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Article

Title: Vegetation cover and grasslands in the vicinity accelerate development of carabid beetle assemblages on restored landfill sites

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DOI: 10.1080/21658005.2015.1068521

Example citation: Rahman, M. L., Tarrant, S., McCollin, D. and Ollerton, J. (2015) Vegetation cover and grasslands in the vicinity accelerate development of carabid beetle assemblages on restored landfill sites. *Zoology and Ecology.* **25**(4), pp. 347-354. 2165-8005.

It is advisable to refer to the publisher's version if you intend to cite from this work.

Version: Accepted version

Note: This is an Accepted Manuscript of an article published by Taylor & Francis in Zoology and Ecology on 11 August 2015, available online: http://www.tandfonline.com/doi/full/10.1080/21658005.2015.1068521.

http://nectar.northampton.ac.uk/8116/

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5   Vegetation cover and grasslands in the vicinity accelerate development of carabid beetle

6   assemblages on restored landfill sites

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27

28   Abstract

29   There is increasing evidence that rare and scarce carabid species of conservation

30   importance are frequent in brownfield sites such as restored landfill. However, this

31   potential has largely been unexplored and was investigated here by examining carabid

32   species composition, richness, and abundance in relation to habitat quality and

33   landscape structure on landfill sites in comparison to paired reference sites of existing

34   wildlife value. Sampling was conducted by collecting carabids in ten pitfall traps set

35   along two 100 m transects on each of nine restored landfill and their paired reference

36   sites in the East Midlands region of the UK. A total of 1014 individuals representing

37   thirty seven carabid species were found during April to September in 2007 and 2008.

38   On the landfill sites, generalist species were common, while no nationally rare or scarce

39   species were found. Neither species richness nor diversity of carabid species was found

40   to be different from that of the reference sites. Seeding during restoration was found to

41   have a strong positive effect on richness and diversity, with seeded landfill sites tending

42   to be similar to reference sites in terms of carabid species composition. Marked

43   differences in diversity and richness were also attributed to variation in the amount of

44   local vegetation cover, with presence of grassland in the surrounding landscape having a

45   positive effect on carabid assemblages. We suggest that initial seeding may be an

46   appropriate conservation strategy to improve beetle diversity and richness, coupled with

47   management in terms of cutting to increase the potential of these sites for carabid

48   conservation at the landscape scale.

49   Keywords: ground beetles, restoration, habitat quality, landscape structure, grassland,

50   landfill.

Introduction

Carabid beetles (Coleoptera: Carabidae) are valuable indicators for habitat restoration research as they are widely distributed and are well known both taxonomically and ecologically, allowing comparisons to be made between a wide range of terrestrial habitats; they are also sensitive to landscape change and disturbance (Butterfield et al. 1995; Niemelä et al. 2002; Rainio and Niemelä 2003; Vanbergen et al. 2005; Karen et al. 2008). Man-made habitats support a rich carabid fauna, including 35% of the rare and scarce carabid species in the UK (Eversham, Roy, and Telefer 1996; Gibbons 1998; Small, Sadler, and Telfer 2002). There is increasing evidence that carabid species of conservation importance are frequent in brownfield sites including former mineral works and landfill sites (Judd and Mason 1995; Eyre, Luff, and Woodward 2003; Morris et al. 2006). These habitats can serve as a novel habitat and have potential multi-functional ecological and cultural roles as both natural conservation sites and amenity land.

Habitat loss from urbanisation and modern intensive agricultural land use practices has contributed to the impoverishment of many invertebrate groups including carabids; thus habitat restoration potentially plays an important role in their conservation (Judd and Mason 1995; Sotherton and Self 2000; Meek et al. 2002). There are approximately 2,200 landfill sites in England and Wales covering 28,000 ha (EA 2006) representing a significant stock of land with conservation potential. Newly restored landfill sites potentially provide suitable habitats for carabids thereby compensating for the reduction of suitable habitat within the local area. It is expected the value of such newly created areas as habitat for carabids will vary depending on species-specific requirements and colonisation ability.

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75   The influence of factors operating at differing scales is key to understanding the

76   composition and diversity of invertebrate assemblages (Söderström et al. 2001;

77   Collinge, Prudic, and Oliver 2003). Community composition of carabid assemblages

78   may be influenced by local habitat to landscape-scale processes (Aviron et al. 2005).

79   Recognising critical local habitat and landscape-level factors is important in developing

80   effective conservation strategies of newly created invertebrate habitat sites. Invertebrate

81   community composition and diversity, including those of carabids, have been found to

82   be positively related to local habitat quality (Weibull, Ostman, and Granqvist 2003)

83   whilst at the landscape scale, carabid assemblages are influenced by the amount and

84   spatial arrangement of habitat patches and the surrounding landscape composition,

85   specifically obstructing linear features such as road density (de la Pena et al. 2003;

86   Aviron et al. 2005), and natural and semi-natural areas in the landscape (Liu et al.

87   2015). Although habitat quality and landscape pattern are known to explain the

88   composition and diversity of other taxa in restored landfill sites (Rahman et al. 2011,

89   2012, 2013; Tarrant et al. 2013), few studies have considered both habitat and landscape

90   factors simultaneously for carabid beetles, particularly on restored landfill sites.

91   In this study, we evaluated the effects of local and landscape factors on diversity

92   and richness of carabids on newly created grassland on restored landfill sites.

93   Specifically, we determined the roles of local factors (vegetation cover, organic matter,

94   and soil bulk density) and landscape factors (grassland, road network, and woodland) on

95   carabid diversity and richness. This study also examined whether initial seeding during

96   restoration, management and age of restored landfill sites have any effect on carabid

97   assemblages.

Material and Methods

Study sites

100 The study was conducted in the four counties of Northamptonshire, Bedfordshire,

101 Warwickshire and Buckinghamshire in the East Midlands region of the UK (Figure 1).

102 Nine restored landfill (henceforth LF) sites were selected randomly from a pool of 42

103 based on minimum size and age of the sites since restoration. The mean size (± SE) of

104 LF sites was 14 ± 3.5 ha. The LF sites, all between 4 and 15 years old, were selected to

105 provide a gradient of ages of restored grassland communities. Three LF sites had been

106 naturally colonised by vegetation and six of the LF sites were seeded by locally

107 available seed mixes. The nine closest grassland sites of recognized nature conservation

108 value, all either designated Local Nature Reserves (LNRs) or Sites of Special Scientific

109 Interest (SSSIs) and not undergoing any specific carabid conservation measures were

110 selected as reference sites (henceforth RF). RF sites were close enough to the LF sites

111 (mean distance = 4.5 ± 3.5 km, range = 1.3 to 11.8 km) to experience similar local

112 climates, to have the same regional pool of species, and to have comparable landscape

113 contexts. Five of the LF sites were managed by mowing during summer and the

114 remainder had no management or grazing; all reference sites were managed by either

115 mowing or grazing.

116 **Sample collection**

117 Two 100 m perpendicular transects crossing each other at the approximate centre point

118 of each site were set out, with directions chosen using randomized bearing tables. Ten

119 (5×2 transects) pitfall traps were set along the two transects using random points which

120 were at least 1 m apart from each other. Each pitfall trap consisted of a plastic cup (5cm

121   diameter, 120ml) sunk flush with the ground. One pitfall per sampling point was half-

122   filled with water (detergent was not added due to restrictions by landfill site operators)

123   and exposed for five days. Longer exposure was not possible due to logistic constraints.

124   Sampling from seven LF sites and their corresponding RF sites were conducted on three

125   occasions April, June, and September 2007. Further, two more LF sites and their

126   corresponding RF sites were surveyed for the same period during 2008 (no sampling

127   could be conducted during 2007 due to flooding on these sites). To reduce the effects of

128   temporal heterogeneity, sampling on both LF and their corresponding RF sites were

129   conducted at the same period of time. All captured carabid beetles were dry mounted

130   and identified to species using Forsythe (1987). Note that it was not our objective to

131   obtain a full faunal list of carabid species for each of these sites, but to use standardised

132   sampling as a means of comparing sites with different land use histories and landscape

133   contexts.

134   **Local habitat and landscape variables**

135   From each of the LF and RF sites, five soil samples from a depth down to 10 cm were

136   collected for soil analysis from random locations along the transects. The variables

137   percentage of organic matter and bulk density were determined following Rowell

138   (1994). Percentage of vegetation cover on each site was also calculated from 10 random

139   1m × 1m quadrats along transects. We used percentage of three non-crop features,

140   grassland, woodland, and road networks, as indicators of the amount and diversity of

141   perennial habitats in the surrounding landscape as perennial features should have a

142   greater influence on composition of invertebrates on newly created habitats. Percentage

143   area of grassland, woodland and a quantitative measure of the road networks, within a 1

144   km radius zone of each site's margins, were determined from Land Cover Map 2000

145   (Raster, 25m resolution) using a Geographical Information System to measure for

146   potential landscape-scale effects (ESRI 1999).

147   **Statistical analysis**

148   All data on species richness and Shannon–Wiener diversity indexes were analysed using

149   total number of species or number of individuals per site. As stated, we aimed at

150   standardised comparison between LF and RF sites, and therefore the short sampling

151   period may have an effect on the study. Carabid species composition and their

152   abundance between site types were represented by non-metric multidimensional scaling

153   (NMDS) by means of Euclidean distance, using the R package "vegan" (Oksanen et al.

154   2013). Generalised Linear Models (GLMs) were constructed to examine the effects of

155   local habitat and landscape parameters, management, method of site plant colonization

156   (seeded or natural), and age of the sites, on species diversity and richness of carabid

157   species assuming Poisson and inverse Gaussian distributions. Within LF sites, we

158   considered a set of models to test whether seeding, management and age of the LF sites

159   have any influence on estimated species richness (Chao, 1984) and diversity. We

160   restricted this analysis to only LF sites because we do not know the age of sites and

161   methods of colonization on RF sites. Further, a separate set of models was constructed

162   for diversity and richness by taking into account site type, year and soil organic matter,

163   bulk density and vegetation cover in the sites, and percentage of grasslands, percentage

164   of woodlands and percentage of road networks in the surrounding landscape. We

165   checked the assumptions of normality of the residuals and homogeneity of the variances

166   using the full models for each response variable. We compared candidate models using

167   Akaike Information Criterion (AIC), one of the most powerful approaches for model

168   selection from a set of alternative plausible models, and which solves the problems of

169   stepwise model selection (Burnham and Anderson 2002; Pinheiro and Bates 2000). Co-

170   linearity among independent variables was examined by Variance Inflation Factors

171   (VIF) and inter-correlated variables with VIF>5 were omitted from the analysis

172   (Crawley 2007). The analysis was carried out in R software (R Development Core

173   Team 2013). Model selection and multi-model inference were implemented using

174   "MuMIn" package in R (Barton 2013). Akaike weights were computed to assess the

175   support in favour of each candidate models. Models with $\Delta AIC_c \leq 2$ were considered to

176   be equally parsimonious (Burnham and Anderson 2002). We used multimode inference

177   to compute the model-averaged estimates of the explanatory variables that had a

178   normalized $\Delta AIC \leq 2$ and their corresponding 95% confidence intervals. The response

179   variable varied with the explanatory variables if 95% confidence interval excludes zero

180   (Burnham and Anderson 2002)

181   **Results**

182   **Carabid composition on LF and RF sites**

183   A total 1014 individuals representing thirty-seven carabid species were identified from

184   nine LF and their corresponding RF sites from 15 survey days. The mean carabid beetle

185   species richness (± SE) per site on LF sites was 7.9 ± 4.3 (mean Shannon-Wiener

186   diversity: 0.51 ± 0.8), whereas that on the RF sites was 9.6 ± 4.0 (mean diversity: 0.41 ±

187   0.1). However, there was no significant difference between carabid species richness

188   (Paired t-test t=-0.82, df=8, p=0.43) nor diversity (Paired t-test t=0.20, df=8, p=0.84)

189   between LF and RF sites.

190   Twenty species were found on both LF and RF sites; five species were found

191   exclusively on LF sites; and 12 species were found exclusively on RF sites (Table 1).

192 However, nationally rare and scarce beetle species were not recorded from LF sites, and

193 only one nationally scarce species (*Carabus monilis*) was found on an RF site. *Amara*,

194 *Harpalus* and *Pterostichus* species were numerous, with 20 species of these three

195 genera contributing 88% of the individuals captured.

196   The NMDS ordination of carabid species showed a clear separation between the

197 sites along the vertical axis (Figure 2). However, six of the nine LF sites were clustered

198 together, indicating that they share many of the carabid species. The RF sites have lower

199 variance in their spread, indicating that increased similarity to one another. Three of the

200 restored landfill sites which were found separated from each other had a lower

201 abundance of carabids, and also those sites were naturally colonized and not managed

202 by mowing.

203 **Effect of seeding, management and age of the LF sites on carabid richness and**

204 **diversity**

205 Within the LF sites, the models of richness that included seeded sites and management

206 independently were found to be most parsimonious (accumulated Akaike weight of

207 0.72). For diversity, the model that included seeded sites had the highest support

208 (Akaike weight of 0.46) and the intercept model was the second ranked model for

209 diversity (Table 2). Models incorporating age of the landfill sites were ΔAIC ≥4 with

210 low Akaike weight in both richness and diversity which indicates no support for the

211 hypothesis that age of the landfill sites has any effect on species richness and diversity.

212 Seeding was found to have a strong positive effect on both richness and diversity

213 (Figure 3), whilst management of the sites have a positive effect on carabid species

214 richness (Table 3).

Effect of local and landscape factors on the richness and diversity of carabids

216   Both species richness and diversity of carabid beetles on the LF and RF sites were

217   related to habitat quality and landscape variables. The richness model that included

218   additive effects of vegetation cover and grassland had the most support (Akaike weight

219   of 0.15). This model was about two-times more likely than the second-ranked model,

220   which considered vegetation cover only. The diversity models that contained vegetation

221   cover, intercept independently and additive effect of vegetation cover and bulk density

222   were found equally likely parsimonious (ΔAIC<2) though vegetation cover had higher

223   support (Akaike weight of 0.11) (Table 4). There was strong relationship between

224   vegetation cover and diversity and richness. Carabid species richness was found to vary

225   with grassland in surrounding landscape (Table 5). We found no evidence of an effect

226   of woodland, site type and bulk density on richness and diversity (Table 5).

Discussion

Composition of carabid species assemblages on LF and RF sites

229   The carabid assemblages of LF sites consisted mostly of opportunistic, generalist, open

230   habitat species, though limited sampling may have excluded rare species, and thus the

231   species collected may not be completely representative of the assemblage in these areas

232   (Lövei and Magura 2011). Generalist species from the genera *Amara*, *Harpalus* and

233   *Pterostichus* were numerous and contributed most to the total abundance across all

234   sites; all are at least facultative consumers of grass seeds (Honek and Jarosik 2003).

235   These findings agree with the results of other studies of derelict urban sites where it was

236   found that *Amara* and *Harpalus* species were numerous (Small, Sadler, and Telfer

237   2002; Eyre, Luff, and Woodward 2003).

238   No differences were found in the carabid assemblage composition between the

239   LF and RF sites, and especially seeded LF sites indicating overlap of species amongst

240   those sites. This reveals an important distinction that seeded LF sites replicate RF sites

241   and therefore seeding has an important influence on carabid community composition.

242   **Seeding, management and age of the LF sites**

243   This study found that carabid colonisation can be enhanced by seeding the newly spread

244   top soil. On restored flood-plain meadows, Woodcock et al. (2008) also found that the

245   structure of beetle assemblages was largely dependent on seed mixture, although

246   management also played an important role. Our findings suggest that restoration using

247   seed mixtures containing grasses and forbs would be expected to provide the greatest

248   resources for beetles, at least at local scales.

249   Grassland management practices, including grazing and cutting, affect ground

250   beetles (Rainio and Niemelä 2003). Because of intensive management and loss of

251   natural and semi-natural habitats, carabids have undergone biotic homogenization with

252   a few common species having become relatively more common at the expense of a large

253   number of rare species, which have become even rarer (Desender, Dufrene, and

254   Maelfait 1994). Management by grazing or mowing in our study sites was found have a

255   positive effect on carabid species richness, echoing the findings of Woodcock et al.

256   (2006). The explanation of a management effect on species richness could relate to

257   timing of management relative to breeding time since the latter is an important factor

258   affecting the survival of carabid populations. In most of the grasslands, cutting or

259   mowing is done in spring time which is the peak breeding season for carabids.

260 However, in the restored LF sites, cutting and mowing usually occurred in summer

261 which may not affect that many carabid species.

262   Wheater and Cullen (1997) found that the age of old limestone quarries was

263 important in determining invertebrate community composition. This may be related to

264 the time available for establishment or a greater degree of stability within the biotic and

265 abiotic components of the site. That age of the LF sites was not found to be an

266 important factor may be due to the limited range of ages we studied, which spanned

267 only 4-15 years. Judd and Mason (1995) stated that invertebrate assemblages on newly

268 restored landfill sites are characterised by carabids as early colonisers. It is expected that

269 early dominance by opportunistic, dispersive, short-lived and generalist invertebrate

270 species will give way to a more stable mix of longer-lived, habitat-specific species as

271 these sites get older.

272 **Effect of local habitat and landscape factors on carabid richness and diversity**

273 We found a stronger relationship between diversity and richness of carabids and local

274 habitat factors compared to landscape factors, supporting the suggestion of Niemelä et

275 al. (2002) that local factors are of primary importance for carabid community

276 composition. Vegetation cover was found to be the most influential factor for richness

277 and diversity of carabids in the study area. Vegetation cover might accelerate the

278 establishment of the carabid community because it provides living space and modifies

279 the microclimate to create a heterogeneous and stratified microenvironment supporting

280 different carabid species. Judd and Mason (1995) also reported that vegetation cover

281 could enhance the invertebrate community on a restored landfill site.

282   Carabid diversity and richness were also found to be related to the percentage of

283   grassland in the surrounding landscape, probably because many carabids have excellent

284   powers of dispersal if there is similar habitat nearby (Judd and Marson 1995). This

285   indicates that the presence of grassland nearby favours movement of species that are

286   open habitat specialists, due to the specific habitat preferences of these species.

287   Landscape containing complex grassland structure should be maintained and

288   sustainably managed as these habitats are important source of carabid species for

289   colonization of newly created sites.

290   **Conclusions**

291   In conclusion, our results showed that the key drivers of carabid assemblage structure

292   were both landscape and local habitat quality variables. More specifically, initial

293   seeding coupled with management practices and vegetation cover of the sites were

294   important at the local scale, whilst presence of grassland was the key landscape variable

295   which dictated carabid composition of the restored sites. Given the effects of the

296   grasslands in the landscape on overall ground beetle composition, future management of

297   non-cropping habitats should be aimed towards creating a more complex landscape

298   structure. In the light of the serious and widespread loss of carabid biodiversity in the

299   UK (Brooks et al. 2012), re-creating clusters of grassland habitat within fragmented

300   landscapes may have potential to enhance carabid conservation (Taboada et al. 2011),

301   even if it is mainly of widespread, generalist carabid species. However, as we stated,

302   more intensive sampling may show that rarer species can be supported in the grassland

303   on restored landfill sites, especially those of greater age. Further detailed long term

304   study to evaluate the success of restoration of landfill sites for carabid beetle

305 assemblages of landfill sites is recommended. Given the reduction and fragmentation of

306 a number of important habitat types, these newly created habitats on restored landfill

307 sites will be increasingly important for enhancing carabid populations, as well as

308 populations of birds, plants and pollinators (Rahman et al. 2011, 2012, 2013; Tarrant et

309 al. 2013). The development of detailed management prescriptions after seeding of

310 restored sites could further enhance the conservation value of restored landfill sites.

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451 **Tables:**

452 Table 1: Carabid beetle species found only on LF sites, only on RF sites, and shared on both LF

453 and RF sites. Total number of individuals sampled is in parentheses.

LF sites	LF sites	RF sites
Agonum assimile	0	6
Amara aenea	11	17
Amara anthobia	1	0
Amara aulica	0	5
Amara convexior	1	1
Amara familiaris	7	18
Amara lunicollis	7	15
Amara ovata	0	1
Amara plebeja	1	3
Bembidion lampros	5	3
Bembidion tetracolum	1	4
Carabus monilis*	0	14
Carabus problematicus	5	2
Carabus violaceus	6	7
Chlaenius nitidulus	0	1
Dyschirius sp.	1	3
Elaphrus riparius	0	1
Harpalus affinis	7	0
Harpalus anxius	6	3
Harpalus rubripes	3	0
Leistus sp.	1	1
Loricera pilicornis	0	3
Microlestes minutulus	4	0
Nebria rufescens	18	25
Nebria sp.	5	0
Ophonus sp.	0	1
Pseudophonus rufipes	9	5
Pterostichus cupreus	195	153
Pterostichus macer	5	11
Pterostichus madidus	35	228
Pterostichus melanarius	65	35
Pterostichus niger	15	1
Pterostichus nigrita	0	15
Pterostichus sp.	0	1
Pterostichus sp.	9	6
Stomis pumicatus	0	1
Unidentified	0	1
Mean species richness per site	7.9 ± 4.3	9.6 ± 4.0
Mean Shannon-Wiener diversity	0.51 ± 0.8	0.41 ± 0.1

454 *Carabus monilis is the only nationally scarce species found in RF sites.

455

456 Table 2: Model selection results for richness and diversity of carabids with seeded sites,
457 management and age of the LF sites

Models	K	AICc	ΔAICc	w_i
Richness				
Seeded	2	44.8	0.00	0.53
Management	2	46.0	1.23	0.29
Seeded+Management	3	48.2	3.38	0.09
Seeded+Age	3	49.6	4.79	0.05
Diversity				
Seeded	3	20.9	0.00	0.46
Null model	2	21.5	0.66	0.33
Management	3	24.2	3.27	0.09
Seeded+Age	4	24.9	3.99	0.06

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459

460   Table 3: Model–averaged estimates of explanatory variables only for LF sites with their respective

461   unconditional standard errors (SE) and 95% confidence intervals (CI) and Relative importance (RI).

462   Estimates in bold indicate a strong effect of that explanatory variable on the response variable.

Variables	Est.	SE	95% CI	RI
Richness				
Null model	1.93	0.13	1.60, 2.25	
Seeded	**1.24**	**0.36**	**0.39, 2.08**	**0.65**
Management	**1.01**	**0.28**	**0.34, 1.68**	**0.35**
Diversity				
Null model	0.34	0.13	0.04, 0.65	
Seeded	**0.63**	**0.25**	**0.05, 1.22**	**0.58**

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473 Table 4: Model selection results for richness and diversity of carabids with their local and

474 landscape parameters. (Parameters: Veg.cover=percentage of vegetation cover,

475 Blk.density=Bulk density)

Models	df	AICc	ΔAICc	w_i
Richness				
Veg.cover+grassland	3	100.15	0.00	0.15
Veg.cover	2	101.42	1.27	0.08
Veg.cover+grassland+woodland	4	101.67	1.52	0.07
Veg.cover+grassland+Site type	4	102.1	1.95	0.06
Veg.cover+grassland+Blk.density	4	102.61	2.46	0.04
Diversity				
Veg.cover	3.00	31.77	0.00	0.11
Null model	2.00	32.42	0.65	0.08
Veg.cover +Blk.density	4.00	32.56	0.79	0.07
Veg.cover+Site type	4	33.8	2.07	0.04

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478 Table 5: Model–averaged estimates of explanatory variables only for both LF and RF sites with their

479 respective unconditional standard errors (SE) and 95% confidence intervals (CI) and Relative

480 importance (RI). (Parameters: Veg.cover=percentage of vegetation cover, Blk.density=Bulk density).

481 Estimates in bold indicate a strong effect of that explanatory variable on the response variable.

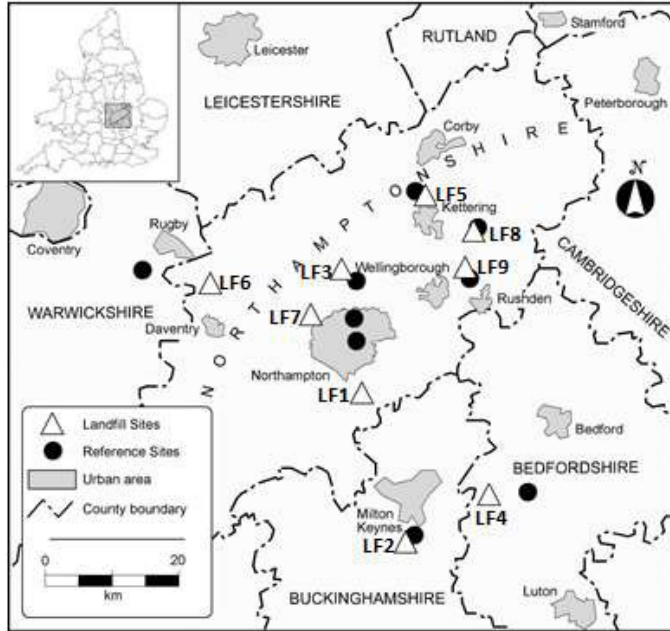| Variables	Est.	SE	95% CI	RI
Richness				
Null model	8.72	0.76	7.24, 10.20	
Veg.cover	**4.72**	**1.35**	**2.06, 7.36**	**1.00**
Grassland	**3.55**	**1.74**	**0.14, 6.95**	**0.78**
Woodland	-1.99	1.59	-5.11,1.13	0.20
Site type	-2.13	1.89	-5.84, 1.58	0.16
Diversity				
Null model	0.35	0.09	0.17,0.52	
Veg.cover	**0.46**	**0.18**	**0.10,0.81**	**0.70**
Blk.desnity	0.25	0.18	-0.10,0.61	0.28

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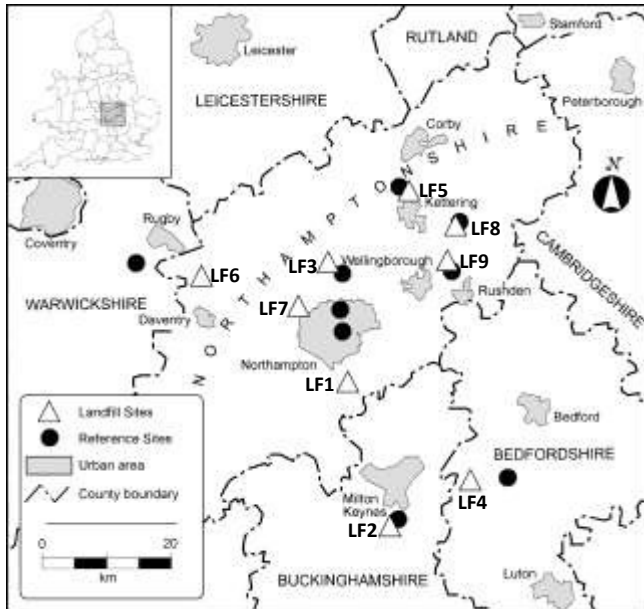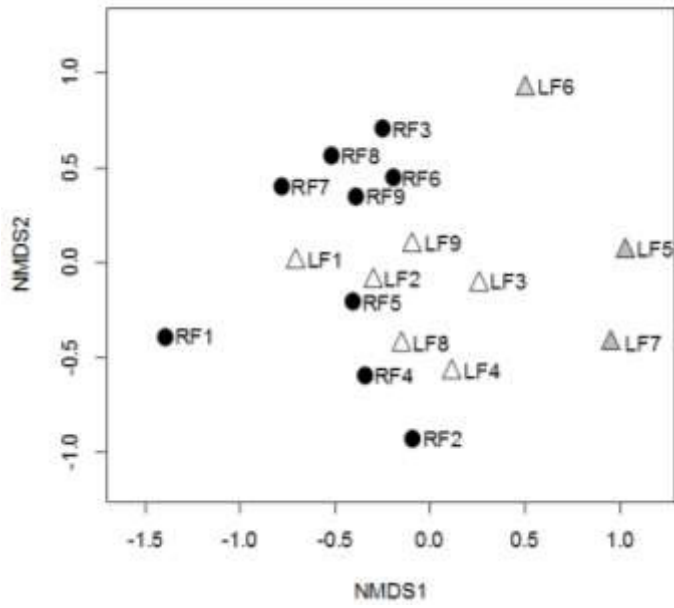
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488 **Figures:**

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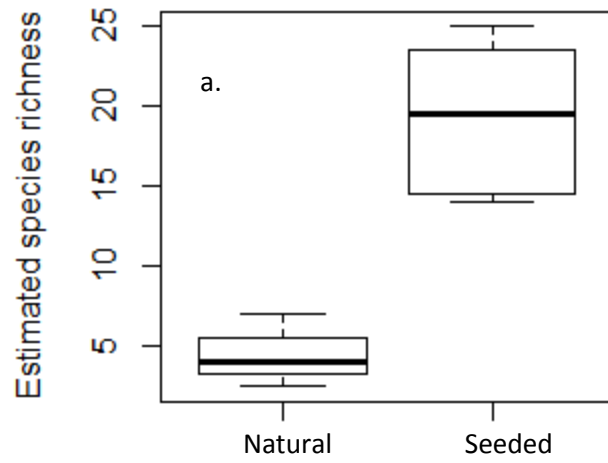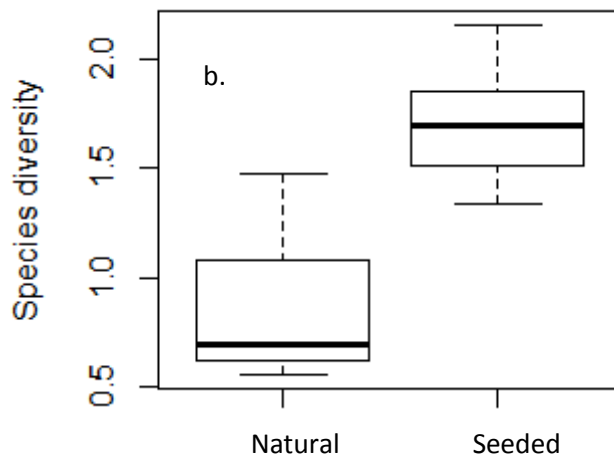508   Figure 2

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Figure 3

525   **Figure Legends**

526   Figure 1: Location of restored landfill (LP, triangles) and reference sites (black dots)

527   studied in Northamptonshire and its surrounding counties.

528   Figure 2: NMDS ordination of carabid species composition and their abundance on nine

529   restored landfill (LF 1-9) sites and nine reference (RF 1-9) sites. Two-dimensions used,

530   S-stress=0.113. Filled triangle=seeded landfill sites; open triangle=naturally colonized

531   landfill sites; filled circles=reference sites.

532   Figure 3: Estimated carabid species richness (a) and Shannon-Wiener diversity (b) on

533   seeded LF and naturally colonized LF sites. The horizontal line shows the median. The

534   bottom and top boxes show the 25th and 75th percentiles respectively. The vertical

535   dashed lines show interquartile range.