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1 Indicators of Diptera diversity in wet grassland habitats are influenced by environmental variability,
2 scale of observation, and habitat type.

3

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10

11 **Highlights**

- 12 • Fine spatial scale analysis is required to investigate dipteran diversity in
13 agri-ecosystems
- 14 • The effectiveness of dipteran surrogate indicators is determined by wet grassland
15 category
- 16 • The scale at which invertebrate diversity is assessed on farmland needs further
17 investigation
- 18 • Marsh fly and hoverfly communities are highly congruent with nine other dipteran
19 families

20

21 **1. Abstract**

22 In low intensity agri-ecosystems such as wet grassland habitats, the inclusion of invertebrates in
23 conservation assessments and monitoring is usually limited to charismatic groups such as bees or
24 butterflies. However, wet grasslands support a wide range of inveterate groups, some of which may
25 exhibit limited movement not generally represented by more mobile groups such as those typically
26 examined. The use of surrogate species which exemplify broader invertebrate diversity has been
27 suggested as a possible means of including these overlooked invertebrates (such as Diptera) in
28 conservation planning within these habitats. Based on collections made by Malaise trap, we utilized
29 two families of Diptera (Sciomyzidae and Syrphidae) as indicators of a wider range of dipteran
30 diversity (nine Diptera families identified to parataxonomic unit level [PUs]) in wet grassland
31 habitats. We examined the role of environmental variability, spatial scale and habitat type on patterns
32 of cross-taxon congruence for all three assemblages. Both environmental correlation and community

33 congruence were significantly stronger among assemblages when examined at low spatial scales;
34 highlighting the need to examine dipteran groups at scales untypical of current agri-environmental
35 assessments; namely field and farm level. Furthermore, when wet grasslands were differentiated into
36 two habitat categories (Sedge and Rush dominated grasslands), the significance of the community
37 congruence increased markedly. This correlation was particularly strong between Sciomyzidae and
38 PUs which demonstrated similar differentiation based on habitat type-implying that assemblages
39 which exhibit comparable ecological partitioning are more likely to be useful surrogates of one
40 another. Correlations between richness, abundance and Shannon's diversity were highly variable
41 among groups, suggesting compositional analysis as the most appropriate examination of dipteran
42 diversity for surrogacy studies. The results indicate that cross-assemblage congruence of Diptera is
43 influenced by similarity of response to environmental variability, scale of observation, and
44 examination of assemblages differentiated into appropriate habitat categories. The results illustrate the
45 need to investigate invertebrate biodiversity surrogates at scales appropriate to the indicator groups
46 and examine congruence among assemblages within specific habitat categories. Such an approach has
47 the potential to maximise gamma diversity in areas where wet grasslands are under threat of
48 intensification or abandonment.

49 Keywords: Biodiversity; Diptera; Sciomyzidae; Syrphidae; Parataxonomy; Community Congruence

50

51 **2. Introduction**

52 European wet grassland habitats are typically low-intensity agricultural systems with semi-natural
53 habitats which support a rich mosaic of plant and animal communities (Bignal, E.M. & McCracken,
54 1996; Bignal and McCracken, 2000; Billeter et al., 2008). While much of the conservation of lowland
55 wet grassland is driven by botanical or ornithological interests, wet grasslands also sustain a high
56 diversity of invertebrates (Drake, 1998; Hayes et al., 2015; Joyce and Wade, 1998; Maher et al.,
57 2014). However, the difficulties associated with collecting comprehensive invertebrate data from
58 habitats have contributed towards the continued limited inclusion of invertebrate groups in broad scale
59 conservation planning and for monitoring conservation objectives (Cardoso et al., 2011). This is
60 particularly relevant in a European agricultural context, where intensification and abandonment of
61 traditional farming practices in areas such as wet grasslands is threatening biodiversity (Henle et al.,
62 2008). The use of invertebrates in the designation and management of agricultural areas considered as
63 being of a high nature value is usually limited (if included at all) to a few well known, easily
64 identifiable, and often iconic groups such as butterflies or bees (Andersen et al., 2004). Other
65 invertebrate groups such as Diptera are largely overlooked despite contributing significantly to the
66 overall biodiversity of such habitats (Keiper et al., 2002).

67 While the need to include a wider suite of invertebrates in conservation strategies for wet grasslands
68 makes ecological sense, the sheer abundance and diversity of groups such as Diptera are perceived as
69 barriers to their inclusion in routine habitat assessments. Alternative approaches such as the use of
70 selected invertebrate groups as biodiversity surrogates for a broader range of taxa has been suggested
71 as a possible means of including invertebrates in conservation and monitoring programs (Anderson et
72 al., 2011; Duelli et al., 1999; Duelli and Obrist, 2003; Hayes et al., 2015). Such biodiversity indicators
73 generally include a well-studied taxon or group of taxa which are ubiquitous within the habitat of
74 interest and can be easily collected and identified (Lindenmayer et al., 2000; McGeoch et al., 2002). A
75 predetermined measure of the diversity of the selected indicator is then used to reflect the diversity of
76 similar, or sometimes different, taxa. One such approach has been the examination of species richness
77 congruence between indicator groups and the taxa they are deemed to represent (Duelli and Obrist,
78 2003; Moreno and Sánchez-rojas, 2007; Prendergast, 1997). However, the species richness approach
79 is considered as having variable outcomes due to its dependency on the pairs of taxa under
80 investigation and it provides little insight into overall species representation and composition (Su et
81 al., 2004).

82 More recently, patterns of congruence derived from community similarity and/or the examination of
83 similarity of community responses to environmental variability have been utilized as approaches to
84 biodiversity surrogacy (Larsen et al., 2012; Paszkowski and Tonn, 2000; Rooney and Azeria, 2015;
85 Rooney and Bayley, 2012; Su et al., 2004). A potential caveat with this method, however, is the effect
86 of spatial scale of observation and habitat differentiation on congruence patterns. Invertebrate
87 diversity may respond to spatial scales not typically considered in conservation strategies (Haslett and
88 Salzburg, 1997; Weaver, 1995), and community composition can be influenced by microhabitat
89 changes across small scales that can have a marked effect on community structures (Cole et al., 2010).
90 In wet grassland habitats, this may be further exacerbated by temporal changes such as periodic
91 inundation in combination with grazing patterns (Carey et al., 2017; Maher et al., 2014; Ryder et al.,
92 2005). Thus, the examination of invertebrate communities may need to be assessed at spatial scales
93 untypical of those employed in conventional biodiversity assessments which are often linked to
94 agri-environmental scheme evaluations at farm or field-level scales.

95 The differentiation of habitats into categorical groups has also been shown to affect congruence
96 patterns with anthropogenic disturbance and ecoregion having a noticeable influence on congruence
97 measures (Ekroos et al., 2013; Myšák and Horsák, 2014; Rooney and Azeria, 2015; Rooney and
98 Bayley, 2012). The selection of invertebrate biodiversity surrogates, therefore, needs to consider
99 carefully determinants such as the distribution of the indicator taxa relative to the scale of the
100 observation, response of the indicator to ecological variance, and possible ecological relationships
101 between the indicator and the wider community it is chosen to represent (McGeoch, 1998; Paoletti,
102 1999).

103 In wet grassland habitats, adult Marshflies (Diptera: Sciomyzidae) are considered as potential
104 bioindicators owing to their ubiquity and ease of capture (Carey and LeRoy et al., 2015; Knutson and
105 Vala, 2011). However, they are known to have highly localised habitat fidelity and exhibit a markedly
106 limited movement (Williams et al., 2010); factors which may restrict their usefulness as surrogates for
107 broader dipteran diversity if the scale of observation utilized is greater than that which accurately
108 reflects their distribution. In contrast to this, adult Hoverflies (Diptera: Syrphidae) are considered
109 suitable bioindicators in agricultural systems (Burgio and Sommaggio, 2007) but are vagile in nature
110 with adults capable of foraging over long distances (Sommaggio, 1999). The use of either of these
111 groups as invertebrate biodiversity surrogates is therefore dependent on the similarity of their
112 response to factors such as spatial scale and habitat differentiation relative to the broader invertebrate
113 diversity for which they are selected to be a proxy

114 Along with these considerations, the identification of multiple and diverse groups such as Diptera to
115 species level usually requires expertise and time which is not readily available in the context of
116 typical designation or monitoring timeframes. Suggested alternatives to this impediment include
117 attempting to rapidly identify several groups of invertebrates using less traditional taxonomic methods
118 (Cardoso et al., 2011). Rapid biodiversity assessment techniques such as parataxonomy
119 (morphospecies) as described by Oliver et al. (1993) utilizes an approach whereby individuals with
120 similar external morphological traits are grouped together as typological units or Parataxonomic Units
121 (PUs) without the use of taxonomic keys. This work can be carried out by individuals with minimal
122 taxonomic training and possibly even through public participation initiatives such as citizen science
123 (Casanovas et al., 2014). Though the method is subject to debate regarding its effectiveness
124 (Thorsten-Krell, 2004; Ward and Stanley, 2004), when executed with caution, and subject to some
125 level of taxonomic verification, it can be utilized to give ecologically relevant outcomes (Cotes et al.,
126 2009; Obrist and Duelli, 2010; Oliver and Beattie, 1996; Ward and Stanley, 2004). Studies of Diptera
127 in wet grasslands which have utilized the two approaches (taxonomic and parataxonomic) have
128 generally focused on richness correlations of all Diptera and not examined community similarity
129 (Hayes et al., 2015; Ryder et al., 2005). Though useful patterns can be derived from such data,
130 especially in terms of family richness and abundances, a more in-depth investigation of a smaller
131 number of dipteran families using more prolonged sampling and intensive sorting methods might be
132 more insightful (Frouz, 1999).

133 Given that Sciomyzidae and Syrphidae fulfil the criteria for suitable bioindicators as outlined by
134 McGeogh (1998), we compared measures of their diversity with a broader assemblage of nine Diptera
135 families identified using parataxonomy. By examining agreement among groups in terms of
136 environmental responses we were able to identify the role that environmental factors play in
137 determining community structure of different dipteran assemblages. We also conducted a hierarchical
138 sampling regime from two wet grassland habitat types (Rush dominated and Sedge dominated wet

139 grasslands) based on samples from individual traps or samples from traps from the same sample patch
140 pooled together. We investigated the role that spatial scale and habitat type played in determining
141 patterns of congruence among the three assemblages (Sciomyzidae, Syrphidae and dipteran PUs)
142 using a range of tests.

143 Our principal objectives were to:

- 144 1. Examine patterns of environmental correlation between the groups at two spatial scales (Trap
145 level and Patch level)
- 146 2. Determine whether cross-taxon congruence among groups was affected by scale of
147 observation
- 148 3. Investigate the role that habitat type plays in contributing to patterns of cross-assemblage
149 congruence

150 The results of this investigation are discussed in the context of selecting suitable invertebrate
151 biodiversity indicators within high nature value agri-ecosystems such as wet grasslands.

152 **3. Materials and Methods**

153 **2.1 Study area**

154 This investigation was undertaken in the west of Ireland in wet grassland habitats defined according to
155 Fossitt (2000). For inclusion in this classification, grass, rush or small sedge cover needs to exceed
156 50% and broadleaf herbs, reeds and larger sedges should not dominate i.e. must be <50%. The
157 broadleaf herb component should also be relatively evenly divided between drier grassland and
158 wetland species. We selected five each of two sub-categories of wet grassland based on their
159 dominant vegetation type i.e. wet grasslands dominated by rushes (Family Juncaceae) and wet
160 grasslands dominated by sedges (Family Cyperaceae). All wet grassland sites in this study were
161 actively managed for livestock grazing and were not subject to intensive cutting regimes or
162 application of fertilizers.

163 **2.2 Diptera sampling and determination**

164 Diptera sampling was undertaken between May 1st and September 4th 2014 using black Malaise traps
165 of Townes design (Townes, 1972). Two traps were placed 20m apart in homogeneous patches of
166 vegetation and away from obvious topographical features such as drainage ditches, wet flushes,
167 hedgerows etc. This method was employed to maximise Diptera collections from within the sample
168 patch rather than as a result of movement between habitats or due to the presence of any obvious
169 ecotonal changes (Carey et al., 2017). Collection heads containing a 70% ethanol solution were

170 positioned in a southerly direction and were collected every 14 days. A portable electric fence was
171 operated to protect the traps from interference by livestock, and vegetation within the enclosure was
172 intermittently shortened and removed to maintain trap efficacy and replicate conditions outside the
173 fenced area. Eleven families of adult Diptera were selected for analysis based on their ease of
174 identification to family level, ubiquity within the habitat, and previous recommendations for use as
175 bioindicators of wetland habitats (Hayes et al., 2015; Speight, 1986). These families were the
176 Dolichopodidae, Empididae, Hybotidae, Limoniidae, Pipunculidae, Scathophagidae, Sciomyzidae
177 Stratiomyidae, Syrphidae, Tabanidae, and Tipulidae.

178 Sciomyzidae and Syrphidae were utilized as the principal biodiversity indicators and identified to
179 species level using Rozkošný (1987) and Vala (1989) for sciomyzids and Ball & Morris (2013) and
180 Stubbs & Falk (2002) for syrphids. The remaining nine families were identified using parataxonomy,
181 a rapid biodiversity assessment method. Non-specialist individuals (undergraduate students hereafter
182 referred to as parataxonomists) utilized a simplified character key derived from Oosterbroek (2007)
183 and Unwin (1981) to assist them in the removal of the remaining nine families from bulk samples.
184 Subsequent to initial sorting to family level, the specimens were categorised into parataxonomic units
185 (PUs) based on their external morphological features without the use of keys (Oliver and Beattie,
186 1996, 1993). Each newly assigned PU was digitally photographed and the image inserted into a shared
187 data base allowing each parataxonomist access to the image for referral. The initial voucher specimen
188 was preserved in 100% ethanol for determination to species level by taxonomists. These voucher
189 specimens were utilized to determine the level of ‘splitting’ whereby a taxonomic species was split
190 into two or more PUs. Oliver and Beattie (1996) recommend that a random subset of ~30 individuals
191 from each PU is also maintained (as vouchers) to determine the degree of ‘lumping’ i.e. when two or
192 more taxonomic species are classed within the same PU. Our study opted to exclude this verification
193 as it would have entailed the identification of over 3000 individual specimens by taxonomists.
194 Instead, only the original voucher specimens were identified by specialists and, therefore, only species
195 splitting and not lumping was accounted for. Omitting the ‘lumping’ protocol due to time constraints
196 is likely to have led to underestimations of species richness, but was countered by the speed of the
197 species determination by specialist taxonomists who needed to verify ~30 times less specimens. This
198 effectively maximised taxonomic input by minimising effort; an important consideration due to the
199 increasingly limited availability of taxonomic specialists (Cardoso et al., 2011) and requirement for
200 rapid assessments of biodiversity in line with typical conservation strategies. The original inventory
201 was then subjected to changes based on the species determinations and the corrected PU data utilized
202 for analysis. The percentage of splitting error for each PU family was reported according to Oliver
203 and Beattie (1996).

204 **2.3 Environmental data**

205 A series of environmental variables was recorded at each site throughout the collection period and
206 mean values calculated. These included vegetation height as well as the percentage cover of grasses,
207 sedges, rushes, moss, moribund material, broadleaf herbs and bare ground. Each of the variables was
208 estimated using five 50 x 50 cm quadrats placed randomly within 10m of each Malaise trap location
209 and within the homogeneous vegetation patch. Soil samples were extracted from the same random
210 sampling areas and mean pH, soil carbon content, and soil moisture were analysed (Anon, 1990). The
211 depth of standing water at each site was recorded in the October following trap removal and prior to
212 any extensive winter inundation which would have rendered water depth measurements unsafe.

213 **2.4 Data analysis**

214 Prior to multivariate analysis invertebrate abundance data was $\log_{10}(x+1)$ transformed to reduce the
215 influence of numerically dominant species and to approximate multivariate normality. Species which
216 were considered outliers (>2.0 standard deviations) using the Sørensen distance measure were also
217 removed (McCune and Mefford, 2011). Environmental data (with the exception of soil pH) was
218 arcsine square root transformed or $\log_{10}(x+1)$ transformed to improve linearity and to approximate
219 normality (McCune and Mefford, 2011).

220 Non-metric multidimensional scaling (NMS) ordinations (McCune and Grace, 2002) of samples was
221 undertaken using the Sørensen distance in PC-Ord v.6 (McCune and Mefford, 2011). Samples which
222 were identified as extreme outliers with standard deviations >3.0 using the Sørensen distance measure
223 were removed prior to NMS ordination. We utilized 250 runs of real data to 250 runs with randomised
224 data to determine the number of significant axes. An orthogonal principal axis output was selected for
225 each NMS to illustrate maximum community variation along axis 1. Environmental data were utilized
226 as a second explanatory matrix and variables with Pearson r^2 values >0.2 overlain as a bi-plot
227 (McCune and Mefford, 2011). Multi-Response Permutation Procedures (MRPP), which are
228 non-parametric procedures for the testing the hypothesis of no difference between two groups, were
229 utilized to examine for significant differentiation between habitat types based on the species/PU
230 composition of each assemblage (McCune and Mefford, 2011).

231 A Principal Components Analysis (PCA) with a cross products matrix based on correlation was
232 utilized to evaluate the environmental data. MRPP was also utilized to examine habitat differentiation
233 based on PCA results. The PC1 score obtained from this analysis was used to rotate the NMS
234 ordination of each assemblage to ensure a standard alignment for comparisons between community
235 structure and NMS axes. Such rotation does not alter the relative position of each sample in species
236 space and allows for comparison between assemblages (McCune and Mefford, 2011; Rooney and
237 Bayley, 2012). Subsequent to rotation, we examined the response of the three assemblages to
238 environmental variables by comparing the Pearson correlation coefficients of each significant axis
239 derived from the NMS using Spearman Rank correlations in PAST (Hammer et al., 2001). All

240 responses were considered at trap scale (each individual Malaise trap [n=20]) and patch scale (where
241 data from pair-wise traps from the same vegetation patch were combined [n=10]).

242 We compared changes in assemblage structure within fields (i.e. between pair-wise traps) using the
243 Sørensen similarity as a measure of differentiation between pair-wise traps. Similarity scores obtained
244 for each assemblage were compared using linear correlation (Pearson's R) to investigate whether
245 patterns of differentiation between pair-wise traps was congruent between assemblages. For each
246 assemblage, we also tested the relationship between the level of community similarity between
247 pair-wise traps and the differentiation of environmental variables between pair-wise traps using
248 Spearman Rank correlations in PAST (Hammer et al., 2001).

249 Community congruence between indicator groups was tested using Partial Mantel tests controlling for
250 geographical and environmental autocorrelation using Sørensen distance measures for species/PU
251 data and Euclidean distance measure for control matrices (McCune and Mefford, 2011). Partial
252 Mantel tests were carried out across all samples, at two spatial scales (patch and field), and within
253 habitat types at trap scale using PC-Ord V.6.

254 Sciomyzidae, Syrphidae and PU richness from each sample was calculated as a proportion of the total
255 richness of each assemblage across all sample sites (Finch and Löffler, 2009). Shannon's entropy
256 (previously known as Shannon's diversity) was utilized as a measure of the diversity of each
257 assemblage (Ellison, 2010; Jost, 2007), along with raw abundance values. We utilized Spearman rank
258 correlations to investigate patterns of cross-taxon congruence of each of these values using PAST
259 (Hammer et al., 2001). Correlations were investigated at two spatial scales (Trap scale [n=20], Patch
260 scale [n=10]) and within habitat types (rush or sedge dominated sites) at trap scale (n=10).

261 **5. Results**

262 **5.1. General results**

263 A total of 105,666 individuals from eleven families of Diptera were collected from the sampling sites
264 and subjected to taxonomic or parataxonomic identification. Sciomyzidae (1,975 individuals) and
265 Syrphidae (9,568 individuals) were determined to species level with 34 and 72 species identified,
266 respectively. This represents 53% of Sciomyzidae and 40% of Syrphidae from the Irish fauna
267 (Chandler et al., 2008).

268 A total of 105 parataxonomic units were identified from the nine remaining Diptera families. Once
269 splitting had been accounted for, this was reduced to 85 with an overall percentage splitting error of
270 24% (Table 1). Dolichopodidae (Long-legged flies) represented the most abundant family (45,337)
271 with Stratiomyidae (Soldierflies) contributing the least number of individuals (685). Scathophagidae
272 (Dung flies) showed the highest percentage splitting error of PU allocation owing to the markedly
273 different body size and colouration attributed to the yellow dung fly (*Scathophaga stercoraria* L.

274 1758). Pipunculidae (Big-headed flies) showed the lowest level of splitting error, though it is likely
 275 that ‘lumping’ of species occurred in the allocation of PUs to this family owing to marked similarity
 276 among sibling species.

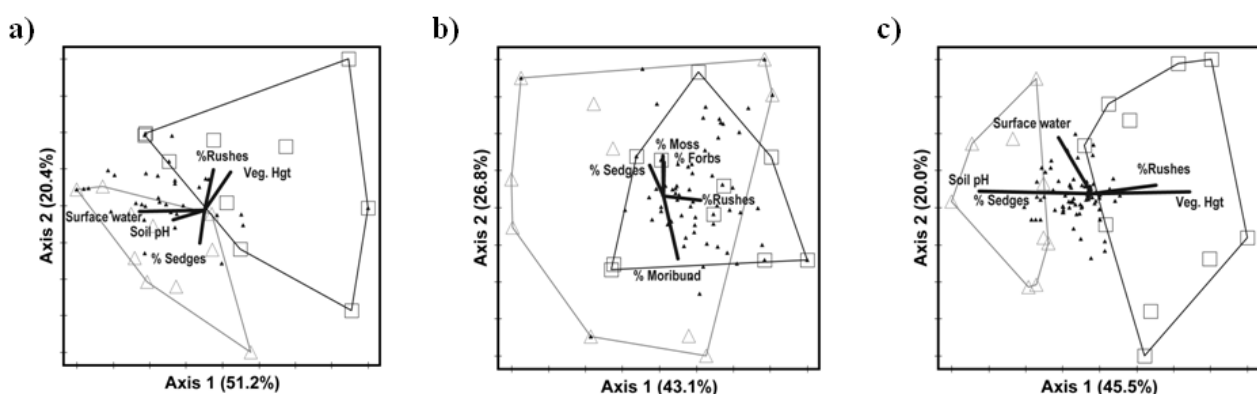
277 Table 1: Number of individuals, species, and Parataxonomic units (PUs) per Diptera family. The
 278 percentage splitting error refers to the proportion of splitting within each family whereby a species
 279 was classified as more than one PU by parataxonomists.

Family (Individuals)	Number of species	Number of PUs	% splitting error
Dolichopodidae (45337)	15	17	13
Limoniidae (13796)	14	15	7
Empididae (11987)	15	18	20
Scathophagidae (8933)	7	14	100
Hybotidae (6098)	12	13	8
Pipunculidae (3129)	4	4	0
Tabanidae (2820)	3	4	33
Tipulidae (1338)	5	8	60
Stratiomyidae (685)	10	12	20
Overall	85	105	24

280

281 5.2. Response to environmental variability

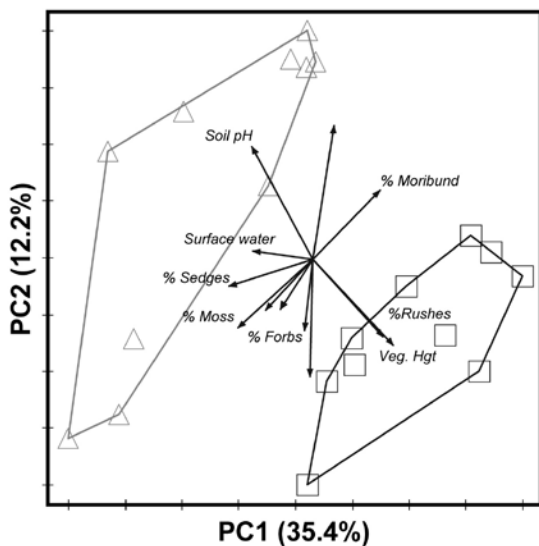
282 NMS ordinations of samples in species/PU-space produced three dimensional solutions which
 283 explained >80% of the variation for each assemblage (Sciomyzidae 86.0%, Syrphidae 84.3%, PUs
 284 80.4%). Environmental variables with a Pearson r^2 score of >0.2 are shown as bi-plots (Figure 1).
 285 Stress levels for each ordination were all <11.5 with values of ~10 are considered suitable for
 286 reasonable interpretation (McCune and Mefford, 2011). Multi-response permutation-procedure
 287 (MRPP) analysis showed significant differences between the two habitat types for all three
 288 assemblages using the Sørensen distance measure. PUs showed the most significant differentiation
 289 between habitat types ($A=0.080$, $P=3 \times 10^{-5}$), followed by Sciomyzidae ($A=0.062$, $P=6 \times 10^{-4}$) and
 290 then Syrphidae ($A=0.030$, $P=0.027$).



291

292 Figure 1: NMS ordinations of samples in species/PU space. Environmental variables with r^2 values
293 >0.2 are shown as bi-plots. The principal orthogonal axes are shown with the percentage of variation
294 associated with each axis. Samples are shown as open squares (rush dominated wet grassland) and
295 open triangles (sedge dominated wet grassland), species/PUs as black triangles. **a)** Sciomyzidae, **b)**
296 Syrphidae and **c)** and PUs. Only axes 1 & 2 are illustrated which explain a cumulative variation of:
297 Sciomyzidae (71.6%), Syrphidae (69.9%); PUs (65.5%). Habitat types were significantly different
298 using MRPP analysis for all three groups **a)** $A=0.062$, $P=6 \times 10^{-4}$ **b)** $A=0.030$, $P=0.02700$ **c)**
299 $A=0.080$, $P=3 \times 10^{-5}$.

300 Principal components analysis of environmental variables showed that PC1 explained 35.4% of the
301 variance between samples (Figure 2). MRPP analysis confirmed that habitats were significantly
302 different from one another based on environmental data ($A=0.249$, $P=2 \times 10^{-5}$). The scores from PC1
303 were utilized to rotate the NMS ordinations for examination of cross-assembly agreement based on
304 environmental correlations. All three assemblages showed significant correlations based on NMS axis
305 one irrespective of spatial scale of observation (Table 2). At trap scale ($n=20$), Sciomyzidae and
306 Syrphidae showed significant correlations across all axes. This was reduced to the first two axes at
307 patch scale. PUs showed significant correlations with the other groups only on axis one. It is worth
308 noting that the negative correlation between Syrphidae and PUs is likely an artefact of the rotation of
309 the NMS using only PC1 scores (Rooney and Bayley, 2012).



310

311 Figure 2: Principal Components Analysis of sampling patches showing the environmental variables
312 most strongly associated with PC1. Samples are differentiated into habitat type (Rush wet grassland:
313 open squares; sedge wet grassland: open triangles). Habitat types were significantly different based on

314 MRPP analysis (A=0.249, P=0.00002). Arrows with no associated environmental variable were
 315 associated with PC2.

316
 317
 318
 319
 320
 321
 322
 323
 324
 325
 326

327 Table 2: Environmental correlations (Spearman's r) between dipteran assemblages based on the axes

	Axis 1			Axis 2			Axis 3		
	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs
Trap scale (n=20)									
Sciomyzidae	1			1			1		
Syrphidae	0.73**	1		0.65*	1		0.73**	1	
PUs	0.98***	0.70**	1	-0.01	-0.53	1	-0.38	-0.07	1
Patch scale (n=10)									
Sciomyzidae	1			1			1		
Syrphidae	0.85***	1		0.79**	1		0.07	1	
PUs	0.76**	0.73**	1	-0.49	-0.80**	1	-0.13	-0.05	1

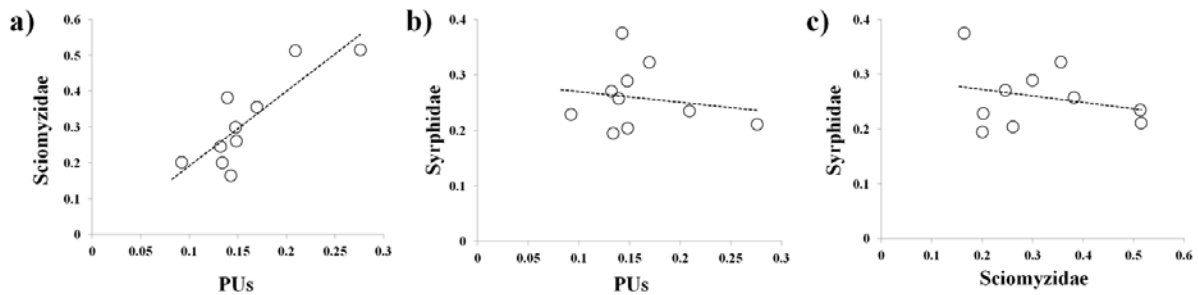
328 of the NMS ordinations rotated by PCA score. (P<0.05*, P<0.01**, P<0.001***)

329

330 **5.3. Community similarity between pair-wise traps**

331 Sciomyzidae and PUs showed a significant relationship in terms of how their assemblages responded
 332 to differentiation between pair-wise traps i.e. increased dissimilarity between pair-wise traps was
 333 congruent among both assemblages (r^2 0.84, $P=0.002$) (Figure 3). None of the measured
 334 environmental variables were significantly correlated with changes in community similarity of
 335 Sciomyzids or PUs between pair-wise traps i.e. none of the measured variables appeared to
 336 significantly affect differences in community structure between traps from the same patch. Syrphidae
 337 were not significantly correlated with Sciomyzidae (r^2 -0.23, $P=0.470$) or PUs (r^2 -0.17, $P=0.063$) in
 338 terms of similarity of community differentiation between pair-wise traps. However, differentiation of
 339 Syrphidae between pair-wise traps was significantly correlated with differences in the percentage
 340 cover of broadleaf herbs between pair-wise traps (Spearman's r 0.82, $P=0.004$); a relationship that
 341 was not significant for Sciomyzidae or PUs.

342



343

344 Figure 3: Linear regression illustrating the congruence of changes in assemblage structure between
 345 pair-wise traps using the Sorensen distance measure. Increasing values are indicative of greater
 346 dissimilarity. **a)** Sciomyzidae and PUs were significantly correlated (r^2 0.84, $P=0.002$), **b)** Syrphidae
 347 and PUs (r^2 -0.17, $P=0.0630$) (r^2 -0.23, $P=0.470$) and **c)** Syrphidae and Sciomyzidae (r^2 -0.23,
 348 $P=0.470$) were not significantly correlated.

349 5.4. Cross-assemblage congruence

350 Mantel R values were weak but significant between Sciomyzidae and PUs across all samples but only
 351 at trap level (Table 3). This relationship was maintained even when geographic and environmental
 352 autocorrelation was controlled for. There was also a significant relationship between Syrphidae and
 353 PUs at trap scale but this was not maintained when either geographic or environmental autocorrelation
 354 was controlled for. There were no significant correlations between any of the three assemblages at
 355 patch level. When correlations among assemblages were examined within habitat types, Sciomyzidae
 356 and PUs were significantly congruent within both habitats (Table 4) even when geographic and
 357 environmental autocorrelation were controlled for. Sciomyzidae were also significantly correlated
 358 with Syrphidae in both habitats, however, in the sedge habitats this was a result of geographic and/or
 359 environmental co-response i.e. trap proximity or similarity of environmental variables at the trap

360 locations appeared to influence congruence. Syrphidae were congruent with PUs in Rush dominated
 361 wet grasslands but not in Sedge dominated habitats.

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370 Table 3: Mantel R statistics of assemblage concordance at two spatial scales using the Sorensen
 371 distance measure for species/PU data and the Euclidean distance measure to control for location
 372 (geographic) and environmental variability. (P<0.05*, P<0.01**, P<0.001***)

Controlled effect	None			Geographical			Environmental		
	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs
Trap scale (n=20)									
Sciomyzidae	1			1			1		
Syrphidae	0.08	1		0.03	1		0.06	1	
PUs	0.44**	0.20*	1	0.41**	0.13	1	0.44**	0.15	1
Patch scale (n=10)									
Sciomyzidae	1			1			1		
Syrphidae	-0.16	1		-0.17	1		-0.16	1	
PUs	-0.03	0.12	1	-0.03	0.09	1	-0.01	0.18	1

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385 Table 4: Mantel R statistics of assemblage concordance within habitats using the Sørensen distance
 386 measure for species/PU data and the Euclidean distance measure to control for location and
 387 environmental variability. (P<0.05*, P<0.01**, P<0.001***)

Controlled effect	None			Geographical			Environmental		
	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs	Sciomyzidae	Syrphidae	PUs
Sedge grasslands (n=10)									
Sciomyzidae	1			1			1		
Syrphidae	0.27*	1		0.15	1		0.20	1	
PUs	0.71**	0.04	1	0.68**	0.13	1	0.71**	0.02	1
Rush grasslands (n=10)									
Sciomyzidae	1			1			1		
Syrphidae	0.35*	1		0.24*	1		0.36*	1	
PUs	0.72**	0.51**	1	0.67**	0.42**	1	0.75**	0.47*	1

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5.5. Cross-taxon congruence of richness, abundance and Shannon's entropy

Patterns of cross-taxon congruence were not significant for richness among any of the groups (Table 5). Sciomyzidae and PU abundance was significantly positively correlated across all samples at trap scale (n=20) but not at patch scale (n=10) or within habitat types. Syrphidae and PU abundance was significantly positively correlated, but only within rush dominated wet grasslands. There was also a significantly positive relationship between Shannon's entropy of Syrphidae and PUs across all samples but only at trap scale (n=20).

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424 Table 5: Spearman Rank correlations of richness, abundance and Shannon's diversity (entropy) of each group. (P<0.05*, P<0.01**, P<0.001***)

	Overall (Trap scale n=20)			Overall (Patch scale n=10)			Sedge Habitats (n=10)			Rush Habitats (n=10)		
	Sciomyzidae	Syrphidae	PU	Sciomyzidae	Syrphidae	PU	Sciomyzidae	Syrphidae	PU	Sciomyzidae	Syrphidae	PU
Richness												
Sciomyzidae	/	-0.003	0.261	/	-0.055	0.212	/	-0.6	0.044	/	0.482	0
Syrphidae		/	0.055		/	0.024		/	0.314		/	0.360
PU			/			/			/			/
Abundance												
Sciomyzidae	/	0.002	0.543*	/	0.018	0.045	/	-0.067	0.382	/	0.249	0.079
Syrphidae		/	0.310		/	0.503		/	0.552		/	0.746*
PU			/			/			/			/
Shannon's H' (Entropy)												
Sciomyzidae	/	-0.381	-0.307	/	-0.467	-0.285	/	-0.164	-0.309	/	-0.321	-0.539
Syrphidae		/	0.448*		/	0.587		/	0.224		/	-0.018
PU			/			/			/			/

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427 **6. Discussion**

428 Community congruence between dipteran groups examined at low spatial scales and within district
429 habitat types provided the most useful predictors of selected dipteran diversity. Assemblages which
430 showed similar responses to environmental variables and exhibited shared community differentiation
431 based on habitat type were most significantly correlated. Diptera which display limited movement
432 (Sciomyzidae) were more indicative of changes in wider dipteran community structure (represented
433 by PUs) than those which are generally more mobile in nature (Syrphidae). The results highlight the
434 variation that occurs between groups when a range of statistical expressions and scales of observation
435 are utilized in biodiversity indication. The results also suggest compositional analysis of Diptera as
436 the most appropriate approach to the investigation of this overlooked group as previously suggested
437 (Hughes et al., 2000).

438 Assessments of invertebrate cross-taxon taxon congruence in agricultural systems may be limited in
439 spatial resolution so that patterns can be examined at scales such as field or farm level which
440 coordinate with administrative requirements e.g. agri-environmental payments are allocated at such
441 scales (Anderson et al., 2011; McMahan et al., 2012). While this is a pragmatic approach to aligning
442 assessment and monitoring of biodiversity with policy, the distribution of certain organisms such as
443 Diptera may occur at more restricted scales. Sciomyzidae, for example, have been shown to exhibit
444 habitat fidelity across scales as low as 23m (Williams et al., 2010), and Hughes et al. (2008)
445 determined high levels of dipteran community dissimilarity (0.52 Bray-Curtis) between two Malaise
446 traps sited 25m apart in a meadow.

447 Therefore, the evaluation of patterns of congruence between surrogates and wider diversity of such
448 organisms should occur at scales appropriate to the community distribution of the target taxa
449 (McGeoch, 1998). Subsequent monitoring of such groups which take place at fixed sampling sites can
450 then provide accurate insights into the success of typical conservation strategies such as habitat
451 maintenance or restoration; but inclusive of organisms that inhabit areas at lower spatial scales.

452 In the present study, environmental correlations between groups based on axis 1 of the NMS
453 ordination were of most interest as they explained the largest amount of variation owing to the use of
454 orthogonal principal outputs in the analysis. While all three dipteran assemblages showed some
455 congruence in terms of environmental correlates, the strongest relationship was seen between
456 Sciomyzidae and PUs at trap level. Based on the NMS output, Sciomyzidae and PU assemblages were
457 largely influenced by the same environmental variables (% Rush, % Sedge, Soil pH, vegetation height
458 and surface water depth) whereas Syrphidae were influenced by a different combination of
459 environmental factors (% Rush, % Sedge, % Moss, % Forbs, % Moribund). It is unsurprising that

460 Syrphidae communities responded primarily to variables associated with vegetation type and structure
461 as adults are largely dependent on pollen and nectar resources for food (Ricarte et al., 2011).
462 Sciomyzidae on the other hand are known to respond to factors such as soil moisture, hydroperiod and
463 vegetation type (Maher et al., 2014; Williams et al., 2009b) and exhibit very limited movement within
464 wet grassland habitats (Williams et al., 2010). Their response to factors such as soil pH and water
465 depth suggests that environmental influences that may affect their malacophagous larvae could also
466 influence adult distributions.

467 The strong correlation between PU and Sciomyzidae assemblages in terms of environmental
468 correlates at trap level not only illustrates that PUs are influenced by comparable environmental
469 factors as Sciomyzidae, but that they also respond at similar scales. Fine scale habitat features are
470 known to affect arthropods in grassland systems, especially more sedentary groups (Cole et al., 2010).
471 Within wet grassland habitats, variations in hydrological regime and grazing patterns are known to
472 have significant influences on dipteran diversity – in particular Sciomyzidae (Maher et al., 2014;
473 Ryder et al., 2005; Williams et al., 2009a). Changes in environmental factors across small spatial
474 scales e.g. between pair-wise traps 20m apart, could therefore have a more marked effect on groups
475 such as Sciomyzidae which are characterised as having limited movement. This response to small
476 scale features could be a result of their reduced ability to respond to disturbances or stressors, or
477 alternatively, it may be an indication of the availability of a specific resource located at finer scales.
478 Factors that influence dispersal of Diptera adults include larval development, sexual behaviour and
479 food requirements (Delettre et al., 1998). Analysis of Syrphidae community differentiation between
480 pair-wise traps within each site was not congruent with Sciomyzidae or PUs but was significantly
481 influenced by changes in the percentage cover of broadleaf herbs. As Syrphidae adults are largely
482 dependent of pollen and nectar as food sources (Sutherland et al., 2001) and patches of increased
483 broadleaf herb cover within sites most likely attracted Syrphidae adults, this is not surprising.
484 Sciomyzidae adults, on the other hand, can be restricted to very small areas characterised by dominant
485 vegetation type (Williams et al., 2010) and their distribution may potentially be influenced by the
486 availability of larval food sources i.e. aquatic and semi-aquatic molluscs which often have very
487 clustered distributions (Knutson and Vala, 2011). Sciomyzidae community differentiation between
488 pair-wise traps was anticipated in our results but interestingly was not correlated with any of the
489 measured environmental variables examined in this study. However, the significant correlation
490 between Sciomyzidae and PU community structure between pair-wise traps strongly suggests that the
491 factors contributing towards community differentiation across small spatial scales for these two
492 groups are similar.

493 This result may explain why highly mobile adult Syrphidae were not strongly correlated with the
494 other dipteran assemblages in terms of environmental correlates or community congruence between
495 pair-wise traps as they likely respond to environmental heterogeneity at much broader scales

496 (Hendrickx et al., 2007). In contrast, groups which share a decreased ability to disperse and/or have
497 high habitat fidelity have previously been shown to have increased levels of congruence (Grenouillet
498 et al., 2008). However, such patterns of congruence might only be recognisable at spatial scales where
499 these taxa respond similarly to environmental stressors (McGeoch, 1998).

500 The partitioning of data into biogeographical units, discrete habitat types or subsets of taxa based on
501 community structure is also known to affect congruence patterns between assemblages showing both
502 increased and decreased correlations (Myšák and Horsák, 2014; Pawar et al., 2007; Rooney and
503 Bayley, 2012). In the present study, all three assemblages showed significant community
504 differentiation between the two habitat types, and community congruence between groups within
505 these two wet grassland habitats were markedly more significant than congruence from groups
506 examined at overall wet grassland level. Wet grassland in Ireland is broadly defined, and generally
507 classified as having >50% cover of grasses, small sedges or rushes, with the remaining vegetation
508 comprised of a mixture of wetland and drier grassland herbs (Fossitt, 2000). However, dominant
509 vegetation type is known to significantly contribute towards dipteran community structure (Hughes et
510 al., 2008) and our results imply that assessments of dipteran diversity in wet grassland habitats should
511 consider sub-categories based on dominant vegetation type. Selecting a biodiversity surrogate requires
512 knowledge of the scale of distribution of the proposed surrogate within the habitat of interest and an
513 examination of its relationship with the predicted taxa below this scale (McGeoch, 1998). In the
514 present study, examining the wet grassland habitats as distinct habitat-types increased community
515 congruence among the groups, in particular between Sciomyzidae and PUs which displayed similar
516 differentiation between the two wet grassland habitats. Strong community congruence between groups
517 that share similar responses to environmental variables and gradients such as habitat change has been
518 previously found among arthropods in grassland habitats (Oertli et al., 2005).

519 The examination of Diptera community congruence within these habitat categories at the lowest
520 spatial scale provided the most significant insight into relationships between potential biodiversity
521 surrogates (predictor taxa) and the wider assemblage as represented by PUs (predicted taxa).
522 However, while Sciomyzidae and Syrphidae were both useful surrogates of the PU assemblages in
523 rush dominated habitats, only sciomyzids were significantly congruent with PU assemblages in sedge
524 dominated wet grasslands. The result further emphasises the need to include multiple biodiversity
525 surrogates in evaluations and monitoring rather than reliance on one group (Rooney and Bayley,
526 2012). The results also illustrate the importance of examining community congruence between taxa
527 based on distinct partitioning of communities.

528 Species richness is largely utilized as a measure of the success of conservation actions (Su et al.,
529 2004) and is a relatively straightforward value to communicate to policy makers. However, changes in
530 the species richness of a target group may not be indicative of the response of a wider suite of

531 organisms, especially in agricultural systems (Billeter et al., 2008). Correlations between richness,
532 abundance, and Shannon's entropy in this study varied between groups as is generally reported in
533 studies investigating cross taxon congruence (Báldi, 2003; Hayes et al., 2015; Legakis et al., 2004).
534 Only three significant correlations were recorded using these measures, all of which were found when
535 the lowest spatial scale (trap level) was examined. Hayes et al. (2015) reported a significant
536 relationship between Sciomyzidae richness and overall Diptera PU richness in a comparative study of
537 wet grassland conducted at sampling scales similar to the trap level utilized in this investigation; albeit
538 using a sweep net. Though our study was limited to the use of nine families of Diptera to PU level, it
539 is interesting to note that Sciomyzidae abundance and PU abundance was significantly correlated
540 overall at trap level. In general, the richness and abundance of a group tends to be significantly
541 correlated so it could have been expected that if abundance between two groups was significant, then
542 the richness measures would also have been co-correlated. However, an underestimation of PU
543 richness as a result of the conservative allocation of PUs in this study i.e. lumping, may have
544 contributed towards a lack of significance between the richness of PUs and Sciomyzidae. Maher et al.
545 (2014) illustrated patterns that suggested increased richness and abundance of Sciomyzidae was
546 indicative of decreased abundance of Syrphidae in wet grassland habitats. However, these findings
547 were reflective of reduced flowering plant diversity due to increased hydroperiod which positively
548 affected Sciomyzidae but negatively affected Syrphidae.

549 Syrphidae diversity (Shannon's entropy) and PU diversity (Shannon's entropy) were also significantly
550 correlated; but only at trap scale. It could be considered that the most robust PU measure utilized
551 within these correlations was abundance which was a raw value not subject to interpretation. It is
552 therefore interesting that the most significant correlation among these abundance measures was seen
553 between Syrphidae abundance and PU abundance in rush dominated habitats. This result suggests that
554 within such habitats, areas of rush dominated wet grasslands with high abundances of Hoverflies
555 could be important for maintaining high abundances of other Diptera; a pattern also reflected in the
556 community congruence of these two groups with this habitat type. However, the results also highlight
557 the variability of congruence when using richness, abundance and Shannon's entropy as measures of
558 each group and suggest compositional analysis as a measure of Diptera for surrogacy evaluation.

559 Agricultural landscapes such as those frequent in the west of Ireland are a mosaic of improved
560 grasslands interspersed with pockets of wet grassland habitat. Realistically, land managers may be
561 required to maintain a suite of wet grasslands whilst others are sacrificed to drainage and/or change of
562 use. Dipteran diversity provides one practical way of maximising landscape-level complementarity in
563 the assemblage conserved. The suite of sites to be preserved, which would maximise gamma diversity
564 of the eleven families studied here, could reasonably be predicted by reference to changes in
565 Sciomyzidae composition dissimilarities (if the sites were *Carex* or *Juncus*-dominated) or with
566 reference to Syrphidae composition (if the sites were *Juncus*-dominated).

567 **7. Conclusion**

568 The inclusion of overlooked groups such as Diptera in conservation strategies is unlikely to reach the
569 thresholds of more iconic invertebrate groups so the use of surrogate taxa is increasingly probable. If
570 such action is required, the surrogates selected for representation of wider dipteran diversity need to
571 reflect the distribution of Diptera at both habitat level and at smaller scales within those habitats. Our
572 results demonstrate that the use of community similarity at low spatial scales and within specific
573 habitat types appears to be the most useful surrogate of dipteran diversity in wet grasslands. However,
574 it may well be most beneficial for conservationists to adopt several invertebrate groups should a
575 surrogate approach be utilized to account for the range of dispersal and distribution patterns of wet
576 grassland invertebrates. While a more extensive study of invertebrates in high nature value farming
577 systems is required before a practical solution to invertebrate diversity surrogacy can be selected, in
578 the case of Diptera within wet grassland habitats, our results suggest that both Sciomyzidae and
579 Syrphidae meet these requirements; though the former appear to represent the predicted taxa (PUs)
580 better in this study. In light of the increasing intensification and abandonment of wet grassland
581 habitats, the inclusion of easily captured, readily identified, and ubiquitous groups as seen in this
582 study may provide essential information on the status of dipteran assemblages within these areas and
583 inform for the selection of areas for conservation. We recommend that considering samples from low
584 spatial scales rather than administrative units such as field or farm level in cross-taxon congruence
585 investigations may highlight patterns of correlation among invertebrate groups which are generally
586 overlooked.

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