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1 **Does pollution influence small mammal diet in the field? A metabarcoding approach**
2 **in a generalist consumer.**

3
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18

19 **Abstract:**

20 Mammals are mainly exposed to trace metals via consuming contaminated food. Several
21 studies have demonstrated relationships between metal concentrations in food and in animal
22 tissues. However, potential effects of trace metals on feeding behaviour of wildlife have been
23 poorly documented under field conditions, despite experimental evidence showing that food
24 selection is impacted by resource contamination. Here we test the hypothesis that the diet of
25 a generalist rodent, the wood mouse (*Apodemus sylvaticus*), is altered by soil trace metal
26 contamination in the field. Wood mice were sampled in spring and in autumn along a
27 gradient of soil contamination in the surroundings of a former smelter located in northern
28 France. Available resources in the field were inventoried and the diet of the animals was
29 analysed using DNA “metabarcoding”. We demonstrated that (i) relationship between the
30 resource richness in the diet and their richness in the field was altered by soil metal
31 contamination. Wood mice specialised their diet along the gradient of soil metal
32 contamination for both plant and invertebrate resources in spring. We also showed that (ii)
33 preference for Salicaceae, a plant family accumulating metals, decreased when soil
34 contamination increased. These results suggest that environmental trace metal pollution
35 could act as a force modulating trophic interactions in terrestrial food webs, thereby affecting
36 wildlife exposure to contaminants by trophic route.

37

38 **Keywords:**

39 Biodiversity; Diet diversity; Food selection; Trace metals; *Apodemus sylvaticus*

40

41 **Introduction**

42 Feeding behaviour is determined by life history traits and life stages of animals (Schoener,
43 1971; Pyke et al., 1977), abundance and distribution of food resources in nature (White,
44 2008; Crampton et al., 2011), and food quality (i.e. nutrient and toxin content) (Butet, 1986a;
45 Kerley & Erasmus 1991; Schmidt, 2000; Lewis et al., 2001). All these factors are driven by
46 ecological determinants like season, climate, community interactions or landscape features
47 (Polis et al., 1997; Visser & Both 2005; White, 2008). The optimal foraging theory assumes
48 that animals consume resources providing high energy intake per unit time (Stephens &
49 Krebs, 1986) and that the diet of animals tends to be specialized when food density is high
50 (MacArthur & Pianka, 1966). However, animals require not only energy but also nutrients
51 like vitamins or essential metals. The nutrient requirements can thus hardly be satisfied from
52 a single plant species and thus animals seek a variety of diet for nutrient balance (i.e. nutrient
53 balance hypothesis) (Westoby, 1978). Likewise, Freeland and Janzen (1974) proposed that
54 generalist mammalian herbivores are unable to detoxify large amounts of similar plant
55 secondary metabolites (i.e. those from a single plant species) and thus a variety of plant
56 sources allow them to avoid similar plant toxin load (i.e. detoxification limitation
57 hypothesis). When the use of a resource is disproportional to its availability in the field, the
58 feeding process by which an animal chooses the resource is called “selection” (Johnson,
59 1980; Manly et al., 2002). The nature of selection (i.e. preference or avoidance) reflects the
60 likelihood of a given resource for being chosen when this resource is offered with others
61 (Johnson, 1980). The food selection hence manifests a relationship between individual
62 requirements and resource constraints.

63 Trace metals (TM) are environmental pollutants of concern for both human health and
64 wildlife. They occur often as a legacy of past industrial activities. Even if the emissions of
65 many of them have been reduced in the past decades thanks to environmental regulation and

66 technical improvements at least in so-called developed countries, the pollution still persists
67 over vast surfaces due to non-degradability of metals and to their current emissions in many
68 countries (Kabata-Pendias, 2011; EMEP, 2013). TM pollution may have effects on plants
69 and invertebrates at several levels of organisation, from sub-individual physiological effects
70 reducing survival or reproduction to the modification of community composition and
71 structure (Walker et al., 2012). For instance, a recent meta-analysis about the responses of
72 terrestrial biota to TM pollution -dealing with 206 pollution point sources worldwide-
73 showed a decrease of diversity for most of the taxa (producers, secondary consumers and
74 decomposers) (Kozlov & Zvereva, 2011). In addition, Dazy et al. (2009) demonstrated that
75 plant species richness correlated negatively to TM concentrations in soils in eastern France.
76 Metal pollution can also reduce species richness in earthworm communities (Spurgeon &
77 Hopkin, 1999; Nahmani et al., 2003) and impact the structure and composition of soil macro-
78 fauna communities (Nahmani & Lavelle, 2002). Trophic transfer , i.e. the transfer of matter
79 via food consumption, is generally considered to be the main route for TM from the
80 environment to animals of higher trophic levels (Shore & Rattner, 2001; Smith et al., 2007).
81 For small mammals, several studies have demonstrated relationships between soil pollution
82 by TM, contamination of food, and subsequently, high concentrations of TM in wild animals'
83 tissues (Hunter & Johnson, 1982; Hunter et al., 1987a; Torres & Johnson, 2001; Rogival et
84 al., 2007; van den Brink et al., 2010). Avoidance of TM contaminated food can be a
85 mechanism that mitigates organisms' exposure to contaminants (Gillet & Ponge, 2003). This
86 has been reported for the wood mouse (*Apodemus sylvaticus*) under laboratory settings but
87 not confirmed in natural conditions (Beernaert et al., 2008). To the best of our knowledge,
88 no study has reported variations of richness in the diet and selection as a response to both
89 the variation of resource availability and the contamination of the environment *in natura* in
90 terrestrial ecosystems.

91 One of the main difficulties in assessing wildlife diet is the identification of items actually
92 consumed. Identifying items with the highest taxonomic resolution is almost impossible with
93 classical macro- or microscopy-based methods, which then makes it difficult to match diet
94 with field records of resources. Recently, DNA-based techniques have arisen as a powerful
95 approach for the taxonomic identification. Although DNA-based identification is not novel,
96 current technological developments such as DNA metabarcoding approach enable to
97 simultaneously identify different taxa in environmental samples, like soils or faeces,
98 containing multiple specimens and potentially highly degraded DNA (reviewed in Valentini
99 et al., 2009a; Taberlet et al., 2012; Pompanon et al., 2012). The efficiency of this approach
100 for analysing the diet has been demonstrated in various species including herbivorous
101 mammals, birds and invertebrates (Soininen et al., 2009; Valentini et al., 2009b),
102 insectivorous bats (Bohmann et al., 2011), or omnivorous animals like the brown bear (De
103 Barba et al., 2013).

104 In the present study, we focused on the wood mouse (*Apodemus sylvaticus*), which is a
105 generalist small mammal (Watts, 1968; Hansson, 1985; Butet, 1990) and therefore a model
106 of choice when studying diet plasticity as a response to environmental variations. Indeed,
107 provided its opportunistic feeding behaviour, the diet richness (i.e. total number of resources'
108 species in the diet) of the wood mouse is expected to be positively correlated with resource
109 richness in the field. We hypothesised that: (i) diet richness of the wood mouse would be
110 negatively correlated to TMs contamination of soils given the reported negative effects of
111 pollution on plant and invertebrate richness in community, and (ii) food selection by the
112 wood mouse would be affected by TM contamination in soils possibly due to avoidance
113 behaviour.

114

115 **Materials and methods**

116 **Study sites**

117 This study was carried out in the surroundings of the former lead (Pb) and zinc (Zn) smelter
118 named “Metaleurop Nord” located in northern France (Noyelles Godault, Hauts-de-France,
119 France). The surrounding soils were highly contaminated by TM, mainly cadmium (Cd), Pb,
120 and Zn, over a large area of about 120 km² because of metal-contaminated dust released for
121 decades from this smelter and another one named “Umicore”, located about 4 km east of the
122 first (Sterckeman et al., 2002; Douay et al., 2008; Douay et al., 2009; Waterlot et al., 2016).
123 A study area of 40 km² was defined and divided into 160 sites of 25 ha (500m x 500m)
124 (Fritsch et al., 2010), from which seven are used in the present study (see below). Total TM
125 concentrations in the soil of woody patches ranged from 0.1 to 236.5 mg kg⁻¹ of dry soil for
126 Cd, from 16 to 7331 mg kg⁻¹ of dry soil for Pb, and from 44 to 7264 mg kg⁻¹ of dry soil for
127 Zn in an area of 40 km² in the surroundings of the former smelter (Fritsch et al., 2010). These
128 values largely exceed background TM concentrations in soils of the region Hauts-de-France
129 (Sterckeman et al., 2007). Those TM concentrations in woody patches were used in our
130 modelling (cf. below). The seven sites are located along a soil pollution gradient and
131 composed of three types of dominant habitats (woodland, urban features, arable lands) to
132 provide a wide range of resource richness in the field (for details see Supporting Information
133 SII).

134

135 **Rodent trapping**

136 At each site, we trapped wood mice in spring (April) and in autumn (September and October)
137 2012 according to Fritsch et al. (2011) and in accordance with current French legislation
138 about ethics and use of animals in research. Briefly, small break-back traps were used with
139 peanut as rodent’s bait. Ten lines, composed of 10 traps 3 m spaced each, were used per site
140 and per season. All trap lines were set in woody habitats of the sites and their location was

141 geo-referenced. The trap lines were checked in the morning for three consecutive days and
142 re-set and/or re-baited, if necessary. After being weighed in the field, animals were
143 immediately frozen at -20°C for further analyses.

144 Two hundred and forty-six rodents (117 in spring and 129 in autumn; 116 females and 127
145 males) were used in the study. Age was estimated using body weight according to four
146 classes (approximately, class I < 50 days < class II < 130 days < class III < 230 days < class
147 IV), referring to Vandorpe & Verhagen (1980) and Tête et al. (2014). Age structure differed
148 between seasons: no mouse of age class I was found in spring, and the proportions of animals
149 in the other 3 classes were significantly different ($\chi^2 = 31.04$, p-value < 0.001) such that
150 animals in spring were older than in autumn.

151

152 **Stomach content samples**

153 Consumed items were identified by biomolecular analysis of stomach contents (SC). Wood
154 mice were thawed at room temperature. Their SC was extracted from the body with a spatula,
155 which was thoroughly cleaned with disposable tissue, then washed with ultra-pure water
156 (18.2 M Ω /cm²) and wiped off with other tissue between each extraction. Remaining bait was
157 removed from SC. Each SC was then homogenized and split into two aliquots: one (about
158 10 mg) was stored in 95% ethanol for metabarcoding analysis (see below), the other was
159 reserved for another study. For 13 mice among the 246 rodents, faeces taken from intestines
160 were used as an alternative material for food identification because their stomach was empty.
161 The 13 mice were equitably distributed according to the explanatory factors tested in this
162 study.

163

164 **Metabarcoding analysis**

165 The DNA extraction, amplification and the PCR purification were performed at SPYGEN

166 facilities (www.spygen.com). DNeasy Blood and Tissue Kit (Qiagen GmbH) was used for
167 extracting total DNA, following the manufacturer's instruction. DNA amplification was
168 performed with three sets of primers (Table 1): the P6 loop of the chloroplast *trnL* (UAA)
169 intron *g/h* (Taberlet et al., 2007) was used for identifying general plant species; primer
170 targeting a short fragment of mitochondrial 16S gene (16S mtDNA) was used for arthropods
171 and molluscs DNA (*16SMAV-F/16SMAV-R*) (De Barba et al., 2013); and primer targeting
172 short region of 16S mtDNA for earthworms (*ewD/ewE*) (Bienert et al., 2012). PCR
173 amplifications were carried out on Applied Biosystems Veriti 96 Wells (Life Technologies).
174 The amplification was realized in a final volume of 25 μ L using 3 μ L of DNA extract. Two
175 PCR replicates were performed per each sample. For the amplification of arthropod and
176 mollusc DNA, 2 μ M of a blocking primer for mammal's DNA (*MamMAVBI*; De Barba et
177 al., 2013) was added in the PCR mix. The amplification mixture contained 1 U of AmpliTaq
178 Gold DNA Polymerase (Applied Biosystems), 10 mM of Tris-HCl, 50 mM of KCl, 2.5 mM
179 of MgCl₂, 0.2 mM of each dNTP, 0.2 μ M of group-specific primers, 0.2 μ g/ μ L of bovine
180 serum albumin (BSA, Roche Diagnostic) and ultra-pure water (18.2 M Ω /cm²) to bring each
181 sample to the final volume. The mixture was denatured at 95°C for 10 min, followed by 45
182 cycles of 30 s at 95°C, 30 s at 50°C for *trnL-g/h*, at 55°C for *16SMAV-F/16SMAV-R* and at
183 58°C for *ewD/ewE* and 1 min at 72°C, followed by a final elongation at 72°C for 7 min.
184 Extraction and PCR negative controls were analysed in parallel in order to monitor potential
185 contamination. After amplification, the samples were titrated using capillary electrophoresis
186 (QIAxcel; Qiagen GmbH) and purified using a MinElute PCR purification kit (Qiagen
187 GmbH). Before sequencing, purified DNA was titrated again using capillary electrophoresis.
188 The purified PCR products were pooled in equal volumes, to achieve an expected sequencing
189 depth of 10,000 reads per sample. Libraries were prepared using TruSeq Nano DNA genomic
190 kit (Illumina) and a pair-end sequencing (2x100 bp) was carried out with an Illumina HiSeq

191 sequencer (Illumina) using TruSeq SBS Kit v3 (Illumina) following the manufacturer's
192 instructions. Library preparation and sequencing were performed at Fasteris facilities
193 (www.fasteris.com).

194 Reads were handled by the software Mothur pipeline (Schloss et al., 2009). Briefly, forward
195 and reverse reads were assembled in contig sequences. Then, sequences were filtered based
196 on length (20-90bp for *trnL-g/h*; 36-38bp for *16SMAV*; 69-81bp for *ewD/ewE*), homo-
197 polymer (less than 10 nucleotides) and no ambiguous nucleotides. After de-replication
198 (occurrence count of each different sequence), only unique sequences with a minimum count
199 of 10 (sum of all samples) were used for further analysis.

200

201 **Field inventory of plant and invertebrate resources**

202 Vegetation survey was realized once per site from 4th June to 5th September 2012. Three
203 different strata were defined based on height: tree stratum (woody species > 8 m high), shrub
204 stratum (woody species < 8 m high), and herbaceous stratum. Taxa were identified at species
205 level in the field. Nomenclature of species followed Lambinon et al. (2004) and Dudman &
206 Richards (1997). Cover-abundance of vascular plant species was visually estimated as the
207 vertically projected area following Braun-Blanquet et al. (1952). Vegetation habitats were
208 determined by plants' composition of one or more strata and delineated as polygons with the
209 aid of aerial pictures. The polygons were geo-referenced and digitalized using the QGIS
210 software (<http://www.qgis.org/>). We listed 236 different plant taxa in the field (see SI1 for
211 the taxonomic richness in the field per site and SI2 for the list of the taxa and their abundance
212 per site).

213 Invertebrate fauna was sampled at the same time as the rodent trapping (i.e. in the two
214 seasons). Ground-dwelling invertebrates were captured near rodent traps using three pitfall
215 traps per line. Flying invertebrates were captured with yellow pan traps containing a mix of

216 soap and water. Four yellow pan traps were set in each site. Yellow colour attracts especially
217 flower visiting insects. The traps were checked every morning for three consecutive days.
218 All traps were geo-referenced. Captured invertebrates were conserved in ethanol or in freezer
219 at -20°C and identified to the highest possible taxonomic resolution by morphological
220 characteristics (the main references are Coulon, 2003; Forel & Leplat, 2001; Jeannel, 1941;
221 Trautner & Geigenmueller, 1987). Captured ground-dwelling and flying invertebrates
222 consisted of 88 and 95 taxa, respectively (see SI1 for the taxonomic richness in the field per
223 site and SI2 for the list of the taxa and their abundance per site). Because of the different
224 taxonomic resolution, we used mainly family level (and higher levels) for invertebrate
225 richness in the field (cf below): 24 and 95 families (or higher) in ground-dwelling and flying
226 invertebrates, respectively.

227 We then estimated available resources for wood mice near their trapping location. Although
228 notion of “availability” is distinct from “abundance” in selection studies (Manly et al., 2002),
229 we considered them equivalent for wood mice given its generalist diet behaviour. Plant
230 resource availability was estimated by the cover-abundance of each plant species found
231 within a buffer of 10 m around trap lines (referred to as “buffer” thereafter). This buffer is
232 equivalent to an area of about 1000 m², considered as the average size of wood mouse vital
233 domain (Quéré & Le Louarn, 2011). We considered the data set collected between June and
234 September to be representative of food availability in spring and autumn. Since the traps
235 used to capture invertebrates (pitfall trap and yellow pan trap) were inappropriate for
236 catching earthworms and molluscs, both earthworm and mollusc richness in the field were
237 unknown. Resource availability of ground-dwelling invertebrates in buffers both in spring
238 and in autumn was estimated by the abundance of each taxa found per trap line (sum of the
239 three traps per line), whereas availability of flying invertebrates was based on abundance in
240 the yellow pan trap nearest to the trap line.

241

242 **Diet diversity analyses**

243 Sequence data for diet diversity analyses

244 Diet diversity analyses were performed by employing Molecular Operational Taxonomic
245 Units (MOTUs): groups of DNA sequences clustered according to similarity, which are thus
246 independent of any reference database (Sun et al., 2012). Sequences were clustered for each
247 primer with average neighbour algorithm using Needlman-Wunsch distance. Distances
248 cutting clusters were arbitrarily chosen as 0.032 for sequences obtained from primer *trnL-*
249 *g/h*, as 0.042 for *16SMAV* and as 0.034 for *ewD/ewE* (see SI3 for sequence data of plant,
250 arthropod and earthworm MOTUs). Deagle et al. (2013) showed that the number of
251 sequences obtained from degraded DNA is likely not proportional to the biomass of each
252 taxa really eaten. The quantitative sequence information data were therefore converted into
253 presence/absence of MOTUs using an occurrence threshold of 100 to remove background
254 sequencing data.

255

256 Diet richness and modelling

257 Richness in the diet of each mouse was calculated as the total number of MOTUs per mouse
258 by three types of food: plant, arthropod and mollusc (referred to as “arthropod” thereafter),
259 or earthworm. We assumed that plants potentially consumed by the invertebrates would not
260 artificially increase the number of plant MOTUs consumed by the rodent (i.e. there may be
261 no secondary predation, for detail see Harwood et al., 2001) because the number of plant
262 MOTUs was not significantly different between SCs containing or not containing
263 invertebrate food (arthropod and/or earthworm) (data not shown). The diet richness per
264 mouse was compared among seasons, wood mouse sexes and age-classes, and landscape
265 features using the nonparametric Wilcoxon-Mann-Whitney (for comparison of two

266 categories) or Kruskal-Wallis tests (for more than two categories), due to skewed distribution
267 of MOTUs data. Frequency of occurrence was also compared by chi-square goodness-of-fit
268 test.

269 Relationship between diet richness, richness in the field and TM concentrations in soil was
270 assessed by statistical modelling. In order to estimate the richness in the diet of all mice in a
271 buffer, the total number of MOTUs of all mice captured over a given buffer (referred to as
272 “diet richness” thereafter) was calculated, per type of food or as total diet richness by
273 summing plant, arthropod and earthworm MOTUs. The relationship was analysed using
274 multimodel inference on the basis of the information-theoretic approaches (Burnham &
275 Anderson, 2002). A set of candidate models was established by combinations of several
276 explanatory variables. Second order Akaike Information Criterion (AICc: corrected AIC for
277 small sample size in relation to the number of model parameters) was used for ranking the
278 proposed models. Probability of the “best” model over the set was estimated by Akaike
279 weight (w). In situations where the best model was uncertain (i.e. w of the first ranked model
280 < 0.9), we applied a model averaging approach for reducing model selection uncertainty. A
281 confidence set of candidate models, whose $\Delta AICc < 6$ (difference of AICc from the first
282 ranked model < 6 , which is equivalent to a likelihood ratio from the first ranked model $>$
283 0.05), was re-selected, and each parameter of those re-proposed models was averaged by re-
284 calculated Akaike weight (Burnham & Anderson, 2002). When a given parameter was not
285 included in a proposed model, the value of the parameter was considered as 0 in this model
286 (Burnham & Anderson, 2002; Lukacs et al., 2010).

287 All candidate models were established as generalized linear models (GLM) with logarithm
288 link function for Poisson distribution. Models were built separately per season, given
289 potential seasonal diet variation of the animal (Butet, 1986b; Abt & Bock, 1998), as well as
290 per TM, considering their different effects on organisms and transfer pathways (Eisler, 2000).

291 We used the diet richness in buffer as response variable and number of analysed mice per
292 trap line as one of the explanatory variables. Soil TM concentrations of woody patch
293 characterised by Fritsch et al. (2010) were applied to buffer set in or near a given woody
294 patch. Mice of age class I were discarded from modelling because diet of juveniles could
295 differ from adults (Watts, 1968; Montgomery & Montgomery, 1990). The modelling was
296 carried out on 38 buffers in spring and 37 buffers in autumn. Number of mice used in the
297 analysis was 181 and their number per line ranged from 1 to 6.

298 For plant richness in the diet, combinations of three variables and one interaction were
299 considered as candidate models: number of mice, TM concentrations in soils of woody
300 habitat (referred by the data of Fritsch et al., 2010; see "Study site" above) with a natural
301 logarithm transformation, and plant richness in the field, as well as an interaction between
302 the TM concentrations and the plant richness in the field (i.e. 10 models including the
303 intercept only model). For arthropod richness in the diet, four variables and two interactions
304 were used: mouse number, TM concentrations, both ground-dwelling and flying invertebrate
305 richness in the field, as well as two interactions between the TM concentration and the
306 richness in the field (i.e. 26 models). For earthworm richness in the diet, we used only two
307 variables: mouse number and TM concentrations (i.e. four models). For total diet richness,
308 we used three variables and one interaction: mouse number, TM concentrations, and total
309 richness in the field (sum of plant and both ground-dwelling and flying invertebrate richness
310 at family level), as well as interaction between the TM concentrations and the total richness
311 in the field (i.e. 10 models). Overdispersion (observed variance higher than theoretical one)
312 was checked according to Cameron & Trivedi (1990). Explanatory value of our final models
313 were estimated by deviance R^2 (R^2_D : proportion of deviance explained by the given model)
314 (Zuur, 2009).

315

316 **Food selection analyses**

317 Sequence data for selection analysis and association with field survey data

318 Reference sequences of the species recorded in our study area were taken from the GenBank
319 sequence database (www.ncbi.nlm.nih.gov/genbank). Each sequence extracted from SCs
320 (i.e., query sequence) was compared to the reference sequences for checking their similarity,
321 where we accepted difference of only one nucleotide. When query sequences could match
322 with several reference sequences, they were gathered into one group composed of the
323 corresponding species, called “sequence group (Grp)” (see SI4 for details of sequence
324 groups). Non assigned query sequences were excluded. Query sequences with occurrence
325 lower than 100 were not considered. Food Grp data were finally converted into
326 presence/absence. We represented Grps using both ID number and family name(s)
327 corresponding to their component species. We applied such method only to plant food
328 because almost all invertebrate resources in the field were identified to family level only.

329

330 Plant food selection and modelling

331 Frequency of occurrence of each plant food item (i.e. percentage of number of occurrences
332 of a given item compared to the total number of occurrence of all items, referring to Klare
333 et al., 2011) was calculated at family level using Grp. Food selection was assessed, for each
334 plant food item, per season, comparing the frequency of occurrence and relative proportion
335 of resources in the field (i.e. cover-abundance of a given plant food of all strata compared to
336 the total cover-abundance of all plants of all strata). Plant resource taxa in the field were
337 gathered into corresponding Grp and then into the same plant family. Resource availability
338 was estimated based on the abundance of the plant families in each buffer, summed up per
339 family for all mice in each season. Significance of food selection was evaluated per plant
340 family using Bailey’s confidence intervals at p-value < 0.05 (Bailey, 1980; Cherry, 1996):

341 when relative availability of a given resource was located below or above the confidence
342 interval of its frequency of occurrence, this resource is considered as preferred or avoided,
343 respectively.

344 Preference change according to richness in the field and/or TM concentrations in soil was
345 then assessed by multimodel inference approaches. First, food selection was estimated in
346 each buffer as described above. Buffers where selection for a given resource was observed
347 were considered 1 (i.e. selection for the resource is present), whereas the other buffers were
348 considered 0 (i.e. selection for the resource is absent). We used GLM with logit link function
349 for assessing change of proportion of buffers where preference was observed. There were
350 only one plant family (Salicaceae) for which selection was significantly observed in more
351 than five buffers, allowing statistical analyses. Salicaceae was preferred in spring in six
352 among 38 buffers. Candidate models were established by combinations of three variables
353 and one interaction: mice number in buffer, plant richness in the field, and TM concentrations,
354 as well as an interaction between the richness in the field and the TM concentrations. The
355 modelling was performed for each TM. Model selection methods were the same as described
356 above.

357 All statistical analyses were computed using the statistical software R (ver. 3.4.2; R
358 Development Core Team). GLM was handled by “glm” function in “lme4” package and
359 model averaging by “model.avg” function in “MuMIn” package. Overdispersion checking
360 was carried out by “dispersiontest” function in “AER” package.

361

362 **Results**

363 **Resources richness in the field, trace metals and diet richness**

364 Plant items occurred in the diet of 96.3% of wood mice, arthropod items in 65.9% and
365 earthworm items in 29.3%. The occurrence of earthworm items was significantly lower in

366 forest landscape and significantly higher in urban landscape ($\chi^2 = 7.619$, p-value = 0.022;
367 for details see SI5). The variations of the total richness in the diet and of the relative
368 proportion of the three food item categories (plants, arthropods and earthworms) along the
369 soil contamination gradient are shown in SI6 and SI7, respectively.

370 Number of plant MOTUs per mouse ranged from 0 to 7 and median was 2 MOTUs. It was
371 significantly higher in autumn than in spring ($W = 5394.5$, p-value < 0.001). Number of
372 arthropod MOTUs ranged from 0 to 9 and median was 1 MOTUs. In autumn, the number
373 was significantly lower in age class I than in class II (Kruskal-Wallis $\chi^2 = 7.972$, p-value =
374 0.047) (N.B. no mouse of class I was found in spring). Number of earthworm MOTUs ranged
375 from 0 to 3 and median was 0 MOTUs. The number was significantly higher in urban area
376 than in forest area in spring only (Kruskal-Wallis $\chi^2 = 8.068$, p-value = 0.018) (see SI8).

377 No single “best” model was evidenced and thus model averaging was applied for all sets of
378 models. Complete results of modelling are shown in SI9. The final model for plant richness
379 in the diet in buffer included mouse number, soil TM concentrations, richness in the field
380 and the interaction between the last two for the three TM in the two seasons. Figure 1a and
381 1b show plant richness in the diet according to plant richness in the field and Cd
382 concentration in soils in spring, respectively. The pattern was similar for the other metals.
383 Influence of richness in the field on the diet richness was tiny in spring (Figure 1a), whereas
384 diet richness decreased according to TM in soils regardless of the richness in the field (e.g.
385 from 6 to 4 plant MOTUs along the range of TM concentration; Figure 1b). R^2_D was 0.301,
386 0.313 and 0.307 for Cd, Pb and Zn, respectively. Such tendency changed in autumn (Figure
387 1c and 1d for Cd, similar for other TM). Diet richness was negatively correlated to plant
388 richness when soil contamination was low, while this correlation disappeared, or even
389 reversed, when TM concentration increased (Figure 1c). A similar tendency was predicted
390 for the relationship between diet richness and TM contamination in soils (Figure 1d): diet

391 richness was negatively correlated to TM concentration when plant richness was low and
392 positively correlated when the plant richness was high. R^2_D was 0.624, 0.492 and 0.601 for
393 Cd, Pb and Zn, respectively.

394 The model for invertebrate richness in the diet included all proposed explanatory variables
395 in the two seasons, except the interaction between TMs in soils and richness of ground-
396 dwelling invertebrates for the models for Cd and Zn in autumn. In spring, arthropod richness
397 in the diet increased according to richness of both ground-dwelling and flying invertebrates
398 when soil contamination was low. This correlation disappeared, then reversed, when TM
399 concentration in soils increased. For example, our model for Cd predicted that arthropod
400 MOTUs increased from 2 to 5 along the gradient of ground-dwelling invertebrate richness
401 in the field when Cd concentration in soils was low, whereas arthropod MOTUs decreased
402 from 5 to 3 when Cd concentration was high (Figure 2a; the pattern was similar for flying
403 invertebrates). The relationship between arthropod richness in the diet and TM concentration
404 in soils followed the same tendency (Figure 2b). In autumn, arthropod richness in the diet
405 was explained almost only by mouse number. Neither TM concentration in soils nor richness
406 of both types of invertebrates had sensible influence on diet richness (Figure 2c and 2d). R^2_D
407 for Cd, Pb and Zn was respectively 0.502, 0.472 and 0.495 in spring, and 0.495, 0.504 and
408 0.497 in autumn.

409 The averaging model for earthworm richness in the diet included mouse number and TM
410 concentrations in soils in both seasons. Earthworm richness in the diet slightly decreased
411 along TM gradient (e.g. difference of about 0.5 MOTUs; Figure 3a and 3b), and mouse
412 number on trap line had a larger influence in autumn than in spring. R^2_D for Cd, Pb and Zn
413 was respectively 0.101, 0.056 and 0.075 in spring, and 0.169, 0.158, 0.192 in autumn.

414

415 **Plant food selection**

416 We found 48 Grps in the food of the 246 mice. Sapindaceae, Salicaceae, Cornaceae,
417 Adoxaceae, Asteraceae, and Rosaceae were plant taxa frequently eaten in our area (see SI10
418 for details). Selection for some plant families was observed at the scale of all study sites
419 together and at the two seasons: preference for Fagaceae and avoidance of Urticaceae.
420 Furthermore, selected plant resources changed between the two seasons, for example
421 preference for Salicaceae, Sapindaceae and Rosaceae in spring and preference for Poaceae,
422 Adoxaceae, Asteraceae and Cornaceae in autumn (see SI11 for details).
423 Model averaging was used for the final model for Salicaceae preference in spring (see SI12
424 for complete results). The model included all variables: mouse number, plant richness in the
425 field, TM concentration in soils and the interaction. Preference for Salicaceae was negatively
426 correlated to plant richness in the field when soil contamination was low (e.g. from 0.6 to
427 0.4; Figure 4a). On the other hand, preference for Salicaceae disappeared along the gradient
428 of TM concentration in soils, whatever plant richness in the field was (e.g. preference
429 probability for Salicaceae decreased from 0.6 to 0; Figure 4b). R^2_D of the final model was
430 0.280, 0.165 and 0.271 for Cd, Pb and Zn, respectively.

431

432 **Discussion**

433 We first hypothesised that soil TM contamination would reduce diet richness of wood mouse
434 due to adverse effect of TM on resources' richness in the field. Our study revealed negative
435 correlations between diet richness and TM in soils. This was true for both earthworms and
436 plants in spring and in autumn (although only at low and medium levels of plant richness in
437 autumn), and for arthropods in spring when richness in the field was high. On the contrary,
438 positive correlations were observed, for plants in autumn when plant richness in the field
439 was high, and for arthropods in spring when arthropod richness in the field was low. A
440 negative relationship between diet richness and pollution by TM is expected if resource

441 richness in the field is negatively affected by TM concentrations. Literature about the effects
442 of pollution on various taxonomic groups suggests a global negative effect of pollution on
443 the richness of organisms, and an absence or a positive correlation for a few taxa groups or
444 situations. Although decreasing richness of earthworms with increasing TM concentrations
445 gradients in soils has been widely reported (Spurgeon & Hopkin, 1996, Spurgeon & Hopkin,
446 1999; Pérès et al., 2011), several studies have reported that relationships between species
447 richness of soil macro-fauna and TM contamination is not straightforward (Nahmani &
448 Lavelle, 2002; Gillet & Ponge, 2003; Migliorini, et al., 2004). Moreover, several studies
449 showed that community composition was affected by soil TM concentrations. Tolerance to,
450 and/or accumulation capacity of metals differ according to taxa, organs, and metals (Das et
451 al., 1997; Broadley et al., 2007; Pourrut et al., 2011). Lower resistance to toxic effects can
452 make metal sensitive plants disappear in contaminated areas, which may favour metal
453 tolerant ones, sometimes without modification of taxonomic richness. Such modification of
454 community composition has been reported by several authors in plants (Banášová et al.,
455 2006; Strandberg et al., 2006; Dazy et al., 2009) and in invertebrates (Nahmani & Lavelle,
456 2002; Migliorini et al., 2004; Babin-Fenske & Anand, 2011). In our study area, we found no
457 correlation or even positive trends between TM concentration in soils and both plant and
458 invertebrate richness in the field (unpublished data). However, there were confounding
459 factors in our site like the anthropogenic increase of habitat diversity (see Douay et al., 2009)
460 and possibly of plant richness along the gradient of contamination on the Metaleurop Nord
461 site. Community composition also differed according to soil contamination levels or other
462 soil properties affecting metal bioavailability (unpublished data). Difference in resource
463 composition could alter resource availability and thus modify feeding behaviour and diet
464 composition. Few studies documented the effects of pollution on the diet of terrestrial
465 vertebrates, most of them dealing with diet composition rather than richness or diversity.

466 Clare et al. (2014), describing the diet of insectivorous bats *Myotis lucifugus* in different
467 locations, showed a trend of lower diet species richness in area of lower habitat quality
468 (acidification or organic pollution). Eeva et al. (2005) showed a significant difference in diet
469 composition of nestlings of two insectivorous birds, *Parus major* and *Ficedula hypoleuca*,
470 between unpolluted and TM polluted sites, reflecting differences in resources composition,
471 phenology and feeding ecology among the two bird species: in polluted areas where
472 caterpillars were less abundant, both species ate more beetles and different larvae of flying
473 insects but less moth. The study of Heroldová (2002) demonstrated that the diet of field vole
474 *Microtus agrestis* in forest clearings caused by air pollution was dominated by grasses,
475 reflecting higher abundance of grasses and a poorer diversity of food supply in clearings.
476 Thus, it is suggested that diet richness (as demonstrated in the present study) and/or
477 composition can be affected by pollution induced changes in trophic resources.

478 We also hypothesized that TM contamination would affect food selection by wood mouse.
479 Our model predicts that preference probability for Salicaceae in spring in the highest
480 contaminated sites decreases to become almost null. Avoidance of TM contaminated soil or
481 food has been shown in invertebrates but has rarely been investigated in vertebrates, and is
482 particularly hard to prove under field conditions in comparison with controlled experiments.
483 This behaviour has been observed under laboratory conditions for some invertebrates like
484 isopods or small moths (Odendaal & Reinecke, 1999; Zidar et al., 2004; Scheirs et al., 2006),
485 and for the wood mouse (Beernaert et al., 2008), but mechanisms of such aversion are not
486 yet elucidated. In Beernaert et al. (2008), wood mice chose acorns (*Quercus robur*,
487 Fagaceae) sampled from unpolluted sites rather than ones from metal polluted sites (mainly
488 by Cd, copper, Pb, and Zn), but such avoidance behaviour was no longer detected under field
489 conditions, perhaps due to ecological co-factors such as food shortage, and/or the presence
490 of competitors and predators. Avoidance of contaminated food, leading to shifts in diet, can

491 be one of the mechanisms allowing animals to mitigate their exposure in highly polluted
492 environments (Gillet & Ponge, 2003). Actually, Salicaceae family is considered as TM
493 accumulator and resistant to highly TM contaminated soils (Pulford & Watson, 2003).
494 Migeon et al. (2009) showed that Salicaceae family in the surroundings of Metaleurop Nord,
495 composed of two genera *Salix* and *Populus*, accumulated larger amount of Cd and Zn in their
496 shoot parts than the other families like Fagaceae or Sapindaceae. These two last are also
497 preferred plant families in our study and known as common food of wood mice (i.e. Watts,
498 1968). However, mechanisms of changes in preference remains unclear. The nutritional
499 quality of metal contaminated plant organs has been shown to be lowered, with for instance
500 a decrease of sugar or protein concentrations (Scheirs et al., 2006; Beernaert et al., 2008),
501 features which play an important role for food choice in rodents (Butet, 1990; Kerley &
502 Erasmus, 1991). It is also possible that animals might identify metal contaminated plants due
503 to metal-induced metabolic compounds (Poschenrieder et al., 2006), largely known as
504 compounds limiting feeding (Foley & Moore, 2005). Dearing et al (2000) reported that plant
505 secondary compounds might play more important role than nutrient level in the diet of the
506 generalist woodrat, *Neotoma albigula*. It is therefore supposed that mice could directly or
507 indirectly detect noxious substances (plant toxins or TM) and avoid ingesting such items.
508 Also, Behmer et al. (2005) demonstrated from their experiments using nutritionally balanced
509 synthetic food that high Zn concentration *per se* altered the feeding behaviour of the desert
510 locust *Schistocerca gregaria*. Whatever the mechanism, the hyperaccumulation of metals in
511 plant tissues contribute to defensive effects of plants against natural enemies, including
512 invertebrate herbivory (Poschenrieder et al., 2006; Boyd, 2007). Such defensive effect has
513 not been reported against vertebrates so far, but the disappearance of the preference for
514 Salicaceae family along TM contamination in soils could be associated to its high metal
515 accumulation capacity. Furthermore, plant richness in the diet in autumn increased along the

516 soil TM concentration gradient when richness in the field was high. Apart from changes of
517 resource composition, this pattern could be explained, at least partly, by the nutrient balance
518 hypothesis or the detoxification limitation hypothesis: if nutritional quality of all plant
519 resources was altered (i.e. if nutritional quality was not clearly contrasted among resources)
520 diet richness of animals could increase for completing nutrient and/or for avoiding loads of
521 similar secondary compounds.

522 Contrary to plants, selection for invertebrate food could not be analysed in part because of
523 the primer used for arthropods and molluscs: its short size is more reliable for the analysis
524 of degraded DNA in the case of diet (Taberlet et al., 2012) but the precision in taxonomic
525 assignment was low. We also did not sample earthworms in the field. Moreover, it is difficult
526 to determine selection for invertebrate resources because of their mobility. Foraging strategy
527 of predators can be modified according to mobility of preys (Sih & Christensen, 2001),
528 suggesting that relative quantity in the field is not the only factor to consider when analysing
529 mobile prey selection. Even though we supposed that decreased earthworm diet richness
530 might be linked to their richness in the field, invertebrate food selection in wood mice
531 remains as a further challenge.

532 An important feature of our results is the strong seasonal difference of patterns, for both food
533 selection and correlations with TM contamination. In temperate ecosystems, seasons have a
534 key influence on the dynamics of ecosystems and on the various aspects of biology and
535 ecology of living organisms. Seasonal difference of patterns might result from the adaptation
536 of wood mice feeding behaviour to phenology of resources. Indeed, wood mice consume
537 mainly seeds and other reproductive organs (Watts, 1968; Butet, 1986a), seeking high energy
538 or protein-rich tissues (Butet, 1990). Although parts of plant resources actually consumed
539 could not be distinguished by using DNA metabarcoding, we supposed that parts of plants
540 preferably consumed have changed between spring and autumn. Moreover, the nutritional

541 composition and quality of plants and invertebrates are likely to vary according to seasons.
542 Also, resources functionally important for wood mice might have a strong phenology, such
543 as mushrooms in autumn. However, the molecular primers we used did not target mushrooms,
544 which prevents further conclusion. Finally, TM accumulation in organisms is also subject to
545 seasonality at contaminated site (i.e. Hunter et al, 1987b; Migeon et al., 2009). Seasonal
546 variation of TM accumulation pattern can be related to growth season or physiological
547 changes of each taxa (Kabata-Pendias, 2011; Hussein et al., 2006). Disentangling the
548 combined effects of these factors on the patterns of interest in this study is beyond our present
549 scope, but it is clear that seasonality is a key feature of the interplay between pollution,
550 resource dynamics and consumer feeding behaviour.

551 Our study sheds light on the effects of soil TM contamination on patterns of feeding
552 behaviour of a generalist consumer, by applying DNA metabarcoding for diet identification.
553 We underline potential altering effects of TM on the diet richness, which possibly partly
554 results from modifying food selection, at least for plant resources. Such modification of diet
555 related to habitat contamination might lead to a different pattern of contaminant exposure in
556 the animals. We hence suggest that ecosystem pollution should now be considered as a driver
557 of changes in food web structure and dynamics in time and space.

558

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843 **Data Accessibility:**

844 DNA sequence data, soil TM concentration in buffers, resources availability in buffers, and
845 wood mouse data are available from the metadata portal “dat@osu”;
846 doi:10.25666/DATAOSU-2018-07-17.

847

848 **Supporting Information:**

849 SI1: Information for each study site about TM contamination and dominant landscape
850 feature, as well as richness of plants and invertebrates observed in the field.

851 SI2: Inventory of plant and invertebrate taxa observed in each site.

852 SI3: Sequence data of plant, arthropod and earthworm MOTUs for each mouse.

853 SI4: Sequence data of sequence groups for each mouse.

854 SI5: Frequency of occurrence of plant, arthropod and earthworm items in wood mouse food
855 across several environmental and biological factors.

856 SI6: Variation of the total richness in the diet along the soil contamination gradient.

857 SI7: Relative proportion of the three food item categories (plants, arthropods and
858 earthworms) along the soil contamination gradient.

859 SI8: Plant, arthropod and earthworm richness in the diet of wood mouse across several
860 environmental and biological factors.

861 SI9: Complete data on models of richness in the diet.

862 SI10: Complete data on number and frequency of occurrence of plant food item (sequence
863 group).

864 SI11: Information on food selection for plant families corresponding to sequence groups in
865 the wood mouse in spring and in autumn.

866 SI12: Complete results on plant food selection modelling at buffer scale.

867

868 **Author contributions:**

869 F.R. and R.S. conceived the study; S.O., R.S., C.F. and F.R. designed the study; R.S., C.F.,
870 F.M., T.C. and F.R. performed field samplings and laboratory treatments; B.V. performed
871 bioinformatics treatments; S.O. performed the statistical analyses; S.O., F.R., R.S. and C.F.
872 interpreted the results. S.O. wrote the manuscript under the supervision of F.R, R.S. and C.F.

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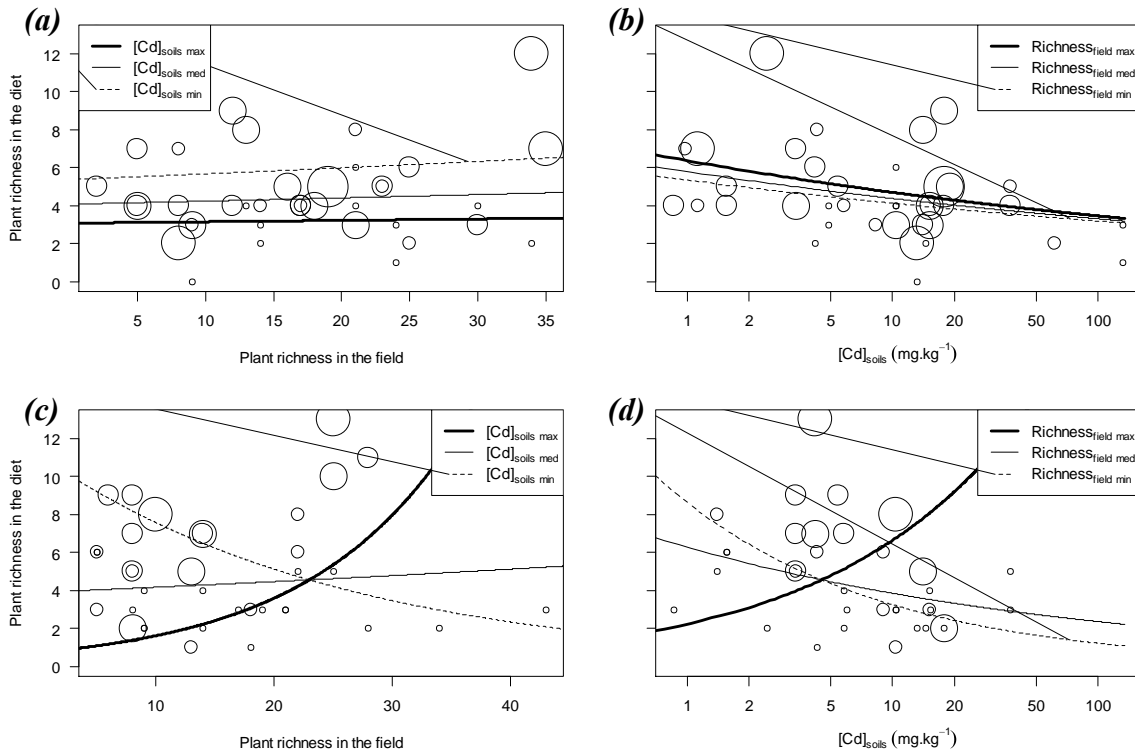
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Table 1: Characteristics of the three primers used to amplify wood mouse stomach content DNA samples.

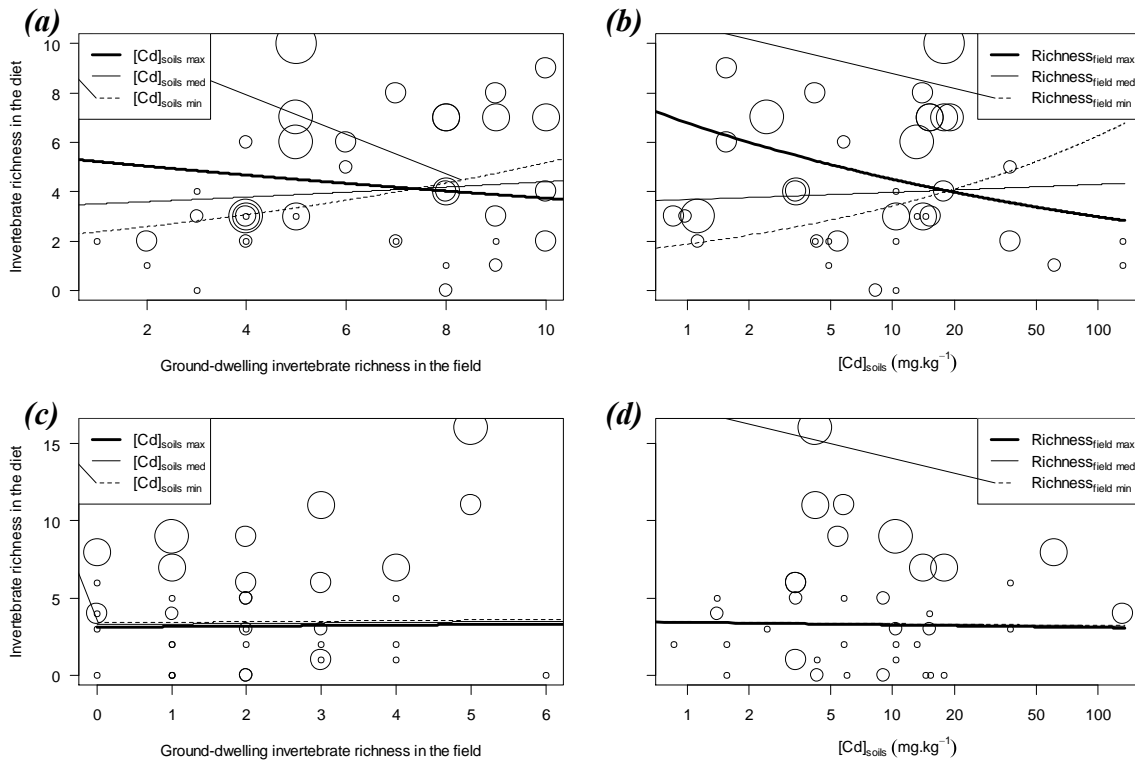
Taxonomic group	DNA type	DNA region	Primer name	Primer sequence 5'-3'	Reference
Plants	Chloroplast	trnL (UAA)	g (<i>forward</i>) h (<i>reverse</i>)	GGGCAATCCTGAGCCAA CCATTGAGTCTCTGCACCTATC	Taberlet <i>et al.</i> , 2007
Arthropods & molluscs	Mitochondrial	16S mtDNA	16SMAV-F 16SMAV-R	CCAACATCGAGGTCRYAA ARTTACYNTAGGGATAACAG	De Barba <i>et al.</i> , 2013
Earthworms	Mitochondrial	16S mtDNA	ewD (<i>forward</i>) ewE (<i>reverse</i>)	ATTCGGTTGGGGCGACC CTGTTATCCCTAAGGTAGCTT	Bienert <i>et al.</i> , 2012

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877 *Figure 1: Plant richness in the diet of the wood mouse along the gradient of plant richness*
 878 *in the field and Cd concentration in soils, in spring (a and b) and in autumn (c and d). Each*
 879 *point represents a buffer and point size is proportional to the number of mice captured in a*
 880 *given buffer. Values predicted from models are indicated by three lines: dotted, normal and*
 881 *bold lines correspond to minimal, median and maximum value of Cd concentration in soils*
 882 *(a and c) or plants richness in the field (b and d), respectively. The number of mice is fixed*
 883 *to the median value for drawing the lines (three mice in spring and two mice in autumn).*
 884 *The patterns are similar for Pb and Zn concentrations in soils.*



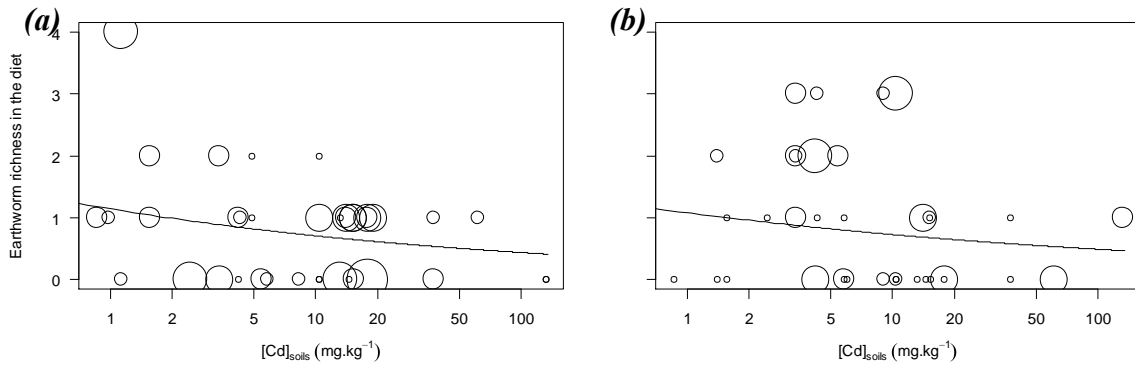
888 *Figure 2: Invertebrate richness in the diet of the wood mouse along the gradient of ground-*
 889 *dwelling invertebrate richness in the field and Cd concentration in soils in spring (a and b)*
 890 *and in autumn (c and d). Each point represents a buffer and point size is proportional to the*
 891 *number of mice captured in a given buffer. Values predicted from models are indicated by*
 892 *three lines: dotted, normal and bold lines correspond to minimal, median and maximum*
 893 *value of Cd concentration in soils (a and c) or ground-dwelling invertebrate richness in the*
 894 *field (b and d), respectively. Mouse number is fixed to the median value for drawing the lines*
 895 *(three mice in spring and two mice in autumn). The patterns of the relation between ground-*
 896 *dwelling invertebrate richness in the diet and in the field are similar for flying invertebrate*
 897 *richness. The patterns are similar for Pb and Zn concentrations in soils.*



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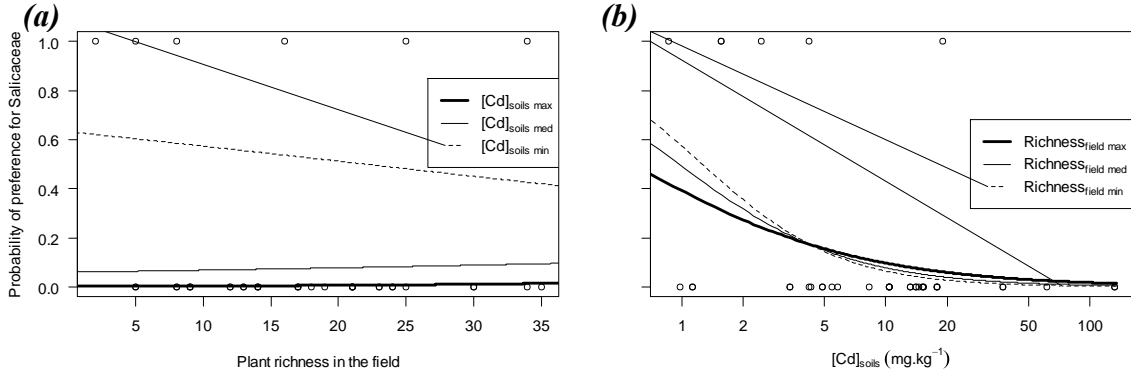
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901 Figure 3: Earthworm richness in the diet of the wood mouse along the gradient of Cd
902 concentration in soils in spring (a) and in autumn (b). Each point represents a buffer and
903 point size is proportional to the number of mice captured in a given buffer. Values predicted
904 from our model are indicated by a line. Mouse number is fixed to the median value for
905 drawing the line (three mice in spring and two mice in autumn). The patterns are similar for
906 Pb and Zn concentrations in soils.



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908

909 *Figure 4: Predicted probabilities of preference for Salicaceae in spring along the gradient*
 910 *of plant richness in the field (a) and Cd concentration in soils (b). Plotted points at*
 911 *probability value 1 indicate buffers where preference for Salicaceae was observed, and*
 912 *points at 0 indicate buffers where preference was not observed. Predicted probability form*
 913 *our model is indicated by three lines: dotted, normal and bold lines correspond minimal,*
 914 *median and maximum value of Cd concentrations in soils (a) or plant richness in the field*
 915 *(b), respectively. Mouse number is fixed to the median value for drawing the lines (three*
 916 *mice in spring and two mice in autumn). The patterns are similar for Pb and Zn*
 917 *concentrations in soils.*



918