

1 **Come to the dark side! The role of functional traits in shaping**
2 **dark diversity patterns of Southeast European hoverflies**

3

4 Short title: Hoverfly dark diversity in SE Europe

5

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21

22 **Abstract**

23 Dark diversity represents the set of species belonging to a species
24 pool but is not locally present, i.e. species that are currently
25 'missing' from a site but have the potential to occur there. The
26 concept allows characterization of the mechanisms determining
27 why species are sometimes absent from an area that seems
28 ecologically suitable for them. Our aim was to assess the dark
29 diversity of hoverflies (Diptera: Syrphidae) in Southeast Europe
30 and to discuss the role of different functional traits that might
31 increase the likelihood of species contributing to dark diversity.
32 Based on expert opinion, the Syrph the Net database and known
33 occurrences of species, we estimated observed, potential and dark
34 diversities within each defined vegetation class for 564 hoverfly
35 species registered in SE Europe. To detect the most important
36 functional traits of species contributing to dark diversity across
37 classes, we used a random forest algorithm and respective statistics
38 for variable importance (%IncMSE). The highest dark diversity
39 was found for Southwest Balkan sub-Mediterranean mixed oak
40 forests, whereas the lowest was in Mediterranean mixed forests.
41 Three larval feeding modes (saproxylic, and phytophagous of
42 bulbs or roots) were found to be most important for determining
43 the probability of a species contributing to hoverfly dark diversity,
44 based on univariate correlations and random forest analysis. This
45 study shows that studying dark diversity might provide important

46 insights into what drives community assembly in this taxon and
47 region, especially its missing components and contributes to more
48 precise conservation prioritization of both hoverfly species and
49 their habitats.

50

51 **KEYWORDS:** disturbance; functional characteristics; insects;
52 missing species; richness; Syrphidae; vegetation classes

53

54 **Introduction**

55 In order to understand and try to alleviate the negative
56 consequences of biodiversity loss, detailed information on different
57 aspects of biodiversity is essential (Hooper *et al.*, 2005; Barton &
58 Evans, 2017). Data on extant biodiversity may not allow
59 interesting patterns related to missing components of communities
60 to be uncovered (Pärtel *et al.*, 2011). Recent findings (Pärtel, 2011,
61 2014; Lewis *et al.*, 2017) have shown that so-called ‘dark
62 diversity’ could reveal new biodiversity patterns that would not be
63 evident from investigating only observed diversity (Ronk *et al.*,
64 2015). In order to examine dark diversity of a target site, the
65 habitat-specific species pool must be established, i.e. the set of
66 species that can inhabit a given area under designated ecological
67 conditions (Cornell & Harrison 2014; Zobel, 2016). The species
68 that are not recorded at a particular site, but that belong to its

69 species pool and could potentially co-occur in the site given its
70 biotic, abiotic processes and dispersal limitations, constitute the
71 dark diversity of that site (Pärtel *et al.*, 2011). In other words, dark
72 diversity represents the absent element of a habitat-specific species
73 pool (Lewis *et al.*, 2017). Importantly, a species pool is not simply
74 the sum of observed and missing species (Fig. 1). Although total
75 observed diversity consists of species that belong to the habitat-
76 specific species pool, it also comprises species that are currently
77 present in a particular community but are not part of the pool.

78

79 Figure 1. Schematic diagram illustrating species pool, observed
80 and dark diversity.

81

82 Hoverflies (Diptera: Syrphidae) play important roles in providing
83 vital ecosystem services such as pollination (Ssymank & Kearns,
84 2009; Petanidou *et al.*, 2011; Jauker *et al.*, 2012), waste
85 decomposition (Gilbert, 1985) and biological control (White *et al.*,
86 1995; Blaauw & Isaacs, 2012; Day *et al.*, 2015). Recent studies
87 have shown that hoverflies can serve as valuable model organisms
88 in terms of studies of climate change (Kaloveloni *et al.*, 2015;
89 Radenković *et al.*, 2017; Miličić *et al.*, 2018), urbanization (Bates
90 *et al.*, 2011; Verboven *et al.*, 2014), landscape structure (Power *et*

91 *al.*, 2016) or land use (Aguirre-Gutiérrez *et al.*, 2015, Földesi &
92 Kovács-Hostyánszki, 2016; Lucas *et al.*, 2017).

93 Southeast Europe (SE Europe) is one of the richest areas globally
94 in terms of hoverfly diversity, mostly due to its complex geology
95 (Cvetković *et al.*, 2015) and climatic diversity (Vukelić *et al.*,
96 2018). Pronounced human-induced environmental changes have
97 taken place in this region and left their mark on its vegetation.
98 Considering the close connection of hoverflies to their habitats,
99 this environmentally heterogeneous region is particularly suitable
100 for examining biological and ecological responses of hoverflies to
101 contrasting habitat types.

102 Ecological studies of hoverflies increasingly use functional traits
103 (i.e. physiological, phenological, morphological or behavioral
104 characteristics), rather than simply focusing on their taxonomic
105 identity. Various authors have examined the response of hoverfly
106 functional diversity to different environmental aspects including
107 land use and land management (Schweiger *et al.*, 2007, de Groot *et*
108 *al.*, 2016, Winsa *et al.*, 2017), climate change (Aguirre-Gutiérrez *et*
109 *al.*, 2016) or habitat heterogeneity (Larrieu *et al.*, 2015). However,
110 which functional traits promote the absence of species from
111 apparently suitable sites (missing species), hence promoting higher
112 levels of hoverfly dark diversity, remains unanswered.

113 This study for the first time tests the hypothesis that functional
114 traits affect dark diversity of hoverflies, revealing ecological
115 patterns reflected in hoverfly communities. Based on known
116 occurrence records of hoverflies in SE Europe, the Syrph the Net
117 (StN) database (Speight *et al.*, 2015), and expert opinion, we aim
118 to assess the dark diversity of hoverflies in this region, as well as
119 discuss the role of different functional traits in explaining the
120 patterns of dark diversity.

121

122 **Methods**

123 **Study area and species occurrences**

124 This study includes all available information on SE European
125 hoverflies. In a geographical sense, this area covers the Balkan
126 Peninsula and the Aegean islands governed by Greece. Based on
127 field collecting in this region and extensive literature review, we
128 compiled a list of hoverfly species occurring in SE Europe.

129 Information on the distribution of registered species was extracted
130 from the database of the Faculty of Sciences, University of Novi
131 Sad, Serbia (FSUNS). This database is a result of the hoverfly
132 monitoring program across investigated area from 1950 to 2017,
133 during which the sampling of hoverflies was conducted using a
134 consistent census protocol (see Radenković *et al.*, 2017 for more
135 details). Additionally, we included data from published material

136 referring to this territory, as well as data obtained from different
137 museum and private collections. Only specimens for which precise
138 distributional data were available were used in our analyses. Exact
139 locality coordinates were checked for accuracy where provided.
140 Records for which only locality names were available were
141 assigned coordinates using Google Earth (Google Inc., 2018). In
142 total, we considered 63814 occurrence records of 564 species.

143

144 **Vegetation types**

145 To assess the distribution of hoverflies within different vegetation
146 types represented in SE Europe, we used the map of natural
147 vegetation of Europe (Bohn *et al.*, 2000/2003). In order to
148 correspond with known biological and ecological characteristics of
149 hoverfly species, we merged certain vegetation classes to generate
150 a total of 11 classes (Fig. 2).

151

152 Figure 2. Vegetation classes in Southeast Europe.

153

154 **Functional traits**

155 Data on functional traits of hoverflies were collected from multiple
156 sources. We used published data (Speight *et al.*, 2015; Speight,
157 2017), expert opinion-based data, and fieldwork experience
158 spanning 35 years regarding biological and ecological

159 characteristics of species. Information on wing length of analyzed
160 species was not available in the literature, but because we
161 considered this trait to be biologically significant, we measured it
162 using a Nikon SMZ 745T stereomicroscope. We measured four
163 specimens of each species (two male and two female), and used the
164 averaged value as a variable in further analyses. Functional traits
165 with multiple non-mutually-exclusive trait states were coded as
166 dummy variables. Additionally, if possible and where justified,
167 categorical traits were transformed into ordinal variables. If the
168 trait state for the given species was unknown, the species was
169 assigned the most common trait state found in other members of
170 that genus. The list of functional traits used in the analyses is
171 presented in Tab. 1.

172

173 Table 1. Functional traits and trait states of hoverflies in SE
174 Europe.

175

176 **Estimation of dark diversity**

177 Species pools for each defined vegetation type separately were
178 compiled based on expert opinion and information from the StN
179 database. Each species was assigned to any
180 major vegetation type in SE Europe defined in this study in which
181 it can occur, taking into consideration its known distribution across

182 Europe, as well as the known biological and ecological preferences
183 of that species (Speight *et al.*, 2015; Speight, 2017).
184 All estimations and statistics were made using the full dataset
185 (across vegetation types). To quantify observed diversity,
186 occurrence records were plotted on the vegetation map using open
187 source GIS software (QGIS Development Team, 2009). Dark
188 diversity was calculated for each vegetation type separately as the
189 difference between the species pool and the observed species
190 belonging to that particular pool (i.e. the number of potential
191 species missing from each pool). Percentage of dark diversity
192 across all vegetation classes was calculated as the ratio between
193 dark diversity and the sum of dark and observed diversity.

194

195 **Statistical analyses**

196 Our goal was to verify if species with certain traits were more
197 likely to contribute to dark diversity. Therefore, our response
198 variable was the proportion of times each species belonged to dark
199 diversity in relation to its potential diversity across vegetation
200 classes, termed herein the ‘dark diversity index’ (DDi). The
201 explanatory variables were functional traits (or trait states) of
202 species.

203 In a first step, we checked for correlations among functional traits.
204 Spearman’s rank correlations were conducted for continuous

205 variable comparisons, Mann-Whitney tests for continuous-
206 categorical variable comparisons, and Eta correlation coefficients
207 were generated for categorical variable comparisons. Values above
208 0.7 were considered indicative of collinearity (Dormann et al.,
209 2013).

210 Next, we assessed the univariate correlation between functional
211 traits and dark diversity; for categorical/binary variables we used
212 the Kruskal Wallis test, whereas Spearman rank correlation was
213 used for continuous and ordinal variables.

214 Even if some traits were not revealed to be important in univariate
215 correlations, they might be important only when other factors are
216 also considered. To establish the unique contribution of each trait
217 to the probability of being part of dark diversity, we used a random
218 forest (RF) regression model (Breiman, 2001), implemented within
219 the randomForest R package (Liaw & Wiener, 2002). In this
220 analysis, the number of trees (ntree) was set to 5000, and the
221 number of predictors sampled for splitting at each node (mtry) was
222 set to 6, which represents the default value suggested for
223 regression RF models, i.e. number of predictor variables/3. Node
224 size (nodesize) was kept as default, i.e. 5 for regression. After
225 running the model, and in order to verify if species with certain
226 traits were more likely to contribute to dark diversity, we
227 quantified the importance of each predictive variable by

228 calculating the Mean Decrease Accuracy (%IncMSE). Merrill
229 (2009) demonstrated that this latter measure provides the highest
230 stability compared to node purity measures.

231

232 **Results**

233 **Species pool, observed species and dark diversity**

234 The number of species representing observed and dark diversity, as
235 well as species complements of the species pools, varied between
236 different vegetation classes (Fig. 3).

237

238 Figure 3. Number of species in dark diversity, observed diversity
239 and in the species for each vegetation class.

240

241 The mean number of species for species pools was 276.1 (+/-39.1
242 SD), for observed diversity it was 242.7 (+/-66.3 SD), and for dark
243 diversity it was 83.0 (+/-39.6 SD) across all vegetation classes. The
244 most diverse species pool (355 species) was in Beech and mixed
245 beech forests (E), whereas Acidophilous oak and mixed oak -
246 hornbeam forests (D) exhibited the most hoverfly-species-poor
247 pool (227 species) (Fig. 3). In terms of numbers of observed
248 species, the richest vegetation class (402 species) was again beech
249 and mixed beech forests (E), and the lowest number of observed
250 hoverfly species (136 species) was recorded from Southwest

251 Balkan sub-Mediterranean mixed oak forests (H). Hoverfly dark
252 diversity was lowest in Mediterranean mixed forests (J) (33
253 species, 11.9%) and in Beech and mixed beech forests (E) (36
254 species, 10.1%), and highest in Southwest Balkan sub-
255 Mediterranean mixed oak forests (H) (162 species, 62.8%) and in
256 Alpine, subalpine and oro-Mediterranean vegetation (A) (140
257 species, 45%).

258

259 **Dark diversity and functional traits**

260

261 After excluding highly correlated variables, we retained 18 traits or
262 trait states for further analyses. Individual correlations between the
263 DDi and each functional trait are shown in Tab. 2.

264 The DDi was found to be significantly correlated with the
265 following variables: four of the larval feeding modes
266 (phytophagous on bulbs, saproxylic, phytophagous on roots, and
267 zoophagous), body length, flight ability, distribution, body pile,
268 body shape, inundation tolerance and duration of larval
269 development. The most significant correlations occurred between
270 DDi and traits related to specific larval food types such as
271 phytophagous on bulbs, saproxylic, phytophagous on roots and
272 zoophagous. In contrast, there does not appear to be a significant

273 correlation between DDi and the saprophagous larval feeding
274 mode.

275

276 Table 2. Correlations between the dark diversity index (DDi) and
277 functional traits.

278

279 Three larval feeding modes were the most significant variables
280 according to %IncMSE values (saproxyllic = 54.2, phytophagous
281 on bulbs = 53.2, phytophagous on roots = 51.9) (Fig. 4), matching
282 the three most significant individual correlations with the DDi
283 (Tab. 2). Flight period, inundation tolerance, body shape and
284 human impact tolerance were also found to be very important
285 explanatory variables contributing to the probability of a species
286 being part of dark diversity, each of which presented a %IncMSE
287 value > 40.0. However, when assessed individually, human impact
288 tolerance and flight period were no longer important explanatory
289 variables.

290

291 Figure 4. Functional traits and trait states showing relative
292 importance (%IncMSE) for dark diversity of hoverflies in
293 Southeast Europe.

294

295 **Discussion**

296 Several studies in recent years have addressed the significance and
297 possible applications of investigating dark diversity (e.g. Pärtel *et*
298 *al.*, 2011; 2014; Ronk, 2016; Lewis *et al.*, 2017). However, to the
299 best of our knowledge, no previous study has addressed the dark
300 diversity of any insect group, which is perhaps unsurprising given
301 that invertebrates are usually neglected in conservation studies
302 (Zamin *et al.*, 2010; Cardoso *et al.*, 2011). Thus, our study
303 represents the first extensive assessment of dark diversity among
304 hoverflies across SE Europe. Furthermore, it reveals which
305 functional traits of hoverflies can be linked to dark diversity,
306 potentially revealing why certain species are often missing from
307 habitat types in which they could potentially thrive.

308

309 **Levels of hoverfly dark diversity across vegetation types**

310 Southwest Balkan sub-Mediterranean mixed oak forests showed
311 the highest level of dark diversity and lowest level of observed
312 diversity. This outcome may be because oak forests are in decline
313 generally all across Europe and most of the respective habitat
314 patches are small and scattered, limiting their carrying capacity and
315 inhibiting the metapopulation dynamics important for sustaining
316 populations. Southwest Balkan sub-Mediterranean mixed oak
317 forests have been exposed to intense anthropogenic pressure over
318 the last 2000 years, subjected to constant felling (Mansourian *et*

319 *al.*, 2013). This pressure has resulted in overt destruction, size
320 reductions and fragmentation of these forests, as well as promoting
321 edge effects (Robinson *et al.*, 1995). Jovičić *et al.* (2017)
322 investigated the effect of landscape structure on the two largest
323 hoverfly genera (*Merodon* Meigen, 1803 and *Cheilosia* Maigen,
324 1822) and suggested that habitat connectivity influences the
325 species composition of habitats. Moreover, it has been
326 demonstrated that edge effects also impact physical characteristics
327 of the environment, leading to increased temperatures and reduced
328 humidity (Murcia *et al.*, 1995). Additional causes of these declines
329 are pathogenic infection of oak species (Jung *et al.*, 2010) and
330 extreme weather (drought or frost) (Thomas, 2008). However, it is
331 human impact that appears to be playing a crucial role in
332 maintaining deciduous oakwood forests. For instance, occasional
333 fires have been shown to promote the occurrence of oak (Abrams,
334 1992), and it seems that the recent decline in applying traditional
335 management strategies (such as burning) has negatively affected
336 oakwood assemblages (Hedl *et al.*, 2010). A second vegetation
337 class that presented high levels of dark diversity was Alpine,
338 subalpine and oro-Mediterranean. Multiple studies have
339 demonstrated the sensitivity of these habitats (Ruiz *et al.*, 2008;
340 Gillaredeli *et al.*, 2013). The main reasons for the vulnerability of
341 this vegetation type are increased human impact through

342 infrastructural development (Pintaldi *et al.*, 2017) and high levels
343 of grazing (Firm *et al.*, 2009). Moreover, patches of alpine and
344 subalpine habitats are usually small and fragmented. Thus,
345 multiple characteristics of the Alpine, subalpine and oro-
346 Mediterranean vegetation class could be responsible for the high
347 dark diversity of hoverflies we report for this class.

348 Mediterranean mixed forests showed the lowest level of dark
349 diversity. Although Mediterranean habitats have been reported as
350 seriously degraded due to intensive human actions (King, 1997;
351 Blondel, 2006), it would seem that they are resilient to existing
352 pressures, at least in terms of their capability of hosting a large
353 proportion of hoverfly species. Many reports (di Castri, 1981;
354 Thompson, 2005) have explained this phenomenon as resulting
355 from long-term adaptation of ecosystems to humans in this region,
356 even going so far as to apply the term “co-evolution”. Large
357 expanses of this vegetation type, which are highly connected,
358 enable the existence of many species, including those that require
359 large areas to maintain viable populations, resulting in only 33
360 species being identified as contributing to dark diversity. Beech
361 and mixed beech forests showed the second lowest level of dark
362 diversity, and presented the largest species pool and greatest
363 observed diversity. This outcome can be explained by the fact that
364 these forests are among the best preserved in SE Europe (Meyer *et*

365 *al.*, 2003), providing suitable microhabitats for both larvae and
366 adults of hoverflies. Additionally, this result may be partially
367 attributable to geographical proximity to degraded oak forests from
368 which hoverflies may have migrated to find more favorable
369 conditions.

370

371 **Functional traits contributing to dark diversity**

372 Three different larval feeding modes were identified as the most
373 significant traits contributing to dark diversity. The importance of
374 larval type and particularly their food preferences has been well
375 established for many other aspects of hoverfly biology and ecology
376 (Schweiger *et al.*, 2007; Haenke *et al.*, 2009), and our results
377 suggest that these factors dictate which species might survive in
378 habitats that are apparently adequate. Larval feeding mode
379 indirectly reflects the level of specialization of a species.
380 Saproxylic hoverfly larvae, as well as phytophagous species that
381 develop in bulbs and roots, are considered specialists (van Veen,
382 2004, Müller *et al.*, 2011). Generalist species are widely regarded
383 as being at an advantage compared to specialists in adapting to
384 changing environmental conditions (Biesmeijer *et al.*, 2006,
385 Barthel *et al.*, 2014). Generalist species are likely favored under
386 conditions of detrimental change and should constitute the majority
387 of observed diversity, whereas specialists may be absent from

388 impacted habitats and contribute towards dark diversity. Different
389 saproxylic hoverflies require different stages of wood decay for the
390 development and nutrition of their larvae (Speight, 1989), which is
391 particularly important in clear-cut forests without tree retention
392 where the microhabitats of these species are directly destroyed
393 (Larrieu *et al.*, 2012). Due to their high level of specialization,
394 saproxylic hoverflies have no alternative for their development.
395 However, changes in forest management (heterogeneity of trees in
396 terms of age and size structure) may increase saproxylic hoverfly
397 diversity (Remeer, 2005). Regarding phytophagous larvae (both
398 those developing in bulbs and in roots), their specialization is
399 reflected in the connection to a specific plant species. Thus,
400 geographical distributions of phytophagous species are limited by
401 the existence of suitable habitat for their host plants, which is
402 particularly important when species are monophagous (Müller *et*
403 *al.*, 2011).

404 With further regard to host dependency, the period during which
405 species fly was also revealed to be of high importance for dark
406 diversity in our RF analysis, but not when tested individually.
407 Flight period of hoverflies is directly linked to food resource
408 availability, with a temporal mismatch between hoverfly activity
409 and when host plants representing their primary food source
410 flower. Increasing temperatures caused by climate change might

411 lead to further phenological asynchrony (Memmott *et al.*, 2007),
412 which would have a serious negative impact on specialist species.
413 Moreover, exploitation of food resources is dependent on other
414 functional traits. Thus, complex interactions between flight period
415 and other traits are likely to have a synergistic effect on dark
416 diversity.

417 Our results also indicate the significance of inundation tolerance
418 for the dark diversity of hoverflies. It is reasonable to assume that
419 species with lower inundation tolerance contribute more to
420 hoverfly dark diversity. Inundation-tolerant species can adapt to
421 wet habitats and achieve higher species richness under those
422 environmental conditions (Keil *et al.*, 2008), reflected in their
423 higher levels of observed diversity. Additionally, inundation
424 tolerance likely plays a crucial role in the ability of species to
425 survive challenging conditions. For example, dark diversity of
426 hoverflies is lowest in Mediterranean mixed forests, even though
427 flooding and extensive land degradation and erosion often occur in
428 that habitat (Poesen & Hooke, 1997).

429 Body shape is significant in shaping patterns of dark diversity
430 because it influences species' dispersal ability, with species
431 contributing to dark diversity in general having lower dispersal
432 capacities (Riibak *et al.*, 2015). Even though other measures
433 directly related to dispersal ability were not identified as being of

434 high importance to dark diversity in our study, body shape still
435 provides useful insights into the potential of a species to disperse.
436 This functional trait contributes to species being able to overcome
437 challenging environmental conditions, as well as to extend their
438 current range and invade new suitable areas. Additionally, species
439 with well-developed dispersal abilities are less affected by habitat
440 fragmentation, as they can migrate between suitable habitat
441 patches (Thomas, 2000). Apart from its link to dispersal ability,
442 body shape might also contribute a hitherto unknown mechanism
443 of physiological or behavioral adaptation important for dark
444 diversity for which we currently have no evidence.

445 Tolerance of human impacts determines the ability of a species to
446 persist in increasingly changing environments where agricultural
447 intensification, intensive grazing, forestry or urbanization are
448 taking place, ensuring resilient species have an advantage over
449 more sensitive ones (Winfree *et al.*, 2011). Evolutionary processes
450 through which resilience develops not only result in species
451 surviving and reproducing in impacted habitats (Souza *et al.*,
452 2014), but also endow robustness against other types of
453 disturbances (Basley *et al.*, 2018). Accordingly, widely distributed
454 generalist species with the ability to thrive in different types of
455 habitats are those most likely to be resilient to human impacts
456 (Schweiger *et al.*, 2007).

457

458 **Application of dark diversity concept in hoverfly conservation**

459 Investigation of dark diversity can contribute to prioritization of
460 conservation efforts of both species and their habitats. Despite this,
461 a small number of research studies has tackled this topic (Yoshioka
462 *et al.*, 2014; Ronk *et al.*, 2015, Lewis *et al.*, 2017, Moeslund *et al.*,
463 2017). These studies highlight two main reasons for studying dark
464 diversity in the light of conservation. Firstly, high levels of dark
465 diversity in certain areas could imply the existence of
466 environmental disruptors. Secondly, apparently suitable areas that
467 exhibit a large portion of missing species have great restorative
468 potential. Thus, assessing dark diversity in these regions could
469 inform to what extent we can expect conservation activities to be
470 prosperous. Bearing this in mind, our results indicate that hoverfly
471 habitats within Southwest Balkan sub-Mediterranean mixed oak
472 forests and Alpine, subalpine and oro-Mediterranean vegetation
473 should be targeted for conservation.

474 Beside contribution to the conservation of habitats, dark diversity
475 studies enable more precise conservation prioritization in respect
476 of species-based approach. Namely, linking functional traits and
477 dark diversity may guide conservation efforts towards more
478 sensitive species. Our study shows that certain traits such as highly
479 specialized larval feeding modes, lack of inundation tolerance or

480 flight period promote dark diversity of hoverflies. Identifying
481 species possessing these traits could be an additional criterion
482 when deciding which species should receive conservation
483 attention.

484

485 **Conclusion**

486 Overall, our study shows that hoverfly dark diversity to varying
487 degrees occurs in every vegetation type in SE Europe. We found
488 larval feeding mode to be the trait of greatest importance to
489 determining species potential to be a part of dark diversity.
490 Establishing which functional traits are responsible for dark
491 diversity can help identify the processes causing species to be
492 absent from their species pool in a given habitat. Additionally,
493 these results contribute to more precise conservation prioritization
494 of both hoverfly species and their habitats.

495

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507 design of the study, MM, SP and PC wrote the paper; AV, MM, SP
508 and BI participated in data collecting; MM, SP and PC performed
509 the analyses, MM, SP and BI prepared the figures; all authors
510 contributed to manuscript with comments and revision.

511

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514

515 **References**

516

- 517 1. Abrams, M. D. (1992) Fire and the development of oak
518 forests. *BioScience*, **42**, 346-353.
- 519 2. Aguirre-Gutiérrez, J., Biesmeijer, J. C., van Loon, E. E.,
520 Reemer, M., WallisDeVries, M. F. & Carvalheiro, L. G.
521 (2015) Susceptibility of pollinators to ongoing landscape
522 changes depends on landscape history. *Diversity and*
523 *Distributions*, **21**, 1129-1140.
- 524 3. Aguirre-Gutiérrez, J., Kissling, W. D., Carvalheiro, L. G.,
525 WallisDeVries, M. F., Franzén, M. & Biesmeijer, J. C.

- 526 (2016) Functional traits help to explain half-century long
527 shifts in pollinator distributions. *Scientific reports*, **6**,
528 24451.
- 529 4. Barthel, A., Kopka, I., Vogel, H., Zipfel, P., Heckel, D. G.
530 & Groot, A. T. (2014) Immune defence strategies of
531 generalist and specialist insect herbivores. *Proceedings of*
532 *the Royal Society B: Biological Sciences*, **281**, 20140897.
- 533 5. Barton, P. S. & Evans, M. J. (2017) Insect biodiversity
534 meets ecosystem function: differential effects of habitat and
535 insects on carrion decomposition. *Ecological*
536 *entomology*, **42**, 364-374.
- 537 6. Basley, K., Davenport, B., Vogiatzis, K. & Goulson, D.
538 (2018) Effects of chronic exposure to thiamethoxam on
539 larvae of the hoverfly *Eristalis tenax* (Diptera, Syrphidae).
540 *PeerJ*, **6**, e4258.
- 541 7. Bates, A. J., Sadler, J. P., Fairbrass, A. J., Falk, S. J., Hale,
542 J. D. & Matthews, T. J. (2011) Changing bee and hoverfly
543 pollinator assemblages along an urban-rural gradient. *PloS*
544 *one*, **6**, e23459
- 545 8. Biesmeijer, J. C., Roberts, S. P., Reemer, M., Ohlemüller,
546 R., Edwards, M., Peeters, T., Schaffers, A. P., Potts, S. G.,
547 Kleukers, R., Thomas, C. D., Settele, J. & Kunin, W. E.
548 (2006) Parallel declines in pollinators and insect-pollinated

549 plants in Britain and the Netherlands. *Science*, **313**, 351-
550 354.

551 9. Blaauw, B. R. & Isaacs, R. (2012) Larger wildflower
552 plantings increase natural enemy density, diversity, and
553 biological control of sentinel prey, without increasing
554 herbivore density. *Ecological Entomology*, **37**, 386-394.

555 10. Blondel, J. (2006) The 'design' of Mediterranean
556 landscapes: a millennial story of humans and ecological
557 systems during the historic period. *Human ecology*, **34**,
558 713-729.

559 11. Bohn, U., Gollub, G., Hettwer, C., Weber, H.,
560 Neuhäuslová, Z., Raus, T. & Schlüter, H. (2000) Karte der
561 natürlichen Vegetation Europas/Map of the Natural
562 Vegetation of Europe. Maßstab/Scale 1: 2,500,000.

563 12. Breiman, L. (2001) Random Forests. *Machine Learning*,
564 **45**, 5-32.

565 13. Cardoso, P., Erwin, T. L., Borges, P. A. & New, T. R.
566 (2011) The seven impediments in invertebrate conservation
567 and how to overcome them. *Biological Conservation*, **144**,
568 2647-2655.

569 14. Cornell, H. V. & Harrison, S. P. (2014) What are species
570 pools and when are they important? *Annual Review of*
571 *Ecology, Evolution, and Systematics*, **45**, 45-67.

- 572 15. Cvetkovic, V., Prelević, D. & Schmid, S. (2016) Geology
573 of South-Eastern Europe. *Mineral and thermal waters of*
574 *Southeastern Europe* (ed. by Papić, P.), pp. 1-29. Springer,
575 Cham.
- 576 16. Day, R. L., Hickman, J. M., Sprague, R. I. & Wratten, S. D.
577 (2015) Predatory hoverflies increase oviposition in
578 response to colour stimuli offering no reward: Implications
579 for biological control. *Basic and applied ecology*, **16**, 544-
580 552.
- 581 17. de Groot, M., Eler, K., Flajšman, K., Grebenc, T.,
582 Marinšek, A. & Kutnar, L. (2016) Differential short-term
583 response of functional groups to a change in forest
584 management in a temperate forest. *Forest Ecology and*
585 *Management*, **376**, 256-264.
- 586 18. Di Castri, F. (1981) Mediterranean-type shrublands of the
587 world. *Mediterranean-type Shrublands* (ed. by Di Castri,
588 F., Goodall, D.W. & Specht R.L.), pp. 1-52, Elsevier,
589 Amsterdam.
- 590 19. Dormann, C. F., Elith, J., Bacher, S., Buchmann, C., Carl,
591 G., Carré, G., García Marquéz, J. R., Gruber, B.,
592 Lafourcade, B., Leitão, P. J., Münkemüller, T., McClean,
593 C., Osborne, P. E., Reineking, B., Schröder, b., Skidmore,
594 A. K., Zurell, D. & Lautenbach, S. (2013) Collinearity: a

- 595 review of methods to deal with it and a simulation study
596 evaluating their performance. *Ecography*, **36**, 27-46.
597
- 598 20. Firm, D., Nagel, T. A. & Diaci, J. (2009) Disturbance
599 history and dynamics of an old-growth mixed species
600 mountain forest in the Slovenian Alps. *Forest Ecology and*
601 *Management*, **257**, 1893-1901.
- 602 21. Földesi, R., Kovács-Hostyánszki, A., Kőrösi, Á., Somay,
603 L., Elek, Z., Markó, V., Sárospataki, M., Bakos, R., Varga,
604 Á., Nyisztor, K. & Báldi, A. (2016) Relationships between
605 wild bees, hoverflies and pollination success in apple
606 orchards with different landscape contexts. *Agricultural*
607 *and Forest Entomology*, **18**, 68-75.
- 608 22. Gilardelli, F., Gentili, R., Prosser, F., Bonomi, C., Varotto,
609 C. & Sgorbati, S. (2013) Ecological and biodiversity
610 gradients across alpine dry grassland habitats: implications
611 for an endangered species. *Nordic journal of botany*, **31**,
612 225-238.
- 613 23. Gilbert, F. S. (1985). Ecomorphological relationships in
614 hoverflies (Diptera, Syrphidae). *Proceedings of the Royal*
615 *society of London. Series B. Biological sciences*, **224**, 91-
616 105.
- 617 24. Google Inc. (2018) Google Earth (Version 5.1.3533.1731)
618 [Software].

- 619 25. Haenke, S., Scheid, B., Schaefer, M., Tschardtke, T. &
620 Thies, C. (2009) Increasing syrphid fly diversity and
621 density in sown flower strips within simple vs. complex
622 landscapes. *Journal of Applied Ecology*, **46**, 1106-1114.
- 623 26. Hedl, R., Kopecky, M. & Komarek, J. (2010) Half a
624 century of succession in a temperate oakwood: from
625 species-rich community to mesic forest. *Diversity and*
626 *Distributions*, **16**, 267–276.
- 627 27. Hooper, D. U., Adair, E. C., Cardinale, B. J., Byrnes, J. E.,
628 Hungate, B. A., Matulich, K. L., Gonzales, A., Duffy J. E.,
629 Gamfeldt, L. & O'Connor, M. I. (2012). A global synthesis
630 reveals biodiversity loss as a major driver of ecosystem
631 change. *Nature*, **486**, 105.
- 632 28. Jauker, F., Bondarenko, B., Becker, H. C. &
633 Steffan-Dewenter, I. (2012) Pollination efficiency of wild
634 bees and hoverflies provided to oilseed rape. *Agricultural*
635 *and Forest Entomology*, **14**, 81-87.
- 636 29. Jovičić, S., Burgio, G., Diti, I., Krašić, D., Markov, Z.,
637 Radenković, S. & Vujić, A. (2017) Influence of landscape
638 structure and land use on *Merodon* and *Cheilosia* (Diptera:
639 Syrphidae): contrasting responses of two genera. *Journal of*
640 *Insect conservation*, **21**, 53-64.

- 641 30. Jung, T., Blaschke, H. & Osswald, W. (2000) Involvement
642 of soilborne *Phytophthora* species in Central European oak
643 decline and the effect of site factors on the disease. *Plant*
644 *Pathology*, **49**, 706-718.
- 645 31. Kaloveloni, A., Tscheulin, T., Vujić, A., Radenković, S. &
646 Petanidou, T. (2015) Winners and losers of climate change
647 for the genus *Merodon* (Diptera: Syrphidae) across the
648 Balkan Peninsula. *Ecological modelling*, **313**, 201-211.
- 649 32. Keil, P., Dziock, F. & Storch, D. (2008) Geographical
650 patterns of hoverfly (Diptera, Syrphidae) functional groups
651 in Europe: inconsistency in environmental correlates and
652 latitudinal trends. *Ecological Entomology*, **33**, 748-757.
- 653 33. King, R. (1997) *Introduction: an essay on*
654 *Mediterraneanism*. The Mediterranean: Environment and
655 Society. (ed. by King, R., Proudfoot, L. & Smith, B.), pp.
656 1-11, Arnold, London.
- 657 34. Larrieu, L., Cabanettes, A. & Delarue, A. (2012) Impact of
658 silviculture on dead wood and on the distribution and
659 frequency of tree microhabitats in montane beech-fir
660 forests of the Pyrenees. *European Journal of Forest*
661 *Research*, **131**, 773-786.
- 662 35. Larrieu, L., Cabanettes, A. & Sarthou, J. P. (2015)
663 Hoverfly (Diptera: Syrphidae) richness and abundance vary

- 664 with forest stand heterogeneity: preliminary evidence from
665 a case study in a montane beech fir forest. *European*
666 *Journal of Entomology*, **112**, 755-769.
- 667 36. Lewis, R. J., de Bello, F., Bennett, J. A., Fibich, P., Finerty,
668 G. E., Götzenberger, L., Hiisalu, I. Kasari, L. Lepš, J.
669 Májeková, M., Mudrák, O., Riibak, K., Ronk, A.,
670 Rychtecká, T., Vitová, A. & Pärtel, M. (2017) Applying the
671 dark diversity concept to nature conservation. *Conservation*
672 *biology*, **31**, 40-47.
- 673 37. Liaw, A. & Wiener, M. (2002) Classification and
674 regression by randomForest. *R news*, **2**, 18-22.
- 675 38. Lucas, A., Bull, J. C., de Vere, N., Neyland, P. J. &
676 Forman, D. W. (2017) Flower resource and land
677 management drives hoverfly communities and bee
678 abundance in seminatural and agricultural grasslands.
679 *Ecology and evolution*, **7**, 8073-8086.
- 680 39. Mansourian, S., Rossi, M. & Vallauri, D. (2013) *Ancient*
681 *Forests in the Northern Mediterranean: Neglected High*
682 *Conservation Value Areas*. Marseille: WWF France.
- 683 40. Memmott, J., Craze, P.G., Waser, N.M. & Price, M.V.
684 (2007) Global warming and the disruption of plant-
685 pollinator interactions. *Ecology Letters*, **10**, 710–717.

- 686 41. Merrill, A. C. (2009) Investigations of Variable Importance
687 Measures Within Random Forests. *All Graduate Theses*
688 *and Dissertations*. 7078.
- 689 42. Meyer, P., Tabaku, V. & Lüpke, B. (2003) Die Struktur
690 albanischer Rotbuchen-Urwälder–Ableitungen für eine
691 naturnahe Buchenwirtschaft: Structural Characteristics of
692 Albanian Beech (*Fagus sylvatica* L.) Virgin Forests–
693 Deductions for Semi-Natural
694 Forestry. *Forstwissenschaftliches Centralblatt*, **122**, 47-58.
- 695 43. Miličić, M., Vujić, A. & Cardoso, P. (2018) Effects of
696 climate change on the distribution of hoverfly species
697 (Diptera: Syrphidae) in Southeast Europe. *Biodiversity and*
698 *Conservation*, **27**, 1173-1187.
- 699 44. Moeslund, J. E., Brunbjerg, A. K., Clausen, K. K., Dalby,
700 L., Fløjgaard, C., Juel, A. & Lenoir, J. (2017) Using dark
701 diversity and plant characteristics to guide conservation and
702 restoration. *Journal of applied ecology*, **54**, 1730-1741.
- 703 45. Müller, J., Stadler, J., Jarzabek-Müller, A., Hacker, H., ter
704 Braak, C. & Brandl, R. (2011) The predictability of
705 phytophagous insect communities: host specialists as
706 habitat specialists. *PLoS One*, **6**, e25986.

- 707 46. Murcia, C. (1995) Edge effects in fragmented forests:
708 implications for conservation. *Trends in ecology &*
709 *evolution*, **10**, 58-62.
- 710 47. Pärtel, M. (2014) Community ecology of absent species:
711 hidden and dark diversity. *Journal of Vegetation Science*,
712 **25**, 1154-1159.
- 713 48. Pärtel, M., Szava-Kovats, R. & Zobel, M. (2011) Dark
714 diversity: shedding light on absent species. *Trends in*
715 *ecology & evolution*, **26**, 124-128.
- 716 49. Pintaldi, E., Hudek, C., Stanchi, S., Spiegelberger, T.,
717 Rivella, E. & Freppaz, M. (2017) Sustainable Soil
718 Management in Ski Areas: Threats and Challenges.
719 *Sustainability*, **9**, 2150.
- 720 50. Poesen, J. W. & Hooke, J. M. (1997) Erosion, flooding and
721 channel management in Mediterranean environments of
722 southern Europe. *Progress in Physical Geography*, **21**, 157-
723 199.
- 724 51. Petanidou, T., Vujić, A. & Ellis, W. N. (2011) Hoverfly
725 diversity (Diptera: Syrphidae) in a Mediterranean scrub
726 community near Athens, Greece. *Annales de la Société*
727 *Entomologique de France*, **47**, 168-175.
- 728 52. Power, E. F., Jackson, Z. & Stout, J.C. (2016) Organic
729 farming and landscape factors affect abundance and

- 730 richness of hoverflies (Diptera, Syrphidae) in grasslands.
731 *Insect Conservation and Diversity*, **9**, 244-253.
- 732 53. QGIS Development Team (2009) QGIS Geographic
733 Information System. Open Source Geospatial Foundation
734 Project. <http://qgis.osgeo.org>
- 735 54. Radenković, S., Schweiger, O., Milić, D., Harpke, A. &
736 Vujić, A. (2017) Living on the edge: Forecasting the trends
737 in abundance and distribution of the largest hoverfly genus
738 (Diptera: Syrphidae) on the Balkan Peninsula under future
739 climate change. *Biological Conservation*, **212**, 216-229.
- 740 55. Reemer, M. (2005) Saproxylic hoverflies benefit by
741 modern forest management (Diptera: Syrphidae). *Journal*
742 *of Insect Conservation*, **9**, 49-59.
- 743 56. Robinson, S. K., Thompson, F. R., Donovan, T. M.,
744 Whitehead, D. R. & Faaborg, J. (1995) Regional forest
745 fragmentation and the nesting success of migratory birds.
746 *Science*, **267**, 1987-1990.
- 747 57. Riibak, K., Reitalu, T., Tamme, R., Helm, A., Gerhold, P.,
748 Znamenskiy, S., Bengtsson, K., Rosén, E., Prentice, H.C. &
749 Pärtel, M. (2015) Dark diversity in dry calcareous
750 grasslands is determined by dispersal ability and stress-
751 tolerance. *Ecography*, **38**, 713-721.

- 752 58. Ronk, A. (2016) Plant diversity patterns across Europe:
753 observed and dark diversity. *Dissertationes Biologicae*
754 *Universitatis Tartuensis*, 300.
- 755 59. Ronk, A., Szava-Kovats, R. & Pärtel, M. (2015) Applying
756 the dark diversity concept to plants at the European scale.
757 *Ecography*, **38**, 1015-1025.
- 758 60. Ruiz, G. M., Freestone, A. L., Fofonoff, P. W., Simkanin,
759 C. (2009) Habitat distribution and heterogeneity in marine
760 invasion dynamics: the importance of hard substrate and
761 artificial structure. *Marine hard bottom communities*, **206**,
762 321-332.
- 763 61. Schweiger, O., Musche, M., Bailey, D., Billeter, R.,
764 Diekötter, T., Hendrickx, F., Herzog, F., Liira, J., Maelfait,
765 J.P., Speelmans, M. & Dziock, F. (2007) Functional
766 richness of local hoverfly communities (Diptera,
767 Syrphidae) in response to land use across temperate
768 Europe. *Oikos*, **116**, 461-472.
- 769 62. Souza, J. M. T. D., Marinoni, R. C. & Marinoni, L. (2014)
770 Open and disturbed habitats support higher diversity of
771 Syrphidae (Diptera)? A case study during three yr of
772 sampling in a fragment of Araucaria Forest in Southern
773 Brazil. *Journal of Insect Science*, **14**, 236.

- 774 63. Speight, M. C. D. (1989) *Saproxylic Invertebrates and*
775 *their Conservation*. Council of Europe, Strasbourg.
- 776 64. Speight, M. C. D., Castella, E. & Sarthou, J. P. StN (2015).
777 In: Syrph the Net on CD, Issue 10. The database of
778 European Syrphidae (ed. by Speight, M. C. D., Castella, E.,
779 Sarthou, J. P. & Vanappelghem, C.), Syrph the Net
780 Publications, Dublin.
- 781 65. Speight, M. C. D. (2017) Species accounts of European
782 Syrphidae (Diptera). Syrph the Net, the database of
783 European Syrphidae, **103**, 1-302, Syrph the Net
784 publications, Dublin.
- 785 66. Ssymank, A. & Kearns, C. (2009) Flies–Pollinators on two
786 wings. Caring for Pollinators: safeguarding
787 agrobiodiversity and wild plant diversity. *Bonn, Bundesamt*
788 *für Naturschutz, German Federal Agency for Nature*
789 *Conservation*, 39-52.
- 790 67. Thomas, C. D. (2000) Dispersal and extinction in
791 fragmented landscapes. *Proceedings of the Royal Society of*
792 *London. Series B: Biological Sciences*, **267**, 139-145.
- 793 68. Thomas, F. M. (2008) Recent advances in cause-effect
794 research on oak decline in Europe. *CAB Reviews:*
795 *Perspectives in Agriculture, Veterinary Science, Nutrition*
796 *and Natural Resources*, **3**, 1-12.

- 797 69. Thompson, J. N. (2005) Coevolution: the geographic
798 mosaic of coevolutionary arms races. *Current Biology*, **15**,
799 R992-R994.
- 800 70. Van Veen, M. (2004) *Hoverflies of Northwest Europe:*
801 *identification keys to the Syrphidae*. KNNV Publishing,
802 Utrecht.
- 803 71. Verboven, H. A., Uyttenbroeck, R., Brys, R. & Hermy, M.
804 (2014) Different responses of bees and hoverflies to land
805 use in an urban–rural gradient show the importance of the
806 nature of the rural land use. *Landscape and Urban*
807 *Planning*, **126**, 31-41.
- 808 72. Winfree, R., Bartomeus, I. & Cariveau, D. P. (2011) Native
809 pollinators in anthropogenic habitats. *Annual Review of*
810 *Ecology, Evolution, and Systematics*, **42**, 1-22.
- 811 73. Vukelić, J., Korijan, P., Šapić, I., Alegro, A., Šegota, V. &
812 Poljak, I. (2018) Forest Vegetation of Hardwood Tree
813 Species along the Mirna River in Istria (Croatia). *South-*
814 *east European forestry*, **9**, 1-16.
- 815 74. White, A. J., Wratten, S. D., Berry, N. A. & Weigmann, U.
816 (1995) Habitat manipulation to enhance biological control
817 of *Brassica* pests by hover flies (Diptera: Syrphidae).
818 *Journal of Economic Entomology*, **88**, 1171-1176.

- 819 75. Winsa, M., Öckinger, E., Bommarco, R., Lindborg, R.,
820 Roberts, S.P., Wärensberg, J. & Bartomeus, I. (2017)
821 Sustained functional composition of pollinators in restored
822 pastures despite slow functional restoration of plants.
823 *Ecology and evolution*, **7**, 3836-3846.
- 824 76. Yoshioka, A., Miyazaki, Y., Sekizaki, Y., Suda, S. I.,
825 Kadoya, T. & Washitani, I. (2014) A “lost biodiversity”
826 approach to revealing major anthropogenic threats to
827 regional freshwater ecosystems. *Ecological indicators*, **36**,
828 348-355.
- 829 77. Zamin, T. J., Baillie, J. E., Miller, R. M., Rodríguez, J. P.,
830 Ardid, A. N. A. & Collen, B. E. N. (2010) National red
831 listing beyond the 2010 target. *Conservation Biology*, **24**,
832 1012-1020.
- 833 78. Zobel, M. (2016) The species pool concept as a framework
834 for studying patterns of plant diversity. *Journal of*
835 *Vegetation Science*, **27**, 8-18.
- 836

837 **Table legends**

838 Table 1. Functional traits and trait states of hoverflies in SE

839 Europe.

840

841 Table 2. Correlations between the dark diversity index (DDi) and

842 functional traits; rs, Spearman's rank correlation coefficient; H,

843 Kruskal-Wallis statistic.* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

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862 **Figure legends**

863 Figure 1. Schematic diagram illustrating species pool, observed
864 and dark diversity.

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866 Figure 2. Vegetation classes in Southeast Europe. A-Alpine,
867 subalpine and oro-Mediterranean vegetation, B-Montane spruce
868 and mixed spruce forests, C-Montane pine forests, D-Acidophilous
869 oak and mixed oak - hornbeam forests, E-Beech and mixed beech
870 forests, F-Thermophilous mixed bitter, pedunculate or sessile oak
871 forests, G-Southeast Balkan sub-Mediterranean mixed oak forests,
872 H-Southwest Balkan sub-Mediterranean mixed oak forests, I-
873 Pannonian lowland mixed oak forests and steppes, J-Mediterranean
874 mixed forests, K-Hardwood alluvial forests, wet lowland forests
875 and swamps.

876

877 Figure 3. Number of species in dark diversity (pale purple),
878 observed diversity (mid purple) and in the species pool (dark
879 purple) for each vegetation class (A-K, see Fig. 1 legend).

880

881 Figure 4. Functional traits and trait states showing relative
882 importance (%IncMSE) for dark diversity of hoverflies in
883 Southeast Europe.

884