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

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Abstract

The ecosystem services offered by pollinators are vital for support-31 ing agriculture and ecosystem functioning, with bees standing out as 32 especially valuable contributors among these insects. Threats such as 33 habitat fragmentation, intensive agriculture, and climate change are 34 contributing to the decline of natural bee populations. Remote sens-35 ing could be a useful tool to identify sites of high diversity before 36 investing into more expensive field survey. In this study, the ability of 37 Unoccupied Aerial Vehicles (UAV) images to estimate biodiversity at 38 a local scale has been assessed while testing the concept of the Height 39 Variation Hypothesis (HVH). This hypothesis states that the higher 40 the vegetation height heterogeneity (HH) measured by remote sensing 41 information, the higher the vegetation vertical complexity and the as-42 sociated species diversity. In this study, the concept has been further 43 developed to understand if vegetation HH can also be considered a 44 proxy for bee diversity and abundance. We tested this approach in 30 45 grasslands in the South of the Netherlands, where an intensive field 46 data campaign (collection of flower and bee diversity and abundance) 47 was carried out in 2021, along with a UAV campaign (collection of true 48 color -RGB- images at high spatial resolution). Canopy Height Mod-49 els (CHM) of the grasslands were derived using the photogrammetry 50 technique "Structure from Motion" (SfM) with horizontal resolution 51 (spatial) of 10 cm, 25 cm, and 50 cm. The accuracy of the CHM 52 derived from UAV photogrammetry was assessed by comparing them 53 through linear regression against local CHM LiDAR (Light Detection 54 and Ranging) data derived from an Airborne Laser Scanner campaign 55 completed in 2020/2021, yielding an R^2 of 0.71. Subsequently, the HH 56 assessed on the CHMs at the three spatial resolutions, using four dif-57 ferent heterogeneity indices (Rao's Q, Coefficient of Variation, Berger-58 Parker index, and Simpson's D index), was correlated with the ground-59 based flower and bee diversity and bee abundance data. The Rao's Q 60 index was the most effective heterogeneity index, reaching high cor-61 relations with the ground-based data (0.44 for flower diversity, 0.47 62 for bee diversity, and 0.34 for bee abundance). Interestingly, the cor-63 relations were not significantly influenced by the spatial resolution of 64 the CHM derived from UAV photogrammetry. Our results suggest 65 that vegetation height heterogeneity can be used as a proxy for large-66 scale, standardized, and cost-effective inference of flower diversity and 67 habitat quality for bees. 68

Keywords: biodiversity, photogrammetry, pollinators, habitat suitabil ity, insect diversity, structural habitat diversity

30

71 1 Introduction

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

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

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

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

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

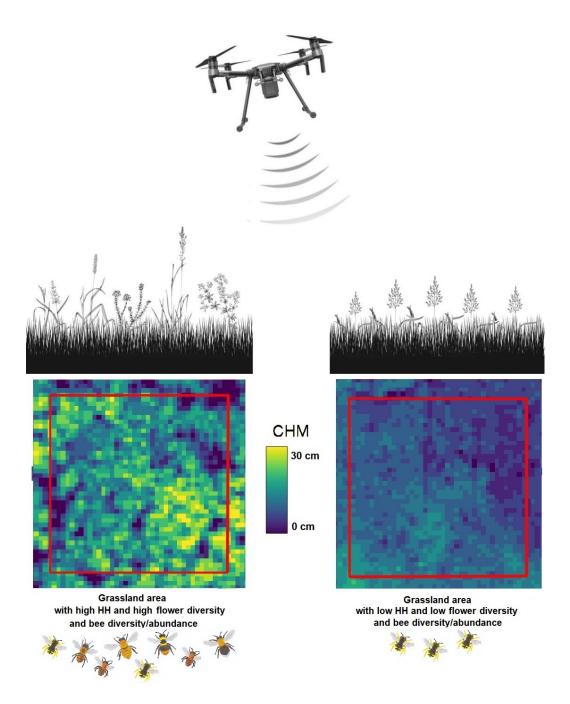


Figure 1: A graphical summary of the main expectations of this study. Grassland ecosystems with high HH (assessed through CHM derived by UAV photogrammetric images) with a complex vertical structure (seen from the side in the upper figure and from above in the lower figure) and high environmental heterogeneity 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

The study areas (approximately 70 km^2 with elevations ranging from 70 to 168 171 m asl) are located in the southeast of the Netherlands, near the village 169 of Gulpen (Fig. 2). Thirty grasslands representing a range of land use inten-170 sities, from nutrient-poor, biodiversity-rich semi-natural grasslands to inten-171 sively fertilized areas, were chosen in order to test the proposed approach. 172 Management of the grasslands included moving (16 sites), grazing (10 sites) 173 and mixed regimes (4 sites), ranging in intensity from one to five uses per 174 year (details in Appendix Table 1). Data collection for this study took place 175

before the first cut but extensive grazing (<2 LSU/ha) had occurred at most 176 grazed plots. Percent herb cover ranged from 0.1% to 69%, with the most 177 dominant species in terms of flower cover being Ranunculus repens, R. acris 178 and R. bulbosus, Leucanthemum vulgare, Trifolium pratense, Bellis perennis 179 and Taraxacum sp. (all >5% of the total flower area over all transects). The 180 study areas are part of the experimental biodiversity area network of the 181 EU Showcase project https://showcase-project.eu/. By selecting semi-182 natural, extensively utilized, and intensely managed grasslands from diverse 183 regions, we reduced spatial clustering of distinct grassland types. 184

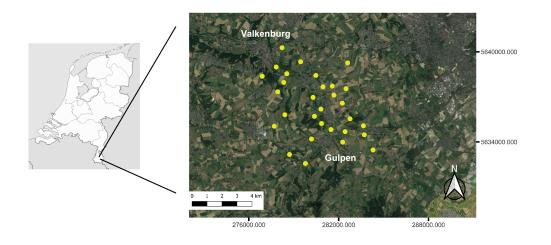


Figure 2: The study areas located in the Southeast of the Netherlands. The 30 plots transects within each study area are indicated by yellow dots (Basemap: Google Earth map as of August 2022).

185 2.2 Field data

$_{186}$ 2.2.1 Collection

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

222 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.

227 2.3 UAV Data Acquisition and Data Processing

The UAV data were acquired simultaneously with the field survey between 228 May 12th and 31st, 2021. A RGB Zenmuse X5 camera (16.0 MP, 17.3 x 229 13.0 mm sensor) with an integrated RTK GPS was carried by the UAV "DJI 230 Matrice 210 RTK". To simplify the production of the final point cloud and 231 the digital elevation model, the images were taken at an overlapping rate of 232 80%. All flights were conducted at a height of approximately 20 m above the 233 ground. The average spatial resolution of the resulting UAV images is 0.5 234 cm. 235

The Agisoft Metashape Professional Edition software was used to analyze 236 and process the UAV images following three main procedural stages: image 237 alignment, dense point cloud creation, and inference of the digital elevation 238 model. In the first step, set with "high" accuracy, the software extracted 239 features within the images and matched them to produce a sparse 3D point 240 cloud. At this stage, the software automatically detected the precise fea-241 tures of the GCP and extracted the GPS coordinates for each of them. We 242 maintained the "high" accuracy setting during the construction of the dense 243 point cloud, which was subsequently exported as a LAS file. The mean point 244 density for all 30 areas was 700 $points/m^2$ while the vertical resolution was 245 around 15 mm. The Digital Surface Model (DSM) was derived at different 246 spatial resolutions (10 cm, 25 cm, and 50 cm) using the "dsmtin" algorithm 247 of the "rasterize canopy" function of the R package "lidR" [68]. This algo-248 rithm uses the Delaunay triangulation method to connect the points in the 249 point cloud, forming a network of non-overlapping triangles. The resulting 250 triangular irregular network (TIN) represents the surface, and rasterization 251 is then applied to convert this TIN into a gridded DSM, providing a compre-252 hensive representation of the terrain and vegetation structure. The Digital 253 Terrain Model (DTM) was derived using the same function but with a prior 254 filtering of the point cloud, selecting the lowest points every 50 cm. Finally, 255 the CHM was derived by taking the difference between the DSM and DTM. 256 The decision to set the finest spatial resolution at 10 cm was primarily driven 257 by computational considerations. 258

259 2.4 Heterogeneity index

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

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

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

where:

268

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

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

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

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

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

$$CV = (SD/\overline{x}) \times 100 \tag{2}$$

276 where:

CV = Coefficient of Variation

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

279

 \overline{x} = mean of the pixel values within a selected area

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

$$BP = \frac{n_{\max}}{N} \tag{3}$$

where:

BP is the Berger-Parker heterogeneity index

 $-n_{\rm max}$ is the abundance of the most dominant pixel value in the data-set

- N is the total abundance of all pixels in the data-set.

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

$$D = \sum_{i=1}^{n} p_i^2 \tag{4}$$

where:

D =Simpson index

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

DSM and DTM with a spatial resolution of 50 cm derived from local LiDAR 297 data collected by an ALS LiDAR campaign carried out between 2020 and 2022 298 (AHN4 data-set, freely available for download here: https://geotiles.nl/) 299 were used to validate the UAV digital models. The LiDAR flight was conducted 300 on February 18th, 2021.DSM and DTM with a spatial resolution of 50 cm 301 derived from local Li-DAR data collected as part of an national ALS LiDAR 302 campaign carried out between 2020 and 2022 (AHN4 data-set, freely available 303 for download here: https://geotiles.nl/) were used to validate the UAV digi-304 tal models. AHN datasets are systematically gathered every few years for all 305 of the Netherlands, by multiple operators and sensors, where the exact spec-306 ifications may vary over time and space. AHN4 pointclouds have a vertical 307 resolution of 13 mm and a density of 10-14 point/m2. In our study area, the 308 LiDAR flight for AHN4 was conducted on February 18th, 2021. During this 309 season, the grassland vegetation is very low, resulting in the DSM and DTM 310 having equal elevations, effectively yielding a CHM value of zero. For this 311 reason, we decided to validate the UAV-DTM with the LiDAR-DTM using 312 10 random points within each study area (300 points in total). Additionally, 313 we validated the CHM over multi-annual visible vegetation-patch (e.g., small 314

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

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

322 2.6 Workflow

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

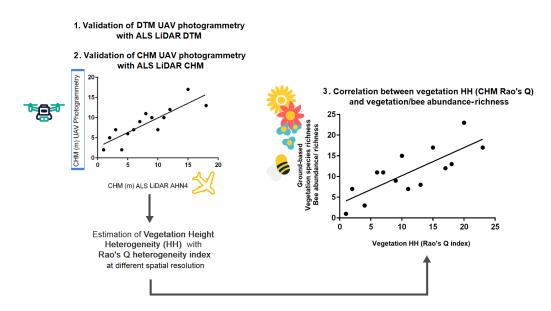


Figure 3: The image shows the workflow of the proposed approach.

333 3 Results

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

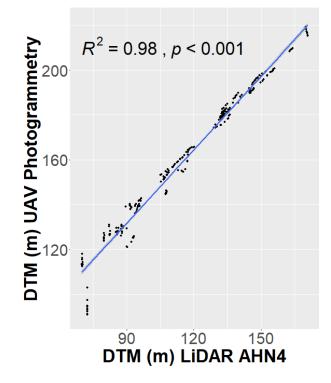


Figure 4: The validation of the DTM derived from UAV photogrammetry with the local LiDAR DTM AHN4 is shown with the blue line.

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

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

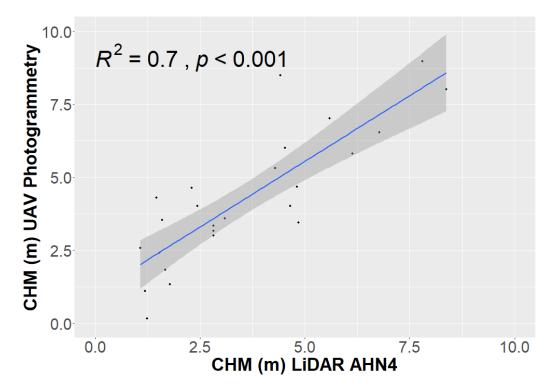


Figure 5: Validation of the CHM derived from UAV photogrammetry with the local LiDAR CHM AHN4.

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

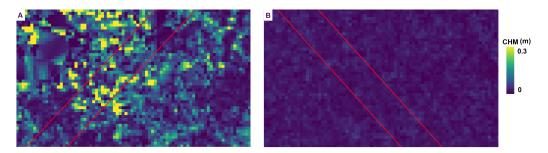


Figure 6: A study area displaying two distinct vegetation structures. The image highlights a central grassy strip, characterized by a higher complexity in vegetation structure and high HH values. Adjacent to it is a grassland area with lower HH values. Sub-figure A showcases the RGB image, while sub-figure 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).

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

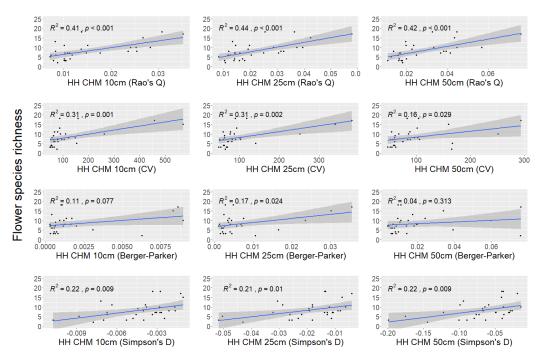


Figure 7: Correlation between the ground-based flower diversity and the HH calculated with the four heterogeneity indices (Rao's Q, CV, Berger-Parker and Simpson's D) derived from UAV CHM at 10 cm, 25 cm and 50 cm

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

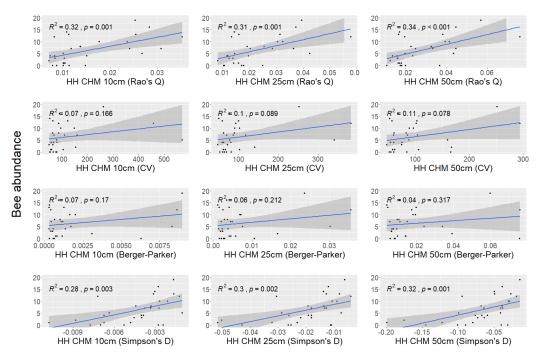


Figure 8: Correlation between ground-based bee abundance and HH calculated with the four heterogeneity indices (Rao's Q, CV, Berger-Parker, and Simpson's D) derived from UAV CHM at 10 cm, 25 cm, and 50 cm.

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

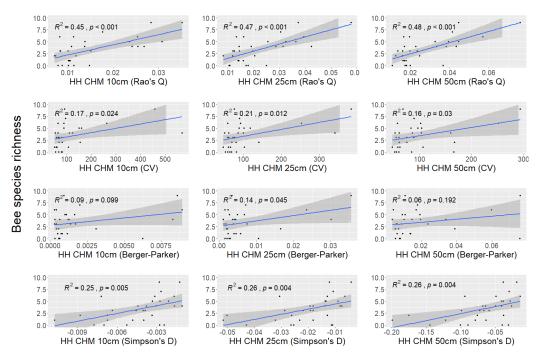


Figure 9: Correlation between ground-based bee diversity and HH calculated with the four heterogeneity indices (Rao's Q, CV, Berger-Parker, and Simpson's D) derived from UAV CHM at 10 cm, 25 cm, and 50 cm.

388 4 Discussion

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

UAV imagery and HH in estimating biodiversity at a local scale, specifically
in grassland ecosystems. The results indicate that vegetation height heterogeneity, as measured through UAV-derived CHMs, can serve as a proxy for
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

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

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

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

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

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

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

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

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

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

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

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

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

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

579 5 Conclusions

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

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

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

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⁹⁵² 10 Author Contribution statement

Michele Torresani: Conceptualization, Methodology, Formal analysis, Writ-953 ing - Original Draft, Visualization. Duccio Rocchini: Conceptualization, 954 Writing - Review & Editing, Visualization, Supervision, Project adminis-955 tration. Giada Ceola: Conceptualization, Methodology, Formal analysis, 956 Visualization. Jan Peter Reinier de Vries: Conceptualization Methodology, 957 Validation, Resources, Data Curation. Hannes Feilhauer: Conceptualization 958 , Writing - Review & Editing, Visualization. Vítězslav Moudrý: Conceptu-959 alization, Writing - Review & Editing, Visualization. Harm Bartholomeus: 960 Conceptualization, Writing - Review & Editing, Data Curation. Michela 961 Perrone: Conceptualization, Writing - Review & Editing, Visualization. 962 Matteo Anderle: Conceptualization, Writing - Review & Editing, Visualiza-963 tion. Hannes Andres Gamper: Conceptualization, Writing - Review & Edit-964 ing, Visualization. Ludovico Chieffallo: Conceptualization, Methodology, 965 Formal analysis, Visualization. Enrico Guatelli: Conceptualization, Visual-966 ization. Roberto Cazzolla Gatti: Writing - Review & Editing David Kleijn: 967 Conceptualization, Data Curation, Writing - Review & Editing, Visualiza-968 tion, Resources, Supervision, Project administration, Funding acquisition. 969