Title: Habitat requirements and natural history of *Tetanocera elata* (Diptera: Sciomyzidae): Case study of a dry meadow in western Ireland

Authors: Allison Bistline-East[1,2,3,4], Daniel Burke[1,2], Christopher D. Williams[5], and Michael J. Gormally[1,2]

[1] Applied Ecology Unit, National University of Ireland Galway, Galway, Ireland

[2] Ryan Institute, National University of Ireland Galway, Galway, Ireland

[3] Current affiliation: Purdue University, 901 W State St. West Lafayette, IN 47907, USA

[4] Corresponding author email: allison@bistlineeast.com

[5] School of Natural Sciences and Psychology, Liverpool John Moores University, UK

Running Title: Key habitat features for *Tetanocera elata*

Abstract

1. Terrestrial slugs are pervasive pests of agriculture throughout temperate regions and have the potential to disrupt the germination of seedlings, cause damage to fruiting bodies of crops, and vector plant pathogens.

2. *Tetanocera elata* Fabricius (Diptera: Sciomyzidae), a widely-distributed Palaearctic species, is an obligate mesoparasitoid and predator of pestiferous slugs including *Deroceras reticulatum* (Müller) (Stylommatophora: Agriolimacidae). It has the potential to be developed as a native natural enemy in a conservation biological control programme as an alternative to chemical molluscicides.

3. To better understand the ecological requirements of this species, a detailed observational study was conducted at a site in the west of Ireland possessing naturally occurring *T. elata* populations.

4. Comparison of local patches where *T. elata* were recovered revealed no association with plant community composition.

5. Taller dead vegetation was associated with *T. elata* presence throughout the site.Within the area of greatest *T. elata* aggregation, there was a significantly greater percentage cover of dead vegetation where *T. elata* occurred. Abundance of *T. elata* was also significantly correlated to hedgerow proximity. Results of this study are directly applicable for the design of a conservation biological control programme which effectively satisfies the principal habitat requirements of *T. elata* populations.

Key words: agroecology, conservation biological control, ecological engineering, hedgerow, mollusc, vegetation

Introduction

Throughout the world, terrestrial slugs (Mollusca: Gastropoda) are serious pests of tillage agriculture. One species in particular, *Deroceras reticulatum* Müller (Stylommatophora: Agriolimacidae), has spread from its native Palaearctic range (Wiktor, 2000) and established populations on all continents except Antarctica (Smith, 1989; Robinson, 1999). While large populations of *D. reticulatum* are regularly associated with agricultural land (Howlett, 2012) they can also successfully occupy a wide range of other habitats, including other frequently-disturbed habitats, such as construction sites and home gardens, as well as natural and seminatural grasslands, (South, 1992). This ability to disperse into other habitats and form aggregations outside of cropping areas makes *D. reticulatum* a particularly difficult pest to target and control. Damage incurred by *D. reticulatum* and other slug pests has been valued at £8 and £10 million (GBP) in the UK (MacDonald, 2009), primarily from feeding damage to seedlings causing crop failure (Hunter, 1968). In the EU there are currently three chemicals approved for slug control: methiocarb, metaldehyde, and ferric phosphate (European Commission, 2016a; European Commission, 2016b; European Commission, 2016c). However, methiocarb use has recently been restricted by the European Union (European Commission, 2014; European Commission, 2018) and metaldehyde will no longer be available for use in the UK beginning in 2020 (HSE, 2018), due to detrimental effects on non-target species and high potential to contaminate waterways in runoff (South, 1992; Cloyd, 2012; Howlett, 2012). There is also mounting evidence that ferric phosphate, currently approved for organic farming, is not effective at significantly reducing slug feeding damage to crops (Iglesias et al., 2001; Speiser & Kistler, 2002; Rae et al., 2009) and may have negative effects on non-target soil arthropods caused by excess iron build-up, especially when coupled with chelating substances (Langan & Shaw, 2006; Edwards et al., 2009).

72   Biological control of slugs offers an alternative to chemical pesticide that can be used

73 in both conventional and organic agriculture. Currently the only widely available biocontrol

74 agent of slugs is the parasitic nematode *Phasmarhabditis hermaphrodita* (Rhabditida:

75 Rhabditidae) (Glen & Wilson, 1997), marketed as Nemaslug (BASF, Ludwigshafen,

76 Germany). While *P. hermaphrodita* is useful in slug control, it is limited in its use as an

77 inundative biological control agent. As such, *P. hermaphrodita* is prone to the same

78 shortcomings of many such "single use" natural enemies which do not persist in the

79 agroecosystem, including high expense, labour-intensive application multiple times per

80 growing season, variable success rates and control levels, and short shelf life (Glen &

81 Wilson, 1997; Speiser et al., 2001; Rae et al., 2007; Howlett, 2012), rendering them an

82 unsustainable long-term solution (Michaud, 2018).

83   In an effort to both develop alternative slug control programmes and advance

84 sustainable agriculture practices, recent research has focused on the potential use of

85 *Tetanocera elata* (Diptera: Sciomyzidae) in a conservation biological control programme.

86 The larval stages of *T. elata* are specifically associated with terrestrial slugs (Knutson et al.,

87 1965). First and second instar larvae parasitise *D. reticulatum* and *Deroceras laeve* Müller as

88 mesoparasitoids which kill the host by the end of the second instar, at which point larvae

89 become free-living predators which feed on an expanded range of slug species (Knutson et

90 al., 1965; Hynes et al., 2014a; D'Ahmed et al., 2019; Bistline-East et al., 2019). Predaceous

91 larvae have the capacity to kill up to 12 prey slugs before beginning pupariation (Hynes et al.,

92 2014a), making them potentially important natural enemies. Due to their particular biological

93 requirements and life cycle (e.g., current challenges in mass rearing [Hynes et al., 2014b;

94 Hynes et al., 2014c], lengthy developmental time [Knutson et al., 1965; Hynes et al., 2014c]),

95 and the nature of cropping areas where slugs are in need of control (open cropping fields

96 from which aerial natural enemies may disperse), *T. elata* may not be viable for use in

97   inundative biocontrol programmes in the same way as *P. hermaphrodita*. Rather, this

98   Palaearctic species is an excellent candidate for conservation biological control. The aim of

99   such a programme would be to enhance agroecosystems in such a way that populations of

100   natural enemies are self-regulating and self-sustaining, as well as maintaining essential

101   habitat features like those promoted for biodiversity under existing agri-environment

102   schemes, providing constant and recurring pest control (Holland et al., 2016). For *T. elata*,

103   this would be accomplished by managing agricultural landscapes which can meet the

104   essential requirements to complete its life cycle after initial populations are introduced or

105   existing natural populations of *T. elata* are augmented.

106   In the case of conservation biological control with a single target species, the

107   biological control output may be maximised by specifically designing habitat features to meet

108   the biological and ecological requirements of the desired natural enemy (Van Driesche &

109   Bellows, 2001; Ramsden et al., 2015; Holland et al., 2016). While there has been much

110   research on the biology, physiology, and biological control potential of *T. elata* in recent

111   years (Hynes et al., 2014a; Hynes et al., 2014b; Hynes et al., 2014c; Bistline-East et al., 2018;

112   D'Ahmed et al., 2019; Bistline-East et al., 2019), the only habitat data for this species to date

113   are provided by a limited number of individual collection records or species lists for the

114   family Sciomyzidae (Chandler, 1972; Blackith et al., 1991; Williams et al., 2007; Williams et

115   al., 2009a; Speight & Knutson, 2012). Amongst the most common habitats where *T. elata* has

116   been collected are fens (Knutson & Berg, 1971), flood plains and callows (Maher et al.,

117   2014), wet grassland (Carey et al., 2017), and on the margins of turloughts – temporary

118   wetlands unique to the west of Ireland (Williams et al., 2009a, 2009b).

119   The current study aimed to identify specific habitat characteristics important to *T.*

120   *elata* populations by undertaking an extensive examination of local habitats within a field site

121   where *T. elata* have been regularly observed. These data may, in turn, be used to inform

122   optimal land management strategies for supporting *T. elata* in a conservation biological

123   control programme.

124

125   **Materials & Methods**

126

127   **Study Site.** Surveys of habitat characteristics took place within a 10.5 ha subsection

128   of Cow Park, Clarenbridge, Co. Galway, Ireland (ITM 541725.671, 720345.825; GPS

129   53°13'49.1"N, -8°52'22.1"W) where *T. elata* adults had been recovered in previous years.

130   The field site (bordered by a river and deciduous woodland on the eastern side and

131   surrounded by hedgerows/scrub; Fig. 1) was comprised largely of a dry meadow with a patch

132   of wet grassland to the north-east of the site (designated as GS2 and GS4 respectively after

133   Fossitt [2000]).

134   *Local habitat selection.* The survey was comprised of a series of 0.5 m x 0.5 m plots

135   of four categorical types placed semi-randomly throughout the study site according to

136   category criteria. Categories of local habitats measured using these observation plots were

137   comprised of areas that appeared visually similar (e.g., had a similar appearance in plant

138   community and structure) to where *T. elata* adults had been collected at Cow Park in previous

139   years ("expected"), and plots that appeared visually unsuitable for *T. elata* based on prior

140   collections ("unexpected"). Expected plots were identified by vegetation structure and

141   species, consisting typically of thick graminoid tussocks in tall stands, while unexpected plots

142   possessed less densely growing vegetation (or even displayed gaps in growth) and were lower

143   growing. Each of these categories was replicated at near (<5 m) and distant (>10 m)

144   proximities to a hedgerow boundary, to provide four categorical types of observational plots

145   (expected/near [E/N], unexpected/near [U/N], expected/distant [E/D], and unexpected/distant

146 [U/D]). Each category had 8 replicates, resulting in 32 observational plots. In addition to the

147 above plots which were selected in May 2017 (before the *T. elata* adult flight period in

148 Ireland [ABE, pers. obs.]), subsequent plots were included in the study as *T. elata* specimens

149 were recovered throughout the summer (see "Ecological Measurements"), regardless of

150 vegetation appearance or proximity to hedgerow, to comprise a fifth treatment category

151 ("TE"). On occasions where *T. elata* collection coincided with previously designated plots,

152 such plots were both assigned a "TE" plot number as well as maintaining their original

153 designation. In this way, observations were made in both *T. elata*-positive and *T. elata*-

154 negative plots. All plots were marked with a bamboo garden stake which remained in place

155 throughout the study to ensure identical areas were observed each time. The boundaries of

156 observational plots were marked using a 0.5 m x 0.5 m wire frame quadrat oriented using the

157 bamboo stake and closest linear feature (e.g., hedgerow or other boundary).

158   **Invertebrate Sampling.** Sampling for *T. elata* specimens and their associated slug

159 larval hosts (*D. reticulatum*) were conducted to determine what effect, if any, the vegetative

160 habitat parameters being monitored had on the presence and abundance of these species.

161 Surveys for invertebrates only took place within the active summer season (June – August

162 2017).

163   <u>Tetanocera elata</u> *adults.* Adult *T. elata* were collected by passing a heavy-duty sweep

164 net (45 cm diameter, mesh size 0.1 mm) in a figure-of-eight motion through vegetation.

165 Collections were made using short transects (approx. 2 m) in randomised directions to cover

166 the entire study site. Transects did not run through observation plots to avoid trampling

167 vegetation, but sweeps extended into transects to cover vegetation by reaching into plots as

168 transects ran adjacent. Sampling was conducted every two weeks during the flight period and

169 occurred typically between 0900 and 1200 hrs. Recovered individuals were collected in

170 barrel-style pooters (Watkins & Doncaster, The Naturalists, Hawkhurst, Kent, UK) and

171 returned to laboratory facilities at the National University of Ireland Galway for examination.

172 Sciomyzidae were examined using a dissecting stereomicroscope (Olympus SZ40, X6.7 to

173 X40 magnification) and species and sex were confirmed morphologically (Rozkoŝný, 1984;

174 Rozkoŝný, 1987). Field locations where *T. elata* were collected were marked as a "TE" plot

175 using a bamboo stake (as described in "Local habitat selection") and numbered

176 chronologically as individuals were recovered throughout the flight period. Where *T. elata*

177 were recovered within approx. 0.5 m of a designated (*a priori*) observation plot, the existing

178 plot was given an additional "TE" designation to indicate *T. elata* presence.

179   *Terrestrial slugs.* Pitfall traps were deployed at the end of the summer (10 August) to

180 survey for terrestrial slug species associated with *T. elata* habitats. To minimise disturbance

181 to study areas, traps were placed at the exterior margins of each observational plot and baited

182 with the intention of attracting slugs from within and around observational areas. Plastic cups

183 (180 ml) were buried with the lip placed level at topsoil and contained a bait comprised of

184 cotton wool soaked in dark beer (Smith & Boswell, 1970). Traps were covered with a

185 corrugated plastic square (10 cm x 10 cm) held in place by two 150 cm nails to keep out

186 precipitation and prevent potential disturbance. Four pitfall traps were set for each plot, one

187 along each edge. Traps were deployed overnight for approximately 18 hours, and all

188 collections were made in a single trapping event. All slugs collected in traps were recorded,

189 including any slugs recovered on the exterior of the cup or on the corrugated plastic cover.

190 Slugs were recorded as either "*D. reticulatum*" or "other". Abundance of ground beetles

191 (Coleoptera: Carabidae), numerous species of which are known to prey on slugs, were also

192 recorded.

193   **Ecological Measurements.**

194   Measurement of plant community composition consisted of both percentage cover

195   and abundance measurements. Percentage cover of plant species was assessed first by making

196   a visual estimation of the proportion of the plot within the quadrat frame occupied by each

197   species visible when viewed top-down. A species list of plants was then generated for each

198   plot by hand-searching within the delineated area, including both visible and understorey

199   plants, and morphologically identifying present species using vegetative keys (Clark, 2015;

200   Streeter et al., 2009; Price, 2016). The relative abundance of each species identified when

201   hand-searching was also ranked and scored using the DAFOR scale (e.g., dominant,

202   abundant, frequent, occasional, or rare [Sutherland, 2006]). Plant community observations

203   were made bi-weekly during the summer flight period (June – August 2017).

204   Vegetation structure was assessed via measurements of the height of live and dead

205   vegetation as well as the depth of the detritus layer in the understorey. Heights of both live

206   and dead vegetation were taken for each plot by lowering a metre stick through vegetation

207   until the base rested on but did not penetrate the topsoil, and then taking the maximum

208   measurement of live (growing) vegetation and dead vegetation *in situ* height at that point.

209   Points were taken near each corner of the quadrat and at a random location within the frame

210   and measurements were subsequently averaged to yield a mean live and mean dead

211   vegetation height per plot. The detritus layer was measured similarly using a tapered garden

212   stake (to better penetrate detritus to reach topsoil without compressing the layer) marked with

213   corresponding measurements (cm). These measurements were completed in five random

214   locations throughout the plot and pooled to give a mean detritus depth per plot. Unlike plant

215   community analysis, structural features were measured only at the start and end of the adult

216   flight season to track the seasonal growth of plants in survey plots.

217   Finally, vegetation "openness" (i.e., thickness or thinness of plant coverage) at each

218   plot was measured at the beginning (16 June) and end (10 August) of the sampling period

219   using the golf-ball method for rangeland assessment described by Schultz *et al.* (2017) to

220   compare changes in plant structure over the peak growing period. Ten golf balls (fluorescent

221   orange for easier visual detection [Links Choice, VA, USA]) were dropped perpendicularly

222   into each plot from a height of approximately 2 m and each was assigned a score based on

223   visibility of the golf balls. Lower scores (e.g., fewer golf balls visible) correspond to a

224   "closed" vegetation structure suggesting more dense growth, while plots with high scores

225   (e.g., higher visibility of golf balls) indicate a more "open" structure. The exception to this

226   was where vegetation had such a closed structure that the balls could not penetrate; in these

227   cases, golf balls were assigned a score of 0 associated with extremely dense vegetation

228   (Schultz et al., 2017).

229   After the end of the peak summer growth season and *T. elata* flight period (September

230   2017), observations continued for percentage cover and height of live and dead vegetation

231   every 6 to 8 weeks, dependent on weather conditions. The 2017 winter season experienced

232   numerous storms in western Ireland, and observations were occasionally delayed when

233   weather conditions were considered to be hazardous or when vegetation was not accessible

234   (e.g., under snow). Observations continued until May 2018, to complete a 12 month survey

235   period of primary parameters.

236   *Indirect parameter assessment.* In addition to direct measurements, other ecological

237   parameters were calculated and examined for their potential influence on *T. elata* presence

238   and abundance: light penetration (L), soil moisture (F), reaction/pH (R), and nitrogen content

239   (N). These criteria were generated using Ellenberg index values for vegetation (Equation 1),

240   adapted by Hill *et al.* (1999) for use in the UK. A weighted average for each plot was

241   produced using flight period measurements, as adapted by Diekmann and Grerup (1998)

242   where x = the median percentage cover for a species of plant in that plot over the duration of

243   the study, y = the Ellenberg index value for the plant. Ellenberg values were calculated using

244   measurements taken during *T. elata* flight period.

$$WA = \frac{(x_1 \times y_1) + (x_2 \times y_2) + \cdots + (x_n \times y_n)}{(x_1 + x_2 + \cdots + x_n)} \qquad \textbf{Eq 1}$$

245

246   **2.4. Statistical Analyses.** Analysis of categorical environmental factors (i.e., distance

247   category from hedgerow and *T. elata* presence/absence) were completed using a Chi-square

248   test. Comparisons of invertebrate collections in pitfall traps were conducted using Wilcoxon

249   rank-sum or t-tests, as appropriate based on normality of data sets. Similarity of plant

250   community (e.g., percent cover) and structure (e.g., vegetation height, depth of detritus,

251   density) were compared between treatment types using individual measurements over the 12-

252   month period using Kruskal-Wallis tests, with *post-hoc* Dunn tests with Bonferroni

253   adjustment for multiple comparisons where Kruskal-Wallis were significant. Comparison was

254   also made between plots pooled by *T. elata* presence or absence in a subset of plots along the

255   east/southeast field margin near the hedgerow boundary ("E/SE subset") (Fig. 1). This subset

256   was isolated because plots along the eastern site margin accounted for nearly all *T. elata*

257   presence, and restricting the analysis to these plots limited potential complicating variables,

258   namely aspect. Additionally, data were restricted to "near" plots due to a significant

259   correlation ($\chi^2_1 = 4.7055$, $P = 0.03$) between distance category and *T. elata* presence (as

260   concluded by Chi-square analysis). These comparisons were performed using Wilcoxon rank-

261   sum tests with continuity correction and approximate P-values to account for rank ties.

262   Principle components analysis (PCA) plotting environmental variables against *T.*

263   *elata* presence/absence was carried out using PC-ORD v.6 (McCune & Mefford, 2011). The

264   primary matrix was comprised of direct and indirect quantitative environmental variables,

265   including: percentage cover of the five most abundant plant species (*Dactylis glomerata* L.

266 [Poales: Poaceae], *Festuca pratensis* Huds. [Poales: Poaceae], *Poa pratensis* L. [Poales:

267 Poaceae], *Centaurea nigra* L. [Asterales: Asteraceae], and *Plantago lanceolata* L. [Lamiales:

268 Plantaginaceae]), percentage cover of dead vegetation, mean height of live and dead

269 vegetation, vegetation thickness, depth of detritus layer, and Ellenberg calculated values

270 (light, moisture, reaction [pH], nitrogen). Measurements were entered as the mean value per

271 plot over the 12 month trial period. The categorical variable of *T. elata* absence (0) or

272 presence (1) comprised the second matrix and was used as a grouping factor. A preliminary

273 PCA was conducted at a site level including all plots, and secondary PCAs were run

274 subsequently using data from the E/SE subset for environmental variables recorded during

275 the flight period and for the 12 month trial. All PCA analyses used a variance/covariance

276 centred matrix with scores calculated based on distance biplot.

277   Following PCA analysis, environmental variables were examined for their influence

278 on *T. elata* abundance in the E/SE subset with a multiple regression analysis, using values of

279 Primary Components 1 and 2 as the independent variables and *T. elata* abundance as the

280 dependent variable. Analyses (with the exception of PCA) were conducted using R (R

281 version 3.2.5, R Core Team 2013, The R Foundation for Statistical Computing, Vienna,

282 Austria) in R Studio.

283

284   **Results**

285

286   **Invertebrate Sampling**

287   During the summer flight period, a total of 32 individual *T. elata* were collected from

288 13 discrete plots within the field site (Table 1). Of the 13 plots where *T. elata* were recovered,

289 seven plots were unique (54%) and six (46%) coincided with both expected and unexpected

290   pre-designated survey plots (Table 1). Only two TE plots occurred distant from the

291   hedgerow, with 85% of TE plots occurring at "near" proximity. Four individuals (12.5%)

292   were recovered at plots designated as having a superficially dissimilar physical appearance

293   ("unexpected") to where *T. elata* had been previously recovered, however 96% were

294   collected near the hedgerow (Table 1), indicating the importance of this feature. Overall,

295   there were significantly greater numbers of *T. elata* captured in "near" proximity to the

296   hedgerow ($\chi^2_1 = 4.7055$, $P = 0.03$), and the majority of captures (87.5%) occurred near the

297   hedgerow comprising the east/southeast margin of the site (Fig. 1). Based on these collection

298   numbers, environmental factors were examined both at site level and for this E/SE subset

299   specifically.

300   Pitfall trapping returned 31 slugs, including one *D. reticulatum*, in addition to 93

301   carabid specimens (Fig. S1). The only *D. reticulatum* recovered was found near the

302   hedgerow. Within the E/SE subset, presence of *T. elata* was not associated with variability in

303   slug density (t = -0.48617, $P = 0.9572$), however there were significantly fewer carabids

304   recovered in plots where *T. elata* were present than in other plots (t = -3.4378, $P = 0.002$),

305   potentially indicating a negative association.

306   **Characterisation of Vegetation**

307   *Plant community.* At site level, a total of 35 plant species/groups were identified

308   across the plots during the 12 month period (Table 2). Grasses occurred at the highest

309   frequency across all plots, the most frequent being *P. pratensis* (100%), *D. glomerata* (97%),

310   and *F. pratensis* (72%). The most frequently occurring forb species throughout the site were

311   *Centaurea nigra* (54%) and *P. lanceolata* (36%) (Table S1). Significant differences were

312   observed between categories for each of these most frequently occurring species (Table S2),

313   however *post-hoc* pairwise comparisons between plot types showed no consistent trends

314 either across species or with respect to *T. elata* presence (Table S3). *Dactylis glomerata* cover

315 was the most variable, while *F. pratensis* showed the least variability across plots (Table S3)

316 The plant community composition described using the DAFOR scale was highly variable

317 between plots (Table S1), and the species present where *T. elata* was recovered varied

318 considerably between plots. Graminoids and forbs comprised similar proportions in all plot

319 categories at site level, while scrub (*Rubus fruticosus*) and other vegetation types (e.g.,

320 *Pteridium aquilinum*, lichens and mosses) were present only in those categories of plots

321 occurring near a hedgerow (Fig. 2A).

322 Vegetation in the E/SE subset demonstrated similar patterns to site-wide frequency

323 and percentage cover. The E/SE subset of observational plots demonstrated similar relative

324 frequencies of these dominant species (100%, 100%, 81%, 38%, and 38% respectively), and

325 dead vegetation was again observed in all plots, with a mean percentage cover of 40.9 ± 3.0

326 SE. These dominant species provided similar coverage across plots pooled according to *T.*

327 *elata* presence/absence (Table 2). As observed at site scale, graminoid and forb vegetation

328 types were represented at similar levels in E/SE subset plots when pooled according to *T.*

329 *elata* presence/absence (Fig. 2B). When pooled in this manner, plots where *T. elata* were

330 present had significantly higher percentage cover of dead vegetation and *P. lanceolata* (W =

331 2268, *P* = 0.0172 and W = 2743.5, *P* < 0.001, respectively); no significant differences were

332 observed in the remaining dominant species (Table 2).

333 *Local structure.* The majority of observational plots (62%), representing plots from

334 every treatment category, received the maximum possible vegetation closeness score (0). The

335 remaining plots were distributed in incrementally increasing categorical scores,

336 corresponding to increasing vegetation openness (Table 3). In plots where *T. elata* specimens

337 were recorded, the median vegetation closeness score was 0 and ranged from 0 to 3.5. A

338 significant difference was observed in median vegetation openness between plot types (χ^2_4 =

339   10.692, $P = 0.0303$) with unexpected/distant plots having more open structure than any other

340   plot type (Table 3), but not when pooled by *T. elata* presence/absence in the E/SE subset (W

341    $= 68$, $P = 0.7855$).

342   At site level, maximum live vegetation height ranged from 1 cm to 150 cm, with a

343   site-wide mean height of 46.8 cm $\pm$ 1.8 SE. In the plots where *T. elata* were recorded, live

344   vegetation height ranged from 3 cm (overwinter) to 139 cm (peak summer). Maximum height

345   was highly variable across plots and treatment types, with a mean live vegetation heights of

346   53.56 cm $\pm$ 11.4 SE (expected/near), 40.21 cm $\pm$ 9.5 SE (unexpected/near), 55.00 cm $\pm$ 12.0

347   SE (expected/distant), 44.84 cm $\pm$ 9.4 SE (unexpected/distant), and 42.18 cm $\pm$ 8.0 SE (TE)

348   (Table 3). Live vegetation height differed between plot categories ($\chi^2_4 = 12.62$, $P = 0.0133$),

349   but the high variance of heights within and between plot categories rendered pairwise

350   comparisons nonsignificant when adjusted (Bonferroni) for multiple comparisons.

351   Maximum dead vegetation height at site-level ranged from 1 cm to 184 cm, with a site

352   mean height of 33.24 cm $\pm$ 1.8 SE (Table 3). In plots where *T. elata* specimens were

353   recovered the average dead vegetation height was 34.01 cm $\pm$ 8.9, with a range from 2 cm to

354   184 cm, representing the second shortest and tallest dead vegetation measurements at the site.

355   The mean height of dead vegetation was greater in areas where *T. elata* were collected during

356   the flight period than in most other plots (Table 3). Dead vegetation height varied

357   significantly between plot categories ($\chi^2_4 = 14.607$, $P = 0.0056$), with significant pairwise

358   differences observed between expected and unexpected/near plots (Z = 3.466, $P_{adj} = 0.0053$)

359   as well as expected/near and unexpected/distant (Z = 2.996, $P_{adj} = 0.0274$). When plots were

360   examined based on *T. elata* presence in the E/SE subset, mean dead vegetation height was

361   greater and live vegetation was shorter on average in plots where *T. elata* was recovered (Fig.

362   3), though neither difference was statistically significant (W = 695, $P = 0.2621$ and W = 778,

363    $P = 0.3573$, respectively).

364   Detritus depth had a site-wide mean of 5.7 cm ± 0.4 SE. In the plots where *T. elata*

365   were recorded, the average depth was 6.00 cm ± 1.0. Measurements of detritus depth varied

366   less than measurements of vegetation height, however there was still enough variability

367   within plots of the same category to render comparisons between categories non-significant

368   ($\chi^2_4 = 6.6312$, $P = 0.1567$) (Table 3). In the E/SE subset, there was no difference in detritus

369   depth between plots pooled by *T. elata* presence/absence (W = 83.5, $P = 0.7855$) (Fig. 3).

370   *Indirect parameters.* Ellenberg values showed small variations between plots due to

371   differences in light, moisture, pH, and nitrogen, however when means were calculated for

372   each treatment there was little variation evident (Table 3). No significant differences existed

373   for indirect parameters between plot types.

374   **Effects of environmental factors on *T. elata* populations**

375   At the site level, significantly greater abundance ($\chi^2_1 = 4.7055$, $P = 0.03$) of *T. elata*

376   was recorded close to the hedgerow in comparison to distant plots. Results of site-level PCA

377   produced two axes which accounted for over 97% of observed variation (83.5% and 13.8%

378   on PC1 and PC2, respectively) in environmental factors, with near complete overlap between

379   plots when grouped by *T. elata* presence/absence (Fig. 4A). When considering data from only

380   the E/SE subset, there was a much clearer separation of groups based on *T. elata*

381   presence/absence. During the flight period, PCA ordination accounted for over 99% of

382   variation, with 82.9% attributed to PC1 and 16.3% explained by PC2 (Fig. 4B). Similarly,

383   ordination of mean data over 12 months explained over 98% of variation; 86.2% represented

384   on PC1 and 11.9% on PC2 (Fig. 4C). Environmental factors with R^2 values > 0.2 were

385   overlaid as biplots on all PCA graphs, however only those related to structure (live and dead

386   vegetation height, detritus depth, and density) resolved, and oriented on PC2 in all cases,

387   indicating the presence of *T. elata* may be positively correlated with the height of dead

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388   vegetation and negatively correlated to live vegetation height. A multiple linear regression

389   using the results of the E/SE subset PCA with 12-month data indicated significant differences

390   in *T. elata* abundance with PC2 (t = 2.512, *P* = 0.02733), however the non-significant global

391   values for the model ($F_{2,12}$ = 3.22, *P* = 0.07594) suggest a lack of power to this relationship.

392

393   **Discussion**

394

395   The primary finding of the current study is that hedgerow proximity is significantly

396   associated with *T. elata* populations. Similar effects may be observed for smaller patches of

397   scrub and treelines within agroecosystems, as they likely provide valuable refugia, however

398   isolated trees have not been observed to convey any benefit for *T. elata* (ABE, pers. obs.).

399   The authors have observed anecdotally that hedgerows may be important features in habitats

400   when collecting *T. elata*, and data presented here comprise the first instance which

401   quantitatively supports this assertion. These results support growing evidence that presence

402   and proximity of hedgerows have significant positive effects on the abundance of natural

403   enemies, with a higher abundance of beneficial species closer to the hedgerow (Morandin et

404   al., 2014; Holland et al., 2016; Garratt et al., 2017). A majority of *T. elata* specimens

405   recovered in the study were collected near the south-eastern hedgerow border, in a grouping

406   comprising the E/SE subset (Fig. 1). There are several possible explanations for this

407   including hedgerow quality, a lack of connectivity between hedgerow sections (e.g., gaps in

408   hedgerows for human or livestock movement or thin growth of hedgerow species), or

409   hedgerow orientation relative to wind patterns and sun exposure. Although specific hedgerow

410   characteristics were not quantified, the hedgerow bordering areas where most *T. elata* were

411   recovered was characterised by a mixture of tall tree species (*Fraxinus* spp., *Acer*

17

412 *pseudoplatauis*, and *Fagus* spp.) commonly found in woodland habitats and smaller hedgerow

413 species (*Crataegus monogyna*), compared to the other hedgerows in this study which were

414 dominated by a single smaller species (*C. monogyna*). Hedgerow quality has been shown to

415 influence invertebrate communities, with richness and abundance of beneficial invertebrate

416 species (e.g., natural enemies and pollinators) increasing with increasing hedgerow quality. I

417 Increased diversity of woody species and trees, hedges laid to prevent gapping, and the

418 maintenance of a healthy understory (through reduced mowing and herbicide/pesticide

419 application) are all known to contribute to increased arthropod biodiversity (Garratt et al.,

420 2017). Localisation of *T. elata* around the southeast hedgerow could also be attributed to a

421 lack of connectivity between hedgerows. Several gaps were observed in the hedgerow at the

422 study site, primarily caused by a footpath leading from the grassland into adjacent woodland

423 on the distal SE margin, and a large gap in the NE corner of the site used for livestock

424 passage to adjacent fields. Patchy or uneven growth was also noted along the northern

425 margin. Due to the sedentary behaviour of adult Sciomyzidae (Williams et al., 2009a;

426 Williams et al., 2009b), the dispersal of *T. elata* to other hedgerows across the site may have

427 been hampered by the lack of a continuous hedgerow, as has been observed for other

428 invertebrates with limited dispersal capacity (Holland et al., 2016).

429   The study also revealed the positive association between *T. elata* presence and dead

430 vegetation height, and an inverse relationship with live vegetation height. This contrasts

431 somewhat with previous research, which while also finding vegetation structure to be an

432 important factor influencing marsh fly communities (Williams et al., 2009a; Williams et al.,

433 2009b), the structure discussed in literature refers to the height of living vegetation. In the

434 current study, while plots in the E/SE subset did not significantly differ from one another in

435 composition/structure based on *T. elata* presence, there was a significantly greater coverage

436 of dead vegetation in plots where *T. elata* were recovered, potentially indicating a build-up of

437 dead vegetation over an extended period of time. This observation coincides with records of

438 *T. elata* being collected from ungrazed or abandoned agricultural land and grasslands

439 (Knutson et al., 1965; Speight, 2004a; Speight & Knutson, 2012). When considering these

440 findings in a three-dimensional scale, the combination of high cover and height suggests a

441 greater overall volume of dead vegetation, again potentially indicative of grazing which is

442 low-intensity or absent. This may be important for a number of reasons, such as providing a

443 more sheltering canopy allowing for development of a mesocosm within, which is more

444 buffered against changes in temperature, humidity, or light penetration (Gontijo, 2019). The

445 larger amounts of decaying vegetation may also influence soil and invertebrate communities

446 and provide desirable shelter and alternative food sources for slugs (Godan, 1983), including

447 *D. reticulatum*. It would, therefore, benefit *T. elata* larvae greatly to also inhabit a similar

448 spatial niche. Adult *T. elata* are known for having high patch fidelity and low dispersal rates

449 (Speight & Knutson, 2012), which suggest if these areas do provide ideal juvenile habitats,

450 the adult life stage will likely remain closely associated. There was no impact detected from

451 detritus levels or vegetation openness as influencing *T. elata* presence, however it should be

452 noted that the majority of plots throughout the study site were dominated by *D. glomerata*, a

453 species that forms dense tussocks over several growing seasons. Tscharntke and Greiler

454 (1995) have suggested that tussock-forming grasses are important for providing shelter for

455 ground-dwelling arthropods, especially as they build up and decompose in regular cycles.

456 Terrestrial slugs, such as *D. reticulatum*, are also known to associate with tussock-forming

457 grasses for the shelter they provide (South, 1965). This tussock structure, combined with high

458 volumes of dead vegetation, could provide valuable refuges for developing *T. elata* larvae.

459 Carey *et al.* (2017) also identified an association of *T. elata* with wet grasslands dominated

460 by *Juncus* spp. While these plants can also form dense tussocks, they do not possess the same

461 amounts of dead vegetation build-up. This association, instead, may be considered similar to

462   that with hedgerows and the shelter and shade supplied by these providing a different type of

463   refugia.

464   While some significant differences were identified between plot categories in the

465   coverage of the most abundant plant species, plant community composition did not show any

466   consistent trends with relation to where *T. elata* were present throughout the study site.

467   Collected individuals were distributed across nearly all *a priori* treatment types (all except

468   unexpected/distant) possessing variable habitat parameters. The clustering of the E/SE subset

469   may be explained in part by the site being bordered to the east by a river (Fig. 1), which

470   likely created a physical boundary for distribution. Conversely, the north border of the site

471   was adjacent to additional grass and pasture fields separated by gappy hedges, which may

472   have allowed for more distribution if *T. elata* populations were present in that area.

473   Plant community composition in wet and dry grasslands can be confidently excluded

474   as an important indicator of the presence and abundance of *T. elata* populations. This result

475   was not unexpected as *T. elata* have been recorded in a variety of habitats (Knutson et al.,

476   1965; Speight, 2001; Williams et al., 2007), which in itself suggests that *T. elata* are able to

477   survive in a range of habitats and do not depend on particular host or shelter plants. This lack

478   of association between *T. elata* and plant species may also be attributed to the distribution

479   and feeding habits of its larval host/prey species (*D. reticulatum*) which is a generalist

480   herbivore (Cook et al., 2000). It is also one of the most widely distributed terrestrial slug

481   species, occupying a range of habitats from frequently disturbed tillage fields to intensively

482   managed agricultural land to abandoned fields (Hunter, 1966; South, 1992). This association

483   is widened still further as *T. elata* larvae mature to predaceous third instars, at which point

484   they are capable of predating several other slug species (Knutson et al., 1965; Knutson &

485   Vala, 2011; Giordani et al., 2014; Bistline-East et al., 2019), none of which have a specialist

486   diet (Hunter, 1968; South, 1992). In addition to the larvae feeding on generalist molluscs,

487   adult *T. elata*, which rely on a largely carbohydrate-based diet, are not known to associate

488   with specific nectar-producing plants and have not been observed feeding on floral or

489   extrafloral resources. Recent research has demonstrated that adult Sciomyzidae including *T.*

490   *elata* may associate opportunistically with specialist and generalist insect herbivores

491   (Hemiptera) to fulfil part or all of their dietary requirements by consuming honeydew

492   (Bistline-East et al., 2018). Such herbivores may be expected to a distribution within a single

493   site ranging from uniform to patchy, depending on the level of homogeneity of the site.

494   However, even when a site may have a patchier distribution of Hemiptera, such as the one in

495   which the current study was conducted, the range of dietary plasticity observed for T. elata

496   means it is unlikely that adult flies were restricted in their distribution based solely on this

497   trophic association.

498   When the Ellenberg index values for secondary habitat parameters were compared

499   based on *T. elata* presence/absence, adult *T. elata* adults did not appear to be influenced by

500   light penetration, soil moisture, soil pH, or nitrogen content throughout the site. However, as

501   these values were calculated indirectly based on plant community, there may be some

502   difference in actual environmental conditions within the understorey in locations where *T.*

503   *elata* occurred compared to the wider site. Future research should aim to measure such

504   factors directly (e.g., through the use of environmental data loggers).

505   The findings of this study (e.g., little to no association with particular plant

506   communities) may in fact be promising for the potential for *T. elata* to be established as a

507   self-sustaining natural enemy in a conservation biological control scheme because it means

508   that the vegetation composition of arable field margins will not likely have to be heavily

509   manipulated to provide a suitable habitat. The correlation of *T. elata* presence to hedgerow

510   proximity is both promising and challenging for its potential future use in biological control.

511   The findings of this study further validate the importance of maintaining hedgerows in field

margins, in this case for the benefit of *T. elata* populations, but hedgerows are also widely

acknowledged for their importance for other natural enemies, pollinators, and biodiversity

within agroecosystems (Thomas & Marshall, 1999; Holland et al., 2016; Garratt et al., 2017;

Van Vooran et al., 2017). In addition, hedgerows have been also shown to provide beneficial

habitats for birds, mammals, and woodland plants (Garratt et al., 2017; Heath et al., 2017;

Brien et al., 2016; Wehling & Diekmann, 2009). As a result, providing a habitat for *T. elata*

can be multifunctional by also increasing biodiversity at the agroecosystem level. Within

agroecosystems lacking hedgerows, it can now be recommended with confidence that

establishment of a hedgerow is likely essential to support populations of this natural enemy.

Although the planting of hedgerows is profitable over time, initial investment is high and

could prove prohibitive for growers. It also takes time to establish high-quality hedgerows,

which may result in introduced *T. elata* populations not initially being self-sustaining. This

should also be considered as a deterrent for hedgerow removal. Aside from the general loss of

biodiversity, the decision to reinstall hedgerows for the purposes of biological control can

expected to be very costly and labour-intensive. Alternatively, *T. elata* population

establishment may be more likely to succeed when introduced into sites which already

benefit from well maintained, mature hedgerows. The current study recovered only two

individuals in the central field of the study site, which indicates *T. elata* adults may not be

inclined to disperse away from the hedgerow with which they are associated. In a biological

control context, this could result in high levels of pest suppression around the edges of a crop

field (e.g., the margin(s) nearest a hedgerow) but insufficient control of pests within the field

itself. This could potentially be counteracted by establishing low hedgerows within tillage

fields, similar to what is done with intercropped beetle banks, however this adds a degree of

logistical difficulty with regard to the continued tillage and harvest of the field.

When considering the implication of vegetation structure for the use of *T. elata* as a self-sustaining biological control agent of slugs, it is important to note that the vegetation structure in the study site, which had not been mown or grazed for at least three years (E. O'Riordan, *pers. comm.*), differs from what would be found in and around tillage fields in agroecosystems. Specifically, the importance of land being undisturbed for extended periods should not be underestimated. Plots where *T. elata* adults were recovered were all characterised by tall, thick dead vegetation, which can only occur if grasses have been allowed to grow and die back over several cycles. If *T. elata* populations are to be established, the landscape requires areas of abandonment (e.g., unmanaged field margins or fallow portions of tillage fields), which may prove difficult or undesirable for some growers. Under the current Green Low-carbon Agri-environmental Scheme (GLAS) in Ireland, farmers are required to graze, mulch, or mow such areas at least once per year to be eligible to receive payment (DAFM, 2015); additionally, the Basic Payment Scheme (BPS) requires farmers to mow or graze grass margins annually (DAFM, 2017). Ultimately, habitat features of importance for *T. elata* populations and other arthropod biodiversity (e.g., ground beetles) should be easily applied to existing agroecological landscapes, especially those which already have some areas of semi-natural habitat, but some accommodations will likely need to be made with regard to the criteria of agri-environmental support schemes.

The identification of these features is the first step to developing a viable conservation biological control scheme (Holland et al., 2016) and lays the groundwork for future conservation biological control development. However, while this is a valuable first step, the authors acknowledge the small scale of the study. It should, therefore, be viewed as a preliminary case study and proof of concept and be used to conduct further wider scale studies in future. While a mild negative correlation was observed in the current study, between carabids and *T. elata*, this was the result of only a single night of sampling. For any

561 true association to be made, a more robust survey of carabid populations is required,

562 preferably across the entire flight period. Further research is also needed to examine what

563 impact slug community composition, density, and distribution have on *T. elata*. It was

564 beyond the scope of this study to directly survey the honeydew producers in the site. It has

565 previously been shown that the chemical composition of honeydew can vary between species

566 of producers (Douglas, 2006). Consequently, honeydew with a different chemical

567 composition could impact the fitness of adult *T. elata*. The presence of a hedgerow rather

568 than a grassy verge at field margins has previously been shown to increase densities of the

569 honeydew producers such as aphids (Hemiptera: Aphidoidea) (Van Vooran et al., 2017) and

570 should be further explored. Hedgerow characteristics were not examined in this study

571 although this could now (based on the results of this project) be an important factor for the

572 success of *T. elata* as a biological control agent.

573

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575

580

581 **Author contributions**

582

583   ABE conceived the overall scope for the studies presented here. ABE and DB

584 designed the vegetation study and conducted field observations. Analysis of vegetation study

585 was examined by DB (summer months) with further analysis by ABE (overall trends), with

586 advice provided by CDW. The manuscript was prepared by ABE with contributions from

587 DB. All research was overseen by MJG.

588

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Table 1. Plot classification and *T. elata* collection locations in Cow Park during the trial period (June – August 2017). Specimens that were recovered in the absence of designated plots were used to establish "unique" plots; others which were recovered within approx. 0.5 m of an *a priori* selected plot were associated with that plot as well as being given a TE designation. For these co-occurring plots, the associated plot is listed.

TE plot	No. *T. elata* collected	Hedgerow proximity	Unique plot?	*a priori* plot type
TE 1	3	Near	Y	-
TE 2	2	Near	Y	-
TE 3	9	Near	Y	-
TE 4	3	Near	N	U/N
TE 5	2	Near	N	E/N
TE 6	1	Distant	N	E/D
TE7	3	Near	N	E/N
TE 8	3	Near	Y	-
TE 9	1	Near	Y	-
TE 10	1	Distant	Y	-
TE 11	1	Near	N	U/N
TE 12	2	Near	Y	-
TE 13	1	Near	N	E/N
Site total	32			

Plot category abbreviations: E/N = expected/near; U/N = unexpected/near; E/D = expected/distant; U/D = unexpected/distant; TE = plot where *T. elata* was recovered

Table 2. Mean percentage cover of the five most abundant plant species and dead vegetation in E/SE subset, pooled based on *T. elata* presence or absence. Statistical comparisons are given per plant species between *T. elata* present and absent plots based on two-sample Wilcoxon tests, and indicated as significant (* $P < 0.05$) and highly significant (** $P < 0.001$).

	Mean Cover (%) ± SE			P-value	Test statistic (W)
	Subset total	***T. elata* present**	***T. elata* absent**		
Dead vegetation	40.9 ± 2.6	44.6 ± 4.0	34.0 ± 2.9	0.0173 *	2268
Dactylis glomerata	29.2 ± 2.0	28.6 ± 3.3	28.2 ± 3.3	0.6260	1250.5
Poa pratensis	12.1 ± 1.7	12.7 ± 2.5	12.0 ± 3.4	0.9960	1941.5
Festuca pratensis	11.6 ± 1.7	9.5 ± 1.5	13.7 ± 5.0	0.5013	2140
Centaurea nigra	1.7 ± 0.8	2.2 ± 1.4	1.0 ± 0.8	0.6940	2085.5
Plantago lanceolata	1.3 ± 0.5	1.6 ± 0.7	0 ± 0	< 0.0001 **	2743.5

Table 3. Summary of vegetation structure by plot category, including mean live and dead vegetation height (12 month and flight period means; min/max), mean depth of detritus layer, median vegetation thickness score (Schultz et al. 2017), and mean calculated Ellenberg values (Hill et al. 1999). Statistically significant differences between plot categories per variable are indicated by the same superscript letter. Pairwise differences are the result of *post-hoc* Dunn tests with Bonferroni adjustment for multiple comparisons following significant Kruskal-Wallis tests for each variable.

Plot category	Mean height (cm) ± SE (min – max)				Mean detritus depth (cm) ± SE	Median structure score	Mean Ellenberg values			
	Live vegetation		Dead vegetation							
	12 mo.	Flight Period	12 mo.	Flight Period			Light (L)	Soil moisture (F)	pH (R)	Nitrogen (N)
E/N	53.56 ± 11.4 (8 – 150)	90.97 ± 9.1 (8 – 150)	43.15^{A,B} ± 12.2 (3 – 184)	59.75 ± 14.4 (8 – 184)	5.65 ± 1.0	0	7.03 ± 0.2	5.10 ± 0.1	6.52 ± 0.2	5.38 ± 0.2
U/N	40.21 ± 9.5 (1 – 125)	69.70 ± 8.0 (2 – 125)	26.75^{A} ± 9.9 (1 – 121)	34.27 ± 12.0 (1 – 121)	5.39 ± 1.6	1.3	7.03 ± 0.1	5.22 ± 0.1	6.25 ± 0.1	5.23 ± 0.2
E/D	55.00 ± 12.0 (3 – 140)	91.86 ± 9.6 (5 – 140)	36.62 ± 11.5 (1 – 136)	52.09 ± 13.5 (1 – 136)	6.79 ± 1.6	0	7.16 ± 0.1	5.21 ± 0.1	6.71 ± 0.1	5.62 ± 0.1
U/D	44.84 ± 9.4 (7 – 110)	71.66 ± 6.4 (9 – 110)	27.09^{B} ± 8.2 (1.5 – 128)	34.94 ± 9.8 (1.5 – 128)	5.11 ± 1.6	0	7.06 ± 0	5.60 ± 0.2	5.96 ± 0.2	5.02 ± 0.1

TE	42.18 ± 8.0 (3 – 139)	78.35 ± 7.3 (8 – 139)	34.01 ± 8.9 (2 – 184)	56.34 ± 10.9 (4.5 – 184)	6.00 ± 1.0	0	7.17 ± 0.1	5.25 ± 0.1	6.61 ± 0.1	5.58 ± 0.2
Site	46.80 ± 1.8	80.48 ± 2.25	33.24 ± 1.8	47.01 ± 3.76	5.97 ± 0.4	0	7.07 ± 0.1	5.27 ± 0.1	6.40 ± 0.1	5.39 ± 0.1

Plot category abbreviations: E/N = expected/near; U/N = unexpected/near; E/D = expected/distant; U/D = unexpected/distant; TE = plot where

T. elata was recovered

[A] $Z = 3.466$, $P = 0.0053$

[B] $Z = 1.437$, $P = 0.0274$

Figure 1. Habitat map of vegetation study site, Cow Park, Clarenbridge, Co. Galway.

Figure 2. Mean proportion of graminoids, forbs, and other growth (*Pteridium aquilinum*, *Rubus fruticosus*) per plot category over 12 months (**A**) at site level (E/N = expected/close; U/N = not expected/close; E/D = expected/distant; U/D = not expected/distant; TE + = *T. elata* present in plot) and (**B**) in the E/SE subset (TE + = *T. elata* present in plot; TE - = *T. elata* absent from plot).

Figure 3. Mean and range of vegetation heights (live and dead) and detritus depth over 12 months in plots from the E/SE subset. Plots are pooled by presence or absence of *Tetanocera elata* (TE).

Figure 4. Graphical representations of PCA ordinations, overlaid with environmental variables which resolved with an R^2 value greater than 0.2. (**A**) Site-level over 12 months; (**B**) E/SE subset in the flight period and (**C**) over 12 months.
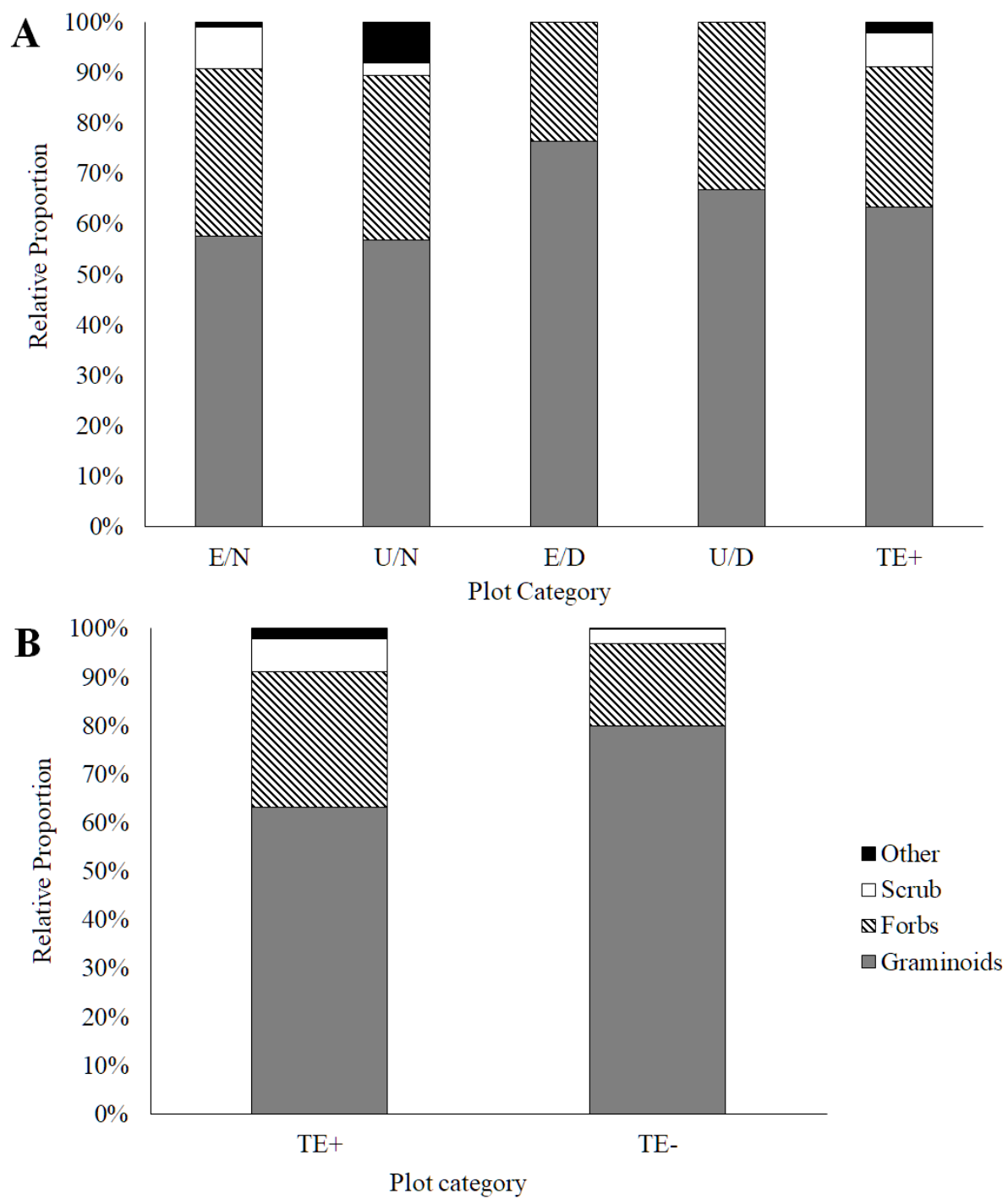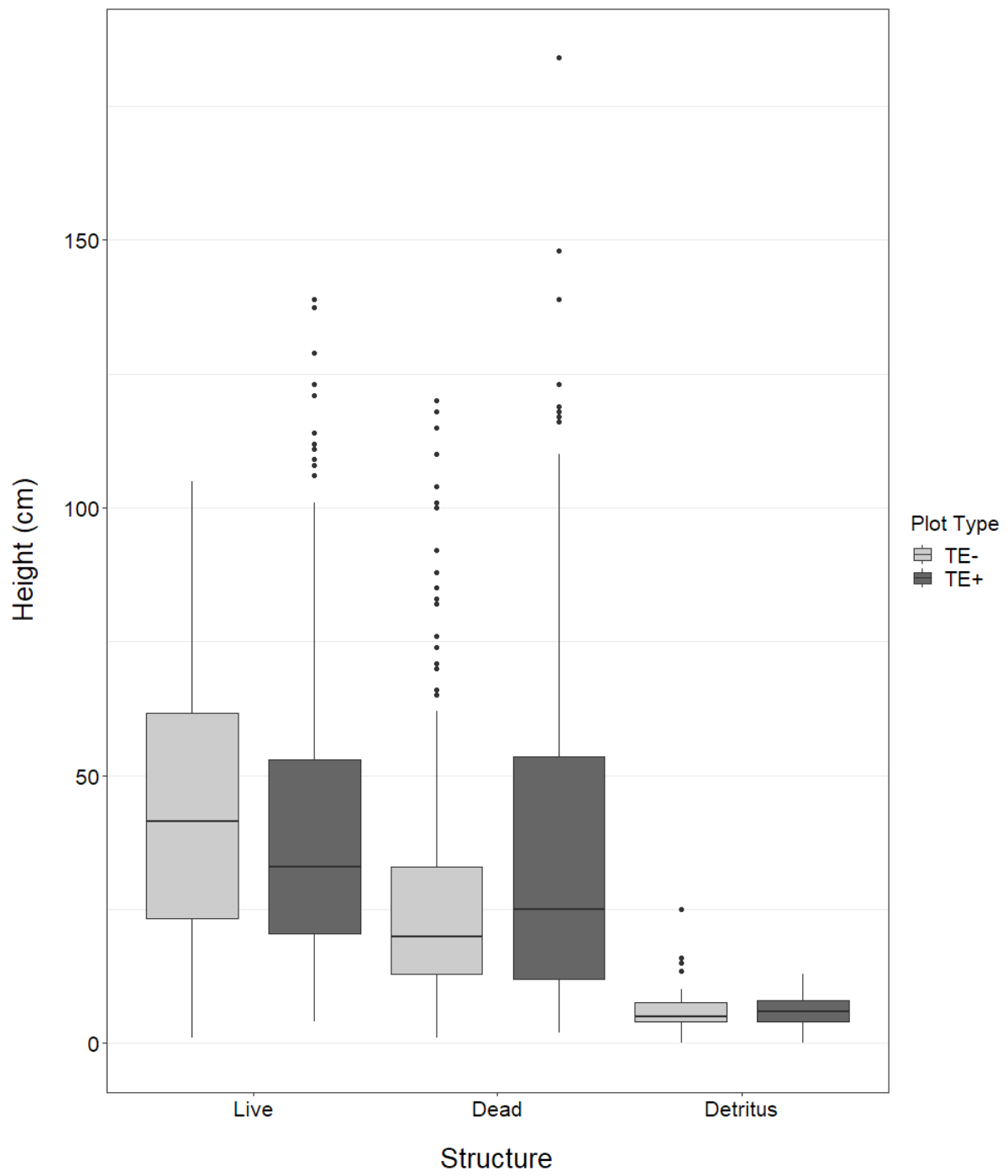
Figure 1

Figure 2

Figure 3

TE_PCA1_ESEplots12moPCA

Figure 4