1 Estimating tree species diversity from space in
2 an alpine conifer forest: the Rao's Q diversity
3 index meets the Spectral Variation Hypothesis

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Abstract

Forests cover about 30% of the Earth surface, they are among the most biodiverse terrestrial ecosystems and represent the bulk of many ecological processes and services. The assessment of biodiversity is an important and essential goal to achieve but it can results difficult, time consuming and expensive when based on field data. Remote sensing covers large areas and provides consistent quality and standardized data, which can be used to estimate species diversity. One method to estimate species diversity from remote sensing data is based on the Spectral Variation Hypothesis (SVH), which assumes that the higher the spectral variation of an image, the higher the environmental heterogeneity and the species diversity of the considered area. SVH has been tested using different spectral heterogeneity (SH) indices and measures, recently the Rao's Q index has been proposed as a new spectral variation measure to be applied to remote sensing data. In this paper, we tested the SVH in an alpine coniferous forest to estimate tree species diversity. We evaluated the performance of the Rao's Q index and compared it with another widely used SH index, the Coefficient of Variation (CV), validating them against values of Shannon's H (used as species diversity index) derived from in-situ collected data. A NDVI time-series (for 2016 and 2017) derived from the Sentinel-2A and 2B and Landsat 8 OLI satellites has been used to test the effect of the spatial grain of both the sensors and to understand the seasonality of the SVH. The results showed that the SVH is season and sensor dependent. For both years and satellites, the relation between Rao's Q and field data reached the highest R^2 between June and July, decreasing towards winter and spring similarly to the NDVI time-series. This relationship could be given because, when NDVI reaches its highest values, it is able to capture small variation in reflectance of different leaf traits typical of specific trees. The relation between field and spectral diversity reached a value of $R^2=0.70$ (2017) and $R^2=0.48$ (2016) for Sentinel-2 and of $R^2=0.42$ (2017) and $R^2=0.47$ (2016) for Landsat 8. CV showed the same NDVI temporal tendency. However, the relation between field-derived Shannon's H and CV was on average lower than that we found for Rao's Q. This research underlined the goodness of the Rao's Q index, the relevance of the NDVI in the study of the SVH and the importance of the multi-temporal approach.

Keywords: alpine ecosystems, biodiversity, environmental heterogeneity, spectral heterogeneity index, remote sensing, time series analysis.

1 Introduction

1.1 Forest biodiversity and remote sensing

Forest biodiversity is the variability among living organisms in forest ecosystems encompassing the diversity of species across forest ecosystems (Whittaker, 1960). It also accounts for the ecological structures, functions and processes in forest ecosystems (Kaennel, 1998), (Innes and Koch, 1998) which play a key role for human well-being. Forests cover approximately 30% of the Earth's land surface (Mace et al., 2012) and provide food, medicines, fuel and other necessities to approximately 1.6 billion people. They support water flow regulation, carbon storage, and services such as habitat preservation (Team et al., 2007), pollution control (Grote et al., 2016), and soil protection and formation (Cunningham, 1963).

Many of the essential benefits deriving from forests depends on their biodiversity (Fleming et al., 2011). Forests are among the most biodiverse terrestrial ecosystems, they have the highest species diversity for many taxonomic groups, and they support about 65 % of the world's terrestrial taxa hosting two-thirds of all plants and animals living on land. (Lindenmayer et al., 2006). Although the importance of biodiversity is well known (Gamfeldt et al., 2008), forest biodiversity is declining due to a series of causes including habitat degradation (Hanski, 2011), unsustainable forest management (Chaudhary et al., 2016), climate change (Bellard et al., 2012), and pollution (McNeely et al., 1992) worldwide.

In the 1960s, Whittaker (Whittaker, 1960) introduced the terms of alpha, beta and gamma biodiversity to measure diversity at different spatial scales. Alpha biodiversity represents the species diversity within a specific area or ecosystem and can be expressed by the number of species (i.e., species richness). However, diversity indices, that also take into account the abundances of species (evenness), such as the Shannon's diversity index (H) (Shannon, 1948) are more widely used (Oldeland et al., 2010), (Nagendra , 2002), (Gorelick et al., 2006).

Species diversity can be directly assessed through field sampling. However, for large areas, this can be costly and time consuming. Therefore, efficient methodologies and tools that estimate species diversity and the related human impact are needed (Nagendra , 2001). Earth observation is a key instrument for monitoring ecosystems and the recent advances in sensor technology (high spatial resolution, broad coverage and high revisit frequency) make its application in highly heterogeneous ecosystems possible and economically acceptable. The assessment of forest biodiversity has been accomplished using different remote sensing data (Nagendra et al., 2010).

For instance, active sensors like LiDAR (Light Detection and Ranging) and radar have been used to evaluate the relationship between 3D structure and vegetation species diversity (Bergen et al., 2009), (Simonson et al., 2012) and animal richness (Muller et al., 2009), (Jung et al., 2012), (Muller et al., 2014). Optical images have been also largely used for this purpose. Digital Aerial photographs have been used to map tropical (Garzon-Lopez et al., 2013) and temperate (Getzin et al., 2012) forests. Hyperspectral images showed excellent results to map some aspects of biodiversity in different forest ecosystems, including tropical (Laurin et al., 2014), rain (Carlson et al., 2007), conifer (Gong et al., 1997) and mixed mountain forests (Schneider et al., 2017). Multi-spectral data from unmanned aerial vehicles (UAV) (Dandois et al., 2015), airborne (Lassau et al., 2005) and from satellite (Rocchini, 2007), (Nagendra and Rocchini, 2008) provided also very interesting results for the assessment of some aspects of forest biodiversity. In this context, the recently launched Sentinel-2 mission, which was developed by the European Space Agency (ESA) as part of the Copernicus program is fundamental with its free and open data access policy. This mission consists of a constellation of two optical satellites (Sentinel-2A and Sentinel-2B) that acquire images with 13 spectral bands (with resolution between 10 and 60 meters) and a revisit frequency of 5 days (at equator). Sentinel-2 has shown promising results in the monitoring of forest ecosystems, in tree species classifications (Immitzer et al., 2016), forest mapping (Puletti et al., 2017), monitoring forest disturbances (Verhegghen et al., 2016) and predicting growing stock volume (Mura et al., 2018), (Chrysafis et al., 2017).

Furthermore, the use of remote sensing data to measure some aspects of biodiversity has been increasingly considered as an elective choice of many worldwide initiatives (Rocchini et al., 2018). In particular the Group on Earth Observations - Biodiversity Observation Network - GEO BON (www.geobon.org) developed the concept of essential biodiversity variable (EBV), a "*derived measurement required to study, report, and manage biodiversity change, focusing on status and trend in elements of biodiversity*" that can be easily defined through satellite information (Jetz et al., 2016).

1.2 The Spectral Variation Hypothesis and the Rao's Q index

Remote sensing data can be used to assess certain aspect of biodiversity through direct and indirect approaches (Turner et al., 2003). Direct approaches require data with high spatial or spectral resolution and aim to map targets (individual species or communities) directly from remote sensing data

143 (Write et al., 2010). Indirect approaches can be of two types (Nagendra ,
144 2001). In the first type, environmental characteristics are derived from re-
145 motely sensed data that are then used to generate species distribution maps
146 based on a priori knowledge (Gillespie et al., 2008). In the second type, di-
147 rect relationships between remotely sensed reflectance values and field-based
148 data of species diversity are produced (Palmer et al., 2002).

149 An example of the latest approach is based on the Spectral Variation
150 Hypothesis (SVH) proposed for the first time by Palmer et al. (2002). This
151 concept hypothesizes that the variability of the spectral response of a re-
152 motely sensed image could be used as a proxy to assess plant biodiversity.
153 Areas with high spectral heterogeneity (SH) in a remotely sensed image cor-
154 respond to a higher number of available ecological niches that can host more
155 species. Therefore, the spectral variation of an image is related to the en-
156 vironmental heterogeneity and could be used as a powerful proxy of species
157 diversity (Palmer et al., 2002).

158 The SVH has been tested in different ecosystems including wetlands
159 (Rocchini et al., 2017), prairie vegetation (Palmer et al., 2002), tropical
160 forests (Féret and Asner, 2014), grasslands (Lopes et al., 2017) and Mediter-
161 ranean vegetation (Levin et al., 2007). Recently Schmidtlein and Fassnacht
162 (Schmidtlein and Fassnacht, 2017) tested the SVH across different habi-
163 tats observing that it does not hold across different ecosystems, stressing
164 its ecosystem dependency. The SVH has been tested using data from air-
165 borne hyperspectral sensors (Oldeland et al., 2010), (Gholizadeh et al., 2018),
166 multi-spectral satellite such as MODIS (Schmidtlein and Fassnacht, 2017),
167 Landsat (Rocchini, 2007), (Levin et al., 2007), QuickBird (Hall et al., 2010),
168 ASTER (Levin et al., 2007) and SPOT (Lopes et al., 2017). These studies
169 showed the strong sensor dependency of SVH resulting from different spatial
170 scales (spatial resolution and image extent) and spectral scales (number of
171 bands, radiometric resolution, band width and spectral range covered).

172 SVH also strongly depends on the index used to calculate the SH. Some
173 indices like the convex hull volume and convex hull area (Gholizadeh et al.,
174 2018) require a multi-dimensional image. Other indices like the coefficient of
175 variation (CV) can be derived from a single band (Madonsela et al., 2017)
176 or from spectral indices, such as the Normalized Difference Vegetation Index
177 (NDVI) (Tucker, C. J., 1979), (Oindo and Skidmore, 2002), (Levin et al.,
178 2007), (Gould et al., 2000).

179 Recently the Rao’s Q index has been proposed as an innovative SH mea-
180 sure in the field of remote sensing (Rocchini et al., 2017). The Rao’s Q
181 index has been developed by Rao (Rao, 1982), and proposed by Botta-Dukat
182 (2005) as a measure of functional diversity in ecology. As stated by Rocchini
183 et al. (2017) “given an image of N pixels, the Rao’s Q is related to the sum

of all the pixel values pairwise distances, each of which is multiplied by the relative abundance of each pair of pixels in the analyzed image. Rao’s Q is the expected difference in reflectance values between two pixels drawn randomly with replacement from the considered evaluated pixels set.” Hence, Rao’s Q considers both the value of the pixel (through the distance/difference between the pixel) and the abundance of the pixel in the image.

The aim of this paper is to test the Spectral Variation Hypothesis in an alpine coniferous forest to estimate tree species diversity. In particular the objective of this research is to evaluate the performance of the Rao’s Q, comparing it with the CV (used as a benchmark) and to validate them against values of Shannon’s H (used as species diversity index) derived from in-situ collected data. The SVH has been tested using an NDVI data-set derived from Sentinel-2 and Landsat 8 for the year 2016 and 2017. This has been done to test the effect of the spatial grain of different sensors and to understand the seasonality of the SVH.

2 Material and methods

2.1 Field data

The study area is located in a coniferous forest at 1100 m a.s.l. in the municipality of San Genesio/Jenesien, in South Tyrol (Italy, Figure 1). The dominant species is *Pinus sylvestris*, followed by *Larix decidua* and *Picea abies* while broadleaved trees such as *Betula alba*, *Corylus avellana*, *Salix caprea* and *Sorbus aucuparia* are accessory species. The forest is particularly dense and characterized by a high canopy closure. We chose a coniferous forest since SVH has never been tested in such ecosystems. Furthermore, the considered area has a particular species diversity gradient, with regions where the overall species diversity is very low (pure pine forest) and areas with a higher mixture of different tree species.

Twenty squared study plots were randomly placed within the forest, with a size for each plot of 1 ha (100m x 100m) defined following similar sampling designs (Schmidtlein and Fassnacht, 2017), (Rocchini, 2007), (Oldeland et al., 2010). The field campaign was carried out in summer 2017. All plots were geo-referenced with a GPS device (spatial accuracy $\pm 3m$) to obtain the exact position of the center and the four corners. Trees with a diameter at breast high (DBH) of at least 5 cm were classified by species. The number of individuals per plot ranged from 466 to 3196 (with a total amount of sampled individuals corresponding to 19827), attaining to a number of species ranging from 4 to 11.

221 Due to the low amount of species (total amount = 15 species) expected in
 222 this type of habitat (Gong et al., 1997), we decided to rely on the Shannon's
 223 H index (Equation 1) as a measure of species diversity calculated for each
 224 plot, starting from the sampled individuals. Shannon's H is one of the most
 225 frequently used ecological index, it is sensitive to both rarity and species
 226 abundance and has been used in different studies as a measure of alpha
 227 diversity (Madonsela et al., 2017), (Oldeland et al., 2010). Strictly speaking,
 228 making use of abundance based measures is expected to improve models of
 229 species versus spectral diversity (Oldeland et al., 2010).

$$H_e = - \sum_{i=1}^n p_i * \ln(p_i) \quad (1)$$

230 where:

231 H_e = Shannon's entropy used in ecology

232 n = total number of individuals

233 p = proportion of individuals attaining to species i relative to the total
 234 number of individuals

235 2.2 Remote sensing data

236 The SVH has been tested using NDVI time-series derived from Sentinel-2
 237 and Landsat 8 OLI for the years 2016 and 2017. The choice to use NDVI has
 238 been based on the assumption of previous studies (Madonsela et al., 2017),
 239 (Gillespie, 2005), (Parviainen et al., 2010) that variability in NDVI is related
 240 to species diversity. NDVI is one of the most widely used remote-sensing
 241 based vegetation indices to quantify the biomass of an ecosystem. NDVI
 242 derived from remotely sensed observations is related to the energy exchanged
 243 in an ecosystem and with primary productivity (Parviainen et al., 2010).
 244 Such relation has been found to be a valid indicator of regional variation
 245 in species diversity. Furthermore we hypothesize that the small variation in
 246 reflectance of different leaf traits, typical of specific trees can be captured by
 247 NDVI (He et al., 2009). All images with the lowest amount of noise (e.g.,
 248 snow, shadows, clouds, aerosols) were used for this purpose (Appendix 1).
 249 Sentinel-2A and 2B satellite images (Level-1C) acquired with the relative
 250 orbit numbers R022 and R065 and provided as 32TPS were downloaded
 251 from the ESA's Sentinel Scientific Data Hub. The Landsat 8 images (Level-
 252 1TP) with a Worldwide Reference System (WRS) path of 192 and 193, and
 253 WRS row of 027 and 028 were obtained from the Earth Explorer Hub of the

254 USGS. Both products were radiometrically calibrated and orthorectified by
 255 the providers using ground control points and digital elevation models. Due
 256 to the different radiometric data formats, we converted Landsat OLI Digital
 257 Numbers (DNs) to top of atmosphere (TOA) reflectance (Equation 2) to
 258 allow comparability with the Sentinel-2 Level-1C data format (Bhardwaj et
 259 al., 2015) .

$$\rho_{\lambda} = \frac{M_{\rho}Q_{cal} + A_{\rho}}{\cos(\theta_{SZ})} \quad (2)$$

260 where:

261 ρ_{λ} = TOA reflectance

262 M_{ρ} = band-specific multiplicative rescaling factor

263 Q_{cal} = quantized and calibrated standard product pixel values

264 A_{ρ} = band-specific additive rescaling factor from the metadata

265 $\cos(\theta_{SZ})$ = cosine of local solar zenith angle

266 NDVI was derived for both sources of images, following the standard
 267 formula:

$$NDVI = \frac{NIR - RED}{NIR + RED} \quad (3)$$

268 where:

269 NIR = Near Infrared band

270 RED = Red band

271 NIR 8th and 5th band were used for the Sentinel-2 and Landsat 8 re-
 272 spectively, and RED 4th band was used for both the satellites. The spatial
 273 resolution of bands 4 and 8 of Sentinel-2 was 10 m, while the spatial resolu-
 274 tion of band 4 and 5 of Landsat 8 was 30m. The derived NDVI had then a
 275 spatial resolution of 10 and 30 m respectively for Sentinel-2 and Landsat 8.

276 2.3 Spectral heterogeneity indices

277 The SH was calculated for all 20 plots for all images and for both satellites
 278 using the derived NDVI images. Two indices were used to calculate the SH:
 279 the Rao's Q and the Coefficient of Variation (CV).

280 The Rao's Q diversity index (Rao, 1982) was proposed by Botta-Dukat
 281 (2005) as a measure of functional diversity in Ecology with the following
 282 formula:

$$Q_e = \sum_{i=1}^{C-1} \sum_{j=i+1}^C d_{ij} * p_i * p_j \quad (4)$$

283 where:

284 Q_e = Rao's Q used in ecology

285 p = relative abundance of a species in a community (C)

286 d_{ij} = distance between the i-th and j-th species ($d_{ij} = d_{ji}$ and $d_{ii} = 0$)

287 i = species i

288 j = species j

289 Botta-Dukat (2005) proposed that d_{ij} can be calculated considering the
 290 differences in character/traits of the considered species, using different dis-
 291 tance functions (Legendre and Legendre, 1998), (Podani et al., 2000) such as
 292 the Euclidean distance divided by the number of the traits/characters:

$$d_{ij} = \frac{1}{n} \sum_{k=1}^n (X_{ik} - X_{jk})^2 \quad (5)$$

293

294 or the mean character difference:

295

$$d_{ij} = \frac{1}{n} \sum_{k=1}^n |X_{ik} - X_{jk}| \quad (6)$$

296

297 where:

298 n = number of traits considered

299 X_{ik} = value of trait k in species i

300 X_{jk} = value of trait k in species j

301 Rocchini et al. (2017) applied the Rao's Q index to optical remote sensing
 302 data, as SH measure, using the distance d_{ij} among pixel values of an image,
 303 and their relative abundance, calculated as:

$$Q_{rs} = \sum_{i=1}^{F-1} \sum_{j=i+1}^F d_{ij} * p_i * p_j \quad (7)$$

304 where:

305 Q_{rs} = Rao's Q applied to remote sensing

306 p = relative abundance of a pixel value in a selected plot image (F)

307 d_{ij} = spectral distance between the i-th and j-th pixel value ($d_{ij} = d_{ji}$
308 and $d_{ii} = 0$)

309 i = pixel i

310 j = pixel j

311 The distance matrix, where the d_{ij} is computed, can be built in differ-
312 ent dimensions, allowing to consider more than one layer/band at time. If
313 only one layer is considered, like in our case where a NDVI data-set was
314 used, d_{ij} can be calculated as a simple Euclidean distance. If more layers
315 are considered, d_{ij} can be computed relying for example on the Euclidean,
316 Manhattan and Canberra distances (Rocchini et al., 2017). The formulas of
317 such distances, with their advantages and disadvantages have been described
318 by Rocchini et al. (2017) together with a straightforward R-package function
319 (*spectralrao()*) to calculate the Q_{rs} in a single or multi-dimensional environ-
320 ment, allowing to calculate the Rao's Q with a moving window of different
321 sizes. In our case we decided to implement the function to obtain a single
322 value of Rao's Q for the whole plot area (100m x 100m in our case) represent-
323 ing our local landscape. This choice was made for an appropriate comparison
324 with the data of species diversity that were collected at plot level.

325 The CV (formula 8) has been also widely used in different researches as
326 SH index (Gholizadeh et al., 2018), (Levin et al., 2007) , and was calculated
327 as:

$$CV = SD/\bar{x} \quad (8)$$

328 where:

329 CV = Coefficient of Variation

330 SD = Standard Deviation of NDVI for each plot image

331 $\bar{x}$ = mean of the NDVI value of each plot image

332 For each plot, the species diversity estimated through the Shannon's H
333 derived from in-situ data and the spectral heterogeneity derived from the

334 two indices for the Sentinel-2 and Landsat 8 images were correlated by linear
335 regression. The approach is summarized in Figure 2.

336 For all the twenty plots, a time-series of NDVI values for the available
337 images was obtained (mean of the pixel of each plot) to understand the
338 temporal variation within the year and the relation to the SVH.

339 3 Results

340 3.1 Sentinel-2

341 Figures 3 A and C (see also Table 1) show the temporal trend of the coefficient
342 of determination (R^2) between Shannon's H derived from field data and two
343 SH indices derived from the Sentinel-2 NDVI images for 2016 and 2017,
344 respectively. Figures 3 B and D show the NDVI time-series as the mean NDVI
345 value of all the twenty study areas for the year 2016 and 2017 respectively.
346 The boxes show the 25th and 75th quantile, the dots represent the outliers
347 (see Appendix 2 for all the sampling plot based NDVI time series). The R^2
348 of Rao's Q and CV had a similar trend, reaching the highest value between
349 June and July (180th-200th day of the year) and decreasing towards winter
350 and spring similarly to the NDVI time-series.

351 For both years, the Rao's Q index showed the highest relation, reaching
352 values of $R^2=0.48$ and $R^2=0.70$ for the year 2016 and 2017, respectively
353 (Table 1). The difference in R^2 between the two years was mainly related to
354 a combined effect of missing images in 2016 (in particular during the peak
355 of NDVI) due to cloudiness and the absence of the Sentinel-2 B satellite
356 (available since July 2017).

357 3.2 Landsat 8

358 Figures 4 A and C (see also Table 2) show the temporal trend of the relation
359 between the Shannon's H derived from field data and the two SH indices
360 calculated from Landsat 8 NDVI images. The NDVI time-series, derived
361 as the mean NDVI value of the twenty plots is shown in Figures 4 B and
362 D for year 2016 and 2017, respectively (see Appendix 2 for all the related
363 linear regressions). Also in this case, the relation between field-derived and
364 remotely derived Rao's Q and CV had a similar trend of NDVI, reaching the
365 highest R^2 value when the vegetation index is at its peak.

366 For both years, irrespective of the sensor, the Rao's Q index showed higher
367 relation with field data in comparison with the CV (Figure 3). For 2017, the
368 relation between field-derived Shannon's H and the indices calculated from

369 Landsat 8 images were lower than those resulting from Sentinel-2 images. On
370 the contrary, no particular differences were observed between the two sensors
371 in 2016. The regression model together with R^2 and p values for all the plots
372 are shown in Appendix 2.

373 Since the analysis are based on testing multiple hypotheses, the p-values,
374 used as a measure of significance, were corrected with the Benjamin-Hochberg
375 correction (Benjamini and Hochberg , 1995). The corrected p-values confirm
376 the strong relation between species and spectral diversity. In fact, the relation
377 between the SH calculated with Rao's Q (for both Sentinel-2 and Landsat
378 8) and the field based Shannon's H were, near or at the NDVI peak period,
379 always significant. The CV did not show the same level of significance, having
380 for some years and sensors (e.g. Landsat 8 in 2017 and Sentinel-2 in 2016)
381 high p-values (Appendix 2).

382 4 Discussion

383 In this paper the Spectral Hypothesis has been tested for the first time in an
384 alpine coniferous forest to estimate the tree species diversity. We tested the
385 goodness of the Rao's Q as a new SH index. Rao's Q showed higher correlation
386 to field measurement than the widely used CV, since it directly accounts
387 for both distance among pixel values as well as their relative abundance in
388 a single formula. The CV showed similar results, with a trend similar to the
389 Rao's Q index but with a lower level of relation compared to the Rao's Q.
390 The CV has been used among the others by Wang et al. (Wang et al., 2018)
391 showing that the relative contributions of richness, evenness, and composition
392 to the spectral reflectance variability, in a hyperspectral data-set of a
393 prairie ecosystem, could not be discriminated.

394 In our research, we also took in consideration the use of Shannon's H as
395 SH index (Rocchini et al., 2017). We realized that this index cannot be used
396 as a measure of spectral in an image with continuous (floating) values such as
397 the NDVI. The pixel values cannot be treated as categories and it is highly
398 unlikely that two pixels will have exactly the same value, thus the calculation
399 result will only depend on the number of pixels considered. As an example
400 having an image of four pixels with the following numbers: IMG1(1, 7, 8 ,
401 10) will lead to the same Shannon's value of an image having the following
402 numbers: IMG2(1, 10, 100, 200), since every value occupies the same area
403 (25%) of the image. In both cases, Shannon's H would be 1.386294. On the
404 contrary Rao's Q takes into account the distance among values besides their
405 relative abundance, reaching a value of 3.5 for IMG1 and a value of 85.9 for

406 IMG2.

407 This paper highlighted the importance of a time-series approach in testing
408 the SVH. Our results showed that the relation between field data and all the
409 SH indices vary strongly within the year. In 2017 for the Rao's Q index,
410 minimum values are generally found in winter ($R^2=0.16$) while maximum
411 values are reached in summer ($R^2=0.7$) when the NDVI reached its peak.
412 Previous studies focused primarily just on a specific period of the year and
413 obtaining in general lower level of relationship. Rocchini et al. (Rocchini,
414 2007) for example tested the SVH to estimate the species richness of a wetland
415 ecosystem in Central Italy using a single image of June 2002 reaching a R^2
416 value of 0.48. Madonsela et al. (Madonsela et al., 2017) compared data
417 of alpha diversity from the Savannah woodland in southern Africa with the
418 spectral variability of single bands and different vegetation indices derived
419 from three Landsat 8 images in March 2016 (end of the wet season), reaching
420 a maximum R^2 value of 0.41.

421 Our results support one of the main outcomes of a study previously car-
422 ried out by Schmidtlein and Fassnacht (2017), who underlined that the re-
423 lation between spectral variability and species is season-dependent. Also
424 Feilhauer and Schmidtlein (2011) showed that the relation between species
425 composition and reflectance changes over time, with the highest correlation
426 near the vegetation optimum. In this paper, a significant relationship be-
427 tween tree species diversity and the SH of NDVI was shown, particularly
428 when the vegetation index reached the highest seasonal values. This is due
429 to the fact that, when NDVI reaches its highest values, it is able to capture
430 small variation in reflectance of different leaf traits typical of specific trees
431 (He et al., 2009). According to Parviainen et al. (2010), species richness,
432 related to the habitat heterogeneity is reflected by the variability of NDVI.
433 Our study was not the first attempt to test the SVH with NDVI data, previ-
434 ous studies tested it, without using a multi-temporal approach: Levin et al.
435 (2007) tested the spectral variation of NDVI images derived from Landsat
436 7 (ETM +), Aster and QuickBird to examine the relationship with plant
437 richness and rarity in Mediterranean forests. Gillespie (2005) predicted the
438 richness of woody-plant species in a tropical dry forest, testing the SVH with
439 a single image of NDVI derived from a Landsat 7 (ETM +) image. The
440 variation of three NDVI images, derived from Landsat 8, has been used by
441 Madonsela et al. (2017) to quantify tree species diversity in Savannah wood-
442 land.

443 This research confirmed that the SVH is scale and sensor dependent: a
444 decrease in spatial resolution influences the spectral heterogeneity creating

445 mixed pixels that can threaten the ability of matching spectral heterogeneity
446 with field heterogeneity. Sentinel-2 data showed better results testing the
447 SVH due to its high-intermediate spatial resolution (10m) peculiar to dis-
448 criminate forest trees. The quick revisit time of the new European satellite (5
449 days at equator with both the Sentinel-2 A and B) gives also the possibility
450 to acquire several images per year, particularly important for the study of
451 the SVH in alpine regions, where the meteorological conditions (for example
452 clouds, haze, snow, topographic effects) are not always optimal. Focusing
453 on the Sentinel-2 results, the difference in R^2 between 2016 and 2017 was
454 evident, underlying the importance of the number of available images for the
455 application of SVH.

456 Concerns may arise about the approach that we used: the Sentinel-2 data
457 could have been re-scaled to 30 meter resolution without using the Landsat-8
458 satellite but, as underlined by Rahbek (2005), the spectral variability depends
459 on the scene and on the sensors; research that deal with the effects of spatial
460 and spectral resolution should not ignore these two important aspects.

461 In this study, the effect of the grain size of sampling units in the study
462 area has not been investigated. The grain effect on the link between SH and
463 species diversity have been examined by different authors: Rocchini et al.
464 (Rocchini et al., 2004), applying the SVH to a wetland ecosystem found out
465 that, the increase of the spatial scale of analysis (from 100m² to 1ha) brings
466 to a higher correlation between spectral heterogeneity and species richness.
467 Similar results were obtained by Oldeland et al. (2010) who estimated vege-
468 tation species diversity in Central Namibia, finding that correlation improves
469 while increasing the window of analysis (from 100m² to 1000m²). This issue
470 called Modifiable Areal Unit Problem (MAUP) that involves many studies of
471 landscape ecology has been well discussed and analyzed by Jelinski and Wu
472 (Jelinski and Wu, 1996). From our side, it was very difficult to study this
473 effect, in particular with the Landsat 8 data that, with a lower resolution
474 (30m) does not allow to test the SVH in smaller areas (already limited using
475 nine pixel).

476 An additional concern that may emerge could deal with the small extent
477 of the study area, a dense alpine coniferous forest dominated mainly by pines,
478 larches and spruces. Other studies tested the SVH in relative small areas,
479 considering a limited number of plots. Gould et al. (2000) tested the SVH in
480 the Hood river region of the central Canadian arctic using 17 plots of 0.5km²
481 size. Rocchini et al. (Rocchini et al., 2004) used 22 plots to test the spectral
482 variation of multispectral images for the estimation of the species diversity in
483 a wetland area in Central Italy. This study represents a first step to under-
484 stand the relation between the spectral variability and the species diversity in
485 an alpine coniferous forest, since no similar studies have been carried out in

486 this ecosystem to date. This point represents a typical bias of any empirical
487 study and the results of this research can probably be applicable to wider
488 areas on the strength of the general relation between SH and species diversity
489 (Rocchini, 2007). As stressed in the introduction, the SVH is not based on
490 catching directly species diversity in the field, but on using indirect measures
491 based on environmental heterogeneity. The spectral variability of a remotely
492 sensed image can be directly related to environmental heterogeneity which
493 in turn might be a proxy of species diversity. Yet the biodiversity of a cer-
494 tain ecosystem is affected by much more than environmental heterogeneity
495 alone; however, as we stated in our objectives, the main aim of this paper
496 was to test the reliability of the SVH as a proxy for tree species diversity in
497 a coniferous forest ecosystem.

498 5 Conclusion

499 This study has focused on the relation between tree species diversity and
500 spectral variation of a multi-temporal NDVI time-series, derived from Sentinel-
501 2 and Landsat 8 satellites, in an alpine coniferous forest ecosystem. The SVH
502 has been tested with the new SH index Rao's Q and compared with the CV,
503 a well known SH index. The Rao's Q performed better than the CV index,
504 particularly the Sentinel-2 data. This research underlined the relevance of
505 the NDVI in the study of the SVH, therefore proving the importance of the
506 multi-temporal approach.

507 As we stressed in the introduction, in the past decades, the SVH has been
508 tested in different ecosystems, with various remote sensing optical data, using
509 several new SH indices reaching in general discrete to positive results similar
510 to those of this research. While the approach being proposed has successfully
511 led to a clear relationship between spectral and field diversity, we are aware
512 that further tests should be conducted in different environments before the
513 approach can be considered as a generalizable method, as also pointed out
514 by Schmidtlein and Fassnacht (2017).

515 The study of species diversity through remote sensing data in complex
516 landscapes such as the Alps and alpine habitats, like the coniferous forests
517 considered in this study, gives the opportunity to rapidly estimate biodiver-
518 sity in highly topographically complex regions to further potentially guide
519 field sampling. The results of this research, can be used in a practical way
520 as a "first filter" in the localization of biodiversity hotspots (Rocchini, 2007)
521 or in the prediction of spatial changes over time.

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Figure 1: The study area located in the municipality of San Genesio-Jenesien (South Tyrol) Italy. The 20 plots are indicated by black squares.

Table 1: R^2 derived from the linear regression between the two SH indices (Rao's Q and CV) and the species diversity (Shannon's H field based) for the Sentinel-2 images. The higher relation values for each year and SH index are in bold.

	Date	Rao's Q	CV
2016	03.20	0.23	0.18
	04.12	0.40	0.37
	05.22	0.27	0.22
	06.22	0.47	0.35
	07.18	0.42	0.32
	08.27	0.32	0.26
	09.06	0.36	0.31
	09.09	0.26	0.23
	09.26	0.29	0.23
	10.16	0.27	0.19
	10.29	0.31	0.27
	12.15	0.31	0.19
2017	01.07	0.34	0.25
	02.16	0.36	0.33
	03.25	0.35	0.31
	03.28	0.36	0.33
	04.14	0.16	0.14
	05.17	0.15	0.09
	05.27	0.37	0.25
	06.13	0.7	0.61
	06.26	0.61	0.54
	07.06	0.61	0.56
	07.08	0.56	0.52
	07.18	0.62	0.56
	08.07	0.62	0.57
	08.22	0.5	0.45
	08.30	0.59	0.55
	09.21	0.43	0.36
	10.09	0.24	0.2
	10.16	0.22	0.16
	10.24	0.36	0.31
	11.15	0.43	0.35
	12.15	0.27	0.18

Table 2: R^2 derived from the linear regression between the two SH indices (Rao's Q and CV) and the species diversity (Shannon's H field based) for the Landsat 8 images. The higher relation values for each year and SH index are in bold.

	Date	Rao's Q	CV
2016	02.24	0.05	0.04
	03.20	0	0.01
	04.12	0.06	0.06
	05.07	0.07	0.06
	06.24	0.48	0.37
	07.10	0.31	0.27
	07.17	0.27	0.23
	09.12	0.14	0.11
	10.05	0.04	0.03
	10.23	0.11	0.1
	10.30	0.12	0.1
	11.15	0	0.01
	12.08	0.06	0.05
	12.17	0.02	0.04
2017	01.02	0.02	0.03
	01.18	0.16	0.13
	01.25	0.05	0.06
	03.14	0.16	0.15
	04.08	0.11	0.11
	05.17	0.11	0.06
	05.26	0.25	0.2
	06.11	0.32	0.25
	06.18	0.42	0.35
	07.04	0.38	0.34
	08.05	0.3	0.26
	08.30	0.21	0.18
	09.22	0.13	0.1
	11.18	0.15	0.13
	12.04	0.12	0.1
	12.20	0.01	0.03

Figure 2: Flowchart representing the approach we used in this study: the linear regressions between field data and remote sensing data were calculated for both sensors (Sentinel-2 and Landsat 8) for the NDVI time-series (2016 and 2017), using two different spectral heterogeneity indices.

Figure 3: Sentinel-2: figures A and C show the time-series of the determination coefficient (R^2) between the field-based Shannon's H and the SH indices for the year 2016 and 2017 respectively. Points indicate the R^2 for the considered day. The line represent the smooth local regressions between the points. Figures B and D show the time-series statistics of mean NDVI value of all the study areas for both years. The boxes show the 25th and 75th quantile, the dots represent the out layers. The single relations for all the points and the NDVI time-series of the other areas are in the Appendix 2 of this paper.

Figure 4: Landsat 8: figures A and C show the time-series of the determination coefficient (R^2) between the field-based Shannon's H and the SH indices for the year 2016 and 2017 respectively. Points indicate the R^2 for the considered day. The line represent the smooth local regressions between the points. Figures B and D show the time-series statistics of mean NDVI value of all the study areas for both the years. The boxes show the 25th and 75th quantile, the dots represent the out layers. The single relations for all the points and the NDVI time-series of the other areas are in the Appendix 2 of this paper.