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Article

Title: Effect of soil conditions and landscape factors on macro-snail communities in newly created grasslands of restored landfill sites in the UK

Creators: Rahman, M. L., Tarrant, S., Ollerton, J. and McCollin, D.

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2 version. Please refer to the published version for the definitive text and findings.

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5 Soil conditions and landscape factors dictate macro-snail communities of newly created
6 grasslands on restored landfill sites in the UK

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9 Md Lutfor Rahman¹, Sam Tarrant², Jeff Ollerton, Duncan McCollin,

10

11 Landscape and Biodiversity Research Group, School of Science and Technology, The

12 University of Northampton, Avenue Campus, Northampton, NN2 6JD, UK

13

14

15 ¹Current Address:

16 International Wildlife Consultants Ltd, PO Box 19, Carmarthen, SA33 5YL, Wales, UK

17

18 ²Current Address:

19 RSPB UK Headquarters, The Lodge, Sandy, Bedfordshire, SG19 2DL, UK

20

21 *Author for correspondence:

22 Md Lutfor Rahman

23 International Wildlife Consultants Ltd.

24 PO Box 19, Carmarthen, SA33 5YL, Wales, UK

25 Phone: +44(0) 1267233684

26 Email: lutforestry@yahoo.com

27

28 **Running Title:** Land snails on restored landfill sites

29

30 Abstract

31 Though restored landfill sites provide habitat for a number of taxa, their potential for
32 land snail remains unexplored. In this study, large-sized land snails (> 5 mm) were
33 surveyed using transect sampling on nine restored landfill sites and nine corresponding
34 nature sites in the East Midlands region of the UK during 2008. The effect of restoration
35 was investigated by examining land snail species composition, richness, and diversity
36 (Shannon Weiner index) in relation to habitat and landscape structure. Thirteen macro-
37 snail species were found in total and rarefied species richness and diversity on restored
38 landfill sites was not found to be statistically different to that of reference sites. One
39 third of the snail species, comprising 30% of total abundance, found in the restored
40 landfill sites were non-native species introduced to the UK. Soil electrical conductivity
41 was the strongest predictor for richness and diversity of land snails. Road density was
42 found to have a positive influence on snail species diversity. Given the high percentage
43 of introduced species detected further research is needed in terms of the management
44 implications of restored landfill sites and the dynamics of native versus non-native
45 species.

46

47 **Keywords:** land snail, waste ground, restoration, grassland, landfill.

48

49 **Introduction**

50 Land snail populations are considered to be relatively stable (Lydeard et al. 2004),
51 however, the abundance of some grassland snail species may have changed mainly due
52 to intensive management practices coupled with habitat loss (Kerney 1999; Martin and
53 Sommer 2004a; Stoll et al. 2009). Many invertebrate species of conservation
54 importance are established on brownfield sites such as landfill (Judd and Mason 1995;
55 Rahman et al. 2015; Tarrant et al. 2013). Therefore, the process of habitat restoration
56 **could be** important for the enhancement of other invertebrate species such as land snails
57 which may have been declined locally or regionally such as *Arianta arbustorum* and
58 *Candidula gigaxii* (Seddon et al. 2014). In England and Wales there are approximately
59 2,200 landfill sites covering *ca.* 28,000 ha (EA 2006) which is large area of land with
60 conservation potential, but which remains largely unexplored for invertebrates such as
61 land snails.

62 As detritivores land snails are an integral part of ecosystems (Caldwell 1993; Kappes et
63 al. 2007) including playing vital roles as food for higher trophic levels (Eeva et al.
64 2010). However, community composition of grassland snails is influenced by habitat
65 variation from the local to landscape-scale (Magnin et al. 1995; Martin and Sommer
66 2004a, **Boschi 2007**). Traditionally, calcium is considered as limiting factor for
67 colonisation and distribution of land snail species richness and density (Ondina et al.
68 1998) although it might be substituted with soil pH or soil buffer system (Cameron et al.
69 1980; **Kappes and Topp 2014**). Less often, soil moisture and litter depth are used to
70 explain any such differences (Juricková et al. 2008; Hettenbergerová et al. 2013).
71 Distance to the habitat border and land use within a radius of 1 km can predict snail
72 assemblages in stable habitats such as old forests (Kappes et al. 2009b; Kappes et al.

2011) although connectivity at the landscape scale may have an even larger influence on land snail colonisation of newly created habitats (Kappes et al. 2009a; Stoll et al. 2009, Knop et al. 2011). Recognising critical local and landscape-level factors is important in developing effective conservation strategies for newly created habitats. However, there is a lack of knowledge of which factors are the most important (Ondina et al. 1998). In forests Martin and Sommer (2004b) noticed that moisture availability can somewhat shadow the expected patterns from pH in a set of different forests, whilst Kappes et al. (2007) found that enhanced habitat quality of coarse woody debris increased snail richness and densities over expectations from soil pH alone in a paired sampling approach. In contrast very little research has been conducted on factors affecting assemblages of snail species on restored grasslands and there is also a lack of baseline information on the diversity of snails of restored landfill sites (Wheater and Cullen 1997; Watters et al. 2005). Detailed studies of the relationship between the composition of land snail communities and their local and landscape habitat can provide an understanding of the impacts of restoration and determine the role of newly created grassland habitats in supporting land snail species. Knop et al. (2011) stated that structural connectivity of grassland habitats is important for increasing the restoration success for snails. We therefore hypothesised that macro-snail species richness and diversity are: 1) positively related with management (in terms of mowing), seeding and the age of the sites, and 2) different local habitat and landscape variables may influence the establishment of snail species on restored landfill sites. The present research aims to reveal any distribution patterns of land snail species and to investigate which local and landscape factors control their diversity and abundance on newly created grassland of restored landfill sites.

97

98 **Methods**

99 *Study sites*

100 The study was conducted in the East Midlands region of the UK in the counties of
101 Northamptonshire, Bedfordshire, Warwickshire and Buckinghamshire. Nine restored
102 landfill sites (hereafter LF) were selected randomly from a set of 42 known LF site in
103 this region (Fig. 1). The sites had similar characteristics and were representative of LF
104 sites within the region. In order to provide a comparison, nine reference sites (hereafter
105 RF) were selected which were the closest recognized protected grassland sites for their
106 nature conservation value, being designated as either Local Nature Reserves (LNRs) or
107 Sites of Special Scientific Interest (SSSIs) for their local or national special natural
108 interest respectively. The RF sites were spatially close enough (mean distance = $4.5 \pm$
109 3.5 km, range = 1.3–11.8 km) to the LF sites so that they experience similar
110 physiography, climate, soil and land use history (see details in Rahman et al. 2015). Six
111 of the restored landfill sites were managed by mowing during the late summer and three
112 sites had no mowing or grazing regime (Table 1).

113 *Sampling method*

114 All land snails along two randomly selected transects of 100 m long x 2 m wide
115 crossing each other at the approximate centre point of the site, were collected by hand
116 from vegetation, soil and sifted ground litter. Surveys were conducted three times from
117 April-September with a regular interval (*ca.* 10 weeks intervals) during 2008 to provide
118 a sampling regime with a good coverage in spring-summer. Each transect was searched
119 extensively by two people (approximately 30-60 minutes). In this study, snails on the

120 soil surface (i.e. micro-snails and also some small size juveniles which were less 5mm)
121 were not considered. However, some small snail species *Vitrea crystallina*, *Euconulus*
122 *fulvus*, *Nesovitrea hammonis* and *Vitrina pellucida* were excluded from further analysis
123 except listing of species as few individuals were found. Our sampling efforts were
124 restricted to spring-summer and we did not include snails of soil as it was not our
125 objective to obtain a full list of land snail species for each of these sites but to use
126 standardised sampling as a means of comparison between LF and RF sites. Both live
127 and dead snails were collected and all snails were preserved for further identification
128 following the method of Kerney and Cameron (1979). Snails were cleaned under
129 running water, and then transferred to 70% alcohol and stored for later identification
130 using Kerney and Cameron (1979) and Cameron (2003). Nomenclature follows
131 Beedham (1972). We also classified snails as native or introduced in our study area
132 based on Kerney (1999) though some of those introduced are quite ancient, dating from
133 Roman times.

134 ***Local 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. Soil moisture
137 content (%), electrical conductivity (microSiemens per centimetre, ($\mu\text{S}/\text{cm}$) which
138 indicates the amount of dissolved minerals present in the soil), stone content (%), litter
139 depth (mm) and pH were determined following Rowell (1994) (Table 2). We used
140 percentage of total area of non-crop features such as grassland, woodland, and road
141 networks as an indicator of the amount and diversity of perennial habitats in the
142 surrounding landscape derived from Land Cover Map 2000 (25 m \times 25 m resolution)

143 (LCM 2000). Percentage of the total area of grassland and woodland and road networks
144 (since road networks may also indicate urban pressure), within a 1 km radius zone of
145 each site's margins were determined using a Geographical Information System to
146 measure for potential landscape-scale effects (ESRI 1999; Table 2).

147

148 *Statistical analysis*

149 We used rarefied number of species for a total of two individual samples using the R
150 package “vegan” (Oksanen et al. 2013; R Development Core Team 2013) and the
151 Shannon diversity index for analysis as we found high differences in number of
152 individual snails in terms of richness between LF and RF sites which been tested with
153 Generalized Linear Models. Land snail species composition and their abundance
154 between site types (LF and RF sites) were expressed by non-metric multidimensional
155 scaling (NMDS) using Euclidean distance, again using the package “vegan” in R. We
156 log-transformed number of recorded individuals because abundance also greatly varied
157 between sites (Table 1). We examined similarities of macro-snail species composition
158 of the two site types by Analysis of Similarities (ANOSIM) using 999 permutations.
159 Generalised Linear Models (GLMs) were constructed to examine the effects of
160 management, method of site colonization (seeded or natural), age of the LF sites
161 affecting richness and diversity. This particular analysis is limited to only LF sites as
162 the method of colonization and age of RF sites were unknown. Furthermore, separate
163 independent models were also built for richness and diversity of both LF and RF sites
164 taking into account local factors (site type, moisture content, soil electrical conductivity,
165 pH, and litter depth) and landscape-scale parameters (percentage area of grasslands,

166 woodlands and road networks) separately assuming a Poisson and inverse Gaussian
167 distribution for richness and diversity respectively as richness are count data and
168 diversity are discrete data. We compared candidate models with null models which is
169 intercept only model using the Akaike Information Criterion (AIC), one of the most
170 powerful approaches for model selection from a set of alternative plausible models and
171 which solves the problems of stepwise model selection and also corrected for small
172 sample sizes (Burnham and Anderson 2002). Model selection and multi-model
173 inference were implemented in R using the “MuMIn” package (Barton 2013). Akaike
174 weights were assessed to find best supported models. The top-ranked models had an
175 Akaike weight >0.05 ; we used multi-model inference to compute the model-averaged
176 estimates of the explanatory variable and 95% confidence interval (Burnham and
177 Anderson 2002). A 95% confidence interval excluding 0 indicated that the response
178 variable varied with the explanatory variable of interest (Burnham and Anderson 2002).

179 **Results**

180 *Snail composition and effects of seeding, management, age*

181 A total of 13 macro-snail species (10 species in LF and 10 species in RF sites) with 838
182 individuals (681 in LF and 157 in RF sites) were recorded from nine LF sites and their
183 corresponding RF sites. Seven species were found both on LF and RF sites, while three
184 introduced species were found exclusively in LF site and three native species found
185 exclusively on RF sites (Table 3).

186 The NMDS ordination of macro-snail composition showed a clear separation between
187 the LF and RF sites along the horizontal and vertical axis primarily due to the high
188 proportion of introduced species in LF sites (ANOSIM test $R=0.25$, $P=0.01$), though

189 there is clear separation of three of the LF sites along the first axis which indicates that
190 those sites share few snail species among themselves (Fig. 2). The RF sites showed low
191 variance in spread which indicated a higher similarity to one another. There was also no
192 significant difference in mean rarefied species richness per site ($P=0.32$) and diversity
193 ($P=0.13$) between LF and RF sites but there was a significant difference in species
194 richness ($P=0.03$) (Table 3). Only the model incorporating seeding variable is most
195 parsimonious for snail species richness. However, we did not find any statistical support
196 for effect of seeding on species richness of native or introduced plant species. However,
197 we found seeding has a positive significant effect on *Candidula intersecta* which is an
198 introduced species ($t=4.05$, $p=0.009$). None of the models incorporating management or
199 age of the LF sites were found to be parsimonious ($AIC >2$) for snail species richness
200 and diversity (Table 4). We did not find any particular species having any effect due to
201 management (mowing) on restored landfill sites.

202 ***Local and landscape factors on snail richness and diversity of both LF and RF sites***

203 GLM analysis and model selections suggested that land snail species richness and
204 diversity were related to both local and landscape factors (Table 5). Both richness and
205 diversity models that included soil conductivity had the highest support (Akaike weight
206 of 0.22 for richness and 0.34 for diversity). However, the model containing soil
207 conductivity and site type was also an equally parsimonious model as the moisture
208 content model for snail species richness (Table 5).

209 At a landscape scale, the snail species richness model that considered a fixed effect of
210 road density had the highest support (Akaike weight 0.19) and the richness model
211 containing an additive effect of road density and woodland, road density and grassland

212 on surrounding landscape were equally parsimonious. The diversity model containing
213 only road density had the highest support (Akaike weight 0.69) (Table 5).

214 We found a positive effect of conductivity on both richness and diversity of snail
215 species but found no evidence of an effect of soil moisture and litter depth on both
216 species richness and diversity. There was a **negative** effect of road density on the
217 **Shannon** diversity index and we found no evidence of an effect of grassland and
218 woodland on either snail richness or diversity (Table 6).

219 **Discussion**

220 *Snail composition and effects of seeding, management, age*

221 Though we only included macro-snails, the land snail species in the present study
222 represent approximately 15% of the total land snail species of the UK suggesting that
223 restored LF sites has potential as habitat for a significant fraction of the species of this
224 taxon. These snails in turn can have roles in the processes of succession and nutrient
225 cycling of these newly created grasslands (Holland et al. 2007). However, one third of
226 the snail species, comprising 30% of total abundance, found in the LF sites were
227 introduced species to the UK. Such non-native land snail species may cause major
228 changes to these novel ecosystems by supressing native species. **Further research is**
229 **needed to assess whether these European 'exotics' have any impact or not (but see**
230 **Holland et al. 2007).**

231 We did not found any variation in snail species richness and diversity due to site
232 management, age of the sites, or whether sites were seeded or not. However, seeding
233 may potentially affect land snail densities through enhancing vegetation cover which

234 may reduce extreme and abrupt changes in the microclimatic conditions, such as high
235 temperatures. In previous studies grasslands subjected to constant grazing had
236 decreased land snail diversity and abundance indicating that high grazing pressure may
237 be detrimental to snails (Cameron and Morgan-Huws 1975; Labaune and Magnin 2002;
238 Ruesink 1995). In our landfill sites mowing is restricted to late summer which might be
239 a reason for no negative effects being detected. However, this may also be due to the
240 limited range of ages (4-15 years), related to the time available for establishment or
241 attaining stability within the biotic and abiotic components of the site.

242 ***Effect of local and landscape factors on richness and diversity of land snail***

243 The conductivity in the study sites ranged from 12-110 μScm^{-1} and pH ranged from 4.2-
244 7.8. A positive relation between electrical soil conductivity and land snail species
245 richness and diversity indicates the gradient of different available minerals is important
246 factors for snail composition though we do not know which minerals are most important
247 in our samples. We recommend further research should be conducted to determine
248 which minerals could be important factors as other researcher have found effects of
249 different minerals (Ondina et al. 1998; Juricková et al. 2008; Horsák 2004).

250 The results from this study confirmed that land snail richness was structured by a
251 gradient of woodland in the vicinity though we did not found strong support for this
252 (Table 6). Small grassland patches such as our recreated grassland on restored landfill
253 sites may benefit from the presence of woodlands in the vicinity for land snail
254 colonisation to take place (Labaune and Magnin 2002) particularly introduced generalist
255 species such as *Candidula intersecta* and *Ceriuella virgate*. Sites near to woodland had
256 higher land snail richness than those of open areas, which indicates the community is

257 enriched due to dispersal if distances to sources of immigration are not too far
258 (Cameron et al. 1980; Magnin et al. 1995). A gradient of road density was also found to
259 be one of the strong predictors for land snail species diversity and some species such as
260 *Trichia striolata* (t=2.14, P=0.03). Many introduced land snails are associated with
261 human habitation and roads or anthropogenic disturbances, therefore the positive
262 influence of roads found on Shannon diversity in this study may reflect greater
263 opportunities for both native (t=3.70, P=0.002) and invasive species but did not find any
264 relationship with invasive species (t=1.66, P=0.12). Though most land snails have a
265 restricted active dispersal capability, passive transport allows them to colonise new
266 habitats (Dörge et al. 1999).

267 In conclusion, the creation (or re-creation) of grassland habitat within fragmented
268 landscapes has potential to enhance biodiversity conservation. However, the high
269 proportion of non-native snail species found in grasslands in this study presents an
270 interesting opportunity to further research the interactions between native and non-
271 native species in terms of their ecology and management.

272

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282

283 **Disclosure statement**

284 There are no potential conflicts of interest of anybody or organisation relevant to this
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286

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406 *Biological Conservation* 126: 166-174.

407

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410 Tables:
 411
 412 Table 1: Abundance (abund), richness and diversity (divers) of native and introduced
 413 snail species in restored landfill (LF). Values in parentheses are their corresponding
 414 reference (RF) sites parameters.

415

	Seeded	Manage	Native Species			Introduced Species		
			abund	richness	divers	abund	richness	divers
1	Yes	Yes	18 (12)	3 (3)	0.65 (1.08)	16 (9)	2 (2)	0.69 (0.69)
2	Yes	Yes	10 (48)	2 (4)	0.69 (0.64)	16 (0)	2 (0)	0.69 (0.00)
3	Yes	Yes	56 (1)	2 (1)	0.21 (0.00)	4 (1)	1 (1)	0.00 (0.00)
4	Yes	No	66 (0)	1 (0)	0.00 (0.00)	1 (0)	1 (0)	0.00 (0.00)
5	No	No	39 (2)	2 (1)	0.12 (0.00)	39 (3)	5 (2)	1.14 (0.64)
6	No	No	12 (0)	4 (0)	0.98 (0.00)	0 (0)	0 (0)	0.00 (0.00)
7	No	Yes	47 (53)	2 (4)	0.29 (0.82)	20 (0)	2 (0)	0.20 (0.00)
8	Yes	Yes	227 (8)	4 (2)	0.21 (0.56)	101 (1)	3 (1)	0.73 (0.00)
9	Yes	Yes	3 (19)	2 (3)	0.64 (0.82)	6 (0)	2 (0)	0.45 (0.00)

416

417

418 Table 2: Mean, Median values with their Minimum (Min) and Maximum (Max) of studied local and
 419 landscape variables

420

Parameters	LF Sites (N=9)				RF sites (N=9)			
	Mean	Median	Min	Max	Mean	Median	Min	Max
Moisture content (%)	29.93	30.89	20.46	40.46	39.07	35.72	29.96	53.04
pH	7.25	7.06	6.42	8.01	7.19	6.99	6.39	8.12
Soil Electrical Conductivity	52.57	48.96	19.46	98.14	41.39	29.12	5.75	104.32
Litter depth (mm)	7.44	5.80	0.80	17.40	5.91	4.00	3.60	10.40
Stone content (%)	6.30	7.21	0.49	12.71	0.81	0.18	0.00	2.55
Woodland (% area)	5.70	2.93	0.41	19.80	3.29	1.92	0.00	11.53
Grassland (% area)	17.98	18.34	7.47	32.13	22.02	16.27	4.83	71.57
Road network (% area)	6.73	5.73	2.44	14.47	8.08	6.75	1.14	19.36

421

422

423 Table 3: List and number of land snail species found only landfill sites (LF), only
 424 reference sites (RF) and species found on both LF and RF sites. Numbers in parentheses
 425 are number of sites, \pm denotes standard error.

Species	LF sites	RF sites
<i>Arianta arbustorum</i>	0	2 (1)
<i>Cepaea hortensis</i>	10 (6)	5 (3)
<i>Cepaea nemoralis</i>	5 (2)	2 (1)
<i>Monacha cantiana</i>	445 (8)	103 (7)
<i>Oxychilus cellarius</i>	0	6 (1)
<i>Succinea putris</i>	0	3 (1)
<i>Trichia hispida</i>	11(4)	2 (2)
<i>Trichia striolata</i>	7 (2)	20 (3)
<i>Candidula gigaxii</i> *	2 (1)	0
<i>Candidula intersecta</i> *	119 (6)	6 (3)
<i>Cernuella virgata</i> *	77 (8)	8 (3)
<i>Helix aspersa</i> *	4 (2)	0
<i>Helix pomatia</i> *	1 (1)	0
<i>Euconulus fulvus</i>	1(1)	0
<i>Nesovitrea hammonis</i>	1 (1)	1 (1)
<i>Vitrea crystallina</i>	0	2 (2)
<i>Vitrina pellucida</i>	1 (1)	0
Mean macro-snail species richness per site	4.78 \pm 0.54	3.00 \pm 0.65
Mean rarefied macro- snail species richness per site	1.52 \pm 0.08	1.28 \pm 0.24
Mean Shannon diversity of macro- snails	0.95 \pm 0.14	0.72 \pm 0.16

426 * Introduced species. Source: Kerney (1999)

427

428 Table 4: Model selection results for richness and diversity with seeded sites,
 429 management and age of the LF sites. K = No. of paramters, AICc = Akaike's
 430 information criterion corrected for small smaple sizes, $\Delta AICc$ = AICc relative to the top
 431 most model, w_i = AICc model weight

Models	K	AICc	$\Delta AICc$	w_i
<i>Richness</i>				
Null model	1	37.3	0.00	0.51
Seeded	2	38.9	1.64	0.22
Age	2	40.5	3.21	0.10
Management	2	40.7	3.37	0.90
<i>Diversity</i>				
Null model	2	-42.9	0.00	0.74
Management	3	-39.2	3.69	0.12

432

433 Table 5: Model selection results for richness, diversity with their local and landscape
 434 parameters. (Parameters: Rdnet=Road network). K = No. of paramters, AICc =
 435 Akaike's information criterion corrected for small sample sizes, $\Delta AICc$ = AICc relative
 436 to the top most model, w_i = AICc model weight

Models	K	AICc	$\Delta AICc$	w_i
<i>Richness</i>				
<i>Local variables</i>				
Conductivity + Site type	3	72.9	0.00	0.22
Conductivity	2	73.0	0.08	0.21
Moisture content	3	74.2	1.31	0.11
<i>Landscape variables</i>				
Rdnet	2	75.3	0.00	0.19
Rdnet + Woodland	3	76.0	0.67	0.14
Null model	1	76.4	1.09	0.11
Rdnet + Grassland	3	76.7	1.36	0.10
<i>Diversity</i>				
<i>Local variables</i>				
Conductivity	4	-89.4	0.00	0.34
Null model	3	-88.0	1.43	0.16
Conductivity + Site type	5	-86.9	2.44	0.10

<i>Landscape variables</i>				
Rdnet	3	-91.9	0.00	0.69
Rdnet + Grassland	4	-88.8	3.09	0.15
Rdnet + Woodland	4	-88.4	3.48	0.12

437

438 Table 6: The explanatory variable selected in GLM for occurrence of and snail species
 439 richness and **Shannon**-diversity on restored landfill sites and reference sites. Numbers
 440 in bold shows response variable varied with the explanatory variable. Est.= Parameter
 441 Estimates, SE = Standard Error, CI = Confidence Intervals.

	Variables	Est.	SE	Lower 95% CI	Upper 95% CI
Richness	Management	0.08	0.32	-0.68	0.83
	Seeding	-0.42	0.31	-1.17	0.33
	Age	0.15	0.33	-0.62	0.93
	Site type	-0.44	0.26	-0.99	0.11
	Conductivity	0.57	0.25	0.04	1.11
	Moisture content	-0.39	0.31	-1.05	0.26
	Road network	0.49	0.25	-0.04	1.03
	Grassland	0.30	0.24	-0.21	0.82
	Woodland	0.38	0.25	-0.15	0.92
Diversity	Management	0.01	0.01	-0.02	0.03
	Conductivity	0.02	0.01	0.00	0.03
	Site type	-0.01	0.01	-0.02	0.01
	Road network	0.02	0.01	0.01	0.04
	Grassland	0.01	0.01	-0.01	0.02
	Woodland	0.00	0.01	-0.01	0.01

442 **Figure Legend**

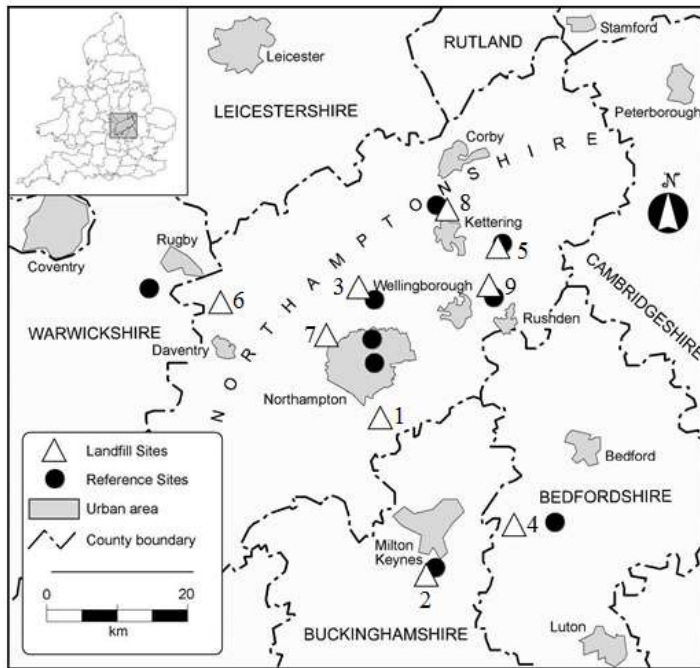
443

444 Fig. 1: Location of studied restored landfill sites (triangles) and their corresponding
 445 reference sites (black dots). **Numbers on the map correspond to descriptions of landfill**
 446 **sites in Table 1.**

447

448 Fig. 2. NMDS ordination of snail species composition and their abundance on LF and
 449 RF sites. Two-dimensions uses, S-stress=0.08. Nine LF and RF site denote LF(1-9) and
 450 RF(1-9) respectively.

451 **Figures:**

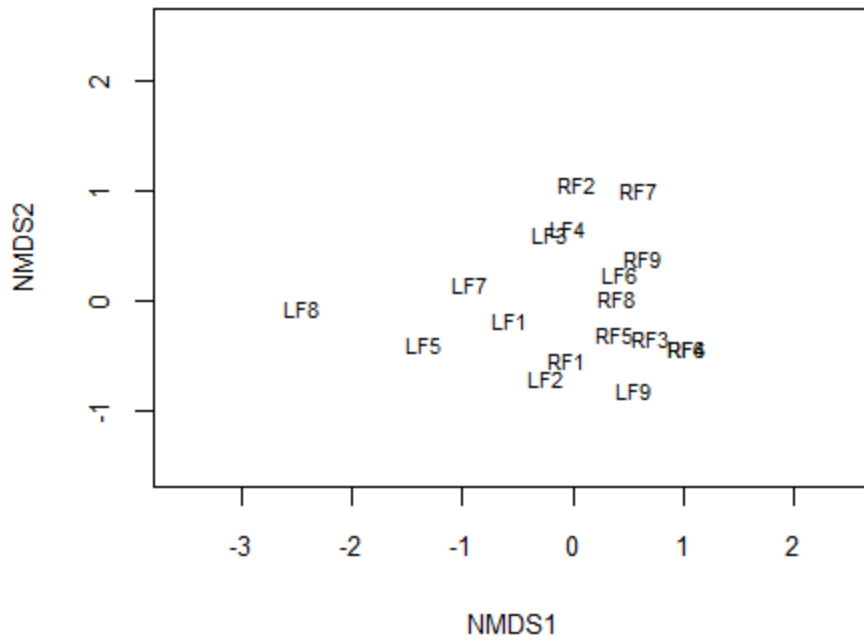


452

453

454 Fig. 1

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459 Fig. 2

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