

1 **Flexible estimation of biodiversity with short-range multispectral imaging**  
2 **in a temperate grassland**

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28 **Abstract**

29 Image sensing technologies are rapidly increasing the cost-effectiveness of biodiversity monitoring  
30 efforts. Species differences in the reflectance of electromagnetic radiation have recently been  
31 highlighted as a promising target to estimate plant biodiversity using multispectral image data.  
32 However, these efforts are currently hampered by logistical difficulties in broad-scale implementation  
33 and their use in characterizing biodiversity at different spatial scales. Here, we investigate the utility of  
34 multispectral imaging technology from commercially available unmanned aerial vehicles (UAVs, or  
35 drones) in estimating biodiversity metrics at short-range (<10 m image recording height) in a temperate  
36 calcareous grassland ecosystem in Oxfordshire, UK. We calculate a suite of moments (coefficient of  
37 variation, standard deviation, skew, kurtosis) for the distribution of reflectance from multispectral  
38 images at five wavelength bands (Blue 450±16 nm; Green 560±16 nm; Red 650±16 nm; Red Edge  
39 730±16 nm; Near Infrared 840±16 nm) and test their effectiveness at estimating ground-truthed  
40 biodiversity metrics from *in-situ* botanical surveys for 37 - 1 m × 1 m quadrats. We find positive  
41 associations between the average coefficient of variation in spectral reflectance and both the Shannon-  
42 Weiner and Simpsons biodiversity indices. Furthermore, we find that the average coefficient of  
43 variation in spectral reflectance is consistent and highly repeatable, across sampling days and recording  
44 heights. Positive associations with biodiversity indices hold irrespective of the image recording height  
45 (2-8 m), but we report reductions in estimates of spectral diversity with increases to UAV recording  
46 height. UAV imaging reduced sampling time by 16-fold relative to *in-situ* botanical surveys. We  
47 demonstrate the utility of multispectral reflectance moments as an indicator of grassland biodiversity  
48 metrics at high spatial resolution using a widely available UAV monitoring system at a coarse spectral  
49 resolution. The use of UAV technology with multispectral sensors has far-reaching potential to provide  
50 cost-effective and high-resolution monitoring of biodiversity in complex environments.

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52 Keywords: Autonomous monitoring, Biodiversity Drone, Remote sensing, Unmanned Aerial Vehicle  
53 (UAV)

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## 56 **1. Introduction**

57 With over 1 million species expected to go extinct by 2100, cost-effectively monitoring biodiversity is  
58 a critical task in the Anthropocene (Díaz et al., 2019). Image sensing technologies, which can be used  
59 to monitor biological systems through the measurement of reflected and emitted radiation, have  
60 emerged as a critical tool that can increase this cost-effectiveness (Turner, 2014). The characterisation  
61 of floral biodiversity with remote sensing, particularly with satellite imagery, is well-established in  
62 biodiversity research (Pettoirelli et al., 2005). Multiple efforts have been made towards using remote  
63 sensing data, particularly at large spatial scales and in forest ecosystems, to estimate plant diversity  
64 (e.g., Frye et al., 2021; Jetz et al., 2016; Tuanmu & Jetz, 2015; Turner et al., 2003). However, there are  
65 limitations in the use of long-range remote sensing, including low spatial resolution that does not  
66 necessarily highlight biodiversity at small spatial scales (Mairota et al., 2015), high sensor costs (e.g.,  
67 \$98,700, Headwall Photonics, 2022) and monitoring costs (e.g., \$60,000, Jet Propulsion Laboratory,  
68 2022), and reliance on publicly available satellite data (e.g., The European Space Agency, 2022).  
69 Flexible application of remote sensing concepts and technology at a wide range of spatial scales, in  
70 complex changing environments, and with increased cost-effectiveness, will provide vital resources for  
71 monitoring biodiversity (Turner, 2014).

72 Reflectance of electromagnetic (EM) radiation beyond the visible range (380-700 nm) has  
73 recently been shown accurate proxy for biodiversity (Cavender-Bares et al., 2020; Wang and Gamon,  
74 2019). The general concept of ‘spectral diversity’ is founded on the principle that, due to differences in  
75 functional form (both growth form and pigmentation), plant species have differential reflectance signals  
76 across the electromagnetic (EM) spectrum (Gamon et al., 1997). Thus, for a multispectral image, the  
77 diversity of spectral reflectance can be a proxy for the number of different plant species, or species  
78 diversity (Gholizadeh et al., 2019; Laliberté et al., 2020). The spectral diversity concept was recently  
79 applied in the hyper-diverse Cape Floristic Region, where destructively sampled leaf reflectance spectra  
80 were used to obtain a robust proxy ( $R^2 > 0.9$ ) of species diversity across 1,267 - 10 m × 5 m quadrats  
81 (Frye et al., 2021). Therefore, integrating sensing data at a range of spatial scales (Laliberté et al., 2020;  
82 Turner, 2014) and the use of spectral surrogates for biodiversity (Frye et al., 2021) can rapidly improve  
83 biodiversity monitoring.

84           Recently, there have been several applications of spectral diversity from close range imaging  
85 data (Gholizadeh et al., 2019; Lopatin et al., 2017). In prairie grassland ecosystems, close associations  
86 have been found between species diversity and spectral diversity, captured using aircraft-mounted  
87 hyperspectral sensors and images at a spatial resolution of 1 m × 1 m (pixel resolution) (Gholizadeh et  
88 al., 2020, 2019, 2018). Gholizadeh et al. (2019) primarily use the average coefficient of variation across  
89 pixels and spectral bands as the metric of spectral diversity, which we also adopt here. Furthermore, at  
90 a fine resolution of < 0.5 cm × 0.5 cm, static monitoring of grassland plots has been used to estimate  
91 not only biodiversity metrics (Imran et al., 2021; Villoslada et al., 2020), but to reconstruct species  
92 percentage cover and extract detailed features of community dynamics (Lopatin et al., 2017). However,  
93 a key limitation of these close-range imaging approaches is their reliance on expensive hyperspectral  
94 sensors (> \$50,000 sensors; Gholizadeh et al., 2019; Imran et al., 2021; Lopatin et al., 2017) and  
95 monitoring (\$1,200 per hour using the CALMIT aerial sensor from Gholizadeh et al., 2019).  
96 Overcoming these cost limitations will facilitate further use of spectral imaging in biodiversity research.

97           Despite advances in image sensing, there is a need for monitoring systems that i) are deployable  
98 in a diverse range of habitats, ii) can span a range of spatial resolutions, iii) are cost-effective, and iv)  
99 user friendly. One potential solution is the use of commercial unmanned aerial vehicles (UAVs), or  
100 drones, which have rapidly increased in popularity over the last decade (Colomina and Molina, 2014).  
101 Here, we investigate the efficacy of multispectral imaging UAV technology in the estimation of  
102 biodiversity in a temperate calcareous grassland. We used a commercially available UAV system with  
103 a five-band multispectral band sensor (Blue 450±16 nm; Green 560±16 nm; Red 650±16 nm; Red Edge  
104 730±16 nm; Near Infrared 840±16 nm) to image 37 - 1 m × 1 m quadrats that were also characterised  
105 using *in situ* biodiversity assessments from botanical surveys. Then, by extracting moments of spectral  
106 diversity from multispectral image data, we estimate spectral diversity and explored associations  
107 between spectral diversity and biodiversity. Our goal is to provide a framework for cost-effective  
108 biodiversity monitoring in complex environments using readily and commercially available  
109 technologies.

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## 111 2. Materials and Methods

### 112 2.1 Study site and in-situ biodiversity data

113 Data collection took place at the five-acre section of the Upper Seeds field site (51°46'16.8"N  
114 1°19'59.1"W) in Wytham woods, Oxfordshire, UK between 16<sup>th</sup> June-14<sup>th</sup> July 2021, the peak of the  
115 growing season. The Upper Seeds site is a recovering and managed calcareous grassland, which was  
116 used for agriculture in the 1950s, before encroaching scrub vegetation was removed and the site was  
117 managed as a grassland beginning in 1978 (Gibson, 1986). Management on upper seeds is implemented  
118 with mowing of the site in mid-July at the peak of the growing season, and again in early October,  
119 coinciding with the end of the growing season. The site has a low average soil depth (300-500 mm),  
120 generally alkaline soils (Gibson and Brown, 1991), a daily average temperature range of -5 °C to 26  
121 °C (2016-2020), a daily total precipitation range of 0-40 mm (2016-2020), and high general  
122 biodiversity, in which graminoids are the dominant functional group (59.1% by biomass). A total of 37  
123 1 m × 1 m experimental quadrats were used in the current study, which display a large degree of  
124 variation in species composition and biomass. There were between 16 and 33 vascular plant species per  
125 m<sup>2</sup>, with a mean richness of 25.77 species and a median richness of 26 species. Total above-ground dry  
126 biomass across quadrats varied between 166.8 g/m<sup>2</sup> and 931.5 g/m<sup>2</sup>, with a mean of 397.9 g/m<sup>2</sup> and  
127 median of 327.2 g/m<sup>2</sup>.

128 We explored biodiversity and spectral diversity associations in the context of two long-term  
129 experiments that aim to explore the response of grasslands to environmental change (full site map in  
130 Fig. S1). These experiments are the Disturbance and Resources Across Global Grasslands (DRAGNet,  
131 n = 20 plots) coordinated research network (<https://nutnet.org/dragnet>) and the global drought network  
132 (DroughtNet, n = 17 plots) coordinated research network (<https://drought-net.colostate.edu/>). All  
133 DRAGNet plots (5 m × 5 m plots) were ambient controls, with no experimental treatments applied prior  
134 to the collection of the data reported here. Each 5 m × 5 m plot from DroughtNet was one of four  
135 experimental treatments: ambient control plots (n = 5), -50% rainfall shelters to simulate drought (n =  
136 5), +50% irrigated plots to simulate increased rainfall (n = 5), and procedural controls (rainfall shelter  
137 with no change to rainfall) (n = 2; three plots were inaccessible for the UAV as the rainfall shelters were  
138 fixed). For analyses, ambient control treatments (n = 25) across the two research networks were pooled

139 as we did not observe substantial differences between biodiversity metrics (Fig. S2). To account for  
140 replicated observations of the same quadrats and estimate the consistency of spectral diversity measures,  
141 we explored quadrat-level variance using intercept-only random effects for the quadrat ID.

142 To estimate the efficacy of multispectral sensors in predicting biodiversity, we collected data  
143 from two sources, *in-situ* biodiversity assessments and UAV derived multi-spectral image data (Fig. 1).  
144 For the *in-situ* assessments, we quantified biodiversity metrics using species-level percentage cover and  
145 dry above-ground biomass data. We estimated percentage cover data for all vascular plant species in a  
146 plot using a 1 m × 1 m gridded quadrat (10 cm grid), focusing on four broad functional groups:  
147 graminoids, legumes, forbs, and woody species (Fig. 1). Because species overlapped spatially,  
148 percentage cover estimates could exceed 100%. Using relative proportions,  $p$ , calculated from  
149 percentage cover estimates, we calculated three biodiversity metrics: i) vascular plant species richness,  
150 ii) the Shannon-Weiner diversity index,  $H$  (equation 1; Shannon & Weaver, 1963), and iii) the  
151 Simpson's diversity index,  $D$  (equation 2; Simpson, 1949):

$$152 \quad H = -\sum p \ln p, \quad \text{Eqn. 1}$$

$$153 \quad D = \sum p^2 \quad \text{Eqn. 2}$$

154 We estimated above ground biomass after UAV image/percentage cover data collection, using  
155 a clip strip of all vascular plant material in 1 m × 0.2 m (DRAGNet; collected from standardised  
156 locations in the plot) or 1 m × 0.25 m (DroughtNet; collected from the centre of each quadrat). Clip  
157 strips were gathered using hand trimmers at a height of 1-2cm above the soil surface. Within one day  
158 of collection, we sorted clip strips in to five functional groups: graminoids, legumes, forbs, woody  
159 species, and bryophytes (not included in species-level percentage cover estimates) and dried them at  
160 70°C for 48hr, before weighing the dry biomass at an accuracy of ±0.1g. The estimates of biomass were  
161 scaled to g/m<sup>2</sup> for analyses.

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## 163 2.2 UAV image data collection

164 To obtain spectral diversity data, we collected image data using manual flights of the DJI Phantom 4  
165 multispectral UAV (<https://www.dji.com/p4-multispectral>). The sensor payload of the DJI Phantom 4

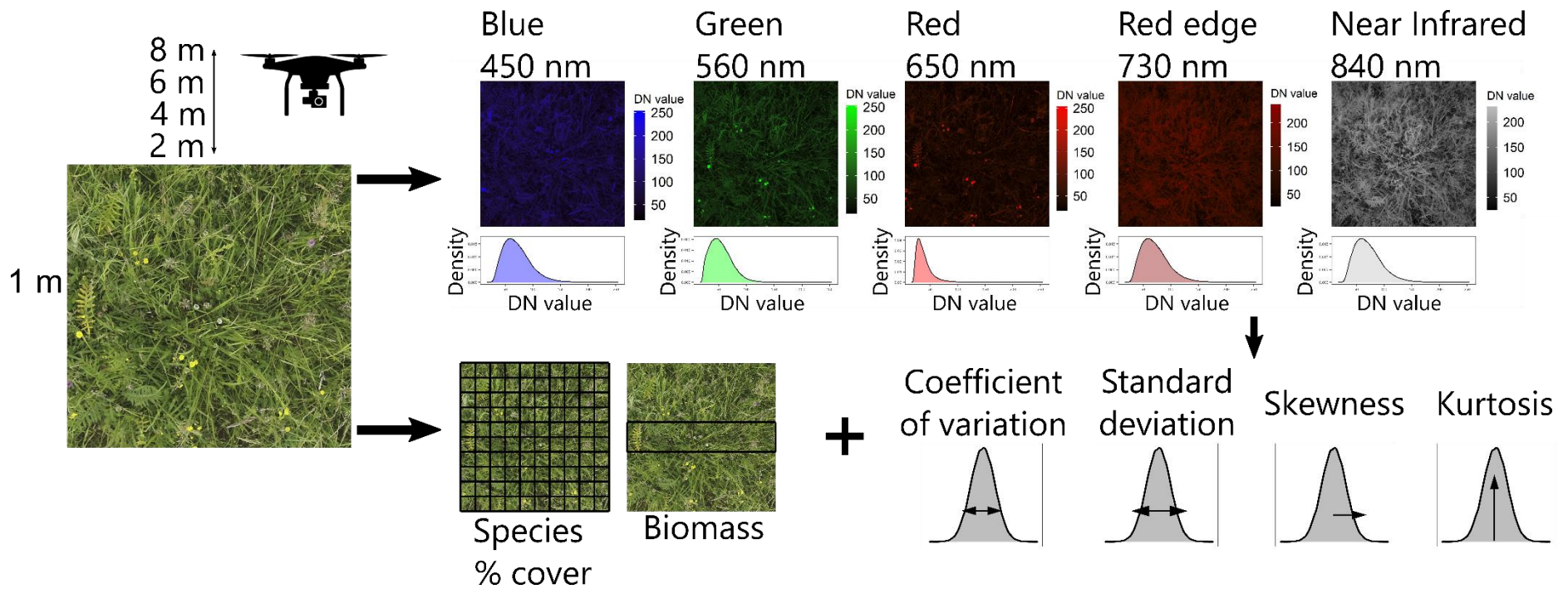
166 multispectral consists of six 4.96 mm × 3.72 mm complementary metal–oxide–semiconductor (CMOS)  
167 sensors: one RGB sensor for visible range colour images, and five monochrome sensors for  
168 multispectral imaging. The five multispectral sensors are sensitive at the following electromagnetic  
169 wavelengths: Blue - 450 nm ± 16 nm, Green - 560 nm ± 16 nm, Red - 650 nm ± 16 nm, Red edge - 730  
170 nm ± 16 nm and Near-infrared - 840 nm ± 26 nm (Fig. 1). Each sensor has an effective resolution of  
171 2.08 MP. All six image sensors are triggered simultaneously when capturing data, with negligible (but  
172 non-0) positional differences between sensors. A dorsal spectral sunlight sensor on the P4 multispectral  
173 sensor provides image exposure compensation of multispectral image data.

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179 **Fig. 1. Schematic for assessing the efficacy of spectral distribution moments for capturing biodiversity in a temperate grassland.** For each  $1\text{m} \times 1\text{m}$   
 180 observation quadrat, we collected both UAV image data (top) and in-situ biodiversity data (bottom). In-situ biodiversity data were collected by botanical surveys  
 181 for vascular plant percentage cover across the quadrat (from which richness, Shannon-Weiner and Simpson's indices were calculated) and using dry above-  
 182 ground biomass for clip strips (area determined by the coordinated research networks DRAGNet and DroughtNet; see 2.1), after UAV images were taken. UAV  
 183 images were collected for each plot at four recording heights (2 m, 4 m, 6 m, and 8 m) across five multispectral bands for which at-sensor radiance Digital  
 184 Number (DN) value distributions were summarised using four moments. Finally, *in situ* biodiversity data were combined with spectral distribution moments to  
 185 examine their potential relationships using a Bayesian linear regression framework.



186 To obtain spectral diversity metrics, we collected multispectral images for each quadrat over  
187 several flights across the sampling period, capturing quadrat-level variability with weather/light  
188 conditions. However, to minimise visual interference (from rain or low sun), all images were taken  
189 during dry weather and between 10:30-15:30 (BST). The corners of each DRAGNet quadrat were  
190 marked with flags (Fig. S1b). For DroughtNet, the quadrat was approximated using the outer edges of  
191 the 5 m × 5 m plot. All images were collected facing the western edge of each plot. We collected images  
192 at increasing approximate image recording heights of 2 m, 4 m, 6 m, and 8 m above the ground to  
193 capture changes in image resolution and consequences for estimating biodiversity (Fig. 1). Flying height  
194 is recorded relative to the UAV's take-off location, and although the topographical variation at the site  
195 is < 5 m, image record heights were approximated using structures of known height (*i.e.*, rainfall  
196 shelters, see Fig. S5). A total of 1,878 individual images were collected for the 37 quadrats over seven  
197 sampling days.

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### 199 *2.3 Image processing*

200 To extract spectral reflectance metrics from the raw image data, we standardised raw images across  
201 quadrats for each sample. The raw images encompassed the full field of view of the sensors, and we  
202 first batch-cropped images with Adobe Lightroom v. 5 (Adobe, 2021) to include only data for the  
203 desired 1 m<sup>2</sup> quadrats. Images were exported as .tif files maintaining at-sensor radiance values with  
204 minimal post-processing. We did not perform post-processing to account for interference from other  
205 sources solar radiation (e.g. Gholizadeh et al., 2019) due to the low recording height of UAV flights in  
206 the current study. Instead we estimated quadrat-level variability in spectral signals across the study  
207 period. The data stored in each .tif image were at-sensor radiance Digital Number (DN) values, which  
208 were used in subsequent analyses. The use of raw DN values was appropriate for the purpose of this  
209 study because we aimed to capture moments of the distribution of DN values in each image. Scaling  
210 DN values to normalised reflectance values was not necessary to explore the reflectance distributions  
211 because relative differences between DN values, and thus distributional moments remain unaltered.  
212 Furthermore, the omission of calibration to reflectance values, which is typically performed with

213 standardised reflectance plates, was more appropriate for the application of the current protocol and  
214 aims *i.e.* deployment in complex environments. Multispectral .tif images were treated as rasters for  
215 further image processing, and all subsequent analysis was carried out using R version 4.0.5 (R Core  
216 Team, 2021).

217 We calculated moments of spectral reflectance for each image using the *raster* package  
218 (Hijmans, 2020). Following Gholizadeh et al. (2019), we calculated the coefficient of variation,  
219 standard deviation, skewness, and kurtosis across raster pixels to capture the shape of the reflectance  
220 distribution (Fig. 1). We averaged moment values of reflectance across all multispectral bands for a  
221 single observation (a given quadrat at a given recording height in each sampling event) to calculate  
222 overall distributional moments. Thus, here we define the spectral coefficient of variation as the mean  
223 coefficient of variation of the spectral reflectance across raster pixels and multispectral bands for a  
224 single image. Observations were discarded if the image recording height was >8 m and replicate images  
225 were not obtained for all quadrats at all image recording heights. Therefore, the final sample size for  
226 the averaged spectral moment data was 193. In addition, to identify the spectral bands that were most  
227 sensitive to biodiversity metrics, we also tested band-level associations, where reflectance distributions  
228 were not averaged across spectral bands for the same raw data, and in this case spectral reflectance  
229 distributions of each band were related to biodiversity indices.

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#### 231 *2.4 Statistical analyses*

232 We explored the efficacy of spectral reflectance distribution moments in describing *in-situ* biodiversity  
233 indices using a Bayesian hierarchical linear regression model selection framework using the *brms*  
234 package (Bürkner, 2017; Fig. 1). All variables were z-scored (mean and variance centered on 0) for  
235 analysis to meet the distributional assumptions of linear regressions. The key response variable was the  
236 spectral coefficient of variation (Gholizadeh et al., 2019), and the key predictor variables were the *in-*  
237 *situ* biodiversity indices. However, we also tested other spectral moment-biodiversity associations,  
238 namely, the skewness of spectral reflectance and biomass.

239 We then estimated the out-of-sample predictive performance of models including biodiversity  
240 indices relative to base models. For each explored pair-wise combination of spectral distribution  
241 moment and biodiversity indices, we performed leave-one-out cross validation with the *loo* criterion  
242 and the expected log-wise predictive density (*elpd*, where  $\Delta elpd$  gives the change in *elpd* relative to  
243 another explanatory model) (Vehtari et al., 2017). Base models did not include any predictor variables,  
244 including only an intercept-only random effect for quadrat. We also investigated the performance of  
245 models including image recording height, and two-way interaction terms between biodiversity indices  
246 and height to explore how image resolution change influenced the efficacy of spectral diversity indices.

247 In addition to models on averaged spectral moments, we also used band-level moments to  
248 investigate the relationship between biodiversity indices and the spectral coefficient of variation for  
249 each individual reflectance band. We included univariate and two-way interaction terms between the  
250 reflectance band and biodiversity indices variables. Finally, because a small number of quadrats used  
251 in the current study were also exposed to long-term drought/irrigation/control treatments, we explored  
252 whether there were differences in average spectral moments between ambient (n = 25 plots), control (n  
253 = 2), irrigated (n = 5) and drought (n = 5) using a categorical predictor for treatment.

254 To account for repeated observations from the same quadrat at different heights or across  
255 sampling events, all models included an intercept-only random effect for the quadrat  $\sigma_{quadrat}$ . From  
256 this random effect, we also estimated the intraclass correlation (ICC) or repeatability (*R*). This term  
257 indicates the proportion of quadrat-level variance  $\sigma_{quadrat}$  with respect to the population-level variance  
258  $\sigma$  (Nakagawa and Schielzeth, 2010). We used this estimate of repeatability enabled us to assess the  
259 consistency of spectral reflectance distributions across observations of the same quadrat.

260 In analyses with average spectral distribution moments, we used weakly informed normal priors  
261 for the population-level intercept and coefficient terms of  $N(0,1)$ . The  $\sigma_{quadrat}$  term was fit using an  
262 exponential prior with a rate of two. For band-level analyses (with a greater number of parameters),  
263 models were fit using  $N(0,0.7)$  intercept/coefficient priors and exponential  $\sigma_{quadrat}$  priors with a rate  
264 of four. Models were run across four serial chains for 2,000 iterations with 1,000 warmup iterations,  
265 and the model's convergence across chains was assessed by inspecting  $\hat{R}$  values (Bürkner, 2017).

### 266 3. Results

267 We found consistent positive associations between the average coefficient of variation in  
268 spectral reflectance and biodiversity, namely, the Shannon-Weiner and Simpson's indices (Fig. 2). The  
269 model including the Shannon-Weiner index and image recording height as univariate terms  
270 outperformed the base model, with  $\Delta elpd = 123.6$  (Table S1). Increases in the Shannon-Weiner index  
271 were associated with increases in the average spectral coefficient of variation ( $\beta_{shannon} = 0.19 [-0.04,$   
272  $0.43]$ , 95% credible intervals; Fig. 2a). Furthermore, as expected, there was a strong negative  
273 association between image recording height and the average spectral coefficient of variation ( $\beta_{height} =$   
274  $-0.28 [-0.31, -0.26]$ ; Fig. 2a). This negative association suggests that the resolution of spectral diversity  
275 decreases rapidly with recording height at this spatial resolution (~ 30 % decrease in scaled spectral  
276 variation per 1 m height increase). The positive association with the spectral coefficient of variation  
277 was stronger for the Simpson's biodiversity index (Fig. 2b). Although the full model including a two-  
278 way interaction between recording height and Simpson's index was the best predictive model, we  
279 selected the model including only univariate effects ( $\Delta elpd = 125.0$ ), because of a lack of a clear  
280 interaction effect (Table S2). Here, a similar pattern with image recording height was accompanied by a  
281 stronger positive association between Simpson's index and spectral coefficient of variation ( $\beta_{simpsons}$   
282  $= 0.33 [0.12, 0.54]$ ; Fig. 2b).

283 Generally, we did not observe associations between the skewness and kurtosis in spectral  
284 reflectance distributions and biodiversity indices (Fig. S3). Furthermore, there was no clear evidence  
285 for a relationship between total above-ground biomass and any of the spectral distribution moments  
286 (Fig. S3). Specifically, although there was increased model predictive performance from models  
287 including biomass, there was no clear relationship between the skewness of spectral reflectance  
288 distribution and biomass ( $\beta_{biomass} = 0.08 [-0.19, 0.35]$ ; Fig. S3; Table S5)

289 In addition to overall effects, in band-level analyses where raw data were not averaged across  
290 bands, there was evidence for an interaction effect between the spectral band and both the Shannon-  
291 Weiner ( $\Delta elpd = 772.0$ ) and Simpson's indices ( $\Delta elpd = 771.5$ ) (Table S3 & S4, respectively).  
292 Generally, the green ( $560 \pm 16$  nm) and red ( $650 \pm 16$  nm) spectral bands displayed higher variability in

293 the coefficient of variation across quadrats, and stronger associations with the Shannon-Weiner and  
294 Simpson's indices (Fig. 3). The Red Edge ( $730\pm 16$  nm) and Near Infrared ( $840\pm 16$  nm) bands exhibited  
295 weaker associations with biodiversity indices (Fig. 3).

296 When assessing the influence of treatment on spectral reflectance, we also observed reductions  
297 in the average spectral coefficient of variation in both drought and procedural control quadrats in  
298 comparison to ambient or irrigated treatments (Fig. S4). However, given the congruence of procedural  
299 control and drought treatments in DroughtNet, both of which are characterised by metal rainfall shelters,  
300 we conclude that the reduction in spectral coefficient of variation in drought and procedural treatments  
301 is likely a result of structural interference from the rain shelter structures (Fig. S5).

302 Finally, we tested the consistency of the spectral coefficient of variation across observation  
303 days and heights for each quadrat in the best predictive Shannon-Weiner and Simpson's index models  
304 (Table S1 & S2, respectively). The average coefficient of variation was highly consistent for each  
305 quadrat when images at different heights or across sampling events were compared (Fig. S6). Both the  
306 Shannon-Weiner and Simpson's models with the average coefficient of variation exhibited quadrat-  
307 level variance that exceeded the population-level variance and a high degree of repeatability (Fig. S5;  
308 0.76 [0.65, 0.85] and 0.72 [0.60, 0.82], respectively).

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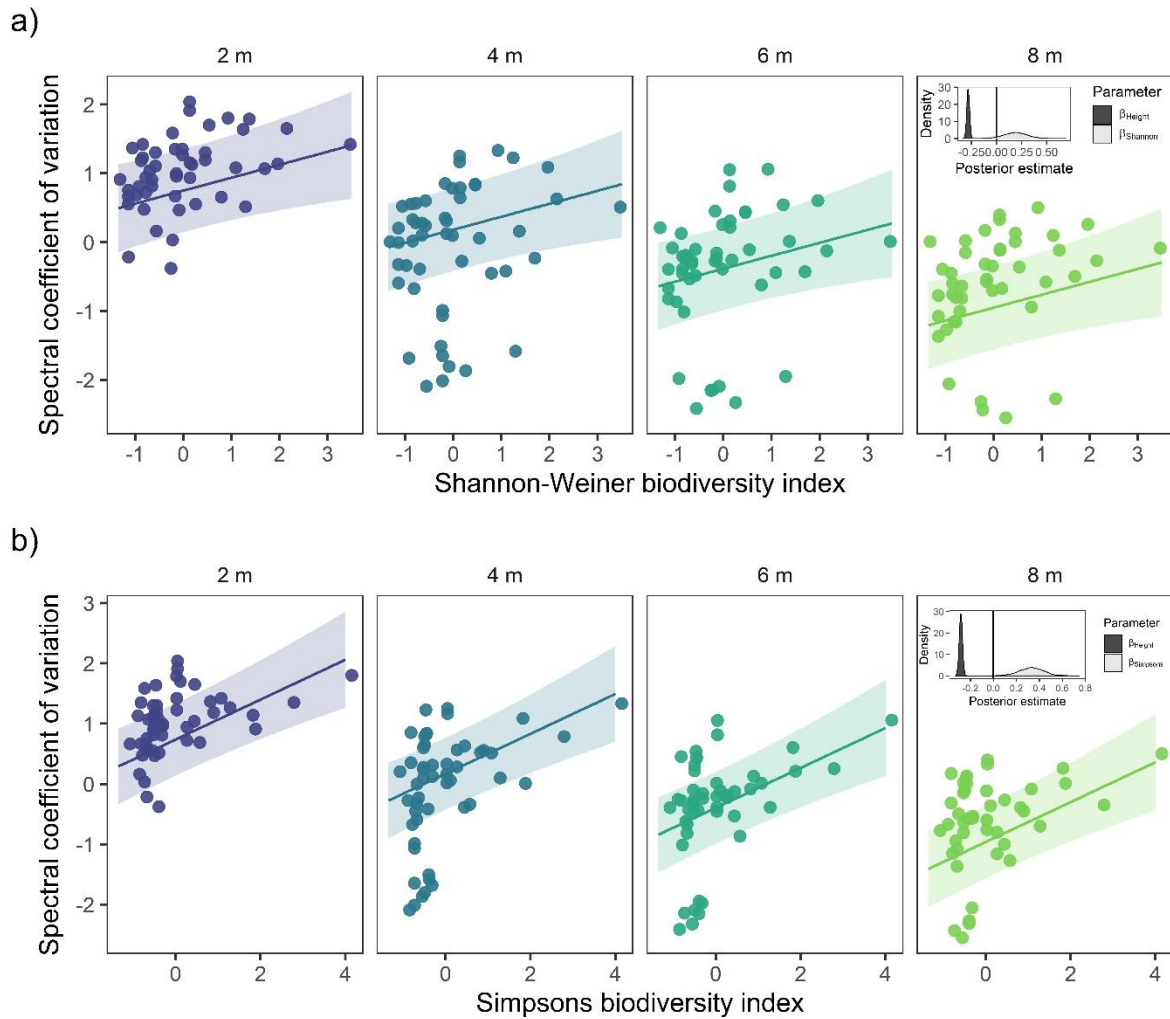
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322 **Fig. 2. Consistent positive associations between biodiversity indices and the average spectral**

323 **coefficient of variation.** The positive association between (a) Shannon-Weiner biodiversity index and

324 (b) Simpsons index, and the average spectral coefficient of variation (averaged across five spectral

325 bands) for different image recording heights: 2 m, 4 m, 6 m, and 8 m (panels). Points are observations

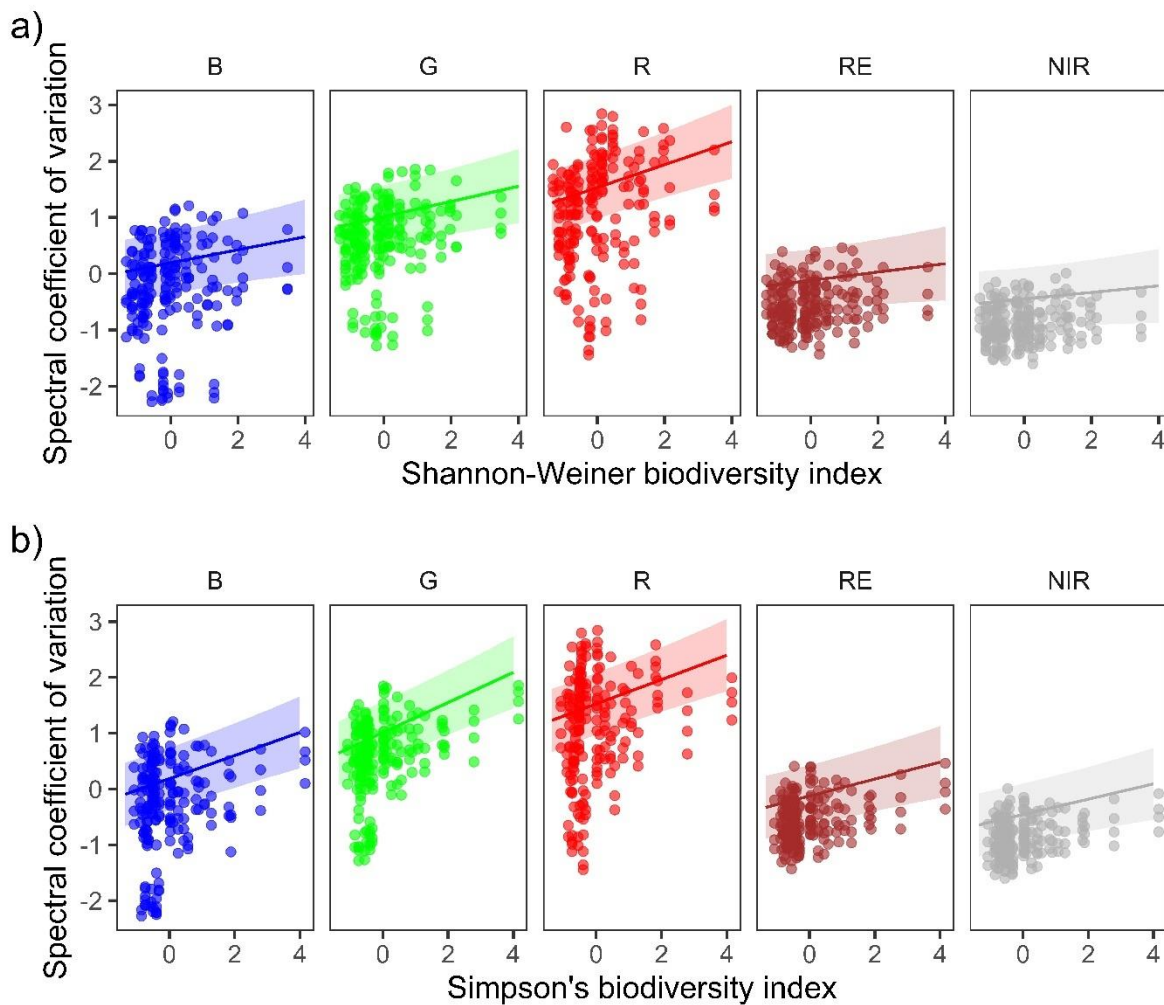
326 from a single quadrat at a given height. Both biodiversity indices and spectral coefficient of variation

327 are z-scored. Lines represented the posterior prediction mean over 4,000 simulations averaged over all

328 quadrats, with the 90% credible intervals. Insets showcase density distributions of the posterior

329 estimates for the image recording height ( $\beta_{height}$ ) and biodiversity indices ( $\beta_{shannon}$  and  $\beta_{height}$ ).

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332 **Fig. 3. Green and Red spectral reflectance bands are the most sensitive to biodiversity indices.**

333 Posterior predictions for the band-level spectral coefficient of variation with the Shannon-Weiner (a)

334 and Simpson's (b) biodiversity indices at an image recording height of 2 m. Here, raw spectral moments

335 were not averaged across spectral bands (as in Fig. 2). Points are raw observations from a single plot,

336 and the colour denotes the spectral band (B = Blue, G = Green, R = Red, RE = Red Edge, NIR = Near

337 Infrared). Both biodiversity indices and spectral coefficient of variation are z-scored. Lines are the

338 posterior prediction mean over 4,000 simulations averaged over plots, with the 90% credible intervals.

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#### 344 4. Discussion

345 Despite rapid technological advancements in image sensing over the last four decades, biodiversity  
346 monitoring is not currently able to track the full extent of human impacts on the biosphere (Wang and  
347 Gamon, 2019; Wilson, 2017). We urgently need more cost-effective and widely available systems to  
348 monitor detailed changes in biodiversity (Turner, 2014; Turner et al., 2003). Here, using a commercially  
349 available short-range Unmanned Aerial Vehicle (UAV, drone), we find a consistent, repeatable  
350 association between variation in spectral reflectance and species diversity in a biodiverse temperate  
351 grassland. The coefficient of variation in spectral reflectance was positively associated with the  
352 Shannon-Weiner and Simpsons indices, and in particular the green and red bands of the electromagnetic  
353 (EM) spectrum were most indicative of grassland biodiversity. Our results build on extensive work in  
354 grassland ecosystems exploring the use of spectral diversity as a surrogate for biodiversity (Frye et al.,  
355 2021; Gholizadeh et al., 2019; Villoslada et al., 2020) and species composition (Lopatin et al., 2017).  
356 However, our research in a diverse temperate grassland community contrast with previous findings that  
357 highlighted limitations to the characterisation of biodiversity using spectral imaging in species-rich  
358 environments (Imran et al., 2021). We highlight the importance of close-range remote sensing for  
359 biodiversity monitoring (Turner, 2014; Turner et al., 2003; Wang and Gamon, 2019). Crucially, we  
360 demonstrate the feasibility of the spectral diversity concept using a commercially available UAV with  
361 a low spectral resolution sensor, which has far-reaching potential as a tool to explore biodiversity  
362 change at high spatio-temporal resolution and in complex environments.

363 The key advantage of using commercially available UAV technology is its cost-effectiveness  
364 relative to reliance on *in-situ* monitoring or the use of long-range or high spectral resolution sensors. In  
365 the current study, where we collected data from 37 quadrats at four different image recording heights,  
366 the total flight time was 134 min. If we reasonably allocate one researcher 60 min to do a full botanical  
367 survey (species percentage cover and biomass clip – ignoring biomass processing of 30 min per sample),  
368 the full *in-situ* sampling time is 37 hr. Thus, remote monitoring would have saved on sampling time by  
369 a factor of 16. In the current study, the reduction in sampling time is of course limited to proxies of  
370 broad biodiversity metrics. However, with increases to sensor resolution and decreasing costs,  
371 reconstructing species-level biodiversity data with flexible remote monitoring may also be possible



372 (Lopatin et al., 2017). Increases in cost-effectiveness may also be increased by automated flight paths  
373 over survey locations, for which unsupervised spectral reflectance data could be collected. Furthermore,  
374 while rapid advancements have been made on spectral diversity, previous studies have utilised high-  
375 resolution multi/hyperspectral sensors that are either immobile, high-cost, or both (Frye et al., 2021;  
376 Gholizadeh et al., 2019; Imran et al., 2021; Lopatin et al., 2017). The current complete monitoring  
377 system is available for purchase for < 10,000 USD, relative to >50,000 USD for many hyperspectral  
378 sensors. Moreover, because the most sensitive spectral reflectance moments for biodiversity indices  
379 were in the green and red bands, which lie within the visible range, we provide evidence that spectral  
380 reflectance from visible-only imaging may be sufficient to act as a proxy for biodiversity indices. The  
381 present method provides a low-cost and mobile solution to rapidly characterise biodiversity in complex  
382 environments.

383         The rapid increase in the public use of drone technology provides an opportunity for a rapid  
384 expansion of detailed biodiversity monitoring at flexible spatio-temporal scales (Colomina and Molina,  
385 2014). For example, drone technology with multispectral imaging sensors has recently been applied to  
386 characterise fungal disease in lemon myrtle trees (Heim et al., 2019) and macroalgal community  
387 structure in intertidal habitats (Tait et al., 2019), when combined with classification algorithms to  
388 disentangle biological communities. These recent findings suggest that drone technology may be  
389 applicable in many ecosystems, when combined with ground-truthed biodiversity data. However,  
390 biodiversity metrics are not the only ecological indicators, and functional traits are also widely used as  
391 ecological indicators of environmental change (*e.g.*, Bjorkman et al., 2018). Because spectral  
392 reflectance is related to functional form (Gamon et al., 1997), the applicability of sensing technology is  
393 not limited to biodiversity, and can also act as a proxy for functional diversity (Frye et al., 2021). In the  
394 current study site, incorporating UAV monitoring to long-term experimental manipulations will enable  
395 the investigation of how environmental disturbances such as drought and nutrient addition influence  
396 biodiversity, functional diversity and community structure.

397         Another advantage of the current study is our use of raw digital reflectance values and moments  
398 of spectral reflectance distributions as opposed to ground-calibrated reflectance values that are typically  
399 applied to long-range multispectral imaging (Schläpfer et al., 2020). We therefore call for the

400 application of the current approach across ecosystems. The public interest in drone technology may also  
401 assist in the development of crowd sourcing or citizen science programs to provide high-resolution  
402 biodiversity monitoring globally. Furthermore, the utility of this approach to gather large amounts of  
403 image data will increase when combined with machine learning algorithms to identify single species or  
404 high-resolution community dynamics. Currently, machine learning has been applied to agricultural  
405 imaging challenges (Heim et al., 2019) and in static species-cover assessments (Lopatin et al., 2017).  
406 Therefore, there is now an opportunity to combine this technology with large UAV-derived datasets.  
407 Ultimately, capitalising on drone technology has the potential to revolutionise biodiversity research.

408         Two key limitations to the current approach that may influence its application in other  
409 environments is the spatial and temporal resolution of data. Integrating spatial scales has long been a  
410 central issue in remote sensing applications (Turner, 2014). We found a negative association between  
411 spectral diversity and recording height. With increases in recording height there are decreases in pixel  
412 resolution, which are then unable to discern micro-structures from plant species that contribute to  
413 spectral reflectance variation. While this negative relationship was insufficient to influence species-  
414 diversity relationships in the current study below 10 m in recording height, this finding suggests that at  
415 greater recording heights associations with high-resolution measures of species diversity may  
416 breakdown. Previous studies have either focussed on short-range/destructive sampling (Frye et al.,  
417 2021; Lopatin et al., 2017) or long-range multispectral imaging (Gholizadeh et al., 2019), and therefore  
418 there is a need to explore species-spectral diversity associations across spatial scales. Recently, novel  
419 dissimilarity approaches have been applied to satellite imaging data at a range of spatial scales (Rossi  
420 et al., 2021). Integrating these approaches using UAV technology in novel habitats with alternate plant  
421 communities will be crucial for future research.

422         Temporal resolution is also a key factor in need of consideration when assessing spectral-  
423 species diversity associations. The data used in the current study represent a ‘static’ measure of  
424 biodiversity at the peak of the growing season in a temperate grassland. However, grassland  
425 communities exhibit a high degree of temporal variability, particularly in response to environmental  
426 drivers (Harrison et al., 2015; Thorhallsdottir, 1990). When implementing unmanned biodiversity  
427 monitoring, this temporal component may hinder the accuracy of spectral diversity measures. A solution

428 developed by Rossi et al., (2021) applies a novel dissimilarity index between pairs of spectral images  
429 over the same region to disentangle temporal components of community change linked to management  
430 and phenology. However, this approach has yet to be applied to images collected using drone  
431 technology in varying habitats.

432

## 433 **5. Conclusions**

434 Taking advantage of technological advancements in unmanned sensing will greatly improve the cost-  
435 effectiveness of biodiversity monitoring. UAVs have the potential to span spatial scales, access  
436 challenging environments, and provide high resolution data on the impact of environmental change on  
437 ecosystems. Our study adds to a growing body of literature highlighting links between spectral and  
438 species diversity. Integrating these patterns at varying spatio-temporal scales and in novel habitats will  
439 provide vital insights to aid in documenting changes in the biosphere.

440

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