Satellite remote sensing of plant functional diversity
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Chapter 2.: Explaining discrepancies between spectral and in-situ plant diversity in multispectral satellite earth observation

Abstract

In light of the ongoing global biodiversity crisis, the urge to monitor and map terrestrial plant biodiversity at large spatial extents has spurred research on adequate quantitative methods. The use of spectral diversity metrics from different remote sensing platforms has emerged as a promising tool for such biodiversity assessments. Satellite remote sensing presents the next frontier for implementation of these methods to assess plant diversity with spatial and temporal continuity at truly regional or global scales. However, the question of what exactly is monitored by spectral diversity metrics from relatively coarse multi-spectral satellite observations has remained largely unanswered.

In this research, we examined which components contribute to satellite remotely sensed spectral diversity. We assessed the relationships between spectral diversity and in-situ taxonomic and trait diversity, and evaluated the role of confounding factors, vegetation cover, and landscape morphology (slope and elevation), in shaping these relationships. Hereto, we used Sentinel-2 imagery and in-situ field trait and species count data collected in the Montesinho-Nogueira Natura 2000 site (Portugal) together with radiative transfer models to quantify the theoretical link between in-situ trait diversity and simulated spectral diversity.

Through the use of linear mixed-effect models, our results highlight that variation in vegetation cover dominates the Sentinel-2's spectral diversity signal (contributing 53-84% of the $R^2_{marginal}$). The vegetation cover component encompasses spatial variability in canopy architecture traits as well as the fraction of bare soil and plant litter spectra. These elements together strongly impact the overall spectral diversity signal, as shown both in our radiative transfer simulations and empirical comparisons. Next to vegetation cover, we found that taxonomic diversity is a significant predictor and covariate of spectral diversity, while the role of leaf trait diversity appeared insignificant in our multispectral dataset.

Variation in vegetation cover dominated the spectral diversity signal in our study while it is not necessarily correlated with plant diversity. We, therefore, recommend that future applications of multi-spectral diversity metrics consider the impact of vegetation cover, including soil variability and the role of morphological traits, in shaping leaf trait - canopy reflectance relationships to better understand the ambiguous performance of spectral diversity as a proxy

of plant diversity. This will result in higher robustness, consistency, and scalability of spectral diversity metrics for predicting in-situ plant diversity across scales, sensors, and ecosystems in regional biodiversity assessments.

2.1.   Introduction

Over the past few decades, the threats to global biodiversity have continued to increase. These threats mostly involve anthropogenic pressures, such as habitat loss and fragmentation, overexploitation, climate change, and pollution (IPBES, 2019; Rands et al., 2010). A large consensus exists on the evident importance of biodiversity for vital Earth system processes and the resilience thereof (Cardinale et al., 2012). Consequently, many national governments have set targets and are committed to halting biodiversity losses, as formulated by the Convention of Biological Diversity in the Aichi Biodiversity Targets (O'Connor et al., 2015; Pettorelli et al., 2016), and the Post-2020 global biodiversity framework (CBD, 2020a, 2020b). To reach these ambitious goals, decision-making needs to be supported with well-understood and reliable indicators to track the current trends and state of biodiversity (Jetz et al., 2016; O'Connor et al., 2015; Pettorelli et al., 2016; Rocchini et al., 2015).

Biodiversity is multidimensional and includes the diversity of species (taxonomic diversity), the genetic variation within them (phylogenetic diversity), and the vast variety of traits, i.e. functional attributes, that each organism possesses (functional diversity) (Anderson, 2018; Gaston, 2010). The interactions and diversity of these traits and attributes within a given community shape ecosystem processes - affecting its productivity, adaptability, vulnerability to disturbances, and general functioning (Cadotte et al., 2011; Duncan et al., 2015; Funk et al., 2016; Isbell et al., 2011; Mori et al., 2013; Ruiz-jaen and Potvin, 2010).

Satellite earth observation has gained increased attention as a global and timely technique for monitoring biodiversity dynamics (O'Connor et al., 2015). Satellite remote sensing uses radiative measurements to provide critical information on the features of the Earth's surface using multiple wavelengths to capture detailed spectral information (Jensen, 2013). However, for a long time, remote sensing operated at coarse spatial and spectral resolutions, which hampered local-scale interpretation for biodiversity (Anderson 2018; Butler 2014). With ongoing advances in satellite sensors, a growing body of research examines the capabilities of satellite remotely sensed spectral information as proxies of biological diversity on the ground (Rocchini, 2007; Schmidtlein and Fassnacht, 2017; Torresani et al., 2019).

The use of spectral diversity, e.g. the variability in spectral information content, has become a widely studied approach for estimating terrestrial plant diversity through remote sensing (Wang and Gamon 2019). Initial research on this approach adopted the spectral variability hypothesis (Palmer et al., 2002) as their main premise (Rocchini, 2007; Rocchini et al., 2004). This hypothesis suggests that the diversity in spectral reflectance of an area is representative of in-situ plant diversity through its measurements of the spatial heterogeneity of the environment (Ewers et al., 2005; Palmer et al., 2002; Rocchini, 2007; Rocchini et al., 2010). Two theoretical concepts are central in the link between spectral diversity and in-situ plant diversity, namely 1) radiative transfer theory on vegetation-light interaction, and 2) the surrogacy hypothesis.

Radiative transfer theory for vegetation physically describes how remotely sensed reflectance is determined by a suite of morphological, biochemical, physiological, and structural

characteristics of plants and the relative position of the sun and sensor (Chandrasekhar, 1960; Ollinger, 2011). Particularly the impacts of leaf biochemical composition and structural properties of vegetation have been emphasized (Feret et al. 2008; Jacquemoud et al. 1996; Jacquemoud et al. 2009). These properties are not only spectrally important but also relate to plant traits that are of ecological importance and thus can shed light on the functional traits, and diversity thereof, present in an ecological community (Damm et al., 2018; Gamon et al., 2019; Kattenborn et al., 2017). Indeed, links between functional diversity and spectral diversity have been established empirically at the leaf level (Schweiger et al., 2018).

While trait ranges and functional diversity have been used to distinguish among plant functional types (van Bodegom et al., 2014; Verheijen et al., 2016), species spectral discrimination has generally been shown to be challenging in multi-spectral settings due to spectral and functional similarities among species and relatively large intra-specific variation in both spectral and functional characteristics (Cochrane, 2000; Hennessy et al., 2020; Price, 1994). Despite these challenges, links between spectral and taxonomic diversity might still apply, but through indirect relationships (Torresani et al., 2019; Villoslada et al., 2020).

The original premise of the spectral variability hypothesis (Palmer et al., 2002) relies on the 'surrogacy hypothesis' which suggests that patterns of biodiversity can be captured through its ecological and physical relationships with environmental heterogeneity (Beier and de Albuquerque 2015; Palmer et al. 2002; Stein, Gerstner, and Kreft 2014; Wang and Gamon 2019). In other words, based on this environmental 'surrogacy', spectral diversity can capture plant diversity indirectly through spectrally observable landscape heterogeneity, as landscape heterogeneity drives plant diversity.

Based on these theoretical concepts, different metrics and platforms have been deployed to quantify spectral diversity and to assess its link to in-situ plant diversity. Over time, studies have used proximal, airborne, and satellite remote sensing to link spectral diversity to either plant taxonomic, phylogenetic, and/or trait diversity (e.g. Dahlin, 2016; Gholizadeh et al., 2018; Lucas and Carter, 2008; Madonsela et al., 2017; Rocchini, 2007; Schmidtlein and Fassnacht, 2017; Schweiger et al., 2018; Torresani et al., 2019; Wang et al., 2018a). They reported both positive correlations and ambiguous relationships between spectral diversity and field-measured plant diversity. Overall, these studies have shown that scale, sensor, metric and season play a role in how well spectral diversity translates to plant diversity (Gholizadeh et al. 2018; Rocchini 2007; Schmidtlein and Fassnacht 2017; Torresani et al. 2019; Wang et al. 2018).

Despite the advances in applying spectral diversity metrics, the question of what exactly is measured in spectral diversity remains largely unanswered. This is particularly the case for multi-spectral satellite remote sensing in which the relatively coarse spatial resolution does not allow for the direct identification of individual plants and canopy crowns. For instance, European Space Agency's (ESA) flagship Sentinel-2 Multi-Spectral Instrument (MSI) presents spectral bands scaled at 10m, 20m, or 60m spatial resolution, which implies that spectral signals are aggregates of multiple canopies. Furthermore, even in densely forested areas, the surface reflectance spectral signal at these scales comprises a number of different constituents, each contributing to the overall signal: vegetation cover (including plant size and density, canopy architecture, and understory layers), the soil/litter/background signal, vegetation biochemical and biophysical composition (trait diversity) as well as landscape morphology (Asner, 1998;

Van Leeuwen and Huete, 1996; Zarnetske et al., 2019). Better characterization and quantification of the components contributing to satellite remotely sensed spectral diversity is therefore needed to further advance the use and interpretation of spectral diversity in assessing in-situ plant diversity.

In this paper, we tested the hypothesis that spectral diversity from multispectral satellite observations is able to capture in-situ measured plant diversity directly, through spectral responses of plant traits as expected based on radiative transfer theory, or indirectly, through environmental 'surrogacy'. Firstly, the theoretical physics-based expectations from radiative transfer were quantified through radiative transfer model (RTM) simulations, providing a theoretical reference. This helped assessing the extent to which modeled spectral diversity based on radiative transfer actually relates to the empirical data; in-situ measured traits as well as Sentinel-2 observed spectral diversity.

Secondly, the empirical relationships between spectral, taxonomic, and trait diversity and confounding factors were examined using Sentinel-2 imagery and in-situ field data collected in the Montesinho Natural Park and Montesinho-Nogueira Natura 2000 sites in northern Portugal. The analysis of bivariate correlations and linear mixed-effect models allowed us to characterize the extent to which observed Sentinel-2 spectral diversity covaries with in-situ observed taxonomic and trait diversity, but also of confounding variation in vegetation cover and landscape morphology. Ultimately, our findings feedback into the discussion on how to assess plant diversity from satellite remote sensing and the considerations needed for spectral diversity approaches in multi-spectral applications.

2.2. Methodology

2.2.1. Study area

The study area includes the Montesinho Natural Park and the Natura 2000 sites of Montesinho-Nogueira (PTZEP002 and PTCON0002), located in the Northeast of Portugal along the Spanish border (latitude 41°70′ - 41°94′N, longitude 6°57′ - 6°90′W) (See Fig. 2.1). With a size of over 1000 km^2, the area plays an important role in the conservation of regionally endemic biodiversity (Aguiar, 2001; Bastos et al., 2018). The study area is a natural mountainous area with an elevation between 371 and 1488m, characterized by high landscape richness and heterogeneous topography (Sil et al., 2016). This corresponds to a relatively wide range of climatic conditions between the highlands versus lowlands respectively with average annual temperature varying between 8.6°C and 12.9°C, and average annual precipitation between 1262mm and 806mm (Sil et al., 2016).

Semi-natural areas comprising of highly diverse shrub communities dominate the landscape, particularly in the highlands (Fonseca et al., 2012). Native deciduous Pyrenean oak forests are well represented, particularly in the Nogueira site (Rego et al., 2011), while Holm-oak woodlands occur mostly in rock outcrops, shallow soils, and steep slopes (Azevedo et al., 2013). The lowlands consist of agriculture intermingled with chestnut groves, while pine forest plantations occur at mid-elevation in the eastern part of the area (Sil et al., 2017). The landscape is heterogeneous, dynamic, and anthropogenic fires play an important role as drivers of landscape change (Azevedo et al., 2011; Sil et al., 2019).
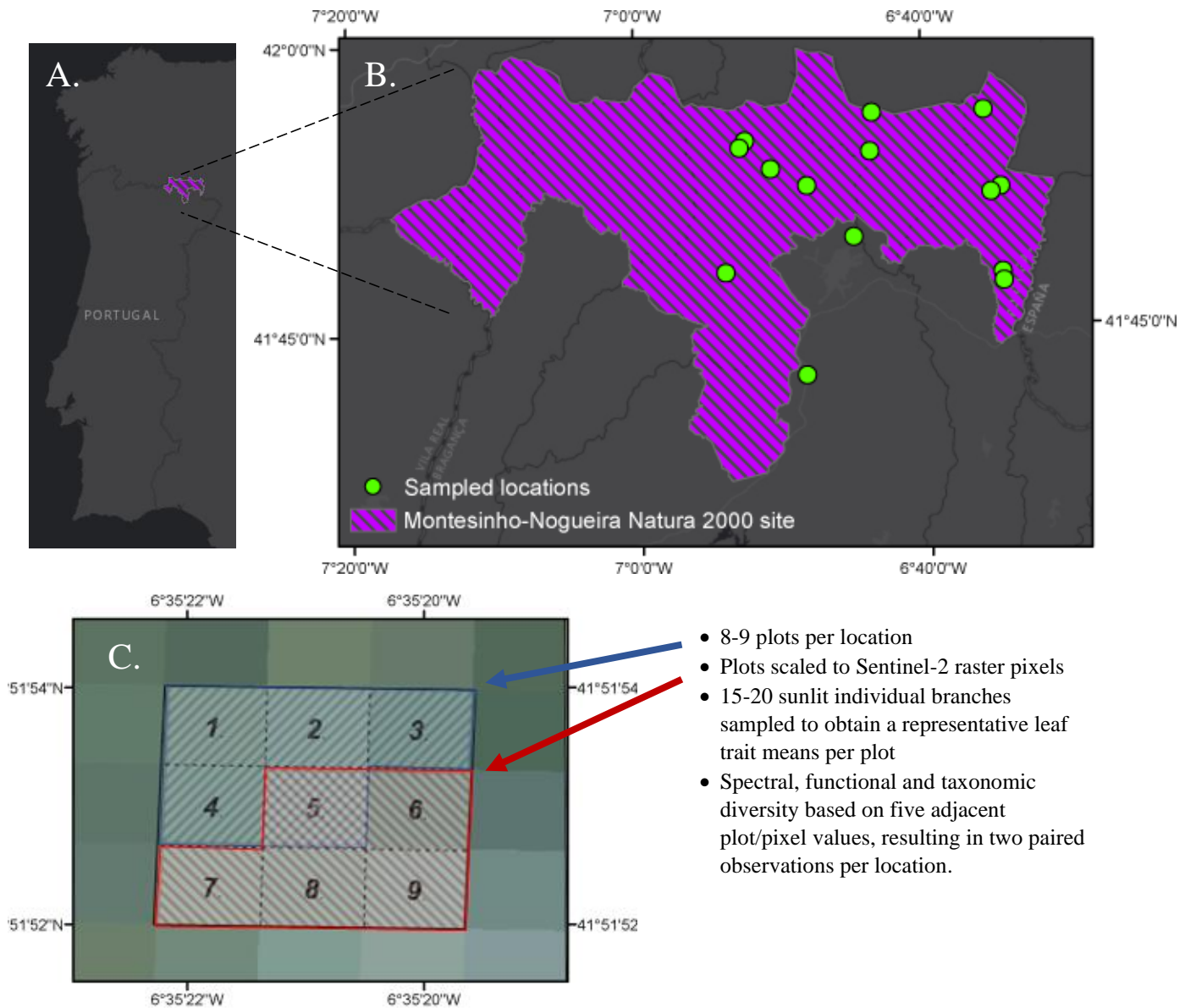
Fig. 2.1 Map of the study area depicting the location of the Montesinho-Nogueira Natura 2000 site in Portugal (panel A.), the distribution of the 14 individual sampling locations characterized by woody vegetation across the wider national park and Natura 2000 site (panel B.), and an exemplar individual location (panel C.) consisting of eight to nine plots scaled and georeferenced to Sentinel-2's pixel raster which is illustrated by surface reflectance at 20m spatial resolution as background. Diversity metrics (spectral, trait, and taxonomic) were calculated per five adjacent plot/pixel values resulting in two paired observations per location. For each plot (total N=115), an average of 17 individual sunlit branches was sampled to collect leaves representative of trait means of the overstory areal composition.

A total of 28 multi-plot sites were sampled across 14 representative vegetated locations in the Montesinho area (Fig. 2.1), covering three different land use types; shrublands, forested areas, and chestnut plantations. Photographs indicative of the different sampling locations and land use types can be found in Suppl. Mat. Fig. S. 1. Each location consisted of multiple adjacent 20m x 20m sampled plots. Each plot (total N = 115) was precisely scaled and georeferenced in the field to match Sentinel-2's pixel raster using a Trimble Catalyst GPS with Global Navigation Satellite System service of <1m precision. Therefore, Sentinel-2 observations

match field measurements in terms of the scale of the plots as well as the size of the clustered plots. Within each plot, we sampled leaves following the sampling protocol described in section 2.2.1. All diversity metrics, both in-situ and remotely sensed, were calculated for clusters of five adjacent plots resulting in two paired observations per location. The diversity metrics quantify the relative distance between trait combinations rather than the individual traits itself. As such, each observation of diversity is unique despite the overlap in pixels illustrated in Fig. 2.1C.

2.2.2. Data collection

2.2.2.1. Field data collection

A field data collection campaign was conducted from 12/06 until 05/07 of 2019. The prime objectives of the campaign were to collect for each plot individually; in-situ leaf trait data, conduct a species inventory, and assess the vegetation cover.

For leaf trait analysis, we collected samples from 15-20 individual branches of the healthy unshaded top of the canopies of each plot to capture a representative plot-level composite trait means. Prior visual and geometric inspection guided sampling choices to select the most dominant canopy types in terms of areal coverage of each plot. Collected leaves were weighted fresh, then transported on ice, and stored in a -18 °C freezer until further analysis in the lab. In total, 1955 leaf samples were analysed to derive four leaf traits (section 2.2.2.2) from 115 plots across 14 locations. Simultaneous with the collection of leaf samples, a species inventory was made for the sampled canopies for each plot of each site. This resulted in an area-based species count survey representative of the top of canopy/overstorey vegetation.

In addition to leaf sampling and the species inventory, we quantified the structural vegetation cover for each plot. Vegetation cover was characterized through the Plant Area Index (PAI), which is defined as the area of living plant material per unit of ground surface area. We used hemispherical photography to specify PAI similar to approaches by Garrigues et al. (2008), Hadi et al. (2017), and Weiss et al. (2004). For consistent measurements across all sites, we took five hemispherical photos per plot, one from the plot centroid and one from the center of each quadrant. Images were retaken in case of the presence of sunbeams or sun fleck problems. After the field campaign, we processed the RGB hemispherical photographs using CAN-EYE v6.4 open-source software to retrieve effective PAI estimates (Weiss and Baret, 2010).

We cross-validated the PAI measurements from hemispherical photography with above and below canopy measurements taken with Photosynthetically Active Radiation (PAR) sensor (Apogee MQ-301; handheld device), quantifying the relative quantity of incident solar radiation absorbed by vegetation. Further, cross-validation was performed through comparison of PAI measurements against the fraction of vegetation through spectral unmixing based on in-situ soil spectral reflectance measurements, and leaf spectral reflectance signals as generated by the PROSPECT model (Lehnert et al., 2018; Sohn and McCoy, 1997). The PAI observations strongly correlated with the PAR measurements as well as with the fraction of vegetation in the spectral signal derived from spectral unmixing (Fig. S. 2. in Suppl. Mat.).

2.2.2.2. Leaf biochemical traits

The collected field samples were analyzed in the lab to obtain relevant plot-level community means of leaf biochemical traits to ultimately assess site-level leaf trait diversity. The selection

of traits was based on 1) their ecological importance in terms of plant functioning (Croft et al., 2017; Damm et al., 2018; Díaz et al., 2016; Wright et al., 2004), and 2) their importance in the spectral response of leaves and our understanding thereof. Specifically, the four selected leaf traits are the input parameters of the widely applied PROSPECT radiative transfer model that links leaf traits and biochemistry to spectral responses using physical laws (Feret et al. 2008; Jacquemoud and Baret 1990).

Chlorophyll A and B content ($\mu g/cm^2$; CAB) is the area-based leaf content of green photosynthetic pigments in chloroplasts. Chlorophyll plays an important role in the photosynthetic capacity and resource strategy of plants (Croft et al., 2017). Carotenoid content ($\mu g/cm^2$; CAR) is the amount of orange and yellow photosynthetic pigments in chloroplasts. CAR serves a number of roles in the functioning of plants, including light harvesting for photosynthesis and dissipating energy to avoid damage by excess light (Croft et al., 2017). Both CAB and CAR were derived using a protocol based on Lichtenthaler (1987).

Equivalent water thickness (g/cm^2; EWT) is the amount of water present in a leaf or canopy divided by its area. EWT plays a role in the physiological plant performance and regulatory mechanisms important for drought and stress tolerance (Damm et al. 2018; Lawlor and Cornic 2002; Saura-Mas 2007; Weiher et al. 1999). Leaf mass per area (g/cm^2; LMA) is the amount of dry mass of a leaf per leaf area. LMA is a key feature in capturing leaf economics, reflecting trade-offs between carbon gain and longevity of a plant (Díaz et al., 2016; Wright et al., 2004). Both EWT and LMA were calculated based on fresh and dry leaf weight and leaf area (as determined in Image J 1.52a software (Schneider, Rasband, and Eliceiri 2012)).

2.2.2.3.   Sentinel-2 surface reflectance

Spectral diversity metrics were calculated based on the optical surface reflectance retrieved by ESA's Sentinel-2 MSI sensor. The Sentinel-2a/b constellation consists of a wide-swath, medium-high spatial resolution (10, 20, and 60m), multi-spectral (13 bands) imager with 5–10 days revisit time (ESA, 2015). Sentinel-2 surface reflectance (level-2a) imagery was downloaded from the Copernicus Scientific Hub which comes as atmospherically corrected by ESA using the Sen2Cor algorithm (Gascon et al., 2014; Louis et al., 2016). Only one scene (29th of July 2019) covering all sampled locations was completely free of quality flags (cloud cover, cloud shadow, cirrus, and other atmospheric contamination) and corresponded with the time of our field campaign. We excluded the 60m bands from the analysis and resampled the 10m bands to 20m spatial resolution using a nearest neighbour resampling method to match the scaling of our 20m×20m georeferenced field plots. This resulted in nine spectrally non-overlapping bands which were used to calculate the spectral diversity metrics (See Table S. 3 for the spectral layout of the Sentinel-2 bands used).

2.2.3.   Data analysis

2.2.3.1.   Diversity metrics

The relationship between plant diversity and spectral diversity is metric-dependent (Gholizadeh et al., 2018). Therefore, we implemented a multi-metric analysis with two of the most commonly used diversity measures in current research on both spectral diversity and trait diversity; Convex Hull Volume (CHV) and Rao's quadratic entropy index (Rao's Q) (Table 2.1). These metrics have been commonly used to capture plant trait diversity (Mouchet et al., 2010; Rocchini et al., 2017; Torresani et al., 2019; Villéger et al., 2008). The use of the same

metrical concepts for both spectral and trait diversity allows for consistency in the analyses. The spectral diversity metrics were based on spectra from pixel-based Sentinel-2 observations (section 2.2.2.3). For the trait diversity metrics, we relied upon plot-wise means of leaf trait samples acquired in-situ, scaled, and georeferenced to the Sentinel-2's pixel observations. The diversity metrics used allow for consideration of abundance. However, since we are using homogenously sized pixel/plot-wise (composite) values, the abundance is equal across plots (abundance = 1).

In addition to these spectral and trait diversity indices, we calculated taxonomic diversity using in-situ plot-wise species count data. We relied on Shannon's H diversity index as a commonly applied indicator of local taxonomic diversity of each site;

$$H = -\sum_{i=1}^{s}(p_i \times \log_2(p_i))$$

where s is the total number of species and P_i the proportion of the community represented by species i. Taxonomic diversity calculations based on Shannon's H were calculated using the scikit-bio 0.5.6 (http://scikit-bio.org/) package in Python. Min-max normalization of all metrics ensured all values were scaled between 0 and 1.

Table 2.1: Overview of the properties of the trait and spectral diversity metrics used in this study.

Metric	Concept	Algorithm	References	
			Spectral Diversity	Trait Diversity
Convex Hull Volume (CHV)	Volume in an n-dimensional space[1]	Quickhull Algorithm	(Dahlin, 2016; Gholizadeh et al., 2018)	(Cornwell et al., 2006)
Rao's quadratic entropy index (Rao's Q)	Mean squared pairwise distances[2]	$Q = \sum_{i=1}^{L-1}\sum_{j=i+1}^{L} d_{ij \times} p_i \times p_j$ $d_{ij} = \text{multivariate distance matrix comprising i-th to j-th pixel}$ $p_{ij} = \text{pixel or plot value abudance } (= 1)$ $L = \text{collective of pixel or plots in site}$	(Rocchini et al., 2017; Torresani et al., 2019)	(Botta-Dukat, 2005)

[1] We used the first three principal components to calculate the convex hull volume to have an equal number of dimensions among spectra or traits metrics.

[2] Traits and spectra were normalized prior to calculation of the index.

2.2.3.2.   Simulating spectral diversity through radiative transfer models

In addition to the empirical data collected, we made use of theoretical models to assess how spectral diversity and the diversity of in-situ measured traits related according to radiative transfer processes. From radiative transfer models, simulated leaf and canopy spectra were generated based on in-situ trait information and, ultimately, used to calculate a simulated spectral diversity based on the metrics described in section 2.2.3.1. The simulated spectral diversity served as a theoretical physics-based reference to evaluate its relationship to in-situ trait diversity based on radiative transfer theory and, in addition, were compared against Sentinel-2 spectral diversity to assess its correspondence to empirical observations.

The simulations were based on two radiative transfer models; the leaf-based PROSPECT-5 model (Feret et al., 2008) and the canopy-scaled PROSAIL model (Jacquemoud et al., 2006). For full compatibility with our empirical observations based on Sentinel-2, we ran a spectral convolution to resample the RTM-simulated spectra to the same spectral band layout as

Sentinel-2 following ESA's spectral response table (ESA, 2020). Based on the plot-wise spectra, the spectral diversity was calculated for five adjacent plots like done for the calculations for the observed Sentinel-2's pixel-based spectra and similar to the trait and taxonomic metrics.

First, we modelled leaf spectra using the commonly applied PROSPECT-5 model (Feret et al., 2008) based on plot-wise aggregated observed trait values. This model uses well-established physical principles of light-matter interaction to simulate how light interacts with a single leaf and serves numerous more complex vegetation radiative transfer model models (such as PROSAIL, INFORM, SCOPE) (Feret et al., 2008). It requires relatively few input parameters to determine spectral responses; leaf structure parameter (N), chlorophyll a+b (CAB), equivalent water thickness (EWT), leaf mass per area (LMA), brown pigment (CBP), and carotenoid concentration (CAR). The spectra were simulated for each plot individually on the basis of the mean leaf trait in-situ plot-wise measurements, with only the leaf structural parameter and brown pigments fixed to N = 1.6, and C_{brown} = 0.01, respectively (Bacour et al., 2002). A strong correlation was expected between leaf trait diversity and spectral diversity calculated with PROSPECT-5 given that we related the diversity of simulated spectra based on in-situ derived trait information to the diversity of that same trait information. Nevertheless, non-linearity in the relationship between traits and spectra and its representation in diversity metrics may have impacted the strength of this correlation (Verrelst et al., 2015).

Secondly, we scaled the leaf trait characteristics to canopy reflectance using the PROSAIL radiative transfer model, which combines the leaf model PROSPECT (Feret et al., 2008; Féret et al., 2017) and the canopy model 4SAIL (Verhoef 1984; Verhoef et al. 2007). Canopy reflectance is more equipped to link directly to the Sentinel-2 observations. However, PROSAIL requires a larger number of parameters beyond what we could collect during the field campaign, with leaf angle distributions and plot-wise soil characteristics not measured. Instead, we modelled canopy reflectance i) by artificially fixing these values (based on Jay et al. 2017; Spitters, Toussaint, and Goudriaan 1986; Bacour et al. 2002), and ii) local optimization of soil and leaf angle parameters to the observed Sentinel-2 spectra through a genetic algorithm (GA). In recent years, GA has been successfully applied to a variety of optimization problems in remote sensing (Fang et al., 2003). The most significant advantage of GA is that it provides a systematic scanning of both the entire solution space as well of local minima to find the global optimum solution (Bozorg-Haddad et al., 2017). An overview of the two implementations of PROSAIL including its input parameters and search ranges is presented in Table S. 4. The spectral diversity obtained from these two implementations of PROSAIL was again compared to the observed Sentinel-2 spectral and in-situ trait diversity.

2.2.3.3. Statistical analysis

Our study design is spatially nested with a total of 28 observations, each consisting of 5 clusters of adjacent plots/pixels, distributed in pairs across 14 independent locations. To avoid autocorrelation in this design, we opted for two solutions; 1) the use of average values between paired observations, and 2) the use of linear mixed-effect models. The first allowed us to integrate paired observations into single independent values without losing information. This increased robustness against outliers in either set of observations and robustness against possible noise in spectral and/or field measurements. Furthermore, it facilitates straightforward bivariate comparisons. The linear mixed-effect approach, on the other hand, allowed us to

maintain power of the nested sample size (N=28) facilitating multivariate analysis while accounting for the effect of location on the empirical relationships (Zuur et al., 2009).

Our statistical analyses consisted of three main parts. In the first part, we evaluated the bivariate relationships between simulated spectral diversity, either at leaf or canopy scale (section 2.2.3.2), compared against the observed Sentinel-2 spectral diversity and in-situ trait diversity. The second part focussed on the empirically observed bivariate relationships between spectral-trait-taxonomic diversity. Both the first and second parts of our analysis relied on bivariate rank correlations operationalized through Spearman's rho (ρ) as the non-parametric and rank-based alternative to Pearson's r (Khamis, 2008; Schober and Schwarte, 2018). Spearman's ρ does not assume normality and is robust against outliers in our small sample size (Fowler, 1987).

The third part assessed the constituents of spectral diversity through a multivariate analysis. We used linear mixed-effect models to assess the relationship between in-situ plant diversity and spectral diversity while accounting for the role of (assumed) confounding factors; vegetation cover and landscape morphology. Models were run using the 'lme4' package in R and model outputs were visualised using the 'sjPlot' package in R v3.6.1 (R Core Team, 2019). The R^2 values implemented were split into marginal and conditional R^2 statistics based on Nakagawa et al. (2017). The underlying assumptions of the linear mixed-effect regression models were checked (Suppl. Mat. Fig. S. 5) and considered as satisfactory. Landscape morphology was quantified as the elevation and slope (resampled to 20m resolution using bilinear interpolation) derived from the Shuttle Radar Topography Mission (25m spatial resolution). Vegetation cover was characterized through Plant Area Index (PAI). Both the mean and variance of landscape morphology and vegetation cover were included in the models. The dependent variable in these mixed-effect models was spectral diversity. The independent variables were the matching metric of in-situ leaf trait diversity in combination with the mean and variance of landscape morphology and vegetation cover. We repeated the same design of models for taxonomic diversity, using Shannon's H index instead of trait diversity metrics.

In addition to the linear mixed-effect models, a relative weights analysis was conducted to assess the relative importance of predictors to supplement effect sizes and statistical significance (Johnson 2000; Kraha et al. 2012). Relative importance is defined as the contribution each predictor makes to the total explained variance – while taking into consideration both its unique contribution and its contribution in the presence of the other predictors (LeBreton et al., 2007). The calculation of relative weights followed Tonidanel and LeBreton (2011).

2.3. Results

2.3.1. Assessing links and discrepancies between spectra and in-situ traits through RTM simulations

When using PROSPECT, a strong relationship was found between simulated spectral diversity and in-situ observed leaf trait diversity (CHV: $\rho=0.72$; Rao's Q: $\rho=0.75$, Fig. 2.2). In contrast to these simulated spectral-leaf trait relationships, no significant correlation was found between PROSPECT-simulated spectral diversity and the observed spectral diversity from Sentinel-2 (CHV: $\rho=0.21$; Rao's Q: $\rho=0.28$, Fig. 2.2). The latter can be understood through the mismatch in scale that exists between the PROSPECT simulated leaf spectra and the actual Sentinel-2 spectra which represent aggregate canopy-soil-understory observations.

When using PROSAIL with fixed estimates of the unmeasured canopy properties, the relationships with in-situ trait diversity weakened (CHV: ρ=0.46; Rao's Q: ρ=0.54, Fig. 2.2), as expected given the additional inclusion of canopy parameters, while the relationship with observed spectral diversity became equivocal (CHV: ρ=0.51; Rao's Q: ρ=0.09, Fig. 2.2). When using an optimized PROSAIL with variable leaf angle distribution and soil characteristics determined by GA optimization, the simulated spectral diversity correlated strongly with observed spectral diversity from Sentinel-2 (CHV: ρ=0.67; Rao's Q: ρ=0.86, Fig. 2.2) while the relationship with in-situ trait diversity was no longer clearly correlated (CHV: ρ=0.23; Rao's Q: ρ=0.07, Fig. 2.2).
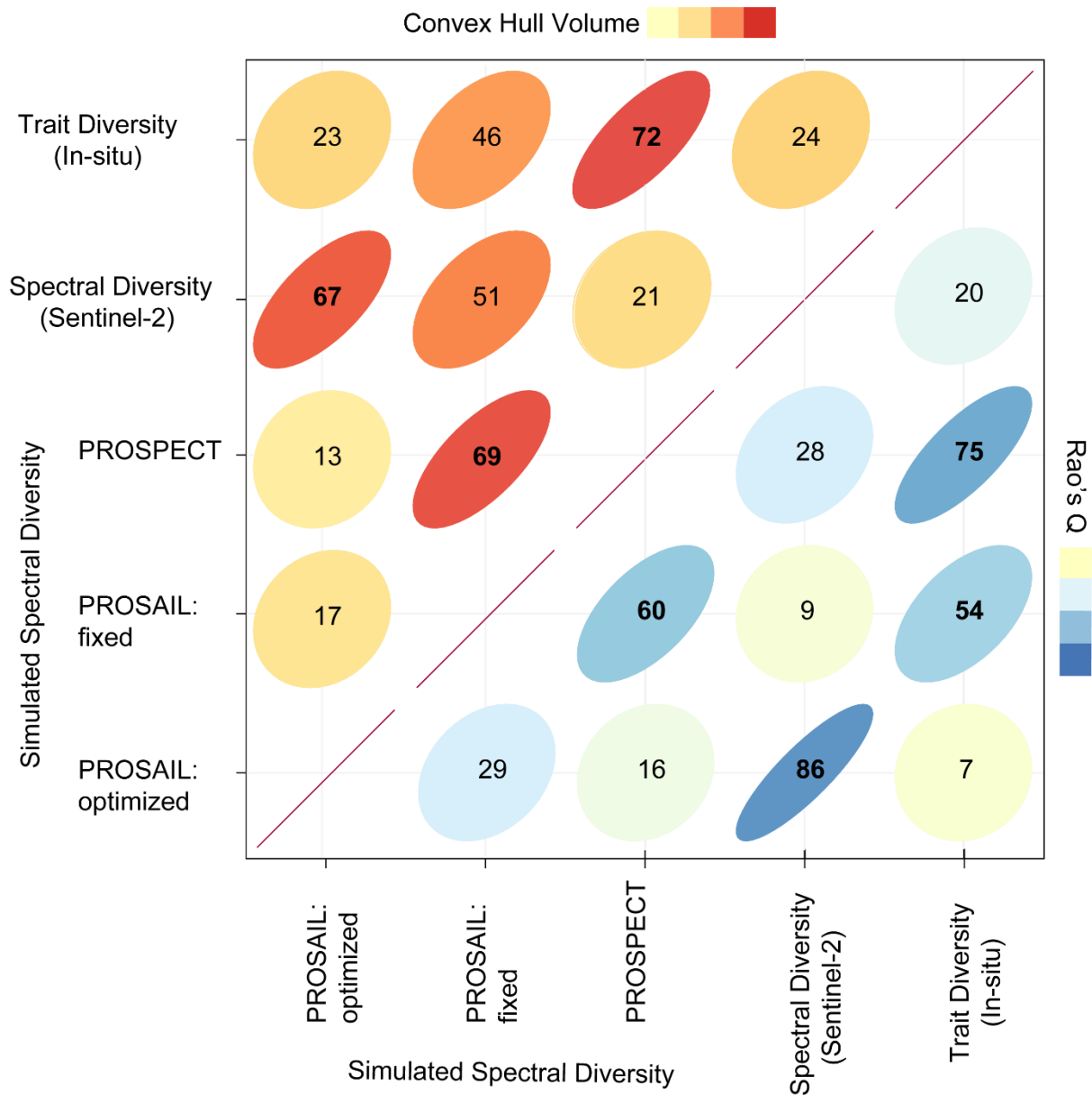

Fig. 2.2: Correlogram presenting the bivariate associations (Spearman's ρ × 100) between in-situ trait diversity, observed Sentinel-2 spectral diversity, and simulated spectral diversity as modelled by PROSPECT, PROSAIL (with fixed parameters), PROSAIL (variable with GA-optimized parameters) respectively. Diversity metrics based on the Convex Hull Volume (CHV) are in the upper left half, and Rao's Quadratic Entropy (Rao's Q) in the lower right half, which are displayed in yellow to red, and yellow to blue, respectively. Values presented in bold are significant (p < 0.05).
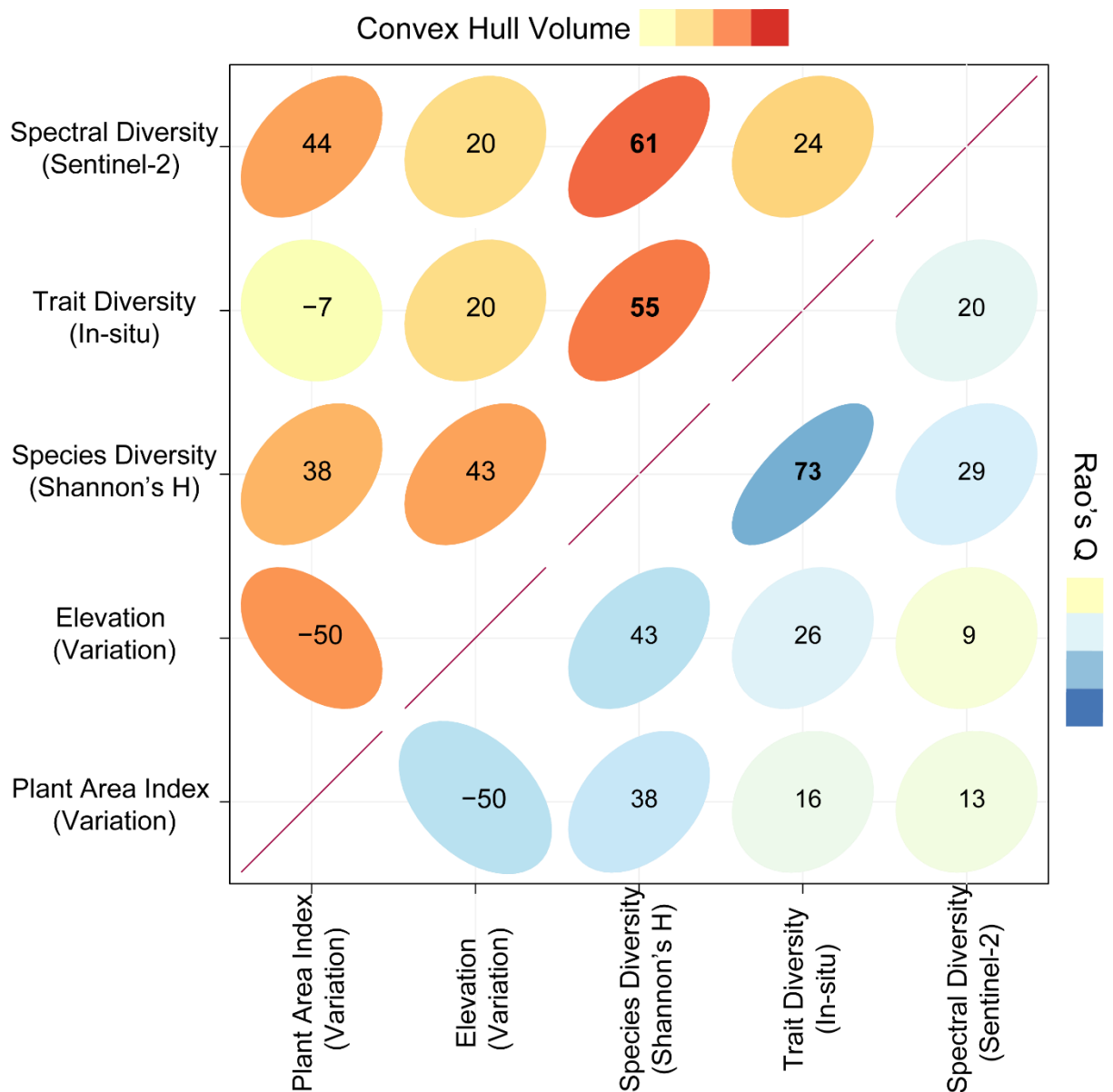
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Fig. 2.3: Correlogram presenting the empirical bivariate relationships (Spearman's $\rho \times 100$) between in-situ plant diversity (Taxonomic; Shannon's H, Traits), the observed Sentinel-2 spectral diversity, and environmental predictors of variation in elevation and variation in vegetation cover (PAI). Diversity metrics based on the Convex Hull Volume (CHV) are in the upper left half, and Rao's Quadratic Entropy (Rao's Q) in the lower right half, which are displayed in yellow to red, and yellow to blue, respectively. Values depicted in bold are considered significant ($p < 0.05$).

2.3.2. Trait-, Taxonomic and Spectral diversity relationships in empirical data

Moving from simulated to entirely empirical data, the field measurements showed that in-situ trait and taxonomic diversity were significantly correlated with each other (CHV: ρ=0.55; Rao's Q: ρ=0.73, Fig. 2.3). We also observed a positive relationship between spectral diversity (CHV) and taxonomic diversity (Shannon's H) (ρ = 0.61, Fig. 2.3) while for Rao's Q this correlation was absent (ρ=0.29). Bivariate correlations between leaf trait diversity and spectral diversity were weak (CHV: ρ=0.25; Rao's Q: ρ=0.20), despite correlations found between

taxonomic diversity and leaf trait diversity and the correlation of taxonomic diversity with spectral diversity (CHV).

Fig. 2.3 also gives insight into relationships between taxonomic, trait, and spectral diversity versus confounding factors of landscape morphology and vegetation cover. No significant correlations were found in our relatively small sample set. However, linear mixed-effect models (section 2.3.3) allowed us to include a larger set of spatially nested observations and to look into the underlying multivariate and potentially confounding relationships with spectral diversity.

2.3.3. Multivariate constituents of spectral diversity through linear mixed-effect models

Fig. 2.4 offers a summarized breakdown of the linear mixed-effect models that assessed the role of plant diversity in combination with landscape morphology and vegetation cover as predictors and constituents of Sentinel-2's spectral diversity. Four linear mixed-effect models were specified with spectral diversity operationalized by either CHV or Rao's Q and relying on either trait or taxonomic diversity as predictors in combination with confounding factors. Each of the individual model summaries is shown in the Suppl. Mat. Table S. 6a-d.
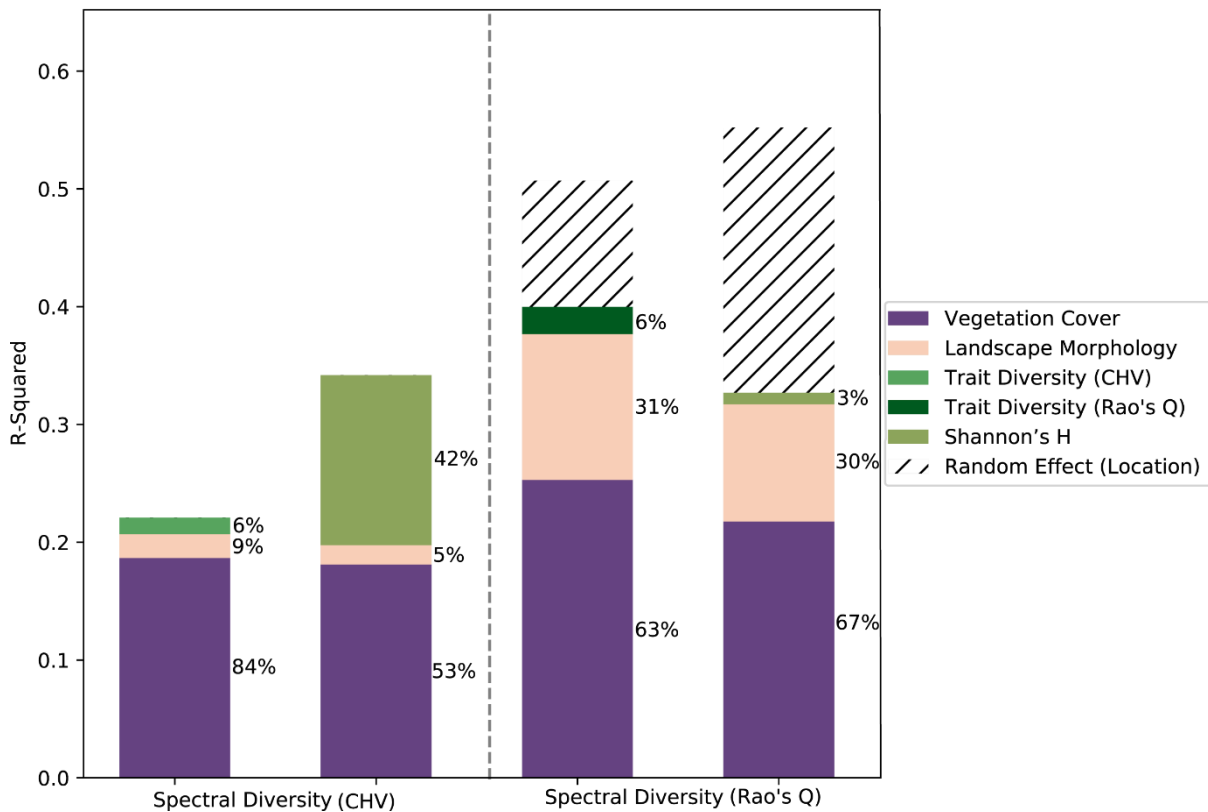

Fig. 2.4: Summary of four linear mixed-effect models with Sentinel-2 observed spectral diversity as the dependent variable and vegetation cover, landscape morphology, and either trait or taxonomic diversity as predictors, while the location was set as the random effect (N=28). Results from the relative weights analyses of each of the four sets of predictors are annotated in percentages and relate to the relative contribution of the fixed effects (excluding random effect).

The role of location as a random effect was minimal for CHV, as revealed by the low values of the random factor intraclass correlation coefficient (ICC) (Table S. 6a-6b). In contrast, Rao's Q revealed moderate effects of location on the proportion of the variance explained, ranging

between one-fifth to one-third of the explained variance (ICC: 0.18-0.33) (Fig. 2.4, Table S. c-6d).

Controlling for the effect of paired observations per location, the models explained between 22% and 40% of the variance found in spectral diversity (Fig. 2.4). These fixed effects were consistently stronger than the random effects of location. Across models, we found that vegetation cover is a particularly significant predictor. The relative weights analysis further highlighted the dominance of vegetation cover in predicting spectral diversity among fixed effects - contributing from 53 to 84% of the explained variance (R^2 $_{Marginal}$).

Trait diversity did not significantly contribute as a predictor of spectral diversity in either CHV or Rao's Q models. The relative importance of leaf trait diversity in explaining variation in spectral diversity was largely inferior to the predictive power of vegetation cover. Shannon's H, on the other hand, constituted as a significant predictor in CHV-based spectral diversity and accounted for as much as 42% of the fixed effects (Fig. 2.4). This was in line with the earlier bivariate correlation found between Shannon's H and spectral diversity (CHV) (Fig. 2.3). No significant role of taxonomic diversity was observed in Rao's Q calculations of spectral diversity. Instead, landscape morphology appeared as a significant explanatory variable in the Rao's Q calculations, with a relative importance accounting for 30-31% of the fixed effects (Fig. 2.4).

2.4. Discussion

This paper examined the question of what exactly is measured in spectral diversity obtained from multi-spectral satellite observations, considering; 1) direct responses of physical mechanisms between plant traits and spectral reflectance, and 2) proxies of plant diversity through environmental heterogeneity. The combination of coordinated field data and Sentinel-2 information allowed for direct empirical comparisons between spectral, taxonomic, and trait diversity, as well as confounding factors, while the RTM simulations evaluated the theoretical physical relationship between spectral and leaf trait diversity based on radiative transfer theory.

2.4.1. Leaf and canopy simulations show large differences in relation to empirical observations

Our empirical observations showed a minimal correlation between in-situ leaf trait diversity and Sentinel-2's spectral diversity. This departed from the strong theoretical relationships of spectral diversity and trait diversity modelled in the leaf-level PROSPECT simulations (ρ=0.72-0.75) (Fig. 2.2) and earlier reported empirical relationships at the leaf scale (Schweiger et al. 2018). Notably, the discrepancy between in-situ leaf trait diversity and simulated leaf spectra versus the actual Sentinel-2 spectral diversity as observed in our study, presents a scaling mismatch between leaf and canopy scales. Sentinel-2 observations represent aggregate complex canopy and background reflectance which limits the transferability of the leaf simulations (PROSPECT) to these empirical multi-spectral observations.

To address the scaling mismatch, PROSAIL simulations were implemented to model canopy reflectance for which we either fixed or varied (using optimization) the missing canopy and soil parameters. Despite the RTM's relative simplicity, our GA-optimized PROSAIL simulations illustrated the possibility to closely model observed Sentinel-2 spectral diversity (ρ=0.67-0.86) based on the inclusion of variability in soil and canopy variables (Fig. 2.2).

Specifically, the differences in the configurations of the RTM simulations used for modelling spectral diversity indicated a trade-off in describing either the in-situ trait diversity or the actual Sentinel-2 spectral diversity (Fig. 2.2). These findings assert the importance of soil and understory variability and canopy traits like leaf angle distribution in shaping and modelling spectral diversity in multi-spectral applications.

2.4.2. Vegetation cover is dominant in spectral diversity signal

The dominance of vegetation cover in the spectral diversity signal is manifested through its significance as a predictor in the linear mixed-effect models. The relative weights analysis highlighted that vegetation cover contributes 53-84% of the explained variance by fixed effects ($R^2_{marginal}$) in the Sentinel-2's spectral diversity signal (Fig. 2.4). Vegetation cover, measured through PAI, influences the extent to which soil, litter, and understory appear in the spectral reflectance signal of a Sentinel-2 pixel. The widely different spectral signatures of soil versus that of vegetation further contribute to the importance of vegetation cover in spectral diversity. Variation in the contribution of soil and vegetation to the overall spectral reflectance can result in relatively large spectral dissimilarities between corresponding plots (Gholizadeh et al., 2018; Villoslada et al., 2020; R. Wang et al., 2018c), even if plots are functionally relatively similar.

Additionally, vegetation cover is tied to the present canopy architecture as dictated by morphological traits. Canopy traits tend to dominate the spectral signal of vegetation across a broad range of the electromagnetic spectrum (Asner, 1998; Gu et al., 2016). Asner (1998) demonstrated that leaf area index (LAI) and leaf angle distribution, as relevant descriptors of the canopy morphology, strongly control the relationship between leaf traits and canopy spectral characteristics. Similarly, Roelofsen et al. (2013) showed the importance of LAI in linking leaf traits to canopy-level reflectance. The multispectral broadband setup may further increase the dominance of canopy, soil, and biomass characteristics as opposed to expressions of biochemical and biophysical leaf traits found in narrower ranges of the electromagnetic spectrum that benefit from hyperspectral measurements (Durán et al., 2019; Schneider et al., 2017; Schweiger et al., 2018).

The dominance of variation in vegetation cover as a driver of the spectral diversity signal requires further attention in future applications of spectral diversity. Diversity in vegetation cover density, biomass, and canopy architecture captured in PAI might not necessarily relate to plant diversity. For example, our dataset revealed no clear bivariate relationships between vegetation cover (variation in PAI) and trait diversity (ρ=-0.07-0.16), or taxonomic diversity (ρ=0.38) (Fig. 2.3). Similar to our findings, yet at a much higher (airborne) spatial resolution, Villoslada et al. (2020) reported that the sensitivity of spectral diversity to vegetation cover density could mask the effect of plant diversity.

In combination, our evidence shows that while spectral diversity approaches allow circumventing the challenges and difficulties in isolating direct plant diversity signals, the approach risks the spectral diversity signal to be dominated by other factors, like vegetation cover (Fig. 2.4), instead of plant diversity directly. Our ability to account for spectrally dominant confounding factors, such as vegetation cover and soil, could improve the robustness and relationship of spectral diversity approaches to in-situ plant diversity across spatial, spectral, geographic, and temporal resolutions.

Several approaches are already in place to assist in further dissection of the spectral signal to isolate plant diversity (Musavi et al., 2015). These techniques include spectral unmixing (Asner and Heidebrecht, 2002; Clasen et al., 2015; Gholizadeh et al., 2019; Sohn and McCoy, 1997), separate vegetation indices (Delegido et al., 2011; Schneider et al., 2017), radiative transfer model inversion (Ali et al., 2020b, 2020a; Brown et al., 2019; Hauser et al., 2021; Rossi et al., 2020), statistical learning (Berger et al., 2021; Durán et al., 2019; Ma et al., 2019) and data fusion approaches (Combal et al., 2002; Hakkenberg et al., 2018; Koetz et al., 2007; Lahoz and Schneider, 2014; Lewis et al., 2012). In Suppl. Mat. S. 7, we elaborate on the potential of these approaches.

2.4.3. Spectral Variability Hypothesis works for taxonomic diversity, the underlying mechanism remains unexplained

Despite the influence of vegetation cover, our empirical results revealed a direct correlation between taxonomic diversity and spectral diversity for CHV calculations ($\rho = 0.61$, Fig. 2.3). Furthermore, linear mixed-effect models and the relative weights analysis confirmed taxonomic diversity as an important contributor (42% of the fixed effects) to the explained variance ($R^2_{marginal}$) in CHV calculations (Fig. 2.4). Although species are spectrally difficult to separate based on multi-spectral broadbands (Cochrane, 2000), our findings suggest that indeed spectral diversity (CHV) can be meaningful as a proxy of taxonomic diversity using Sentinel-2 observations.

The relationship between spectral diversity and taxonomic diversity is often linked to Palmer's (2002) Spectral Variation Hypothesis which states that the spectral diversity in an area is expected to relate to the diversity of species through environmental heterogeneity (Dauber et al., 2003; Ewers et al., 2005; Palmer et al., 2002). Empirical demonstration of this relationship in satellite-based multispectral applications was found by, e.g. Torresani et al. (2019), Madonsela et al., (2017), and Rocchini et al. (2004; 2007). The underlying argumentation builds on the surrogacy hypothesis which suggests that higher environmental variation relates to higher species richness (Beier and de Albuquerque 2015; Stein, Gerstner, and Kreft 2014; Wang and Gamon 2019). However, thus far, studies on the spectral variability hypothesis tended to omit a characterization of the exact drivers of 'surrogacy' that align spectral diversity, environmental heterogeneity, and the diversity of species.

Here, we confirm the correlation of spectral diversity (CHV) and taxonomic diversity in a mountainous and heterogenous Mediterranean landscape using Sentinel-2 observations ($\rho = 0.61$; relative importance of 42% for $R^2_{marginal}$). Despite our attempt to incorporate leaf trait diversity and confounding factors in multivariate models, the exact workings of this relationship remain unexplained in our study. While taxonomic and leaf trait diversity were strongly correlated in-situ, only the former showed a correlation with spectral diversity. In addition, neither variation in elevation or vegetation cover were clearly correlated to taxonomic diversity as confounding mechanisms of 'surrogacy' (Fig. 2.4).

Hence, further research is needed to explore the exact mechanisms through which the relationship between spectral and taxonomic diversity is established. A more elaborate analysis of components of environmental heterogeneity at relevant spatial scales may allow a better understanding of the stronger association of taxonomic diversity compared to leaf trait diversity. For instance, taxonomic diversity observed here might covary with spectral diversity metrics through unmeasured canopy structure traits, not related to PAI, or through aspects of

environmental heterogeneity not considered here. Given that we could explain only a moderate share of variance in spectral diversity (R^2 Marginal = 0.22-0.40), the role of variables like fire regimes, plant phenology, grazing, dead biomass, and soil types in multi-spectral spectral diversity deserves further attention (Pausas and Vallejo, 1999; Schneider et al., 2017; Vivian and Cary, 2012; Zarnetske et al., 2019). Despite our substantial field campaign efforts, our sample size remained relatively small for multivariate analyses (N=28).

Differences in the significance of predictors depending on the metric (CHV versus Rao's Q) underpin that the choice of metric is highly relevant in the relationship between spectral diversity and in-situ plant diversity (see also Gholizadeh et al. 2018 for hyperspectral observations). Noteworthy in understanding differences between CHV and Rao's Q is that the latter also incorporates elements of divergence in quantifying diversity (Botta-Dukat, 2005; Mouchet et al., 2010). Furthermore, Wang, Gamon, Schweiger et al. (2018) highlighted the role of species evenness in metrics of spectral diversity. The authors suggested that the combined spectral effects of species richness and evenness are better captured with Shannon's H index than the trait diversity metrics as expressed by CHV. Moreover, data dimensionality plays a role in the patterns observed (Gholizadeh et al., 2018). Our operationalization of CHV runs on three axes (PCA dimensions) of spectra and traits while Rao's Q uses all standardized traits and spectral bands. The latter therefore does not reduce data dimensionality and could give more weight to signals that are expressed across a broad range of the observed spectral bands/trait space.

2.4.4. Absence of leaf trait diversity in spectral diversity signal

We expected trait diversity and spectral diversity to relate based on radiative transfer theory. Our simulations (Fig. 2.2) confirmed a direct theoretical relationship, yet only at the leaf level as much less association was observed when scaled to the canopy level. In addition, given the correlation between taxonomic and trait diversity in-situ, an indirect relationship was still expected. After all, environmental heterogeneity creates diversity in the optimal environmental fitness and conditions of resource availability (Dézerald et al., 2018; Kraft et al., 2008; Read et al., 2014). Nevertheless, the overall relationship of leaf trait diversity with spectral diversity appeared to be insignificant, even in consideration of confounding variables of vegetation cover and landscape morphology (Fig. 2.3 and Fig. 2.4).

Based on the aforementioned arguments, we forward three non-mutually exclusive explanations for the observed underrepresentation of leaf trait diversity and the dominance of the vegetation cover signal in the Sentinel-2 spectral diversity measurements; (1) the spectral dominance of variability in soil and morphological traits (Asner, 1998; Gholizadeh et al., 2018), (2) the underrepresentation of leaf optical properties at low vegetation density (Asner, 1998; Baret et al., 1994), and (3) the limitations of the multispectral broadbands at aggregate 20m resolution (Nagendra et al., 2010; Rocchini, 2007).

2.4.5. Expanding spectral diversity applications

The sites considered in this study consist of multiple land-cover types, growth forms, and a relatively heterogeneous abiotic environment to provide an important test for the implementation of spectral diversity metrics needed in biodiversity assessments at larger scales (Dahlin, 2016; R. Wang et al., 2018c). Stratification of our sampled sites in different levels of vegetation cover density, fractional cover, growth form, or land use type might have helped to

further examine the importance of each of the above-mentioned explanations. Our sample size posed limitations on the type of analyses possible and the conclusions to be drawn. The sampling effort was strongly constrained by the labour-intensiveness of comprehensive field campaigns at the scale of satellite remote sensing. Future research efforts and/or combined analyses of the data already out there might provide further stratification of spectral diversity relationships and ensure a better representation across time and biomes.

Further testing across different ecosystem types remains crucial. We anticipate the constituents of spectral diversity to be ecosystem-dependent, at least for multispectral spectral diversity metrics (Schmidtlein and Fassnacht, 2017). For instance, in some ecosystem types, variation in vegetation density might align more strongly with leaf trait diversity (Sheil and Bongers, 2020; Walter et al., 2020), while in other ecosystems vegetation cover density could mask or be ordinal to the effect of plant diversity (Hauser et al., 2021; Villoslada et al., 2020). Moreover, denser ecosystem types tend to allow for multiple-scattering, enhancing the contribution coming from leaf-level biochemical and biophysical information within canopy scale reflectance (Asner, 1998; Baret et al., 1994). Even temporally, within the same ecosystem, the impact of vegetation cover on the signal of plant diversity in spectral diversity metrics may vary. For instance, in Torresani et al. (2019)'s temporal study in an alpine conifer forest, the highest correlations between spectral diversity and in-situ plant diversity were found at the peak of the growing season in June and July.

The spectral resolution of the sensors also plays a role in our ability to detect in-situ plant diversity from remote sensing, as repeatedly pointed out in previous studies (Nagendra et al., 2010; Rocchini, 2007). Sentinel-2 MSI's spectral configuration offers rich spectral coverage compared to its predecessors yet is still largely inferior to hyperspectral sensors. While the spectral responses of leaf traits included here (LMA, EWT, CAB, CAR) show sensitivity to the wavelengths present in Sentinel-2's MSI sensor (de Sá et al., 2021; Gu et al., 2016; Verrelst et al., 2019b), the aggregation of signals across 20m spatial resolution through multi-spectral broadbands could have reduced the ability to pick up leaf trait diversity. Nevertheless, the forthcoming launch of hyperspectral satellite imagers (e.g. EnMAP, SBG, CHIME; Cavender-Bares, Gamon, and Townsend 2020) offers prospective that might benefit plant diversity assessments from space with more versatile spectral and spatial resolutions allowing for more detail.

2.5.   Conclusions

While the spectral variability hypothesis has been proposed to capture patterns of plant diversity through the relationship between environmental 'surrogacy' drivers and spectral drivers, the identity of those environmental drivers had not yet been characterized. Our analysis shows that vegetation cover served as the most dominant constituent in Sentinel-2 observed spectral diversity, thus providing important insights in the working of the spectral variability hypothesis. Effects of plant functional diversity, here examined as leaf trait diversity, remained obscured due to the dominant impacts of vegetation cover, possibly due to the low vegetation density in various plots of this study and the spectral dominance of canopy traits in multi-spectral configurations.

In contrast, spectral diversity based on Sentinel-2 observations was capable of predicting taxonomic diversity in accordance with the spectral variability hypothesis. Nevertheless,

despite our consideration of leaf trait diversity and confounding factors of vegetation cover and landscape morphology, the exact workings (or 'surrogacy') of this relationship remained hidden and will require a broader investigation of environmental heterogeneity and canopy traits.

We recommend that future applications of satellite-based spectral diversity metrics consider the impacts of vegetation cover. As illustrated in our study, variation in vegetation cover can dominate the spectral diversity signal while it is not necessarily correlated with plant diversity. We, therefore, think it is important to account for vegetation cover to compare across ecosystems and study areas and understand the potentially ambiguous performance of spectral diversity as a proxy of plant diversity. In the future, a practice of better characterization of constituents of spectral diversity may result in more robust interpretations, consistency, and scalability of spectral diversity metrics in predicting in-situ plant diversity across scales, sensors, ecosystems, and other external drivers for regional biodiversity assessments.