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Grassland vertical height heterogeneity predicts flower and bee diversity: an UAV photogrammetric approach

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1 Grassland vertical height heterogeneity  
2 predicts flower and bee diversity: an UAV  
3 photogrammetric approach

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## Abstract

The ecosystem services offered by pollinators are vital for supporting agriculture and ecosystem functioning, with bees standing out as especially valuable contributors among these insects. Threats such as habitat fragmentation, intensive agriculture, and climate change are contributing to the decline of natural bee populations. Remote sensing could be a useful tool to identify sites of high diversity before investing into more expensive field survey. In this study, the ability of Unoccupied Aerial Vehicles (UAV) images to estimate biodiversity at a local scale has been assessed while testing the concept of the Height Variation Hypothesis (HVH). This hypothesis states that the higher the vegetation height heterogeneity (HH) measured by remote sensing information, the higher the vegetation vertical complexity and the associated species diversity. In this study, the concept has been further developed to understand if vegetation HH can also be considered a proxy for bee diversity and abundance. We tested this approach in 30 grasslands in the South of the Netherlands, where an intensive field data campaign (collection of flower and bee diversity and abundance) was carried out in 2021, along with a UAV campaign (collection of true color -RGB- images at high spatial resolution). Canopy Height Models (CHM) of the grasslands were derived using the photogrammetry technique "Structure from Motion" (SfM) with horizontal resolution (spatial) of 10 cm, 25 cm, and 50 cm. The accuracy of the CHM derived from UAV photogrammetry was assessed by comparing them through linear regression against local CHM LiDAR (Light Detection and Ranging) data derived from an Airborne Laser Scanner campaign completed in 2020/2021, yielding an  $R^2$  of 0.71. Subsequently, the HH assessed on the CHMs at the three spatial resolutions, using four different heterogeneity indices (Rao's Q, Coefficient of Variation, Berger-Parker index, and Simpson's D index), was correlated with the ground-based flower and bee diversity and bee abundance data. The Rao's Q index was the most effective heterogeneity index, reaching high correlations with the ground-based data (0.44 for flower diversity, 0.47 for bee diversity, and 0.34 for bee abundance). Interestingly, the correlations were not significantly influenced by the spatial resolution of the CHM derived from UAV photogrammetry. Our results suggest that vegetation height heterogeneity can be used as a proxy for large-scale, standardized, and cost-effective inference of flower diversity and habitat quality for bees.

**Keywords:** biodiversity, photogrammetry, pollinators, habitat suitability, insect diversity, structural habitat diversity

# 71 1 Introduction

72 In the last decades, we have witnessed a decrease in plant and insect biodi-  
73 versity in agricultural landscapes, resulting in the loss of benefits for crops  
74 and humans [31, 28]. The causes of this can be found in changes of land  
75 use causing habitat loss and fragmentation [27, 69, 76], increasingly inten-  
76 sive agriculture, and climate change [71]. All these factors have affected the  
77 presence of particular niches for different types of insects [31]. Yet insect pol-  
78 linators are essential for the maintenance of wild plant species, contributing  
79 to cultural ecosystem services and agricultural yields [6, 18]. They play a  
80 crucial role in the long-term sustainability of plant communities, and their  
81 loss can lead to a decline in plant diversity, altering vegetation composition  
82 [84]. The economic value of insect pollinators is immense, with estimates sug-  
83 gesting that they contribute to global food production worth more than 150  
84 billion euros per year [21, 20, 54]. Therefore, insect pollinators are essential  
85 for maintaining the health and productivity of both agricultural and natu-  
86 ral ecosystems, as well as for ensuring a continued provisioning of ecosystem  
87 services [30].

88 Earth observation and remote sensing data have become valuable tools  
89 for estimating different aspects of biodiversity worldwide [63, 65]. Significant  
90 advancements in sensor technology (with increased spatial and spectral res-  
91 olution) and vectors (able to cover large areas with higher revisit frequency)  
92 have made remote sensing rapid and cost-effective to obtain extensive envi-  
93 ronmental data at various temporal and spatial scales [7]. Over the past few  
94 years, there has been a development of different methods and techniques uti-  
95 lizing remote sensing data to assess biodiversity at various spatial levels [7].  
96 Some of these approaches rely on indirect associations between the variability  
97 of remotely sensed information and species diversity [82, 81]. Notably, recent  
98 investigations have specifically concentrated on exploring the link between  
99 LiDAR data and species diversity. This approach, called "Height Variation  
100 Hypothesis" (HVH), states that, in a considered ecosystem, the higher the  
101 vegetation height heterogeneity (HH) assessed by LiDAR information, the  
102 higher the availability of different niches that can host more diverse species.  
103 Vertical vegetation structure, which encompasses aspects of habitat hetero-  
104 geneity, plays a critical role in supporting biodiversity. It is considered one  
105 of the drivers of biodiversity, directly influencing species distribution and  
106 diversity, population dynamics, and ecological interactions [41]. By provid-  
107 ing a variety of microhabitats and vertical niches, the vertical vegetation  
108 structure offers opportunities for different species to find suitable habitats  
109 and resources, promoting species coexistence and enhancing overall biodi-  
110 versity. It contributes to ecosystem stability and resilience, making it a key

111 component in conservation and management efforts aimed at preserving and  
112 enhancing biodiversity in various ecosystems [26]. Torresani et al. [80, 79]  
113 tested this approach positively in different forested areas using both Airborne  
114 Laser Scanning (ALS, where the LiDAR sensor is mounted on an aircraft)  
115 and space-borne GEDI (Global Ecosystem Dynamics Investigation) LiDAR  
116 data [16, 14, 34, 53] for the assessment of tree species diversity. Tamburlin et  
117 al. [72] also tested the methodology in forested areas using ALS LiDAR data,  
118 showing that the Canopy Height Model (CHM) is the most appropriate Li-  
119 DAR metric for an accurate estimation of vegetation height heterogeneity  
120 and inference of species diversity. The approach has been used not only to  
121 assess vegetation diversity but also to estimate animal diversity, different  
122 studies showed that the variability in habitat structure has a significant ef-  
123 fect on the bird diversity in both agricultural and forest ecosystems [2, 43].  
124 However, there is limited research specifically on the correlation between veg-  
125 etation structure and insect diversity, particularly at a fine scale observed in  
126 grasslands.

127 In this paper, we aim to test this approach in a grassland ecosystem to  
128 understand if the vegetation grassland HH assessed through remote sensing  
129 techniques can be considered a proxy for flower diversity and subsequently  
130 for bee diversity and abundance. As grassland vegetation structures occur  
131 at very fine spatial scales, there is a need for structural information at a  
132 very high spatial resolution. While there have been a few studies [40] explor-  
133 ing the use of LiDAR for grassland characterization, the limited available  
134 evidence introduces uncertainty regarding its effectiveness in this context.  
135 ~~Furthermore ALS data depend on a dedicated aircraft campaign, and for~~  
136 ~~this reason, they might be relatively expensive.~~ Furthermore, while ALS  
137 data depend on a dedicated aircraft campaign and may involve higher costs,  
138 operational testing of our hypothesis on Unoccupied Aerial Vehicles (UAVs)  
139 data might provide a practical and scalable approach. The recently devel-  
140 oped technology centered around these new vectors, specifically photogram-  
141 metry that employs structure-from-motion algorithms, has resulted in the  
142 creation of highly precise orthomosaics and 3D information across vast areas  
143 at a relatively low expense, with spatial resolutions ranging from centime-  
144 ters to millimeters suitable to derive information on vegetation structure [1].  
145 Previous researches [77, 33, 38, 9, 10] has demonstrated that UAV imagery  
146 can be utilized to gauge vegetation attributes, including diversity, species,  
147 and plant species distribution, as well as to map and track invasive species.  
148 In this context, our prior study [77] established, in the same study area, a  
149 positive correlation between flower cover, estimated through UAV images,  
150 and bee diversity, further emphasizing the versatility of UAV technology in  
151 understanding and quantifying key ecological relationships.

152 The aim of this study is to test whether we can estimate flower diversity  
153 and bee abundance and diversity by testing the Height Variation Hypothesis  
154 in as highly dense and fine structured ecosystem such as grasslands by us-  
155 ing 3D information derived with photogrammetric analysis using UAV RGB  
156 (true-colored) images at high spatial resolution (Figure 1). Specifically, we  
157 assessed the HH with four different heterogeneity indices (Rao's Q, Coeffi-  
158 cient of Variation - CV -, Berger-Parker index and Simpson's D index) using  
159 CHM data derived from UAV photogrammetric analysis previously validated  
160 with local ALS ~~LiDAR~~ data. Successively, we correlated the derived HH with  
161 field-derived flower and bee diversity (species richness) and abundance. Fi-  
162 nally, we investigated the influence of varying spatial resolutions (10 cm, 25  
163 cm, and 50 cm) on the observed relationships. Our study focuses on grass-  
164 lands located in the southeastern region of the Netherlands, which exhibit a  
165 range of management intensities, resulting in varying degrees of flower cover.

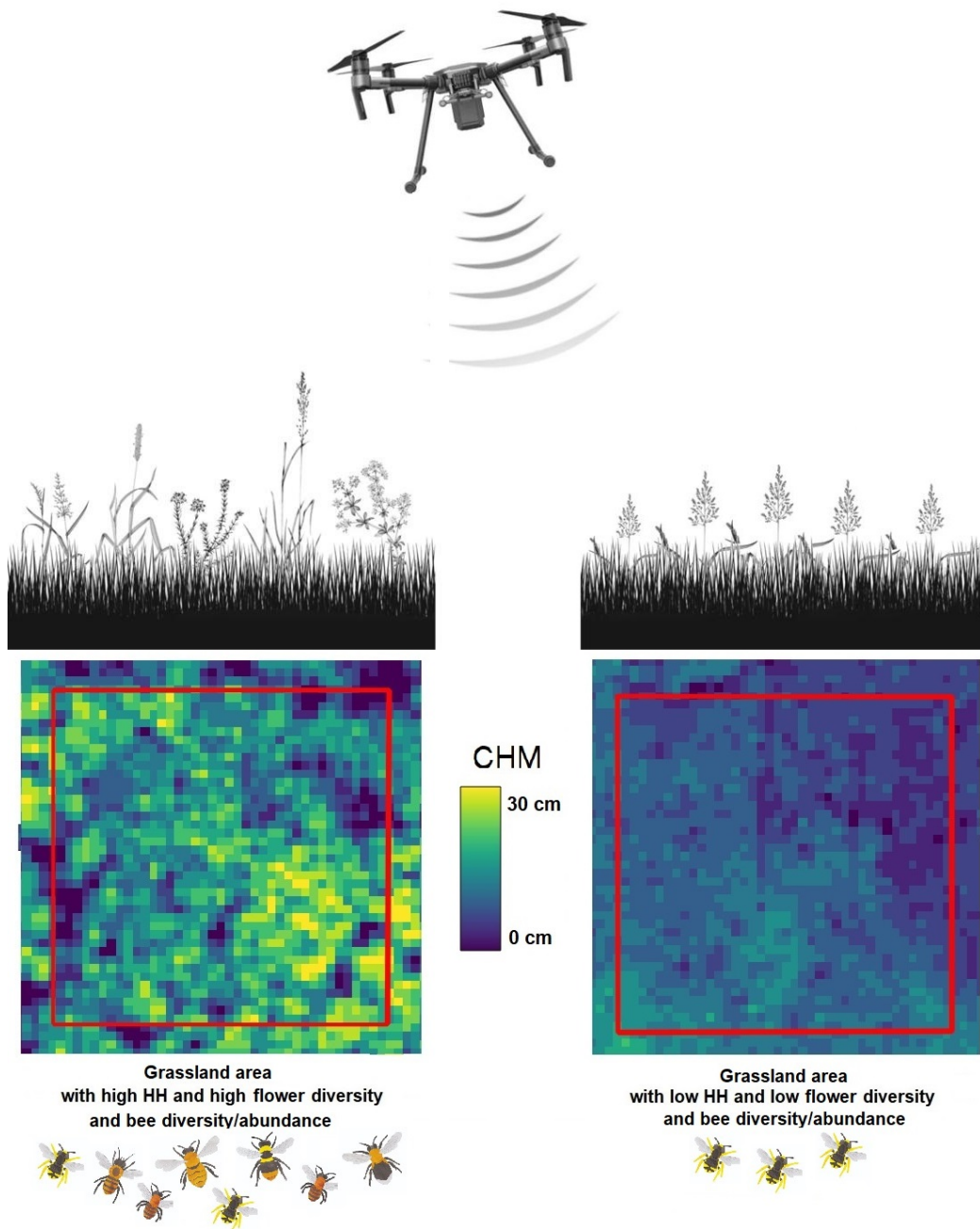


Figure 1: A graphical summary of the main expectations of this study. Grassland ecosystems with high HH (assessed through CHM derived by UAV photogrammetric images) with a complex vertical structure (seen from the side in the upper figure and from above in the lower figure) and high environmental heterogeneity are expected to have a high flower diversity and high bee diversity and abundance (figure on the left). On the other hand, grassland areas with low HH might have lower flower diversity and bee diversity and abundance (figure on the right).



166 **2 Materials and Methods**

167 **2.1 Study areas**

168 The study areas (approximately 70 km<sup>2</sup> with elevations ranging from 70 to  
169 171 m asl) are located in the southeast of the Netherlands, near the village  
170 of Gulpen (Fig. 2). Thirty grasslands representing a range of land use inten-  
171 sities, from nutrient-poor, biodiversity-rich semi-natural grasslands to inten-  
172 sively fertilized areas, were chosen in order to test the proposed approach.  
173 Management of the grasslands included mowing (16 sites), grazing (10 sites)  
174 and mixed regimes (4 sites), ranging in intensity from one to five uses per  
175 year (details in Appendix Table 1). Data collection for this study took place  
176 before the first cut but extensive grazing (<2 LSU/ha) had occurred at most  
177 grazed plots. Percent herb cover ranged from 0.1% to 69%, with the most  
178 dominant species in terms of flower cover being *Ranunculus repens*, *R. acris*  
179 and *R. bulbosus*, *Leucanthemum vulgare*, *Trifolium pratense*, *Bellis perennis*  
180 and *Taraxacum sp.* (all >5% of the total flower area over all transects). The  
181 study areas are part of the experimental biodiversity area network of the  
182 EU Showcase project <https://showcase-project.eu/>. By selecting semi-  
183 natural, extensively utilized, and intensely managed grasslands from diverse  
184 regions, we reduced spatial clustering of distinct grassland types.

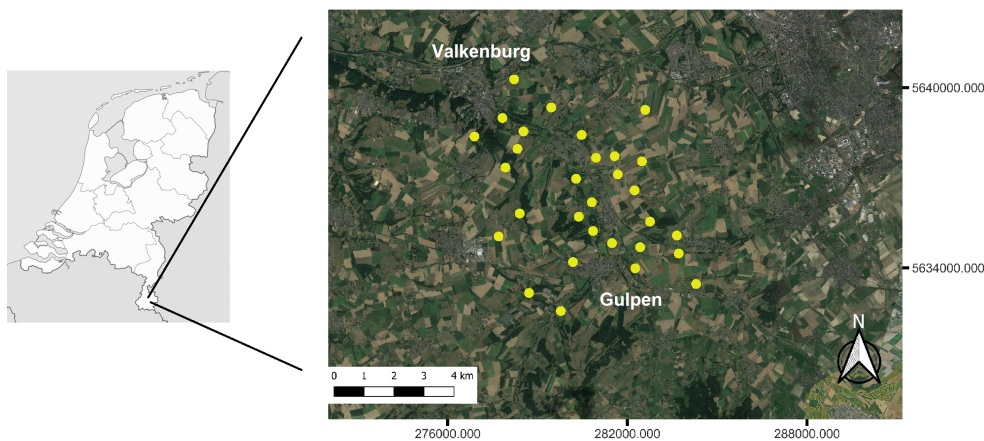


Figure 2: The study areas located in the Southeast of the Netherlands. The 30 plots transects within each study area are indicated by yellow dots (Basemap: Google Earth map as of August 2022).

## 185 2.2 Field data

### 186 2.2.1 Collection

187 In each study area, a transect measuring 150 m by 1 m was established  
188 and divided into three equal sections of 50 m. These transects were visibly  
189 marked with Ground Control Points (GCP) plates that could be identified  
190 by UAV imagery. GCP were positioned from the edge to the center of the  
191 grassland, covering differences in elevation heterogeneity within the grass-  
192 land helping successively to find our sampling locations on the images. To  
193 ensure a sampling of distinct bee populations, adjacent transects were gen-  
194 erally separated by distances greater than 500 m [56]. Previous studies [56]  
195 have shown that, although large-bodied bees like bumblebees can forage at  
196 distances of a few kilometers, their primary foraging distances are shorter,  
197 typically ranging between 250 m and 550 m. Smaller wild bees tend to for-  
198 age even closer to their nests. Along each transect, surveys were conducted  
199 for both bees and flowers. Transect walks, a standard method for studying  
200 plant-pollinator associations, were used to count both wild bees and honey-  
201 bee (*Apis mellifera*) [83]. The transects were surveyed by two observers who  
202 counted all bees within a meter in front of them while slowly walking along  
203 the transect for 15 min, excluding the time required for handling caught  
204 specimens. Species were identified using identification keys specific to Dutch  
205 Apidae [17, 46, 47]. While distinctive species could be identified in the field,  
206 other specimens were collected and identified in the laboratory using stereo-  
207 microscopes and, in some cases, expert consultation. Flower surveys were  
208 conducted in each transect, generally on the same day as the bee surveys,  
209 following the methodology described by Schepers et al. [70]. However, due to  
210 logistical constraints, some grasslands were surveyed one or two days before  
211 or after the bee surveys. Subsequent to the bee surveys, flower surveys were  
212 conducted in each transect at which the number of flowers within the 150 m  
213 x 1 m transect was counted per species [70]. Hence, only flowering species  
214 richness was recorded and abundance was measured in terms of flowering.  
215 Flower surveys were generally conducted on the same day as the bee sur-  
216 veys, but due to logistical constraints, some grasslands were surveyed one or  
217 two days before or after the bee surveys (details in Appendix Table 1). The  
218 surveys were conducted between May 12th and 31st, 2021, from 10 a.m. to  
219 5 p.m., under favorable weather conditions, which included dry conditions,  
220 more than 50% sunlight, temperatures of at least 15 degrees Celsius, and  
221 wind speeds below 2 Beaufort.

## 2.2.2 Ground-based diversity indices

The ground-based flower diversity was calculated using the species richness, namely the number of different flower species per plot transect. Also for the characterization of bee diversity, we relied on species richness. Bee abundance was defined as the total number of bees counted along each transect.

## 2.3 UAV Data Acquisition and Data Processing

The UAV data were acquired simultaneously with the field survey between May 12th and 31st, 2021. A RGB Zenmuse X5 camera (16.0 MP, 17.3 x 13.0 mm sensor) with an integrated RTK GPS was carried by the UAV "DJI Matrice 210 RTK". To simplify the production of the final point cloud and the digital elevation model, the images were taken at an overlapping rate of 80%. All flights were conducted at a height of approximately 20 m above the ground. The average spatial resolution of the resulting UAV images is 0.5 cm.

The Agisoft Metashape Professional Edition software was used to analyze and process the UAV images following three main procedural stages: image alignment, dense point cloud creation, and inference of the digital elevation model. In the first step, set with "high" accuracy, the software extracted features within the images and matched them to produce a sparse 3D point cloud. At this stage, the software automatically detected the precise features of the GCP and extracted the GPS coordinates for each of them. We maintained the "high" accuracy setting during the construction of the dense point cloud, which was subsequently exported as a LAS file. The mean point density for all 30 areas was 700 points/m<sup>2</sup> while the vertical resolution was around 15 mm. The Digital Surface Model (DSM) was derived at different spatial resolutions (10 cm, 25 cm, and 50 cm) using the "dsmtin" algorithm of the "rasterize\_canopy" function of the R package "lidR" [68]. This algorithm uses the Delaunay triangulation method to connect the points in the point cloud, forming a network of non-overlapping triangles. The resulting triangular irregular network (TIN) represents the surface, and rasterization is then applied to convert this TIN into a gridded DSM, providing a comprehensive representation of the terrain and vegetation structure. The Digital Terrain Model (DTM) was derived using the same function but with a prior filtering of the point cloud, selecting the lowest points every 50 cm. Finally, the CHM was derived by taking the difference between the DSM and DTM. The decision to set the finest spatial resolution at 10 cm was primarily driven by computational considerations.

## 259 2.4 Heterogeneity index

260 HH was calculated using the CHM at different spatial resolutions (10 cm, 25  
261 cm, and 50 cm) with four different heterogeneity indices: Rao's Q index, the  
262 CV, the Berger-Parker index, and the Simpson's D index [64].

263 The Rao's Q index, originally developed by Rao [55], was later recom-  
264 mended by Botta-Dukát [5] as a functional diversity index in ecology. Sub-  
265 sequently, Rocchini et al. [62] introduced this measure as a heterogeneity  
266 index for remote sensing data, employing the following equation 1:

$$Q = \sum_{i,j=1}^N d_{ij} \times p_i \times p_j \quad (1)$$

267 where:

268 Q = Rao's Q index, used in remote sensing application

269  $p_i = p_j = 1/N$  = relative abundance of pixel i, j in a selected area (i.e.  
270 in our case, raster over the transects) composed of N pixels

271  $d_{ij}$  = distance/dissimilarity between pixel i and j ( $d_{ij} = d_{ji}$  and  $d_{ii} = 0$ )

272 We determined  $d_{ij}$  as the Euclidean distance using a solitary layer (CHM  
273 raster).

274 The CV, widely employed as a measure of heterogeneity in various eco-  
275 logical studies [22, 35], is calculated using the following equation 2:

$$CV = (SD/\bar{x}) \times 100 \quad (2)$$

276 where:

277 CV= Coefficient of Variation

278 SD= Standard Deviation of the pixel values within a selected area

279  $\bar{x}$  = mean of the pixel values within a selected area

280 The Berger-Parker index is often used as a heterogeneity index in eco-  
281 logical studies and also with remote sensing data, it provides a measure of  
282 species/pixel dominance within a given community/data-set [86]. It has been  
283 calculated using the following equation 3:

$$BP = \frac{n_{\max}}{N} \quad (3)$$

284 where:

285 BP is the Berger-Parker heterogeneity index  
 286 -  $n_{\max}$  is the abundance of the most dominant pixel value in the data-set  
 287 -  $N$  is the total abundance of all pixels in the data-set.

288 The Simpson's D index is a diversity assessment measure frequently em-  
 289 ployed in ecology [33, 13]. It can also serve as a heterogeneity measure with  
 290 remote sensing data, relying solely on the relative abundance of pixels within  
 291 the specific plot or area [64]. It is calculated as (equation 4):

$$D = \sum_{i=1}^n p_i^2 \quad (4)$$

292 where:

293  $D$  = Simpson index

294  $n$  = total number of pixel's value

295  $p_i$  = relative abundance of a pixel value in a CHM raster plot

## 296 2.5 Validation of the UAV DTM and CHM

297 DSM and DTM with a spatial resolution of 50 cm derived from local LiDAR  
 298 data collected by an ALS LiDAR campaign carried out between 2020 and 2022  
 299 (AHN4 data-set, freely available for download here: <https://geotiles.nl/>)  
 300 were used to validate the UAV digital models. The LiDAR flight was conducted  
 301 on February 18th, 2021. DSM and DTM with a spatial resolution of 50 cm  
 302 derived from local Li-DAR data collected as part of an national ALS LiDAR  
 303 campaign carried out between 2020 and 2022 (AHN4 data-set, freely available  
 304 for download here: <https://geotiles.nl/>) were used to validate the UAV digi-  
 305 tal models. AHN datasets are systematically gathered every few years for all  
 306 of the Netherlands, by multiple operators and sensors, where the exact spec-  
 307 ifications may vary over time and space. AHN4 pointclouds have a vertical  
 308 resolution of 13 mm and a density of 10-14 point/m<sup>2</sup>. In our study area, the  
 309 LiDAR flight for AHN4 was conducted on February 18th, 2021. During this  
 310 season, the grassland vegetation is very low, resulting in the DSM and DTM  
 311 having equal elevations, effectively yielding a CHM value of zero. For this  
 312 reason, we decided to validate the UAV-DTM with the LiDAR-DTM using  
 313 10 random points within each study area (300 points in total). Additionally,  
 314 we validated the CHM over multi-annual visible vegetation-patch (e.g., small

315 shrubs) that could be visible in both the UAV-CHM and LiDAR-CHM. We  
 316 randomly selected a point over each multi-annual visible vegetation for each  
 317 study area (29 points in total) and correlated the digital models using linear  
 318 regression.

319 For both the DTM and CHM, the coefficient of determination ( $R^2$ ) was  
 320 used to estimate the goodness of fit of the model, while the  $P$  value was used  
 321 to measure its statistical significance.

## 322 2.6 Workflow

323 The approach proposed in this study is summarized in Figure 3. Firstly  
 324 (point 1), we validated the UAV DTM and CHM with DTM and CHM derived  
 325 from the local ALS LiDAR data. Then (point 2), for each transect, we  
 326 estimated HHs using the UAV CHM data at different spatial resolutions (10  
 327 cm, 25 cm, and 50 cm) with four different heterogeneity indices (Rao's Q  
 328 index, CV, Berger-Parker index and Simpson's D index). Subsequently, we  
 329 performed linear regression analyses to correlate the HHs with the ground-  
 330 based flower and bee diversity and bee abundance. The coefficient of deter-  
 331 mination ( $R^2$ ) was used to estimate the goodness of fit of the model, while  
 332 the  $P$  value was used to measure its statistical significance.

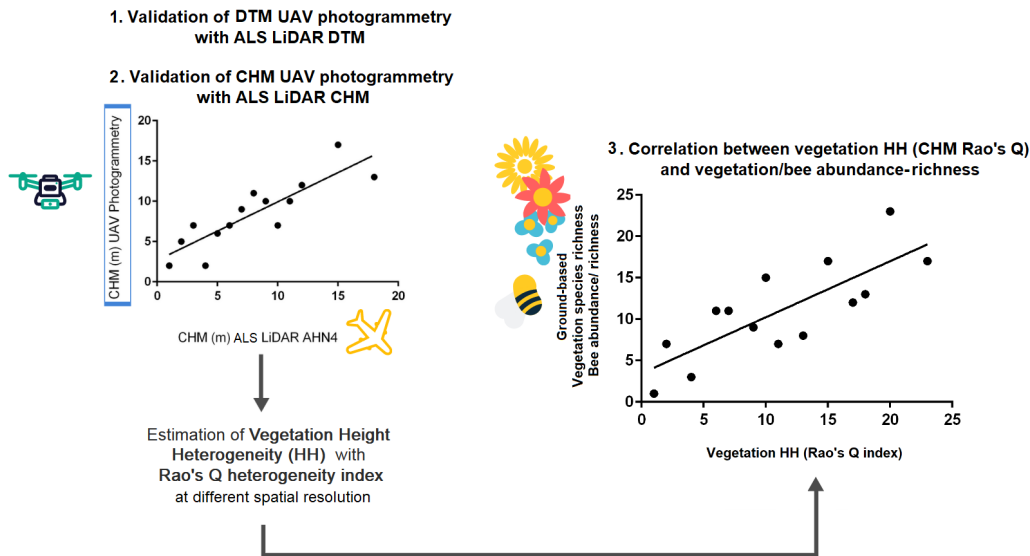


Figure 3: The image shows the workflow of the proposed approach.

333 **3 Results**

334 The validation of the DTM derived from UAV photogrammetry with local  
335 ALS DTM LiDAR data (AHN4 data-set) at a spatial resolution of 50 cm  
336 is shown in Figure 4. The linear regression analysis yielded a positive rela-  
337 tionship and strong correlation between the two variables. The correlation  
338 between the two variables is significant (p-value < 0.05), with a goodness of  
339 fit of 0.98. The UAV-derived DTM tends to be higher than the LiDAR DTM  
340 with a systematic average offset of 44 m (calculated as the difference of the  
341 mean's datasets).

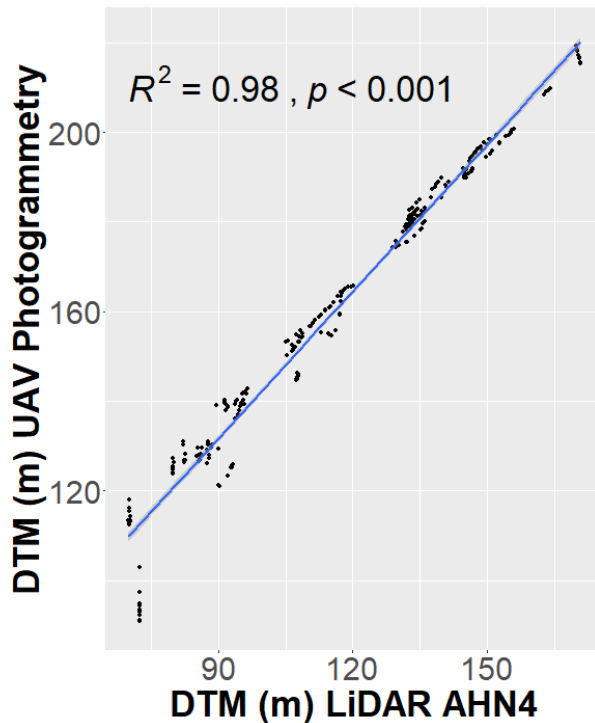


Figure 4: The validation of the DTM derived from UAV photogrammetry with the local LiDAR DTM AHN4 is shown with the blue line.

342 The validation of the CHM derived from UAV photogrammetry with lo-  
343 cal ALS CHM LiDAR data (AHN4 data-set) at a spatial resolution of 50  
344 cm is shown in Figure 5. Similar to the DTM, the linear regression analysis  
345 shows a positive relationship, and the UAV CHM tends to overestimate the  
346 LiDAR CHM with an offset of 1,002 m. This offset may be attributed to  
347 various factors, including seasonality differences (LiDAR data were collected  
348 in February during the leaf-off season, while photogrammetric data were ac-  
349 quired in early spring in May), data processing (methodological distinction

350 arises from the inability to directly calculate the DTM with photogramme-  
 351 try that was derived from the DSM) and differences in the used processing  
 352 algorithms employed for DTM and DSM assessment. Despite the presence  
 353 of this offset, the correlation between the two variables remains statistically  
 354 significant ( $p$ -value  $< 0.05$ ), and the linear model exhibits a commendable  
 355 goodness of fit at 0.71.

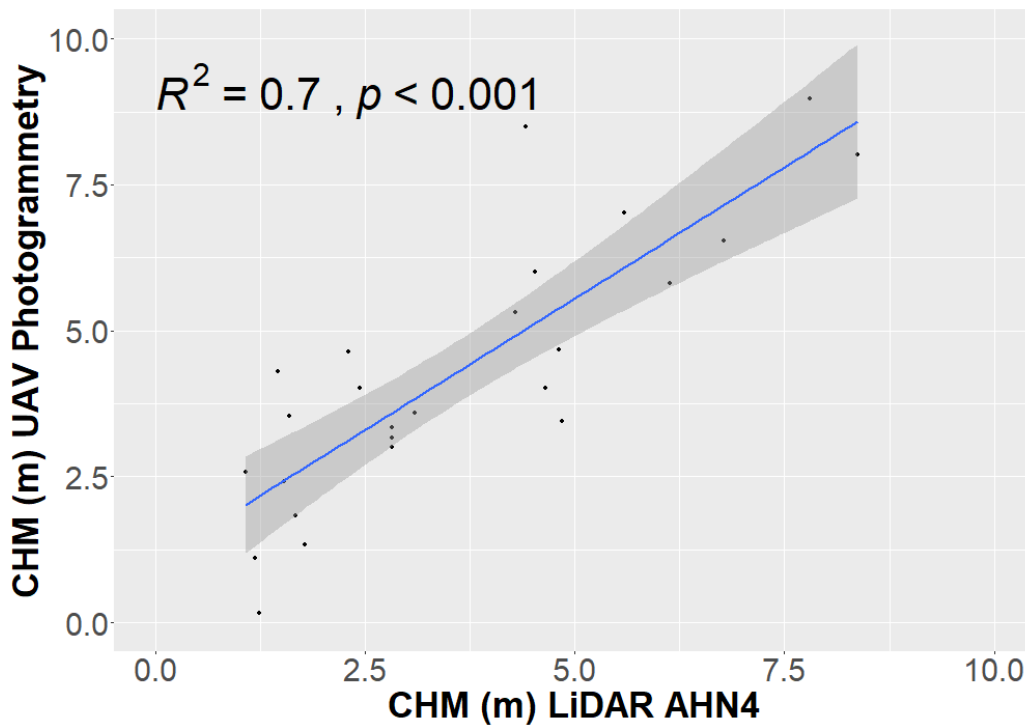


Figure 5: Validation of the CHM derived from UAV photogrammetry with the local LiDAR CHM AHN4.

356 Figure 6 shows a study area with two different vegetation structure. In  
 357 the middle of the figure is shown a stripe of grass characterized by a higher  
 358 vegetation structure complexity and high HH while on the side grassland  
 359 with low HH. Sub-figure A shows the RGB image, sub-figure B the CHM  
 360 derived from the photogrammetric point cloud showed in sub-figure C.



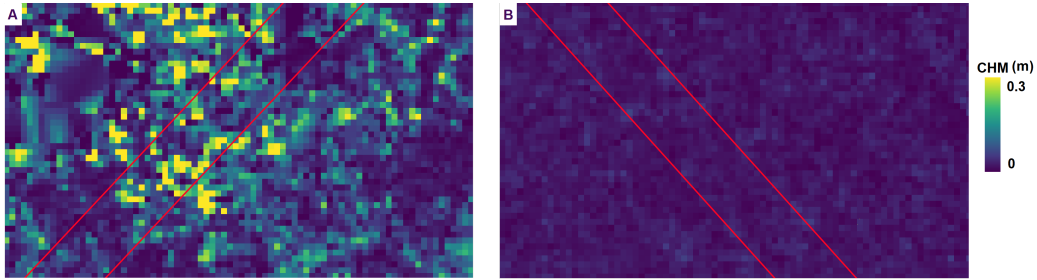


Figure 6: A study area displaying two distinct vegetation structures. The image highlights a central grassy strip, characterized by a higher complexity in vegetation structure and high HH values. Adjacent to it is a grassland area with lower HH values. Sub-figure A showcases the RGB image, while sub-figure B showcases the CHM derived from the photogrammetric point cloud featured in sub-figure C. Two transects (in red) characterized by different height heterogeneity. Sub-figure A shows a CHM of a transect characterized by high height heterogeneity (heterogeneous CHM ranging from 0 to 0.3 m), while sub-figure B shows a transect with low height heterogeneity (homogeneous CHM with values ranging from 0 m to 0.1 m).

361 The correlation between the flower diversity and calculated HH with dif-  
 362 ferent heterogeneity indices (Rao's Q index, CV, Berger-Parker, and Simp-  
 363 son's D) using the CHM at 10 cm, 25 cm, and 50 cm derived from UAV  
 364 photogrammetry is shown in Figure 7. All the correlations are positive and  
 365 significant, except when the HH was calculated with the Berger-Parker index  
 366 using a CHM of 10 cm and 50 cm. The highest  $R^2$  values were obtain when  
 367 the HH was calculated with the Rao's Q index. In this case, the coefficient of  
 368 determination range between 0.41 (UAV CHM spatial resolution of 10 cm)  
 369 and 0.44 (UAV CHM spatial resolution of 25 cm).

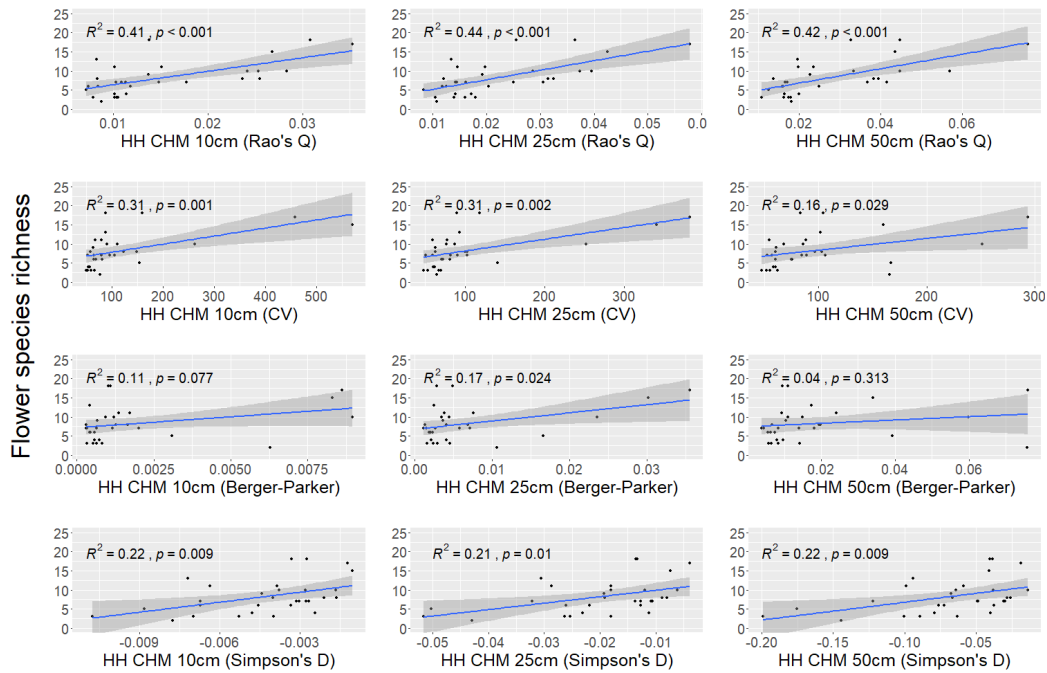


Figure 7: Correlation between the ground-based flower diversity and the HH calculated with the four heterogeneity indices (Rao's Q, CV, Berger-Parker and Simpson's D ) derived from UAV CHM at 10 cm, 25 cm and 50 cm

370 Figure 8 shows the correlation between the bee abundance and the HH  
 371 calculated with different heterogeneity indices (Rao's Q index, CV, Berger-  
 372 Parker, and Simpson's D) using the CHM at 10 cm, 25 cm, and 50 cm derived  
 373 from UAV photogrammetry. In this case, the correlations are all positive, and  
 374 significant only when the HH was calculated with the Rao's Q and Simpson's  
 375 D indices. Generally, the  $R^2$  values are lower than the ones derived from the  
 376 correlation between HH and flower diversity. Higher  $R^2$  are associated with  
 377 HH calculated using the Rao's Q index. The coefficient of determination  
 378 ranges between 0.31 (UAV CHM spatial resolution of 25 cm) and 0.34 (UAV  
 379 CHM spatial resolution of 50 cm).

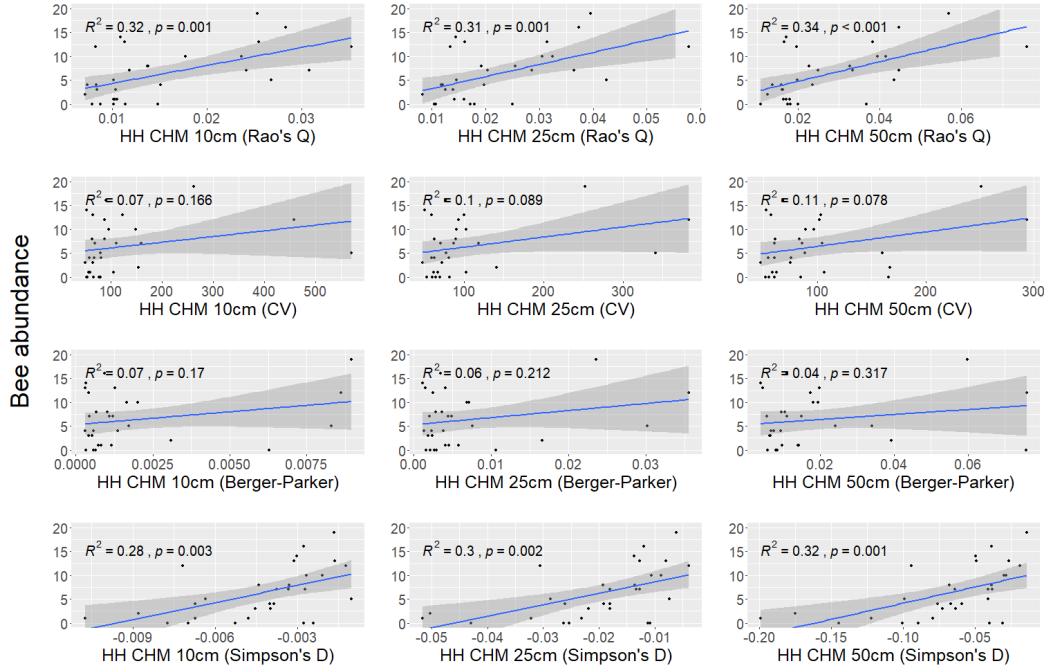


Figure 8: Correlation between ground-based bee abundance and HH calculated with the four heterogeneity indices (Rao's Q, CV, Berger-Parker, and Simpson's D) derived from UAV CHM at 10 cm, 25 cm, and 50 cm.

380 Finally, the correlation between bee diversity and HH calculated with dif-  
 381 ferent heterogeneity indices (Rao's Q index, CV, Berger-Parker, and Simp-  
 382 son's D) using the CHM at 10 cm, 25 cm, and 50 cm derived from UAV  
 383 photogrammetry is shown in Figure 9. Also in this case, positive correla-  
 384 tions persist, with the Rao's Q index yielding the highest  $R^2$  values, while  
 385 the Simpson's D index shows a comparatively modest correlation with HH.  
 386 They are significant, except when the HH was calculated with the Berger-  
 387 Parker index (with CHM at 10 cm and 50 cm).

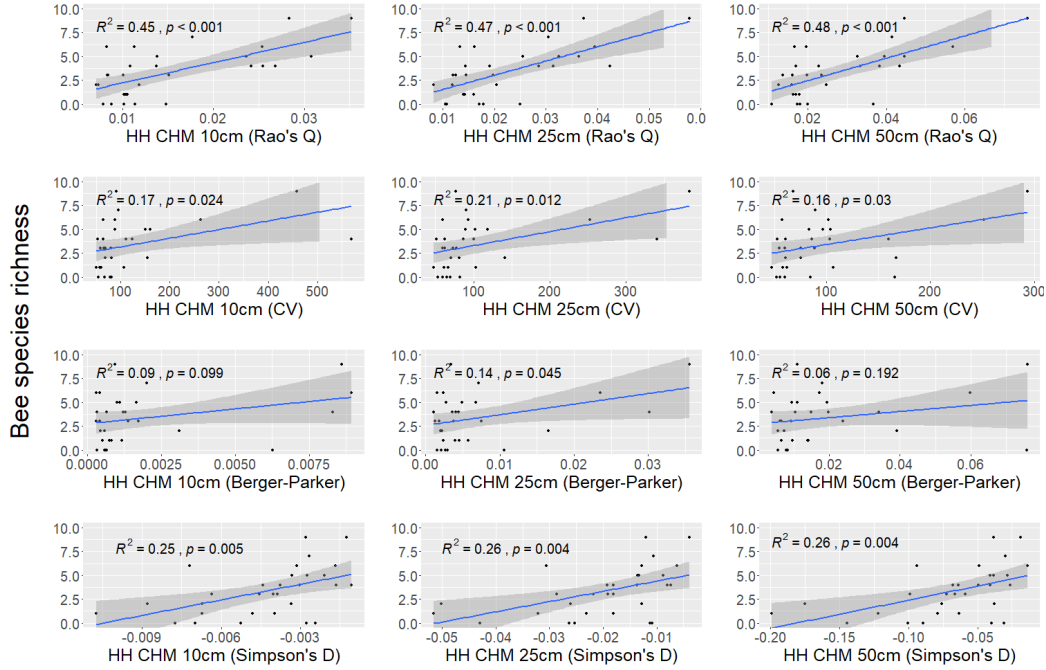


Figure 9: Correlation between ground-based bee diversity and HH calculated with the four heterogeneity indices (Rao's Q, CV, Berger-Parker, and Simpson's D) derived from UAV CHM at 10 cm, 25 cm, and 50 cm.

## 388 4 Discussion

389 This paper introduces a new approach to estimate flower diversity, which  
 390 can be used as an indicator of bee abundance and diversity in grassland  
 391 ecosystems. Our study builds upon previous studies [77] that identified  
 392 UAV images, analyzed through various machine learning algorithms, as a  
 393 reliable proxy for bee diversity and abundance. However, with this inno-  
 394 vative HVH approach, we delve deeper into unraveling the intricate rela-  
 395 tionship between grassland structural heterogeneity and its impact on bee  
 396 diversity. The method utilizes UAV RGB images to create a 3D model of the  
 397 vegetation structure through photogrammetric analysis. By applying differ-  
 398 ent heterogeneity indices, we derived information on vegetation HH, which  
 399 showed a positive correlation with ground-based measures of flower diver-  
 400 sity, bee diversity, and bee abundance. These findings serve as a proof of  
 401 concept, demonstrating the potential of UAV imagery to accurately evaluate  
 402 the habitat structure as a crucial element of grassland habitat quality for  
 403 bees. The findings of this study provide valuable insights into the use of

404 UAV imagery and HH in estimating biodiversity at a local scale, specifically  
405 in grassland ecosystems. The results indicate that vegetation height hetero-  
406 geneity, as measured through UAV-derived CHMs, can serve as a proxy for  
407 flower diversity and, consequently, bee diversity and abundance.

#### 408 **4.1 Height Variation Hypothesis in grassland ecosys-** 409 **tem: Advantages, Contrasts, and Ecological Impli-** 410 **cations**

411 The proposed approach relies on the theory behind the HVH which, unlike  
412 its counterpart (the Spectral Variation Hypothesis -SVH-), offers several sig-  
413 nificant advantages. Being based on vertical structural heterogeneity, the  
414 HVH is not susceptible to certain factors such as the spectral resolution of  
415 the optical images [60, 45], by noise introduced by the soil properties which  
416 can negatively affect the accuracy of biodiversity assessments [22] and by the  
417 atmospheric conditions such as haze, aerosols, and cloud cover [61].

418 This study represents the first validation of the HVH with UAV images,  
419 the results showed that the use of the photogrammetric analysis offer significant  
420 advantages for biodiversity assessment also in grasslands. This study pro-  
421 vides a novel application of the HVH with UAV images in grasslands. The  
422 results indicate the potential of photogrammetric analysis for biodiversity as-  
423 sessment in grasslands, contributing to the understanding of vegetation struc-  
424 ture and its relationship with biodiversity. As shown in other studies [50, 85],  
425 the high-resolution cameras mounted to UAVs allow capturing of detailed im-  
426 ages, enabling the assessment of fine-scale heterogeneity of intensively and  
427 extensively managed grasslands. The proposed approach highlights the ca-  
428 pability of UAVs to assess grassland vegetation structure and heterogeneity,  
429 providing detailed information about the vertical complexity and variability  
430 of the vegetation, critical information for understanding ecosystem dynamics,  
431 biodiversity, and habitat suitability for various organisms [50].

432 Other approaches have been developed to assess these aspects by us-  
433 ing UAV data; recent studies for example focused on the evaluation of flower  
434 abundance as a proxy for diversity and abundance of bees [77, 11]. These ap-  
435 proaches often rely on machine learning algorithms, which necessitate metic-  
436 ulously curated and representative training data-sets that, due to their time-  
437 consuming and resource-intensive nature, can potentially hinder scalability  
438 and applicability in certain contexts [8]. Moreover, the representativeness  
439 of the training data-set is critical to ensure the generalizability of the algo-  
440 rithm’s performance. These challenges can impede the scalability and appli-  
441 cability of machine learning-based approaches under conditions, where there

442 are no comprehensive and diverse training data-sets [74]. Furthermore, ma-  
443 chine learning algorithms may exhibit limitations in their ability to capture  
444 the full complexity of ecological dynamics. They rely on patterns and asso-  
445 ciations learned from the training data-sets, which may not encompass the  
446 entirety of the intricate relationships within an ecosystem. Consequently, the  
447 predictive power of machine learning models may be limited when confronted  
448 with novel or complex ecological scenarios that deviate from the patterns rep-  
449 resented in the training data-set [48].

450 The findings obtained by our analytical approach hold significant rele-  
451 vance for ecological studies for multiple reasons. Understanding the vertical  
452 complexity and variability of grassland vegetation provides insights also into  
453 habitat heterogeneity and resource availability for various organisms, includ-  
454 ing plants, insects, birds, and small mammals [24, 3, 49]. Different species  
455 have specific habitat preferences and requirements based on their vertical  
456 distribution within the grassland. Assessing grassland structure helps to  
457 understand the composition, distribution, and abundance of species within  
458 the ecosystem [52]. It would be intriguing to explore whether there exists a  
459 correlation between grassland structure and the various ecosystem processes  
460 and services such as nutrient cycling, carbon storage, water infiltration, and  
461 energy fluxes. If such a correlation is found, our approach could be uti-  
462 lized to achieve more precise mapping of these significant ecosystem services,  
463 surpassing the current methods employed. Additionally, the information on  
464 grassland structure can be integrated with other environmental data, such as  
465 soil properties and landscape features, to gain a more holistic understanding  
466 of the ecological dynamics and drivers in grassland ecosystems [67]. Further-  
467 more, the proposed approach could be used to assess changes in grassland  
468 structure as a results of land management practices, ecological succession,  
469 and of the impacts of disturbances such as grazing, fire, or land-use changes.  
470 Monitoring and understanding these structural changes are essential for ef-  
471 fective conservation and management of grassland ecosystems [32, 25, 15].

## 472 **4.2 UAVs in Bee Habitat Monitoring: Challenges and** 473 **Prospects**

474 UAV-based methods have emerged as promising tools for monitoring habitat  
475 quality for bee pollinator communities, primarily due to their affordability  
476 [23]. These methods allow different operators, including researchers, farmers,  
477 and ecologists, to acquire high spatial resolution data from various sensors  
478 simultaneously, covering extensive areas within a short time of data collec-  
479 tion. Furthermore, the "on-demand" approach facilitated by UAVs enables

480 capturing specific stages of vegetation phenology, such as flowering time, par-  
481 ticularly in regions characterized by high cloud cover [44, 12]. These capabil-  
482 ities provide valuable insights into the temporal dynamics of plant-pollinator  
483 interactions. However, despite their potential, several challenges must be ad-  
484 dressed before UAV-based methods can be routinely deployed at large spatial  
485 scales. Challenges arising may involve issues related to data processing, sen-  
486 sor calibration, image analysis algorithms, and the development of standard-  
487 ized protocols to ensure data comparability and reliability. ~~Overcoming these~~  
488 ~~hurdles will be crucial for realizing the full potential of UAV-based approaches~~  
489 ~~in ecological research and monitoring.~~ Addressing these challenges paves the  
490 way for a visionary application, where UAVs, equipped with advanced sen-  
491 sors, facilitate large-scale macroecological studies. This approach enables  
492 real-time data acquisition, enhancing our understanding of spatial patterns,  
493 biodiversity dynamics, and ecosystem processes across diverse landscapes.

494 The impact of the spatial resolution of UAV data on the correlation be-  
495 tween grassland structural metrics (such as HH) and flower and bee diversity,  
496 as well as bee abundance was investigated in this study. Based on our re-  
497 sults, the spatial resolution of UAV data does not play a critical role in the  
498 correlations between vegetation assessment variables (such as flower diver-  
499 sity, bee abundance, and bee diversity) and HH calculated using different  
500 heterogeneity indices. The correlations between these variables remain posi-  
501 tive and significant across different spatial resolutions (10 cm, 25 cm, and  
502 50 cm) derived from UAV photogrammetry. This finding aligns with the  
503 results reported in several other studies examining the influence of spatial  
504 resolution on vegetation assessment using UAV imagery. For instance, [37]  
505 demonstrated that species classification in a heterogeneous grassland using  
506 high spatial resolution UAV imagery was not significantly affected by spatial  
507 resolution. Similarly, the impact of spatial resolution on the classification  
508 of vegetation types in highly fragmented planting areas based on UAV hy-  
509 perspectral images was found to be limited [36]. Different studies [29, 11]  
510 highlighted that the use of micro-UAV with relatively low spatial resolution  
511 still provide valuable information for assessing vegetation structure and for  
512 long-term monitoring purposes. On the other hand, it is important to note  
513 that the relationship between the high spatial resolution of optical remote  
514 sensing data and its correlation with ground-based ecological data is a com-  
515 plex matter [42]. Different studies [77, 60, 66] have shown that higher spatial  
516 resolution can lead to higher correlations with ground-based data. It is re-  
517 cognized that images with coarse spatial resolution may integrate the spectral  
518 signature of various vegetation elements, making it challenging to identify  
519 boundaries between spatial entities and potentially resulting in mixed sig-  
520 nals at the pixel scale [45, 19]. These results imply that drone flights can

521 also be conducted at higher altitudes and thus cover larger areas in a single  
522 flight (at a lower spatial resolution), enabling more efficient data collection.

### 523 4.3 Insights from Heterogeneity Indices

524 Regarding the evaluation of the use of different heterogeneity indices, our  
525 results demonstrated the usefulness of the Rao's Q index in assessing the  
526 vegetation HH across plots areas of intensive and extensive grassland man-  
527 agement. This heterogeneity index, widely used as a spectral heterogeneity  
528 index in studies on SVH [39, 62, 75, 51] offers the advantage of coupling  
529 both the relative abundance and the pixel values (as quantified by the Eu-  
530 clidean distance between the pixel values) [78], thus capturing the complete  
531 structural information derived from the heterogeneity of the photogrammet-  
532 ric outcomes. This index, when applied with a single layer or raster as in  
533 our study, can effectively serve as a proxy for heterogeneity by narrowing it  
534 down to variance using half of the squared Euclidean distance ( $1/2 d_{ij}^2$ ) (for  
535 further details on the mathematical characteristics of Rao's Q, we refer to  
536 [62, 57, 59, 58]). On the other hand, other indices evaluated in our work,  
537 proved rather inefficient in assessing HH: the CV rely only on the distance  
538 between the pixel values while the Simpson's D and the Berger-Parker index  
539 rely solely on the relative abundance of CHM pixels within a specific raster or  
540 an area of interest [62]; given the exceptional precision of our photogrammet-  
541 ric point cloud, approximately 15 mm, the likelihood of distinct pixels sharing  
542 identical values is for this reason significantly minimized. Consequently, they  
543 fail to adequately characterize the entire heterogeneity of vegetation heights,  
544 which depend on both the actual values of vegetation height and their distri-  
545 bution and relative frequency. One concern in this study revolves around the  
546 utilization of the CHM as the sole metric for assessing the HH, without con-  
547 sidering other metrics or additional digital layers, such as optical data that  
548 might be related to vegetation structure. The decision to focus solely on the  
549 CHM had two main reasons. Firstly, the primary objective of this study was  
550 to investigate the feasibility of utilizing RGB UAV images to assess vege-  
551 tation structure complexity for estimating HH and flower and bee diversity  
552 and bee abundance. Secondly, choice was guided by the findings of Tam-  
553 burlin et al. [72], who, testing the HVH with LiDAR data, evaluated various  
554 LiDAR metrics (such as entropy and standard deviation of point cloud dis-  
555 tribution, percentage of returns above mean height) for HH estimation and  
556 demonstrated that the CHM was the most effective metric to characterize  
557 vegetation HH.

558 Another concern that could arise is related to the accuracy of the UAV  
559 derived CHM. While the CHMs derived from UAV photogrammetry showed



560 a robust correlation with local CHM LiDAR data, there may still be some  
561 differences in accuracy and precision. We acknowledge that photogramme-  
562 try techniques may not capture true ground points accurately, especially in  
563 areas with dense and short grass. One possible way to enhance the precision  
564 of our approach could be the utilization of LiDAR technology mounted on  
565 UAVs that can provide more precise and detailed measurements of vegetation  
566 structure and topography [4], offering valuable information on floral resources  
567 and bee foraging habitats. However, it is worth noting that LiDAR-equipped  
568 UAVs are currently considered expensive, which can limit their widespread  
569 use. Furthermore recent studies indicate that these systems may not neces-  
570 sarily exhibit significantly improved performance in acquiring accurate DSMs  
571 within closed vegetation canopies [73]. It is important to clarify that our pri-  
572 mary interest lies in assessing the vertical variation within the point cloud  
573 rather than obtaining absolute values for ground surface measurements. To  
574 address this concern, we employed a methodology focused on analyzing the  
575 amount of variation in vertical points rather than relying on precise ground  
576 measurements, allowing to evaluate the relative differences in elevation val-  
577 ues between different areas, which can still provide valuable insights into the  
578 landscape dynamics and terrain characteristics.

## 579 5 Conclusions

580 This study demonstrates the potential of UAV imagery and the HVH con-  
581 cept for estimating biodiversity at a local scale in grassland ecosystems. The  
582 results suggest that vegetation HH, as assessed through UAV-derived CHMs,  
583 can serve as a reliable proxy for flower diversity, bee diversity, and abundance.  
584 The use of UAVs, with the ability to assess species diversity and provide  
585 information on grassland structure, offers a cost-effective and standardized  
586 approach to monitor and manage grassland ecosystems, providing valuable  
587 information for conservation efforts and advancing ecological research. While  
588 our study serves as an initial application, further analysis in diverse grassland  
589 areas using various heterogeneity indices is necessary to establish the gener-  
590 alizability of the approach. Additionally, this approach could be extended to  
591 assess biodiversity not only of bees but also of other insects such as spiders or  
592 butterflies. Further analysis could focus on integrating optical information,  
593 such as flower cover estimation, or spectral variability data, with structural  
594 information from UAVs enhancing the depth of biodiversity characterization.  
595 We propose that ecologists, botanists, and farmers can employ our approach,  
596 utilizing UAV images and photogrammetric analysis in order to assess habi-  
597 tat heterogeneity, as a preliminary analysis for the estimation of bee diversity

598 and abundance.

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## 602 **7 Additional Information**

603 The authors declare no competing interests.

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## 608 **9 Data availability**

609 The datasets used and/or analysed during the current study available from  
610 the corresponding author on reasonable request.

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