados e a reflectância foliar. As estimativas dos traços usando PLSR apresentaram boas precisões com baixa taxas de erros, com melhores coeficientes de determinação para características como LMA e LDMC. O modelo PLSR explicou apenas 11% da variação na razão clorofila a e clorofila b, enquanto a razão clorofila e carotenóides explicaram 63% da variação. Os modelos explicaram mais as características da estrutura foliar LA (81%), LMA (87%) e LDMC (88%). O agrupamento usando traços observados dividiu as espécies em 10 grupos com diferenças significativas na área foliar e matéria seca foliar entre grupos. E foram formados 8 grupos para os traços estimados, em que houve maior variação de traços. Os dois agrupamentos apresentaram uma alta correlação (correlação cofenética, r = 0.77) e em ambos, as espécies coletadas em locais diferentes ficaram no mesmo grupo. Em relação ao espaço multidimensional, três componentes principais representaram 78% e 94% da variação da refletância e dos caracteres foliares, respectivamente. Para a análises dos traços, primeiro eixo foi fortemente associado aos pigmentos, enquanto os demais eixos estão associados às estruturas foliares e altura das espécies. Para o espectro foliar, os comprimentos de onda da borda vermelha e da região do infravermelho próximo foram associados ao primeiro eixo, e o outro eixo está relacionado aos comprimentos de onda da região do visível. As ordenações de traços e reflectância foliar apresentaram concordância (Procrustes r = 0,59; m^{12} = 0.644; p = 0,001) e em ambos as espécies ficaram menos dispersas, com algumas delas apresentaram variação intraespecífica. Nossos resultados mostraram a possibilidade de caracterização funcional de espécies de vegetação de altitude por meio de traços estimados ou reflectância foliar. Além disso, os resultados também indicaram uma maior variação dos traços e das propriedades entre espécies do que dentro das espécies indicando uma relação da composição das espécies e da função das comunidades de vegetação de altitude.

Palavras-chave: Campo de altitude. Reflectância foliar. Traço funcional. Tipos óticos. Espectroscopia foliar

ABSTRACT

MÜLLER, Larissa Areal de Carvalho, D.Sc., Universidade Federal de Viçosa, September, 2022. **How can leaf spectroscopy contribute to the functional characterization of high-altitude vegetation?** Adviser: Andreza Viana Neri. Co-adviser: Cibele Hummel do Amaral.

This work aimed to investigate the functional characteristics of Neotropical Altitude vegetation species through the relationship between functional traits and leaf spectroscopy. We collected leaf reflectance, leaf pigments (chlorophyll a, chlorophyll b, total chlorophyll, carotenoid), leaf area (LA), leaf mass per area (LMA), leaf dry matter content (LDMC), and height of every ten individuals of 32 species along Pedra do pato peak in Serra do Brigadeiro. We estimated the leaf traits from the leaf reflectance using partial least squares regressions (PLSR). We evaluated the quality of the estimates through the coefficient of determination (R^2), the root mean square of the prediction error (RMSEP), and the % RMSEP (RMSEP as a percentage of the characteristic). After the estimates, we performed a hierarchical cluster analysis with the estimated values and the observed traits. We defined the number of groups formed using a hybrid cut. We compared the dendrograms and performed the Kruskal-Wallis's test to analyze the variation of traits between the groups of each cluster. Subsequently, we analyzed the distribution of species in the multidimensional space of the principal component analysis for the observed traits and leaf reflectance. Trait estimates using PLSR showed good accuracy with low error rates, with better determination coefficients for traits such as LMA and LDMC. The PLSR model explained only 11% of the variation in the chlorophyll a and chlorophyll b ratio. In comparison, the chlorophyll and carotenoid ratio explained 63%. The models explained more the characteristics of leaf structure LA (81%), LMA (87%), and LDMC (88%). Clustering using observed traits divided the species into ten groups with significant differences in leaf area and dry matter content. Moreover, eight groups were formed based on estimated traits, with more substantial variation. The two clusters showed a high correlation (cophenetic correlation, r = 0.77), and the species collected in separate places were in the same group. Regarding the multidimensional space, three principal components represented 78% and 94% of the reflectance variation and leaf characters, respectively. The first axis was strongly associated with pigments for trait space. In contrast, the other axes were associated with leaf structures and species height. In leaf reflectance space, the wavelengths of red-edge and near-infrared region correlated with the first axis, and the other axis was related to the wavelengths of the visible region. Trait ordinances and leaf reflectance showed agreement (Procrustes r = 0.59; m^{12} = 0.644; p = 0.001). In both species, they were less dispersed, with some of them showing intraspecific variation. Our results showed the possibility of functional characterization of highland vegetation species through estimated traits or leaf reflectance. Furthermore, the results also indicated a greater variety of traits and properties between species than within species, indicating a relationship between species composition and the function of altitude vegetation communities.

Keywords: Campos de Altitude. Leaf reflectance. Functional trait. Optical types. Leaf spectroscopy

SUMÁRIO

INTRODUÇÃO GERAL

Compreender os traços funcionais das espécies e da comunidade permite a investigação dos mecanismos e efeitos por trás das interações e da montagem da comunidade (MCGill et al., 2006). As características foliares das espécies estão relacionadas às condições já estabelecidas, mas os processos pelos quais a comunidade atinge seu nível ótimo podem variar entre as características (Dong et al., 2020). As propriedades bioquímicas e biofísicas das plantas representam seus traços funcionais e estão relacionadas a todos os filtros que selecionaram as espécies até agora (Lavorel and Garnier, 2002; Pérez-Harguindeguy et al., 2013). Estes traços também estão relacionados aos padrões espectrais da vegetação, o que permite a caracterização funcional em diferentes escalas temporais e espaciais (Homolová et al., 2013; Wang and Gamon, 2019).

A capacidade de inferir os traços funcionais está atrelado às interações entre traços foliares com a energia eletromagnética (REM) que é refletida em assinaturas espectrais (Serbin and Townsend, 2020). Alguns traços foliares podem conferir à planta um comportamento espectral, que pode ser analisada pela espectrometria, uma ferramenta que vem ampliando seu uso em diversas áreas (Helsen et al., 2021; Serbin and Townsend, 2020). A folha - principal órgão fotossintetizante das plantas vasculares- absorve a energia proveniente da luz no intervalo espectral do visível (VIS, entre 0,4-0,7 µm), através das clorofilas e outros pigmentos, e reflete parte desta energia, na região do infravermelho (NIR, entre 07-1,3µm) (Gates et al., 1965; Knipling, 1970). Ela é formada por tecidos com superfícies irregulares e, internamente, apresenta espaços preenchidos por ar intercalados por células com paredes celulares, presença de pigmentos e de água. Devido a essas características, a “luz”, ou a energia radiante, interage com essas estruturas, sendo absorvida, transmitida ou refletida de maneiras diferentes, dando a cada planta comportamentos espectrais diferentes (Gates et al., 1965; Knipling, 1970). O uso inicial do sensoriamento remoto da vegetação permitia detectar a saúde das plantas pelas diferenças nas cores das folhas ou das quedas das mesmas, mais ainda limitante na classificação de tipos funcionais (Ustin and Gamon, 2010). Com o avanço da tecnologia e da ecologia, o sensoriamento passou a ser usado para diferentes abordagens pra se investigar a diversidade da vegetação: mapeamento de habitat, mapeamento de espécies, estimativas de traços funcionais e diversidade espectral (Homolová et al., 2013; Wang and Gamon, 2019). Estimar ou relacionar as propriedades espectrais com as características funcionais permite mapear a distribuição e a variação de traços que podem estar relacionados a montagem das comunidades (Serbin and Townsend, 2020).

A caracterização funcional em tipos funcionais tem sido uma alternativa para simplificar e facilitar a interpretação das respostas das espécies e/ou comunidade (Funk et al., 2016), além de possibilitar estabelece comparações com diferentes floras e biomas (Pierce et al., 2017). Embora amplamente utilizadas, as classificações funcionais convencionais utilizadas como estratégias de CSR ou formas de crescimento podem não ser suficientes para representar a variação funcional encontrada nas comunidades (Matos et al., 2021; Roth et al., 2016; Van Cleemput et al., 2021). Muitas vezes, categorias funcionais são reduzidas em comunidades devido baixa variabilidade de espécies do que variabilidade funcional, como por exemplo formas de vida em *grasslands* (Funk et al., 2016; Zakharova et al., 2019). Ou não representar as respostas à seca em comunidade de altitude (Matos et al., 2021). Sendo assim, o uso de abordagens com base em traços contínuos pode ser mais sutil a mudanças do que tipos funcionais convencionais (Zakharova et al., 2019).Desta forma, as a espectroscopia pode contribuir para a construção da classificação da vegetação em tipos funcionais, trazendo novas abordagens como os tipos ópticos (Ustin and Gamon, 2010; Wang and Gamon, 2019).Es tipos óticos seria a caracterização das espécies com base nos seus comportamentos espectrais, que combinam não só informações bioquímicas e estruturais , como filogenéticas (Schweiger et al., 2018) Portanto, combinar abordagens estatísticas de dados de espectro para delinear grupos funcionais pode integrar as duas disciplinas e contribuir para o conceito de tipos funcionais ópticos (Roth et al., 2016; Ustin and Gamon, 2010; Van Cleemput et al., 2021).

No entanto, para de fato globalizar a caracterização funcional espectralmente, é necessário aumentar estudos em regiões tropicais e com espécies não-arbóreas (Van Cleemput et al., 2021), visto que padrões funcionais entre arbóreas e não-arbóreas pode ser diferente (Díaz et al., 2016; Roelofsen et al., 2014). Além da altura e caule serem perceptivelmente diferentes, certos trade-off, como altura e massa de sementes e LMA são diferentes entre herbáceas e árvores (Díaz et al., 2016). Além dessa variação funcional, a compreensão global dos espectros foliares na abordagem ecológica precisa de mais esforços para amostrais características funcionais tradicionais e dados espectrais de ambientes com espécies com diferentes histórias evolutivas para melhorar os modelos globais (Helsen et al., 2021 ; Streher et al., 2020; Van Cleemput et al., 2018).Desta forma a caracterização funcional dos ecossistemas de montanha neotropicais, representados principalmente por espécies não arbóreas, podem ajudar aprimorando as relações entre espectro-traços.

Esses ecossistemas apresentam uma relação solo-vegetação-atmosfera particular (Aparecido et al., 2018). Além disso, a vegetação depende de fatores como neblina e baixas temperaturas e estar sujeita a condições de vento e alta radiação (Aparecido et al., 2018; Christmann and Oliveras, 2020; Körner, 2003). Toda essa particularidade confere um alto grau de vulnerabilidade a esses ecossistemas, o que evidencia a necessidade de investigar as respostas das espécies por meio de características, para antecipar as consequências das mudanças climáticas (Christmann and Oliveras, 2020).Os processos ecossistêmicos que envolvem a vegetação de altitude ainda são poucos conhecidos e somado à vulnerabilidade às mudanças climáticas, há uma grande importância e urgência em um maior conhecimento das relações presentes entre a vegetação desses ambientes montanhosos. Além disso, esperamos que com o estudo das relações ecológicas e a integração com as características espectrais seja aperfeiçoado a obtenção de informações ecológicas. Assim, nosso objetivo é investigar a contribuição da espectroscopia foliar para a abordagem funcional de espécies de vegetação de altitude respondendo as seguintes perguntas:

1. É possível fazer previsões de características foliares a partir da refletância foliar com alta precisão para vegetação não arbórea em Alta altitude? A relação entre os espectros foliares e as características foliares é a mesma de outras vegetações não arbóreas?

2. Como as espécies são agrupadas de acordo com suas características? Esses agrupamentos são diferentes ao usar características previstas por espectros foliares?

3. Como as espécies estão distribuídas em espaços multidimensionais espectrais e funcionais? Quão bem as formas de vida são caracterizadas por variações nas características funcionais e informações sobre os espectros das folhas?

Chapter one: Relationships of leaf spectroscopy and high-altitude neotropical vegetation leaf traits.

ABSTRACT

The functional characterization of the vegetation utilizing spectroscopy is promising because of its ability to estimate functional traits accurately and the possibility of up-scaling. However, there are few statistical models, such as PLSR, for tropical and non-arboreal species. Thus, we evaluated PLSR models to estimate leaf traits from the leaf reflectance of species from neotropical high-altitude vegetation. We explored the ability of PLSR models to assess pigment content, LMA, LDMC, and LA using the leaf reflectance of 32 *campos de altitude* species distributed in different communities. The PLSR model estimates showed good accuracy, with better determination coefficients for traits such as LMA and LDMC. However, the PLSR model explained only 11% of the variation in the chlorophyll a and chlorophyll b ratio. The chlorophyll and carotenoid ratio explained 63% of the variation. The models explained more about the leaf structure characteristics LA (81%), LMA (87%), and LDMC (88%). Our results contribute to understanding the spectra-trait relationships for tropical and non-arboreal species and extend the sampling effort for species with different adaptations and evolutionary histories.

INTRODUCTION

The traits of plants in the community can represent responses to both biotic or abiotic factors and the effects of species on ecosystems (Funk et al., 2016; Lavorel and Garnier, 2002). Thus, they provide information on the different ecological processes of the ecosystem and improve the understanding of the assembly of plant communities (Funk et al., 2016). Furthermore, the relationship between leaf traits to spectral properties has long been known (Homolová et al., 2013, Ustin and Gamon, 2010). However, the remote sensing approach was primarily used to classify and map vegetation on different temporal and spatial scales (Ustin and Gamon, 2010; Wang and Gamon, 2019).

The spectral properties of plants are related to their leaf structures, such as cellulose, soluble nutrients, and pigments (Knipling, 1970). For example, the energy absorption by chlorophyll and other pigments results in a low reflectance in the visible spectral region (VIS, between 400-700 nm). As the electromagnetic energy reaches the leaf can be absorbed, transmitted, or reflected by other structures such as irregular surfaces, air-filled spaces interspersed, pigments, and water, resulting in different spectral signatures (Gates et al., 1965; Knipling, 1970). With the advancement of technology and ecology, remote sensing has allowed different approaches to investigate vegetation diversity: habitat mapping, species mapping, functional trait estimates, and spectral diversity (Ustin and Gamon, 2010; Wang and Gamon, 2019). In addition, estimating or relating spectral properties to functional traits allows mapping the distribution and variation of traits related to community assembly (Ustin and Gamon, 2010; Wang and Gamon, 2019).

Different approaches evaluate the relationship between spectral and vegetation characteristics (Helsen et al., 2021). Functional vegetation patterns are well estimated in tree and shrub species by different techniques, such as RTM or empirical, with regressions having better accuracies in the estimations (Helsen et al., 2021; Homolová et al., 2013; Van Cleemput et al., 2021). Within regressions, models using Partial Least-Squares Regression (PLSR) have been vastly employed (Serbin et al., 2019; Van Cleemput et al., 2018; Wang and Gamon, 2019). Trait estimations using PLSR are more accurate and transferable to different biomes Helsen et al. (2021). Also, it allows the definition of functional groups (Rebelo et al., 2018; Van Cleemput et al., 2021) and retrieves intraspecific leaf variations, which integrates biochemical and biophysical information in the estimated traits (Helsen et al., 2021; Van Cleemput et al., 2021).

However, the relationship between spectra properties and structural traits, such as LMA and LDMC, is still poorly explored for herbaceous and grass species and studies in tropical and arid areas (Streher et al., 2020; Van Cleemput et al., 2018). Furthermore, the global understanding of leaf spectra in the ecological approach needs more efforts to sample both traditional functional characteristics and spectral data from environments with species with different evolutionary histories and leaf spans, to improve global models (Helsen et al., 2021; Streher et al., 2020).

Thus, we investigated the relationships between foliar spectra and foliar characteristics of vegetation species from neotropical high-altitude ecosystems, addressing the following questions: Can leaf reflectances of these species predict their traits? Which spectral regions are related to the estimates of the functional traits? Our objective is to verify the potential of leaf reflectance in estimating the functional traits of highland species through the statistical method of partial least squares regression (PLSR).

MATERIALS AND METHODS

Study area

The study area was along the peak of Pedra do Pato (1900 m), in the State Park of Serra do Brigadeiro (PESB) in the Atlantic Rainforest domain. The Serra do Brigadeiro State Park (PESB) was created in 1996 by decree 38.319 and is in the Minas Gerais Forest area (between meridians 42º40' and 40º20' West and parallels 20º 33' and 21º 00' South). The park has the third-highest peak in Brazil. It encompasses a range of mountains that are part of the Serra da Mantiqueira, with the most elevated parts reaching 1,985 m above sea level (Safford, 1999). Therefore, the study area has a great diversity of species and habitats in a short altitude gradient (Tinti et al., 2015).

Data sampling

We selected three sites along the elevation (from 1600 to 1900m) based on different inclinations and soil depths. At the beginning of the rainy season (October 2020), We selected species of the higher vegetation cover/abundance in each site (32 in total) based on the frequency and cover collected from phytosociological data. From ten individuals of species, we collected the individual's height (in situ) and three to five healthy and mature leaves during the rainy season. We sampled some species twice since they occur in two different sites. First, the leaves were packed in moist paper bags, sealed in a plastic bag, and stored in a thermal box until transported to the laboratory. Then, the material was kept in a refrigerator at 4 °C for a minimum of 12 h to reach complete turgidity before measurement. After 12 hours, we weighed the leaves to collect the water-saturated fresh mass (g), the leaf reflectance, and the leaf pigments. After the leaf reflectance and pigment collection, the leaves were dehydrated and weighed again.

Leaf spectroscopy data collection

We collected the leaf reflectance using a portable non-imaging spectrometer: FieldSpec® 4 Hi-Res (Analytical Spectral Devices, Boulder, CO, United States). The equipment consists of a probe (pistol) and a clip of leaves, ensuring orthogonal spectra are obtained, under the same lighting conditions and with little influence from the atmosphere, considering the probe contact. It can record the reflectance of targets in the optical, electromagnetic spectrum range between 350 and 2500 nm, with spectral resolutions of 3 nm for shorter waves (350-700 nm) and 10 nm for long wavelengths, collecting a total of 2151 bands (Danner et al., 2015). The spectra collection was in the adaxial portion of the leaves, excluding the midrib.

Functional and pigments traits data

After each leaf reflectance collection, we measure the leaf traits: leaf area (LA), leaf thickness (LT), fresh leaf weight, and dry leaf weight. The leaf area was collected using scanned images of the leaves and calculated using ImageJ software. Following the (Pérez-Harguindeguy et al., 2013) protocols, leaf dry matter content (LDMC) was the oven-dry mass (mg) of a leaf and divided by its water-saturated fresh mass (g). The Leaf mass per area was its oven-dry mass (g) divided by the one-sided area of a fresh leaf (m^2). After 12 hours in a refrigerator at 4 °C, we collected the pigment content from leaf discs (7 mm in diameter) of two leaves per individual. The leaf discs were placed in a 5 mL DMSO solution saturated with CaCO3 (5 g L−1), where they remained for 48 h in the dark (Santos et al., 2008). The absorbance of the samples at

665, 645, and 480 nm was determined in a 10-mm quartz cuvette using a Genesys 10UV spectrophotometer (Thermo Scientific). The calculation of chlorophyll a, b, and carotenoids were according to (Wellburn, 1994) using various solvents with spectrophotometers of different resolutions.

Statistical analyses

We used Partial Least Squares Regression (PLSR) to estimate leaf characteristics from leaf reflectance data. PLSR is a commonly used statistical approach in spectroscopy as it reduces the number of predictors (e.g., spectral features) with high collinearity to a limited number of components. It is a multivariate regression, similar to the PCA, based on the construction of prediction models taking into account the structures of the X and Y matrices. They are modeled by the product of two smaller matrices, the scores and the loads. The loading matrices are calculated so that residuals X and Y are small, while at the same time, the correlation between scores X and Y is maximized (HAALAND; THOMAS, 1988, Wold et al., 2001). In our case, matrices (X) with leaf spectra and (Y) with leaf data.

Based on the protocol proposed by Burnett et al._(2021) using their tutorial R package “spectratrait”, we split the spectra and trait dataset into 80% to use in the production of regression (calibration dataset) and 20% for further model validation. The splitting used the create_data_split() using the species to guide the data splitting, so all species were in both datasets. The selection of the number of final components was through data permutation with a maximum of 20 models for each of the series of components. For permutation, we divide the calibration dataset into 70% for training the model and 30% for testing. The permutation happens until finding the lowest average predictive performance (PRESS) statistical value calculated by t-test. After defining an ideal number of components, we re-adapt the model using the entire calibration set and the validation dataset split at the beginning of the analysis to evaluate the generated model. As recommended, we use the coefficient of determination (R^2), the root mean square of the prediction error (RMSEP), and the percentage of this error concerning the amplitude of the observed traits (%RMSEP).

We also evaluated the influence of variables on the model projection (VIP), whose value indicates the importance of a specific wavelength to predict the trace of interest. VIP contributes to assessing which spectral regions are essential for prediction and possible covariance with other traits based on known absorption features (e.g., @serbin.etal_2014) or plotting coefficients and VIPs of various traits together to explore similarity (for example,@ely.etal_2019).

RESULTS

Our models showed low prediction errors (RMSEP and %RMSEP) and a considerable prediction potential (Table 1). The performance, however, varied with the coefficient of determination (R^2) from 0.11 to 0.89. In addition, the models showed low prediction errors from 5.15 to 0.17 for the estimation of pigments. However, the coefficient of determination varied between predictions ranging from 0.11 to 0.70.

Table 1. The partial least-squares regression (PLSR) estimations for each leaf trait. RMSEP is the root mean square error (RMSE) measured error using the test data, and the RMSEP percentage (%RMSEP) shows the error of each model as a percentage of the observed data range. Finally, R^2 shows the goodness of fit between the observations and the predicted values of each model.

Traits	Number of latent variables	RMSEP (%RMSEP)	R^2
log LMA (g/m^2)	14	0.18 (8.77%)	0.87
LDMC (mg/g)	13	43.98 (10.08%)	0.88
log LA (cm^2)	14	0.62 (12.43%)	0.81
Chl/Car (mg/cm^2)	15	0.17 (11.31%)	0.63
Chl a/Chl b	6	0.79 (11.31%)	0.11
Carotenoids	16	0.72(12.62%)	0.70
Total Chlorophyll	14	5.15 (16.72%)	0.51

The best model performance was for carotenoid estimation, in which the model could explain 70% of the leaf content variation, followed by chlorophyll estimates with an R^2 of 0.51. However, the prediction for chlorophyll a/chlorophyll b had lower, and only 11% of the variation was incorporated into the model. The models for the leaf area, mass per area, and dry matter (LDMC) traits presented a high percentage of explanations of variation (Table 1). All prediction models show a coefficient superior to

80% of the explanation. The proportion of prediction error (%RMSEP) was also relatively low for structural and biochemical traits.

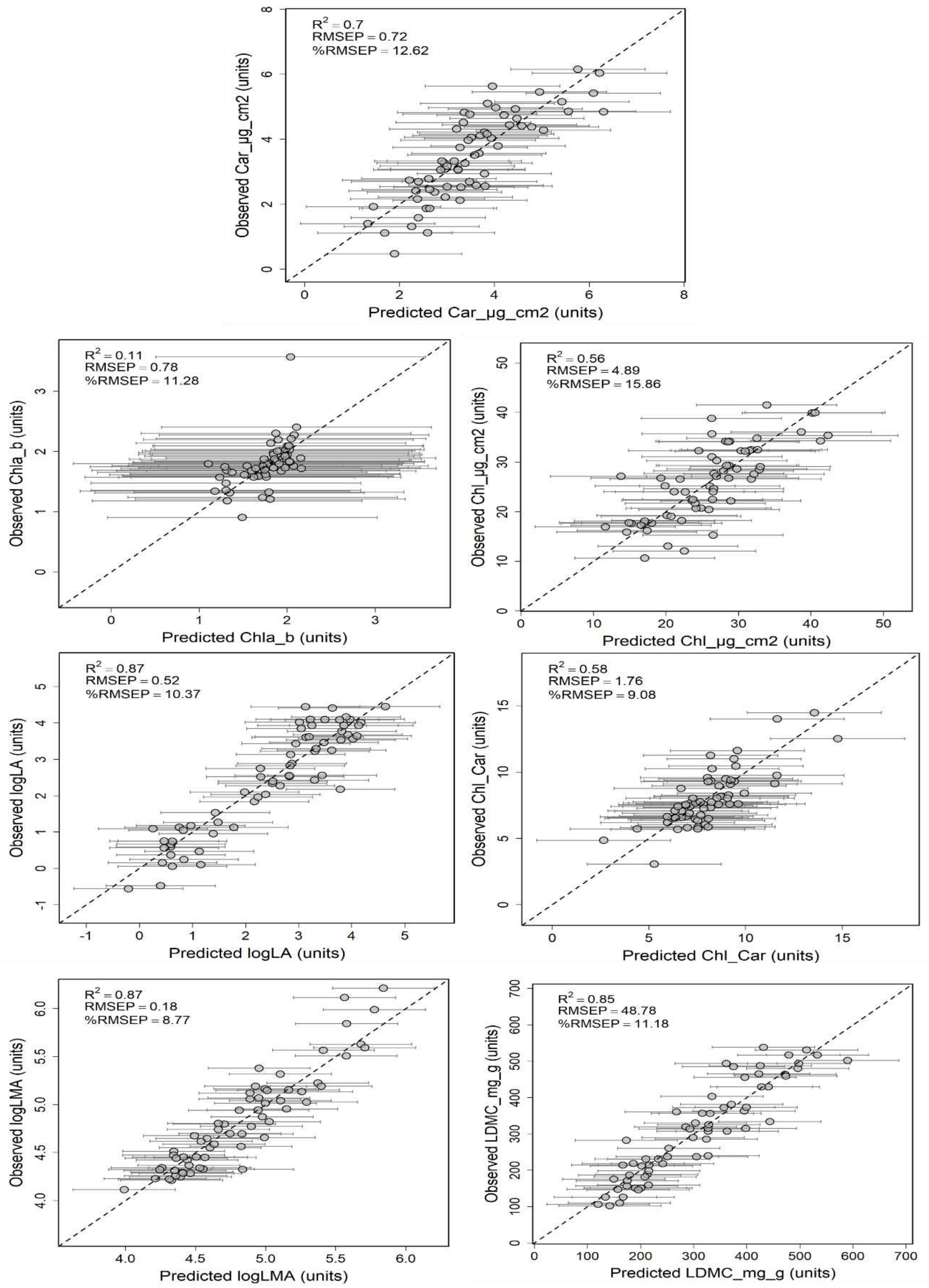

Figure 1.

Traits plots as Observed versus predicted values from leaf-level reflectance using partial least-squares regression (PLSR) models. Final validation plot resulting from model validation using the validation dataset. The relationship between observed (measured) and predicted trait values is shown.

The regions that contributed to the projections varied according to traits. The variable influence on the projection (VIP) presented high values for all models in the red-edge wavelengths (700-750 nm). For leaf photosynthesis pigments, the VIP values were higher for visible wavelengths (500-700 nm). The carotenoid models and the chlorophyll a/chlorophyll b ratio showed VIP values greater than 0.8 for some near-infrared wavelengths (1000-1500 nm), indicating the high importance of the wavelength in the prediction of these biochemical properties. As for the leaf structure models, in addition to the visible wavelength region, the near-infrared wavelength was also crucial, mainly between 1300 nm and 1400 nm, followed by the SWIR regions (700-1900 nm).

DISCUSSION

Our results showed a high predictive ability for the leaf traits of neotropical high-altitude non-tree species, contributing to expanding the sampling efforts of leaf traits and spectra for tropical regions (Streher et al., 2020; Van Cleemput et al., 2018). Although the prediction models showed a considerable performance variation, these variations agree with the literature (Van Cleemput et al., 2018).

Overall, leaf structural characteristics models showed higher determination coefficients than leaf biochemical properties (Table 1). The models for the biochemical data had low prediction error rates (RMSEP and %RMSEP). However, they had a variation in the coefficients of determination (R^2) values, indicating low to moderate capture of the variation (Van Cleemput et al., 2021). The lowest values of R^2 were for the proportion of Chlorophyll a/Chlorophyll b, in which the model explained only 11% of the variation, possibly due to the reduced range of values found (Van Cleemput et al., 2021). The carotenoids and chlorophyll content models adjusted about 70% and 51% of the variance, while chlorophyll/carotenoids were 63% of the variation predicted by the model. The estimates for leaf chlorophyll content to moderate performance were similar to that found in the literature for rangeland vegetation (Van Cleemput et al., 2018).

They were similar regarding the wavelengths that contributed to the construction of the pigments model. The highest values of VIP were in the visible region, related to the absorption of energy for photosynthesis by the pigments (Gates et al., 1965; Huang et al., 2015). Also, it is usually used in hyperspectral indices to monitor chlorophyll (Ustin et al., 2009).

Estimated relationships between structural traits are still poorly explored, especially for herbaceous and shrub species (Van Cleemput et al., 2018). Therefore, our work contributes to increasing efforts to understand the relationships between spectral data and leaf characteristics that vary according to vegetation types (Roelofsen et al., 2014; Van Cleemput et al., 2018). Our estimates of structural traits (leaf area, leaf dry matter content, Leaf mass per area) showed low values of prediction errors and high performance with R^2 coefficient values greater than 80%, indicating a high predictive power. Despite not contributing directly to the spectral signature, the performance of the Leaf area model was high compared to what has already been found in other herbaceous species (Van Cleemput et al., 2018). This high performance may be due to the relationship between the trait and the LMA trait, as the regions that contributed to the leaf area models were similar to the LMA prediction model (Figure 3). Furthermore, the leaf area is closely linked to the LMA calculation (Pérez-Harguindeguy et al., 2013; Van Cleemput et al., 2018).

The LMDC and LMA traits models were similar to those already made for *Campos rupestre* (Streher et al., 2020) and other herbaceous species (Van Cleemput et al., 2021). Therefore, the presented results are similar to the estimations for *Campos rupestre* species (Streher et al., 2020). However, the coefficients of determination (R^2) for the traits in the present study were relatively higher than those for *campos rupestres* (LMA-R^2=0.87; LDMC R^2 = 0.88, for the present study; LMA R^2=0.60, LDMC R^2=0.68) (Streher et al., 2020). For the leaf mass estimations difference may be a consequence of the difference in amplitude of the traits since we logarithmized the LMA values, while in the work of Streher et al. (2020), the values were not transformed.

Beyond the performance, the VIP values of the LMA and LMDC models showed similar patterns to those of the models for *campos rupestres*. For the LMDC models and the Streher et al. (2020) models, the wavelengths that contributed to the estimations were mainly the red-edge wavelengths (700-750 nm) of the red-edge region and the region around 1440 nm. While for the LMA model, the red limit region had the highest VIP value, followed by the red region. These similarities of our models with the models for *campos rupestre* species may indicate similar relationships between leaf spectra and leaf traits for these vegetations, which may facilitate the transferability of models (Helsen et al., 2021).

CONCLUSION

We aimed to investigate the relationships between the biochemical and morphological characteristics of the leaf reflectance of *Campos de altitude's* species. In this way, we reasonably estimated the species traits related to pigments, such as chlorophyll and carotenoids, and structural traits, such as LMA and LMDC.

Although the models presented good performance, the chlorophyll estimations presented low coefficients of determination due to the low variation found in the raw values. These low coefficients suggest limited future estimation using the coefficients in other species. Therefore, it deserves further study.

Besides the estimations, the regions that contributed to the models underlined the relationship between traits and reflectance. For example, the visible (400nm to 750nm) and infrared regions were significant for all estimations indicating that the estimated traits carry information from different regions.

Despite the limitations in chlorophyll estimations, our results highlight that trait estimations using leaf reflectance can be used for Campos de altitude species. Furthermore, it reinforces using spectral information as a proxy for functional traits. In this way, our results contribute to understanding the relationships between spectroscopy and traits, estimating traits through leaf reflectance at altitude species.

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Chapter two: The functional and spectral characterization of species response in Neotropical highland vegetation.

ABSTRACT

The classification of plant species into functional types is an approach that allows relating different floras, regions, and scales. With this in mind, leaf spectroscopy can contribute to classifying species' functional responses since it aggregates biochemical and biophysical information. The vegetation of mountainous ecosystems has a high degree of vulnerability, mainly because of its dependence on certain biotic and abiotic factors. Thus, we investigated how species in different communities functionally group based on traits measured and traits predicted by leaf reflectance. We collected pigment content, LMA, LDMC, and LA using the leaf reflectance of 32 high-altitude field species distributed in different communities in the Campos de altitude ecosystem. The species were distributed into ten groups based on the observed traits, with significant differences in leaf area and dry matter. For the estimated traits, eight groups were formed, with significant differences in all traits. In both clusters, the species were well dispersed, which showed an interspecific variation of species in these communities. Most species collected in different locations showed a low intraspecific variation as they were in the same clusters. The dendrograms presented a high correlation (entanglement = 0.052, cophenetic correlation = 0.77), indicating similar clusters. Our results reinforced the possibility of functional groupings with data estimated by leaf reflectance. Moreover, it evidenced the use of this approach in the study of species responses.

INTRODUCTION

The functional characterization of vegetative communities into functional types has been an alternative to simplify and facilitate the interpretation of species and community responses (Funk et al., 2016). Also, it enables comparisons with different floras and biomes (Pierce et al., 2017). Furthermore, it assumes that species would have the same functional response under the same environmental conditions, i.e., functional similarity de Bello et al. (2021). However, although widely used, conventional functional classifications used as CSR strategies or growth forms may not be sufficient to represent the functional variation found in communities (Matos et al., 2021; Roth et al., 2016; Van Cleemput et al., 2021). Often, functional categories are low in communities due to lower species variability than functional variability, e.g., grassland life forms (Funk et al., 2016; Zakharova et al., 2019). Therefore, continuous trait-based approaches can be more subtle to changes than conventional functional types (Zakharova et al., 2019).

Remote sensing tools contribute to the construction of vegetation classification into functional types, bringing novel approaches such as optical types (Ustin and Gamon, 2010; Wang and Gamon, 2019). Optical types are group definitions through spectral information (Ustin and Gamon, 2010; Van Cleemput et al., 2021). That is, the spectral information of the vegetation would not only reflect the variations in biochemical and biophysical properties and the ecological relationships that govern these characteristics (Ustin and Gamon, 2010). In this case, the optical types relate to spectral and ecological theories (Ustin and Gamon, 2010).

Also, leaf spectroscopy has the advantage of continuous data that information from functional, taxonomic, and phylogeny (Schweiger et al., 2018; Ustin and Gamon, 2010; Wang and Gamon, 2019). Therefore, combining statistical approaches from spectra data to delineate functional groups can integrate the two disciplines and contribute to the concept of optical functional types (Roth et al., 2016; Ustin and Gamon, 2010; Van Cleemput et al., 2021). Likewise, the definition of functional types using spectroscopy may offer advantages over identifying species response groups, as the classification would be more related to the biochemical and structural characteristics of the vegetation Schweiger et al. (2017). This approach helps to fill gaps in the functional characterization of neotropical mountain ecosystems, represented mainly by non-tree species. These ecosystems have a unique soil-vegetation-atmosphere relationship (Aparecido et al., 2018). Furthermore, vegetation depends on factors such as fog and low temperatures and is subject to windy conditions and high radiation (Aparecido et al., 2018; Christmann and Oliveras, 2020; Körner, 2003). All this particularity confers a high degree of vulnerability to these ecosystems, which highlights the need to investigate species' responses through characteristics, to anticipate the consequences of climate change (Christmann and Oliveras, 2020).

We investigated how species in different communities functionally cluster in the present study based on measured traits and traits predicted by leaf reflectance. Based on the characteristics of the species found in *Campos de altitude*, how are these species associated? How similar are the functional responses? Do the associations vary between raw traits and the traits estimated by PLSR?

MATERIALS AND METHODS

Study area

The study area was along the peak of Pedra do Pato (1900 m), in the State Park of Serra do Brigadeiro (PESB) in the Atlantic Rainforest domain. The Serra do Brigadeiro State Park (PESB) was created in 1996 by decree 38.319 and is in the Minas Gerais Forest area (between meridians 42º40' and 40º20' West and parallels 20º 33' and 21º 00' South). The park has the third-highest peak in Brazil. It encompasses a range of mountains that are part of the Serra da Mantiqueira, with the most elevated parts reaching 1,985 m above sea level (Safford, 1999). Therefore, the study area has a great diversity of species and habitats in a short altitude gradient (Tinti et al., 2015).

Data sampling

We selected three sites along the elevation (from 1600 to 1900m) based on different inclinations and soil depths. At the beginning of the rainy season (October 2020), We selected species of the higher vegetation cover/abundance in each site (32 in total) based on the frequency and cover collected from phytosociological data. From ten individuals of species, we collected the individual's height (in situ) and three to five healthy and mature leaves during the rainy season. We sampled some species twice since they occur in two different sites. First, the leaves were packed in moist paper bags, sealed in a plastic bag, and stored in a thermal box until transported to the laboratory. Then, the material was kept in a refrigerator at 4 °C for a minimum of 12 h to reach complete turgidity before measurement. After 12 hours, we weighed the leaves to collect the water-saturated fresh mass (g), the leaf reflectance, and the leaf pigments. After the leaf reflectance and pigment collection, the leaves were dehydrated and weighed again.

Leaf spectroscopy data collection

We collected the leaf reflectance using a portable non-imaging spectrometer: FieldSpec® 4 Hi-Res (Analytical Spectral Devices, Boulder, CO, United States). The equipment consists of a probe (pistol) and a clip of leaves, ensuring orthogonal spectra are obtained, under the same lighting conditions and with little influence from the atmosphere, considering the probe contact. It can record the reflectance of targets in the optical, electromagnetic spectrum range between 350 and 2500 nm, with spectral resolutions of 3 nm for shorter waves (350-700 nm) and 10 nm for long wavelengths, collecting a total of 2151 bands (Danner et al., 2015). The spectra collection was in the adaxial portion of the leaves, excluding the midrib.

Functional and pigments traits data

After each leaf reflectance collection, we measure the leaf traits: leaf area (LA), leaf thickness (LT), fresh leaf weight, and dry leaf weight. The leaf area was collected using scanned images of the leaves and calculated using ImageJ software. Following the (Pérez-Harguindeguy et al., 2013) protocols, leaf dry matter content (LDMC) was the oven-dry mass (mg) of a leaf and divided by its water-saturated fresh mass (g). The Leaf mass per area was its oven-dry mass (g) divided by the one-sided area of a fresh leaf (m^2). After 12 hours in a refrigerator at 4 °C, we collected the pigment content from leaf discs (7 mm in diameter) of two leaves per individual. The leaf discs were placed in a 5 mL DMSO solution saturated with CaCO3 (5 g L−1), where they remained for 48 h in the dark (Santos et al., 2008). The absorbance of the samples at 665, 645, and 480 nm was determined in a 10-mm quartz cuvette using a Genesys 10UV spectrophotometer (Thermo Scientific). Furthermore, the calculation of chlorophyll a, b, and carotenoids were according to (Wellburn, 1994) using various solvents with spectrophotometers of different resolutions.

Statistical analysis

Traits estimations

Estimating traits by leaf reflectance was using Partial Least-Squares Regression (PLSR). The PLSR is a commonly used approach in spectroscopy since it reduces the number of predictors with high collinearity (hyperspectral wavelengths) to a limited number. The new components (latent variables) also exhibit high covariance with the response variable (Wold et al., 2001). Our PLSR model was developed following the procedure proposed by Burnett et al. (2021). As recommended, we used the coefficient of determination (R^2), root mean square error of prediction (RMSEP), and % RMSEP (RMSEP as a percentage of the trace) to evaluate each trace prediction model. For ease of understanding, the estimated traits will be referred to as PLSR traits.

Clustering analyses

To investigate the grouping of species, we use species values in consideration of the site/community of occurrence (site-specie specific). First, the species-site values were calculated for traits measured and PLSR traits. Next, we computed dissimilarity matrices for both traits' datasets with Euclidean distance between observations. Next, we perform the clustering analysis; in this case, we used Hierarchical cluster analysis with Ward's method (Ward, 1963) with the R "stats" package. Before the dissimilarity matrix calculation and clustering, leaf area and Leaf mass per area (LMA) were log-transformed, and all functional and PLSR traits were centered and scaled. We evaluated the clustering quality by the correlation of our cluster tree and the cophenetic distance (Farris, 1969). Then, we define the number of clusters using a hybrid adaptive branch pruning implemented in the "dynamicTreeCut" R Package (Langfelder et al., 2016). We set the minimum number of observations per group to two. Finally, we estimate the differences between the clusters for each trait for the two clusters formed with the Kruskal-Wallis and Dunn's tests.

Comparison between clusters

We checked the alignment quality of the cluster's dendrogram using the R package "dendextend" (Galili et al., 2022). We applied different untangle methods to find optimal alignment, indicated by the entanglement metric. This metric ranges from zero to one, and the smaller the value, the better the alignment quality. The best alignment layout was visualized in a tanglegram (Scornavacca et al., 2011). We also statistically compare through a global comparison of dendrograms and cophenetic correlation.

RESULTS

Groups

The clustering of the species-site measured traits resulted in 10 groups with a moderate structure in the trait data (cophenetic correlation of 0.54). The groups did not present significant differences in pigment content. However, structural characteristics such as leaf area and dry matter content did (Figure 1). For example, the leaf area of cluster 3 (*Cryptangium comatum*, *Gaylussacia densa*, *Hesperozygis nitida*) was lower than cluster 8 (*Pleroma manicatum*) and cluster number 4 *(Hippeastrum glaucescens*, *Zygopetalum maculatum*). In addition, cluster number 4 has lower leaf dry matter content than cluster number 1(*Croton splendidus*, *Gaultheria serrata*, *Vellozia candida*, *Vellozia variegata*) and 3.

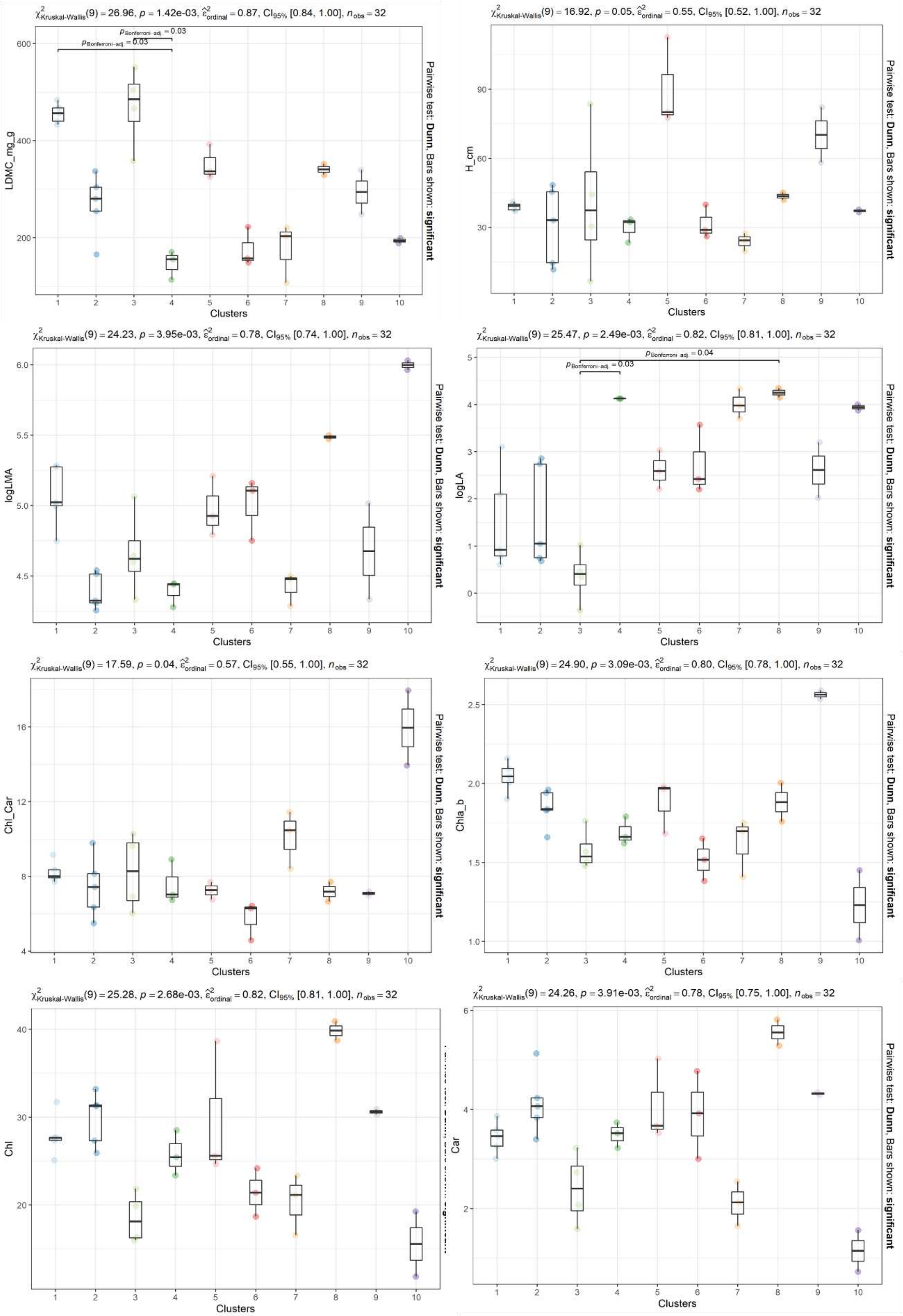

Figure1. Boxplots show the distributions of functional traits for each cluster in the species-site measured traits dendrogram. The boxes show the 25th through 75th quantiles.

The clustering of estimated traits resulted in 8 groups with a moderate structure in the trait data (cophenetic correlation of 0.59). Unlike the previous cluster, the groups from the PLSR trait showed more differences in their trait values (Figure 2). The predicted values for structural leaf trait were also the ones that presented more differences between groups.

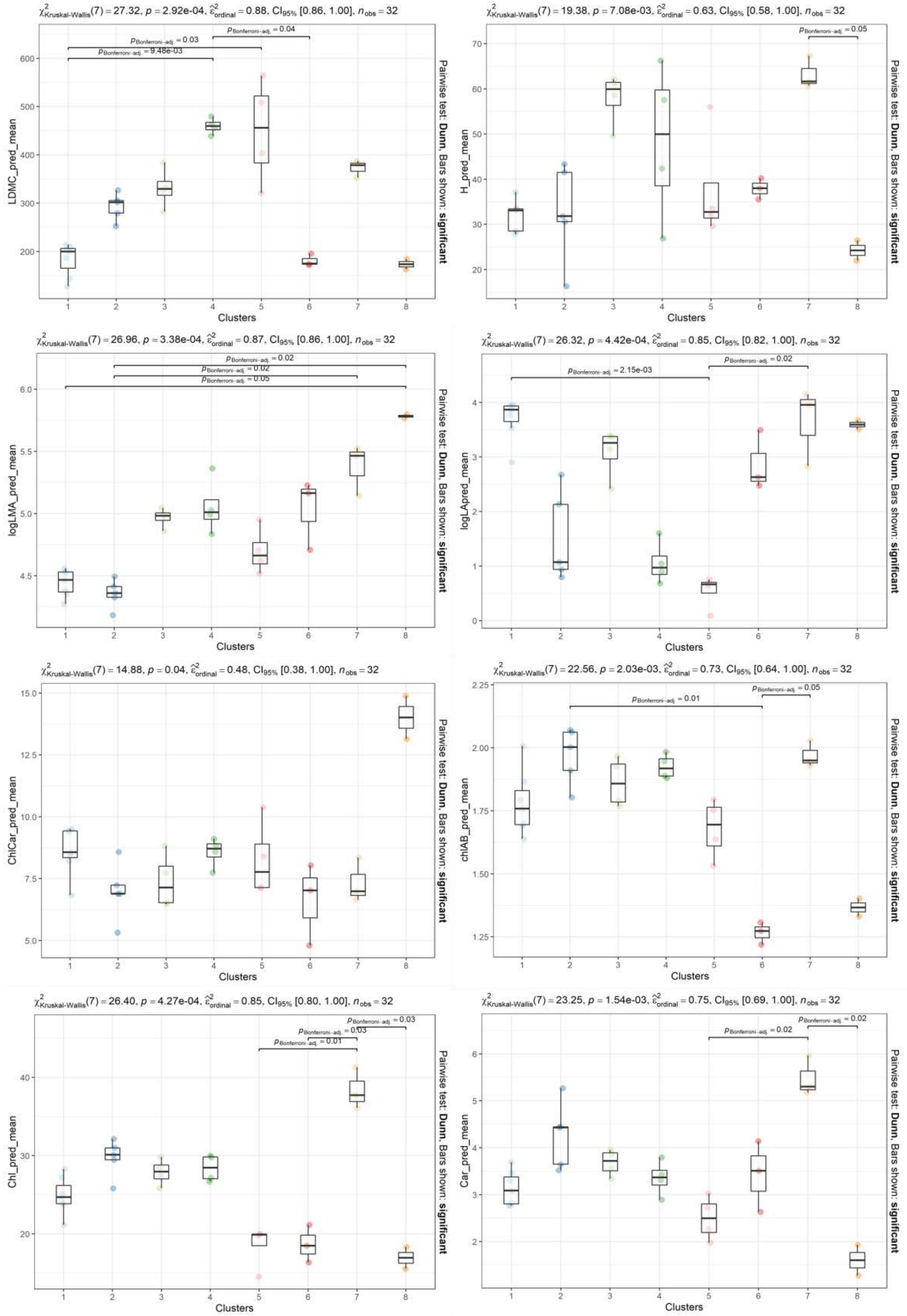

Figure2. Boxplots show the distributions of functional traits for each cluster species-site PLSR traits dendrogram. The boxes show the 25th through 75th quantiles. The Pairwise test was Dunn's, and the shown bars are the significant differences.

Comparison between groups with different trait data

The dendrograms formed from the clusters showed good alignment with an entanglement of 0.052 and a cophenetic correlation of 0.77. Both metrics indicate similar representations between the groups using measured and predicted traits (PLSR). Some sub-trees were present in both dendrograms (Figure 3), like the *Dyckia cinerea* cluster and *Pleroma manicatum*.

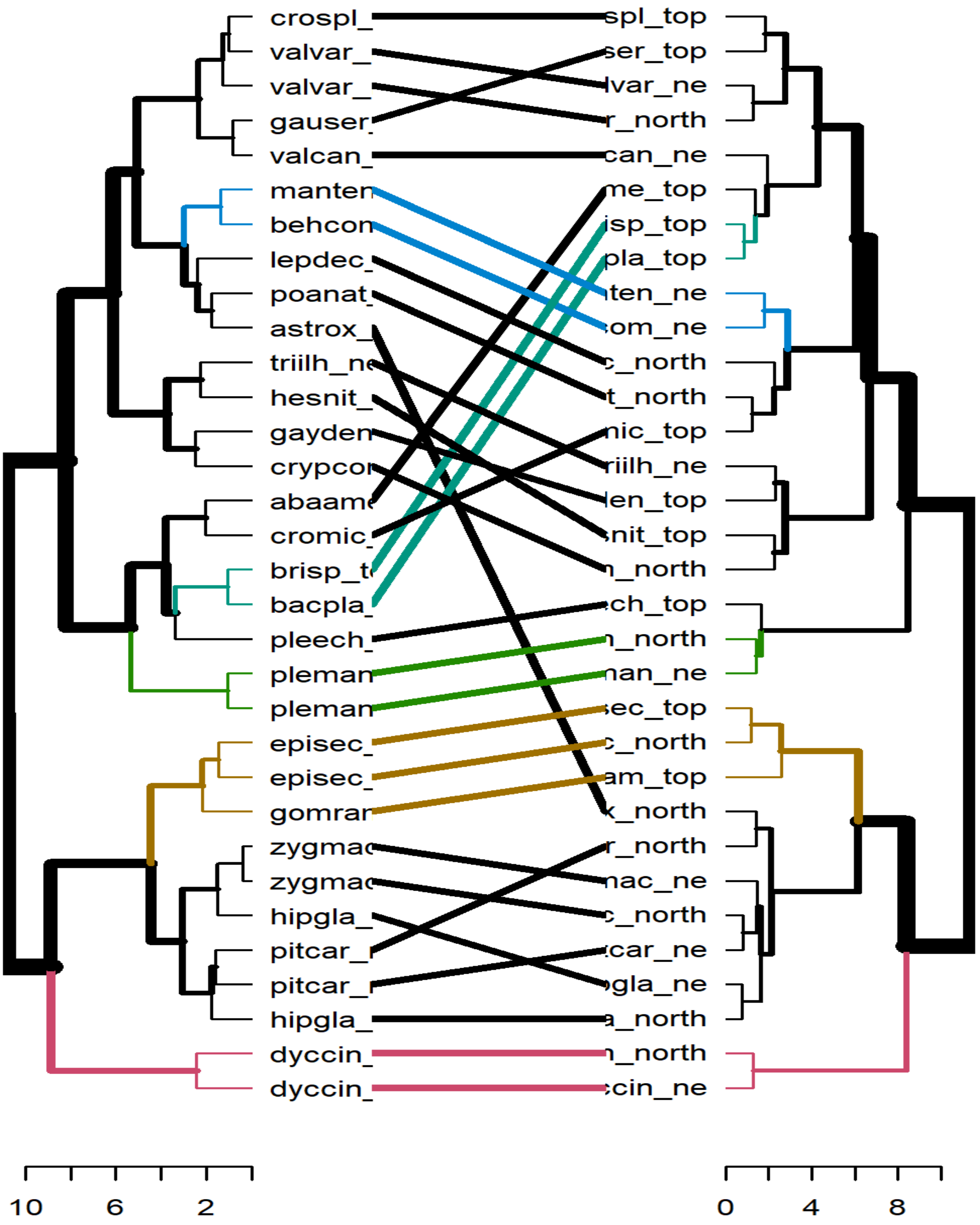

Figure 3. Tanglegram between the dendrograms resulting from the hierarchical clustering based on measured functional (left) and optical traits (right); the connecting lines are colored to highlight two sub-trees in both dendrograms. The color of the branches shows the two common sub-trees

DISCUSSION

Our results showed the ability to use estimated leaf spectra data to analyze ecological relationships between species (Rebelo et al., 2018; Van Cleemput et al., 2021). The dendrogram formed by the estimated traits showed a significant correlation with the dendrogram of the observed characteristics. Furthermore, the groups formed by the observed and estimated traits showed variations, mainly in characters related to leaf structures, such as LA and LCMD.

The functional variations within the clusters were different for the observed and estimated traits. Among the groups of traits observed, there was variation in leaf area and dry matter content. As for the assessed traits, all traits showed some variation between groups. Even so, the groups show a variation reflecting variation in SLE and size (Díaz et al., 2016; Wright et al., 2004).

Interspecific variations caused the functional variations found in both clusters. Species collected at separate locations were within the same groups, indicating low functional variability (Roth et al., 2016; Van Cleemput et al., 2021). For example, *Dyckia cinerea* was collected at sites north and northwest of the peak, where the depth and slope of the sites change. Despite the variation in environmental conditions, they were in the same group for both groups with observed and estimated data.

In addition to similarities within species, the groups also showed similarities in taxonomically related species. For example, *Pleroma* and *Vellozia* were in the same or close groups. This more significant variation between species may indicate different assembly processes of these communities (Scherrer et al., 2019). For example, species of the same genus or the same species collected at separate locations are influenced by environmental filtering resulting in trait convergence, as reported by Scherrer et al. (2019). However, when we reduced the scale, a few species from the exact location were grouped, showing a limitation in similarity (Scherrer et al., 2019).

Environmental factors influence vegetation, such as high radiation and shallow soil linked to drought (Safford, 1999b). Moreover, plants have different strategies to deal with these constraints, as Matos et al. (2021) reported. The cluster analysis also showed that some species have entirely different sets of characteristics from the others, such as *Dyckia cinerea* and *Pleroma manicatum*. Thus, these species formed groups with only them included. This separation into single groups may indicate the functional originality of these species, as seen in other communities of *campos de* *altitude* species, indicating an essential contribution to functional diversity (Matos et al., 2021).

CONCLUSION

We aimed to understand how *Campos de altitud*e species are associated based on their functional responses. Furthermore, we also investigate the potential of leaf spectroscopy in these functional comparison studies. As a result, we observed similar functional responses between species using raw traits and traits estimated from leaf reflectance.

In general, the associations of species based on traits are not necessarily related to where they were established since species from different places were part of the same group. In addition, we observed various responses when evaluating the number of groups formed and the variations in traits between them. Moreover, even species collected in different sites were grouped into the same group.

We found that the traits estimated through leaf reflectance also provide information about the functional responses. The cluster analysis showed the raw data, and the data estimated by the PLSR presented groups composed of the same species. With either raw traits or estimated traits, the groups show that some species, such as Dickyia, present a very different set of traits from others, forming a unique group.

Thus, our results indicated the ability to infer the functional responses of highland grassland species using traits estimated from the leaf reflectance. Furthermore, it improves the studies at different scales of remote sensing. This way, the characteristics estimated using spectral patterns provide useful functional information for studying high-altitude vegetation communities.

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Chapter three: Response of Campos de altitude species in functional and spectral space

ABSTRACT

There are still gaps in understanding the relationships between traits and spectra for herbaceous and subshrub species and how they can contribute to the functional characterization of neotropical species. Therefore, we compared the distribution of specie traits and leaf reflectance in multivariate spaces and the contribution of their Raunkiær life forms in this distribution. We collected chlorophyll content, carotenoids from LMA, LDMC, LA, and leaf reflectance from 32 species from *Campos de Altitude* distributed in different communities. First, we did a principal component analysis to create the multidimensional space. Then, we performed a PERMANOVA followed by BETADISPER to assess the relationship of variation in the multivariate area to Raunkiær life forms. The three main components represented 78% and 94% of the variation of leaf reflectance and traits, respectively. The first axis was strongly associated with pigments for the trait space. In contrast, the other axes were associated with leaf structures and height. In the leaf spectra, the wavelengths of the red edge and the near-infrared region were associated with the first axis, and the other axis is related to the wavelengths of the visible region. The comparison between the ordinates revealed a significant agreement. The relationship of the multivariate space of the two ordinates with the Raunkiær plant forms was significant only for the calculated variation of trait and life forms ($R^2 = 0.32$). However, the BETADISPER analysis showed that there are no differences in dispersion between the groups, suggesting that the difference found in PERMANOVA is due, in part, to the change in the characteristic composition of the species between the different life forms. Our results showed that most species' functional variations are linked to resource allocation, also indicated by spectral variations. Furthermore, variations in characteristics and spectra better represent interspecific variation than life forms.

INTRODUCTION

The leaf characteristics of the species that make up a community are related to the established conditions (Dong et al., 2020; MCGILL et al., 2006). In other words, plant biochemical and biophysical properties represent their functional characteristics that result from different biotic and biotic interactions (Dong et al., 2020; Lavorel and Garnier, 2002; MCGILL et al., 2006). Therefore, understanding species characteristics allows for investigating the mechanisms and effects behind community assembly and species interactions.

The plant's biochemical and biophysical properties are also related to leaf spectral patterns of vegetation, which allows functional characterization at different temporal and spatial scales (Homolová et al., 2013; Wang and Gamon, 2019). Leaf spectroscopy can improve the functional description of species as it integrates functional, taxonomic, and phylogeny information (Schweiger et al., 2018; Ustin and Gamon, 2010; Wang and Gamon, 2019). The spectral characteristics can discriminate indicated species of ecosystems (Amaral et al., 2018; Asner and Martin, 2011; Rebelo et al., 2018), functional variations of different life forms Van Cleemput et al. (2021), in addition to mapping phylogenetic signatures (Diniz et al., 2021).

However, to truly globalize the spectral characterization, it is necessary to increase studies in tropical regions and non-tree species since functional patterns between arboreal and non-arboreal are different (Díaz et al., 2016). For example, in addition to height and stem being perceptibly different, some trade-offs, such as seed height and mass and LMA, are different between herbaceous and arboreal (Díaz et al., 2016). Therefore, the global understanding of leaf spectra in the ecological approach needs more efforts to sample traditional functional characteristics and spectral data from environments with species with different evolutionary histories and leaf spans, to improve global models (Helsen et al., 2021; Streher et al., 2020; Van Cleemput et al., 2018).

Thus, studies on the functional characterization of neotropical mountain ecosystems, represented mainly by non-tree species, can contribute to filling these gaps. The vegetation in these environments depends on factors such as fog and low temperatures to survive. In addition, they are subject to intense radiation and strong winds (Aparecido et al., 2018; Christmann and Oliveras, 2020; Körner, 2003). This strong relationship to their environment gives this vegetation a high degree of vulnerability (Aparecido et al., 2018; Christmann and Oliveras, 2020). However, the species' responses in these ecosystems are scarce, highlighting the need to investigate responses to anticipate the consequences of climate change (Christmann and Oliveras, 2020).

Therefore, we investigated the variation of Campos de altitude species' functional traits and leaf reflectance patterns to answer the questions: 1) How are species distributed in spectral and functional multidimensional spaces?

Based on functional traits and leaf reflectance, are there any patterns in species distribution in these multidimensional spaces? Could these patterns be related to life forms or where the species were found? Are the distribution patterns in the trait space similar to those in the spectral space?

MATERIALS AND METHODS

Study area

The study area was along the peak of Pedra do Pato (1900 m), in the State Park of Serra do Brigadeiro (PESB) in the Atlantic Rainforest domain. The Serra do Brigadeiro State Park (PESB) was created in 1996 by decree 38.319 and is in the Minas Gerais Forest area (between meridians 42º40' and 40º20' West and parallels 20º 33' and 21º 00' South). The park has the third-highest peak in Brazil. It encompasses a set of mountains that are part of the Serra da Mantiqueira, with the most elevated parts reaching 1,985 m above sea level (Safford, 1999). Therefore, the study area has a great diversity of species and habitats in a short altitude gradient (Tinti et al., 2015).

Data sampling

We selected three sites along the elevation (from 1600 to 1900m) based on different inclinations and soil depths. At the beginning of the rainy season (October 2020), We selected species of the higher vegetation cover/abundance in each site (32 in total) based on the frequency and cover collected from phytosociological data. From 10 individuals of species, we collected the individual's height (in situ) and three to five healthy and mature leaves during the rainy season. We sampled some species twice since they occur in two different sites. First, the leaves were packed in moist paper bags, sealed in a plastic bag, and stored in a thermal box until transported to the laboratory. Then, the material was kept in a refrigerator at 4 °C for a minimum of 12 h to reach complete turgidity before measurement. After 12 hours, we weighed the leaves to collect the water-saturated fresh mass (g), the leaf reflectance, and the leaf pigments. After the leaf reflectance and pigment collection, the leaves were dehydrated and weighed again.

Leaf spectroscopy data collection

We collected the leaf reflectance using a portable non-imaging spectrometer: FieldSpec® 4 Hi-Res (Analytical Spectral Devices, Boulder, CO, United States). The equipment consists of a probe (pistol) and a clip of leaves, ensuring orthogonal spectra are obtained, under the same lighting conditions and with little influence from the atmosphere, considering the probe contact. It can record the reflectance of targets in the optical, electromagnetic spectrum range between 350 and 2500 nm, with spectral resolutions of 3 nm for shorter waves (350-700 nm) and 10 nm for long wavelengths, collecting a total of 2151 bands (Danner et al., 2015). The spectra collection was in the adaxial portion of the leaves, excluding the midrib.

Functional and pigments traits data

After each leaf reflectance collection, we measure the leaf traits: leaf area (LA), leaf thickness (LT), fresh leaf weight, and dry leaf weight. The leaf area was collected using scanned images of the leaves and calculated using ImageJ software. Following the (Pérez-Harguindeguy et al., 2013) protocols, leaf dry matter content (LDMC) was the oven-dry mass (mg) of a leaf and divided by its water-saturated fresh mass (g). The Leaf mass per area was its oven-dry mass (g) divided by the one-sided area of a fresh leaf (m^2). After 12 hours in a refrigerator at 4 °C, we collected the pigment content from leaf discs (7 mm in diameter) of two leaves per individual. The leaf discs were placed in a 5 mL DMSO solution saturated with CaCO3 (5 g L−1), where they remained for 48 h in the dark (Santos et al., 2008). The absorbance of the samples at 665, 645, and 480 nm was determined in a 10-mm quartz cuvette using a Genesys 10UV spectrophotometer (Thermo Scientific). Furthermore, the calculation of chlorophyll a, b, and carotenoids were according to (Wellburn, 1994) using various solvents with spectrophotometers of different resolutions.

Statistical Analysis

We performed a Principal component Analysis to obtain the trait space variation in the trait measured and leaf reflectance. The similarity between the functional and optical trait space was assessed through Procrustes and protest. This analysis evaluates the significance of the agreement between the matrices. We performed a PERMANOVA followed by BETADISPER (Anderson and Walsh, 2013) to assess the relationship of the variation in the multivariate space with other factors such as Raunkiær life forms and communities. We utilized the function “adonis” and “betadisper” from the “vegan” package (Oksanen et al., 2022)

RESULTS

Principal component analyzes showed that 78% of the species trait variations could be reduced to three axes. The first axis is strongly associated with pigments, while the other axes are associated with leaf structures and height (Figure 1).

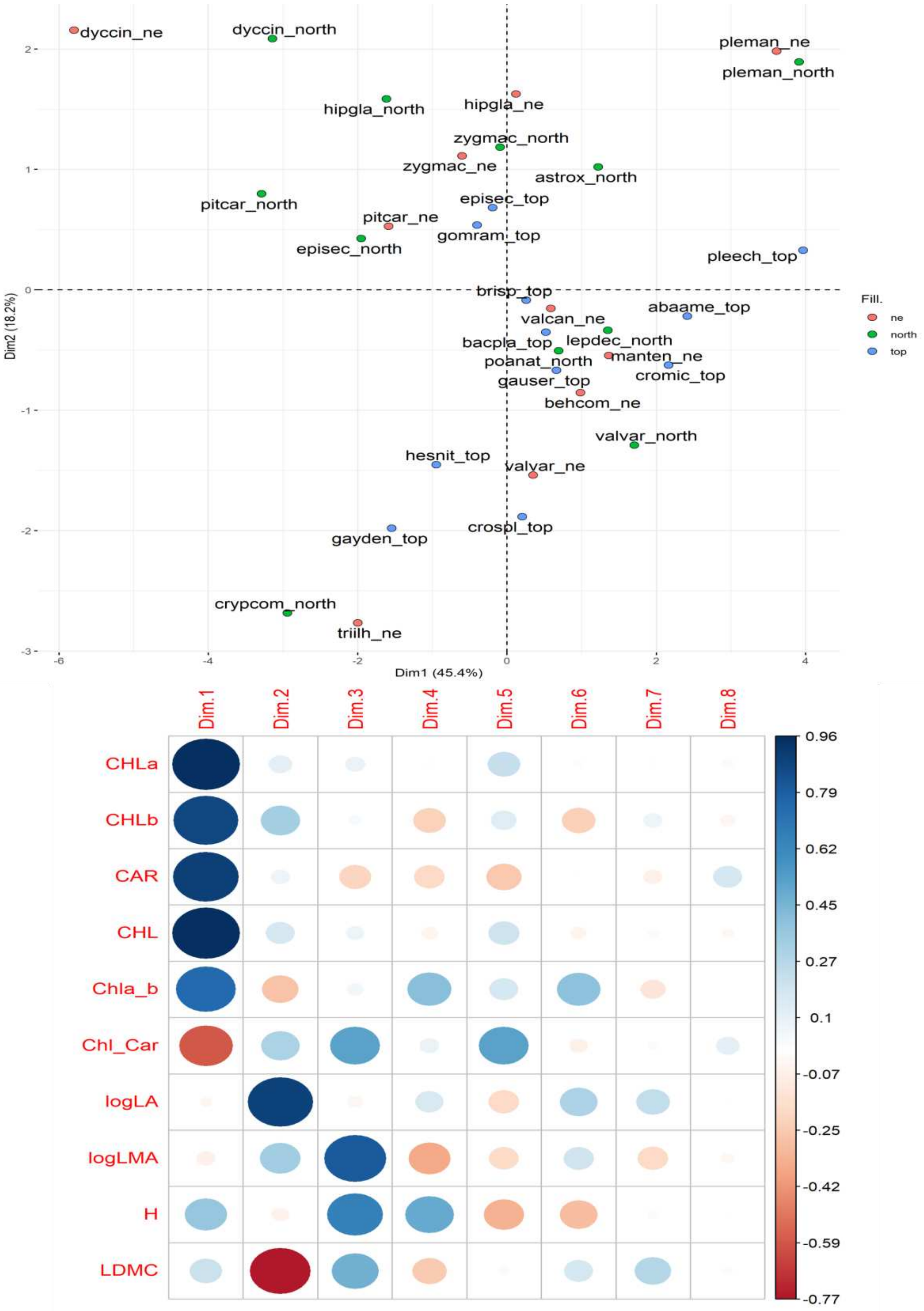


Figure 1. Principal component description: the correlation between principal components and the traits.

On the other hand, leaf reflectance ordination showed that the first three axes account for 94% of the leaf reflectance variation (Figure 2). The first axis is associated with the near-infrared and shortwave wavelengths, and the other is related to the visible region’s wavelengths. The comparison between the multidimensional spaces revealed a significant agreement with the smallest residual value of the sum of squares of 0.644.

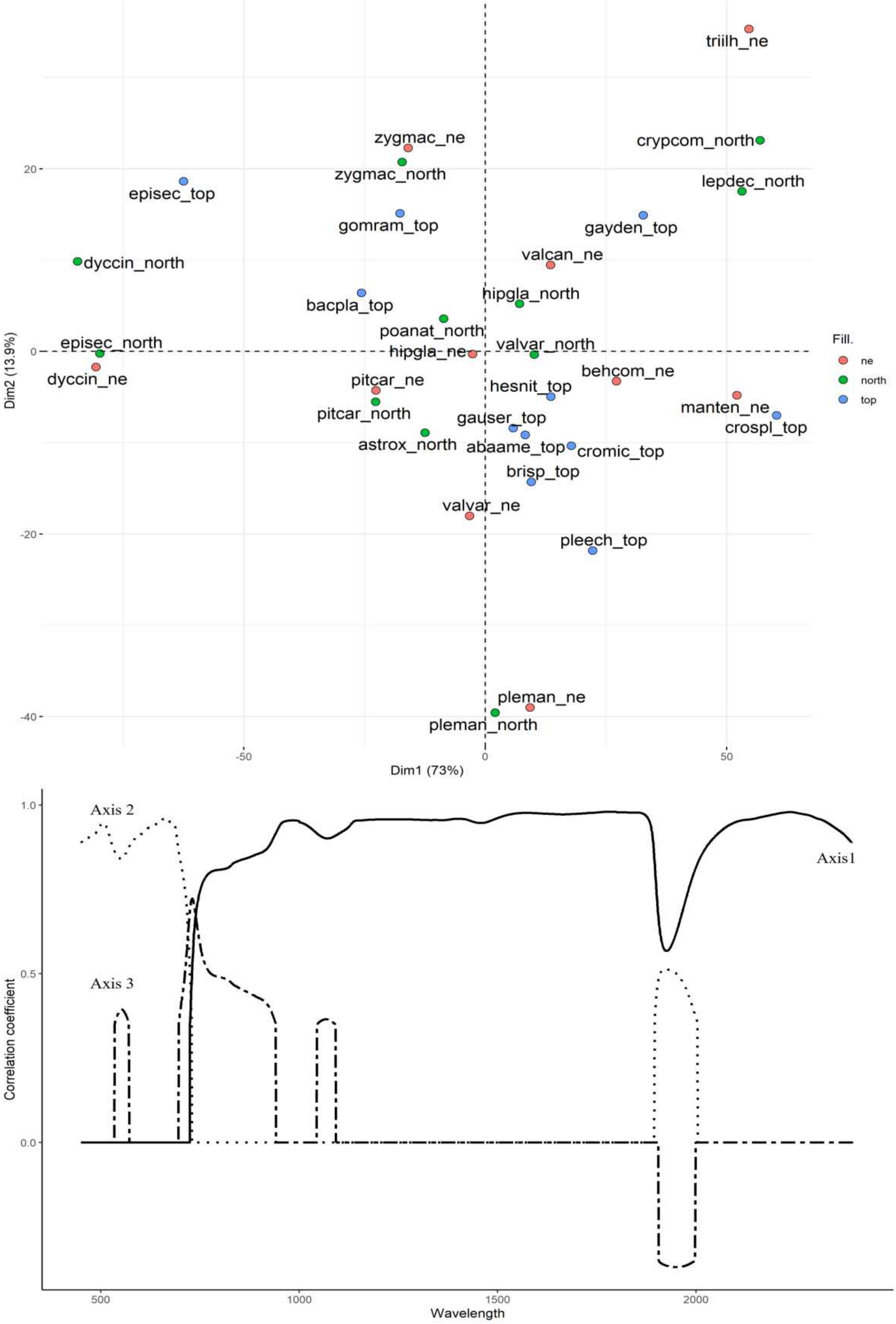
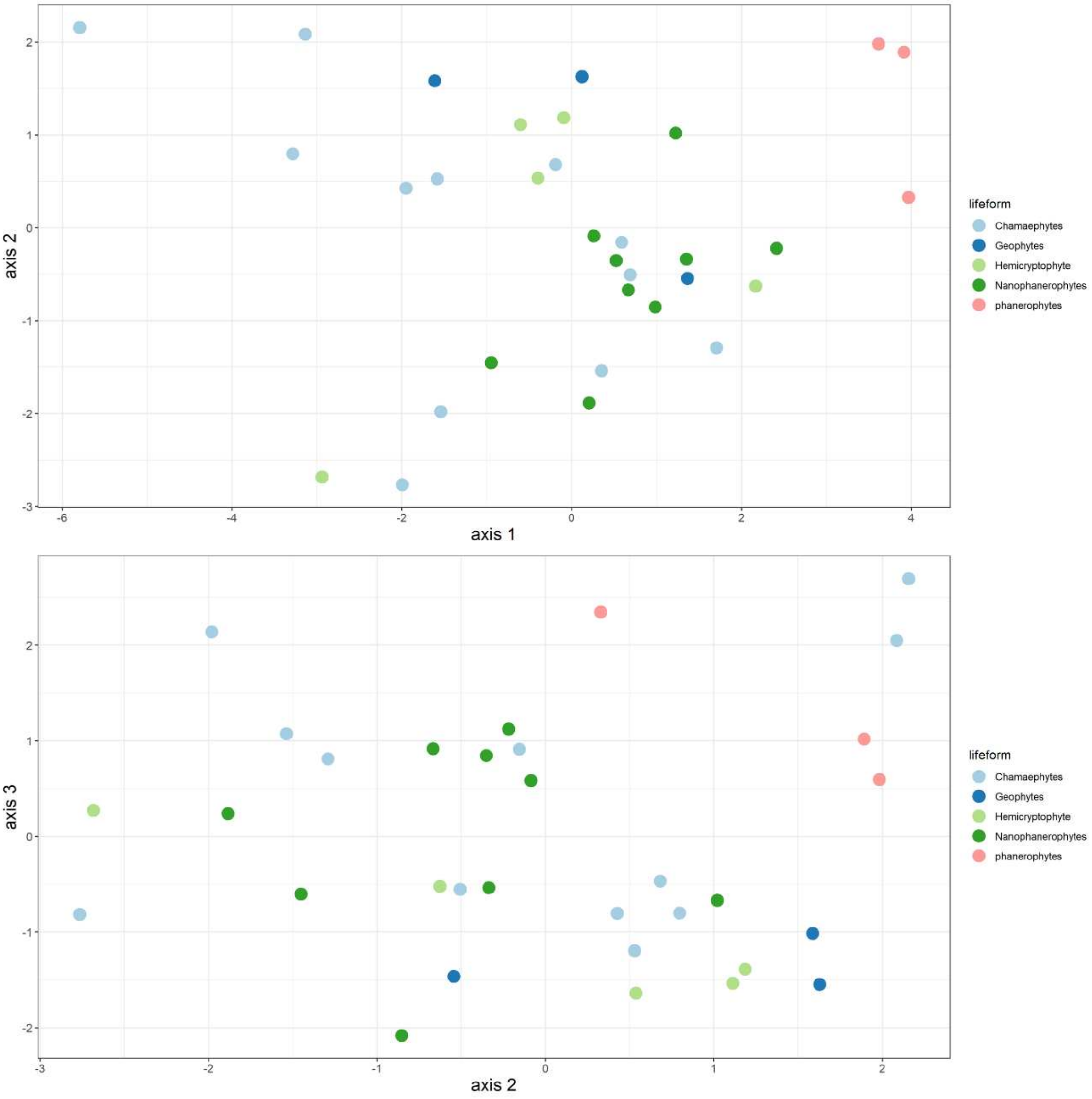


Figure 2. The correlation between principal components and the wavelength.

Concerning the multidimensional space and life forms, the forms of life overlapped in functional and spectral traits (Figure 3). That is, they did not disperse, forming groups related to category. This distribution was also reinforced by the PERMANOVA (table1 significant only for the calculated variation of traits and life forms (R^2 = 0.32). However, the BETA analysis indicated that the variance between the groups is homogeneous; thus, the difference found in PERMANOVA is due, in part, to the change in species composition within the categories of life forms.



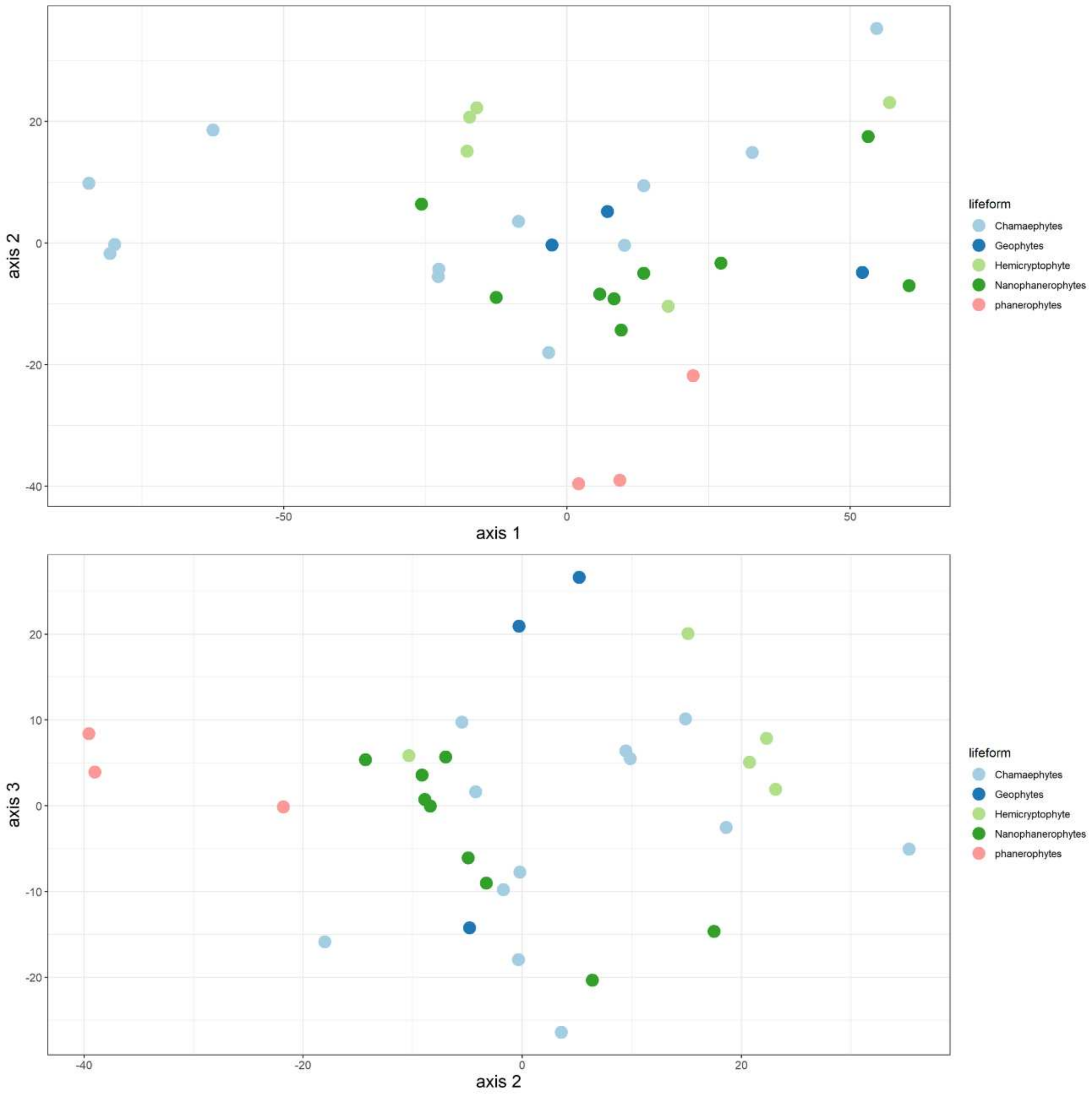


Figure 3. The distribution of life forms in functional and spectral multidimensional space.

Table 1. R^2 and p values of in-group PERMANOVA, indicating the relationship between functional trait variation and leaf reflectance.

Groups	Functional trait		Leaf Reflectance	
	R^2	p-value	R^2	p-value
Plant form (n=5)	0.31472	0.002 **	0.25525	0.055
site (n=3)	0.04915	0.478	0.01849	0.847

DISCUSSION

The multivariate spaces for the observed characteristics and leaf reflectance show significant agreement, reinforcing the relationship between spectral properties and characteristics of this vegetation (Roth et al., 2016; Streher et al., 2020; Van Cleemput et al., 2021). In this way, the species distribution in the two spaces can similarly represent the species' responses (Roth et al., 2016; Van Cleemput et al., 2021). Also, these multivariate spaces showed a relative concentration of species and that life forms do not represent this distribution.

All species traits are associated with at least one of the three axes of the trait multidimensional space. The ones that most contributed to the variation found, represented by the first axis, were the variation of leaf pigments, followed by leaf area and dry matter content. These patterns indicate trait coordination to resource allocation, reflecting the leaf economic spectrum (Reich, 2014; Wright et al., 2004). They also might reflect the trait-trait correlations that allow the persistence of non-tree vegetation under harsh conditions (Caminha-Paiva et al., 2021). The first axis might be related to the resource acquisition-conservative represented by the photosynthetic pigments, followed by mechanical support (LDMC, LA), which Caminha-Paiva et al. (2021) noted in *Campos rupestre* vegetation.

The distribution of species in the trait space shows a variety of strategies, despite a minor variation in the pigment axis. Regarding pigments, most species have a more restricted distribution with reduced metabolic activity (lower chlorophyll values per leaf unit) (Caminha-Paiva et al., 2021; Wright et al., 2004), but more considerable investment in protection (chlorophyll/carotenoids ratio) (Sims and Gamon, 2002). Nevertheless, the mechanical support, the species display more investment in leaf structure (LDMC) instead of light interception (Leaf area) (Caminha-Paiva et al., 2021; Matos et al., 2021). These patterns reflect the different ecophysiological responses to high radiation and drought found in high-altitude vegetation (Liu et al., 2020). This vegetation is under harsh conditions regarding high radiation and wind, influencing water absorption (Aparecido et al., 2018). Therefore, there is more investment in leaf internal structures and decreasing leaf area as drought tolerance and resource-conservative strategy (Liu et al., 2020; Matos et al., 2021).

The multidimensional space represented by the leaf reflectance presents a significant agreement with the area represented by the leaf traits and the height of the species. The most variation was in the near-infrared and shortwave infrared regions (Figure 2), also reported in other studies (Roth et al.,2016). The patterns found in this region are linked to variations in internal structures and leaf surfaces (Gates et al., 1965; Heim et al., 2015; Slaton et al., 2001) which reflects the leaf economic spectrum (Reich, 2014; Wright et al., 2004) and the tolerance to drought (Matos et al., 2021). In addition, variations in leaf surface and internal structures may be related to water absorption strategies of high-altitude vegetation (Boanares et al., 2018; Vitarelli et al., 2016) and in response to high radiation (Liu et al., 2020).

The species distributions in both ordinations underlined the ecophysiological strategies and a lower intraspecific variation (Van Cleemput et al., 2021). Some species collected in two different sites were close to each other, like *Pleroma manicatum* (pleman_ne; pleman_north) and *Zygopetalum maculatum* (zygmac_ne; zygmac_north). Nevertheless, some species showed an intraspecific variation. The species *Dyckia cinerea* (dycinin_ne,dycinin_north), *Pitcairnia cf. carinata*(pitcar_ne,pitcar_north), and *Hippeastrum glaucescens* (hipgla_ne, hipgla_north)change position along the first axis (related to pigments).

As in other works, variations in spectral properties or traits capture the more functional interpretation of the species than others among commonly used functional classifications in Roth et al. (2016) and Van Cleemput et al. (2021). For example, Raukiaer's categories showed a significant relationship only with the ordering of traits. However, this difference would be related to species variation within this classification. It is possible to see in the scatter plot that species that are from different life forms are relatively close. Other functional categorizations, such as CSR strategies, were insufficient to explain altitude species' functional variation (Matos et al., 2021). Therefore, the functional characterization of these vegetations is more effective using models based on traits.

CONCLUSION

Our main objective was to investigate the distribution of species in a multidimensional space composed of their characteristics. Considering the traditional functional traits and the leaf reflectance, which is related to leaf properties, the distribution of most species in both multidimensional spaces showed a concentrated pattern. However, some species, such as *Dyckia cinerea* and *Pleroma manicatum*, show a set of traits that are more different than the others, presenting a more peripheral position in multidimensional spaces.

However, species distribution patterns in the two multidimensional spaces show more variations than species distribution according to the collection site or Rakier's hunger for life.

Furthermore, both the space composed of functional traits and the spectral behavior of the species showed a correlation, which reinforces the idea of the relationship between leaf spectrum and traits, enabling its use in functional ecology studies through remote sensing.

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CONCLUSÃO GERAL

Nossos resultados mostram uma variação na performance de estimativas e traços de espécies que não são arbóreas. Apesar dos valores de erros serem baixos, as variações dos pigmentos foram relativamente baixas o que afetou a performance do modelo, principalmente os modelos para clorofila. Para os outros traços, as espécies apresentaram uma variação que permitiu estimativas com uma melhor performance, como para os traços de área foliar e LMA. Além desses resultados, os nossos modelos mostram semelhanças com outros que foram feitos para uma vegetação de montanha, campos rupestres. Essa semelhança pode indicar similaridades nos modelos o que pode permitir a transferência de modelos entre essas vegetações

Nossas análises de agrupamento evidenciaram as relações entre traços funcionais tradicionalmente calculados daqueles estimados através do espectro. Ambos apresentaram concordância no agrupamento das respostas das espécies. Os grupos formados mostraram uma maior variação na resposta entre espécies que dentro das espécies nessa comunidade de campos de altitude. Mesmo com uma variação maior entre espécies, os agrupamentos também evidenciaram a variação intraespecífica da espécie *Hippeastrum glaucescens*. Além disso, espécies de mesmos gêneros, também agruparam junta, o que pode indicar uma convergência funcional desse grupo.

Os espaços multidimensionais tanto dos traços observados quando das reflectância foliar mostraram que a a maioria das espécies dos campos de altitude possuem conjunto de traços caracterizado como conservador. A distribuição das espécies não foi muito dispersa no espaço, mas algumas espécies estavam mais na região periférica, indicando uma combinação de traços e de padrões espectrais diferentes do que as demais.

Foi possível mostrar que os padrões espectrais estão ligados ao espaço de características da espécie, permitindo que teorias ecológicas e estatísticas comumente usadas em ecologia ampliem a compreensão da diversidade da comunidade vegetal. Além disso, também reforça o poder das abordagens de dados contínuos na ecologia funcional.

Nossos resultados mostraram a possibilidade de caracterização funcional de espécies de vegetação de altitude por meio de traços estimados ou reflectância foliar. Além disso, os resultados também indicaram uma maior variação dos traços e das propriedades entre espécies do que dentro das espécies indicando uma relação da composição das espécies e da função das comunidades de vegetação de altitude. Desta forma, os traços estimados usando padrões espectrais trazem informações funcionais uteis para o estudo das comunidades de vegetação de altitude.

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