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Shinji Ozaki, Clémentine Fritsch, Benoît Valot, Frédéric Mora, Thierry Cornier, et al.. How Do Richness and Composition of Diet Shape Trace Metal Exposure in a Free-Living Generalist Rodent, Apodemus sylvaticus. Environmental Science and Technology, 2019, 53 (10), pp.5977-5986. 10.1021/acs.est.8b07194 . hal-02316627

HAL Id: hal-02316627
https://hal.science/hal-02316627

Submitted on 7 Feb 2022

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How do richness and composition of diet shape trace metal exposure in a free-living generalist rodent, *Apodemus sylvaticus*.

Shinji Ozaki[*†], *Clémentine Fritsch*[†], *Benoit Valot*[†], *Frédéric Mora*[‡], *Thierry Cornier*[§], *Renaud Scheifler*[†#] *and Francis Raoul*[†#]

† Laboratoire Chrono-environnement, UMR 6249 CNRS/Université Bourgogne Franche-Comté UsC INRA, 16 route de Gray, 25030 Besançon Cedex, France

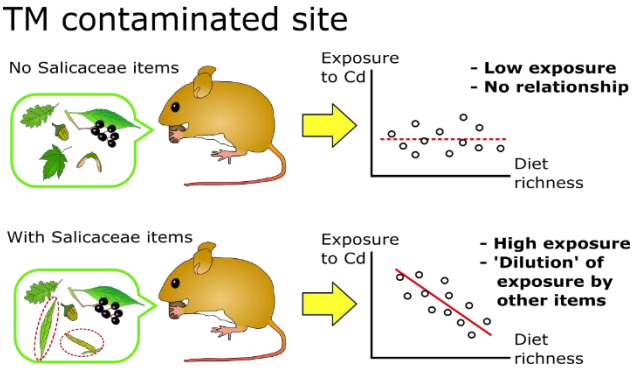
‡ Conservatoire Botanique National de Franche-Comté, Observatoire Régional des Invertébrés, 7 rue Voirin, 25000 Besançon, France

§ Centre régional de phytosociologie agréé Conservatoire Botanique National de Bailleul, Hameau de Haendries, F-59270 Bailleul, France.

KEYWORDS

Food identification; Metabarcoding; Trophic transfer; Wildlife; *Salicaceae*.

15 **Abstract Art**



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Abstract

Exposure of terrestrial mammals to chemical contaminants like trace metals (TMs) is considered to be mainly based on trophic transfer. Although relationships between TM transfer to animals and identity of contaminated food have been studied, the variation of the TM transfer with respect to diet diversity has been poorly documented. In this study, the oral exposure to TMs of wood mice *Apodemus sylvaticus* was investigated with respect to both the number of different items, i.e. diet richness, and the identity of items determined by metabarcoding from their stomach content, i.e. diet composition. The results showed that consuming Salicaceae, a known cadmium accumulator plant family, significantly increased exposure to cadmium and zinc. However, an increase in diet richness minimized exposure to cadmium when mice consumed Salicaceae items. This strongly suggests that TM accumulator items can lead to a high oral exposure to TMs but that such high exposure due to TM accumulator items can be "*diluted*" by diet richness due to other low accumulator items. Our results clearly indicate that both the presence of certain items in the diet and diet richness are important determinants of exposure to TMs in generalist animals, which matches the predictions of the "*diet dilution hypothesis*".

Introduction

Trace metals (TMs) are naturally occurring substances in trace amounts, in a proportion below 0.1%, in natural media.[1] Although some of them are essential for life, TMs can pose risks to both wildlife and human health at elevated concentrations resulting from anthropogenic activities.[2] The assessment and the mitigation of environmental risk of TM contamination of ecosystems require both a sound understanding and an accurate estimate of the exposure of free-living organisms to TMs. The exposure of wild mammals to TMs occurs mainly through consumption of contaminated food.[3] The composition of diet has been focused on as an important aspect of the variation of exposure of small mammals to TMs. For instance, earthworms or snails, organisms that may accumulate TMs at high concentrations in their tissues, have been considered to be important contributors for exposure to TMs in mammals consuming them.[4–6] However, mammals can consume simultaneously numerous different items. Although ingesting some metal accumulator items leads to an increase in exposure, many other less TM accumulator items could reduce total TM concentrations in the diet. A wide variety of items consumed may result in two opposite effects: increased opportunity of consuming TM accumulators or reduced TM concentrations of ingested food. However, few studies have assessed the accurate diet composition and richness related to the TM contamination in mammals,[7] and, to our knowledge, no study have investigated the relationships between the oral exposure of mammals to TMs and such diet richness.

The accurate determination of items actually consumed is a major challenge in wildlife diet assessment because identification at high taxonomic resolution is almost impossible with classical macro- or microscopy-based methods. However, current technological development such as high-throughput next generation sequencing has led a rise of new DNA-based techniques

as a powerful approach for multispecies identification using degraded DNA extracted from wide range of environmental samples, i.e. soil, water or feces (*eDNA metabarcoding*).[8–12] The efficiency of the eDNA metabarcoding has been demonstrated in diet analysis in various mammals.[13–17] This method is expected to identify in detail the diet composed of numerous items in generalist small mammals like the wood mouse (*Apodemus sylvaticus*),[18–21] a rodent widely spread in Europe[22] and frequently used for monitoring of environmental metal pollution,[5,6,23–25] and then to allow associating the oral exposure of the rodent to TMs and both the composition and the richness of their diet.

The present study analyzed the relationship between exposure to TMs of wood mice captured along a gradient of soil TM contaminations in a smelter-impacted area and their diet determined by metabarcoding from their stomach content. We hypothesized that (1) the consumption of (hyper-)accumulator items like earthworms induces a high exposure of small mammals to TMs and that (2) such exposure is shaped by the diet richness due to a "dilution" effect where high exposure related to accumulator items is reduced by the consumption of less accumulator items in case of high diet richness.

Materials and methods

Study Area. The study area was located in Northern France (Noyelles Godault, Hauts-de-France, France). Due to metal containing dust released from the former lead (Pb) and zinc (Zn) smelter "Metaleurop Nord", the soils surrounding the smelter are highly contaminated by TMs, especially cadmium (Cd), Pb and Zn.[26–29] Total TM concentrations in soils of woody habitats in an area of 40km^2 around Metaleurop Nord range from 0.1 to 237 mg kg^{-1} of dry soil for Cd, from 16 to 7331 mg kg^{-1} for Pb, and from 44 to 7264 mg kg^{-1} for Zn, which dramatically exceed the

concentrations in a reference site (0.9-2, 43-200 and 89-278 mg kg^{-1} of dry soil for Cd, Pb, and Zn, respectively) from the same region.[30] The present study was undertaken on seven sites of 500m x 500m, located along a soil contamination gradient and composed of three types of dominant habitats (for details see Supporting Information Table S1). TM concentrations in soils of woody habitats, expressed as mg kg^{-1} of dry soil, were based on data published in Fritsch et al.[30] and used for statistical analyses.

Sample Collection. *Rodent Trapping*. Wood mice were captured in spring (April) and in autumn (September and October) 2012, in accordance with current French legislation about ethics and use of animals in research. In each season and each site, 10 trap lines composed of 10 small break-back traps (3 m spaced each) were used with peanut as rodent's bait following Fritsch et al.[31] All trap lines were set in woody habitats and their position was geo-referenced. The trap lines were checked in the morning for 3 consecutive days and re-set and/or re-baited, if necessary. Captured animals were immediately frozen and stored at -20ºC for further analyses in the laboratory. For this study, 200 wood mice composed of 95 females, 103 males and two individuals whose sex could not be identified were used. The sex-ratio was not significantly different from 1:1 (chi-squared = 0.32, *p-value* = 0.57). One hundred and three mice were taken in spring and 97 in autumn. The ratio between the two seasons was not significantly different from 1:1 (chi-squared = 0.18, *p-value* = 0.67).

Stomach Content Extraction. Stomach content (SC) was extracted with a spatula from each rodent's body thawed at room temperature. After removing remaining bait, each SC was homogenized and split into two aliquots. One aliquot (about 10 mg) was stored in 95 % ethanol for the metabarcoding analysis. The other was used for measuring TM concentrations which

were used to estimate exposure of mice to metals *via* the food ingested within the last few hours. The spatula was thoroughly cleaned with disposable tissues, then washed with ultra-pure water (18.2 MΩ/cm^2 by Millipore Milli-Q Integral 3) and wiped off with other tissue between each extraction.

Metal Analysis. The SCs for TM concentration analysis were dried at 50°C in an oven until obtaining constant mass. The dried samples were digested in HNO_3 (67-69 %; Fisher Scientific Bioblock, ultratrace quality Optima) with H_2O_2 (Fisher Scientific Bioblock) by using Digiprep (SCP Sciences). The samples were then diluted by adding ultra-pure water (18.2 MΩ/cm^2). TM concentrations were measured with an inductively coupled plasma mass spectrometry (ICP-MS: X Series II, ThermoFischer Scientific) and expressed as μg g^{-1} of dry mass (DM). Analysis accuracy was checked by using certified reference materials (INCT-OBTL-5: Oriental Basma Tobacco Leaves). Mean and standard deviation of recovery rate for Cd, Pb and Zn were respectively 107.2 ± 4.7, 83.6 ± 9.6 and 100.7 ± 9.1 %.

Diet Assessment Using Metabarcoding Analysis. *DNA Extraction, Amplification and Sequencing*. The DNA extraction, amplification and the PCR purification were performed at SPYGEN facilities (www.spygen.com). DNeasy Blood and Tissue Kit (Qiagen GmbH) was used for extracting total DNA, following the manufacturer's instruction. DNA amplification was performed with three sets of primers: primer targeting the P6 loop of the chloroplast *trnL* (UAA) intron *g*/*h*[32] was used for identifying general plant species, primer targeting a short fragment of mitochondrial 16S gene (16S mtDNA) was for arthropod and mollusk DNA (*16SMAV-F*/*16SMAV-R*)[16], and primer targeting a short region of 16S mtDNA for earthworm (*ewD*/*ewE*)[33]

(for details of those primers, see Supporting Information Table S2). PCR amplifications were carried out on Applied Biosystems Veriti 96 Wells (Life Technologies). The amplification was realized in a final volume of 25 µL using 3 µL of DNA extract. Two PCR replicates were performed per each sample. For the amplification of arthropod and mollusc DNA, 2 µM of a blocking primer for mammal's DNA (*MamMAVB1*[16]) were added in the PCR mix. The amplification mixture contained 1 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems), 10 mM of Tris-HCl, 50 mM of KCl, 2.5 mM of $MgCl_2$, 0.2 mM of each dNTP, 0.2 µM of group-specific primers, 0.2 µg/µL of bovine serum albumin (BSA, Roche Diagnostic) and ultra-pure water (18.2 MΩ/cm2) to bring each sample to the final volume. The mixture was denatured at 95°C for 10 min, followed by 45 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 58°C for *ewD*/*ewE* and 1 min at 72°C, followed by a final elongation at 72°C for 7 min. Extraction and PCR negative controls were analyzed in parallel in order to monitor potential contamination. After amplification, the samples were purified using a MinElute PCR purification kit (Qiagen GmbH). Before sequencing, purified DNA was titrated using capillary electrophoresis (QIAxcel; Qiagen GmbH). The purified PCR products were pooled in equal volumes, to achieve an expected sequencing depth of 10,000 reads per sample. Libraries were prepared using TruSeq Nano DNA genomic kit (Illumina) and a pair-end sequencing (2x100 bp) was carried out with an Illumina HiSeq sequencer (Illumina) using TruSeq SBS Kit v3 (Illumina) following the manufacturer's instructions. Library preparation and sequencing were performed at Fasteris facilities (www.fasteris.com).

Bioinformatics. Reads were handled by the software Mothur pipeline.[34] Forward and reverse reads were assembled in contig sequences. Sequences were filtered based on length (20-90bp for *trnL-g*/*h*; 36-38bp for *16SMAV*; 69-81bp for *ewD*/*ewE*), homo-polymer (less than 10 nucleotides)

148 and no ambiguous nucleotides. After de-replication (count occurrence of each different
149 sequence), only unique sequences with a minimum count of 10 (sum of all samples) were used
150 for further analyses. The data was applied both to diet richness estimation and to identification of
151 food composition.

152 *Diet Richness Estimation.* Molecular Operational Taxonomic Units (MOTUs) were used for
153 measuring diet richness. Sequences were clustered for each primer with average neighbor
154 algorithm using Needlman-Wunsch distance. The cutoff values were chosen as effective
155 clustering on the basis of relationships between number of clusters and distance: 0.032, 0.042,
156 and 0.034 for sequences obtained from the primers *trnL-g/h*, *16SMAV* and *ewD*/*ewE*,
157 respectively. After using an occurrence of 100 as a threshold to remove background sequencing
158 data, the sequence data were converted into presence/absence of MOTUs because the number of
159 sequences from degraded DNA is likely not proportional to the biomass of each taxa really
160 eaten.[35]

161 *Food Composition.* For the detail of food identification method, see Ozaki et al.[36] Briefly, each
162 sequence extracted from SCs (i.e., query sequence) was compared with reference sequences of
163 species recorded in the study area,[36] taken from the GenBank sequence database
164 (www.ncbi.nlm.nih.gov/genbank). When query sequence matched with more than one reference
165 sequence, query sequences were gathered into one group composed of the corresponding species,
166 and then gathered at the family level. The sequences were finally converted into
167 presence/absence of each plant family in SCs. Invertebrate resources in the field were identified
168 mainly at family level, which could not allow to identify invertebrate food. We used only the
169 presence/absence of arthropod and mollusk items (referred to as only "*arthropod*" hereinafter)
170 and earthworm items in SCs as food composition.

Statistical Analysis. Statistical analyses were carried out on the 200 mice. As TM concentrations in SCs could be influenced both by soil TM contamination levels through food and ingestion of soils,[37,38] we aimed to reduce potential bias related to the level of environmental contamination when quantifying oral exposure as follows. For each TM, a linear positive relationship was observed between the concentrations in SCs and in soils of the woody habitat where each mouse was captured (logarithmically transformed concentrations, *p-values* of ANOVA < 0.05; for details see Supporting Information Figure S1). The residuals of these linear models were referred to as "*exposure*" hereinafter and used in following statistical analyses.

Exposure to TMs and items consumed. The exposure was compared between the two seasons, and presence and absence of each identified item in SCs by the non-parametric Wilcoxon-Mann-Whitney test. Among the identified items consumed by wood mice (for details see Ozaki et al.[36]), only items whose occurrence was higher than 10 in the diet of the 200 mice were used in this comparison between presence/absence of items to ensure statistical power of tests: arthropods, earthworms, Betulaceae (e.g. genura *Alnus*, *Betula*), Fagaceae (*Fagus*), Oleaceae (*Fraxinus*), Poaceae (*Arrhenatherum*, *Holcus Lolium*, etc), Rosaceae (*Crataegus*), Sapindaceae (*Acer*), Salicaceae (*Populus*, *Salix*), and the sequence group composed of Adoxaceae (*Sambucus*), Asteraceae (*Hedera*) and Cornaceae (*Cornus*) items (referred to as "*group AAC*" hereinafter). When exposure was significantly related to several variables tested, we used conditional inference trees (CIT[39]) for ranking their importance for the exposure to TMs. This is a recursive binary partitioning analysis that studies significant univariate splits over all possible splitting variables. The splitting variable the most significantly associated to response value will be chosen. Those steps are recursively performed to the two split data, until no significant

194 difference are observed. Permutation tests by Strasser and Weber[40] are applied for the splitting
195 tests. In this study, CIT was performed across binary partitioning variables: season (spring or
196 autumn) and the presence/absence of the 10 types of items mentioned above. Sites were
197 considered to be random effects for exposure in the Wilcoxon-Mann-Whitney test and CIT
198 because of potential effects linked to each site. Those tests were executed for the residuals of the
199 random effect model, i.e. the model with only site as random effect. As all items were used as
200 partitioning variables without taking into account the result of each Wilcoxon-Mann-Whitney
201 test, *p-value* of CIT was not adjusted by Bonferroni correction.

202 If CIT suggested an influence of TM of hyper-accumulator items, we then checked whether
203 exposure was explained by the importance of the given items in the diet. In general, ascertaining
204 abundance information using metabarcoding of environmental DNA (eDNA) still lacks
205 consistent evidence due to multiple factors distorting the eDNA–biomass relationship, such as
206 difference in origin (species, organ, etc.) and stability of eDNA and primer bias in
207 amplification.[41] This is particularly true when comparison of different taxa, identified using
208 different primers, is an objective. We calculated the proportion of DNA reads of hyper-
209 accumulator items suggested by CIT in the SC over the total number of DNA reads. In doing so,
210 we did not expect strong bias in comparison because (i) each hyper-accumulator was identified
211 using a single primer and (ii) we assumed that its DNA stability would remain comparable
212 among samples. We therefore assumed that changes in the biomass of a hyper-accumulator
213 ingested would be reflected by changes in the proportion of its DNA sequences over the total
214 number of DNA sequences.

215 Relationships between exposure and the proportion of sequences of the given hyper-
216 accumulator items within SCs containing the given items was analyzed using a linear mixed

model with the site as random effect. The variations explained by the fixed and random effects were estimated by marginal R^2 (R^2_m) and conditional R^2 (R^2_c), respectively.[42] Relationships between the proportion of the given items and diet richness was also checked using a linear mixed model.

Diet of wood mice. Co-occurrence of items, which could mislead into a fault interpretation in the relationships between exposure and a given item consumed, was checked by CIT: Frequency of occurrence of each plant item was assessed across season and the presence/absence of the other plant items in SCs. In addition, relationship between occurrence of items and TM concentration in soils was analyzed by generalized linear models with logit function for binary data. Probability for occurrence of a given item was compared to a gradient of each TM concentrations in soils. Significance of each model was checked by the likelihood-ratio test compared to the null model.[43] Meanwhile, diet richness across season was checked by the Wilcoxon-Mann-Whitney test. Each correlation between TM in soils and diet richness was analyzed by the non-parametric Spearman correlation test.

Exposure to TMs and diet richness. Number of plant, arthropod, and earthworm MOTUs in SC were defined as three independent diet richness, and the sum of the three richness as total diet richness. A linear mixed model with the site as random effect was built and analyzed for the exposure to each TM with respect to the total diet richness and to the three richness. The relationships between the exposure, the diet richness, and the presence of specific items were analyzed by model-based recursive partitioning (MOB[44]). The principle of MOB is the same as CIT, i.e. recursive binary partitioning of data by statistical approach, but each terminal node is associated to a model rather than to a variable like CIT. After establishing a model and deciding partitioning variables to involve, MOB assesses whether the model parameters are stable with

respect to partitioning variables. If there is a significant instability,[45,46] the analysis selects the partitioning variable associated with the highest instability and computes the split point (for quantitative variables) or split class (for qualitative variables) which optimizes the parameter stability. Those steps are repeated in the two split data, until instability is not significant. Finally, significance of explanatory variables in each terminal node was assessed by a type III ANOVA (*p-value*$_{node}$). In our case, MOB was executed on the basis of mixed linear models.[47] Partitioning variables were the same as for CIT, and *p-value* of partitioning test was not adjusted. The variations explained by the fixed and random effects of the whole model were estimated by R^2_m and R^2_c, respectively.[42] Explanatory value of the fixed effects in a terminal node (R^2_{node}) was also calculated.

All statistical analyses were computed using the statistical software R (ver. 3.4.2; R Development Core Team). The mixed linear models were carried out based on "*lme*" function in "*nlme*" package. CIT was performed by "*ctree*" function in "*partykit*" package, and MOB based on linear mixed models was performed by "*lmertree*" function in "*glmertree*" package.

Results

Diet of mice. *Occurrence of items*. The frequency of occurrence of plant, arthropod and earthworm items in stomach content of mice is 96.5, 72.5, and 27.0 %, respectively. No significant difference between the two seasons was observed. Frequencies of occurrence of Fagaceae, Oleaceae, Salicaceae, and Sapindaceae items were significantly higher in spring than in autumn (*p-value* < 0.001 for each). Conversely, Poaceae and AAC group items were more frequent in autumn (*p-value* < 0.001 for each). The details of the results are provided in Supporting Information Table S3.

The frequency of occurrence of each item was also dependent on the presence of other items. In spring, the frequency of occurrence of Salicaceae items in SCs was significantly and negatively related to the presence of Sapindaceae items (Supporting Information Figure S2a; *p-value* < 0.001). The frequency of occurrence of Salicaceae items was also significantly and negatively related to the presence of the group AAC items when Sapindaceae items were absent in spring (*p-value* < 0.024). This trend was similar for the frequency of occurrence of Sapindaceae items: in spring, the frequency of occurrence of Sapindaceae items was significantly lower when Salicaceae items were present in SCs, followed by the presence of the group *AAC* items only when Salicaceae items were absent (Supporting Information Figure S2b; *p-value* < 0.001 and = 0.019, respectively). The frequency of occurrence of Fagaceae items in autumn was significantly higher when Betulaceae items were present in SCs (Supporting Information Figure S2c; *p-value* < 0.001). Frequency of occurrence of Oleaceae items was significantly higher when Sapindaceae items were present in SCs (Supporting Information Figure S2d; *p-value* < 0.01). When Sapindaceae items were absent, the frequency of occurrence of Oleaceae items was higher when Fagaceae items were present in SCs (*p-value* = 0.01).

Along the gradient of soil TM concentration, the occurrence of both arthropod and earthworm items did not significantly differ (data not shown). The occurrence of both Salicaceae and Fagaceae items significantly reduced along the gradient of soil TM concentrations only in spring (Supporting Information Figure S3a and S3b; *p-value* < 0.05 for each of the three TMs in soils), whereas the occurrence of Sapindaceae items significantly increased along the gradient (Supporting Information Figure S3c and S3d; *p-value* < 0.05) in both spring and autumn. The occurrence of the group AAC items significantly increased along the gradient only in autumn (Supporting Information Figure S3e; *p-value* < 0.05).

286 *Diet richness*. Plant, arthropod and earthworm diet richness ranged from 0 to 6 MOTUs
287 (median: 2), from 0 to 9 (median: 2), and from 0 to 3 (median: 0), respectively. Total diet
288 richness, as well as both plant and arthropod diet richness, were significantly higher in autumn
289 than in spring (data not shown, W = 3463, *p-value* < 0.001; W = 3646, *p-value* < 0.001; W =
290 4190.5, *p-value* = 0.04, respectively). No significant correlation between the three diet richness
291 values was observed, either in spring or in autumn (Supporting Information Figure S4a and S4b).
292 Only plant diet richness in spring significantly decreased along the gradient of TMs in soils
293 (Spearman's ρ from -0.26 to -0.19, *p-value* < 0.05 for the three TMs; Figure S4a).
294 **Exposure to TMs and items consumed.** Cd, Pb and Zn concentrations in SCs ranged from
295 0.03 to 47.1 (median: 0.93), from 0.03 to 194.2 (median: 3.70) and from 5.3 to 337.5 (median:
296 71.1) µg g^{-1} DM, respectively. The TM concentration in SCs and the exposure were significantly
297 higher in spring than in autumn for the three TMs (*p-value* < 0.05; for details see Supporting
298 Information Figure S5). The exposure to Cd was significantly higher when Salicaceae items were
299 present in SCs and significantly lower when AAC plants were present in SCs (W = 2559 and
300 5986; *p-value* < 0.05, respectively; Supporting Information Figure S6a). The exposure to Zn was
301 significantly higher when Salicaceae items or Oleaceae items were present in SCs (W = 3073
302 and 524; *p-value* < 0.05, respectively; Figure S6b). The exposure to Pb was not influenced by
303 any item consumed.
304 CIT analysis revealed that season was the most important variable influencing the exposure of
305 rodents to Cd (*p-value* < 0.001), followed by the presence of Salicaceae items in SC in spring
306 (Figure 1a; *p-value* = 0.01). The exposure to Cd was the highest when Salicaceae items were
307 present in SC in spring, followed by when Salicaceae item were absent in spring, and the lowest
308 in autumn. For Zn, season was the most important variable (*p-value* < 0.01), and the presence of

Salicaceae items in SC determined the exposure to Zn only in autumn (Figure 1b; *p-value* < 0.05). The exposure to Zn was the highest in spring, followed by when Salicaceae items were present in SCs in autumn, and the lowest when Salicaceae item were absent in SCs in autumn. No significant partitioning variable was observed for exposure to Pb.

Only exposure to Cd was positively explained by the proportion of sequence number of Salicaceae items (Figure 2a; *p-value* < 0.05). R^2_m and R^2_c were 0.079 and 0.251, respectively. The proportion of sequence number of Salicaceae items was negatively correlated with diet richness of plant (Figure 2b; *p-value* < 0.01; $R^2_m = 0.154$; $R^2_c = 0.280$).

Exposure to TMs and diet richness. Exposure to Cd was significantly and negatively correlated, although weakly, only with the diet richness of earthworms (*p-value* = 0.04) but not with other richness values ($R^2_m = 0.021$ and $R^2_c = 0.103$; data not shown). The relationship between exposure to Cd and the total diet richness was the most significantly conditioned by the presence/absence of Salicaceae items in SCs (the first oval in the upper part of Figure 3a; *p-value* < 0.001). When Salicaceae items were present in SCs, the exposure was negatively correlated with the diet richness (the scatter plot in the lower part of Figure 3a linked to "Salicaceae" by the line with "yes"; $p\text{-value}_{node} < 0.01$, $R^2_{node} = 0.184$). Season also significantly conditioned the relationship only for SCs in which Salicaceae items were absent (*p-value* < 0.01). In spring, the presence of earthworm items significantly conditioned the relationship for SCs in which Salicaceae items were absent (*p-value* = 0.03). In autumn, the presence of Poaceae items significantly conditioned the relationship for SCs in which Salicaceae items were absent (*p-value* = 0.03). When both Salicaceae and Poaceae items were absent in SCs in autumn, the exposure was positively correlated with the diet richness ($p\text{-value}_{node} = 0.04$, $R^2_{node} = 0.110$). The other cases showed no significant relationship between the exposure and the total richness. The

relationship between exposure to Cd and the three diet richness values was the most significantly conditioned by presence/absence of Salicaceae items in SCs (Figure 3b; *p-value* < 0.001). The exposure was negatively correlated with diet richness of plant when Salicaceae items were present in SCs ($p\text{-}value_{node}$ < 0.001, R^2_{node} = 0.220). Season significantly conditioned the relationship when Salicaceae items were absent in SCs (*p-value* = 0.03) but the exposure showed no significant relationship with any diet richness. R^2_m and R^2_c of the models for the total richness were 0.196 and 0.275 and for the three richness were 0.191 and 0.276, respectively.

No significant relationship was observed between the exposure to Zn and both the total and the three richness. However, the relationship between the exposure to Zn and the total diet richness was significantly conditioned by season (Figure 3c; *p-value* < 0.01). The exposure was positively correlated with the total diet richness in autumn ($p\text{-}value_{node}$ = 0.04, R^2_{node} = 0.106). The relationship between the exposure to Zn and the three diet richness was significantly conditioned by season (*p-value* < 0.01), followed by the presence of Betulaceae items in SC in autumn (Figure 3d; *p-value* = 0.02). The exposure to Zn was significantly and negatively correlated with the diet richness of earthworm when Betulaceae items were present in SCs in spring ($p\text{-}value_{node}$ = 0.01, R^2_{node} = 0.687; it is noteworthy that only two SCs among 12 ones contained 2 different earthworm items). R^2_m and R^2_c of the models for the total richness were 0.060 and 0.187 and for the three richness were 0.098 and 0.196, respectively.

No significant relationship was observed between the exposure to Pb and both the total and the three richness. There was no significant conditioning factor for exposure to Pb.

Discussion

Our study shed light on the role of diet richness and composition of food on the exposure to TMs in a generalist species. Our results revealed that high diet richness reduces exposure to TMs, particularly in case of consumption of at least one hyper-accumulator taxa, which however depends on both the metal considered and type of items consumed.

Consuming plant items belonging to Salicaceae family significantly raised the trophic exposure of wood mice to both Cd and Zn. Although precaution should be taken in interpreting relationships between number of DNA sequences and biomass consumed (e.g. Deiner et al.[41]), a high proportion of Salicaceae family plants in food, which may be considered as a proxy of greater biomass of Salicaceae family plants in the SC, also increased exposure to Cd. Our findings indicated that consuming Salicaceae plants items increased trophic exposure of mice to Cd. Salicaceae family actually includes taxa accumulating Cd to high concentrations in their tissues like leaves (e.g. genera *Salix* and *Populus*),[48] and their importance on the exposure of mammals to Cd has been suggested. For instance, Nolet et al.[49] showed positive correlations between Cd concentrations in tissues (hairs and kidneys) of beavers *Castor fiber* and in their main food item, bark of genera *Salix* and *Populus*. These genera also accumulate Zn. Brekken and Steinnes[50] argued that genera *Salix* and *Populus* could be the most important Cd and Zn contributors for moose *Alces alces* in a metal-contaminated area. Migeon et al.[51] reported higher concentrations of Cd and Zn in both leaves and stems of genera *Salix* and *Populus* than in the other woody species around Metaleurop Nord. The authors also demonstrated their high bioconcentration factors (i.e. ratio of metal concentrations in leaves and in soils) which reached 2 and 1.2 for Cd and Zn, respectively. Our study confirmed that Salicaceae items were obviously important contributors of Cd and, to a lesser extent of Zn, to wood mice Furthermore, our results demonstrated that high Cd concentration due to Salicaceae items was “diluted” by other plant

items, potentially less Cd accumulators than Salicaceae items. Such phenomenon was postulated by Boyd[52] under the name of "*diet dilution hypothesis*": trophic exposure of vertebrate herbivores to TMs by high metal accumulator plants would be diluted by consuming low accumulator resources. Indeed, high diet richness of plant might lead to dilute the proportion of Salicaceae items in SCs in our analyses. While this possibility has been suggested in other field studies (e.g. Martens and Boyd[53]), we clearly demonstrated, to our knowledge for the first time, the validity of this hypothesis under field conditions.

However, we failed to identify potential contributor of exposure to Pb. Migeon et al.[51] demonstrated an interspecific variation of Pb concentrations in plant tissues over our study area, although less contrasted than for Cd or Zn. One possibility could be an effect of soil ingested by mice. Quantity of soil ingested by small rodents represents only a few percent of dry mass in the diet, which was quantified to be maximum of 2% for the white-footed mouse.[37] Lead bioconcentration factors of woody plants in our study area were very low, ranging from 0.005 to 0.11,[51] suggesting a low mobility of Pb towards plants. The same pattern was observed over this area for invertebrates (earthworms, mollusks, arthropods) which exhibited low biota-to-soil concentration ratios for Pb (mostly lower than 0.1, and lower than for Cd and Zn).[54,55] It is thus possible that a large part of Pb in SCs could be derived from soils and that it may obscure any effect of dietary richness or composition on Pb concentration in SCs. Effects of geophagy on exposure to TM and/or accumulation of TM in animals remain as a further issue that undoubtedly deserves attention.

On the other hand, consuming invertebrate items, especially earthworms that are well-known as high TM accumulator species,[56] did not increase the exposure of wood mice to TMs in our study. Accumulation patterns in earthworms exposed to soils can be strongly modified by

environmental factors like pH or organic matter content in soils,[57–59] which may explain the discrepancy between our results and the literature emphasizing the importance of earthworms on the exposure of mammals to TMs.[5,6,60] Further, trophic availability of metals accumulated in the various diet items can be different according to their sequestration forms in item tissues, which may also or rather lead to such discrepancies with literature.[61] Moreover, some invertebrates show high metal accumulation capacity, e.g. snails, isopods, or spiders,[62–66] whereas others, e.g. Coleoptera,[65] accumulate less metals in their tissues. For instance, Fritsch et al.[54] showed that trophic transfer of TMs to blackbirds *Turdus merula* was less important in habitats linked with a diet based on earthworms than in habitats linked with a diet based on other invertebrates in the surroundings of Metaleurop Nord. They also demonstrated close to or even higher transfer factors (i.e. metal concentration in items divided by metal concentration in soils) of some other invertebrate taxa than earthworms for Cd and Pb. In the present study, invertebrate items could not be specified further than large categories like "arthropods and mollusks" or "earthworms", and our low taxonomic resolution of invertebrate items could make unclear the role of animal matters on the TM exposure of mammals.

Few studies have focused on the relationship between exposure to TMs and diet richness. To our knowledge, only Orłowski et al.[7] showed significant correlations between the number of items in the diet of nestling rooks *Corvus frugilegus* and accumulation of TMs in their tissues. In that study, Pb concentrations in the liver were negatively correlated with the number of vegetal items, while Cd concentrations were positively correlated with the number of animal items. However, oral exposure to TMs is not always correlated with TM accumulation in tissues. In the study of Godwin et al.[67] for example, TM concentrations in stomach contents and in tissues of tree swallow *Tachycineta bicolor* nestlings of the age of 14 days showed no correlation for Ni,

Sr, and Zn, negative correlations for Mo and Cu, and correlations depending on year and/or tissue for other elements including Cd and Pb. TM accumulation in animal tissues is determined by several other factors than oral exposure, like species, age, sex and exposure by other routes.[68] Assimilation of elements via oral route is modulated in intestines by composition of micro- and macro-nutrients, such as iron or calcium.[3,69] Metal speciation is also another factor affecting intestinal assimilation of TMs. Chunhabundit et al.[70] reported higher intestinal assimilation of Cd in human intestinal cell from animal-based food than from vegetal-based food. Taking into account those nutrient factors modulating bioavailability of TMs, diet diversity and composition possibly play different roles over the exposure to TMs and the TM accumulation.

Even though an important functional role of diet richness on the exposure to TMs is shown here, further investigations should be carried out for elucidating the variation of the oral exposure in space and time. First, metal concentrations in both invertebrates and plants are largely variable according to their organ, size, season, age and/or developmental stage,[71,72] which are undetectable by DNA based method. Although the metabarcoding is a powerful food identification technique compared to the classical microscopic method or the nitrogen and carbon isotopic approach, accessing information other than taxonomy of items, such as quantity ingested, organs consumed and their TM concentrations, should be included in further studies. Second, mechanisms underlying the relationship between the diet dilution and the diversity of available food in the field remain unclear. The causes and consequences of food composition and food selection depend on the foraging behavior of a given animal, which is also determined by several factors such as balance between nutrients and toxins in food or avoidance of risks by parasites and/or predators.[73] In our study, occurrences in SCs of Salicaceae and Sapindaceae, two plant families preferentially consumed by wood mice,[36] were likely to switch along the gradient

446 of soil contamination: Occurrence in SCs of Salicaceae items decreased, whereas occurrence of
447 Sapindaceae items increased. This result could be due to a change in the preference of wood
448 mice for Salicaceae items along the gradient of soil TM contamination.[36] The composition of
449 available food in the field and the soil TM contamination may also be considered as to be
450 important factors determining the foraging behavior. Although our finding supported the diet
451 dilution hypothesis, the generality of this phenomenon can depend on several biological and
452 environmental conditions, which should be studied further.

453 To conclude, the level of exposure of small mammals to TMs is influenced not only by the
454 presence of certain items in the diet but also by the diet richness. The functional role of the diet
455 richness on exposure to TMs observed in the present study concurs with the diet dilution
456 hypothesis. Challenging such hypotheses about the functional role of biodiversity on the trophic
457 transfer of pollutants and investigating trait-based mechanisms are possible if ingested food is
458 identified in details. Although the metabarcoding approach allows identifying the taxonomy of
459 food under field conditions, associating life history traits and chemical composition of each item
460 and clarifying mechanisms of foraging behavior of animals will improve chemical risk
461 assessment in wildlife. Further perspectives also deal with the quantification of trophic links to
462 address the functional significance of trophic interactions for the transfer of matter, energy, and
463 essential elements versus the transfer of toxicants, and the evolutionary trade-offs between
464 acquiring nutrients but being exposed to toxic substances.
465

Figure 1. Conditional inference trees (CIT) for (a) the exposure to Cd and (b) the exposure to Zn, i.e. residuals of linear model between logarithmically-transformed concentrations of TMs in stomach content and in soils (µg g^{-1} DM). Partitioning variables significantly selected for splits (univariate *p-value* < 0.05) are listed in inner nods (represented as ovals) with *p-value* and their splitting criteria are listed on the lines connecting variables. Presence or absence of a given item in stomach content of wood mice is represented by "*yes*" or "'*no*", respectively. Boxplot in terminal node describes the residuals of the given TM for each final classification group with sample size, and median of the classification group is mentioned below.

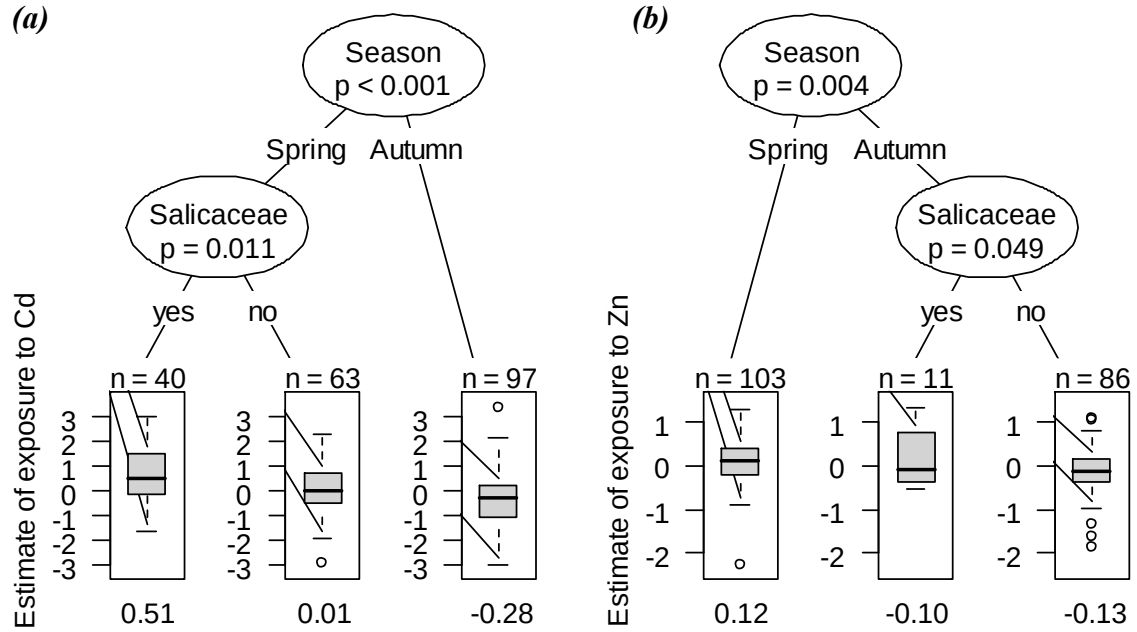


Figure 2. (a) Exposure of the wood mice to Cd along the gradient of proportion of Salicaceae items' sequence number in stomach content, considered as a proxy of biomass consumed and (b) the proportion of Salicaceae items' sequences along the gradient of diet richness of plant. Plotted points indicate stomach contents. Values predicted from our models are indicated by lines. Different color lines represent different sites (random effect). Variation explained by fixed and random effects are respectively explained by marginal and conditional R^2 (R^2_m and R^2_c) in the figures.

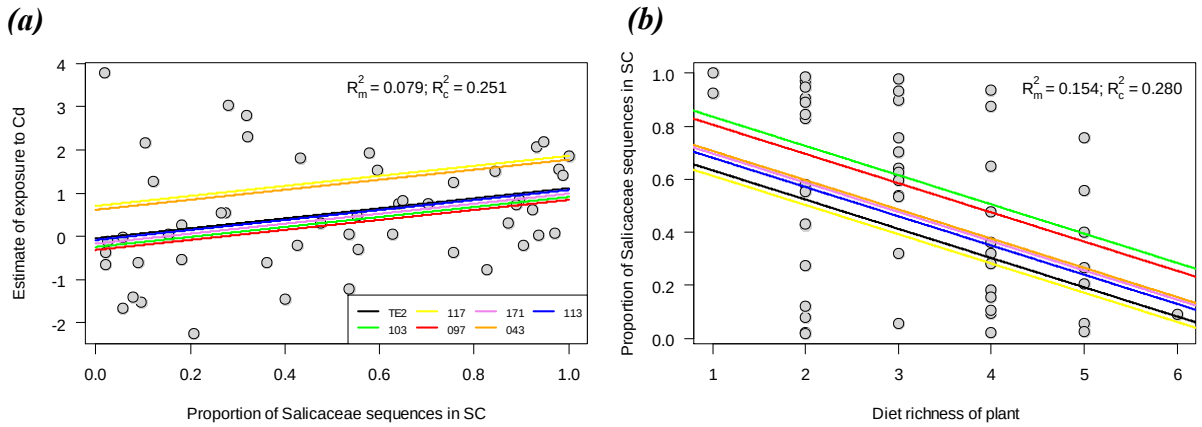
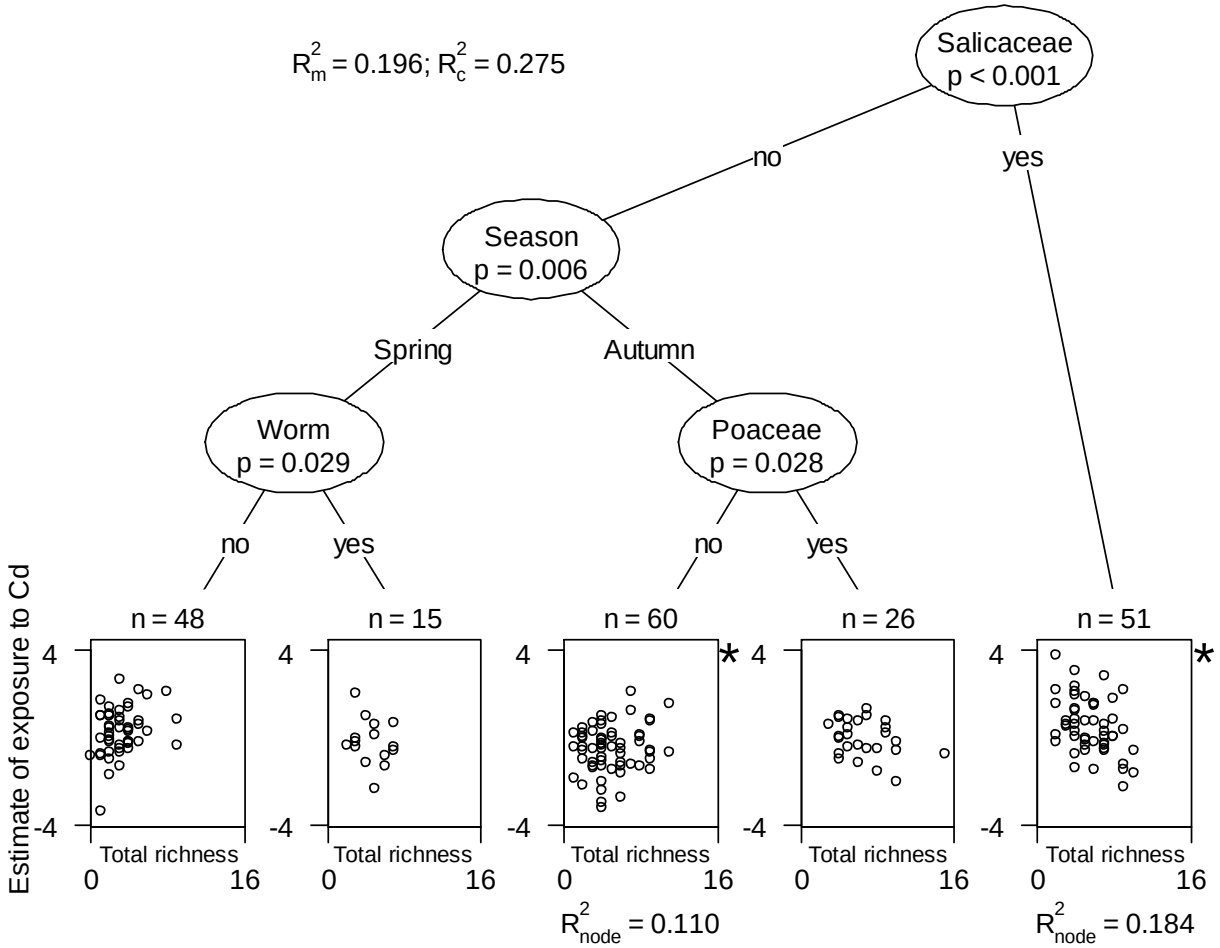
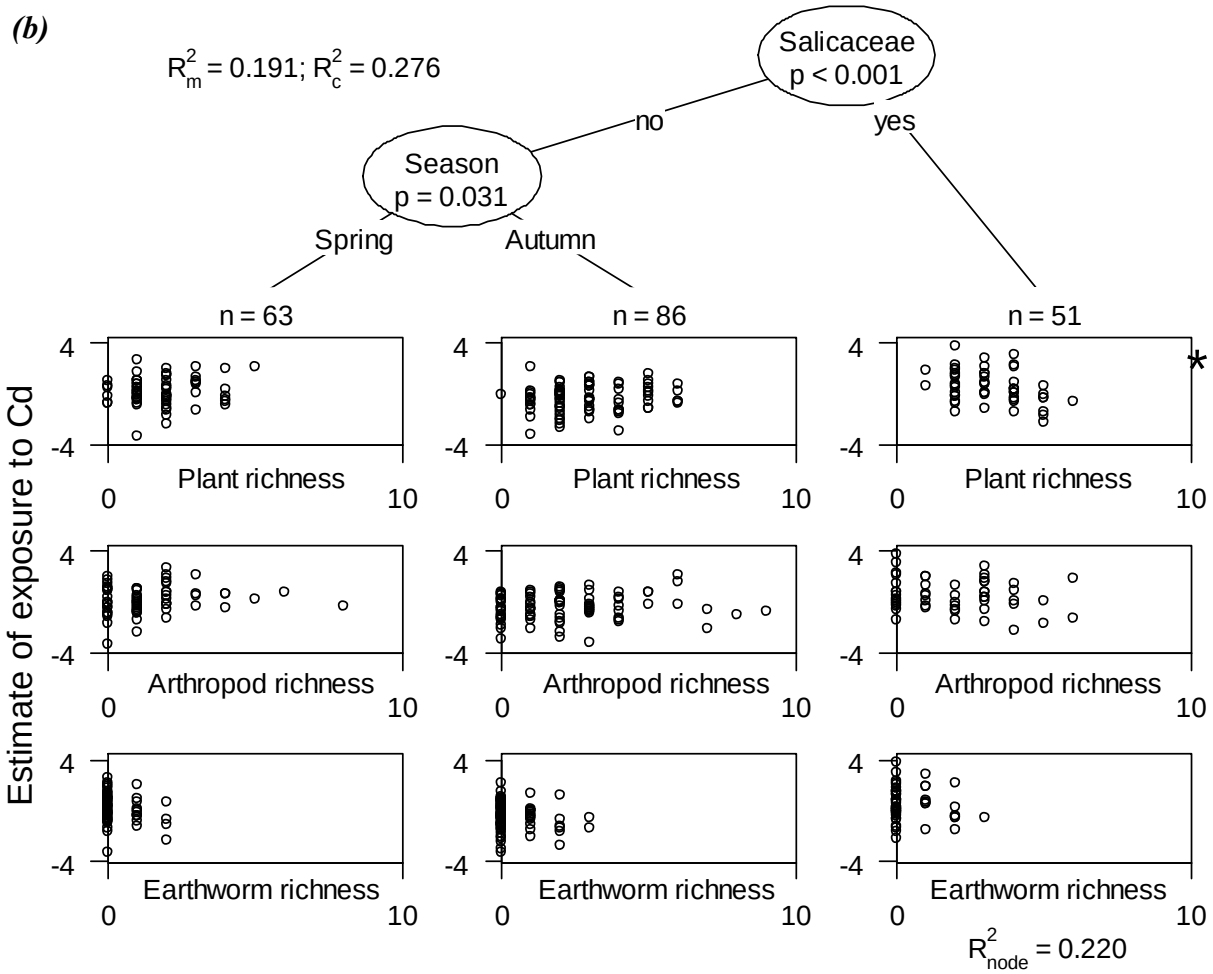


Figure 3. Model-based recursive partitioning (MOB) on mixed linear models for the exposure to TMs, i.e. residuals of linear model between logarithmically-transformed concentrations of TMs in stomach content and in soils ($\mu g\ g^{-1}$), with respect to either three diet richness of plant, arthropod and earthworm MOTUs or the sum of them (i.e. total diet richness): exposure to Cd with respect to the total diet richness (a) and to the three diet richness (b), exposure to Zn with respect to the total diet richness (c) and to the three diet richness (d). Partitioning variables significantly selected for splits (univariate *p-value* < 0.05) are listed in inner nods (represented as ovals) with *p-value* and their splitting criteria are listed on the lines connecting variables. Presence or absence of a given item in stomach content of wood mice is represented by "*yes*" or "*no*", respectively. Scatter plots in terminal nodes describe relationships between the response variable (y axis) and each explanatory variable (x axes, from bottom line to top, earthworm, arthropod and plant diet richness). An asterisk (*) denotes significant correlation by type III ANOVA. R^2 of the linear model for partitioned data (R^2_{node}) is mentioned below only if significant correlation was observed. The variation explained by the fixed and the random effects (R^2_m and R^2_c, respectively) are mentioned at the top of the figure.

(a)

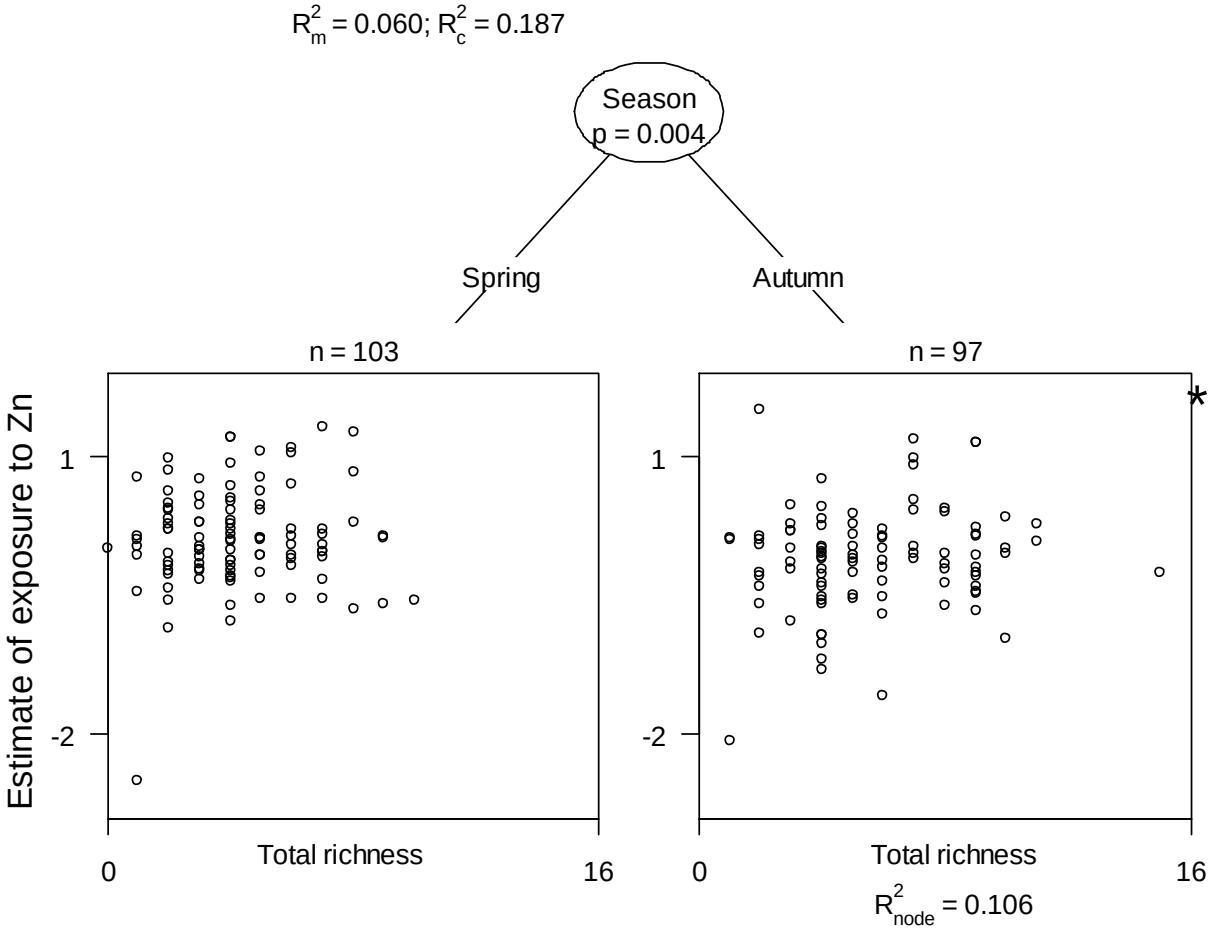

$R^2_m = 0.196$; $R^2_c = 0.275$
Salicaceae
p < 0.001
no
yes
Season
p = 0.006
Spring
Autumn
Worm
p = 0.029
Poaceae
p = 0.028
no
yes
no
yes
n = 48
n = 15
n = 60
n = 26
n = 51
Estimate of exposure to Cd
4
-4
Total richness
0
16
*
*
$R^2_{node} = 0.110$
$R^2_{node} = 0.184$

500


(b)
R^2_m = 0.191; R^2_c = 0.276
Salicaceae
p < 0.001
no
yes
Season
p = 0.031
Spring
Autumn
n = 63
n = 86
n = 51
Estimate of exposure to Cd
Plant richness
Arthropod richness
Earthworm richness
R^2_{node} = 0.220

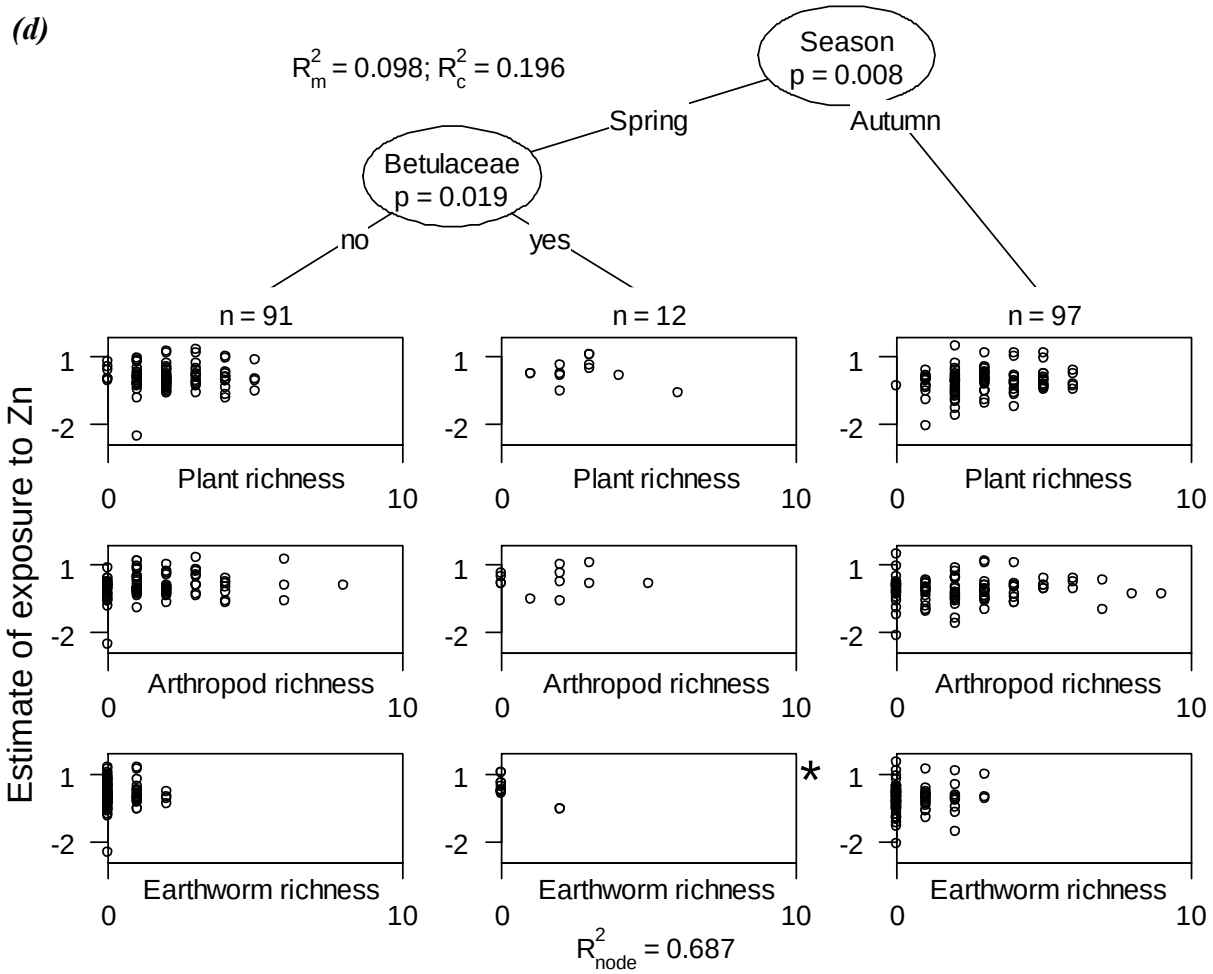
501

(c)


$R^2_m = 0.060$; $R^2_c = 0.187$
Season
p = 0.004
Spring
Autumn
n = 103
n = 97
Estimate of exposure to Zn
1
-2
0
16
Total richness
*
$R^2_{node} = 0.106$

502

(d)


R^2_m = 0.098; R^2_c = 0.196
Season
p = 0.008
Spring
Autumn
Betulaceae
p = 0.019
no
yes
n = 91
n = 12
n = 97
Estimate of exposure to Zn
Plant richness
Arthropod richness
Earthworm richness
*
R^2_{node} = 0.687

503

Supporting Information

Figure S1: Relationships between concentrations of TMs in SCs and in soils.

Figure S2: Conditional inference tree for frequency of occurrence of Salicaceae, Sapindaceae, Fagaceae and Oleaceae items in SCs of wood mice.

Figure S3: Predicted probabilities for occurrence of items consumed in SCs along a gradient of Cd concentration in soils by generalized linear models.

Figure S4: Correlation matrix for TM concentrations in soils and dietary richness of plant, arthropod and earthworm MOTUs.

Figure S5: Concentrations of the three TMs in SCs of wood mice and exposure to the TMs in spring and autumn.

Figure S6: Significant differences in exposure to TMs between the presence/absence of plants items in SCs.

Table S1: Information about each study site about TM contamination and landscape dominant habitats.

Table S2: List of primers used for identifying food.

Table S3: List of frequency of occurrence of items in SCs.

AUTHOR INFORMATION

Corresponding Author

* Phone number: +33 (0)3 81 66 65 98; E-mail address: shinji.ozaki@univ-fcomte.fr.

Notes

# both authors contributed equally to supervising this work

ACKNOWLEDGMENT

This study was financially supported by the project BIOTROPH, co-funded by the *Agence De l'Environnement et de la Maîtrise de l'Energie* (ADEME; contract No.1172C0030) and the *Conseil Régional du Nord-Pas de Calais* (CRNPC; orders No.12000921 and 14001044; joint call with the *Fondation pour la Recherche sur la Biodiversité*). The first author was financially supported by a grant from the *Conseil Régional de Franche-Comté* (contract No. 2015C-06107). The authors gratefully thank Cécile Grand from ADEME for fruitful scientific discussion. We also thank Eva Bellemain and Alice Valentini from SPYGEN Company for their help in DNA analyses and interpretations. We finally thank Nadia Crini, Caroline Amiot, Dominique Rieffel and Anne-Sophie Prudent for their precious assistance.

AUTHOR CONTRIBUTIONS

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

REFERENCES

(1) Madrid, L. "Heavy Metals": Reminding a Long-Standing and Sometimes Forgotten

Controversy. *Geoderma* **2010**, *155* (1–2), 128–129. https://doi.org/10.1016/j.geoderma.2009.11.031.

(2) Walker, C. H.; Hopkin, S. P.; Sibly, R. M.; Peakall, D. B. *Principles of Ecotoxicology*, 4th ed.; CRC Press: Boca Raton, 2012.

(3) Shore, R. F.; Rattner, B. A. *Ecotoxicology of Wild Mammals*; Ecotoxicology and Environmental Toxicology Series; John Wiley and Sons: New York, 2001.

(4) Hamers, T.; van den Berg, J. H. J.; van Gestel, C. A. M.; van Schooten, F.-J.; Murk, A. J. Risk Assessment of Metals and Organic Pollutants for Herbivorous and Carnivorous Small Mammal Food Chains in a Polluted Floodplain (Biesbosch, The Netherlands). *Environ. Pollut.* **2006**, *144* (2), 581–595. https://doi.org/10.1016/j.envpol.2006.01.020.

(5) Rogival, D.; Scheirs, J.; Blust, R. Transfer and Accumulation of Metals in a Soil–diet–wood Mouse Food Chain along a Metal Pollution Gradient. *Environ. Pollut.* **2007**, *145* (2), 516–528. https://doi.org/10.1016/j.envpol.2006.04.019.

(6) van den Brink, N.; Lammertsma, D.; Dimmers, W.; Boerwinkel, M.-C.; van der Hout, A. Effects of Soil Properties on Food Web Accumulation of Heavy Metals to the Wood Mouse (*Apodemus Sylvaticus*). *Environ. Pollut.* **2010**, *158* (1), 245–251. https://doi.org/10.1016/j.envpol.2009.07.013.

(7) Orłowski, G.; Kamiński, P.; Kasprzykowski, Z.; Zawada, Z. Relationships between Stomach Content and Concentrations of Essential and Non-Essential Elements in Tissues of Omnivorous Nestling Rooks *Corvus Frugilegus*: Is the Size and Composition of Stomach Content Relevant. *Folia Zool.* **2013**, *62*, 282–289.

(8) Valentini, A.; Pompanon, F.; Taberlet, P. DNA Barcoding for Ecologists. *Trends Ecol. Evol.* **2009**, *24* (2), 110–117. https://doi.org/10.1016/j.tree.2008.09.011.

(9) Pompanon, F.; Deagle, B. E.; Symondson, W. O. C.; Brown, D. S.; Jarman, S. N.; Taberlet, P. Who Is Eating What: Diet Assessment Using next Generation Sequencing. *Mol. Ecol.* **2012**, *21* (8), 1931–1950. https://doi.org/10.1111/j.1365-294X.2011.05403.x.

(10) Taberlet, P.; Coissac, E.; Hajibabaei, M.; Rieseberg, L. H. Environmental DNA. *Mol. Ecol.* **2012**, *21* (8), 1789–1793.

(11) Taberlet, P.; Coissac, E.; Pompanon, F.; Brochmann, C.; Willerslev, E. Towards next-Generation Biodiversity Assessment Using DNA Metabarcoding. *Mol. Ecol.* **2012**, *21* (8), 2045–2050.

(12) Bohmann, K.; Evans, A.; Gilbert, M. T. P.; Carvalho, G. R.; Creer, S.; Knapp, M.; Yu, D. W.; de Bruyn, M. Environmental DNA for Wildlife Biology and Biodiversity Monitoring. *Trends Ecol. Evol.* **2014**, *29* (6), 358–367. https://doi.org/10.1016/j.tree.2014.04.003.

(13) Soininen, E. M.; Valentini, A.; Coissac, E.; Miquel, C.; Gielly, L.; Brochmann, C.; Brysting, A. K.; Sønstebø, J. H.; Ims, R. A.; Yoccoz, N. G. ; Taberlet, P. Analysing Diet of Small Herbivores: The Efficiency of DNA Barcoding Coupled with High-Throughput Pyrosequencing for Deciphering the Composition of Complex Plant Mixtures. *Front. Zool.* **2009**, *6* (1), 16. https://doi.org/10.1186/1742-9994-6-16.

(14) Bohmann, K.; Monadjem, A.; Lehmkuhl Noer, C.; Rasmussen, M.; Zeale, M. R. K.; Clare, E.; Jones, G.; Willerslev, E.; Gilbert, M. T. P. Molecular Diet Analysis of Two African Free-Tailed Bats (Molossidae) Using High Throughput Sequencing. *PLoS ONE* **2011**, *6* (6), e21441. https://doi.org/10.1371/journal.pone.0021441.

(15) Ait Baamrane, M. A.; Shehzad, W.; Ouhammou, A.; Abbad, A.; Naimi, M.; Coissac, E.; Taberlet, P.; Znari, M. Assessment of the Food Habits of the Moroccan Dorcas Gazelle in M'Sabih Talaa, West Central Morocco, Using the *trn*L Approach. *PLoS ONE* **2012**, *7* (4),

591 e35643. https://doi.org/10.1371/journal.pone.0035643.
592 (16) De Barba, M.; Miquel, C.; Boyer, F.; Mercier, C.; Rioux, D.; Coissac, E.; Taberlet, P. DNA
593 Metabarcoding Multiplexing and Validation of Data Accuracy for Diet Assessment:
594 Application to Omnivorous Diet. *Mol. Ecol. Resour.* **2013**, *14* (2), 306–323.
595 https://doi.org/10.1111/1755-0998.12188.
596 (17) Soininen, E. M.; Ravolainen, V. T.; Bråthen, K. A.; Yoccoz, N. G.; Gielly, L.; Ims, R. A.
597 Arctic Small Rodents Have Diverse Diets and Flexible Food Selection. *PLoS ONE* **2013**, *8*
598 (6), e68128. https://doi.org/10.1371/journal.pone.0068128.
599 (18) Watts, C. H. S. The Foods Eaten by Wood Mice (*Apodemus Sylvaticus*) and Bank Voles
600 (*Clethrionomys Glareolus*) in Wytham Woods, Berkshire. *J. Anim. Ecol.* **1968**, *37* (1), 25–
601 41.
602 (19) Butet, A. Régime Alimentaire D'une Population de Mulots Sylvestres (*Apodemus*
603 *Sylvaticus* L., 1758), Dans Une Lande Xéro-Mésophile En Cours de Recolonisation
604 Végétale. *Bull. Ecol.* **1986**, *17*, 21–37.
605 (20) Rogers, L. M.; Gorman, M. L. The Diet of the Wood Mouse *Apodemus Sylvaticus* on Set-
606 aside Land. *J. Zool.* **1995**, *235* (1), 77–83.
607 (21) Abt, K. F.; Bock, W. F. Seasonal Variations of Diet Composition in Farmland Field Mice
608 *Apodemus* Spp. and Bank Voles *Clethrionomys Glareolus*. *Acta Theriol. (Warsz.)* **1998**, *43*
609 (4), 379–389.
610 (22) Quéré, J.-P.; Le Louarn, H. *Les rongeurs de France : Faunistique et biologie*, 2nd ed.;
611 Quae: Versailles, 2011.
612 (23) González, X. I.; Aboal, J. R.; Fernández, J. A.; Carballeira, A. Considerations on the
613 Sample Size of Wood Mice Used to Biomonitor Metals. *Sci. Total Environ.* **2006**, *366* (2–

614 3), 910–914. https://doi.org/10.1016/j.scitotenv.2005.10.003.
615 (24) Sánchez-Chardi, A.; Peñarroja-Matutano, C.; Ribeiro, C. A. O.; Nadal, J. Bioaccumulation
616 of Metals and Effects of a Landfill in Small Mammals. Part II. The Wood Mouse,
617 *Apodemus Sylvaticus*. *Chemosphere* **2007**, *70* (1), 101–109.
618 https://doi.org/10.1016/j.chemosphere.2007.06.047.
619 (25) Tête, N.; Durfort, M.; Rieffel, D.; Scheifler, R.; Sánchez-Chardi, A. Histopathology
620 Related to Cadmium and Lead Bioaccumulation in Chronically Exposed Wood Mice,
621 *Apodemus Sylvaticus*, around a Former Smelter. *Sci. Total Environ.* **2014**, *481*, 167–177.
622 https://doi.org/10.1016/j.scitotenv.2014.02.029.
623 (26) Sterckeman, T.; Douay, F.; Proix, N.; Fourrier, H.; Perdrix, E. Assessment of the
624 Contamination of Cultivated Soils by Eighteen Trace Elements around Smelters in the
625 North of France. *Water. Air. Soil Pollut.* **2002**, *135* (1–4), 173–194.
626 (27) Douay, F.; Pruvot, C.; Roussel, H.; Ciesielski, H.; Fourrier, H.; Proix, N.; Waterlot, C.
627 Contamination of Urban Soils in an Area of Northern France Polluted by Dust Emissions
628 of Two Smelters. *Water. Air. Soil Pollut.* **2008**, *188* (1–4), 247–260.
629 https://doi.org/10.1007/s11270-007-9541-7.
630 (28) Douay, F.; Pruvot, C.; Waterlot, C.; Fritsch, C.; Fourrier, H.; Loriette, A.; Bidar, G.; Grand,
631 C.; de Vaufleury, A.; Scheifler, R. Contamination of Woody Habitat Soils around a Former
632 Lead Smelter in the North of France. *Sci. Total Environ.* **2009**, *407* (21), 5564–5577.
633 https://doi.org/10.1016/j.scitotenv.2009.06.015.
634 (29) Waterlot, C.; Pruvot, C.; Bidar, G.; Fritsch, C.; De Vaufleury, A.; Scheifler, R.; Douay, F.
635 Prediction of Extractable Cd, Pb and Zn in Contaminated Woody Habitat Soils Using a
636 Change Point Detection Method. *Pedosphere* **2016**, *26* (3), 282–298.

https://doi.org/10.1016/S1002-0160(15)60043-1.

(30) Fritsch, C.; Giraudoux, P.; Cœurdassier, M.; Douay, F.; Raoul, F.; Pruvot, C.; Waterlot, C.; Vaufleury, A. de; Scheifler, R. Spatial Distribution of Metals in Smelter-Impacted Soils of Woody Habitats: Influence of Landscape and Soil Properties, and Risk for Wildlife. *Chemosphere* **2010**, *81* (2), 141–155. https://doi.org/10.1016/j.chemosphere.2010.06.075.

(31) Fritsch, C.; Cœurdassier, M.; Giraudoux, P.; Raoul, F.; Douay, F.; Rieffel, D.; Vaufleury, A. de; Scheifler, R. Spatially Explicit Analysis of Metal Transfer to Biota: Influence of Soil Contamination and Landscape. *PLoS ONE* **2011**, *6* (5), e20682. https://doi.org/10.1371/journal.pone.0020682.

(32) Taberlet, P.; Coissac, E.; Pompanon, F.; Gielly, L.; Miquel, C.; Valentini, A.; Vermat, T.; Corthier, G.; Brochmann, C.; Willerslev, E. Power and Limitations of the Chloroplast *trn*L (UAA) Intron for Plant DNA Barcoding. *Nucleic Acids Res.* **2007**, *35* (3), e14. https://doi.org/10.1093/nar/gkl938.

(33) Bienert, F.; De Danieli, S.; Miquel, C.; Coissac, E.; Poillot, C.; Brun, J.-J.; Taberlet, P. Tracking Earthworm Communities from Soil DNA. *Mol. Ecol.* **2012**, *21* (8), 2017–2030. https://doi.org/10.1111/j.1365-294X.2011.05407.x.

(34) Schloss, P. D.; Westcott, S. L.; Ryabin, T.; Hall, J. R.; Hartmann, M.; Hollister, E. B.; Lesniewski, R. A.; Oakley, B. B.; Parks, D. H.; Robinson, C. J.; Sahl, J. W.; Stres, B.; Thallinger, G. G.; Van Horn, D. J.; Weber, C. F. Introducing Mothur: Open-Source, Platform-Independent, Community-Supported Software for Describing and Comparing Microbial Communities. *Appl. Environ. Microbiol.* **2009**, *75* (23), 7537–7541. https://doi.org/10.1128/AEM.01541-09.

(35) Deagle, B. E.; Thomas, A. C.; Shaffer, A. K.; Trites, A. W.; Jarman, S. N. Quantifying

Sequence Proportions in a DNA-Based Diet Study Using Ion Torrent Amplicon Sequencing: Which Counts Count? *Mol. Ecol. Resour.* **2013**, *13* (4), 620–633. https://doi.org/10.1111/1755-0998.12103.

(36) Ozaki, S.; Fritsch, C.; Valot, B.; Mora, F.; Cornier, T.; Scheifler, R.; Raoul, F. Does Pollution Influence Small Mammal Diet in the Field? A Metabarcoding Approach in a Generalist Consumer. *Mol. Ecol.* **2018**, *27* (18), 3700–3713. https://doi.org/10.1111/mec.14823.

(37) Beyer, W. N.; Connor, E. E.; Gerould, S. Estimates of Soil Ingestion by Wildlife. *J. Wildl. Manag.* **1994**, *58* (2), 375–382. https://doi.org/10.2307/3809405.

(38) Smith, K. M.; Abrahams, P. W.; Dagleish, M. P.; Steigmajer, J. The Intake of Lead and Associated Metals by Sheep Grazing Mining-Contaminated Floodplain Pastures in Mid-Wales, UK: I. Soil Ingestion, Soil–metal Partitioning and Potential Availability to Pasture Herbage and Livestock. *Sci. Total Environ.* **2009**, *407* (12), 3731–3739. https://doi.org/10.1016/j.scitotenv.2009.02.032.

(39) Hothorn, T.; Hornik, K.; Zeileis, A. Unbiased Recursive Partitioning: A Conditional Inference Framework. *J. Comput. Graph. Stat.* **2006**, *15* (3), 651–674.

(40) Strasser, H.; Weber, C. On the Asymptotic Theory of Permutation Statistics. *Math. Methods Stat.* **1999**, *8*, 220–250.

(41) Deiner, K.; Bik, H. M.; Mächler, E.; Seymour, M.; Lacoursière-Roussel, A.; Altermatt, F.; Creer, S.; Bista, I.; Lodge, D. M.; de Vere, N.; Pfrender, M. E.; Bernatchez, L. Environmental DNA Metabarcoding: Transforming How We Survey Animal and Plant Communities. *Mol. Ecol.* **2017**, *26* (21), 5872–5895. https://doi.org/10.1111/mec.14350.

(42) Nakagawa, S.; Schielzeth, H. A General and Simple Method for Obtaining R^2 from

683 Generalized Linear Mixed-Effects Models. *Methods Ecol. Evol.* **2013**, *4* (2), 133–142.
684 https://doi.org/10.1111/j.2041-210x.2012.00261.x.
685 (43) Zuur, A. F. *Mixed Effects Models and Extensions in Ecology with R*; Statistics for biology
686 and health; Springer: New York, 2009.
687 (44) Zeileis, A.; Hothorn, T.; Hornik, K. Model-Based Recursive Partitioning. *J. Comput.*
688 *Graph. Stat.* **2008**, *17* (2), 492–514.
689 (45) Zeileis, A. A Unified Approach to Structural Change Tests Based on ML Scores, F
690 Statistics, and OLS Residuals. *Econom. Rev.* **2005**, *24* (4), 445–466.
691 (46) Zeileis, A.; Hornik, K. Generalized M-Fluctuation Tests for Parameter Instability. *Stat.*
692 *Neerlandica* **2007**, *61* (4), 488–508.
693 (47) Fokkema, M.; Smits, N.; Zeileis, A.; Hothorn, T.; Kelderman, H. Detecting Treatment-
694 Subgroup Interactions in Clustered Data with Generalized Linear Mixed-Effects Model
695 Trees. *Working Paper 2015-10. Working Papers in Economics and Statistics, Research*
696 *Platform Empirical and Experimental Economics, Universität Innsbruck.* 2015, p
697 http://EconPapers.RePEc.org/RePEc:inn:wpaper:2015-10.
698 (48) Pulford, I. D.; Watson, C. Phytoremediation of Heavy Metal-Contaminated Land by
699 Trees—a Review. *Environ. Int.* **2003**, *29* (4), 529–540. https://doi.org/10.1016/S0160-
700 4120(02)00152-6.
701 (49) Nolet, B. A.; Dijkstra, V. A.; Heidecke, D. Cadmium in Beavers Translocated from the
702 Elbe River to the Rhine/Meuse Estuary, and the Possible Effect on Population Growth
703 Rate. *Arch. Environ. Contam. Toxicol.* **1994**, *27* (2), 154–161.
704 (50) Brekken, A.; Steinnes, E. Seasonal Concentrations of Cadmium and Zinc in Native Pasture
705 Plants: Consequences for Grazing Animals. *Sci. Total Environ.* **2004**, *326* (1–3), 181–195.

https://doi.org/10.1016/j.scitotenv.2003.11.023.

(51) Migeon, A.; Richaud, P.; Guinet, F.; Chalot, M.; Blaudez, D. Metal Accumulation by Woody Species on Contaminated Sites in the North of France. *Water. Air. Soil Pollut.* **2009**, *204*, 89–101. https://doi.org/10.1007/s11270-009-0029-5.

(52) Boyd, R. S. Hyperaccumulation as a Plant Defensive Strategy. In *Plants that hyperaccumulate heavy metals*; Brooks, R. R., Ed.; CAB International: Oxford, 1998; pp 181–201.

(53) Martens, S. N.; Boyd, R. S. The Defensive Role of Ni Hyperaccumulation by Plants: A Field Experiment. *Am. J. Bot.* **2002**, *89* (6), 998–1003.

(54) Fritsch, C.; Coeurdassier, M.; Faivre, B.; Baurand, P.-E.; Giraudoux, P.; van den Brink, N. W.; Scheifler, R. Influence of Landscape Composition and Diversity on Contaminant Flux in Terrestrial Food Webs: A Case Study of Trace Metal Transfer to European Blackbirds Turdus Merula. *Sci. Total Environ.* **2012**, *432*, 275–287. https://doi.org/10.1016/j.scitotenv.2012.06.004.

(55) Mourier, B.; Fritsch, C.; Dhivert, E.; Gimbert, F.; Cœurdassier, M.; Pauget, B.; Vaufleury, A. de; Scheifler, R. Chemical Extractions and Predicted Free Ion Activities Fail to Estimate Metal Transfer from Soil to Field Land Snails. *Chemosphere* **2011**, *85* (6), 1057–1065. https://doi.org/10.1016/j.chemosphere.2011.07.035.

(56) Nahmani, J.; Hodson, M. E.; Black, S. A Review of Studies Performed to Assess Metal Uptake by Earthworms. *Environ. Pollut.* **2007**, *145* (2), 402–424. https://doi.org/10.1016/j.envpol.2006.04.009.

(57) Janssen, R.; Posthuma, L.; Baerselman, R.; Den Hollander, H. A.; Van Veen, R. P.; Peijnenburg, W. J. Equilibrium Partitioning of Heavy Metals in Dutch Field Soils. II.

729 Prediction of Metal Accumulation in Earthworms. *Environ. Toxicol. Chem.* **1997**, *16* (12),
730 2479–2488.
731 (58) Morgan, J. E.; Morgan, A. J. Earthworms as Biological Monitors of Cadmium, Copper,
732 Lead and Zinc in Metalliferous Soils. *Environ. Pollut.* **1988**, *54* (2), 123–138.
733 https://doi.org/10.1016/0269-7491(88)90142-X.
734 (59) Nannoni, F.; Rossi, S.; Protano, G. Soil Properties and Metal Accumulation by
735 Earthworms in the Siena Urban Area (Italy). *Appl. Soil Ecol.* **2014**, *77*, 9–17.
736 https://doi.org/10.1016/j.apsoil.2014.01.004.
737 (60) Ma, W. Heavy Metal Accumulation in the Mole, *Talpa Europea*, and Earthworms as an
738 Indicator of Metal Bioavailability in Terrestrial Environments. *Bull. Environ. Contam.*
739 *Toxicol.* **1987**, *39* (6), 933–938.
740 (61) Vijver, M. G.; van Gestel, C. A. M.; Lanno, R. P.; van Straalen, N. M.; Peijnenburg, W. J.
741 G. M. Internal Metal Sequestration and Its Ecotoxicological Relevance: A Review.
742 *Environ. Sci. Technol.* **2004**, *38* (18), 4705–4712. https://doi.org/10.1021/es040354g.
743 (62) Hopkin, S. P. *Ecophysiology of Metals in Terrestrial Invertebrates*; Elsevier Applied
744 Science: London, 1989.
745 (63) Janssen, M. P. M.; Bruins, A.; De Vries, T. H.; Van Straalen, N. M. Comparison of
746 Cadmium Kinetics in Four Soil Arthropod Species. *Arch. Environ. Contam. Toxicol.* **1991**,
747 *20* (3), 305–312.
748 (64) Dallinger, R. Strategies of Metal Detoxification in Terrestrial Invertebrates. In
749 *Ecotoxicology of Metals in Invertebrates*; Dallinger, R., Rainbow, P. S., Eds.; Lewis
750 Publishers: London, 1993; pp 245–280.
751 (65) Heikens, A.; Peijnenburg, W. J. G. M.; Hendriks, A. J. Bioaccumulation of Heavy Metals

752 in Terrestrial Invertebrates. *Environ. Pollut.* **2001**, *113*, 385–393.
753 (66) Hendrickx, F.; Maelfait, J.-P.; Langenbick, F. Absence of Cadmium Excretion and High
754 Assimilation Result in Cadmium Biomagnification in a Wolf Spider. *Ecotoxicol. Environ.*
755 *Saf.* **2003**, *55* (3), 287–292. https://doi.org/10.1016/S0147-6513(02)00129-X.
756 (67) Godwin, C. M.; Smits, J. E. G.; Barclay, R. M. R. Metals and Metalloids in Nestling Tree
757 Swallows and Their Dietary Items near Oilsands Mine Operations in Northern Alberta.
758 *Sci. Total Environ.* **2016**, *562*, 714–723. https://doi.org/10.1016/j.scitotenv.2016.04.069.
759 (68) Gall, J. E.; Boyd, R. S.; Rajakaruna, N. Transfer of Heavy Metals through Terrestrial Food
760 Webs: A Review. *Environ. Monit. Assess.* **2015**, *187* (4). https://doi.org/10.1007/s10661-
761 015-4436-3.
762 (69) Nordberg, G. F.; Fowler, B. A.; Nordberg, M.; Friberg, L. T. *Handbook on the Toxicology*
763 *of Metals*, 4th ed.; Academic Press: London, 2014.
764 (70) Chunhabundit, R.; Srianujata, S.; Bunyaratvej, A.; Kongkachuichai, R.; Satayavivad, J.;
765 Kaojarern, S. Cadmium Bioavailability from Vegetable and Animal-Based Foods Assessed
766 with in Vitro Digestion/Caco-2 Cell Model. *J. Med. Assoc. Thai.* **2011**, *94* (2), 164–171.
767 (71) Hare, L. Aquatic Insects and Trace Metals: Bioavailability, Bioaccumulation and Toxicity.
768 *Crit. Rev. Toxicol.* **1992**, *22*, 327–369.
769 (72) Kabata-Pendias, A. *Trace Elements in Soils and Plants*, 4th ed.; CRC Press: Boca Raton,
770 2011.
771 (73) Singer, M. S.; Bernays, E. A. Understanding Omnivory Needs a Behavioral Perspective.
772 *Ecology* **2003**, *84* (10), 2532–2537.
773