RESEARCH ARTICLE

Assessing plant trait diversity as an indicators of species α- and β-diversity in a subalpine grassland of the Italian Alps

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Abstract

As the need for ecosystem biodiversity assessment increases within the climate crisis framework, more and more studies using spectral variation hypothesis (SVH) are proposed to assess biodiversity at various scales. The SVH implies optical diversity (also called spectral diversity) is driven by light absorption dynamics associated with plant traits (PTs) variability (which is an indicator of functional diversity) which is, in turn, determined by biodiversity. In this study, we examined the relationship between PTs variability, optical diversity and α- and β-diversity at different taxonomic ranks at the Monte Bondone grasslands, Trentino province, Italy. The results of the study showed that the PTs variability, at the α scale, was not correlated with biodiversity. On the other hand, the results observed at the community scale (β-diversity) showed that the variation of some of the investigated biochemical and biophysical PTs was associated with the β-diversity. We used the Mantel test to analyse the relationship between the PTs variability and species β-diversity. The results showed a correlation coefficient of up to 0.50 between PTs variability and species β-diversity. For higher taxonomic ranks such as family and functional groups, a slightly higher Spearman's correlation coefficient of up to 0.64 and 0.61 was observed, respectively. The SVH approach was also tested to estimate β-diversity and we found that spectral diversity calculated by Spectral Angle Mapper showed to be a better proxy of biodiversity in the same ecosystem where the spectral diversity approach failed to estimate α-diversity. These findings suggest that optical and PTs diversity approaches can be used to predict species diversity in the grasslands ecosystem where the species turnover is high.

Introduction

Biodiversity is an essential component for estimating ecosystem functioning and ecosystem services (Record et al., 2020), particularly important within the accelerated climate change context (Lavorel, 2019; Sala et al., 2000).

Also, changes in agricultural practices over the last few decades, involving modification of biogeochemistry by large-scale irrigation, fertilization and pesticide application, as well as changes in human activities have altered biodiversity patterns, directly affecting the individual species abundances and distributions (Record et al., 2020;

Walther et al., 2002). Such modifications have accelerated biodiversity loss at unprecedented rates and caused a considerable loss of landscape diversity, particularly for grassland ecosystems (Bolch et al., 2020; Stevens, 2018). Monitoring biodiversity is always considered challenging because it requires extensive field surveys, which are limited in their ability to sample over large regions. In this context, the effective monitoring of the grassland biodiversity can be carried out using its optical properties acquired by remote optical sensors (Gholizadeh et al., 2020; Record et al., 2020; Reinermann et al., 2020).

Reflectance of the vegetation is determined by the biophysical and biochemical properties of leaves and canopy (Homolova et al., 2013; Ma et al., 2020). Individual plant species respond in their own way to incoming solar radiation according to their pigment, biochemical content, and leaf and canopy structure. Based on this, variability in the canopy reflectance might provide information about the presence of different plant species within the measurement footprint. In the literature, such reflectance variation is defined as 'optical diversity' (also called spectral diversity). As the number of plant species increases for a given area, the spectral diversity observed from that area is also expected to increase (Heumann et al., 2015; Nagendra, 2001; Palmer et al., 2002; Rocchini et al., 2004). Some parts of the spectrum are characterized by a strong absorbance due to the leaf chlorophyll pigment (particularly the red domain) shown to be one of the key spectral areas for biodiversity detection (Blanco-Sacristán et al., 2019; Imran et al., 2021).

The spectral differences among plants thus capture functional differences in chemical, anatomical and morphological traits. It follows that spectral variability describes the pattern of spectral space occupied by a canopy and provides an integrated measure of the functional variability of phenotypes within plant communities (Schweiger et al., 2018). Plant traits (PTs) variability (also called plant functional diversity, Ma et al., 2020) is defined as the range and dispersion of the biochemical and biophysical PTs including pigment content (e.g. chlorophyll, carotene and xanthophyll), leaf essential elements and plant leaf traits. The optical diversity approach is based on variation in spectral reflectance retrieved by remote sensing, which can, in turn, be related to species diversity, genetic diversity and functional diversity. Relatively few studies have explored the relationships among spectral diversity, PTs diversity and taxonomic diversity in grassland ecosystems (Schweiger et al., 2018; Zhao et al., 2021). However, most studies have relied on optical models and optical trait indicators to assess PTs variability, rather than directly measuring biochemical and biophysical PTs (e.g. pigments, element concentration and PTs) in field samples (Durán et al., 2019; Torresani et al., 2021).

During the last decade, the optical diversity approach has been tested to retrieve α- and β-diversity in different ecosystems, such as grasslands (Aneece et al., 2017; Aneece & Epstein, 2015; Gholizadeh et al., 2019; Möckel et al., 2016; Wang et al., 2018) and forests (Khare et al., 2019; Schäfer et al., 2016; Torresani et al., 2019; Zhao et al., 2016). A few recent studies (Durán et al., 2019; Torresani et al., 2021) were focused on the relationship between PTs variability, and both spectral and plant diversity in forest ecosystems. Durán et al. (2019) combined the imaging spectroscopy and *in situ* foliar traits to estimate remotely-sensed functional diversity in tropical forests. Concerning α-diversity, several studies have reported that the optical diversity can be used as a proxy of plant diversity in various ecosystems and at various spatial scales (Conti et al., 2021; Gholizadeh et al., 2019; Imran et al., 2021; Möckel et al., 2016; Wang et al., 2018; Wang & Gamon, 2019). But at the same time, a few studies (Conti et al., 2021; Fassnacht et al., 2022; Gamon et al., 2020; Imran et al., 2021; Rocchini et al., 2010, 2016) found that the optical diversity approach application is not straightforward and not applicable across all spatial scales and ecosystems. However, these studies highlighted important aspects such as, for example, the scale-dependence of the observations and the spectral regions which are more correlated with biodiversity. The spectral variability is not only due to the leaf and canopy structure, because reflectance is also influenced by biochemical processes and phenological processes (e.g. senescence, Aneece & Epstein, 2015). The high number of species and the presence of non-photosynthetic elements and structural heterogeneity at the canopy level hamper the optical detectability of species diversity and the links between PTs variability and α- and β-diversity (Imran et al., 2021).

Regarding the β-diversity, the optical diversity approach has been used to estimate species turnover by analysing distance decay models disclosing floristic dissimilarities and spectral distances. Marzialetti et al. (2021) studied the relationship between field pairwise (Jaccard and Bray-Curtis) and spectral pairwise (Euclidean distance) measures and reported the effectiveness of the distance decay model to describe coastal landscape β-diversity on natural as well as on invaded dunes. Similarly, Rocchini et al. (2009) examined the spectral diversity approach to estimate β-diversity at different taxonomic ranks (plant species, genera and families) at the regional scale and demonstrated that a lower taxonomic rank is important when changes in the taxonomic composition are examined spatially using remotely-sensed data.

The aim of this study was to understand the links between PTs variability and species diversity at different taxonomic ranks to clarify possible reasons for the limitations of the optical sampling approach in monitoring α-

biodiversity (Imran et al., 2021). As a matter of fact, the applicability of the optical diversity approach to estimate taxonomic diversity is based on the hypothesis (in this study called plant traits variability hypothesis – PTVH, Fig. 1) that, as optical diversity is determined by PTs (biochemical and biophysical) variability, PTs variability, in turn, should be an indicator of species diversity. In this paper, we check links between taxonomic biodiversity, PTs variability and spectral diversity to better understand the limitations of the optical sampling approach in estimating grassland α-biodiversity. Additionally, another aim of this paper was to explore the relationship between spectral diversity and species β-diversity. In this context, the specific research objectives of the study were: (1) to investigate the links between α-diversity and both biochemical and biophysical PTs variability; (2) to explore the links between β-diversity and measured pairwise distances of the biochemical and biophysical PTs; and (3) to analyse the performance of spectral diversity proxies to estimate β-diversity using the measured pairwise distances.

Materials and Methods

Study area

The study area is situated in the Italian subalpine vegetation belt at the Viote del Monte Bondone, Trentino province, Italy (IT-MBo; 46°00′ N 11°02′ E; 1480–1550 m a.s.l.). The site is located on a plateau and is managed as an extensive grassland, with low mineral and one cut per year around mid-July at the green biomass peak time (Sakowska et al., 2019). The study site hosts a FLUXNET eddy covariance tower for measuring ecosystem-scale carbon, water and energy fluxes together with a number of ecosystem variables needed to understand the exchange processes. Within the last few years, a few intensive campaigns focused on determining biophysical and biodiversity traits were carried out at IT-MBo (Sakowska et al., 2015, 2016, 2019). Initially, 27 randomly distributed vegetation plots were included in the study, but five plots were discarded after the quality check of the hyperspectral images (visual evaluation to identify and remove any images that appeared blurred or out of focus). Eventually, in the 22 investigated plots of the IT-MBo plateau, the species richness that ranged from 2 to 17 within the 0.25×0.25 m area is presented in Figure 2.

Field data collection

Vegetation surveys within each region of interest (ROI, 0.25×0.25 m) were carried out by a trained person who listed all the species along with their per cent cover within the ROIs based on visual estimation (Kent & Coker, 1992). In each of the investigated plots, biomass samples were collected in sealed plastic bags and kept at low temperatures (4°C) until further processing. In the laboratory, the sampled biomass was well mixed and divided into two portions: one portion was used to measure the variability of the biophysical PTs, while the second portion was stored at −80°C for further determination of the foliar biochemical PTs variability. To calculate the biophysical PTs variability, we divided the first portion into eight subsamples which were separately weighted to obtain the fresh leaf mass (FLM, g), then scanned to obtain the leaf area (LA, cm^2) and finally oven-dried at 65°C for 48 h and weighted again to obtain the leaf dry mass (LDM, g). Specific LA (SLA, cm^2 g^{-1}) was calculated for each subsample as the ratio of LA to LDMs. The variability of the leaf water content (LWC, %) was also determined. The LWC was calculated
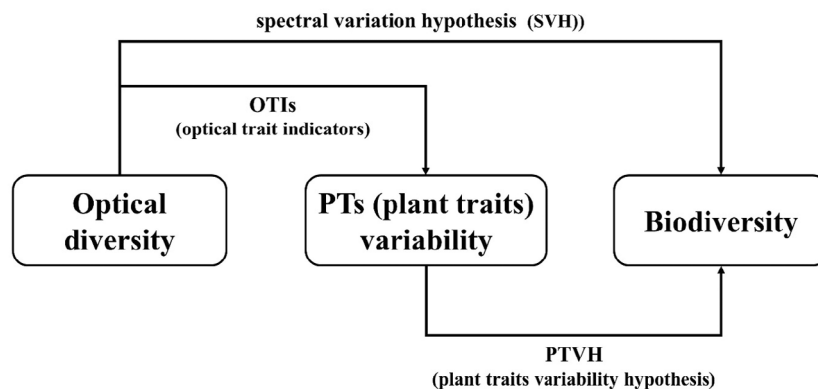

Figure 1. Workflow of the optical approach to estimate plant diversity using the spectral variation hypothesis (SVH) and plant traits variability hypothesis (PTVH).
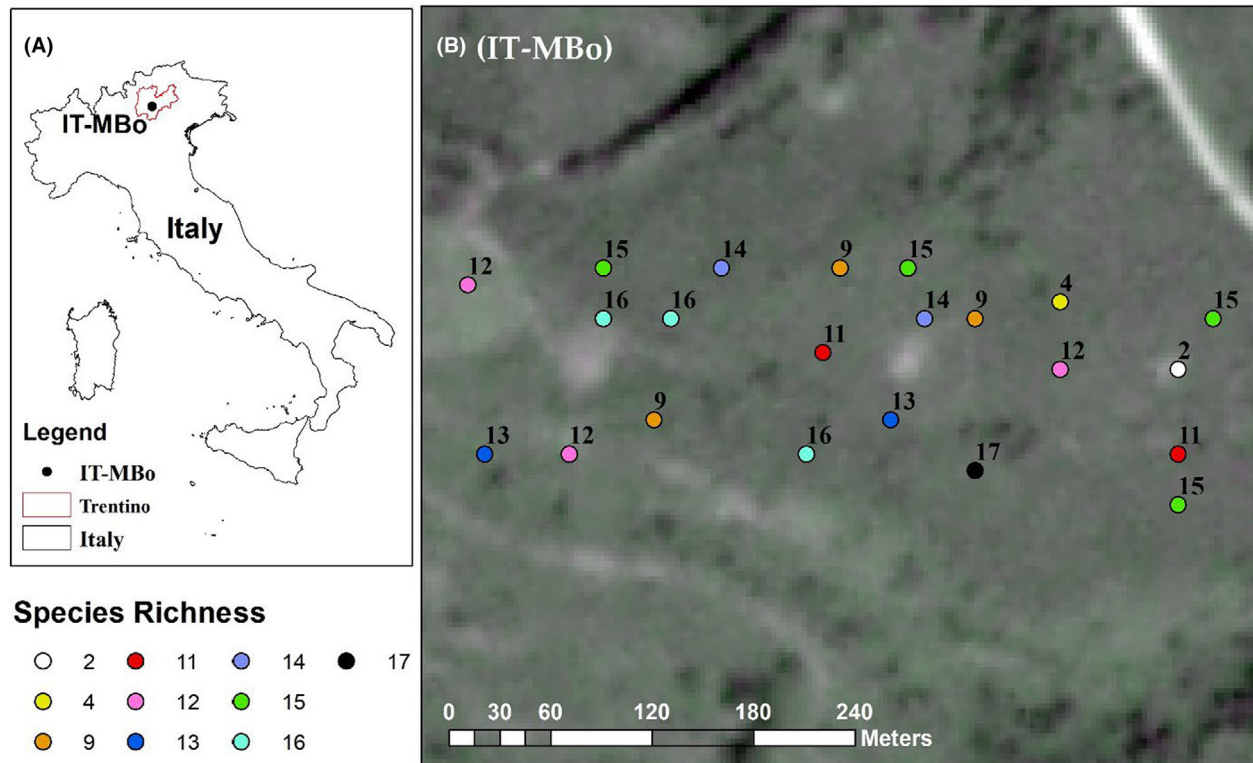
Figure 2. (A) Location of the Monte Bondone study area (IT-MBo) in the Italian Alps. (B) The IT-MBo sampling plots ($n = 22$) located in the natural grassland ecosystem.

as a difference between the FLM and LDM divided by FLM and multiplied by 100.

The second portion which was stored at $-80°C$ was used to measure the biochemical PTs variability: the portion was divided into 10 subsamples and the PTs concentration (mg g^{-1} dry matter) was determined for each subsample, including chlorophyll-*a* (chl*a*), chlorophyll-*b* (chl*b*), β-carotene (bcar), lutein (lut), neoxanthin (neox), violaxanthin (violax) and antheraxanthin (anther) by using high-performance liquid chromatography. The variability of the essential elements content including carbon (C), hydrogen (H), nitrogen (N), sulphur (S) and oxygen (O) was also assessed. The foliar biochemistry analysis was performed by Analytical (Limerick, Ireland) by means of the ICS-3000 Ion Chromatography System (Dionex, CA, USA) and the Vario MACRO Cube elemental analyzer (Elementar Analysen systeme GmbH, Elementar-Strasse 1, D-63505, Langenselbold, Germany).

We collected top-of-canopy level spectral data for the investigated plots (0.25 × 0.25 m) with an imaging spectrometer (SPECIM IQ; Specim Ltd., Oulu, Finland) mounted on a tripod. The spatial sampling of the hyperspectral images was 512 pixels per line and the spectral resolution (full width at half maximum) of

7 nm, with 204 bands across the spectral range of 397–1004 nm. The canopy height was not consistent between the plots (varied from 0.3 to 1.2 m); therefore, the hyperspectral images were collected *c.* 0.7 m from the canopy level to capture a squared footprint of *c.* 0.55 × 0.55 m. Within the image footprint, a 0.25 × 0.25-m frame was placed to define the ROIs used for post-processing and spectral diversity metrics calculations.

Measures of species diversity

In this study, α-diversity and β-diversity for all taxonomic ranks from species to family and function group were considered to capture biological variation at different spatial extents.

Species α-diversity

α-Diversity is the diversity within sampling units, which are usually spatial subunits within a region or landscape (Shannon, 1948). To calculate the α-diversity (expressed as Shannon's index), species per cent cover information was used by using the following equation:

$$H' = -\sum_{i=1}^{S} p_i \ln(p_i), \qquad (1)$$

where p_i is the proportion of individuals that belong to species i and S is the number of species in the sample.

Species β-diversity

β-Diversity (Whittaker, 1960) was calculated by generating the pairwise distance matrix based on pairwise dissimilarity methods (Li et al., 2021). Jaccard dissimilarity index (J) quantifies the pairwise dissimilarity between vegetation plots as the ratio between the number of species shared between the vegetation plots and the number of species that are unique to each plot (Marzialetti et al., 2021). The formula suggested by Jaccard (1912) implies relativization of the ratio between the number of species in common and the number of species that are unique to each plot.

$$J(i,j) = 1 - \frac{a_{ij}}{(a_{ij} + b_i + c_j)}, \qquad (2)$$

where a is the number of species shared between i and j vegetation plots, b is the number of unique species in the i vegetation plot, c is the number of unique species in the j plot.

Measures of biochemical and biophysical PTs variability

The PTs β-diversity was calculated based on Euclidean distance obtained from the biochemical and biophysical PTs. Euclidean distance represents the geometric distance between two points in multidimensional space. However, it is not well suited to data such as species abundances due to its lack of a maximum and its high susceptibility to large differences (Hoffmann et al., 2019). Before calculating the pairwise Euclidean distances of PTs, we standardized each of the variables to a mean of 0 and a standard deviation (SD) of 1 and then we used the following equation:

$$d_{i,j} = \sqrt{\sum (X_{iz} - X_{jz})^2}, \qquad (3)$$

where i and j are the plots being compared and z refers to the variables.

Measures of spectral diversity

To compare the spectral diversity to the taxonomic β-diversity index, spectral β-diversity was calculated using the Spectral Angle Mapper (SAM; Kruse et al., 1993). To depict the spectral diversity, SAM measures the angle between two spectral signatures: when the mean reflectance of all pixels of a plot is similar to the mean of all plots then the SAM values will be smaller for those plots and vice versa. In the spectral images, we observed some over-illuminated pixels due to illumination artefacts can distort the true spectral signatures of the vegetation, leading to inaccurate assessments of species diversity. The advantage of using SAM to calculate spectral diversity in different ecosystem types is its simplicity and insensitivity to the illumination variations present in the images (Cho et al., 2009; Gholizadeh et al., 2019; Sanjoto et al., 2022; Thornley et al., 2022). SAM was calculated by using the 'dist.speclib' function of the 'hsdar' R package (Lehnert et al., 2018) using the following equation:

$$SAM = \cos^{-1}\left(\frac{\sum_{i=1}^{sb} t_i r_i}{\sqrt{\sum_{i=1}^{sb} t_i^2}\sqrt{\sum_{i=1}^{sb} r_i^2}}\right), \qquad (4)$$

where sb is the number of bands. t_i and r_i are the reflectance of target and reference spectrum in band i, respectively.

Statistical analyses

The variability of biochemical and biophysical PTs was measured by using SD of the normalized (by range) values from the 10 subsamples of PTs in each plot by using the 'decostand' function of the 'vegan' R package (Oksanen et al., 2020). To access the performance of PTVH for α-diversity, we opted for a direct measure of linear association between α-diversity (measured by Shannon's index) and biochemical and biophysical PTs variability (measured by SD) within each plot. This approach allows us to capture potential linear dependencies between these variables, which also aligns with the existing literature (Aneece et al., 2017; Wang et al., 2018).

To investigate the relationship between PTs diversity (measured by Euclidean distances of all PTs) and taxonomic β-diversity (measured by Jaccard dissimilarity index), Mantel permutation test (Mantel, 1967) was used which is commonly used in ecological studies to determine the association between two datasets, for example, the species diversity and PTs diversity metrics. The relationship between spectral β-diversity measured as SAM and taxonomic β-diversity was also analysed by using Mantel statistics. For both α- and β-diversity analyses, we presented the results of both Spearman and Pearson correlation coefficients. All statistical analyses were performed with the statistical software R (version 3.6.1) (R Core Team, 2019).

Results

The species distribution in the investigated plots expressed as a proportion (in percentage) is presented in Figure 3. The highest number of recorded species within the 0.25 × 0.25-m area was 17, and it was observed in plot 09 where the dominating species were *Festuca nigrescens* Lam. and *Leontodon hispidus* L. with a contribution of *c.* 18 and 20%, respectively. In general, a very high species richness (ranging from 2 to 17, in 0.25 × 0.25 m area) was observed considering the relatively small area compared to the other studies (Aneece et al., 2017; Peng et al., 2019; Wang et al., 2018 where 1, 0.5 and 1 m² plots were considered, respectively). Among the 22 investigated plots, only two plots (20 and 22) were showing less than five species. The distribution of the family and functional group taxonomy level is presented in Figures S1 and S2.

Biochemical and biophysical PTs variability across the study plots

The summary of the results of biochemistry analysis and biophysical PTs of the biomass samples are shown in Table 1. The chl*a* and chl*b* content ranged from 1.04 to
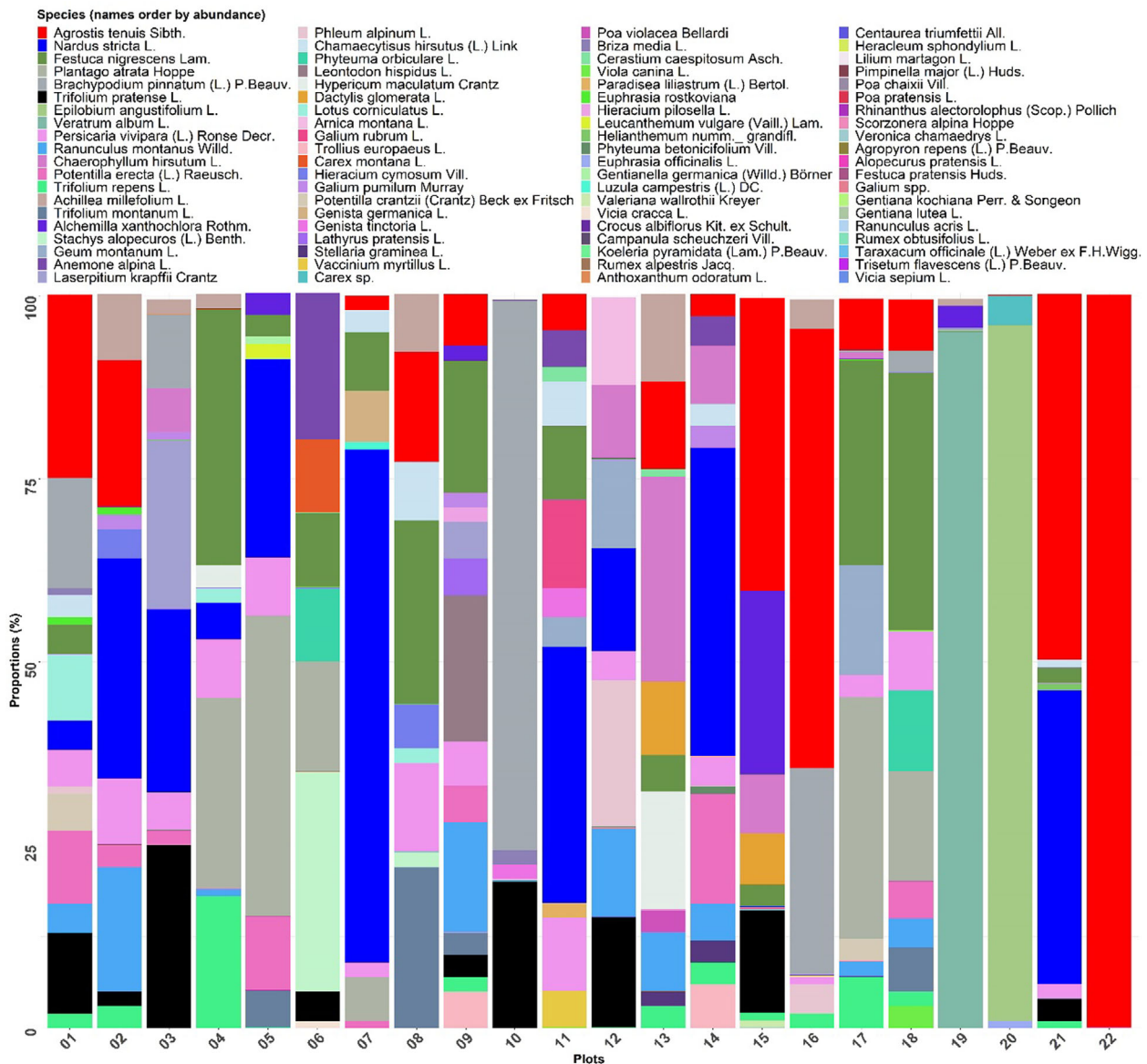

Figure 3. Species distribution (in %) within each plot. The species names were defined in the field when we collected biomass samples and the names were standardized according to the 'theplantlist' database (Version 1.1; http://www.theplantlist.org/).

Table 1. Descriptive statistics of the biochemical and biophysical plant traits (PTs).

Biochemical and biophysical PTs	Min	Max	Mean
Biochemical PTs (mg g^{-1})			
Chlorophyll *a*	1.04	4.92	2.16
Chlorophyll *b*	0.53	2.50	1.08
β-carotene	0.12	0.52	0.25
Lutein	0.13	0.41	0.26
Neoxanthin	0.04	0.17	0.07
Violaxanthin	0.01	0.20	0.09
Antheraxanthin	0.01	0.03	0.02
Carbon	45.75	48.65	47.35
Hydrogen	5.27	5.91	5.62
Nitrogen	1.23	2.28	1.64
Sulphur	0.08	0.20	0.14
Oxygen	36.82	42.02	39.73
Biophysical PTs			
Specific leaf area (cm^2 g^{-1})	105.51	218.55	163.44
Leaf water content (%)	54.49	78.55	69.02

4.92 and 0.53 to 2.50 with a mean of 2.16 and 1.08 mg g^{-1}, respectively. The values of bcar and lut varied from 0.12 to 0.52 and 0.13 to 0.41 mg g^{-1}. For the neox, violax and anther PTs, low differences were observed between the maximum and the minimum values with mean values of 0.07, 0.09 and 0.020 mg g^{-1}, respectively. The values of C and O ranged between 45.75 to 48.65 and 36.82 to 42.02%, with a mean of 47.35 and 39.73%. As concerns biophysical PTs, SLA values varied from 105.51 to 218.55 cm^2 g^{-1} and LWC values ranged from 54.49 to 78.55%.

The variability of the chlorophyll PTs was higher compared to the carotene and xanthophyll PTs. Out of the chlorophyll pigments, chla showed the highest SD range and showed to be a highly heterogeneous metric (Fig. 4A). In the xanthophyll pigments, we observed some outlier values in the SD, but the median SD was always lower than the median SD of chla and bcar. Out of the biochemical elements, N showed the highest variation within SD varying from 0.24 to 0.44 (Fig. 4A). The variation in the biophysical PTs including SLA and LWC is also presented in Figure 4B, where the SLA showed a higher variation compared to the LWC in the study area.

α-Diversity and PTs diversity

The correlation analysis showed a non-significant correlation between PTs variability (at local scale, measured as SD) and species α-diversity (Shannon's index) for all three taxonomy ranks (Table 2). The results are very similar to the ones obtained with spectral diversity metrics at the local scale (which also failed to estimate α-diversity in the same ecosystem) which were presented previously in Imran et al. (2021). All the metrics based on biochemical PTs variability failed to estimate biodiversity and hardly any of the metrics was correlated with α-diversity (Table 2). Both Spearman and Pearson correlation coefficients showed non-significant correlations across the different taxonomic ranks (species, family and functional group).

Biochemical and biophysical PTs distances versus species turnover

The correlation between Jaccard species dissimilarities versus PTs Euclidean distance showed that the taxonomic β-diversity was correlated with PTs variability among plots, at the spatial scale. For taxonomic β-diversity at the species level, the variation of the chlorophyll PTs and xanthophyll PTs including lut and neox showed a higher significant (*P* < 0.01) correlation with β-diversity (Fig. 5A,B,D and E) compared to beta carotene and other xanthophyll PTs (Fig. 5C,F and G). The strength of the correlation between the taxonomic β-diversity and biochemical PTs variability varied depending on the analysed PT. Some PTs, such as chla, chlb, lut and neox, were correlated with the taxonomic β-diversity with $\rho \geq 0.3$, while other PTs, such as bcar, violax and anther, showed to be not linked with the β-diversity. The correlations between the variation of leaf element content and Jaccard dissimilarity also gave some evidence of the connection between PTs diversity and taxonomic β-diversity. Among the investigated leaf elements, the strongest correlation with β-diversity was found for N and O (Fig. 5J,L). In case of the other elements, the correlation with taxonomic β-diversity was negligible: for example, C showed ρ value of 0.2 (Fig. 5H) while H and S showed values around zero (Fig. 5I,K).

The correlation between biophysical PTs diversity calculated from the Euclidean distances and the taxonomic β-diversity was slightly higher when the canopy dry biomass variability was related to β-diversity (Fig. 6C), compared to SLA (Fig. 6A). The LWC diversity, on the other hand, showed no correlation with taxonomic β-diversity.

The Mantel correlation analysis (Spearman and Pearson) between Jaccard species dissimilarities and biochemical and biophysical PTs Euclidean distance showed similar results compared to the taxonomic β-diversity at the species level. A slight drop in correlation values was generally observed for the dissimilarity matrices calculated from the species family-level data and a further decrease in ρ values was observed when considering dissimilarity matrices at higher taxonomy levels (functional group, Table 3). Among the investigated biochemical PTs, chla and β-carotene showed the highest correlations with the taxonomic β-diversity at the family rank compared to other investigated pigments which showed ρ values

<0.30. Among the xanthophyll PTs, the variability of violax and anther showed an overall non-significant correlation with taxonomic β-diversity. At higher taxonomic levels (functional groups), ρ has not exceeded the value of 0.3 for any investigated pigment. The N and O variability showed a correlation between taxonomic β-diversity for species family rank with ρ value 0.44 and 0.52, respectively, while a slight decrease in the ρ values was observed when a higher taxonomic rank (functional group) was considered. The lowest ρ value between β-diversity and biochemical PTs variability at both taxonomic ranks was observed for H. The biophysical PTs diversity showed a very weak correlation except for the dry biomass which showed a significant ($P < 0.001$) correlation for the family and functional group taxonomic rank.

Spectral distances versus species turnover

The analysis of the relationship between spectral diversity and taxonomic β-diversity (Fig. 7) showed a significant ($P < 0.001$) correlation with ρ value of 0.48 and 0.47 for the species and family taxonomic ranks, respectively, and only a slight decrease in ρ values was observed for the functional group taxonomic ranks with $\rho = 0.41$.

Discussion

The potential of the spectral diversity approach to estimate the taxonomic diversity at α- and β-diversity scales has been recently explored, and many authors (Aneece et al., 2017; Marzialetti et al., 2021; Peng et al., 2019; Wang et al., 2018) used the SVH approach to study either
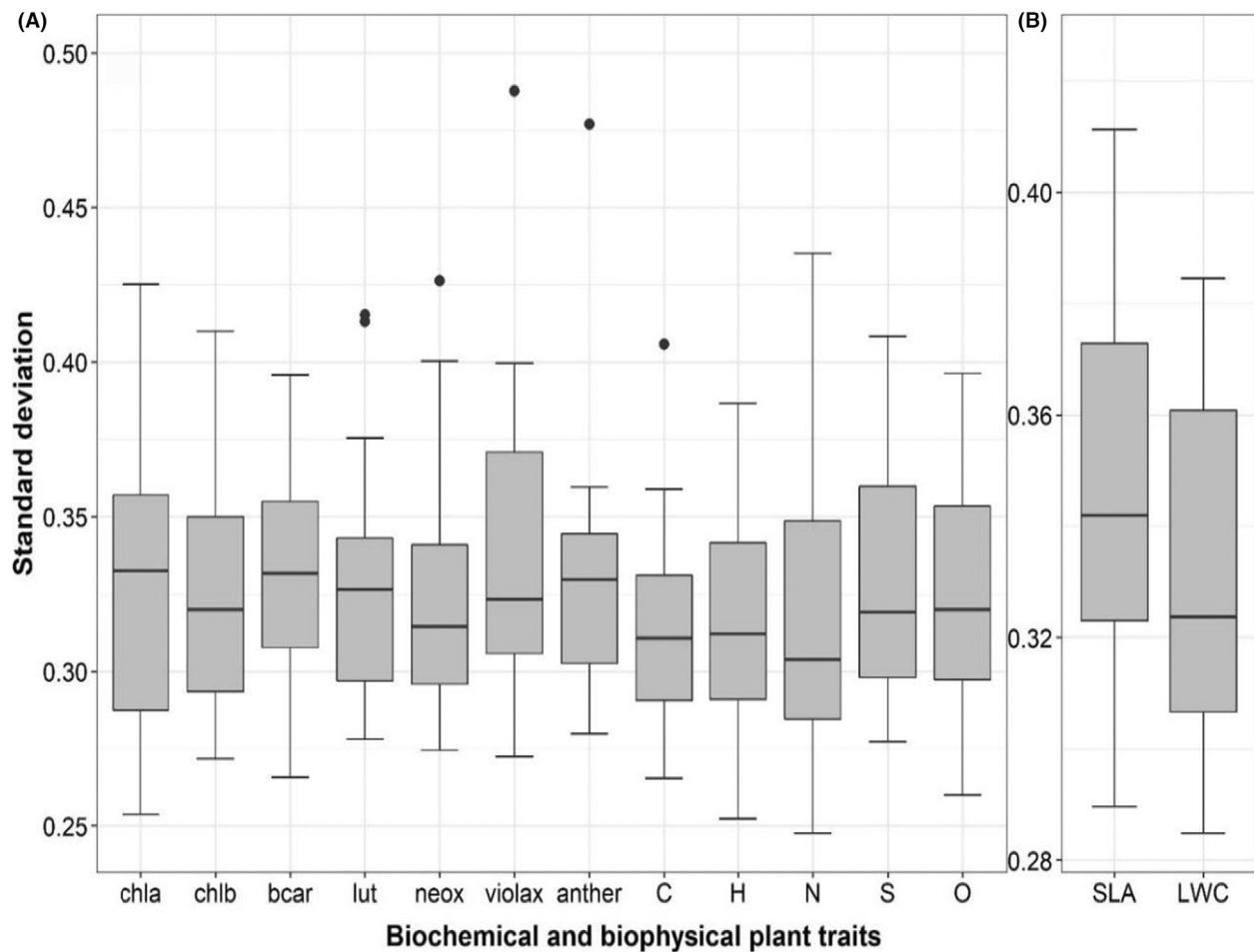

Figure 4. Box plots of the biochemical (A) and biophysical (B) plant traits (PTs) standard deviation (SD) among the investigated plots, measured from the 10 subsamples of PTs in each plot. The grey box represents the interquartile range, with a horizontal line showing the median. Each whisker extends to the highest value that is within 1.5 times the interquartile range. The black points indicate outlier values in the PTs measurement (Wickham, 2007). The abbreviations of the x-axis labels are: anther, antheraxanthin; bcar, β-carotene; C, carbon; chla, chlorophyll a; chlb, chlorophyll b; H, hydrogen; lut, lutein; LWC, leaf water content; N, nitrogen; neox, neoxanthin; O, oxygen; S, sulphur; SLA, specific leaf area; violax, violaxanthin.

Table 2. Summary of correlation analysis for α-diversity (Shannon's index) at three taxonomy ranks versus biochemical and biophysical variability (measured as standard deviation, SD).

Biochemical and biophysical PTs	Shannon's index		
	Species	Families	Functional group
Biochemical PTs (mg g^{-1})			
Chlorophyll *a*	$-0.07^{n.s}$ [$0.19^{n.s}$]	$-0.02^{n.s}$ [$0.08^{n.s}$]	$0.05^{n.s}$ [$0.13^{n.s}$]
Chlorophyll *b*	$-0.15^{n.s}$ [$0.03^{n.s}$]	$-0.23^{n.s}$ [$-0.15^{n.s}$]	$0.07^{n.s}$ [$-0.02^{n.s}$]
β-Carotene	$-0.07^{n.s}$ [$0.02^{n.s}$]	$-0.23^{n.s}$ [$0.03^{n.s}$]	$0.17^{n.s}$ [$0.18^{n.s}$]
Lutein	$-0.07^{n.s}$ [$0.09^{n.s}$]	$-0.13^{n.s}$ [$-0.04^{n.s}$]	$-0.09^{n.s}$ [$-0.03^{n.s}$]
Neoxanthin	$-0.08^{n.s}$ [$0.07^{n.s}$]	$-0.08^{n.s}$ [$-0.04^{n.s}$]	$0.14^{n.s}$ [$0.14^{n.s}$]
Violaxanthin	$0.09^{n.s}$ [$0.11^{n.s}$]	$-0.11^{n.s}$ [$0.01^{n.s}$]	$0.03^{n.s}$ [$0.15^{n.s}$]
Antheraxanthin	$0.15^{n.s}$ [$0.05^{n.s}$]	$-0.03^{n.s}$ [$-0.02^{n.s}$]	$-0.14^{n.s}$ [$-0.01^{n.s}$]
Carbon	$-0.11^{n.s}$ [$-0.23^{n.s}$]	$-0.07^{n.s}$ [$-0.2^{n.s}$]	$0.06^{n.s}$ [$-0.15^{n.s}$]
Hydrogen	$-0.06^{n.s}$ [$-0.11^{n.s}$]	$-0.08^{n.s}$ [$0.05^{n.s}$]	$-0.22^{n.s}$ [$-0.13^{n.s}$]
Nitrogen	$-0.02^{n.s}$ [$-0.17^{n.s}$]	$-0.02^{n.s}$ [$-0.08^{n.s}$]	$-0.06^{n.s}$ [$-0.31^{n.s}$]
Sulphur	$-0.06^{n.s}$ [$0.05^{n.s}$]	$-0.16^{n.s}$ [$-0.14^{n.s}$]	$-0.04^{n.s}$ [$-0.06^{n.s}$]
Oxygen	$-0.1^{n.s}$ [$-0.17^{n.s}$]	$-0.06^{n.s}$ [$-0.03^{n.s}$]	$0.01^{n.s}$ [$-0.07^{n.s}$]
Biophysical PTs			
Specific leaf area (cm^2 g^{-1})	$-0.09^{n.s}$ [$-0.09^{n.s}$]	$0.11^{n.s}$ [$-0.01^{n.s}$]	$-0.35^{n.s}$ [$-0.29^{n.s}$]
Leaf water content (%)	$0.13^{n.s}$ [$0.09^{n.s}$]	$0.24^{n.s}$ [$0.24^{n.s}$]	$0.24^{n.s}$ [$0.3^{n.s}$]

Values outside brackets represent the Spearman correlation (ρ), while values inside brackets indicate the Pearson's correlation (r). Asterisk indicates the significance of correlation: n.s: not significant.

man-made simpler ecosystems or relatively low diverse plant communities. However, the SVH approach has also been criticized for being unstable and not reliable in every context, due to the differences in the level of heterogeneity of the investigated ecosystems (Conti et al., 2021; Imran et al., 2021; Schmidtlein & Fassnacht, 2017). Canopy structure modify optical diversity patterns because high structural diversity and complex vertical structure may express high spectral diversity even when species diversity is low and vice versa (Conti et al., 2021; Imran et al., 2021).

PTs variability as a proxy of species diversity

In this study we used the PTVH to bridge spectral diversity to biodiversity. We found that the relationship between PTs variability and species diversity strongly varies across the diversity scales. Presumably due to the complex nature of the canopies in the investigated ecosystem, we found a very weak correlation between PTs variability and taxonomic α-diversity. The very weak relationships between PTs variability and species α-diversity suggested that the relationship may be impaired by other sources of variability such as, for example, the presence of the non-photosynthetic elements (e.g. flowers, stems and dead material) which might hamper not only the optical diversity approach to estimate diversity (Imran et al., 2021) but also the PTs variability link to the α-diversity. PTs

diversity plays a crucial role in shaping optical diversity, the relationship between PTs diversity and grassland α-diversity can vary depending on various ecological factors such as species composition, species interactions and environmental gradients. Therefore, the absence of correlation between these two diversity measures suggests that other ecological processes and mechanisms beyond PTs diversity may be driving the observed patterns of taxonomic α-diversity. Overall, there is some promise for the use of spectral data to estimate biodiversity in grasslands and more work is needed before the optical approach can be used with more confidence (Thornley et al., 2023). Conversely, we observed higher correlation between taxonomic β-diversity and the Euclidean distances of the PTs (Figs. 5 and 6; Table 3) compared to the correlation between PTs variability and α-diversity (Table 2). Furtherly, we also observed correlations between spectral β-diversity measured as SAM between the investigated plots and taxonomic β-diversity (species, family and functional group ranks) with ρ value of 0.48, 0.47 and 0.41, respectively (Fig. 7).

The results of the study showed that the relationship between PTs variability and biodiversity varied across the biodiversity spatial scales. The analysis of the Mantel test showed that a moderate correlation can be found between the PTs variability or spectral diversity with taxonomic β-diversity at IT-MBo grassland site. Our analysis showed that the variability at the spatial scale of leaf N content among the plots (as measured by pairwise Euclidean
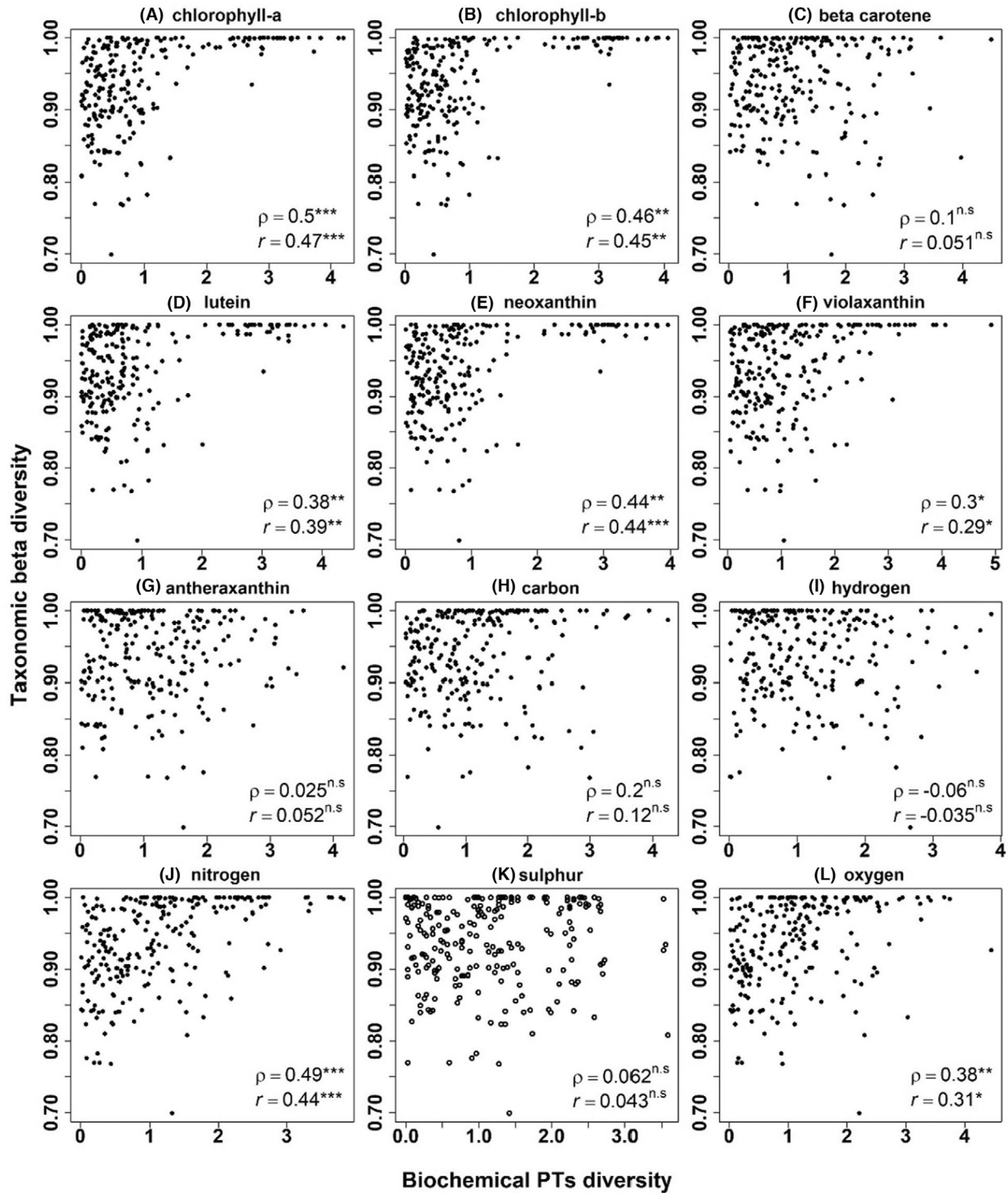
Figure 5. Mantel correlations (Spearman [ρ] and Pearson [r]) between the biochemical plant traits (PTs) diversity (measured by Euclidean distance of various PTs) and species β-diversity (measured by Jaccard dissimilarity index). Asterisk indicates the significance of correlation: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; n.s: not significant.
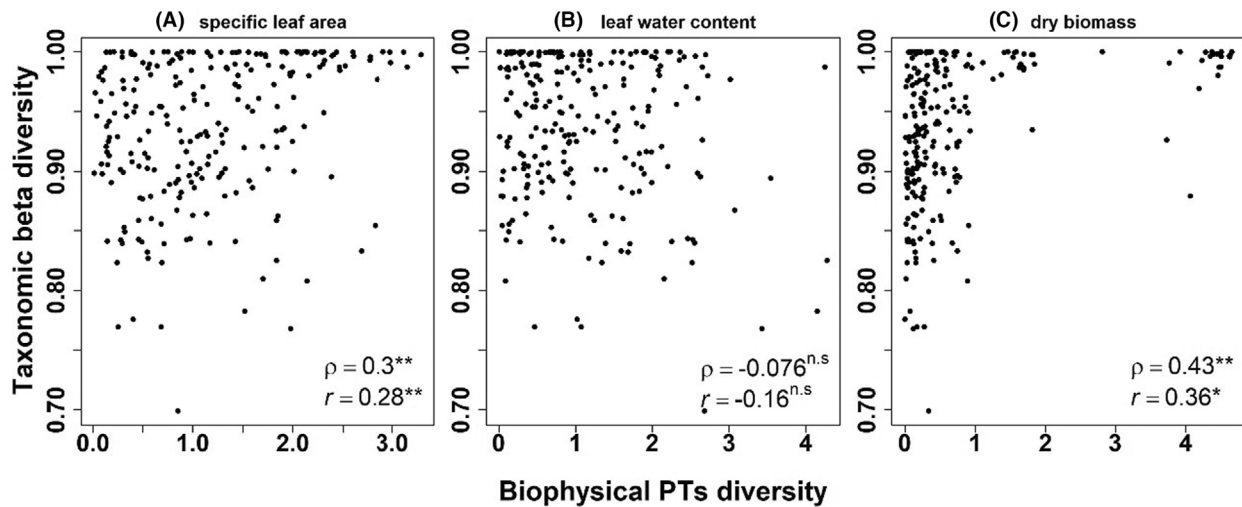
Figure 6. Mantel correlations (Spearman [ρ] and Pearson [r]) between the biophysical plant traits (PTs) [(A) specific leaf area – SLA, (B) leaf water content – LWC and (C) dry biomass] diversity (measured by Euclidean distance of biophysical PTs) and species β-diversity (measured by Jaccard dissimilarity index). Asterisk indicates the significance of correlation: **$P < 0.01$; *$P < 0.05$; n.s: not significant.

Table 3. Summary of correlation analysis for β-diversity at higher taxonomy levels (measured by Jaccard dissimilarity index) versus biochemical and biophysical variability (measured as Euclidean distance).

Biochemical and biophysical PTs	Shannon's index	
	Families	Functional groups
Biochemical PTs (mg g^{-1})		
Chlorophyll a	0.33* [0.3*]	0.19$^{n.s}$ [0.21$^{n.s}$]
Chlorophyll b	0.29* [0.3*]	0.24* [0.25$^{n.s}$]
β-Carotene	0.28* [0.27*]	0.3* [0.36*]
Lutein	0.32* [0.33*]	0.27* [0.3*]
Neoxanthin	0.28* [0.29*]	0.16$^{n.s}$ [0.22$^{n.s}$]
Violaxanthin	0.16$^{n.s}$ [0.13$^{n.s}$]	−0.05$^{n.s}$ [−0.06$^{n.s}$]
Antheraxanthin	0.03$^{n.s}$ [0.04$^{n.s}$]	−0.1$^{n.s}$ [−0.14$^{n.s}$]
Carbon	0.14$^{n.s}$ [0.12$^{n.s}$]	0.12$^{n.s}$ [0.13$^{n.s}$]
Hydrogen	0.08$^{n.s}$ [0.08$^{n.s}$]	0.01$^{n.s}$ [0.01$^{n.s}$]
Nitrogen	0.44** [0.42**]	0.29* [0.32*]
Sulphur	0.26* [0.29**]	0.3** [0.36**]
Oxygen	0.52*** [0.52***]	0.48*** [0.54***]
Biophysical PTs		
Specific leaf area (cm^2 g^{-1})	0.17* [0.17*]	0.14$^{n.s}$ [0.16*]
Leaf water content (%)	−0.06$^{n.s}$ [−0.1$^{n.s}$]	0.09$^{n.s}$ [0.1**]
Dry biomass	0.64*** [0.52***]	0.61*** [0.65***]

Values outside brackets represent the Spearman correlation (ρ), while values inside brackets indicate the Pearson's correlation (r). Asterisk indicates the significance of correlation: ***$P < 0.001$; **$P < 0.01$; *$P < 0.05$; n.s: not significant.

distances) was correlated (ρ = 0.48) with β-diversity (calculated using species pairwise distance matrix). More specifically, N content (in this unfertilized grassland) was directly correlated with β-diversity, differently from what

suggested by Humbert for fertilized grasslands (Humbert et al., 2016).

The relationship between the variability of chlorophyll traits and β-diversity is in line with Torresani et al. (2021). The variation of canopy brown pigment (Cbrown), carotenoids (car) and chlorophyll a and b (Cab) was shown to be an accurate proxy of tree species diversity (Torresani et al., 2021), although uncertainties in the estimation of leaf chemistry were deducted from physical model inversion and not from direct observations. The optical diversity approach, investigating the spectral variation of the canopy reflectance, can provide significant information on productivity, functional diversity and, in turn, on grassland biodiversity (Sakowska et al., 2019). However, our study shed light on the complexities associated with assessing diversity using remote observations or laboratory measurements of in situ samples, especially when studying ecosystems characterized by inherent variability. However, our study revealed that most assessments of diversity from remote observations or from laboratory measurements of in situ samples are rather uncertain and more research needs to be conducted to quantify such uncertainties.

Our study is significantly contributing to the analysis and explanations of the limits of the optical diversity approach that describes the extent and filling pattern of the spectral space occupied by a plant community. Such an approach is expected to describe 'ecosystem functional complexity' (Schweiger et al., 2018) which stems from biodiversity. But why is the optical approach sometimes not showing satisfactory performances for α-biodiversity estimations in, for example, high biodiversity and highly heterogeneous
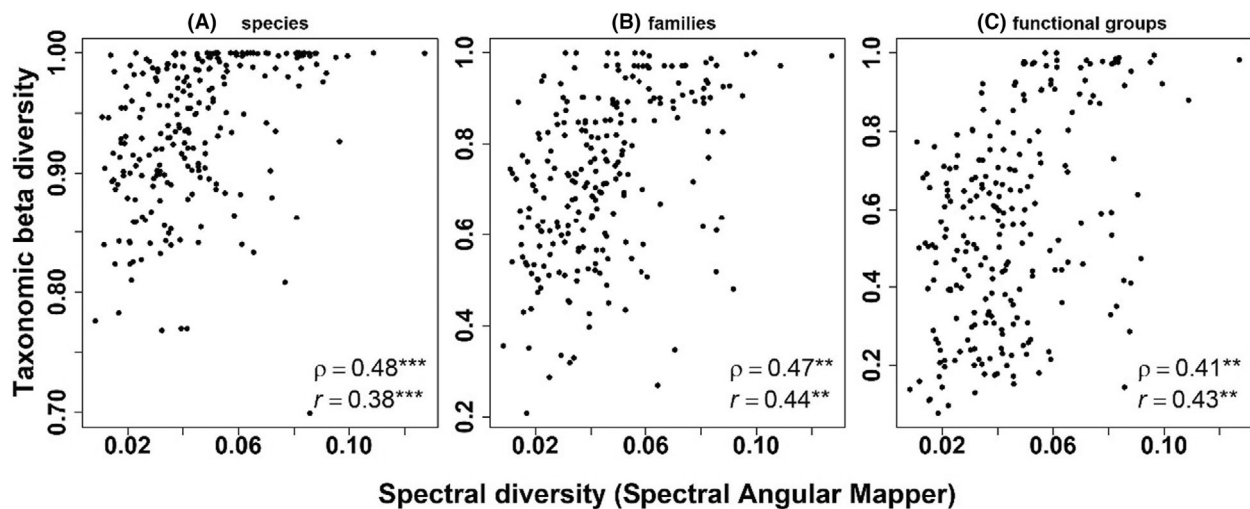
Figure 7. Mantel correlations (Spearman [ρ] and Pearson [r]) between spectral diversity measured by Spectral Angle Mapper (SAM) of average reflectance of each plot and species β-diversity measured by Jaccard dissimilarity index. Different panels represent different taxonomic ranks: (A) species; (B) families; (C) functional groups. Asterisk indicates the significance of correlation: ***$P < 0.001$; **$P < 0.01$.

ecosystems (as in Imran et al., 2021)? Our results are only preliminary – considering the small study area – but they indicate that the complex relationships between functional diversity and biodiversity may play a significant role.

Conclusions

In several studies, the relationship between spectral diversity and plant diversity has been observed, but it has also been demonstrated to be unstable (Imran et al., 2021; Schmidtlein & Fassnacht, 2017). This might be due to site-specificity and in particular to the differences in the level of heterogeneity of the investigated ecosystems, which influence the ability to detect PTs variability and reflect the inherent variability that can vary across different empirical contexts.

In this paper, we demonstrated that: (1) PTs variability is not correlated with α-biodiversity in a species-rich semi-natural subalpine grassland site where, in previous study (Imran et al., 2021), the optical diversity approach was not able to reliably detect α-biodiversity; (2) PTs diversity at the spatial scale (for chlorophyll, xanthophyll and nitrogen content) is linked with β-biodiversity; and (3) β-diversity has a statistically significant relationship with spectral variability since plant compositional turnover monotonically increases with increasing spectral distance among sites. Our preliminary results indicate that the poor performance of optical diversity proxies in estimating biodiversity in the investigated grasslands might be partly due to the complex relationships between functional diversity and biodiversity.

Author Contributions

HAI, LV, DR, MD, and DG conceived the ideas and designed methodology; HAI, LV, MS, MD, and DG collected the data; HAI and LV analysed the data; HAI, LV, and KS led the writing of the manuscript. All authors contributed critically to the draft and gave final approval for publication.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Species distribution at family rank (in %) within each plot.
Figure S2. Species distribution at functional group rank (in %) within each plot.