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

Short title: Hoverfly dark diversity in SE Europe

Marija Miličić*[1,3], Snežana Popov[2], Ante Vujić[2], Bojana Ivošević[1], Pedro Cardoso[3]

[1]BioSense Institute – Research Institute for Information Technologies in Biosystems, University of Novi Sad, Trg Dr Zorana Đinđića 1, 21000 Novi Sad, Serbia

[2]Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia

[3]LIBRe – Laboratory for Integrative Biodiversity Research, Finnish Museum of Natural History, University of Helsinki, Helsinki, Finland

*Corresponding author. Email: marija.milicic@biosense.rs, Trg Dr Zorana Đinđića 1, 21000 Novi Sad, Serbia

Abstract

Dark diversity represents the set of species belonging to a species pool but is not locally present, i.e. species that are currently 'missing' from a site but have the potential to occur there. The concept allows characterization of the mechanisms determining why species are sometimes absent from an area that seems ecologically suitable for them. Our aim was to assess the dark diversity of hoverflies (Diptera: Syrphidae) in Southeast Europe and to discuss the role of different functional traits that might increase the likelihood of species contributing to dark diversity. Based on expert opinion, the Syrph the Net database and known occurrences of species, we estimated observed, potential and dark diversities within each defined vegetation class for 564 hoverfly species registered in SE Europe. To detect the most important functional traits of species contributing to dark diversity across classes, we used a random forest algorithm and respective statistics for variable importance (%IncMSE). The highest dark diversity was found for Southwest Balkan sub-Mediterranean mixed oak forests, whereas the lowest was in Mediterranean mixed forests. Three larval feeding modes (saproxylic, and phytophagous of bulbs or roots) were found to be most important for determining the probability of a species contributing to hoverfly dark diversity, based on univariate correlations and random forest analysis. This study shows that studying dark diversity might provide important insights into what drives community assembly in this taxon and region, especially its missing components and contributes to more precise conservation prioritization of both hoverfly species and their habitats.

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

Introduction

In order to understand and try to alleviate the negative consequences of biodiversity loss, detailed information on different aspects of biodiversity is essential (Hooper *et al*., 2005; Barton & Evans, 2017). Data on extant biodiversity may not allow interesting patterns related to missing components of communities to be uncovered (Pärtel *et al*., 2011). Recent findings (Pärtel, 2011, 2014; Lewis *et al.*, 2017) have shown that so-called 'dark diversity' could reveal new biodiversity patterns that would not be evident from investigating only observed diversity (Ronk *et al*., 2015). In order to examine dark diversity of a target site, the habitat-specific species pool must be established, i.e. the set of species that can inhabit a given area under designated ecological conditions (Cornell & Harrison 2014; Zobel, 2016). The species that are not recorded at a particular site, but that belong to its species pool and could potentially co-occur in the site given its biotic, abiotic processes and dispersal limitations, constitute the dark diversity of that site (Pärtel *et al*., 2011). In other words, dark diversity represents the absent element of a habitat-specific species pool (Lewis *et al*., 2017). Importantly, a species pool is not simply the sum of observed and missing species (Fig. 1). Although total observed diversity consists of species that belong to the habitat-specific species pool, it also comprises species that are currently present in a particular community but are not part of the pool.

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

Hoverflies (Diptera: Syrphidae) play important roles in providing vital ecosystem services such as pollination (Ssymank & Kearns, 2009; Petanidou *et al*., 2011; Jauker *et al*., 2012), waste decomposition (Gilbert, 1985) and biological control (White *et al*., 1995; Blaauw & Isaacs, 2012; Day *et al*., 2015). Recent studies have shown that hoverflies can serve as valuable model organisms in terms of studies of climate change (Kaloveloni *et al*., 2015; Radenković *et al*., 2017; Miličić *et al*., 2018), urbanization (Bates *et al*., 2011; Verboven *et al*., 2014), landscape structure (Power *et* *al*., 2016) or land use (Aguirre-Gutiérrez *et al*., 2015, Földesi & Kovács-Hostyánszki, 2016; Lucas *et al*., 2017).

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

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

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

Methods

Study area and species occurrences

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

Information on the distribution of registered species was extracted from the database of the Faculty of Sciences, University of Novi Sad, Serbia (FSUNS). This database is a result of the hoverfly monitoring program across investigated area from 1950 to 2017, during which the sampling of hoverflies was conducted using a consistent census protocol (see Radenković *et al*., 2017 for more details). Additionally, we included data from published material referring to this territory, as well as data obtained from different museum and private collections. Only specimens for which precise distributional data were available were used in our analyses. Exact locality coordinates were checked for accuracy where provided. Records for which only locality names were available were assigned coordinates using Google Earth (Google Inc., 2018). In total, we considered 63814 occurrence records of 564 species.

Vegetation types

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

Figure 2. Vegetation classes in Southeast Europe.

Functional traits

Data on functional traits of hoverflies were collected from multiple sources. We used published data (Speight *et al.*, 2015; Speight, 2017), expert opinion-based data, and fieldwork experience spanning 35 years regarding biological and ecological characteristics of species. Information on wing length of analyzed species was not available in the literature, but because we considered this trait to be biologically significant, we measured it using a Nikon SMZ 745T stereomicroscope. We measured four specimens of each species (two male and two female), and used the averaged value as a variable in further analyses. Functional traits with multiple non-mutually-exclusive trait states were coded as dummy variables. Additionally, if possible and where justified, categorical traits were transformed into ordinal variables. If the trait state for the given species was unknown, the species was assigned the most common trait state found in other members of that genus. The list of functional traits used in the analyses is presented in Tab. 1.

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

Estimation of dark diversity

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

Europe, as well as the known biological and ecological preferences of that species (Speight *et al*., 2015; Speight, 2017).

All estimations and statistics were made using the full dataset (across vegetation types). To quantify observed diversity, occurrence records were plotted on the vegetation map using open source GIS software (QGIS Development Team, 2009). Dark diversity was calculated for each vegetation type separately as the difference between the species pool and the observed species belonging to that particular pool (i.e. the number of potential species missing from each pool). Percentage of dark diversity across all vegetation classes was calculated as the ratio between dark diversity and the sum of dark and observed diversity.

Statistical analyses

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

In a first step, we checked for correlations among functional traits. Spearman's rank correlations were conducted for continuous variable comparisons, Mann-Whitney tests for continuous-categorical variable comparisons, and Eta correlation coefficients were generated for categorical variable comparisons. Values above 0.7 were considered indicative of collinearity (Dormann et al., 2013).

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

Even if some traits were not revealed to be important in univariate correlations, they might be important only when other factors are also considered. To establish the unique contribution of each trait to the probability of being part of dark diversity, we used a random forest (RF) regression model (Breiman, 2001), implemented within the randomForest R package (Liaw &Wiener, 2002). In this analysis, the number of trees (ntree) was set to 5000, and the number of predictors sampled for splitting at each node (mtry) was set to 6, which represents the default value suggested for regression RF models, i.e. number of predictor variables/3. Node size (nodesize) was kept as default, i.e. 5 for regression. After running the model, and in order to verify if species with certain traits were more likely to contribute to dark diversity, we quantified the importance of each predictive variable by calculating the Mean Decrease Accuracy (%IncMSE). Merrill (2009) demonstrated that this latter measure provides the highest stability compared to node purity measures.

Results

Species pool, observed species and dark diversity

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

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

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

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

Dark diversity and functional traits

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

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

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

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

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

Discussion

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

Levels of hoverfly dark diversity across vegetation types

Southwest Balkan sub-Mediterranean mixed oak forests showed the highest level of dark diversity and lowest level of observed diversity. This outcome may be because oak forests are in decline generally all across Europe and most of the respective habitat patches are small and scattered, limiting their carrying capacity and inhibiting the metapopulation dynamics important for sustaining populations. Southwest Balkan sub-Mediterranean mixed oak forests have been exposed to intense anthropogenic pressure over the last 2000 years, subjected to constant felling (Mansourian *et* *al.*, 2013). This pressure has resulted in overt destruction, size reductions and fragmentation of these forests, as well as promoting edge effects (Robinson *et al.*, 1995). Jovičić *et al.* (2017) investigated the effect of landscape structure on the two largest hoverfly genera (*Merodon* Meigen, 1803 and *Cheilosia* Maigen, 1822) and suggested that habitat connectivity influences the species composition of habitats. Moreover, it has been demonstrated that edge effects also impact physical characteristics of the environment, leading to increased temperatures and reduced humidity (Murcia *et al.*, 1995). Additional causes of these declines are pathogenic infection of oak species (Jung *et al.*, 2010) and extreme weather (drought or frost) (Thomas, 2008). However, it is human impact that appears to be playing a crucial role in maintaining deciduous oakwood forests. For instance, occasional fires have been shown to promote the occurrence of oak (Abrams, 1992), and it seems that the recent decline in applying traditional management strategies (such as burning) has negatively affected oakwood assemblages (Hedl *et al.*, 2010). A second vegetation class that presented high levels of dark diversity was Alpine, subalpine and oro-Mediterranean. Multiple studies have demonstrated the sensitivity of these habitats (Ruiz *et al.*, 2008; Gillaredeli *et al.*, 2013). The main reasons for the vulnerability of this vegetation type are increased human impact through infrastructural development (Pintaldi *et al*., 2017) and high levels of grazing (Firm *et al*., 2009). Moreover, patches of alpine and subalpine habitats are usually small and fragmented. Thus, multiple characteristics of the Alpine, subalpine and oro-Mediterranean vegetation class could be responsible for the high dark diversity of hoverflies we report for this class.

Mediterranean mixed forests showed the lowest level of dark diversity. Although Mediterranean habitats have been reported as seriously degraded due to intensive human actions (King, 1997; Blondel, 2006), it would seem that they are resilient to existing pressures, at least in terms of their capability of hosting a large proportion of hoverfly species. Many reports (di Castri, 1981; Thompson, 2005) have explained this phenomenon as resulting from long-term adaptation of ecosystems to humans in this region, even going so far as to apply the term "co-evolution". Large expanses of this vegetation type, which are highly connected, enable the existence of many species, including those that require large areas to maintain viable populations, resulting in only 33 species being identified as contributing to dark diversity. Beech and mixed beech forests showed the second lowest level of dark diversity, and presented the largest species pool and greatest observed diversity. This outcome can be explained by the fact that these forests are among the best preserved in SE Europe (Meyer *et* *al*., 2003), providing suitable microhabitats for both larvae and adults of hoverflies. Additionally, this result may be partially attributable to geographical proximity to degraded oak forests from which hoverflies may have migrated to find more favorable conditions.

Functional traits contributing to dark diversity

Three different larval feeding modes were identified as the most significant traits contributing to dark diversity. The importance of larval type and particularly their food preferences has been well established for many other aspects of hoverfly biology and ecology (Schweiger *et al.*, 2007; Haenke *et al.*, 2009), and our results suggest that these factors dictate which species might survive in habitats that are apparently adequate. Larval feeding mode indirectly reflects the level of specialization of a species. Saproxylic hoverfly larvae, as well as phytophagous species that develop in bulbs and roots, are considered specialists (van Veen, 2004, Müller *et al.*, 2011). Generalist species are widely regarded as being at an advantage compared to specialists in adapting to changing environmental conditions (Biesmeijer *et al.*, 2006, Barthel *et al.*, 2014). Generalist species are likely favored under conditions of detrimental change and should constitute the majority of observed diversity, whereas specialists may be absent from impacted habitats and contribute towards dark diversity. Different saproxylic hoverflies require different stages of wood decay for the development and nutrition of their larvae (Speight, 1989), which is particularly important in clear-cut forests without tree retention where the microhabitats of these species are directly destroyed (Larrieu *et al*., 2012). Due to their high level of specialization, saproxylic hoverflies have no alternative for their development. However, changes in forest management (heterogeneity of trees in terms of age and size structure) may increase saproxylic hoverfly diversity (Remeer, 2005). Regarding phytophagous larvae (both those developing in bulbs and in roots), their specialization is reflected in the connection to a specific plant species. Thus, geographical distributions of phytophagous species are limited by the existence of suitable habitat for their host plants, which is particularly important when species are monophagous (Müller *et al*., 2011).

With further regard to host dependency, the period during which species fly was also revealed to be of high importance for dark diversity in our RF analysis, but not when tested individually. Flight period of hoverflies is directly linked to food resource availability, with a temporal mismatch between hoverfly activity and when host plants representing their primary food source flower. Increasing temperatures caused by climate change might lead to further phenological asynchrony (Memmott *et al.*, 2007), which would have a serious negative impact on specialist species. Moreover, exploitation of food resources is dependent on other functional traits. Thus, complex interactions between flight period and other traits are likely to have a synergistic effect on dark diversity.

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

Body shape is significant in shaping patterns of dark diversity because it influences species' dispersal ability, with species contributing to dark diversity in general having lower dispersal capacities (Riibak *et al.*, 2015). Even though other measures directly related to dispersal ability were not identified as being of high importance to dark diversity in our study, body shape still provides useful insights into the potential of a species to disperse. This functional trait contributes to species being able to overcome challenging environmental conditions, as well as to extend their current range and invade new suitable areas. Additionally, species with well-developed dispersal abilities are less affected by habitat fragmentation, as they can migrate between suitable habitat patches (Thomas, 2000). Apart from its link to dispersal ability, body shape might also contribute a hitherto unknown mechanism of physiological or behavioral adaptation important for dark diversity for which we currently have no evidence.

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

Application of dark diversity concept in hoverfly conservation

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

Beside contribution to the conservation of habitats, dark diversity studies enable more precise conservation prioritization in respect of species-based approach. Namely, linking functional traits and dark diversity may guide conservation efforts towards more sensitive species. Our study shows that certain traits such as highly specialized larval feeding modes, lack of inundation tolerance or flight period promote dark diversity of hoverflies. Identifying species possessing these traits could be an additional criterion when deciding which species should receive conservation attention.

Conclusion

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

Acknowledgments:

We kindly thank Dr. David Wagner for useful comments that significantly improved the quality of this paper and John O’Brien for English proofreading. This work was funded by the Ministry of Education, Science and Technological Development of the Republic of Serbia, Grants No. OI173002 and III43002, the Provincial Secretariat for Science and Technological Development of the Republic of Serbia, Grant No. 0601-504/3 and H2020 Project ANTARES, Grant No. 664387.

Authors' contribution: AV, PC and MM participated in the design of the study, MM, SP and PC wrote the paper; AV, MM, SP and BI participated in data collecting; MM, SP and PC performed the analyses, MM, SP and BI prepared the figures; all authors contributed to manuscript with comments and revision.

Conflict of interest disclosure: We certify that we have no actual or potential conflict of interest.

References

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

(2016) Functional traits help to explain half-century long shifts in pollinator distributions. *Scientific reports*, **6**, 24451.

4. Barthel, A., Kopka, I., Vogel, H., Zipfel, P., Heckel, D. G. & Groot, A. T. (2014) Immune defence strategies of generalist and specialist insect herbivores. *Proceedings of the Royal Society B: Biological Sciences*, **281**, 20140897.
5. Barton, P. S. & Evans, M. J. (2017) Insect biodiversity meets ecosystem function: differential effects of habitat and insects on carrion decomposition. *Ecological entomology*, **42**, 364-374.
6. Basley, K., Davenport, B., Vogiatzis, K. & Goulson, D. (2018) Effects of chronic exposure to thiamethoxam on larvae of the hoverfly *Eristalis tenax* (Diptera, Syrphidae). *PeerJ*, **6**, e4258.
7. Bates, A. J., Sadler, J. P., Fairbrass, A. J., Falk, S. J., Hale, J. D. & Matthews, T. J. (2011) Changing bee and hoverfly pollinator assemblages along an urban-rural gradient. *PloS one*, **6**, e23459
8. Biesmeijer, J. C., Roberts, S. P., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., Schaffers, A. P., Potts, S. G., Kleukers, R., Thomas, C. D., Settele, J. & Kunin, W. E. (2006) Parallel declines in pollinators and insect-pollinated

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

9. Blaauw, B. R. & Isaacs, R. (2012) Larger wildflower plantings increase natural enemy density, diversity, and biological control of sentinel prey, without increasing herbivore density. *Ecological Entomology*, **37**, 386-394.
10. Blondel, J. (2006) The ‘design’of Mediterranean landscapes: a millennial story of humans and ecological systems during the historic period. *Human ecology*, **34**, 713-729.
11. Bohn, U., Gollub, G., Hettwer, C., Weber, H., Neuhäuslová, Z., Raus, T. & Schlüter, H. (2000) Karte der natürlichen Vegetation Europas/Map of the Natural Vegetation of Europe. Maßstab/Scale 1: 2,500,000.
12. Breiman, L. (2001) Random Forests. *Machine Learning,* **45**, 5-32.
13. Cardoso, P., Erwin, T. L., Borges, P. A. & New, T. R. (2011) The seven impediments in invertebrate conservation and how to overcome them. *Biological Conservation*, **144**, 2647-2655.
14. Cornell, H. V. & Harrison, S. P. (2014) What are species pools and when are they important? *Annual Review of Ecology, Evolution, and Systematics*, **45**, 45-67.

15. Cvetkovic, V., Prelević, D. & Schmid, S. (2016) Geology of South-Eastern Europe. *Mineral and thermal waters of Southeastern Europe* (ed. by Papić, P.), pp. 1-29. Springer, Cham.

16. Day, R. L., Hickman, J. M., Sprague, R. I. & Wratten, S. D. (2015) Predatory hoverflies increase oviposition in response to colour stimuli offering no reward: Implications for biological control. *Basic and applied ecology*, **16**, 544-552.

17. de Groot, M., Eler, K., Flajšman, K., Grebenc, T., Marinšek, A. & Kutnar, L. (2016) Differential short-term response of functional groups to a change in forest management in a temperate forest. *Forest Ecology and Management*, **376**, 256-264.

18. Di Castri, F. (1981) Mediterranean-type shrublands of the world. *Mediterranean-type Shrublands* (ed. by Di Castri, F., Goodall, D.W. & Specht R.L.), pp. 1-52, Elsevier, Amsterdam.

19. Dormann, C. F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., García Marquéz, J. R., Gruber, B., Lafourcade, B., Leitão, P. J., Münkemüller, T., McClean, C., Osborne, P. E., Reineking, B., Schröder, b., Skidmore, A. K., Zurell, D. & Lautenbach, S. (2013) Collinearity: a

review of methods to deal with it and a simulation study evaluating their performance. *Ecography*, **36**, 27-46.

20. Firm, D., Nagel, T. A. & Diaci, J. (2009) Disturbance history and dynamics of an old-growth mixed species mountain forest in the Slovenian Alps. *Forest Ecology and Management*, **257**, 1893-1901.
21. Földesi, R., Kovács-Hostyánszki, A., Kőrösi, Á., Somay, L., Elek, Z., Markó, V., Sárospataki, M., Bakos, R., Varga, Á., Nyisztor, K. & Báldi, A. (2016) Relationships between wild bees, hoverflies and pollination success in apple orchards with different landscape contexts. *Agricultural and Forest Entomology*, **18**, 68-75.
22. Gilardelli, F., Gentili, R., Prosser, F., Bonomi, C., Varotto, C. & Sgorbati, S. (2013) Ecological and biodiversity gradients across alpine dry grassland habitats: implications for an endangered species. *Nordic journal of botany*, **31**, 225-238.
23. Gilbert, F. S. (1985). Ecomorphological relationships in hoverflies (Diptera, Syrphidae). *Proceedings of the Royal society of London. Series B. Biological sciences*, **224**, 91-105.
24. Google Inc. (2018) Google Earth (Version 5.1.3533.1731) [Software].

25. Haenke, S., Scheid, B., Schaefer, M., Tscharntke, T. & Thies, C. (2009) Increasing syrphid fly diversity and density in sown flower strips within simple vs. complex landscapes. *Journal of Applied Ecology*, **46**, 1106-1114.

26. Hedl, R., Kopecky, M. & Komarek, J. (2010) Half a century of succession in a temperate oakwood: from species-rich community to mesic forest. *Diversity and Distributions*, **16**, 267–276.

27. Hooper, D. U., Adair, E. C., Cardinale, B. J., Byrnes, J. E., Hungate, B. A., Matulich, K. L., Gonzales, A., Duffy J. E., Gamfeldt, L. & O'Connor, M. I. (2012). A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature*, **486**, 105.

28. Jauker, F., Bondarenko, B., Becker, H. C. & Steffan-Dewenter, I. (2012) Pollination efficiency of wild bees and hoverflies provided to oilseed rape. *Agricultural and Forest Entomology*, **14**, 81-87.

29. Jovičić, S., Burgio, G., Diti, I., Krašić, D., Markov, Z., Radenković, S. & Vujić, A. (2017) Influence of landscape structure and land use on Merodon and Cheilosia (Diptera: Syrphidae): contrasting responses of two genera. *Journal of Insect conservation*, **21**, 53-64.

30. Jung, T., Blaschke, H. & Osswald, W. (2000) Involvement of soilborne *Phytophthora* species in Central European oak decline and the effect of site factors on the disease. *Plant Pathology*, **49**, 706-718.

31. Kaloveloni, A., Tscheulin, T., Vujić, A., Radenković, S. & Petanidou, T. (2015) Winners and losers of climate change for the genus *Merodon* (Diptera: Syrphidae) across the Balkan Peninsula. *Ecological modelling*, **313**, 201-211.

32. Keil, P., Dziock, F. & Storch, D. (2008) Geographical patterns of hoverfly (Diptera, Syrphidae) functional groups in Europe: inconsistency in environmental correlates and latitudinal trends. *Ecological Entomology*, **33**, 748-757.

33. King, R. (1997) *Introduction: an essay on Mediterraneanism*. The Mediterranean: Environment and Society. (ed. by King, R., Proudfoot, L. & Smith, B.), pp. 1-11, Arnold, London.

34. Larrieu, L., Cabanettes, A. & Delarue, A. (2012) Impact of silviculture on dead wood and on the distribution and frequency of tree microhabitats in montane beech-fir forests of the Pyrenees. *European Journal of Forest Research*, **131**, 773-786.

35. Larrieu, L., Cabanettes, A. & Sarthou, J. P. (2015) Hoverfly (Diptera: Syrphidae) richness and abundance vary

with forest stand heterogeneity: preliminary evidence from a case study in a montane beech fir forest. *European Journal of Entomology*, **112**, 755-769.

36. Lewis, R. J., de Bello, F., Bennett, J. A., Fibich, P., Finerty, G. E., Götzenberger, L., Hiisalu, I. Kasari, L. Lepš, J. Májeková, M., Mudrák, O., Riibak, K., Ronk, A., Rychtecká, T., Vitová, A. & Pärtel, M. (2017) Applying the dark diversity concept to nature conservation. *Conservation biology*, **31**, 40-47.
37. Liaw, A. & Wiener, M. (2002) Classification and regression by randomForest. *R news*, **2**, 18-22.
38. Lucas, A., Bull, J. C., de Vere, N., Neyland, P. J. & Forman, D. W. (2017) Flower resource and land management drives hoverfly communities and bee abundance in seminatural and agricultural grasslands. *Ecology and evolution*, **7**, 8073-8086.
39. Mansourian, S., Rossi, M. & Vallauri, D. (2013) *Ancient Forests in the Northern Mediterranean: Neglected High Conservation Value Areas*. Marseille: WWF France.
40. Memmott, J., Craze, P.G., Waser, N.M. & Price, M.V. (2007) Global warming and the disruption of plant-pollinator interactions. *Ecology Letters*, **10**, 710–717.

41. Merrill, A. C. (2009) Investigations of Variable Importance Measures Within Random Forests. *All Graduate Theses and Dissertations*. 7078.

42. Meyer, P., Tabaku, V. & Lüpke, B. (2003) Die Struktur albanischer Rotbuchen-Urwälder–Ableitungen für eine naturnahe Buchenwirtschaft: Structural Characteristics of Albanian Beech (*Fagus sylvatica* L.) Virgin Forests–Deductions for Semi-Natural Forestry. *Forstwissenschaftliches Centralblatt*, **122**, 47-58.

43. Miličić, M., Vujić, A. & Cardoso, P. (2018) Effects of climate change on the distribution of hoverfly species (Diptera: Syrphidae) in Southeast Europe. *Biodiversity and Conservation*, **27**, 1173-1187.

44. Moeslund, J. E., Brunbjerg, A. K., Clausen, K. K., Dalby, L., Fløjgaard, C., Juel, A. & Lenoir, J. (2017) Using dark diversity and plant characteristics to guide conservation and restoration. *Journal of applied ecology*, **54**, 1730-1741.

45. Müller, J., Stadler, J., Jarzabek-Müller, A., Hacker, H., ter Braak, C. & Brandl, R. (2011) The predictability of phytophagous insect communities: host specialists as habitat specialists. *PLoS One*, **6**, e25986.

46. Murcia, C. (1995) Edge effects in fragmented forests: implications for conservation. *Trends in ecology & evolution*, **10**, 58-62.

47. Pärtel, M. (2014) Community ecology of absent species: hidden and dark diversity. *Journal of Vegetation Science*, **25**, 1154-1159.

48. Pärtel, M., Szava-Kovats, R. & Zobel, M. (2011) Dark diversity: shedding light on absent species. *Trends in ecology & evolution*, **26**, 124-128.

49. Pintaldi, E., Hudek, C., Stanchi, S., Spiegelberger, T., Rivella, E. & Freppaz, M. (2017) Sustainable Soil Management in Ski Areas: Threats and Challenges. *Sustainability*, **9**, 2150.

50. Poesen, J. W. & Hooke, J. M. (1997) Erosion, flooding and channel management in Mediterranean environments of southern Europe. *Progress in Physical Geography*, **21**, 157-199.

51. Petanidou, T., Vujić, A. & Ellis, W. N. (2011) Hoverfly diversity (Diptera: Syrphidae) in a Mediterranean scrub community near Athens, Greece. *Annales de la Société Entomologique de France*, **47**, 168-175.

52. Power, E. F., Jackson, Z. & Stout, J.C. (2016) Organic farming and landscape factors affect abundance and

richness of hoverflies (Diptera, Syrphidae) in grasslands. *Insect Conservation and Diversity*, **9**, 244-253.

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

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

63. Speight, M. C. D. (1989) *Saproxylic Invertrebrates and their Conservation*. Council of Europe, Strasbourg.

64. Speight, M. C. D., Castella, E. & Sarthou, J. P. StN (2015). In: Syrph the Net on CD, Issue 10. The database of European Syrphidae (ed. by Speight, M. C. D., Castella, E., Sarthou, J. P. & Vanappelghem, C.), Syrph the Net Publications, Dublin.

65. Speight, M. C. D. (2017) Species accounts of European Syrphidae (Diptera). Syrph the Net, the database of European Syrphidae, **103**, 1-302, Syrph the Net publications, Dublin.

66. Ssymank, A. & Kearns, C. (2009) Flies–Pollinators on two wings. Caring for Pollinators: safeguarding agrobiodiversity and wild plant diversity. *Bonn, Bundesamt für Naturschutz, German Federal Agency for Nature Conservation*, 39-52.

67. Thomas, C. D. (2000) Dispersal and extinction in fragmented landscapes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **267**, 139-145.

68. Thomas, F. M. (2008) Recent advances in cause-effect research on oak decline in Europe. *CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources*, **3**, 1-12.

69. Thompson, J. N. (2005) Coevolution: the geographic mosaic of coevolutionary arms races. *Current Biology*, **15**, R992-R994.

70. Van Veen, M. (2004) *Hoverflies of Northwest Europe: identification keys to the Syrphidae*. *KNNV* Publishing, Utrecht.

71. Verboven, H. A., Uyttenbroeck, R., Brys, R. & Hermy, M. (2014) Different responses of bees and hoverflies to land use in an urban–rural gradient show the importance of the nature of the rural land use. *Landscape and Urban Planning*, **126**, 31-41.

72. Winfree, R., Bartomeus, I. & Cariveau, D. P. (2011) Native pollinators in anthropogenic habitats. *Annual Review of Ecology, Evolution, and Systematics*, **42**, 1-22.

73. Vukelić, J., Korijan, P., Šapić, I., Alegro, A., Šegota, V. & Poljak, I. (2018) Forest Vegetation of Hardwood Tree Species along the Mirna River in Istria (Croatia). *South-east European forestry*, **9**, 1-16.

74. White, A. J., Wratten, S. D., Berry, N. A. & Weigmann, U. (1995) Habitat manipulation to enhance biological control of *Brassica* pests by hover flies (Diptera: Syrphidae). *Journal of Economic Entomology*, **88**, 1171-1176.

75. Winsa, M., Öckinger, E., Bommarco, R., Lindborg, R., Roberts, S.P., Wärnsberg, J. & Bartomeus, I. (2017) Sustained functional composition of pollinators in restored pastures despite slow functional restoration of plants. *Ecology and evolution*, **7**, 3836-3846.

76. Yoshioka, A., Miyazaki, Y., Sekizaki, Y., Suda, S. I., Kadoya, T. & Washitani, I. (2014) A "lost biodiversity" approach to revealing major anthropogenic threats to regional freshwater ecosystems. *Ecological indicators*, **36**, 348-355.

77. Zamin, T. J., Baillie, J. E., Miller, R. M., Rodríguez, J. P., Ardid, A. N. A. & Collen, B. E. N. (2010) National red listing beyond the 2010 target. *Conservation Biology*, **24**, 1012-1020.

78. Zobel, M. (2016) The species pool concept as a framework for studying patterns of plant diversity. *Journal of Vegetation Science*, **27**, 8-18.

Table legends

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

Table 2. Correlations between the dark diversity index (DDi) and functional traits; rs, Spearman's rank correlation coefficient; H, Kruskal-Wallis statistic.* $p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Figure legends

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

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

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

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