Biodiversity and functioning of terrestrial food webs : application to transfers of trace metals.

Shinji Ozaki

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THESE DE DOCTORAT

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École doctorale n°554

Environnements – Santé

Doctorat de Sciences de la Terre et de l'Environnement

Par

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Biodiversité et fonctionnement des réseaux trophiques terrestres : Application aux transferts d'éléments traces métalliques.

Thèse présentée et soutenue à Besançon, le 18 juin 2019

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Abstract

Effects of biodiversity on zoonotic disease transmission is one of the currently hot scientific topics about the functional roles of biodiversity on ecosystem functioning and services. High diversity in host community can increase (amplification effect) or reduce (dilution effect) the transmission of pathogens, due to context-dependent differences in life history traits between hosts. Trace metals (TMs) circulate from resources to consumers via trophic links. Given the variety of life history traits within resources, it is hypothesized that their diversity would play functional roles on the transfer of TMs to consumers. The aim of this work was to test this hypothesis and to determine the underlying mechanisms, using the wood mouse (Apodemus sylvaticus) as a model consumer. Sampling was undertaken around the former smelter Metaleurop Nord in Northern France where soils were contaminated by cadmium (Cd), lead (Pb) and zinc (Zn).

Analyses about mice resources' distribution in the field (plants and invertebrates) showed that diversity was not, or slightly positively, correlated with soil TM contamination levels. Community composition of resources was also slightly modified according to soil physico-chemical properties including TM concentrations. Diversity and composition of invertebrate communities were more influenced by diversity of plants than by soil physico-chemical properties.

Plant richness in the diet was positively correlated to plant richness in the field in autumn, but not in spring. The seasonal pattern was opposite for invertebrates. Some plant resources, such as Salicaceae, Sapindaceae or Adoxaceae families, were preferred by mice but with seasonal differences. Soil TM contamination reduced mice preference for Salicaceae plants in spring and also modified relationships between richness in the field and diet richness at both seasons.

The type of food items consumed by mice affected their trophic exposure to TMs. Consumption of Salicaceae resources, known as TM accumulator plants, increased exposure to Cd and Zn. However, high exposure to Cd induced by Salicaceae was dampened when a large number of other resources were consumed.

Concentrations of TMs in plant leaves increased along the gradient of soil TM concentrations in both Salicaceae and Sapindaceae, and concentrations of Cd and Zn in leaves were higher in Salicaceae than in Sapindaceae. Higher bioconcentration factor was observed for Cd in leaves of Salicaceae than in those of Sapindaceae. No change in bioconcentration factor was observed in relation to biodiversity.

We finally demonstrated that oral exposure to Pb and accumulation of Cd in the liver and in kidneys of mice decreased along the gradient of plant richness in the field, suggesting a dilution effect. Occurrence of some resources in the field, such as Adoxaceae, Cornacae or Salicaceae families, reduced exposure to TMs, whereas occurrence of Asteraceae family increased accumulation of TMs. Occurrence of these resources was related to the gradient of plant richness.

To sum up, we showed that transfer of TMs from soils to mice was controlled by a complex combination of environmental and biological factors, including soil TM contamination, life history traits of resources, and feeding behavior of wood mice. In most cases, these factors were related to biodiversity. A dilution of TMs transfer to wood mice by a high resource diversity has been shown. This work paves the way for nature-based solutions for the control of metal pollution impacts on wildlife.

Keywords: Biodiversity, ecosystem functioning, dilution effect, ecotoxicology, trace metals, communities, trophic exposure, stomach content, metabarcoding, food identification, food webs, trophic response, food selection, diet dilution hypothesis, hyper accumulator, bioaccumulation, Apodemus sylvaticus.

Résumé

Les effets de la biodiversité sur le risque de transmission de maladies zoonotiques est l'un des sujets scientifiques débattus sur les rôles fonctionnels de la biodiversité sur le fonctionnement et les services des écosystèmes. Une grande diversité de la communauté d'hôtes peut augmenter (effet d'amplification) ou réduire (effet de dilution) la transmission des agents pathogènes, en raison des différences contextes-dépendantes des trais d'histoire de vie entre les hôtes. Les éléments traces métalliques (ETM) circulent des ressources alimentaires aux consommateurs par les liens trophiques. Étant donné la variété des traits d'histoire de vie des ressources, il est supposé que la diversité des ressources joue un rôle fonctionnel dans le transfert des ETM au sein des réseaux trophiques. Le but de cette thèse est de tester cette hypothèse et de déterminer les mécanismes sous-jacents en utilisant le mulot sylvestre (Apodemus sylvaticus) comme modèle biologique. L'échantillonnage a été effectué autour de l'ancienne fonderie Metaleurop Nord dans le nord de la France où les sols sont contaminés par du cadmium (Cd), du plomb (Pb) et du zinc (Zn).

Les analyses sur la répartition des ressources des mulots, plantes et invertébrés, ont montré que la diversité n'était pas, ou légèrement positivement, corrélée aux niveaux de contamination des ETM dans les sols. La composition des ressources sur le terrain a été légèrement modifiée en fonction des propriétés physico-chimiques des sols, y compris la concentration en ETM. La diversité et la composition des invertébrés étaient plus influencées par la diversité des plantes que par les propriétés physico-chimiques des sols.

La richesse en plantes dans le régime alimentaire des mulots est corrélée positivement à la richesse en plantes sur le terrain à l'automne, mais pas au printemps. La tendance saisonnière était opposée pour les invertébrés. Certaines ressources végétales, comme les Salicaceae, les Sapindaceae ou les Adoxaceae, sont préférablement consommées par les mulots, mais avec une différence saisonnière. La préférence pour les Salicaceae est réduite le long du gradient de contamination en ETM dans les sols au printemps. Les relations entre la richesse sur le terrain et la richesse dans le régime alimentaire ont également été affectées aux deux saisons.

Le type de ressources consommées par les mulots influence leur exposition aux ETM. La consommation de Salicaceae, plantes accumulatrices d'ETM, augmente l'exposition au Cd et au Zn. Cependant, l'exposition au Cd par ingestion de Salicaceae est réduite lorsqu'un grand nombre d'autres ressources est consommé.

Les concentrations en ETM dans les feuilles de Salicaceae et de Sapindaceae augmentent le long du gradient de contamination dans les sols. Les concentrations en Cd et Zn dans les feuilles de Salicaceae sont plus élevées que celles des feuilles de Sapindaceae. Les facteurs de bioconcentration pour le Cd sont plus élevés dans les feuilles de Salicaceae que dans celles de Sapindaceae. Aucun changement du facteur de bioconcentration n'a été observé par rapport à la biodiversité.

Il a finalement été démontré que l'exposition au Pb et l'accumulation en Cd dans le foie et dans les reins des mulots diminuent le long du gradient de richesse des plantes sur le terrain, ce qui suggère un effet de dilution. La présence de certaines ressources sur le terrain, telles que les Adoxaceae, les Cornacae ou les Salicaceae, réduit l'exposition aux ETM, tandis que la présence d'Asteraceae augmente l'accumulation des ETM. La présence de ces ressources est liée au gradient de richesse en plantes sur le terrain.

En résumé, il a été montré que le transfert des ETM des sols aux mulots est contrôlé par une combinaison complexe de facteurs environnementaux et biologiques, comme la contamination des sols par les ETM, les traits d'histoire de vie des ressources et le comportement alimentaire des mulots. Dans la plupart des cas, ces facteurs sont liés à la biodiversité des resources. La dilution du transfert des ETM par la biodiversité des ressources a été démontrée. Ces travaux permettent d'envisager des approches écologiques pour le contrôle des impacts de la pollution par les métaux sur la faune.

Mots-clés: Biodiversité, fonctionnement des écosystèmes, effet de dilution, écotoxicologie, éléments traces metalliques, communautés, exposition trophique, contenu stomacal, metabarcoding, identification des aliments, réseau trophique, réponse trophique, sélection alimentaire, diet dilution hypothesis, hyper-accumulateur, bioaccumulation, *Apodemus sylvaticus*.

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Part I. Introduction

I.1 Biodiversity and ecosystem functions

I.1.1 Roles of biodiversity on ecosystem functioning

"*Biodiversity*" represents the variety of living organisms or traits at several levels of organization from genes to ecosystems. Although the term "biodiversity" is currently recognized in the general grand public, its appearance in the scientific literature was recent. Harper and Hawksworth (1994) pointed out that the term first appeared in the planning of the "*National Forum on BioDiversity*" by Walter C. Rosen in 1986. The proceeding of the forum was then published under the editorship of E. O. Wilson in 1988, entitled "*Biodiversity*". However, even before the appearance of the term "biodiversity", scientists had conceptualized the variety of life and used the term "*biological diversity*" in a variety of contexts. Norse (1986) first explicitly expanded and dissected "biological diversity" into three components: genetic diversity (i.e. variety within species), species diversity (i.e. variety of species) and ecological diversity (i.e. variety of species composition between communities). This concept was mirrored to the definition of biological diversity in the Convention on Biological Diversity at the United Nations Conference on the Environment and Development "The Earth Summit" in 1992: "*the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part; this includes diversity within species, between species and of ecosystems*". This definition of biological diversity is now widely referred as the definition of "biodiversity".

Numerous studies have shown that biodiversity is widely affected by human activities. Indeed, humans have altered the global environment by transforming landscapes, increasing atmospheric dioxide carbon concentration, altering accessible surface fresh water, polluting environments, etc. (Soulé, 1991; Vitousek et al., 1997). As a consequence, the current rate of species extinction is much higher than during pre-human period (Pimm et al., 1995), and biodiversity continues to decline (Butchart et al., 2010). Concern about biodiversity loss has led scientists to focus on its consequences in the ecosystem. Actually, loss of certain species from ecosystems can lead a physical formation of habitats (i.e., ecosystem engineers; Jones et al., 1994) or a radical change in structure of an ecological community (i.e. keystone species; Power et al., 1996). These examples show that disappearance of some specific species can substantially alter structure and functioning of whole ecosystems. However, scientists have also focused on a more specific question: does the variety and variability of organisms itself matter for the functioning of ecosystems?

During the last two decades, both theoretical and experimental studies have focused on relationships between biodiversity and multiple "*ecosystem functions*" which are defined as "*ecological processes that control the fluxes of energy, nutrients and organic matter through an environment*" (Cardinale et al., 2012). As the field of study on the ecosystem functions

developed, interests in essential benefits of ecosystems to the humanity, namely "*ecosystem services*", was also raised. The basic idea of the ecosystem services is anthropocentric: "*the benefits to humanity obtained from the conditions and process though which ecosystems sustain*" (Daily, 1997). The Millennium Ecosystem Assessment (2005) highlighted distinct focus of the two research fields: the effects of biodiversity on ecosystem functions and on ecosystem services. The Millennium Ecosystem Assessment (2005) also pooled the services into four categories: provisioning, regulating, cultural and supporting services. The provisioning services (i.e. the production of renewable resources like food, wood, fresh water supply to humans) and regulating services (climate regulation, pest or disease control) are related to ecosystem functions and thus frequently studied for their relationships with biodiversity. Based on the large amount of scientific publications, it is now sufficiently evident that biodiversity per se influences, or is strongly correlated with, certain provisioning and regulating services (Table I.1). For example, increasing diversity of plants enhances both wood biomass production (Piotto, 2008) and carbon sequestration (Cardinale et al., 2011), increases nutrient mineralization and soil organic matter (Quijas et al., 2010), enforces resistance to invasion by exotic plants (Levine et al., 2004) and decreases prevalence of plant pathogens (Quijas et al., 2010). Conversely, some effects of biodiversity on ecosystem regulating services, like controlling animal disease prevalence (Keesing et al., 2010), have shown opposite results or discordance between prediction and observation. Actually, the last point, i.e. a protective effect of biodiversity on disease risk in animals and humans, is one of topics hotly debated in the current scientific community.

Table I.1. Balance of evidence linking biodiversity to ecosystem services (adapted from Cardinale et al., 2012). Data are summarized as follows: green, actual data relationships agree with predictions (whether service increases or decreases as diversity increases); yellow, Data show mixed results; red, data conflict with predictions. Exp, experimental; N, number of data points; Obs, observed; SPU, service providing unit (where natural enemies include predators, parasitoids and pathogens).

Category of service	Measure of service provision	SPU	Diversity level	Study type	*N*	Relationship	
						Predicted	Actual
Provisioning							
Crops	Crop yield	Plants	Genetic	Exp	575	↗	↗
			Species	Exp	100	↘	↗↘
Fisheries	Stability of fisheries yield	Fish	Species	Obs	8	↗	↗
Wood	Wood production	Plants	Species	Exp	53	↗	↗
Fodder	Fodder yield	Plants	Species	Exp	271	↗	↗
Regulating							
Biocontrol	Abundance of herbivorous pests (bottom-up effect of plant diversity)	Plants	Species	Obs	40	↗	↗
		Plants	Species	Exp	100	↗	↗
		Plants	Species	Exp	287	↘	↗↘
		Plants	Species	Exp	100	↘	0
	Abundance of herbivorous pests (top-down effect of natural enemy diversity)	Natural enemies	Species/trait	Obs	18	↗	↗
		Natural enemies	Species	Exp/Obs	266	↗	↗
		Natural enemies	Species	Exp	38	↘	↗↘
	Resistance to plant invasion	Plants	Species	Exp	120	↗	↗
	Disease prevalence (on plants)	Plants	Species	Exp	107	↗	↗
	Disease prevalence (on animals)	Multiple	Species	Exp/Obs	45	↘	↗↘
Climate	Primary production	Plants	Species	Exp	7	↘	0
	Carbon sequestration	Plants	Species	Exp	479	↗	↗
	Carbon storage	Plants	Species/trait	Obs	33	↘	↗↘
Soil	Soil nutrient mineralization	Plants	Species	Exp	103	↗	↗
	Soil organic matter	Plants	Species	Exp	85	↗	↗
Water	Freshwater purification	Multiple	Genetic/species	Exp	8	↘	0
Pollination	Pollination	Insects	Species	Obs	7	↘	↗↘

I.1.2 Functional effects of biodiversity on zoonotic disease risk

The hypothesis about the influence of ecological community diversity on the transmission of pathogens traces back to more than a century. Brumpt (1944) pointed out that protective effects of domestic animals on humans against mosquito bites had been reported as early as 1903. However, the concept of the zooprophylaxis, "*the use of wild or domestic animals, which are not the reservoir hosts of a given disease, to divert the blood-seeking mosquito vectors from the human*" (WHO, 1982), has been practiced long time in various parts of the world to protect people from malaria, often thanks to cattle which serves as the most suitable hosts (Service, 1991). For example, Dobson et al. (2006) suggested that cows have been considered to be a sacred animal in India for a long time, probably thanks to their protective effect against malaria.

Recently, potential effects of biodiversity on disease risk has become in the limelight in the scientific community, especially due to concern about the biodiversity loss. One of classic scientific investigation about the effects of species diversity on disease transmission is about Lyme disease in the forests of the northeastern United States. On the basis of previous theoretical, observational, and simulation studies about the ecology of Lyme disease in those forests (e.g. Ostfeld, 1997), protective effect of diversity of vertebrate hosts against Lyme disease risk in humans was proposed and then considered to be one of functional effects of the diversity in the ecological community, under the name of "*dilution effect*" (LoGiudice et al., 2003; Ostfeld and Keesing, 2000a, 2000b; Schmidt and Ostfeld, 2001).

A few years later, Keesing et al. (2006) redefined the dilution effect in the eco-epidemiological context: "*the net effect of species diversity reducing disease risk by any of a variety of mechanisms for both vector-borne and non-vector-borne diseases*". The opposite of the dilution effect, namely "*amplification effect*", was also defined in the same paper as "*an increase in disease risk when species diversity increases*". Several researches have reported so far that biodiversity loss tends to increase pathogen transmission and disease incidence (i.e. dilution effect) across different ecological systems that vary in type of pathogen, host, ecosystem and transmission mode (Keesing et al., 2010; Table I.2). However, although to a lesser extent, some cases of amplification effect have been reported in the literature. Debates about the generality of the dilution effect continue to rise steadily. For example, the meta-analysis conducted by Salkeld et al. (2013) using 13 published and some unpublished studies on different diseases showed that the overall relationship between biodiversity and disease risk was weekly negative but highly variable among studies. On the other hand, the meta-analysis conducted by Civitello et al. (2015) using more than 200 zoonoses and other diseases demonstrated consistent evidence for dilution effects in diverse host communities, independently host or parasite type. Ostfeld and Keesing (2012) calculated the degree to which the dilution and amplification effects occur on the basis of the review by Cardinale et al. (2012) about the consequence of biodiversity loss: 80% of studies about the relationship between biodiversity and the disease transmission showed a statistically significant negative relationship (i.e. dilution effect), whereas 12% showed a significant positive relationship (i.e. amplification effect), and 8% of studies showed no significant relationship.

Table I.2. Examples of diseases for which species loss leads to increases in total transmission (from Keesing et al., 2010).

Disease	Reference
Amphibian limb malformation	Johnson et al., 2009
Bacteriophage of Pseudomonas	Dennehy et al., 2007
Coral diseases	Raymundo et al., 2009
Fungal disease of Daphnia	Hall et al., 2009
Hantavirus disease	Clay et al., 2009; Dizney and Ruedas, 2009; Tersago et al., 2008
Helminthic parasite of fish	Kelly et al., 2009
Lyme disease	Brunner and Ostfeld, 2008; Keesing et al., 2009; LoGiudice et al., 2008
Malaria	Carlson et al., 2009
Puccinia rust infection of ryegrass	Roscher et al., 2007
Schistosomiasis	Johnson et al., 2009
Trematode diseases of snails and birds	Kopp and Jokela, 2007; Thieltges et al., 2008, 2009
West Nile fever	Allan et al., 2009; Ezenwa et al., 2006; Koenig et al., 2010; Swaddle and Calos, 2008

I.1.3 Ecological conditions determining the dilution effect

In fact, researchers recognized at the early stage some ecological conditions under which a dilution effect could occur (Ostfeld and Keesing, 2000b; Schmidt and Ostfeld, 2001). The possibility of the amplification effect were also thought when these conditions are not met (Ostfeld and Keesing, 2000a; N.B. the term "*rescue effect*" was used in this paper instead of the term "amplification effect"). Large attention of researchers has thus focused on ecological conditions determining the occurrence of dilution effect and the generality of those condition in nature. Ostfeld and Keesing (2012) summarized on the basis of the literature three main conditions under which a dilution effect is expected to occur: (i) "*hosts differ in quality for pathogens or vectors*", (ii) "*higher quality hosts tend to occur in species-poor communities, whereas lower quality hosts tend to occur in more diverse communities*", and (iii) "*lower quality hosts regulate abundance of high-quality hosts or of vectors, or reduce encounter rates between these (high-quality) hosts and pathogens or vectors*". In the case of Lyme disease in northeastern United States, for example, Lyme disease is due to a bacterium *Borrelia burgdorferi* (pathogen) and is transmitted to humans through blacklegged ticks *Ixodes scapularis* (vector) infected by the bacterium. Blacklegged ticks are generalist and thus feed on several birds and mammals (hosts). Ticks become infected only when they feed on hosts holding *B. burgdorferi*. Among potential hosts present in the forests, ticks feeding on the white-footed mouse *Peromyscus leucopus* become more likely infected than ticks feeding on other animals (i.e. the first condition: difference in quality for pathogens or vectors; represented by green-and-yellow circles in Figure I.1). Because of the reliance of ticks on access to vertebrate hosts and high mortality when they fail to find a host, tick abundance is thought to be correlated with

abundance of hosts. In this case, an increase in biodiversity could lead to two opposite outcomes, depending on the total density of both more and less competent hosts (Figure I.2; Dobson, 2004; Rudolf and Antonovics, 2005). However, the mortality of ticks due to host grooming is more important in other animals than in the white-footed mouse. Other hosts contribute more importantly than the white-footed mouse to a decrease of tick density (i.e. the third condition: *regulation by low quality hosts in abundance of vectors*; represented by blue circles in Figure I.1). The white-footed mouse is widespread and can habit even within fragmented landscapes, while its density is reduced in communities where species richness and/or abundance of non-mouse animals is high (e.g., Nupp and Swihart, 2000). Communities with low diversity are more likely to contain high density of the white-footed mouse and low density of other animals (i.e. the second condition: difference in sensibility to biodiversity loss). As a consequence, when biodiversity declines, the higher quality host remains, while lower quality hosts disappears.

The generality of the difference in quality for pathogens or vectors (i.e. the first condition) has been demonstrated in a variety of zoonotic diseases, whereas the generality of the second and third conditions remains to be evaluated. However, the species that tend to disappear from ecological communities with low diversity are species which reduce pathogen transmission (i.e. general aspect of the second condition; Keesing et al., 2010). Indeed, certain life-history treats, like lifespan and trade-offs between reproductive effort and survival, might be linked to both resilience to anthropogenic forces (i.e. biodiversity loss) and permissiveness to pathogens (Previtali et al., 2012). Furthermore, the first and second conditions are sufficient when a given transmission is frequency dependent, regardless of changes in abundances of individual species (Figure I.2; Dobson, 2004; Rudolf and Antonovics, 2005). The third condition of the dilution effect is required only when a transmission is density dependent and when the total abundance of all potential hosts is positively correlated to biodiversity in parallel. However, frequency-dependent transmission is thought to characterize most vector-borne diseases because a vector's biting rate is assumed to be a determining factor (Ostfeld and Keesing, 2012). This suggests the third condition is not mandatory in several cases. Ostfeld and Keesing (2012) thus concluded that the dilution effect generally occurs in a variety of natural and constructed systems thanks to general aspects of its conditions.

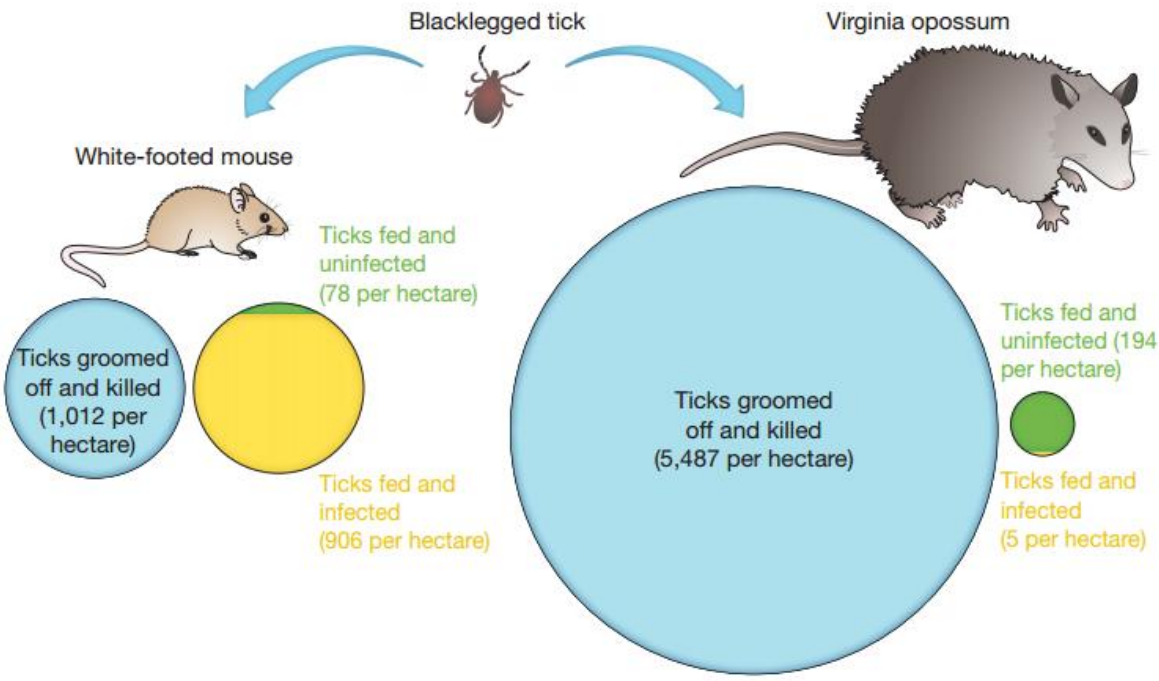


Figure I.1. Schema for roles of two types of host species in the transmission of Lyme disease in the northeastern United States (from Keesing et al., 2010). Lyme disease is transmitted to humans by the bite of an infected blacklegged tick. Immature ticks can acquire the infection if they feed on an infected host and can become infectious to humans if they subsequently survive to the next life stage. Green and yellow circles show the mean number of ticks per hectare fed by hosts; yellow shading shows the proportion of ticks infected after feeding. Blue circles show the mean number of ticks per hectare groomed off and killed by hosts. Ticks that attempt to feed on white-footed mice are likely to be less infected and more killed, whereas ticks that attempt to feed on Virginia opossums are likely to be less infected and more and killed. Ticks that feed on mice are highly likely to become infected with the bacterium that causes Lyme disease, whereas those that feed on opossums are not.

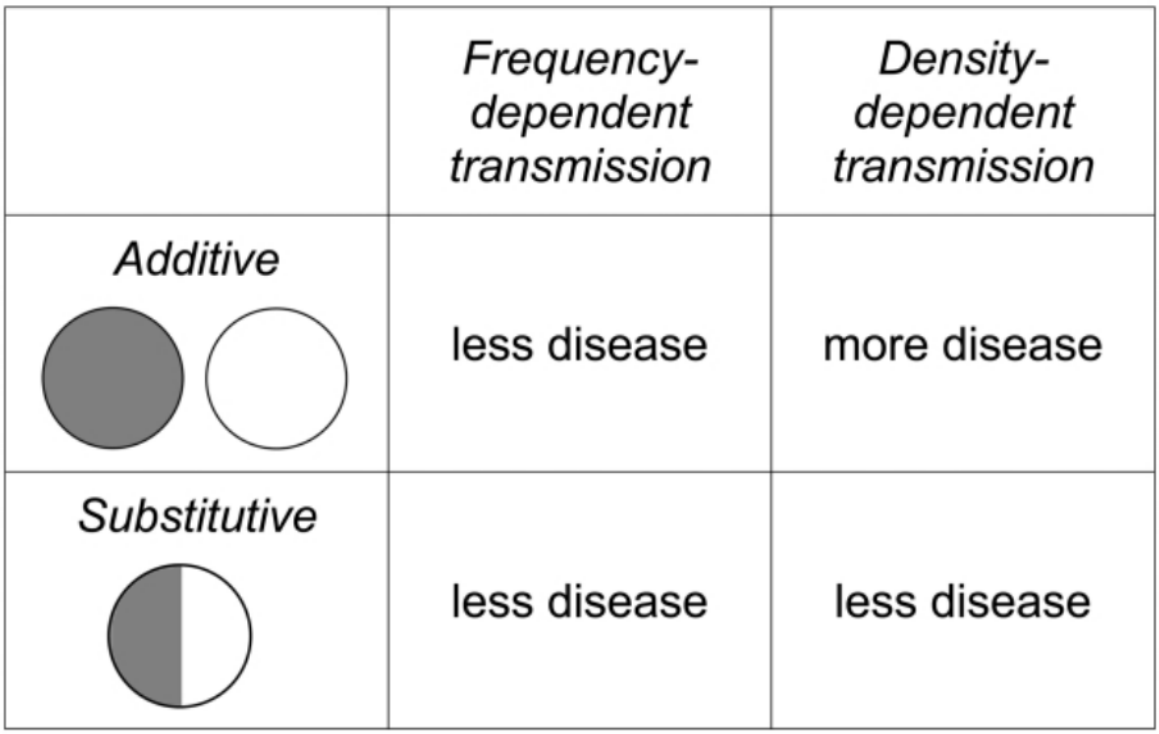


Figure I.2. Effect of transmission mode on disease levels in a target population (shaded) which is more competent in transmission of pathogen following the addition of a second species which is less competent (unshaded), i.e., an increase in diversity(from Rudolf and Antonovics, 2005). The circles represent the density of the populations. Whether an increase in diversity is additive (i.e. the second population increases the total density; top) or substitutive (i.e. a part of the density of the first population is substituted by the second population; bottom), diversity is the same. The outcomes in the two left-hand panels represent the outcomes in the case of the frequency-dependent transmission. The outcomes in the right-hand panels represent the outcomes in the case of the density-dependent transmission.

I.1.4 Possibility of a dilution effect for transfer of contaminants.

Chemical substances can be circulated from one to another organism, via trophic links between them. It is probably relevant to ask whether a kind of "dilution" occurs for a flux of chemical substances in food webs in an ecological community encompassing a variety of organisms. Given that chemical contaminants can cause some threats on health of organisms and/or ecosystems, a protecting role of biodiversity could be proposed as a new ecosystem service or function. To answer this question, it is now necessary to enlighten the mechanisms of the transfer and the fate of chemical contaminants in ecosystems, with a focus on trace metals, and to compare those mechanisms to the ecological conditions of the dilution effect.

I.2 Trace metals

I.2.1 Trace metals: metallic elements occurring in trace amounts in nature

A metal is defined as an element with lustrous appearance, high electrical conductivity and chemical reactions as cations. Elements considered to be metals can be identified within the periodic system (Table I.3). Elements belonging to the same vertical column in the periodic table share similar chemical properties. The first two left columns show respectively elements readily yield mono- and divalent cations. Most of them are commonly found in surface waters and soils, like sodium (Na), potassium (K), magnesium (Mg) and calcium (Ca). The following 10 columns list the transition elements. From left to right, these elements show less tendency to form cations and share electrons with other elements. Elements placed at right side are nonmetal elements, like carbon (C), nitrogen (N), oxygen (O), phosphorus (P) or sulfur (S). Some elements placed between metals and nonmetals are called metalloids, like boron (B), silicon (Si) or arsenic (As), which show characteristics of both metals and nonmetals.

Table I.3. Periodic table of the elements. Those considered to be metal are colored in pink. Non-metal elements are colored in blue. Metaloids are in green. Elements naturally present on the Earth are in bold.

H																	**He**
Li	**Be**											**B**	**C**	**N**	**O**	**F**	**Ne**
Na	**Mg**											**Al**	**Si**	**P**	**S**	**Cl**	**Ar**
K	**Ca**	**Sc**	**Ti**	**V**	**Cr**	**Mn**	**Fe**	**Co**	**Ni**	**Cu**	**Zn**	**Ga**	**Ge**	**As**	**Se**	**Br**	**Kr**
Rb	**Sr**	**Y**	**Zr**	**Nb**	**Mo**	**Tc**	**Ru**	**Rh**	**Pd**	**Ag**	**Cd**	**In**	**Sn**	**Sb**	**Te**	**I**	**Xe**
Cs	**Ba**	*	**Hf**	**Ta**	**W**	**Re**	**Os**	**Ir**	**Pt**	**Au**	**Hg**	**Tl**	**Pb**	**Bi**	**Po**	**At**	**Rn**
Fr	**Ra**	**	Rf	Db	Sg	Bh	Hs	Mt	Ds	Rg	Cn	Nh	Fl	Mc	Lv	Ts	Og

* Lanthanide Series	**La**	**Ce**	**Pr**	**Nd**	**Pm**	**Sm**	**Eu**	**Gd**	**Tb**	**Dy**	**Ho**	**Er**	**Tm**	**Yb**	**Lu**
** Actinide Series	**Ac**	**Th**	**Pa**	**U**	**Np**	**Pu**	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

The term "*heavy metals*" is often used in the literature for referring to metallic elements, sometimes including metalloids, with a relatively high density of them showing toxic effects on organisms at low concentration. A metal which specific gravity is approximately five or higher is widely considered as to be "heavy" (Parker, 2003). However, atomic mass or metal

density is not related to their biological effects, and the definition of heavy metals based on atomic mass have been criticized. In Europe, for example, the heavy metals in the context of hazardous wastes are defined by the Commission decision of 3 May 2000 as follows: "*('heavy metal' means) any compound of antimony, arsenic, cadmium, chromium(VI), copper, lead, mercury, nickel, selenium, tellurium, thallium and tin, as well as these materials in metallic form, as far as these are classified as dangerous substances*". Otherwise, other terms like "*trace elements*" or "*trace metals*" are also used for referring to toxic metallic elements. Trace metals are considered as to be metallic elements among trace elements, and the latter can be defined as elements that occur in amounts less than 0.1% (1000 mg/kg) in the Earth's crust or in biological materials (Kabata-Pendias, 2011). It is worth to note that metals considered as trace metals depend on study field. For example, iron (F) is "trace" in biological materials but not "trace" in geochemistry. In the present thesis, the term "trace metals (TMs)" is used on the basis of the definition above in the context of biological sciences, i.e. all metals except Ca, K, Na and Mg.

According to this definition, not all TMs are *a priori* toxic for life beings. Some TMs, such as cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), and zinc (Zn), are essential elements and required for various biochemical and physiological functions of organisms (WHO, 1996). Each essential element has however a window of essentiality (i.e. a range of optimal intakes by organisms for maintaining their biological performance; Figure I.3a), and both a deficiency and an over-intake cause biochemical or physiological dysfunctions. Some other TMs such as cadmium (Cd) or mercury (Hg) are non-essential for organisms. They have no established biological functions and an over-intake of those TMs above a certain threshold can cause dysfunctions. (Figure I.3b).

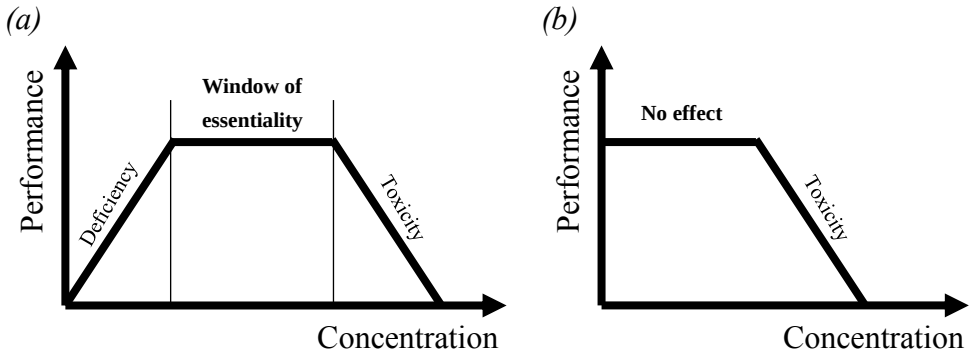


Figure I.3. Schematic diagram showing the relationship between the performances of an organism. (e.g. growth, survival, fecundity) and the amount of an essential element (a) or an non-essential element (b) taken by the organism (modified after Walker et al., 2012).

I.2.2 Contamination of ecosystems by TMs

All metals, except some elements produced by humans in laboratory like rutherfordium, are naturally present on the Earth. An occurrence of high TM concentrations in the environment can be simply due to a natural weathering that releases TMs from ore bodies (e.g. Hågvar and Abrahamsen, 1990) or other natural sources like volcanic emissions or wild forest fires (Nriagu, 1979, 1989). However, human activities are responsible for a large part of global emissions of TMs. Several industrial activities indeed release a variety of TMs (Table I.4), and emissions of TMs from industries, especially emissions of cadmium (Cd), lead (Pb) and zinc (Zn), occupy a high rate of their global atmospheric cycle compared with natural fluxes (Nriagu, 1989; Table I.5). TMs in environments are then partly assimilated in organisms. Metal uptake by organisms is usually not a linear function of their concentrations in the ambient environment. The uptake depends on many factors such as mobility of TMs in environments, exposure routes, assimilability of TMs though biological membranes, and potential antagonisms with other metals (Eisler, 2000; Kabata-Pendias, 2011; Walker et al., 2012). In terrestrial ecosystems, plants are contaminated by TMs due to foliar deposits or through root system from soils (Kabata-Pendias, 2011). Terrestrial vertebrates and invertebrates are contaminated by TMs via three routes: oral, respiratory or cutaneous routes (Walker et al., 2012), among which exposure by oral route, i.e. consuming contaminated food, is the principal route for wild mammals (Shore and Rattner, 2001).

When a harmful substance is assimilated in the organism, some detoxification reactions are triggered off. For TMs which are non-biodegradable (i.e. not break down by organisms), binding active metals with specific proteins and/or ligands, depositing those inactivated metals for a long-term storage, and/or excreting metals outside of body are the main mechanisms of detoxification (Walker et al., 2012). Like the difference in the uptake of TMs from the ambient environment, detoxification reactions also depend on homeostatic mechanisms, which are conditioned by age, sex, interaction with other elements, nutrient state and assimilated elements (Nordberg et al., 2014). When organisms develop a storage strategy, they show a high accumulation of TMs in their body. Some plants are known as "*accumulator*" or even "*hyper-accumulator*" of TMs because of their high metal concentrations in tissues (i.e. absolute concentrations), their high bioconcentration factor (i.e. ratio of metal concentrations between in tissues and in soil), or their shoot-to-root quotient of metal concentrations (van der Ent et al., 2013). For example, *Thlaspi* (currently *Noccaea*) *caerulescens* and *Arabidopsis halleri*, both belonging to Brassicaceae family, have been well reported as Cd hyper-accumulator (e.g. Abe et al., 2008; Bert et al., 2003; Lombi et al., 2000) (Table I.6). About 450 angiosperm species have been identified hyper-accumulators of several elements (e.g. As, Cd, Co, Cu, Mn, Ni, Pb, or Zn) until 2011 (Rascio and Navari-Izzo, 2011). As new reports about hyper-accumulators continue to accrue, it is expected that many yet unidentified hyper-accumulator plants exist in

nature. Likewise, some invertebrate taxa are likely to accumulate a large amount of metals in their tissues such as hepatopancreas under non-toxic forms. For example, Lumbricidae, i.e. earthworms, are often referred to as having a high TM accumulation capacity (e.g. Hopkin, 1989; Morgan and Morgan, 1988). Isopoda (e.g. woodlice) have shown higher body concentrations of TMs, especially Cd, than other invertebrates independently of soil TM contamination levels (e.g. Heikens et al., 2001; Hunter et al., 1987a) (Table I.7). Efficiency in detoxification reactions of organisms, e.g. limiting metal uptake, storage metals and and/or excreting in their feces, is linked to their tolerance to the environmental TM contamination (Clemens et al., 2002; Gall et al., 2015).

Table I.4. Occurrence of metals (including some metalloids) or their compounds in effluents from various industries (from Nagajyoti et al., 2010).

Industry	Metals																											
	Al	Ag	As	Au	Ba	Be	Bi	Cd	Co	Cr	Cu	Fe	Ga	Hg	In	Mn	Mo	Os	Pb	Pd	Ni	Sb	Sn	Ta	Ti	Tl	U	V	W	Zn
Mining operations and ore processing	X		X					X						X		X	X		X								X	X		
Metallurgy and electroplating		X	X			X	X	X		X	X			X	X				X		X								X	X
Chemical industries	X		X		X			X		X	X	X	X	X				X	X				X	X	X		X			X
Dyes and pigments	X		X					X			X	X							X			X			X	X				
Ink manufacturing									X		X	X		X							X									
Pottery and poccelain			X							X												X					X			
Alloys						X							X		X			X		X				X						
Print					X					X								X	X						X					X
Photography		X		X				X		X							X			X							X			
Glass			X		X				X												X				X			X		
Paper mills	X									X	X			X						X		X		X	X			X		
Leather training	X		X		X					X	X	X		X																X
Pharmaceuticals	X										X	X	X	X				X						X						
Textiles	X		X		X			X			X	X		X				X			X	X								
Nuclear technology					X			X							X												X			
Fertilizers	X		X					X		X	X	X		X		X				X	X									X
Chlor-alkali production	X		X					X		X		X		X		X				X			X							X
Petroleum refining	X		X					X		X		X	X	X					X		X									X

Table I.5. Natural versus anthropogenic emissions of some TMs and metalloids to the atmosphere in 1983 (from Nriagu, 1989). The median values with the ranges (in parentheses) of estimated emissions are shown in the unit of 10^6 kg yr^{-1}.

Trace metal	Anthropo-genic source	Natural source	Total emission	Natural/total emissions*
As	19 (12–26)	12 (0.86–23)	31 (13–49)	0.39
Cd	7.6 (3.1–12)	1.3 (0.15–2.6)	8.9 (3.2–15)	0.15
Cr	30 (7.3–54)	44 (4.5–83)	74(12–137)	0.59
Cu	35 (20–51)	28 (2.3–54)	63 (22–105)	0.44
Hg	3.6 (0.91–6.2)	2.5 (0.10–4.9)	6.1 (1.0–11)	0.41
Mn	38 (11–66)	317 (52–582)	355 (63–648)	0.89
Mo	3.3 (0.79–5.4)	3.0 (0.14–5.8)	6.3 (0.93–11)	0.48
Ni	56 (24–87)	30 (3.0–57)	86 (27–144)	0.35
Pb	332 (289–376)	12 (0.97–23)	344 (290–399)	0.04
Sb	3.5 (1.5–5.5)	2.4 (0.07–4.7)	5.9 (1.6–10)	0.41
Se	6.3 (3.0–9.7)	9.3 (0.66–18)	16 (2.5–24)	0.58
V	86 (30–142)	28 (1.6–54)	114 (32–220)	0.25
Zn	132 (70–194)	45 (4.0–86)	177 (74–280)	0.34

*: Median values only

Table I.6. Plant species that had relatively high (≥ 30.0 mg kg^{-1}) and low (≤ 3.0 mg kg^{-1}) Cd concentrations in their shoots (from Abe et al., 2008).

Scientific name	Family name	Cd concentration (mg kg^{-1} DW)
Plant species with relatively high Cd concentrations		
Cichorium intybus	Compositae	77.0
Matricaria chamomilla	Compositae	64.4
Polygonum thunbergii	Polygonaceae	56.2
Cyperus brevifolius var. *leiolepis*	Cyperaceae	48.3
Sisymbrium orientale	Cruciferae	48.2
Sisymbrium altissimum	Cruciferae	48.2
Picris echinoides	Compositae	41.0
Cyperus globosus	Cyperaceae	39.6
Sonchus asper	Compositae	37.0
Malva sylvestris	Malvaceae	33.5
Bidens frondosa	Compositae	31.0
Onopordum acanthium	Compositae	30.0
Plant species with relatively low Cd concentrations		
Oryza sativa (cv. Milyang 42)	Gramineae	0.8
Panicum dichotomiflorum	Gramineae	1.0
Dactylis glomerata	Gramineae	1.2
Plantago virginica	Plantagineaceae	1.4
Panicum bisulcatum	Gramineae	1.5
Oenothera biennis	Onagraceae	1.8
Digitaria ciliaris	Gramineae	1.9
Sesbannia exaltata	Leguminosae	2.1
Echinochloa crus-galli var. *prachicola*	Gramineae	2.1
Coix lacryma-jobi	Gramineae	2.2
Sorgum bicolor (cv. Hybrid sorghum)	Gramineae	2.2
Panicum virgatum	Gramineae	2.2
Panicum dilatatum	Gramineae	2.2
Trifolium repens	Leguminosae	2.7
Poa pratensis	Gramineae	2.7
Setaria faberi	Gramineae	3.0

DW, dry weight.

Table I.7. Regression equations, $log(C_o) = log(a) + b \cdot log(C_s)$, with the standard deviation (S.D.) for metal concentrations in invertebrates and the statistical comparison of the accumulation levels of the taxonomic groups at the $P < 0.05$ level[a] (form Heikens et al., 2001).

Metal & group	Regression equation			Statistical comparison of accumulation levels								
Cd	log a ± S.D.	b ± S.D.	r^2	α↓β→	ara	chi	cole	coll	diplo	diplu	for	iso	lum
Arachnida	0.9 ± 0.1	0.47±0.08	0.37	ara									
Chilopoda	1.0 ± 0.3	−0.14 ± 0.26*	0.07	chi	×								
Coleoptera	−0.1 ± 0.1	0.60 ± 0.01	0.45	cole	<	×							
Collembola	0 .0 ± 0.2	0.77 ± 0.20	0.43	coll	<	×	>						
Diplopoda	0.6 ± 0.2	0.60 ± 0.14	0.73	diplo	=	×	>	>					
Diplura	0.7 ± 0.1	0.56 ± 0.11	0.89	diplu	=	>	>	>	=				
Formicidae	1.1 ± 0.2	−0.04 ± 0.19*	0.00	for	×	=	×	×	×	=			
Isopoda	1.5 ± 0.3	0.09 ± 0.24*	0.02	iso	×	>	×	×	=	=	>		
Lumbricidae	1.1 ± 0.1	0.39 ± 0.09	0.54	lum	=	>	>	×	>	=	×	=	
Cu	log a ± S.D.	b ± S.D.	r^2	α↓ β→	ara	cole	coll	diplo	for	iso	lum		
Arachnida	1.1 ± 0.1	0.35 ± 0.06	0.45	ara									
Coleoptera	0.8 ± 0.2	0.31 ± 0.07	0.36	cole	<								
Collembola	1.3 ± 0.3	0.32 ± 0.11	0.43	coll	=	>							
Diplopoda	1.9 ± 0.1	0.26 ± 0.06	0.76	diplo	>	>	>						
Formicidae	0.5 ± 0.3	0.41 ± 0.14	0.32	for	<	=	<	<					
Isopoda	1.9 ± 0.5	0.24 ± 0.19*	0.20	iso	>	>	>	=	>				
Lumbricidae	0.6 ± 0.1	0.46 ± 0.06	0.75	lum	<	=	<	<	=	<			
Pb	log a ± S.D.	b ± S.D.	r^2	α↓ β→	ara	chi	cole	coll	diplo	For	iso	lum	
Arachnida	0.2 ± 0.3	0.49 ± 0.07	0.52	ara									
Chilopoda	−0.5 ± 1.3	0.47 ± 0.39*	0.27	chi	<								
Coleoptera	−1.9 ± 0.3	0.98 ± 0.09	0.78	cole	×	=							
Collembola	−1.5 ± 0.8	1.13 ± 0.23	0.72	coll	×	×	>						
Diplopoda	−0.7 ± 0.8	0.84 ± 0.22	0.78	diplo	=	>	>	=					
Formicidae	−0.3 ± 0.6	0.63 ± 0.17	0.48	for	=	>	>	×	=				
Isopoda	3.0 ± 0.9	−0.10 ± 0.24*	0.05	iso	×	>	×	×	=	×			
Lumbricidae	−0.3 ± 1.4	0.62 ± 0.58*	0.09	lum	=	>	>	<	=	=	=		
Zn	log a ± S.D.	b ± S.D.	r^2	α↓ β→	ara	chi	cole	coll	diplo	for	iso	lum	
Arachnida	1.8 ± 0.2	0.34 ± 0.05	0.49	ara									
Chilopoda	2.1 ± 0.9	0.18 ± 0.28*	0.10	chi	=								
Coleoptera	1.5 ± 0.4	0.24 ± 0.11	0.13	cole	<	<							
Collembola	0.1 ± 1.0	0.78 ± 0.38*	0.32	coll	×	×	×						
Diplopoda	2.2 ± 0.6	0.29 ± 0.19*	0.35	diplo	>	>	>	×					
Formicidae	2.7 ± 0.7	−0.04 ± 0.20*	0.00	for	×	=	>	×	<				
Isopoda	2.8 ± 0.6	0.09 ± 0.17*	0.09	iso	=	=	>	×	=	>			
Lumbricidae	1.8 ± 0.1	0.17 ± 0.03	0.70	lum	<	=	=	×	<	=	<		

[a] C_o = internal metal concentration (µg/g dry wt.), C_s = total soil concentration (µg/g dry wt.), * = b is not significant different from zero. The number of samples can be found in Table 1. The regression equation of a taxonomic group α is compared with the regression equation of a group β. × α and β are not parallel, the level of the regression lines cannot be compared; = α and β are not significant different from each other; < α is significant lower than β; > α is significant higher than β. Arachnida (ara), Chilopoda (chi), Coleoptera (cole), Collembola (coll), Diplopoda (diplo), Diplura (diplu), Formicidae (for), Isopoda (iso), Lumbricidae (lum).

I.2.3 Effects of TM contamination on biodiversity

Once contaminants enter an ecosystem, each population of organisms shows different dynamics: a disappearance, a decline until another stable size, or possibly an increase (Walker et al., 2012; Figure I.4), or an absence of observable effect. For example, Vidic et al. (2006) showed that a low species richness in a community, which was composed of hyper-accumulator plants during a period of high TM pollution, had recovered after a reduction in emissions of TMs from a lead smelter in Slovenia. The consequence of the environmental TM contamination on the diversity and composition of the community depends, at least partly, on the sensibility of each initial population to contaminants. Indeed, Dazy et al. (2009) showed that abundance of plants in a former coke-factory site in France depended on both soils TM contamination

levels and plant antioxidant systems against reactive oxygen species (ROS) provoked by TMs, whereas Nahmani and Lavelle (2002) observed in northern France a higher richness of soil macro invertebrates in Zn polluted areas than in unpolluted areas, due to a settlement of metal tolerant arthropod taxa. In some cases, rather composition of community than species richness or biomass has shown a clear change along TM soil contamination gradients (e.g. Migliorini et al., 2004; Strandberg et al., 2006) (Table I.8).

However, the relationship between biodiversity and the TM contamination is complicated because of the influence of other environmental factors. For instance, changes in plant abundance near a copper smelter in Chile were explained by total soil nitrogen rather than soil pH and copper content (Ginocchio, 2000), while species richness of plants was not correlated with TM content of soil but with other properties (e.g. carbonate content, conductivity of the soil, and soil C/N ratio) in an ancient mining area in Germany (Becker and Brändel, 2007). In addition to such soil physico-chemical factors, vegetation can be another factor modifying diversity and composition of terrestrial invertebrates. Although being not still verified in a TM contaminated site, the diversity of the vegetation has been shown to play a determinant role, as habitat and/or resources, on the diversity and the composition of invertebrate community in both grassland and woodland (e.g. Haddad et al., 2001; Scherber et al., 2014; Siemann, 1998). On the other hand, some plants can be introduced by humans in a TM contaminated site for the phytoremediation, i.e. the use of plants to remove pollutants from the environment or to render them harmless (Salt et al., 1998). In this case, a change in vegetation may be not correlated directly with TM contamination levels but influenced by introduction of certain plants, usually high TM accumulators, by humans. Some meta-analyses have shown that, despite a generally negative impact of air TM pollution on biodiversity, the effects of pollution are highly heterogeneous depending on characteristics of the specific pollutant of concern and contamination context (i.e. type, amount of emission, duration of impact on biota), the affected organism (i.e. trophic group, life history), and the ambient environment (Kozlov and Zvereva, 2011; Zvereva and Kozlov, 2010, 2012; Zvereva et al., 2008) (Figure I.5).

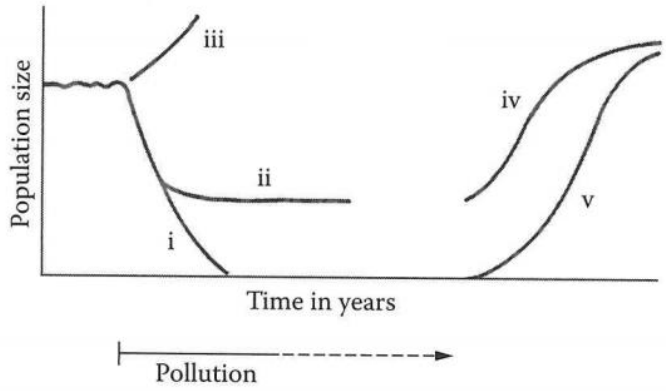


Figure I.4. Possible responses of population size to pollution (taken form Walker et al., 2012). The number of some species can decline to zero (curve i) or to a lower level (curve ii). The number can even increase (curve iii). If the pollution is chronic and resistance evolves within the population, the number can recover from the levels to which it was depressed by the pollution (curve iv) or even from the disappearance through immigration and recolonization (curve v).

Table I.8. Taxa distribution in the shooting range and control sites (S and N represent the number of taxa and total abundance at each site) (form Migliorini et al., 2004)). Pb HNO3-extractable fractions in soil from sampling sites were Contr.2 < Contr.1 < C < A < B < G < D < F < E.

	A	B	C	D	E	F	G	Contr. 1	Contr. 2
Protura			1	1	12				
Diplura	3	2	2	1	4		3		
Collembola	455	203	127	347	628	197	299	159	266
Psocoptera	20	10	5	7	8	12	10	13	4
Hemiptera	172	1	30	44	69	3		6	3
Thysanoptera	4	3	1	4	2			2	2
Coleoptera	3	1		6	2	2	4	1	3
Formicidae	1	16	15	3	8	1		12	
Diptera (larvae)	15	11	5	3	4	12	3	7	6
Holometabola (larvae)	8	7	8	8	11	4	2	10	6
Araneidae	3	2		7	1	3			
Acarida	972	710	1171	920	892	817	1005	1191	671
Isopoda		2	1		4	3	5		5
Diplopoda		1	1	1	1	3	5	2	1
Chilopoda	1		1		2	1	8		
Symphyla	1	1	1	1			1	4	13
Pauropoda	41	20	2	9	36	11	21	18	17
S	14	15	15	15	16	13	12	12	12
N	1699	990	1371	1362	1684	1069	1366	1425	997

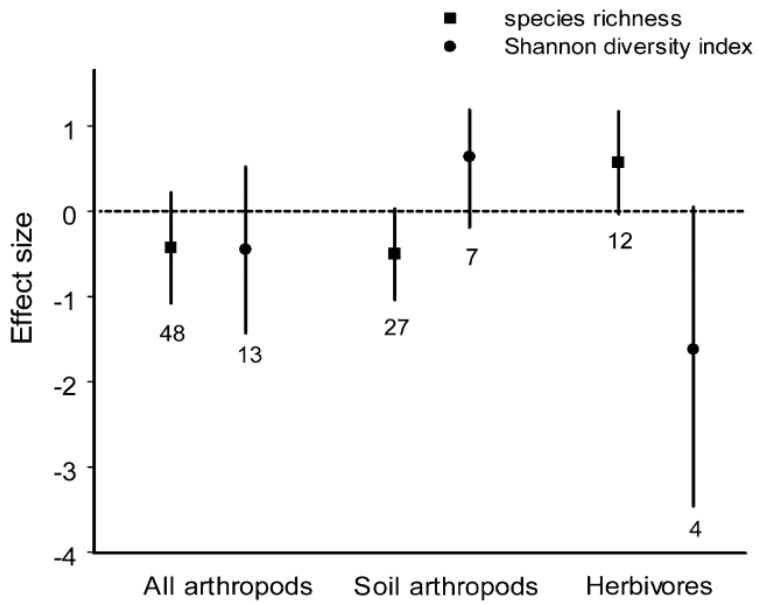


Figure I.5. Pollution effects (mean effect sizes, 95% confidence intervals, and sample sizes) on species richness and diversity of terrestrial arthropods: overall effect and effects on soil arthropods and herbivores (from Zvereva and Kozlov, 2010).

I.2.4 TMs and trophic levels

As TMs are transferred form the ambient environment to terrestrial consumers and predators mainly through the oral route, the transfer can be influenced by trophic levels to which their resources belong. An increase in element concentration in one trophic level relative to the previous one, namely "*biomagnification*", is well known for many organic contaminants, especially in birds or mammals in aquatic ecosystems (Gray, 2002). However, most of metallic contaminants do not show biomagnification as clearly as organic contaminants even in aquatic ecosystems (Burger, 2008; Goodyear and McNeill, 1999; Gray, 2002). A variation of TM concentration in organism is actually often larger within a trophic level than between trophic levels. Nonetheless, resources belonging to different trophic levels could lead to different effects on the oral exposure of their predators to TMs. For example, concentrations of Cd and Cu in small mammals' tissues were higher in insectivorous shrews *Sorex araneus* than in herbivorous or granivorous small mammals, *Microtus agrestis* or *Apodemus sylvaticus*, in the surrounding of a refinery in the United Kingdom (Hunter and Johnson, 1982). Higher accumulation of TMs in insectivorous or carnivorous small mammals than in herbivorous ones have been reported in other studies (e.g. Fritsch et al., 2011; Hunter et al., 1989; Smith and Rongstad, 1982; Veltman et al., 2007; Wijnhoven et al., 2007). Although studies about the relationship between metal transfer and diet diversity are quite rare, Orłowski et al. (2013) showed correlations between number of different taxa observed in stomach content and

concentration of TMs in different tissues of nestling rooks *Corvus frugilegus*. In their study, the number of different plant taxa ingested was negatively correlated to concentrations of Pb in liver, whereas the number of invertebrate families in stomach content was positively correlated to concentrations of Co and Cd in their kidneys. Those results indicate a potential difference between plant and animal food in the trophic transfer of TMs.

Furthermore, TMs can also affect interactions between prey and predator. Several studies in aquatic ecosystems have recently highlighted "*info-disruption*": an interference by a pollutant in the chemical communication networks that inform organisms about their biotic and abiotic environments (Lürling and Scheffer, 2007). The disruption induced by TMs in anti-predator reactions, such as predator avoidance behavior, has been widely observed in fish, amphibians, or aquatic invertebrates (e.g. Lefcort et al., 1998, 2000; Michels et al., 2000; Scott and Sloman, 2004; Scott et al., 2003). A decrease in predator avoidance behavior of prey could result in an increase in predation of prey more affected by TMs (Boyd, 2010). However, such disruptions can be due to exposure to water-borne TMs rather than to dietary exposure (Scott et al., 2003), and similar effects have not been reported for terrestrial communities. On the contrary, the "*elemental defense hypothesis*", i.e. certain elements present at high concentrations in an organism's tissues may protect it from natural enemies (Boyd, 2004), has been widely demonstrated in terrestrial ecosystems but not in aquatic ecosystems. Boyd (2007) reviewed papers concerting the elemental defense hypothesis and pointed out that high concentrations of many elements such as As, Cd, Ni, Se and Zn in plants could play a defensive role against natural enemies like herbivorous animals and pathogens. Indeed, high metal concentration in food can modify food preference of terrestrial invertebrates. For instance, avoidance for leaves contaminated by TMs (Cu and Cd) has been reported in woodlice *Porcellio* sp. (Crustacea, lsopoda) under laboratory conditions (Odendaal and Reinecke, 1999; Zidar et al., 2004), whereas feeding and oviposition of the leaf-mining fly *Chromatomyia milii* (Agromyzidae, Diptera) decreased as concentration of Cd in the grass *Holcus lanatus* (Poaceae) increased under an alternative choice experiment (Scheirs et al., 2006a). Such aversion for contaminated food was also found in vertebrates. Actually, Beernaert et al. (2008) studied and demonstrated such aversion in the wood mouse (*Apodemus sylvaticus*) under an alternative choice experiment in laboratory by using acorns of the oak *Quercus robur* (Fagaceae) taken from both TM contaminated and non-contaminated sites in Belgium. The mechanisms of those avoidance for TM contaminated food remain to be discussed, and several possibilities have been suggested, such as disturbance in nutrient content (Beernaert et al., 2008; Scheirs et al., 2006a), metal-induced metabolic compounds (Poschenrieder et al., 2006), or even detection of TMs per se in food (Behmer et al., 2005). Whatever the mechanisms are, terrestrial animals could have a tendency for preference of low or non-contaminated resources rather than high TM contaminated resources.

I.3 The scientific issue of the present thesis: a potential dilution effect in the transfer of TMs in food webs

Although details of mechanisms are different between the transfer of TMs and the transmission of pathogens, there are comparable points: both pathogens and TMs might cause harmful effects in organisms, both the prevalence and the TM body burden differ among hosts and resource species, and both the transmission of pathogens and the transfer of TMs are carried out through physical contacts between organisms belonging to different categories of species (i.e. host and vector; trophic levels). Those comparable points support the hypothesis that diversity of resources might condition the variation of oral exposure to TMs. However, the dilution effect in the transmission of pathogens requires the three conditions for the generality of its occurrence in nature. It is thus important to determine conditions under which a dilution effect may generally occur in the transfer of TMs.

I.3.1 Comparison of mechanisms between the transmission of pathogens and the transfer of TMs.

For illustrating similarities between the transmission of pathogens and the transmission of TMs, the latter is compared to the transmission of Lyme disease. In the case of Lyme disease, the agent causing harmful effects is the bacterium *B. burgdorferi*, which is transmitted by the general feeding tick, *I. scapularis*, and threatens the human health. In the case of the environmental TM contamination, the harmful agent is one or some TMs, which are transferred through food webs. The Lyme disease risk in humans can also be estimated by the density of infected ticks by *B. burgdorferi*, whereas the TM contamination of the predator can be evaluated on the basis of the TM concentrations in the given animals. The sources of the harmful agents for the vectors are available animal hosts in the case of Lyme disease and available resources in the case of environmental TM pollution. Thus, the diversity involved in the dilution effect of the transmission of *Borrelia*, i.e. the diversity of hosts available, corresponds to the diversity of available resources in the case of the transfer of TMs.

I.3.2 How could a dilution effect occur in the transfer of TMs?

In the transfer of TMs, the first condition of the dilution effect in the transmission of pathogens (i.e. difference in quality of hosts for pathogens) can correspond to a difference in TM accumulation capacity of resources. When the first condition is not met, there is no effect of biodiversity on the transfer of TMs (Figure I.6). The second condition (i.e. difference in sensibility of hosts to biodiversity loss) can stay the same: a difference in sensibility to biodiversity loss. For an animal which diet is proportional to density of available resources in the field, the two conditions are enough for the occurrence of dilution effect: the given animal

would consume more importantly hyper TM accumulator resources in diversity-poor communities where those accumulator resources dominate. However, when an animal shows preference for certain resources, preference can influence more importantly the proportion of the two types of resources in the diet than density in the field. In this case, another condition is required: "*low TM accumulator resources, at least some of them, should be preferably consumed*". Those conditions correspond respectively the third condition of the dilution effect in the transmission of pathogens (i.e. regulation by low quality hosts in encounter rates between high quality hosts and pathogens). The second condition is slightly modified: "*occurrence, but not density, of low accumulator resources decreases when biodiversity declines, whereas TM hyper accumulator resources are omnipresent insensibly to biodiversity loss*". When the three conditions are met, low accumulator resources would be consumed in diversity-rich communities due to their presence and preference of the animal for the resources, whereas hyper accumulator resources would be consumed only in diversity-poor communities where preferred resources (i.e. low accumulator resources) are absent (Figure I.7a). When the second and third conditions are not totally met, a relationship between biodiversity loss and the transfer of TMs could theoretically result in a positive correlation (i.e. amplification effect) or no correlation. On the other hand, the three hypothetical conditions do not predict what would occur when both preferred and non-preferred resources are present in a community. In this case, the transfer of TMs is directly determined by the structure and composition of the diet (Figure I.7b). A potential role of available resources in the field on the transfer of TMs depends on whether and how the structure and composition of the diet are determined by available resources in the field.

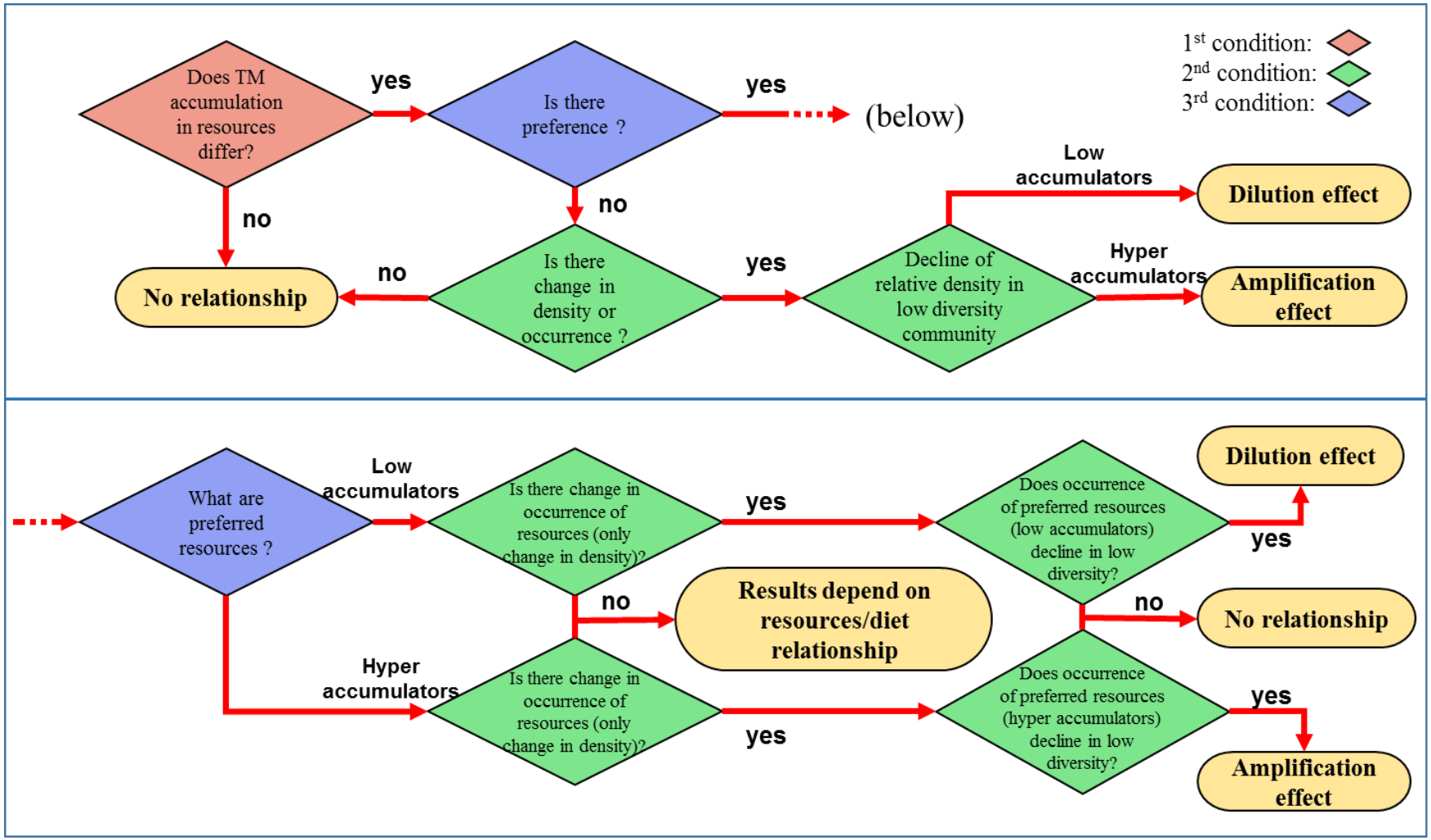


Figure I.6. Flowchart illustrating the three conditions of the dilution effect in the transfer of TMs and potential consequences.

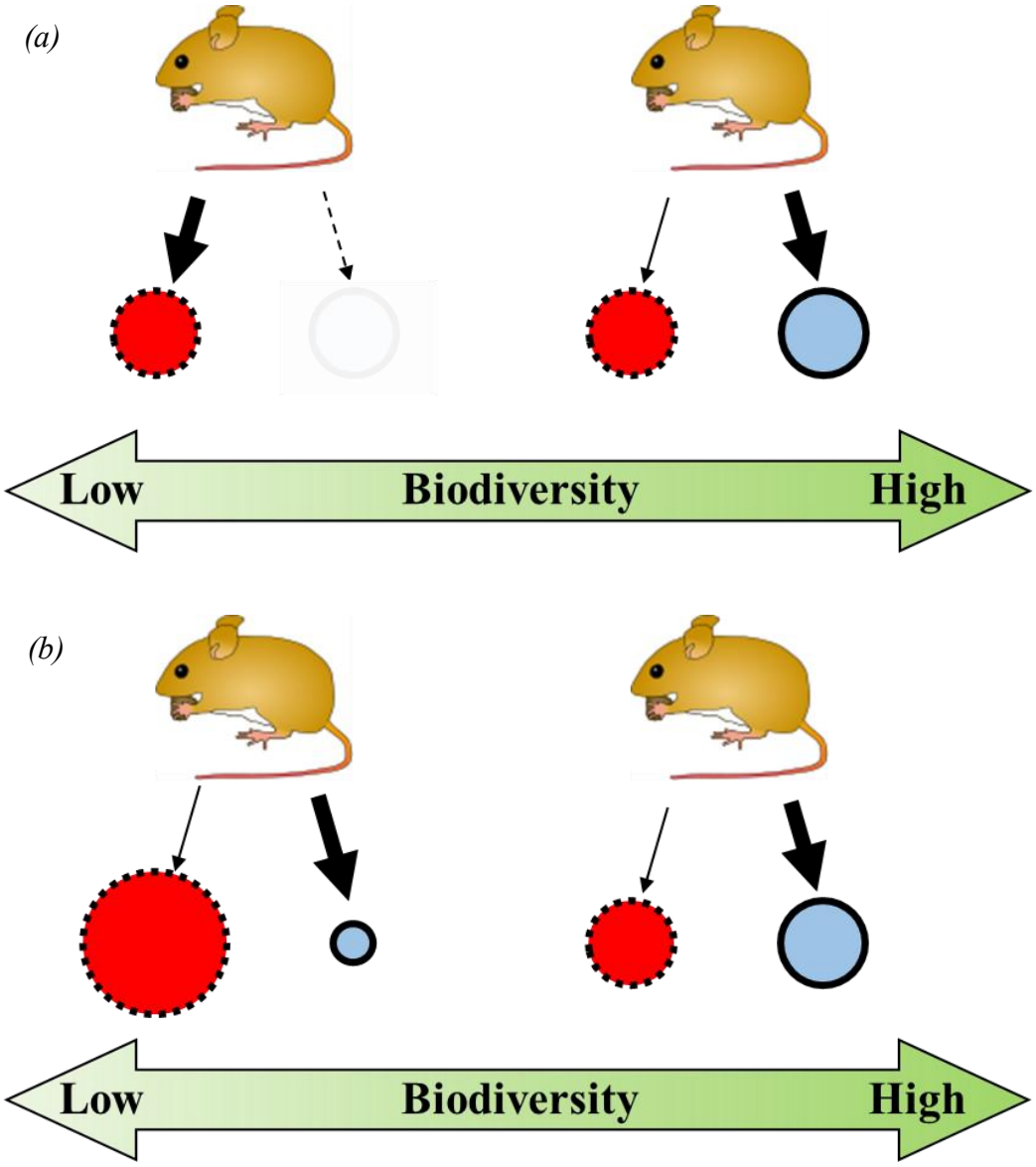


Figure I.7. Schemas for hypothetical situations of the dilution effect in the transfer of TMs in low and high resources diversity. Low accumulator resources preferred by mice are represented by blue plain circles, whereas hyper accumulator resources not preferred by mice are represented by red dotted circle. (a) When biodiversity loss affects occurrence of preferred resources (i.e. decline of resource richness), mice cannot consume preferred resources and thus consume not preferred resources in low diversity community. (b) When biodiversity loss affects only relative density of preferred resources (i.e. decline of evenness), mice consume preferred resources even in low diversity community.

I.3.3 Are the three hypothetical conditions met in nature?

Several studies have shown a difference in TM accumulation capacity among species, and the first condition can be widely met in nature. However, decline in occurrence of low accumulator resources could not be totally evident. Although hyper accumulators are more likely to remain in TM contaminated sites and hence in diversity poor communities, composition and diversity of resources could widely vary in TM contaminated sites due to initial composition of species, different sensibility to TMs among species and other factors determining ecological niche of each species. The second condition therefore remains to be dealt with. The third condition remains as the most important issue: does an animal involved show preference for low contaminated food under field conditions? Although avoidance for TM contaminated food has been shown in some terrestrial invertebrates, only the wood mouse was investigated and showed such avoidance under laboratory conditions among terrestrial mammals. In addition, this study used only one resource, the acorn, for demonstrating the avoidance of the wood mouse, whereas several resources can be consumed by the rodent in the field. However, mammals would seek a variety of food for maintain nutrient balance from different resources (i.e. nutrient balance hypothesis; Westoby, 1978) and/or for minimizing one kind of plant natural toxin form one resources (i.e. detoxification limitation hypothesis; Freeland and Janzen, 1974). Although not evident, it is supposed that mammals could choose low contaminated resources under field conditions. Furthermore, Boyd (1998) suggested that the elemental difference by TM hyper-accumulator plants might be less efficient against vertebrate herbivory, due to their mixed food with other non-accumulator resources (i.e. "*diet dilution hypothesis*"). This hypothesis suggests that high oral exposure due to hyper accumulator resources could be diluted by other low accumulator resources simultaneously consumed. Even in communities where both preferred and non-preferred resources are present, higher diet diversity might reduce oral exposure to TMs, provided that higher diversity of resources increases higher diet diversity.

Although other possibilities cannot be rejected as the general relationship between biodiversity loss and the transfer of TMs in food webs, a dilution effect may be assumed to be more likely to occur for terrestrial mammals which potentially show an avoidance for TM contaminated food, like the wood mouse. Nonetheless, examining the three conditions as underlying mechanisms is also important. This requires details in ecology of an animals and chemical properties of TMs involved in.

I.4 Biological and chemical models in the present thesis

For assessing the dilution effect in transfer of TMs in food webs, the wood mouse (*Apodemus sylvaticus*) is chosen as biological model, whereas Cd, Pb and Zn are chosen as metal contaminants in the present thesis. The ecology of the rodent with a focus on its diet ecology, some chemical properties of the three TMs, as well as critical values of the TMs for the wood mouse are described.

I.4.1 Ecology of the wood mouse

The wood mouse is a murid rodent widely spread in Europe, having large and protruding eyes, developed ear flaps and a long tail with short fur (Figure I.8). The length from nose to anus is about 90 mm and the average weight is about 23g for adults, but body size can vary according to the latitude: for example, its weight is about 18-20g at latitude 50° north, but reaches 30g at 40° north in Europe (Alcántara, 1991). The wood mouse has a breeding season from March to October in Europe. However, offspring born in spring more rapidly grows than offspring born in summer (Butet and Paillat, 1997). Previous studies have shown a slightly favorable sex ratio for males (52–60%) (Butet and Paillat, 1997).

Figure I.8. The wood mouse Apodemus sylvaticus (pictures from Clémentine Fritsch, co-supervisor of the present thesis).

The mouse shows high adaptability to a wide range of habitat, e.g. farmland, woodland, urban area, and hence is considered as to be a pioneer of recolonization (Halle, 1993). The home range of the wood mouse ranges from 300 to 14,000m^2 for males and from 300 to 8,000m^2 for females (Benhamou, 1991; Green, 1979; Rogers and Gorman, 1995). In fact, several studies have shown a narrower home range in females than in males, but the dimension varies according to habitats and food availability in the field. The average size of home range is from 1000 to 2000m^2 (Quéré and Le Louarn, 2011). The wood mouse is a nocturnal rodent and most frequently active in the first hour of darkness (Miller and Elton, 1955). Furthermore, the wood mouse shows a high arboreality (i.e. capacity of tree climbing) in all seasons, which allows

them to exploit food resources above ground and to escape predators. In the study of Buesching et al. (2008), climbing was frequently observed at heights of 1m but reached up to 20m off the ground. Males are more arboreal than females (Buesching et al., 2008; Montgomery, 1980).

The diet of the wood mouse is mainly granivorous. However, importance of seeds in the diet varies according to seasons and habitats. In the study of Watts (1968) for example, seeds of different species constituted from 5 % (in May) to 99 % of ingested volume (in February) in mixed deciduous woodland in England, whereas seeds constituted from 8 % (in April) to 80 % of volume (in November) in a heathland in France (Butet, 1986a). Fruits like berries are also consumed in season (Watts, 1968). To a lesser extent, other plant parts like flowers or leaves also occur in the diet, particularly in spring (Butet, 1986a; Green, 1979; Watts, 1968). However, roots have been rarely observed in the diet (Hansson, 1971). Likewise, mushrooms have been reported in the diet with quite low abundance or frequency (Hansson, 1971; Watts, 1968). Composition of plant resources is highly diverse. Some examples are ash (genus *Fraxinus*, Oleaceae), beech (*Fagus*, Fagaceae), oak (*Quercus*, Fagaceae), maple (*Acer*, Sapindaceae), elm (*Ulmus*, Ulmaceae), pine (*Pinus*, Pinaceae), elderberry (*Sambucus*, Adoxaceae), blackberry (*Rubus*, Rosaceae), dog rose (*Rosa*, Rosaceae), sorrel (*Rumex*, Polygonaceae), oat (*Avena*, Poaceae), meadow-grass (*Poa*, Poaceae), bracken (*Pteridium*, Dennstaedtiaceae) and moss. On the other hand, invertebrates of different taxa are a part of the diet, mainly from spring to early autumn (Butet, 1986a; Hansson, 1971; Watts, 1968). They can represent even up to 80% of diet volume in spring (e.g. Watts, 1968), but the proportion varies according to habitats and seasons. Several studies have reported a compensation between seeds and invertebrates in the diet: a high proportion of invertebrates in the diet is normally observed form April to October, while a high proportion of seeds is observed from November to March (Butet, 1986a; Hansson, 1971; Watts, 1968). In general, invertebrate resources are not actively sought but occasionally found when animals seek plant resources (Butet and Paillat, 1997). Invertebrate resources are composed of different insect larva, coleopteran adults and/or earthworms, but composition highly differs between studies. Consumption of other vertebrates (e.g. bird eggs or nestlings) has also been reported, but only by a quite few studies (e.g. Bailey, 1970). It is worth to note that a large proportion of food is usually plant and animal unidentified matter.

Contrary to the composition, only a few studies have shown other characteristics of the diet of the wood mouse. Daily food consumption under natural conditions are unknown, but Hunter et al. (1987b) reported 10g dry weights of daily food consumption in average, ranging from 8.5 to 12g, under laboratory conditions. Similarly, daily water intake was estimated to be 200 ml kg^{-1} day^{-1} for laboratory mice (Shore and Douben, 1994a). Number of items consumed (i.e. diet richness) under natural conditions has been rarely measured. Hansson (1971) showed that number of items, which consisted of grand categories like “seeds”, “insects”, “earthworms” or “herbs”, was two per mouse in average in all seasons. Basically, there is no difference in the

diet between males and females (e.g. Butet, 1986b). On the other hand, a little difference in the diet between adults and young has sometimes been recorded: invertebrate items can be more frequently or abundantly eaten by adults than juveniles (Montgomery and Montgomery, 1990; Watts, 1968).

I.4.2 Chemical properties and toxicology of Cd, Pb and Zn

Cadmium (Cd): Cd is a silver-white lustrous metal which has an atomic weight of 112.4, an atomic number of 48, and a density of 8.7 g cm^{-3}. Cd belongs to the International Union of Pure and Applied Chemistry (IUPAC) group 12 and has similar chemical properties to the elements of the same group: Zn and mercury. Global supply of Cd reached 22 000 metric tons in 2002, and mainly used for nickel-cadmium batteries or pigments (Nordberg et al., 2014). Concentration of Cd in non-polluted natural soils is less than 1 mg kg^{-1} dry soil (Nordberg et al., 2014). However, the worldwide average of concentrations of Cd in soils is 0.41 mg kg^{-1} dry soil and widely differ between continents (Kabata-Pendias, 2011). The soil remediation intervention value for Cd, above which the functional properties of the soil is seriously impaired or threatened, is 12 mg kg^{-1} dry weight in a standard soil, i.e. 10% organic matter and 25% clay (VROM, 2000). Mobility of Cd in soils depends on several factors. For instance, Cd activity is strongly affected by pH whereas phosphate added to soils immobilizes the Cd (Kabata-Pendias, 2011).

Cd in soil is absorbed in plants though both root and leaf systems but accumulated mainly in root tissues even when Cd enters via foliar systems (Kabata-Pendias, 2011). For mammals or birds, Cd is absorbed through both inhalation and ingestion. Although absorption is more efficient from lungs (15-50% average 25 % of the inhaled particles) than from the gastrointestinal tract (1-12%, average 5% of the ingested dose), ingestion is the main route for wild mammals (Shore and Rattner, 2001). The absorption of cadmium from the gastrointestinal tract has been influenced by other elements or nutritional composition of food. At moderate doses of Cd, for example, the presence of divalent and trivalent cations, such as Ca, Cr, Mg, and Zn, and/or dietary fiber may decrease gastrointestinal Cd uptake (Andersen et al., 2004; Flanagan et al., 1978; Foulkes, 1985). Furthermore, influence of iron stored in body on intestinal absorption of Cd was also observed in humans (Flanagan et al., 1978). Absorbed Cd is then transported through blood and accumulated mainly in lungs, kidneys and liver (Nordberg et al., 2014; Shore and Rattner, 2001). After oral exposure, the highest accumulation of Cd is observed in the liver and kidneys, and lower levels spread throughout the rest of the body in both humans and animals (Kotsonis and Klaassen, 1978; Weigel et al., 1984). Liver and kidney Cd concentrations are comparable after short-term exposure (Andersen et al., 2004; Jonah and Bhattacharyya, 1989), but the concentration in kidneys exceeds the concentration in liver following prolonged exposure (Kotsonis and Klaassen, 1978). Cd is hardly excreted and thus

retained for a long time in the body. For example, half-life of Cd in mice are approximately 200-700 days (Nordberg et al., 2014).

General symptoms of Cd toxicity in plants are growth retardation, root damage and chlorosis of leaves (Das et al., 1997; Kabata-Pendias, 2011). Symptoms of Cd in vertebrates can differ according to exposure routes. Exposures to cadmium through inhalation can result in inflammation and minimal fibrosis, and in serious cases, pneumonia or emphysema, whereas oral exposure to Cd can result in necrosis and degeneration of the liver and/or kidneys, necrosis and ulceration in the stomach and intestines, severe anemia and decrease in motor activity (Faroon et al., 2012; Nordberg et al., 2014). In addition, decrease in immune response and dysfunction in developing nervous system, as well as skeletal effects such as decrease in bone mineral density, impaired mechanical strength, and increase of fractures, have also been observed (Faroon et al., 2012).

Lead (Pb): Pb is a white-gray soft metal and has an atomic weight of 207.2, an atomic number of 82, and a density of 11.3 g cm^{-3}. Pb belongs to the IUPAC group 14, where carbon, silicon, germanium and, tin are also found. The International Lead and Zinc Study Group (ILZSG, 2018) estimated that the annual world mine production of Pb would reach approximately 4.90 million metric tons in 2018. Batteries, mainly for vehicles but also for electricity backup systems and industrial batteries, are the predominant use of Pb (Nordberg et al., 2014). The history of Pb pollution and the description about Pb poisoning date back to the antiquity (Nriagu, 1996, 1998). The today's worldwide average of concentrations of Pb in soils is 27 mg kg^{-1} dry soil (Kabata-Pendias, 2011). The soil remediation intervention value for Pb is 530 mg kg^{-1} dry weight in the standard soil (VROM, 2000).

Pb is considered to be the less mobile metal in soils, but certain soil and plant factors, such as pH, soil P content or organic ligands, can promote Pb uptake by plants (Kabata-Pendias, 2011). Pb is mainly absorbed in plants though root system and stored mainly in roots because its translocation from roots to shoots is limited (Kabata-Pendias, 2011; Pourrut et al., 2011). Consumption of contaminated food is generally the main route for exposure of animals to Pb, and absorption from ingested food ranges 2-16% of ingested dose in adult mammals (Shore and Rattner, 2001). A variety of other factors are known to influence the absorption of ingested Pb, including the chemical form of the ingested lead, the presence of food in the gastrointestinal tract, and nutritional content of food such as Ca, Fe, Zn, fat, protein, food diet fiber, vitamins D and E (e.g. Abadin et al., 2007; Eisler, 2000; Nordberg et al., 2014). Pb absorbed from the gastrointestinal tract is translocated though blood first to soft tissues like liver and kidneys, then to bone where more than 90% of Pb is retained for a long time (Nordberg et al., 2014; Shore and Rattner, 2001). In mammals, Pb in bone is comparatively stable with a half-life of several years, whereas Pb in liver and kidneys have a half-life of about 3-4 weeks (Scheuhammer, 1987).

Pb is excreted mainly in urine or feces and, to a lesser extent, through other routes like saliva, sweat, hair or nails (Nordberg et al., 2014).

Exposure to high amounts of Pb alters uptake of nutrient elements, water absorption, and decrease total protein content at biochemical scale, which leads to inhibit growth, respiration and photosynthesis in plants (Kabata-Pendias, 2011; Pourrut et al., 2011). Exposure of mammals to high amounts of Pb can induce dysfunction in brain cardiovascular, hematological renal and musculoskeletal systems, disturbances in hormonal and immunological parameters, alteration in vitamin D metabolism, neurobehavioral dysfunctions (Abadin et al., 2007; Eisler, 2000). Furthermore, mice exposed to Pb during pregnancy had offspring showing renal proliferative lesions and renal tumors (Waalkes et al., 1995), whereas rodents exposure to Pb during various developmental periods also showed various types of alterations in sexual maturation of both males and females (Dearth et al., 2004; Iavicoli et al., 2004).

Zinc (Zn): Zn is a silver-gray metal which has an atomic weight of 65.4, an atomic number of 30, and a density of 7.1 g cm^{-3}. Zn belongs to the IUPAC group 12 like Cd. The ILZSG, (2018) estimated that the annual world mine production of Zn was forecast to rise to 14.0 million metric tons in 2018. Zn is used as protective coating for other metals by galvanizing but also as alloying (Nordberg et al., 2014). Zn is the 25th most abundant element in Earth's crust where the average content is 78 mg kg^{-1} (Nordberg et al., 2014). The worldwide average of concentrations of Zn in soils is 70 mg kg^{-1} dry soil (Kabata-Pendias, 2011). The soil remediation intervention value for Zn is 720 mg kg^{-1} dry weight in the standard soil (VROM, 2000).

The dominant factor determining the distribution of Zn in soils is pH, whereas soil type, soil moisture, and clay and organic matter contents also affect Zn distribution in soils (Broadley et al., 2007; Kabata-Pendias, 2011). Zn is an essential metal for living organisms and plays essential metabolic, and thus uptake of Zn can be regulated in both plants and animals (Eisler, 2000; Kabata-Pendias, 2011; Nordberg et al., 2014; Shore and Rattner, 2001). The rate of Zn absorption form soils however differs among plant species, and is also conditioned by soil pH and composition of nutrients, particularly the presence of Ca (Broadley et al., 2007; Kabata-Pendias, 2011). Zn is mobile from old leaves to generative organs when Zn is sufficiently supplied, whereas Zn is less mobile and stays in mature leaves under Zn-deficiency conditions (Kabata-Pendias, 2011). In mammals, 5-40% of ingested Zn is absorbed across gastrointestinal tract under regulations in homeostatic ways (Shore and Rattner, 2001). After ingestion, Zn is absorbed across several active membranes and finally excreted primarily in the feces (Nordberg et al., 2014). Although Zn is distributed widely in the body, the highest concentrations are generally found in liver, bone, kidneys, muscle and pancreas (Shore and Rattner, 2001).

Zn deficiency rather than Zn toxicity have been widely reported in both plants and animals. However, environmental Zn pollution causes several symptoms of plants related to

disturbance in uptake of other elements like P, Fe, Mg, and Mn. Contamination of plants by Zn thus results in reduction in yields, stunted growth, chlorosis through reductions in chlorophyll synthesis and chloroplast degradation (Broadley et al., 2007; Kabata-Pendias, 2011). In mammals, despite homeostatic regulation in the body, excess oral exposure to Zn can cause anemia, depression in growth, and especially symptoms related to nutritional disturbance (Shore and Rattner, 2001). Pancreatic, gastrointestinal, kidney, pancreas and liver damage can also be found in animals ingesting zinc for some months (Roney et al., 2005).

I.4.3 Critical values for the three TMs for exposure and contamination of rodents

Critical values of both exposure to TMs and TM body burden are found in the literature. The median lethal dose (LD_{50}) for oral exposure of rodents to Cd was between 530-790 µmol kg^{-1} body weights (BW) as $CdCl_2$ solution in water (Andersen et al., 1988; equivalent to 60-90 µg g^{-1} BW of Cd). Assuming the average body weight of 23g (cf. 1.4.1 Ecology of the wood mouse), this LD_{50} is equivalent to 1380-2070 µg of Cd for an individual wood mouse. Contrary to Cd, health effects after a single exposure to Pb have been rarely reported in the literature. The Joint FAO/WHO Expert Committee on Food Additives (JECFA; 2000) pointed out that lowest observed lethal doses in animals after short-term oral exposure to Pb range from 300 to 4000 µg g^{-1} BW. Although Zn is an essential metal, high exposure to Zn is also deadly. LD_{50} for oral exposure to Zn widely depends on its forms: LD_{50} for oral exposure of laboratory mice to Zn is 337 µg g^{-1} BW as zinc sulfate, 86 µg g^{-1} BW as zinc acetate, 605 µg g^{-1} BW as zinc chloride and 204 µg g^{-1} BW as zinc nitrate (Domingo et al., 1988).

Critical values of chronic exposure to those TMs largely vary depending on targeted effects on health, duration of exposure, health conditions of animals, and methods of exposure (e.g. TMs in water or in food, nutritional compositions of daily food, etc.). For example, chronic oral intake of 10 ppm of $CdCl_2$ solution in water (equivalent to 2.0 µg g^{-1} BW day^{-1}, assuming the estimated daily water intake of 200 ml g^{-1} BW day^{-1}; cf. 1.4.1 Ecology of the wood mouse) for 280 days increased mortality by leukemia virus, probably due to immune dysfunction, in laboratory mice (Blakley, 1986), whereas chronic exposure to 5 ppm of Cd as $CdCl_2$ in food for 250 days decreased femur calcium content in laboratory female mice only undergoing repeated pregnancy periods (Bhattacharyya et al., 1988; equivalent to 1.8 µg g^{-1} BW day^{-1} with the daily food consumption reported in the same publication). Even chronic exposure to 5 µg mL^{-1} of Cd as $CdCl_2$ solution in water for 21 days could decrease humoral immune response in laboratory female mice (Blakley, 1985; equivalent to 1.0 µg g^{-1} BW day^{-1}). Based on the literature about effects of oral exposure of laboratory rodents to Cd for some months, Shore and Douben (1994a) argued that the lowest observable adverse effect level (LOAEL) for chronic oral exposure to Cd can be estimated to be approximately 3.5-7.5 µg g^{-1} BW day^{-1} (equivalent to Cd

concentration in food of 8.1-17.3 µg g^{-1} dry mass, assuming the average daily food intake of 10g dry weights and the average body weight of the wood mouse; cf. 1.4.1 Ecology of the wood mouse). The LOAEL for chronic oral exposure to Pb can be estimated to be approximately 5-15 µg g^{-1} BW day^{-1} for rodents (Shore and Douben, 1994b; equivalent to Pb concentration in food of 11.5-34.5 µg g^{-1} dry mass for an average wood mouse). Critical values for chronical exposure to Zn are higher than the two other TMs thanks to homeostatic regulation. The no observed adverse effect level (NOAEL) for oral exposure to Zn could be 104 µg g^{-1} BW day^{-1} (Maita et al., 1981; equivalent to Zn concentration in food of 239.2 µg g^{-1} DW day^{-1} for an average wood mouse).

TM body burden in mammals is examined mainly through concentrations of TMs in their "*critical organ*", i.e. the organ which first attains its critical concentration of the metal. Several studies have compared concentrations of Cd, Pb and/or Zn in critical organs, liver and kidneys, of wood mice taken from control and TM contaminated sites (Table I.9). Histopathological alterations produced by TMs have also been reported in wood mice from. For example, Tête et al. (2014a) observed a significant correlation between soil TM contamination levels and histological alterations like liver necrosis in mice taken from the surrounding of a former Pb and Zn smelter in France. Sánchez-Chardi et al. (2009) reported higher proportion of mice with liver damage (21 out of 24 mice) in the vicinity of leachates containing a high concentration of organic and metallic contaminants than in a reference site (11 out of 25) in Spain. Furthermore, Tersago et al. (2004) demonstrated an increase in proportions of early apoptotic white blood cell along a gradient of soil and liver Cd concentrations in wood mice taken from the vicinity of a nonferrous smelter in Belgium and concluded negative effects TMs on the immune function under field conditions. On the basis of the literature, Shore and Douben (1994a) argued that the LOAEL for Cd in liver and kidney of laboratory rodent could be approximately 15 and 105 µg g^{-1} dry weight, respectively. The Pb poisoning effects are associated with concentrations of 7 µg g^{-1} µg g^{-1} of wet weight (WW) in liver and 20 µg g^{-1} of WW in kidney (Scheuhammer, 1987). These values can be respectively equivalent to 25 and 70 µg g^{-1} DW, assuming that concentration in tissue given as wet weight could be converted to dry weights by multiplying by 3.5 (Shore and Douben, 1994b). However, diagnostic signs appear when Pb concentration in kidneys is above 25 µg g^{-1} in mammals (Ma, 1989), and this concentration can be considered as to be the LOAEL for Pb in kidney of rodents (Shore and Douben, 1994b). Contrary to the two non-essential TMs, critical values of Zn in tissues are not found, to the best of my knowledge, in the literature. Actually, several studies have pointed out that concentrations of Zn in tissues do not dramatically differ between control and contaminated sites (e.g. in Table I.9).

Table I.9.Mean value of Cd, Pb and Zn concentrations in the liver and the kidney of the wood mouse, as well as concentration in soils (mg kg^{-1} dry weight) reported in some studies across Europe. References showing values of TM concentration are mentioned in the table. If any, the name of other organs in which a given study measured TM concentrations are mentioned.

	Cd			Pb			Zn			Other organs	Reference
	Liver	Kidney	Soil	Liver	Kidney	Soil	Liver	Kidney	Soil		
Control site	1.4	5.1	1.4	0.1	1.0	99.8	-	-	-		(Tête et al., 2014a)
Site near Metaleurop Nord	5.6	16.5	44.6	1.3	24.8	2871.9	-	-	-		
Control site*	2.8	12.4	1.6	0.2	2.7	110.0	55.8	46.1	183.0		Fritsch et al., 2010a
Site near Metaleurop Nord*	5.2	30.9	59.3	3.9	39.7	1357.0	53.5	53.6	1802.0		
Floodplain area with TM contaminated sediments	-	0.2	1.2	-	1.6	57.9	-	68.2	207.0		van den Brink et al., 2010
Site near a zinc-smelter	-	9.9	2.2	-	1.9	61.9	-	122.0	154.0		
Control site	1.8	15.1	0.5	0.2	0.7	50.8	80.2	99.0	103.0	Hair, lung, muscle	Beernaert et al., 2007
Site near nonferrous metal industry	31.4	105.5	26.3	0.6	3.0	693.3	97.8	121.0	224.7		
Control site	2.3	21.0	0.5	0.2	0.7	50.8	-	-	-		(Berckmoes et al., 2005; Scheirs et al., 2006b)
Site near nonferrous metal industry	41.4	135.7	26.3	0.8	2.8	693.3	-	-	-		
Control site	0.3	0.9	-	0.4	0.7	-	162.1	142.8	-		Sánchez-Chardi and Nadal, 2007**; Sánchez-Chardi et al., 2009
Vicinity of leachates	0.4	1.4	0.6	0.7	1.1	< 0.5	200.5	135.9	1.2		
Control site	0.3	1.1	0.3	0.4	1.2	31.0	-	-	-	Bone, muscle	Milton et al., 2002, 2004
Mining site in Cwmsymlog	0.5	1.4	0.8	3.4	43.0	12550.0	-	-	-		
Mining site in East Halkyn	3.0	13.0	126.0	4.7	6.8	17410.0	-	-	-		
Control site	0.4	2.0	0.8	-	-	-	-	-	-	Pancreas	(Hunter et al., 1987c, 1989)
Cu-Cd alloying plant	18.2	41.7	15.4	-	-	-	-	-	-		

*: Median values are mentioned in the Table.

**: Reference only for soil TM concentration data.

I.4.4 TMs in wild wood mice and its food

Variation in TM accumulation in the wood mouse is expected to depend on food variability, i.e. resource variation in space and time. Hunter et al. (1987b) estimated daily Cd and Cu intake of three small mammals throughout a year in contaminated grasslands in the surrounding of a refinery site in north-west England by (i) determining their food according to broad categories (e.g. seed, fruit, invertebrate) based on residuals of resources ingested found in feces and (ii) calculating TM contents of each item with TM concentration values in resources in the same sites which had been measured in their previous studies (Hunter et al., 1987a, 1987c). Exposure of the wood mouse to TMs was lower than for the two other small mammals, *M. agrestis* and *S. araneus*. The authors argued that by discarding high contaminated food, like invertebrates which accounted for less than 5 % by weight of the diet of the wood mouse throughout the year, the wood mouse could be less exposed to TMs than the others. Rogival et al. (2007) compared concentrations of five elements, As, Cd, Cu, Pb and Zn, in liver, kidneys and bone of the wood mouse and in its two primary food items, acorn and earthworm, to evaluate element transfer in soil-resource-wood mouse food chain in the vicinity of an active non-ferrous metallurgic plant in Belgium. The authors observed significant positive correlations between Cd, Pb and Zn concentrations in soil-resource and resource-wood mouse tissues. They also concluded that the earthworm was more important for oral exposure to TMs than the acorn. Furthermore, van den Brink et al. (2011) studied Cd accumulation in small mammals in relation to habitat use and food, using and stable isotope signatures of C and N. Their results indicated a major influence of food on Cd accumulation in kidney of the wood mouse, possibly due to high proportion of earthworm in the diet. These studies clearly demonstrate the importance of identity of food for both exposure and accumulation. However, to the best of my knowledge, no study has measured directly oral exposure of the free-living wood mouse to TMs by measuring ingested materials such as stomach content. Only few examples have actually measured oral exposure in terrestrial wildlife, such as Godwin et al. (2016) who measured TM concentrations in stomach content of nestling tree swallows *Tachycineta bicolor* near oilsand mining areas. Determining details of food consumed by the wood mouse and associating to a real measure for oral exposure still remain as to be investigated.

I.5 Outline and objectives of this thesis

I.5.1 The global hypothesis and issues remaining to dealt with in the present thesis.

In the present thesis, **it is hypothesized that higher diversity of resources of the wood mouse would reduce the transfer of the three TMs (i.e. Cd, Pb and Zn) to the mouse**, on the basis of possible differences in occurrence between resources with high and low TM accumulation capacity and potential aversion for TM contaminated food. In parallel, other possibilities, i.e. amplification effect or no relationship, should not be rejected because of uncertainty about sensibility of resources to diversity loss and about aversion of the wood mouse for hyper accumulator resources under field conditions. The present thesis thus considers not only a correlation between diversity of resources and transfer of TMs, but also the relevance of the three conditions under which a dilution is expected to occur: (i) the difference in TM accumulation capacity among the resources available in the field, (ii) the difference in the sensibility of the resources to biodiversity loss, and (iii) the difference in the food preference of the wood mouse between hyper and low accumulator resources.

In order to verify those conditions, several issues remain to be dealt with. **What are resources really consumed by the wood mouse?** Because of its wide range of potential food, the composition of the diet of the wood mouse varies according to habitats and seasons. Identifying details of food really consumed is essential. **Does the wood mouse show really preference for certain resources?** Although some studies have suggested preference for some resources, details of preferred resources in relation to their density in the field still remain unclear. Determining such preference is a crucial point for the conditions of the dilution effect. **Is there a difference in TM accumulation among resources really consumed by the wood mouse?** Despite several studies suggests a potential difference in TM concentrations between resources, it is not certain that such difference would be observed within resources really consumed by the wood mouse, especially between preferred or not preferred resources. **Do the diversity and the composition of the diet of the wood mouse play a filtering role in the transfer of TMs to the mouse?** The hypothetical three conditions do not cover the case in which both hyper accumulator and low accumulator resources are present. However, it is possible that both the resource–diet and diet–exposure relationships could be involved in the mechanisms of the dilution effects. This issue about a potential role of the diet can be subdivided into two parts: (a) **what is the relationship between resources really consumed and their availability in the field?** (b) **What is the relationship between the diet and the exposure to TMs?** The last but not the least issue is the relationship between biodiversity loss and environmental TM contamination. Contrary to the case of the transmission of pathogens, TMs can be one of potential causes for biodiversity loss. It is important to survey **whether and how**

the composition and diversity of the available resources for the wood mouse are modified in a TM contaminated site.

I.5.2 Outline of the present thesis

The present thesis consists of four main parts: "General Introduction", "General Materials and Methods", "Results" and "General Discussion". The **General Introduction** provides the global hypothesis about functional effects of biodiversity on the transfer of TMs on the basis of the dilution effect in the transmission of pathogens and its underlying mechanisms. The **General Materials and Methods** part provides an overview about the data and analyses used for dealing with each issue. On the basis of the flux of chemical contaminants from environment to the wood mouse (Figure I.9), five chapters are constructed in the **Results** part as follows:

Chapter 1: Relationship between resources in the field and TMs in soils – Composition and diversity of resources potentially consumed by the wood mouse, plants and invertebrates available in the field, are examined along a gradient of soil TM contamination levels. It is supposed that both composition and diversity of resources would be modified along the gradient of soil TM levels and/or along a gradient of some soil physico-chemical properties affecting concentrations of TMs in resources, i.e. soil properties modifying bioavailability of TMs (cf. 1.4.2). Due to potentially different roles of plant and invertebrate resources on exposure to TMs (cf. 1.2.4; 1.4.4), potential changes in plants and invertebrates are separately examined. Furthermore, as vegetation can be a determining factor for invertebrate community (cf. 1.2.3), the relationship between composition and/or diversity of plants and that of invertebrates is also examined even though no study have investigated this point in a TM contaminated site. This chapter thus assesses (i) **whether and how diversity and composition of plant communities are affected by soil TMs and/or some soil physico-chemical properties that may influence bioavailability** and (ii) **whether and how diversity and composition of invertebrate communities are indirectly influenced by the vegetation in a TM contaminated site**.

Chapter 2: Relationship between the diet of the wood mouse and the resources in the field – Composition and diversity of food consumed by the wood mouse are compared to resources available in the field for determining, if any, resources preferentially consumed by the rodent. Even if the preference for certain resources have been documented elsewhere in the literature, this could be different in the present study because of its high adaptability for food and habitat and of the metallic contamination of the area (cf. 1.4.1). Even though no study has demonstrated the relationship between diversity of available resources and of the diet, it is expected that they are positively correlated for seeking nutrient balance and/or minimizing plant natural toxin effects (cf. 1.3.3). However, TMs themselves may perform as modifying factor of feeding behavior (1.2.4). Preference and the relationship between the available resources and

the diet may be different along a gradient of environmental TM contamination levels. This chapter thus assesses (i) **what are the resources, if any, preferably consumed by the wood mouse**, and (ii) **whether and how the relationship between resources available in the field and consumed, as well as preference of the rodent, are modified by the soil TM contamination**.

Chapter 3: Relationship between the exposure to TMs and the diet of the wood mouse – The variation of exposure to TMs is analyzed with respect to the diet of wood mice. The wood mouse is expected to be highly exposed to TMs when it consumes resources accumulating TM in body like earthworms (cf. 1.4.4). However, given the wide range of the diet of the wood mouse (cf. 1.4.1) and the large variety in TM accumulation capacity of resources (cf. 1.2.2), it is required to verify which resources really contribute to exposure of the wood mouse. On the other hand, the number of resources consumed (i.e. diet richness) can play a functional role on exposure to TMs (cf. 1.2.4), such as a dilution of TMs in the diet (cf. 1.3.3). This chapter thus assesses (i) **what resources of the wood mouse are, if any, responsible for high exposure to TMs**. (ii) **Whether and how does high diet richness influence oral exposure to TMs**?

Chapter 4: Composition of elements in some resources of the wood mouse – Concentrations of TMs and of other elements in some resources are measured for assessing **how TM concentrations in resources really consumed by the wood mouse differ along a gradient of environmental TM contamination**. It also remains to be investigated **whether and how biochemical composition is modified in resources along the gradient of TM contamination**, especially if preference for these resources and/or the resource–diet relationship is modified by the TM contamination (1.4.4). Furthermore, one could not reject the possibility that bioavailability of TMs could vary along a gradient of biodiversity, due to modification de some physico-chemical properties of soils by biodiversity, especially diversity of plants like quantity of nutrients in soils (cf. 1.1.1; 1.4.2). **Bioconcentration factor of TMs in resources are calculated and compared along the gradient of biodiversity in this chapter**.

Chapter 5: The exposure to TMs and the TM contamination of the wood mouse tissues in relation to the diversity of resources in the field – The general hypothesis of this thesis is investigated, i.e. **how do vary exposure to TMs and TM accumulation of the wood mouse with respect to the diversity of the resources available in the field**? Exposure to TMs and TM accumulation actually observed in the wood mouse are also compared to their toxicological threshold values (cf. 1.4.3). On the other hand, although decline in biodiversity can be closely related to TM contamination, effects of TMs on diversity is highly variable (1.2.3). The second condition of the dilution effect is also investigated in this chapter: **whether and how occurrence of preferred resources determined in the second chapter is modified in relation to biodiversity of resource**. **Exposure to TMs and TM accumulation are**

furthermore compared between presence and absence of the preferred resources, to indirectly assessing resources involved in the dilution and/or amplification effects.

Apart from the chapter 4, each chapter is represented in the form of a scientific article with both summary and highlight translated into French. Finally, the results from all of the five chapters are synthesized in the **General Discussion** part. Main results of each chapter are first summarized. The functional role of the diversity and composition of the diet of the wood mouse by the second and third chapters is then integrated in the results by the fifth chapter. This leads to examine the general mechanisms underlying the relationship between diversity of resources and the transfer of TMs. Other environmental and biological factors potentially controlling the underlying mechanisms of the dilution effect are discussed. As a conclusion, after examining a possibility of a dilution effect in other animals and other ecosystems, this thesis answers the question of whether the functional role of diversity of resources on the transfer of TMs is, if any, able to be proposed as a new ecosystem service as a conclusion.

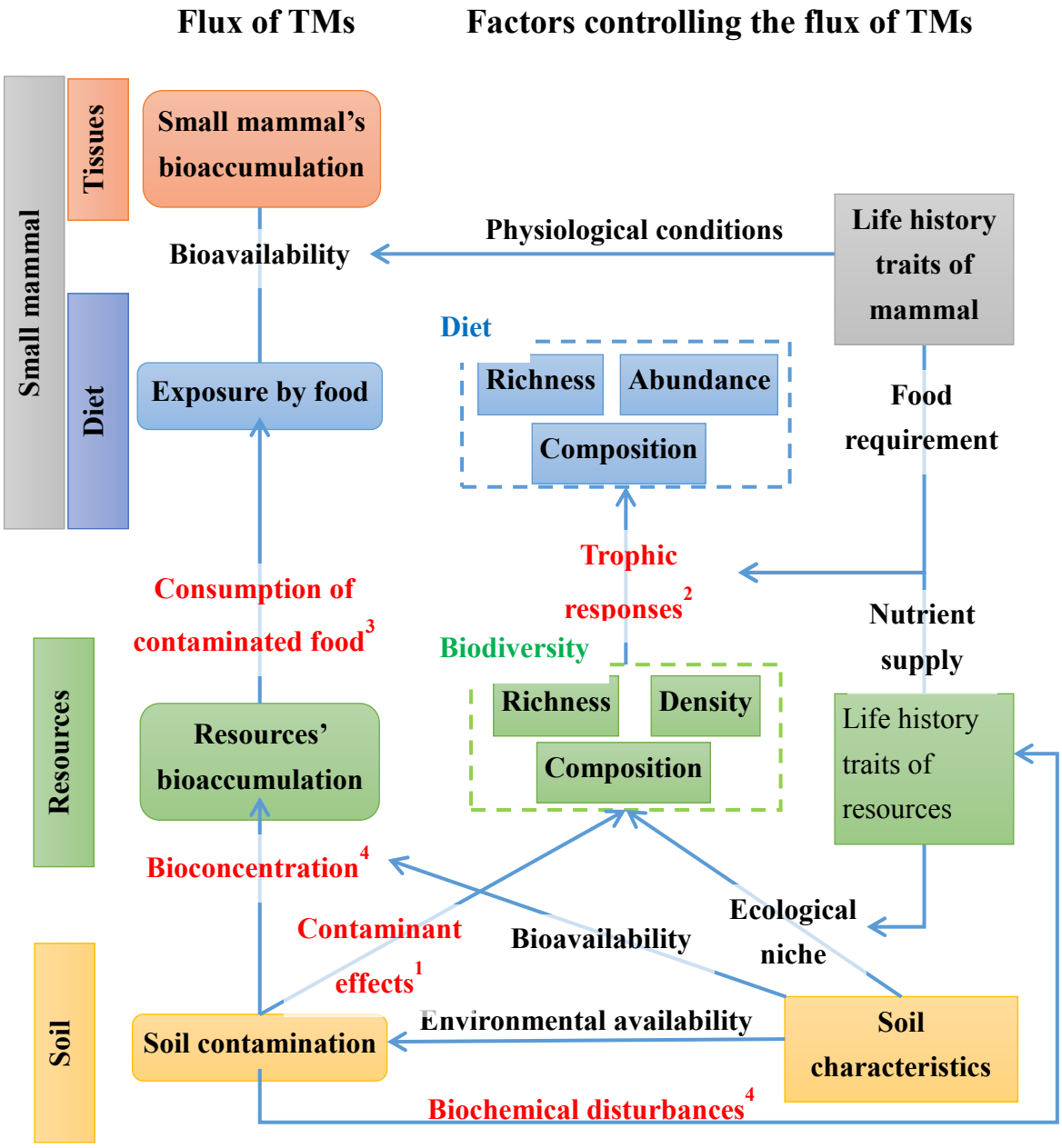


Figure I.9. Diagram about the flux of TMs in the terrestrial ecosystem from the soil to the small mammal through the trophic route. Rounded rectangles represent steps of the flux of TMs, whereas rectangles represent environmental or biological factors controlling the flux. Underlying mechanisms are represented by blue arrows. The hypothetical mechanism of this thesis, dilution effect, is represented by red dotted arrows directly caused by biodiversity or indirectly through some modifications of the diet. Factors directly analyzed in the present thesis were indicated in red with superscript numbers referring to chapters.

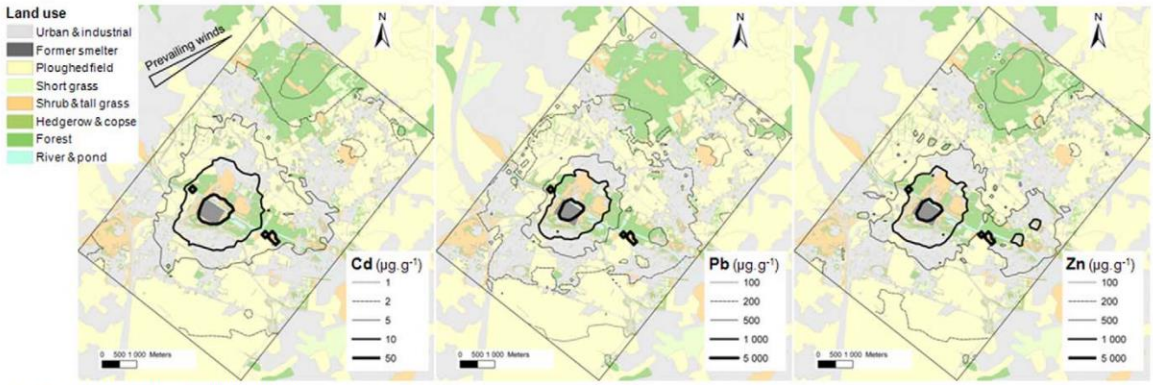
Part II. General Materials and Methods

II.1 Study area: "*Metaleurop Nord*" in northern France

The present thesis was carried out in the surroundings of the former Pb and Zn smelter named "*Metaleurop Nord*" located in Northern France (Noyelles Godault, Hauts-de-France; 50°25'42 N and 3°00'55 E). The general context of the study area is described below.

II.1.1 Soil TM contamination in the surroundings of Metaleurop Nord

The Metaleurop Nord smelter was created in 1894 and closed in 2003. For more than a century, the pyro-metallurgic process had generated large quantities of dust. Despite the implementation of technical improvements during the 1970s, the smelter had released a large quantity of TMs contained in industrial dust. In 2002, for example, about 1.0 ton of Cd, 16.9 tons of Pb and 31.6 tons of Zn were released from the smelter (DRIRE, 2003). Soils of a large area around of the smelter was thus highly contaminated by TMs, due to metal in dust from this smelter and from another large Zn smelter, namely "*Umicore*", which has been working since 1869 in the neighboring town Auby. Actually, the dust emission of the two smelters has affected an area of around 120 km^2 (Douay et al., 2008). Several studies have revealed a high degree of soil contamination by TMs, mainly Cd, Pb and Zn. (Sterckeman et al., 2002a) showed that concentration of Cd, Pb and Zn in agricultural top soils reached 21, 1132 and 2167 mg kg^{-1} of dry soil, respectively. Sterckeman et al. (2000) reported 67, 4890 and 2685 mg kg^{-1} dry soil of Cd, Pb and Zn, respectively, on grasslands. In woody habitats (e.g. hedges, groves or small woods), total concentrations of Cd, Pb and Zn reached even 236, 7331 and 7264 mg kg^{-1} of dry soil, respectively, in a highly contaminated site (Fritsch et al., 2010b). The studies of (Fritsch et al., 2010b, 2011) demonstrated that soil TM concentrations draw concentric circles around Metaleurop Nord, levels decreasing with distance to the smelter, and with an enhancement of the contamination in downwind areas (Figure II.1). Moreover, some hot spots of concentration values corresponding to dredged sediment deposits were highlighted. It is also worth to note that soils in the surrounding of the smelter often show a trace of anthropization, i.e. a deposit or embankments related to human activities such as brick, concrete or waste rock. Douay et al. (2009) indeed observed medium to high degree of soil anthropization in more than 50% of woody habitats. The soil TM concentration values are quite higher than concentrations in non-contaminated soils in the same region. Indeed, Sterckeman et al. (2007) reported that median of concentrations in surface of several types of soils far from contamination resources (e.g. factories, traffic roads or urbanized areas) were 0.4, 29.7 and 67.1 mg kg^{-1} dry soil for Cd, Pb and Zn, respectively, in the administrative region Nord-Pas-de-Calais (N.B. the former name of the region Hauts-de-France).



doi:10.1371/journal.pone.0020682.g001

Figure II.1. Iso-concentration lines of predicted total Cd, Pb and Zn concentrations in topsoil from Metaleurop-impacted area (mg g-1 dry soil). (from Fritsch et al., 2011).

Effects of TMs in soils on wildlife have also been investigated in this TM contaminated area for several purposes. Ecotoxicological works on plants have measured TM concentrations in plant tissues observed in the study area. For example, Migeon et al. (2009) compared TM concentration in leaves and stems of 25 woody species present in this area to seek good candidates for phytoremediation programs. They demonstrated high Cd and Zn concentrations in Salicaceae family plants, especially in leaves of hybrid popular *Populus tremula x Populus tremuloides* with up to 950 mg kg^{-1} DM of Zn and 44 mg kg^{-1} DM of Cd. They also reported generally low and species independent concentration of Pb in woody plant tissues. For herbaceous plants, Deram et al. (2006, 2007) sampled a perennial grass which tolerates high TM concentrations in shoots, *Arrhenatherum elatius*, and measured their metal bioaccumulation capacity to estimate their potential for phytoremediation operations. In their study, up to 100 mg kg^{-1} DM of Cd was observed in shoots of *A. elatius*. These studies have clearly demonstrated a large variation of TM contaminations of plants in this area, but effects of TMs on diversity or composition of plants in this area remains unclear.

On the other hand, some studies about invertebrates have been focused on effects of soil TM contamination on diversity, and complicated relationships between soil TM contamination levels and composition and/or diversity of invertebrates have been reported. Grelle et al. (2000) studied change in community of Isopoda and Chilopoda in sites contaminated by TMs. In their study, no clear change in abundance or species structure was observed, and the authors suggested an important role of vegetation on the invertebrate community. Pérès et al. (2011) examined different earthworm community descriptors such as total abundance, ecological and specific structure with respect to soil contamination by polycyclic aromatic hydrocarbon or metal in five different sites in France, including the surroundings of Metaleurop Nord. No relationship was observed between earthworm abundance and metal contamination, but the proportion of the different ecological groups of earthworms was impacted by the TM

contamination in Metaleurop site (Figure II.2). These results indicate that diversity and composition of invertebrates are not, at least not only, controlled by TM contamination.

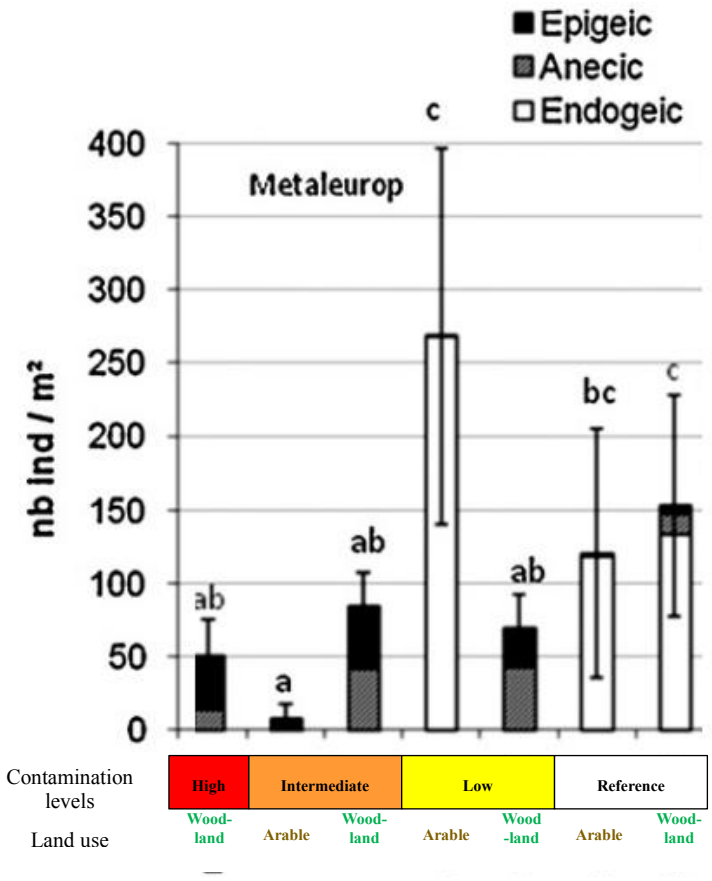


Figure II.2. Abundance of earthworms (number of individuals m^{-2}) and the different proportions of ecological groups sampled in 2009 in Metaleurop site (adapted from Pérès et al., 2011). Sampling were carried out in seven plots: four metal contamination levels (High, intermediate, low levels and reference) and two types of land use (woodland and arable). Different letters indicate the significant effect of treatment within the studied sites (p-value < 0.05).

Studies about vertebrates like mammals and birds have been investigated for body burden of metals and its, physiological effects. For example, concentrations of TMs in body or other responses of wild small mammals, notably wood mice, to soil TM have also been revealed, such as metallothionein levels in liver and kidney (Fritsch et al., 2010a), variation in some body condition indices (Tête et al., 2013), or histological alterations in liver (Tête et al., 2014a). These studies have however shown the responses of wild animals to soil TM contaminations are not straightforward. On the other hand, Fritsch et al. (2011) measured TM concentrations in liver of small mammals *Myodes glareolus* and *Crocidura russula* and in soft body of snails *Cepaea* sp. and *Oxychilus draparnaudi* sampled in woody habitats and estimated effects of landscape on transfer of TMs. Fritsch et al. (2012) studied bioaccumulation of Cd and Pb in

blood and feathers of blackbirds *Turdus merula* with respect to landscape of habitats, composition and contamination of their food. They suggested that landscape influenced TM transfer though diet composition, spatial and foraging behavior.

II.1.2 Study sites of the present thesis

The present thesis was undertaken on six sites of 25 ha (500m x 500m) among 160 sites which were located within an area of 40 km^2 (5 x 8 km) including Metaleurop in its center. Each site was assigned by number (from 20 to 197) and numerous studies have been realized in some of the 160 sites (e.g. (Douay et al., 2009; Fritsch et al., 2010b, 2011, Tête et al., 2013, 2014a); N.B. the term "*square*" was used in those studies, but the term "site" is used in the present thesis) (Figure II.3). Another site of 25 ha was located about 1km northeast of the former smelter, referred to as "*TE2*", was also included as a control site (Figure II.4). The seven sites were chosen for representing both a gradient of TM soil concentrations and different landscape feature types (Table II.1) which had been characterized in previous studies (Douay et al., 2009; Fritsch et al., 2010b). The soil properties used in this thesis were taken from previous studies (cf. below).

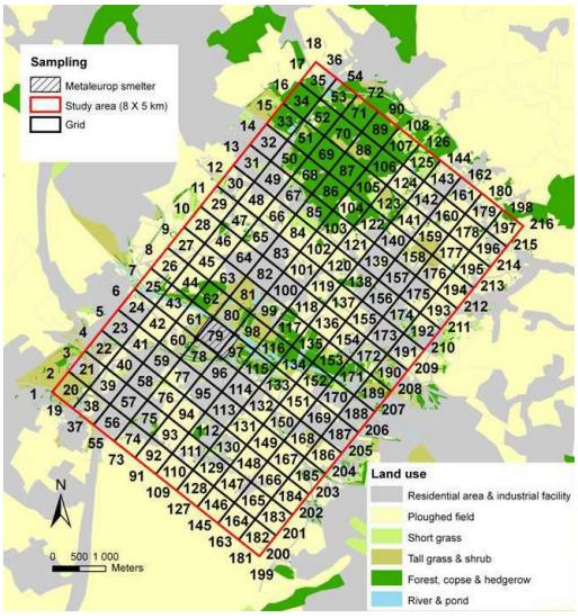


Figure II.3. Subdivision of the area of 40 km^2 into 160 sites in the surroundings of Metaleurop Nord (from Tête, 2014).

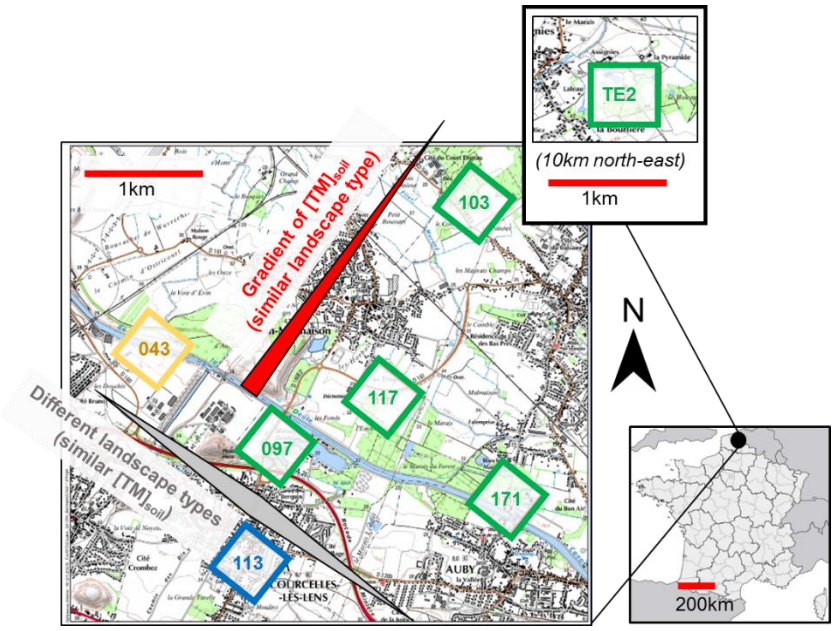


Figure II.4. The seven study sites of the present thesis. The sites are represented by squares with each name. Colors of square represent different types of landscape feature (green: forest; orange: arable; blue: urban).

Table II.1. Soil TM concentrations in the study area(from Fritsch et al., 2010b).

Soil TM concentration (mg kg^{-1} dry soil)		TE2	103	117	097	171	043	113
$[Cd]_{soils}$	Minimum	0.86	1.5	3.6	15.3	4.9	1.3	4.4
	Median	1.4	4.3	9.1	48.3	7.5	15.2	11.5
	Maximum	2.4	6.0	17.8	236.5	14.5	42.7	13.0
$[Pb]_{soils}$	Minimum	43.3	237.5	244.7	658.5	287.6	105.0	266.6
	Median	107.4	267.2	512.0	1295.3	584.0	323.1	678.9
	Maximum	199.8	333.0	859.8	6809.4	2063.3	1028.9	806.0
$[Zn]_{soils}$	Minimum	89.3	114.4	302.8	1069.3	487.2	153.9	414.7
	Median	168.8	352.7	555.8	1874.7	1362.7	512.8	1001.2
	Maximum	277.7	407.5	958.5	7263.5	2451.5	1549.6	1170.4
Soil contamination level		"Control*"	+	++	+++	++	++	++
Landscape feature		Forest	Forest	Forest	Forest	Forest	Arable	Urban

*: TM concentrations as close as possible to regional background concentrations (Sterckeman et al., 2002b)

II.2 Field sessions of the present thesis

The present thesis uses data collected from four field sessions carried out in the surroundings of Metaleurop Nord (Table II.2). The first field work was carried out in spring 2012, from 3rd to 12th April for (1) capturing rodents and (2) establishing inventory of invertebrates in the field. The second field work was carried out in summer 2012, from 4th June to 5th September, for establishing (3) inventory of plants in the field. The third field work was carried out in autumn 2012, from 23rd September to 5th October, for the same objectives as the first sampling session. The three sessions were realized through the scientific program "BIOTROPH". The fourth field work was realized in spring 2017 during the present thesis, from 10th to the 21st of April, with the aim of (4) collecting some resources in the field and then measuring concentrations of TMs and other elements in them.

Table II.2. Period and tasks realized in each session of field works.

Session No.	Period	Rodent trapping	Invertebrate inventory	Plant inventory	Resource sampling
1	2012/04/03-12	X	X	-	-
2	2012/06/04-09/05	-	-	X	-
3	2012/09/23-10/05	X	X	-	-
4*	2017/04/10-21	-	-	-	X

*: Session in which the PhD student participated

II.2.1 Rodent trapping

Rodents were captured in accordance with current French legislation about ethics and use of animals in research. Break-back traps were used with peanuts (*Arachis hypogaea*, Fabaceae) as bait (Figure II.5). In each season and each site, 10 trap lines, each of which were composed of 10 traps at 3m intervals, were set in woody habitats following Fritsch et al. (2011). Their position was geo-referenced. The trap lines were checked in the morning for three consecutive days and re-set and/or re-baited, if necessary. After being weighed in the field, the captured animals were immediately frozen and stored at -20 ºC for further analyses. Age of mice was estimated by using a correlation between body weights and eye lens' weight (Tête et al., 2014b) and another correlation between eye lens' weight and age (Vandorpe and Verhagen, 1980). Age was expressed as days and using four age classes defined by Vandorpe and Verhagen (1980): approximately, class I < 50 days (corresponding to 13.8 g of body weight) < class II < 130 days (20.0 g) < class III < 230 days (23.9 g) < class IV.

In total, 305 wood mice (134 mice in spring and 171 in autumn), 44 bank voles (*Myodes glareolus*), 31 greater white-toothed shrews (*Crocidura russula*) and 6 other shrews (*Sorex* sp.)

were captured in the two field sessions (Table II.3). The 305 mice were composed of 139 females and 160 males, as well as six individuals which sex could not be identified. The sex ratio was not significantly different from 1:1 (chi-squared = 1.47, p-value = 0.22).

Figure II.5. The break-back trap (picture from Ozaki et al., 2015).

Table II.3. Rodents captured in the two sessions in 2002.

Sites	Wood mouse (*A. sylvaticus*)	Bank vole (*M. glareolus*)	Greater white-toothed shrew (*C. russula*)	Shrew sp. (*Sorex* sp.)	Total
TE2	**37**	**16**	**1**		**54**
Spring	*22*	*3*	*1*		*26*
Autumn	*15*	*13*			*28*
103	**62**	**10**	**2**	**6**	**80**
Spring	*15*	*5*	*2*		*22*
Autumn	*47*	*5*		*6*	*58*
117	**53**				**53**
Spring	*20*				*20*
Autumn	*33*				*33*
097	**40**	**4**	**7**		**51**
Spring	*22*	*2*			*24*
Autumn	*18*	*2*	*7*		*27*
171	**48**	**11**	**3**		**62**
Spring	*29*	*7*			*36*
Autumn	*19*	*4*	*3*		*26*
043	**47**	**3**	**3**		**53**
Spring	*22*	*2*			*24*
Autumn	*25*	*1*	*3*		*29*
113	**18**		**15**		**33**
Spring	*4*		*2*		*6*
Autumn	*14*		*13*		*27*
Total	**305**	**44**	**31**	**6**	**386**

II.2.2 Field inventory of invertebrates

Invertebrate fauna was sampled by two types of trapping: the pitfall trap (Figure II.6a) and the yellow pan trap (Figure II.6b). The pitfall trap is one of the oldest and the most frequently used methods for sampling epigeic invertebrates (Leather, 2005). This trap commonly samples highly active and mobile invertebrates like beetles (Coleoptera), wandering spiders (e.g. Aranae), or ants (Formicidae, Hymenoptera) (Greenslade, 1964; Uetz and Unzicker, 1976). On the other hand, the pan trap is frequently used for insects flying above or between plants within the understory (Leather, 2005). Trapping color plays a determinant role in the effectiveness with which different insect groups are caught, and yellow color is efficient for catching a wide range of flower visiting insects and predators or parasitoids of them (Kirk, 1984).

Sampling in the field was realized with the help of the "*Conservatoire Botanique National de Franche-Comté – Observatoire Régional des Invertébrés* (ORI: http://cbnfc-ori.org/)". A pitfall trap was composed of an 800ml polypropylene beaker with neither roof nor preservative fluid. Three pitfall traps were placed in line at 15m intervals near each trap line for rodents. In total, 70 lines composed of 210 pitfall traps were used in each season. A yellow pan trap was filled with soap mixed water. Four yellow pan traps were set per site and per season. In total, 28 plates were used in each season. Some of them were set near the trap line for rodents, but the others were set far from the trap lines. Locations of the two types of traps were geo-referenced and checked every morning for three consecutive days. Captured invertebrates were conserved in ethanol or in freezer at -20°C. Captured invertebrates were then identified by ORI in laboratory at the lowest possible taxonomic levels by morphological characteristics. The main references were Coulon (2003), Forel and Leplat (2001), Jeannel (1941), and Trautner and Geigenmueller (1987). Springtails (mainly Collembola) were then removed from the inventory, because of their small body size: on the one hand it was supposed that wood mice could not consume them, on the other hand a large number of springtails were locally captured, and thus could distort biodiversity comparisons. In total, 88 taxa mainly at species level for Carabidae and at family level for others were captured by pitfall traps, and 95 taxa mainly at family level were captured by yellow pan traps. Fauna captured by each type of traps were considered to be ground-dwelling invertebrates and flying ones, respectively.

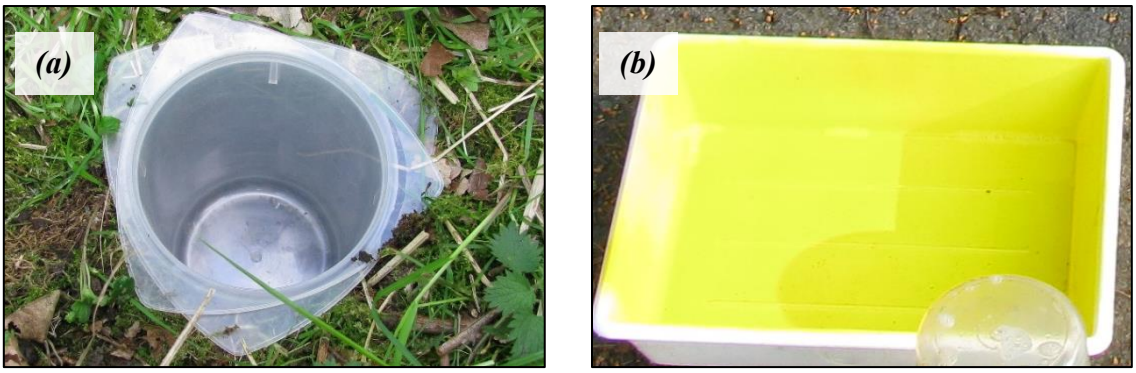


Figure II.6. (a) The pitfall trap and (b) the yellow pan trap.

II.2.3 Field inventory of plants

Vegetation survey was realized once in each site by "*Conservatoire Botanique National de Bailleul* (CBNBL: https://www.cbnbl.org/)". Three different strata of vascular plants were defined based upon height: tree stratum (woody species > 8 m high), shrub stratum (woody species < 8 m high), and herbaceous stratum. Taxa were then identified at species level in the field. Nomenclature of species followed Lambinon et al. (2004), except nomenclature for species of the genus *Taraxacum* (Dudman and Richards, 1997). Cover-abundance of vascular plant species was visually estimated as the vertically projected area following Braun-Blanquet et al. (1952). Vegetation habitats were determined by plants' composition of one or more strata and delineated as polygons with the aid of aerial pictures. The polygons were geo-referenced and digitalized using the QGIS software. In total, 236 different plant taxa (principally at species level) were listed as rodents' potential resources.

II.2.4 Field sampling of plants and invertebrates

According to the results of the analyses on diet of wood mice and importance in exposure to TMs, (cf. chapter 2 and chapter 3 of the Results part, respectively), as well as distribution in the study sites, plant species potentially important for diet and exposure to TMs were selected: sycamore maples (*Acer pseudoplatanus*, Sapindaceae), goat willows (*Salix caprea*, Salicaceae) and Poplar trees (*Populus* sp, Salicaceae).

Sampling points were chosen in some "*woody patches*" where soil TM concentrations had been studied by (Fritsch et al., 2010b). From the seven sites, 38 sampling points were chosen due to no or little change of habitat since 2010 and presence of target plant species. Those sampling points are referred to as "*patch*" hereinafter. Only leaves of sycamore maples, goat willows and poplar, as well as female catkins of goat willow, were available in the sampling period. For each species five trees were randomly selected per patch. When there were less than five trees in patch, sampling was carried out from all available trees, increasing quantity of

organs taken from one tree. Three pairs of leaves (sycamores) or three leaves (willows and poplars) from terminal bud, considered as to be young ones, were picked at about 1-2m height with polyethylene gloves. The samples were packed in polyethylene bags and were stored at -20 °C and transported to the laboratory.

II.3 Chemical analyses in laboratory

Samples taken from the field were processed in chemical analyses for measuring: (1) concentration of TMs and (2) carbon and nitrogen contents, or (3) identifying food of wood mice (Table II.4). The mouse samples were treated during the "BIOTROPH" program, and the samples taken from the field work in 2017 were treated during the present thesis. Concentrations of elements obtained in chemical analyses were logarithmically transformed for statistical analyses below.

Table II.4. Summary for chemical analyses.

Samples	TM concentrations	C and N content	Metabarcoding
Liver & kidney of wood mice	X	-	-
Stomach content of wood mice	X	-	X
Plant resources (*A. pseudoplatanus*; *S. caprea*; *Populus* sp.)	X	X	-

II.3.1 Preparation for chemical analyses

In Chrono-Environment laboratory, the wood mice were thawed at room temperature. Their liver and kidneys were extracted from the body and used for measuring TM concentrations (cf. 2.3.2 Measuring TM concentrations). TM concentrations in livers and kidneys were measured in 303 out of 305 captured mice (N.B. TM concentrations were measured in both organs in 303 mice, another was used only for concentrations in liver, and the other was used only for concentrations in kidney). Stomach and digestive tract were also removed from the body of a subsample of 246 mice chosen among 305 to get a balanced sample size among sites. Mice from each site were nevertheless randomly chosen. Stomach content (SC) was then extracted with a spatula. Remaining bait (i.e. peanut) was distinguished by its white color and removed from SC (Figure II.7). Each SC was then homogenized and split into two aliquots. One of about 10 mg was stored in 95% ethanol for metabarcoding analysis (cf. 2.3.4 Identification of wood mouse's diet), the other was reserved for measuring TM concentrations (cf. 2.3.2 Measuring TM concentrations). When quantity of SC was not sufficient for the two analyses, SC was used only for the metabarcoding analysis. TM concentrations in SCs were measured for 204 mice, whereas food identification was carried out for 246 mice, among which feces of 13 mice were used as an alternative material when stomach of mouse was empty. The used spatula was thoroughly cleaned with disposable tissue, then washed with ultra-pure water (18.2 MΩ/cm^2 by Millipore Milli-Q Integral 3) and wiped off

with other tissue after each extraction. PhD student participated to extraction of some SCs form body.

On the other hand, the leaves and/or catkins of plants collected in the field work in 2017 were gathered both by patch and by species. They were then freeze-dried and ground to powder in a mortar. The freeze-dried powders were used for both measuring TM concentrations (cf. 2.3.2 Measuring TM concentrations) and C and N contents (cf. 2.3.3 Measuring carbon and nitrogen content in plants). These operation were carried out by PhD student at Chrono-Environment laboratory.

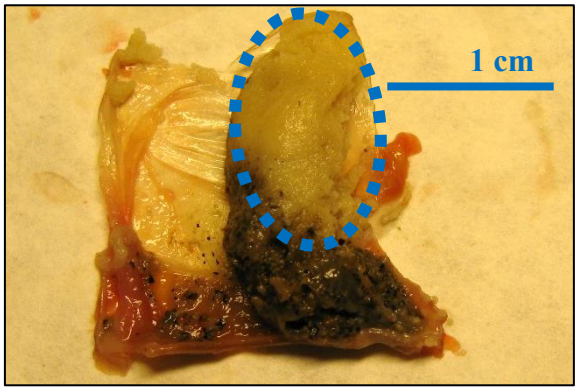


Figure II.7. Stomach content of wood mouse. White colored material (blue dotted circle) is considered as bait for rodents (peanut, Arachis hypogaea, Fabaceae) and removed from stomach content before chemical analyses.

II.3.2 Measuring metal concentrations

Concentrations of elements in stomach contents (SCs) of wood mice: The samples were dried at 50°C in oven until obtaining a constant mass, digested in HNO_3 (67-69 %; Fisher Scientific Bioblock, ultratrace quality (gamme Optima)) with H_2O_2 (Fisher Scientific Bioblock) by using a Digiprep (SCP Sciences), and diluted by adding ultra-pure water. The concentrations of nine elements (As, Cd, Cr, Cu, Fe, Mo, Pb, Se, Zn) were measured with an inductively coupled plasma mass spectrometry (ICP-MS: X Series II, ThermoFischer Scientific) and expressed as micrograms per grams of dry mass ($\mu g.g^{-1}$ DM), using certified reference materials (INCT-OBTL-5: Oriental Basma Tobacco Leaves) for checking analysis accuracy. PhD student participated to these operations at Chrono-Environment laboratory.

Concentrations of elements in liver and kidneys of wood mice: The organs were dried at 60 °C in oven and digested in HNO_3 by using a Digiprep (SCP Sciences). The samples were then diluted by adding ultra-pure water. The concentrations of 14 elements (Al, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, P, Pb, Se, Si, Zn) were measured with an inductively coupled argon plasma atomic emission spectrometry (ICP-AES: ICAP 6500 Radial Thermos) and expressed as $\mu g.g^{-}$

[1] DM. Analysis accuracy was checked by using certified reference materials (TORT-3: Lobster Hepatopancreas and DOLT-5: Dogfish Liver; National Research Council, Canada). Measurement of TM concentration in organs were realized at Chrono-Environment laboratory by the technical services.

Only concentrations of Cd, Pb and Zn in SCs were used in the following analyses. On the other hand, only concentrations of Cd and Zn in liver and kidneys were used in following analyses because Pb concentrations in the two organs were below the detection limit in more than 60 % of the samples.

Concentrations of elements in plants (leaves and/or female catkins): The freeze-dried powders were digested and diluted in the same ways as SCs. The concentrations of 25 elements (Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, P, Pb, S, Sb, Se, Si, Sn, Sr, Ti, Zn) were measured with the ICP-MS using INCT-OBTL-5 as certified reference materials. The results were expressed as µg g^{-1} DM. PhD student participated these operation at Chrono-Environment laboratory.

Critical values for concentrations of TMs in SC, liver and kidneys: On the basis of the literature (cf. 1.4.3 Critical values for the three TMs for exposure and contamination of the wood mouse), the concentrations in SCs of 3.5, 11.5 and 239.2 µg g^{-1} DM are considered to be critical values for oral exposure to Cd, Pb and Zn, respectively (LOAELs for Cd and Pb as well as NOAEL for Zn; Table II.5). Moreover, Cd concentrations of 15 and 105 µg g^{-1} DM in liver and kidney, as well as Pb concentrations of 25 µg g^{-1} DM in both liver and kidney, are considered to be critical values for TM accumulation in body of the wood mouse (LOAELs). Wood mice could be thus considered to be "at risk" for toxic effects when concentrations of TMs in SC, liver or kidneys were above those values.

Table II.5. LOAELs for The TMs in tissues and SCs of the wood mouse (µg g^{-1} DM).

TM	Exposure	Accumulation	
(*µg g^{-1} dry matter*)	SC	Liver	Kidney
Cd	3.5*	15.0*	105.0*
Pb	11.5**	25.0**	25.0**
Zn	239.2***	-	-

*: Shore and Douben, 1994a
**: Shore and Douben, 1994b
***: Maita et al., 1981

II.3.3 Measuring carbon and nitrogen content in plants (leaves and/or female catkins)

The freeze-dried powders were analyzed for initial percent of C, N and S using an automated elemental analyzer (Vario Max CNS, Elementar Analysensysteme, GmbH) with

sulfadiazine as standard. After estimating the C/N ratio, quantity of C, N, and S in plant tissues were calculated by multiplying their initial percent by sample's mass. C, N and S concentrations in plant tissues (expressed as mg g^{-1} DM) were then adjusted by referring to the value of S concentration in the same tissues measured by ICP-MS, and the N/P ratio was calculated. Concentration of 25 elements and contents of C and N were measured in leaves of *A. Pseudoplatanus* (N = 34), *S. caprea* (N = 13) and *Populus* sp. (N = 7), as well as in female catkins of *S. caprea* (N = 13).

II.3.4 Identification of wood mouse's diet

Food of the wood mouse was determined using the DNA-based identification method namely "*metabarcoding*". Various DNA-based identification strategies have been developed first for organisms for which morphology-based identification are difficult, such as viruses, bacteria and protists, and then extended to macro-organisms (Taberlet et al., 2012a). Hebert et al. (2003) proposed a taxonomic identification of species focusing on a DNA region as a standard marker, "*DNA barcode*". Although the DNA barcoding *sensu stricto* corresponds to the identification of the species level using a single standardized DNA primer (e.g. COI; *rbc*L) to a single specimen, this approach has been extended to "*DNA metabarcoding*" which refers to an identification of multiple species from a single bulk sample or "*environmental DNA (eDNA) metabarcoding*" when DNA of several species is extracted from an environmental sample such as soil, water, air or feces of animals (Figure II.8; Taberlet et al., 2012b).

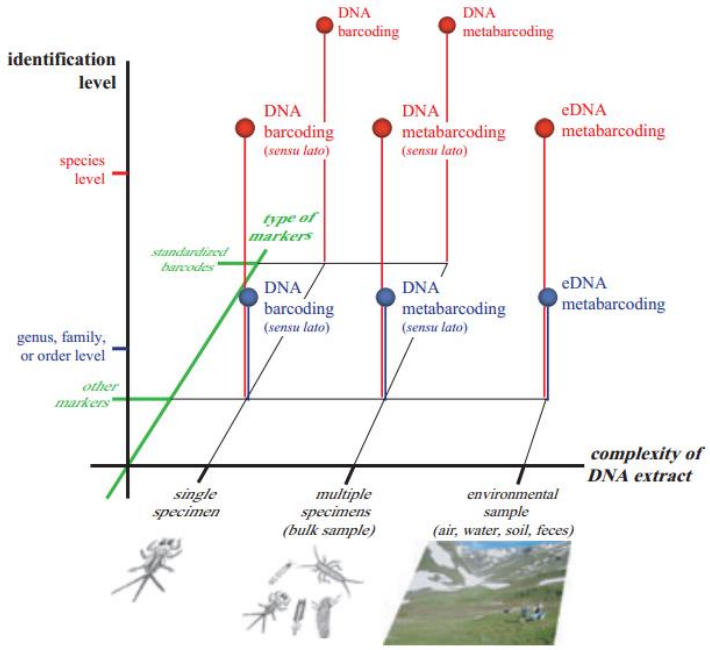


Figure II.8. Suggested terminology for DNA-based taxa identification according to the type of marker used, the level of identification and the complexity of template DNA (from (Taberlet et al., 2012b).

Several studies have applied metabarcoding to investigate the diet of various species. Valentini et al. (2009a) for example assessed diet of herbivorous species such as golden marmot *Marmota caudata*, brown bear *Ursus arctos* (N.B. the brown bear in their study area, Deosai National Park, Pakistan is mainly vegetarian), capercaillie *Tetrao urogallus*, grasshoppers *Chorthippus biguttulus* and *Gomphocerippus rufus*, snail *Helix aspersa*, and slugs *Deroceras reticulatum* and *Arion ater*, through DNA extracted from their feces. Soininen et al. (2009) compared the metabarcoding method to a traditional micro-histological identification method for stomach contents of two vole species *Microtus oeconomus* and *Myodes rufocanus* and concluded that more detailed and relatively unbiased results were obtained from the metabarcoding method. Diet of carnivorous, insectivorous or omnivorous animals has also been assessed by the metabarcoding approaches, such as leopard cat *Prionailurus bengalensis* (Shehzad et al., 2012), free-tailed bats *Chaerephon pumilus* and *Mops condylurus* (Bohmann et al., 2011), or brown bear form an area where the bear is omnivorous (De Barba et al., 2013). Actually, the DNA-based identification releases researchers from the problems inherent to morphological taxonomic identification by microscopes, such as requirement of expertise, time-consuming tasks, or morphological limitations for identification of too little fragmented or totally digested items (Pompanon et al., 2012; Valentini et al., 2009b). However, eDNA is also characterized by possible degradation, i.e. DNA molecules are cut into small fragments, and the identification by eDNA requires primers targeting much shorter DNA regions than the common standardized DNA primers (Taberlet et al., 2012b). Furthermore, estimation of species

abundance though the eDNA metabarcoding remains as a challenge. Although a positive correlation between relative number of reads and relative rank abundance of resources can be probably observed, multiple factors in the field (e.g. origin, state, fate of each eDNA) and/or the laboratory (e.g. primer bias in match with target DNA region or in DNA amplification) normally interfere with such correlation (Deiner et al., 2017). Indeed, Deagle et al. (2013) showed that the number of sequences obtained from degraded DNA is likely not proportional to the biomass of each taxa really eaten. On the other hand, it is also possible that DNA fragments of prey remaining in its consumer would be detected when the diet of predator digesting this consumer was assessed by the metabarcoding approach. This is because the eDNA metabarcoding can detect even very short and degraded DNA. Such detection of prey remains, namely "*secondary predation*", cannot be distinguished from DNA of items directly consumed and potentially leads false food composition of target animal (Harwood et al., 2001). Despite those disadvantages, the eDNA metabarcoding approach was applied to SCs of mice for determining their food as accurately and specifically as possible.

The DNA extraction, amplification and the PCR purification were performed at SPYGEN facilities (http://www.spygen.com/) through the scientific program BIOTROPH. DNeasy Blood and Tissue Kit (Qiagen GmbH) was used for extracting total DNA, following the manufacturer's instruction. Primers for DNA amplification were chosen with respect to the omnivore diet of the wood mouse (Table II.6): the P6 loop of the chloroplast trnL (UAA) intron g/h (Taberlet et al., 2007) was used for identifying general plant species; primer targeting a short fragment of mitochondrial 16S gene (16S mtDNA) was used for arthropods and mollusks DNA (16SMAV-F/16SMAV-R) (De Barba et al., 2013); and primer targeting short region of 16S mtDNA for earthworms (ewD/ewE) (Bienert et al., 2012). 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 mollusk DNA, 2 µM of a blocking primer for mammal's DNA (MamMAVB1; De Barba et al., 2013) was 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 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 titrated using capillary electrophoresis (QIAxcel; Qiagen GmbH) and purified using a MinElute PCR purification kit (Qiagen GmbH). Before sequencing, purified DNA was titrated again using capillary

electrophoresis. 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 (https://www.fasteris.com/dna/).

Table II.6. The primers used for the metabarcoding identification of food.

Analyzed taxonomic group	DNA type	DNA region	Primer name	Primer sequence 5'-3'	Reference
Plant	Chloroplast	*trn*L (UAA)	g *(forward)*	GGGCAATCCTGAGCCAA	Taberlet et al., 2007
			h *(reverse)*	CCATTGAGTCTCTGCACCTATC	
Arthropod & mollusk	Mitochondrial	16S mtDNA	16SMAV-F	CCAACATCGAGGTCRYAA	De Barba et al., 2013
			16SMAV-R	ARTTACYNTAGGGATAACAG	
Earthworm	Mitochondrial	16S mtDNA	ewD *(forward)*	ATTCGGTTGGGGCGACC	Bienert et al., 2012
			ewE *(reverse)*	CTGTTATCCCTAAGGTAGCTT	

Reads were handled by the software Mothur pipeline (Schloss et al., 2009) at Chrono-Environment laboratory by research collaborator. Forward and reverse reads were assembled in contig sequences. Then, 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) and no ambiguous nucleotides. After de-replication (occurrence count of each different sequence), only unique sequences with a minimum count of 10 (sum of all samples) were kept.

II.4 Factors studied

II.4.1 Estimation of resource diversity

Biodiversity in the broad sense is the number, abundance, composition, spatial distribution, and interactions of genotypes, populations, species, functional types and traits, and landscape units in a given system (Díaz et al., 2006; Figure II.9). Although numerous diversity indices have been proposed, no single index adequately summarizes biodiversity (Hurlbert, 1971; Purvis and Hector, 2000). Choice of diversity index is thus sometimes problematic. Species is often the fundamental unit of biodiversity, and species richness (i.e. number of different species) is the simplest metric to represent species diversity and widely applied to ecological studies (Magurran, 2003; Whittaker, 1972). There are furthermore numerous compound indices combining measures of richness and abundance. Foremost ones are the Shannon's diversity index ($-\sum P_i \ ln(P_i)$, where P_i is the relative abundance of species i, i.e. proportional abundance of species i in relation to total abundance of all species) and the Simpson's diversity index ($1/\sum P_i^2$). These measures can be derived from the same basic generalized entropy formula (Hill, 1973): $H_a = (ln\sum P_i^a)/(1-a)$, and its effective number (also called "*Hill numbers*"): $N_a = exp(H_a) = (\sum P_i^a)^{1/(1-a)}$. The parameter a, namely "*order*", determines the sensitivity of the measure to the relative abundance of community. The measure with high value of a takes into account only high abundant species (Jost, 2006). Actually, species richness corresponding to the Hill number 0 (i.e. $a = 0$) counts only number of species whatever their abundance is, value of Simpson's diversity index corresponding to the Hill number 2 (i.e. $a = 2$) is little dependent of species of low relative abundance.

In the present study, species richness was used as a proxy for the number of total available resources of wood mice. Richness of resources in each site was provided in Table II.7. Simpson's diversity index was also used as a proxy for the number of abundant resources (Jost, 2006). When richness was 0 or 1, Simpson's diversity index (called simply "*Simpson's index*" hereinafter) was also considered to be 0 or 1, respectively. The indices were hereinafter referred to as "*diversity indices*".

The diversity indices for ground-dwelling invertebrates were estimated on the basis of numbers captured per trap line (i.e. sum of numbers captured in 3 pitfall traps set for 3 days), while the indices for flying invertebrates were estimated on the basis of numbers captured per yellow pan trap (i.e. sum of numbers captured from a pan trap for 3 days). As most of invertebrates were identified at family taxonomic levels, the indices were calculated at family or higher taxonomic levels (24 taxa and 95 taxa at family or higher levels in ground-dwelling and flying invertebrates, respectively). The diversity indices for plants were measured based on number and cover-abundance (m^2) of plants present in an area of 1000 m^2 around trap lines for rodents, considered to be a minimum area of vital domain of wood mice (Quéré and Le Louarn,

2011). This area of 1000 m^2 around trap lines is hereinafter referred to as "*buffer*". As plants were identified to species, the indices for plants were calculated at species level (236 taxa at species level). Plant data collected between June and September were regarded to be similarly representative for their availability in the two seasons, supposing that available plant species and relative cover-abundance would not substantially differ between the two seasons. As pitfall traps were set near the trap lines for rodents, it is easy to associate the diversity indices of plants to the indices of ground-dwelling invertebrates. On the contrary, some of yellow pan traps were set far from the trap lines. For assessing the relationship between the diversity indices of plants and the indices of flying invertebrates (i.e. chapter 1), diversity indices of plants was also estimated in an area of the same surface (i.e. 1000 m^2) around yellow pan traps, referred to as "*buffer around pan trap*" hereinafter.

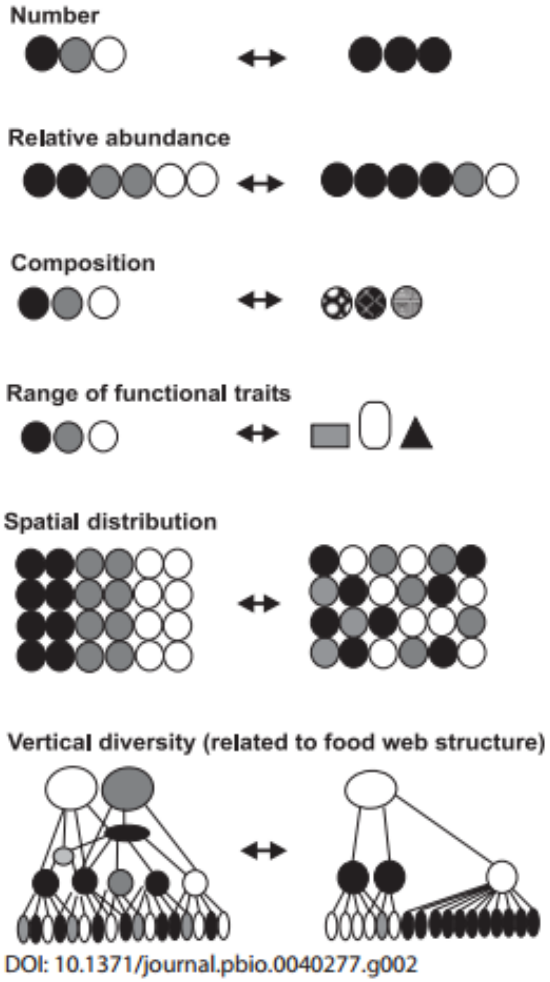


Figure II.9. The different components of biodiversity (from Díaz et al., 2006). All of these components can be affected by human intervention (arrows), and in turn have repercussions for ecosystem properties and services. Symbols represent individuals or biomass units. Symbols of different shades represent different genotypes, phenotypes, or species.

Table II.7. Richness of plants and invertebrates in each site.

	TE2	103	117	097	171	043	113
Tree stratum richness	15	7	9	7	14	14	-*
Shrub stratum richness	23	14	13	11	16	10	7
Herbaceous stratum richness	70	58	56	68	71	45	33
Ground-dwelling invertebrates richness at different levels (and at family level)	32 (13)	41 (18)	36 (16)	32 (18)	19 (11)	43 (20)	16 (10)
Spring	*27 (11)*	*33 (15)*	*32 (13)*	*32 (18)*	*17 (11)*	*36 (19)*	*11 (8)*
Autumn	*13 (8)*	*16 (10)*	*14 (11)*	*4 (4)*	*8 (5)*	*14 (10)*	*10 (8)*
Flying invertebrates richness at family level	64	64	57	54	43	66	65
Spring	*48*	*50*	*44*	*43*	*32*	*44*	*41*
Autumn	*46*	*51*	*29*	*35*	*31*	*51*	*52*

*: No survey was carried out because there were few trees.

II.4.2 Estimation of spatial variation in resource composition (i.e., beta diversity)

In addition to diversity in species at local level (i.g., *alpha diversity*), spatial variation in species composition among sites, namely "*beta diversity*", is an important aspect in biodiversity (Whittaker, 1972). Such change in community composition occurs along a given spatial or temporal gradient and/or may be understood as variation among sampling units (Anderson et al., 2011). Beta diversity patterns result from two distinct processes: "*species replacement*", also called "*turnover*", refers to the fact that species tend to replace each other, whereas "*richness difference*", also called "*nestedness*", refers to the fact that number of species may differ among communities due to their available niches (Carvalho et al., 2013). Like alpha diversity indices, summarizing the concept of beta diversity is also complicated, and various measures have been proposed so far (Anderson et al., 2011; Tuomisto, 2010a, 2010b). One of the interesting approaches is to use the total variance of the site-by-species community data (Legendre and De Cáceres, 2013; Legendre et al., 2005). Actually, Legendre (2014) demonstrated that beta diversity based on dissimilarity matrix among communities can be partitioned into matrices representing species replacement and richness difference, each of which can be analyzed in relation to explanatory environmental variables.

In the present thesis, the two types of dissimilarity matrices were built from the composition of each plant strata and of ground-dwelling invertebrates in buffers. Abundance (cover-abundance or number of effective) was converted to presence-absence because binary dissimilarity coefficients produce more interesting results than quantitative indices when species are largely different among communities (Legendre, 2014). The dissimilarity matrices were then partitioned into their two components, dissimilarity matrices for replacement and for richness difference (Legendre, 2014). In a similar way, the two types of dissimilarity matrices

were built from the composition of flying invertebrates at yellow pan traps and plants at buffers *around pan trap.* Those two types of matrices were hereinafter referred to as "*beta diversity matrices*".

II.4.3 Estimation of diet diversity

Diet diversity analyses were performed by employing Molecular Operational Taxonomic Units (MOTUs): groups of DNA sequences clustered according to similarity, which are thus independent of any reference database (Sun et al., 2012). Sequences were clustered for each primer with average neighbor algorithm using Needlman-Wunsch distance. Distances cutting clusters were chosen as 0.032 for sequences obtained from primer trnL-g/h, as 0.042 for 16SMAV and as 0.034 for ewD/ewE based on the relationship between number of clusters and distance. As estimating abundance still remains as a challenge in metabarcoding approach (cf. 2.3.4 Identification of wood mouse's diet), only diet richness, i.e. number of different items consumed by mice, was measured. The data about number of sequence reads were converted into presence/absence of MOTUs after removing from the data sequences which number of reads was below 100. Diet richness of mouse was calculated as the total number of MOTUs per mouse for three types of food: plant, arthropod and mollusk (referred to as "arthropod" thereafter), or earthworm. A potential secondary predation was checked by comparing plant richness in the diet between mice consuming and not consuming invertebrate items, assuming that plants remaining in invertebrates might increase plant richness in the diet if secondary predation really occurs. As no significant difference was observed in plant diet richness by the nonparametric Wilcoxon-Mann-Whitney test (Figure II.10; W= 5622.5 and p-value = 0.82), the possibility of the second predation was not taken into account in the present thesis.

II.4.4 Estimation of diet composition

In addition to diet richness, items consumed by each mouse were identified. Reference sequences of the species recorded in the study area were taken from the GenBank sequence database (https://www.ncbi.nlm.nih.gov/genbank/). Each sequence extracted from SCs (i.e., query sequence) was compared to the reference sequences for checking their similarity, where difference of only one nucleotide was accepted. When query sequences could match with several reference sequences, they were gathered into one group composed of the corresponding species, called "sequence group (Grp)". Non assigned query sequences were excluded. Query sequences with occurrence lower than 100 were not considered. Food Grp data were finally converted into presence/absence. The Grps were represented by using both ID number and family name(s) corresponding to their component species. Such method could be used only for plant food because almost all invertebrate resources in the field were identified to family level

only. Only presence/absence of arthropod and earthworm items was thus taken into account for invertebrate food.

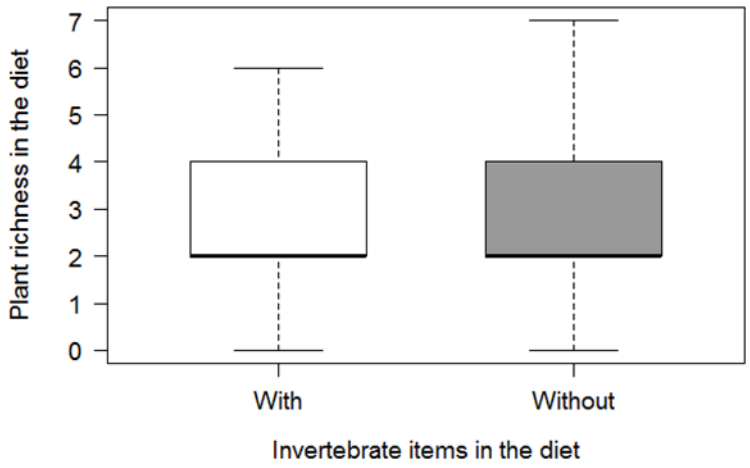


Figure II.10. Plant richness in the diet between wood mice consuming (unshaded; N = 187) and not consuming invertebrate items (shaded; N = 59). There is no significant difference in the plant richness in the diet (Wilcoxon-Mann-Whitney's W= 5622.5; p-value = 0.82).

II.4.5 Estimation of exposure to TMs and accumulation of TMs in organs of mice

To take environmental TM contamination into account when studying the contamination of animals, exposure to TMs and accumulation of TMs in organs were estimated based on residuals of a linear model (LM) built for TM concentrations in SCs or organs in relation to soil TM concentrations and other variables which differ according to chapters. (cf. 2.5 Statistical analyses for details of estimation in each chapter). These calculations for exposure and accumulation were hereinafter referred to as "*exposure*" and "*accumulation*", respectively.

To relate TM concentrations in mice with the diversity indices calculated at buffer scale (i.e. in the chapter 5), the median value of TM concentrations in SCs of the wood mice was first calculated per trap line. The TM concentrations in soils of woody patch (Fritsch et al., 2010b) were then linked to buffers corresponding to the given patch. The link between TM concentrations in soils and diversity indices was used in the chapter 2. On the other hand, when exposure to TM was calculated for each individual mouse (i.e. in the chapter 3), the TM concentrations in soils of woody patches were applied to mice taken from buffers corresponding to the given patch.

II.5 Statistical analyses

In each chapter, different statistical analyses were used. In addition to the statistical analyses commonly used in ecology, e.g. general linear model (LM), generalized linear model (GLM), linear mixed model (LMM) or redundancy analysis (RDA), some other statistical analyses not frequently used, at least not for all field of ecology, were also applied. Statistical analyses used in each chapter are then overviewed with a brief description of the concepts of these analyses. Number of mice and/or buffers used in each chapter are also provided. It is worth to note that these numbers differ between analyses due to availability of data. For instance, only mice which diet was analyzed and in parallel which were taken from buffers where resources are inventoried were used for the relationship between resource and diet (chapter 2), whereas only mice which diet and exposure were both analyzed were used for the relationship between exposure and diet (chapter 3), whatever the buffer from which they were taken.

II.5.1 Chapter 1:

Soil TM concentrations, soil pH and organic carbon content in soils represented as g kg^{-1} dry soil which was considered as a proxy of the organic matter (OM) content, were chosen as soil properties related to TM transfer from soils to resources. Correlations between diversity indices of both plants and invertebrates, including logarithmically transformed abundance of resources, and the soil properties (pH and both logarithmically transformed soil TM concentrations and OM content) were checked by the Pearson's correlation test. Effects of the soil properties on diversity indices of resources were assessed using the RDA. Furthermore, potential independent effects of either soil properties or plant diversity on composition of invertebrates, i.e. beta diversity of invertebrates, were assessed by a type of RDA, namely distance-based RDA (dbRDA), which was separately applied on "species replacement" and "richness difference" matrices. Community composition data with a large number of zeros, i.e. absences, is treaded by correspondence analysis and canonical correspondence analysis (CCA), in which rare species contribute more importantly to the results than frequent species (Legendre and Legendre, 2012), or by principal component analysis and RDA after appropriate data transformation (e.g., chord, Hellinger or chi-square transformation; Legendre and Gallagher, 2001). However, other types of dissimilarity measures are difficult to be, or even unable to be, treated by CCA or RDA with a transformation. The dbRDA allows to compute RDA based on such dissimilarity measures (Legendre and Anderson, 1999). Operationally, a principal coordinate analysis is carried out for a dissimilarity matrix on beta diversity, and those principal coordinates are used as response matrix in a RDA. A pre-transformation of dissimilarity matrix for binary data (i.e., presence/absence) by square-root is recommended (Legendre, 2014). Like RDA, a forward selection procedure can be carried out for determining significant explanatory

variables (Borcard et al., 2011) and the proportion of variance explained by the selected explanatory factors is indicated by an adjusted R^2 (R^2_{adj}) (Peres-Neto et al., 2006).

II.5.2 Chapter 2:

Food preference was assessed by comparing items in the diet of mice and their availability in the field. Selection can be defined as a feeding process of the animal in which "*resources are used disproportionally to their availability*" (definition by Manly et al., 2002). A resource is considered as to be "preferred" if its usage exceeded its availability and "avoided" if the reverse is true. It is worth to note that the "abundance" is not the same as the "availability" in the food selection study. The abundance is "*the quantity of that component (i.e. the given resource) in the environment, as defined independently of the consumer*", whereas the availability is "*its (i.e. the given resource's) accessibility to the consumer*" (definition taken from Johnson, 1980). In this present thesis, however, the availability is considered to be the same as the abundance, given the generalist diet of the wood mouse and the accessibility of the resources considered. Several studies have proposed so far different "*indices of selection*", also called "*indices of selectivity*" or "*electivity indices*", based on proportions of a given resource in the diet and in the field (Lechowicz, 1982). Each of these indices provides a rank of items form the most avoided item (the most negative value) to the most preferred item (the most positive value). Johnson (1980) also provided an alternative approach for the selectivity of food based on a comparison of ranks for the usage and the availability of the resources. Those approaches are useful to compare selection in different sites if the same resources are available for all sites. However, when available resources are largely different between sites, those approaches based on rank are not relevant. Another approach for selection analysis is to use a hypothesis test and/or confidence intervals to assess "significance" of preference or avoidance for each resources (e.g. Bailey, 1980; Goodman, 1965; Quesenberry and Hurst, 1964). Cherry (1996) compared different confidence interval methods on the basis of their length and the two types of errors and concluded that the Bailey's confidence intervals (Bailey, 1980) showed the most consistent performance. In this present study, the last approach, i.e. Bailey's confidence intervals, was thus applied to each of all identified items.

As the diet was represented by presence/absence of plant items, frequency of occurrence of each plant food item (i.e. percentage of number of occurrences of a given item compared to the total number of occurrence of all items; Klare et al., 2011) was calculated after gathering the items represented by Grp into family level. Cover-abundance of plant resource species in the field were also gathered into corresponding Grp. Only plant resources which Grp was observed in the diet were then gathered into the plant family. The frequency of occurrence of plant food and relative proportion of resources in the field (i.e. cover-abundance of a given plant food of all strata compared to the total cover-abundance of all plants of all strata) were used for

food selection per season: For each plant family, Bailey's confidence intervals of frequency of occurrence were calculated and compared with relative proportion of resources in the field. When relative availability of a given resource was located below or above the confidence intervals, this resource is considered as preferred or avoided, respectively.

A potential preference change in relation to soil TM contamination levels was measured at buffer scale. Preference for each item was measured in each buffer per season. Presence/absence of preference for an item in buffers along a gradient of logarithmically transformed soil TM concentrations was treated by GLM with logit link function for assessing change in proportion of buffers where preference for the given item was observed. Number of mice in buffer, plant richness in the field, and logarithmically transformed soil TM concentrations, as well as an interaction between the richness in the field and the soil TM concentrations were considered as explanatory variables. This modelling was carried out only for items for which preference or avoidance was observed at least in five buffers among 38 buffers in spring or 37 buffers in autumn, for higher statistical power. On the other hand, the relationship between the diet and the available resources was also analyzed at buffer scale. For each type of items (i.e. plants, arthropods, and earthworms), the number of different MOTUs in the diet of mice for a given buffer was considered as diet richness at buffer scale. Change in the diet richness was assessed in relation to the gradient of the logarithmically transformed soil TM concentrations and the resource richness in the field, as well as their interactions, using GLM with logarithm link function for Poisson distribution. The modelling was carried out using 38 buffers in spring or 37 buffers in autumn. Number of mice from the 75 buffers was 181, and number per buffer ranged from 1 to 6. Overdispersion (i.e. observed variance higher than theoretical one) was checked according to Cameron and Trivedi (1990). After the statistical inference (cf. below), explanatory value of our final models were estimated by deviance R^2: proportion of deviance explained by the given model (Zuur, 2009).

Statistical inference was based on "*model averaging*" in information-theoretic approaches (Burnham and Anderson, 2002). A set of candidate models is compared based on Kullback–Leibler (K-L) information: the model with the least K-L information loss is considered as the best model. The Akaike Information Criterion (AIC; Akaike, 1973) is quite often used for comparing such information loss in the model set. The model with the smallest AIC is considered as the best model, and difference of AIC value from the smallest AIC, represented as ΔAIC are pivotal for ranking the models according to K-L information loss (Burnham et al., 2011). Akaike weight of each candidate model, which is considered to be a probability of the best model over the model set, is also often calculated. In many cases, however, selecting one model as the best model is not certain, especially when AIC of other models are close to the smallest AIC value. Model averaging is considered to be one of the solutions for reducing such model selection uncertainty. In model averaging, each parameter of all candidate models is

averaged by Akaike weight (Burnham and Anderson, 2002). There are two types of averaging: (1) average of a parameter is carried out over all candidate models, and the value of the parameter is considered as 0 in models in which the given parameter is not included; (2) average of a parameter is carried out only over candidate models in which the given parameter is included. The first averaging approach was used in the present thesis because of less model selection bias by the first approach (Burnham and Anderson, 2002).

In this present thesis, "*second order AIC*", also called "*corrected AIC* (*AICc*)", was used instead of AIC because of small sample size in relation to the number of model parameters (Burnham and Anderson, 2002). A model averaging approach was used when the Akaike weight of the best model < 0.9. In this case, a model averaging was applied only for a confidence set of candidate models showing $\Delta AICc < 6$, which is equivalent to a likelihood ratio from the first ranked model > 0.05 (Burnham and Anderson, 2002). Each parameter of the confidence set of models was averaged by re-calculated Akaike weight.

II.5.3 Chapter 3:

Exposure to TMs per individual mouse was estimated by TM concentrations in SCs and in soils using 200 mice. The exposure to each TM was compared between the two seasons and presence/absence of each identified item in SCs by the Wilcoxon-Mann-Whitney test. Items which occurrence in the 200 mice was higher than 10 (i.e. more than 5 % of the 200 mice) were used in this chapter to improve statistical power of tests. When exposure was significantly related to several tested variables, conditional inference trees (CIT) were used for ranking their importance. The CIT is a recursive binary partitioning analysis which seeks significant univariate splits over all possible splitting variables (Hothorn et al., 2006). The most significant variable (i.e. the variable with the lowest *p-value*) is chosen for splitting. These steps are performed recursively to the two daughters split data, until no significant difference is observed. The splitting test is performed by the permutation tests developed by Strasser and Weber (1999). Whatever the result of each Wilcoxon-Mann-Whitney test is, all items and season were considered as partitioning variables, and *p-value* for partitioning in CIT was not adjusted by Bonferroni correction.

Co-occurrence of items was also checked by CIT to avoid any misleading interpretation in the relationships between exposure and items consumed. For frequency of occurrence of each item, season and presence/absence of each other items were applied as partitioning variables. On the other hand, probability for occurrence of each item in the diet was analyzed along a gradient of logarithmically transformed soil TM concentrations using GLM with logit function for binary data. Significance of GLM was checked by the likelihood-ratio test compared to null model (Zuur, 2009).

The relationship between exposure to TMs and diet richness was analyzed by LMM with the site as random effect. LMM were built for each TM as response variable and total diet richness (sum of richness for plants, invertebrates and earthworms) or each one of the three richness as independent explanatory variables. Furthermore, potential modification in the LMM by presence of certain items in the diet was assessed using model-based recursive partitioning (MOB). The principle of the MOB is the same as the CIT, i.e. recursive binary partitioning of data by statistical approach, but each terminal node is associated to a single model (Zeileis et al., 2008). A pre-established model, e.g. linear or generalized linear models, is checked to assess whether parameters of explanatory variable(s) significantly differ when the data is split by one of the partitioning variables involved. If there are several significant partitioning (Zeileis, 2005; Zeileis and Hornik, 2007), the analysis selects the partitioning variable associated with the lowest *p-value*. The steps will be performed recursively to the two daughter data, until no significant partitioning will be observed. The significance of the explanatory variable(s) in each terminal node is calculated by a type III ANOVA, and coefficient of determination R^2 in each terminal node, represented R^2_{node} in this present thesis, is also calculated. Mixed models can be also applied to the MOB (Fokkema et al., 2015). MOB was carried out for each of the LMM above. Partitioning variables for MOB were the same as the partitioning variables used for the CIT above. Finally, explanatory value for fixed effects of the whole model (i.e. all terminal nodes and partitioning) was estimated by marginal R^2 (R^2_m), whereas explanatory value for random effects was estimated by conditional R^2 (R^2_c) (Nakagawa and Schielzeth, 2013).

II.5.4 Chapter 4:

The concentration of each of the 27 elements (i.e. the 25 elements measured by ICP-MS and C and N), C/N ratio and N/P ratio in leaves were compared between the three species by the non-parametric Kruskal-Wallis test. Concentrations of each element, C/N ratio and N/P ratio between leaves and female catkins of *S. caprea* were compared by the Wilcoxon-Mann-Whitney test.

Principal component analysis (PCA) was applied to concentrations of all elements in leaves of the three species to describe correlations between each element. Concentrations of elements in plant organs were logarithmically transformed because of their skewed distributions and then scaled to unit variance before executing PCA. Centroid positions of three species and 95% confidence ellipses for those centroid positions were projected on dimensionally reduced space. Moreover, correlations between concentrations of Cd, Pb and Zn in leaves and concentrations of each of the other elements in leaves were checked by the Pearson's correlation test for each species.

Concentrations of Cd, Pb and Zn in leaves in relation to soil TM concentrations were analysed by LM. Logarithmically transformed soil TM concentrations due to skewed

distributions, three plant species and their interactions were used as explanatory variables. Significance of explanatory variables in each LM was checked by ANOVA. If assumptions of linear regression were not met, the Spearman's correlation test was applied for each species.

Bioconcentration factors for Cd, Pb and Zn in leaves were compared between the three species by the Kruskal-Wallis test. Possible change in bioconcentration factors for the three TMs along the gradient of diversity indices of plants in the field was assessed using leaves of *A. pseudoplatanus* by LM or the Spearman's correlation test, depending on assumptions of linear regression.

II.5.5 Chapter 5:

Exposure to TMs was estimated by TM concentrations in SCs and in soils, season, and their interaction using 169 mice taken form 72 buffers. Accumulation of TMs was also estimated by TM concentrations in SCs and in soils, season, mouse age and their interactions. Both exposure and accumulation in relation to resource richness in the field were assessed for each TM using LMM with the site as random effect. An optimal random effect structure was checked following Zuur (2009). When optimal model included a random effect, significance was checked by the likelihood ratio test compared to null model and R^2_m and R^2_c were calculated. When optimal model did not include any random effect, significance was tested by ANOVA type III, and the coefficient of determination R^2 was then calculated.

On the other hand, resources potentially involved in dilution and/or amplification effects were estimated as follows: both exposure and accumulation were compared between presence/absence of some resources in the field by the Wilcoxon-Mann-Whitney test, assuming that occurrence of resources potentially involved in these effects would significantly modify exposure and/or accumulation. This analysis was carried out only for resources considered to be preferred in the chapter 2, to match the third condition of the dilution effect. For each of the resources related to a significant difference in exposure or accumulation, the probability of occurrence in the diet along a gradient of resource diversity indices was analyzed using GLM with logit function for binary data. R^2_D was finally calculated when the model was significant under the likelihood ratio test.

Part III. Results

III.1 Chapter 1

Abstract and keywords in French.

Résumé :

La pollution par les éléments traces métalliques (ETM) peut affecter la diversité des plantes et des animaux. Dans la plupart des études, l'impact de la contamination par les ETM a été estimé séparément sur les communautés végétales et animales. Cependant, le rôle fonctionnel de la végétation sur la diversité et/ou la composition des invertébrés est bien documenté. Il est supposé que des changements dans la diversité et/ou la composition des invertébrés par une contamination par les ETM pouvaient s'expliquer non seulement par les effets des ETM eux-mêmes, mais aussi par des modifications des communautés végétales, qui constituent leur habitat et/ou leur nourriture. Comme les propriétés du sol peuvent exercer une influence importante sur les communautés végétales et la biodisponibilité des ETM, la teneur en matière organique (MO) et le pH des sols ont été pris en compte, avec les concentrations en ETM dans les sols, dans cette étude. Le présent travail a étudié les modifications de la diversité et la composition des invertébrés par rapport aux propriétés des sols, y compris la contamination par les ETM, et par les changements de diversité végétale sur un site contaminé par les ETM. L'hypothèse est que la diversité végétale est un facteur qui influence plus la diversité des invertébrés au niveau local (diversité alpha) et la variation spatiale de leur composition (diversité bêta) que les propriétés des sols et les concentrations en ETM. Les résultats ont montré que la diversité végétale expliquait d'une façon égale ou plus importante les diversités alpha et bêta des invertébrés que les propriétés des sols et les concentrations en ETM. Les résultats montrent également que certains groupes trophiques d'invertébrés sont plus impliqués que d'autres dans le renouvellement d'espèces dans les communuatés d'invertébrés marcheurs et volants le long des gradients de propriétés des sols et de diversité végétale. La diversité et la composition de la végétation devraient être prises en compte dans les études sur les impacts de la contamination environnementale sur les communautés d'invertébrés.

Mots-clés : Diversité alpha, diversité beta, biodiversité, fonctionnement des écosystèmes, composition des communautés, éléments traces métalliques.

Scientific manuscript in preparation for publication

Vegetation shapes diversity and composition of aboveground invertebrates more than soil properties and pollution on a metal contaminated site

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Abstract

Pollution by trace metals (TMs) has been shown to affect diversity of plants and animals. In most of studies, the impact of TM contamination has been estimated separately on plant and animal communities. However, the functional role of vegetation on invertebrate diversity and/or composition is well documented. It is supposed that changes in invertebrate diversity and/or composition due to TM contamination would be explained not only by effects of TMs but also by plant communities, which constitute their habitat and/or food. As soil properties can exert an important influence on both plant communities and bioavailability of TMs, organic matter content (OM) and pH have been considered together with TM concentrations in soils in this study. The present work therefore studied the changes in diversity and composition of aboveground invertebrates in relation to both soil properties and TM contamination and plant diversity in a TM contaminated site. Plant diversity was hypothesized to be a factor explaining more the variation of invertebrates at local level (i.e. alpha diversity) and the spatial variation in their composition (i.e. beta diversity) than soil properties and TM concentrations. Plant diversity evenly or more importantly explained both alpha and beta diversity of invertebrates than soil properties and TM concentration. Furthermore, trophic groups of invertebrates could be involved in their species turnover along the gradients of soil properties and/or of plant diversity in both ground-dwelling and flying invertebrates. Diversity and composition of vegetation should be taken into account when addressing impacts of environmental contamination on invertebrate communities.

Highlights

- Alpha and beta diversity of invertebrates were analyzed in relation to both soil properties and TM concentrations and alpha diversity of plants in a TM contaminated site.
- Diversity of plants did not show any correlation with soil TM concentrations or soil properties.
- Plant diversity evenly or more importantly explained alpha and diversity of both flying and ground-dwelling invertebrates than soil properties and TM concentrations.
- Plant diversity also evenly or more importantly explained beta diversity of invertebrates than soil properties.
- Species turnover of taxa along gradients of soil properties and/or of plant diversity could be related to trophic groups in both ground-dwelling and flying invertebrates.

Keywords

Alpha diversity, beta diversity, biodiversity, ecosystem functioning, community composition, trace metals.

Introduction

Trace metals (TMs) naturally occur in the environment but high concentrations emitted into the environment through various anthropogenic activities can be associated to deleterious effects on ecosystems. Although technical and regulatory improvements have considerably reduced over decades the release of many TMs from industrial activities in developed countries (EMEP, 2013), pollution by some TMs and legacy pollution of those non-degradable elements still widely persist in nature and affect ecosystems. Harmful effects of TM contaminations have been observed at several biological levels (Walker et al., 2012). Ecosystems undergoing strong disturbances display many negative responses, one of which is the decrease of biodiversity (Odum, 1985). Diversity indices are indeed commonly used as indicators for estimating pollutant's effects in ecosystems (e.g. Zvereva et al., 2008; Zvereva and Kozlov, 2012, 2010). In terrestrial ecosystems, impacts of metal contamination on biodiversity have been studies in both plants and animals. It has been reported that diversity of plants is frequently affected by soil TM contamination (e.g. Bes et al., 2010; Dazy et al., 2009; Ginocchio, 2000; Vidic et al., 2006). However, responses in plant diversity to soil TM contamination vary according to elements, vegetation types, and/or diversity indices (Zvereva et al., 2008). Change in plant composition rather than in plant diversity along a gradient of TM soil contamination levels has also been reported (e.g. Strandberg et al., 2006). A similar framework has been highlighted about the relationship between biodiversity of animal communities and TM contamination. Some studies have clearly shown negative correlations between diversity of invertebrates and soil TM contaminations (e.g. Paoletti et al., 1988; Read et al., 1987; Spurgeon and Hopkin, 1996), whereas changes in invertebrate community structure and composition rather than in diversity indices have also been reported in other studies (e.g. Babin-Fenske and Anand, 2011; Migliorini et al., 2004; Nahmani and Lavelle, 2002).

Most of time in the previous studies, impacts of TMs have been estimated separately on plants and animals. However, interactions between plants and animals could be involved in effects of environmental TM contamination on each of them (Bol'shakov et al., 2001; Eeva et al., 2012; Storm et al., 1993). Positive effects of a diverse vegetation on both above- and under-ground, as well as on both herbivorous and predatory invertebrates, have been widely demonstrated in experimental and observational studies in grassland systems (e.g. Brose, 2003; Haddad et al., 2001; Knops et al., 1999; Scherber et al., 2010; Siemann, 1998) and, to a lesser extent, under woodland conditions (e.g. Fraser et al., 2007; Humphrey et al., 1999; Scherber et al., 2014; Sobek et al., 2009). Although underlying mechanisms relating ecosystem biodiversity and functioning are still debated (Borer et al., 2012; Haddad et al., 2009), a diverse plant community could provide a variety of resources for a greater number of herbivore species (Hutchinson, 1959), or more refuges and more stable prey availability for predators, supporting higher diversity and abundance of animals (Root, 1973). Potential effects of vegetation on

composition of invertebrate communities in TM contaminated sites have been commented in some ecotoxicological studies (e.g. Grelle et al., 2000; Nahmani and Rossi, 2003). However, at least to our knowledge, no study has clearly demonstrated how importantly plants would contribute in shaping invertebrate community changes compared to soil TM contamination effects. Evaluating effects of vegetation on invertebrate community in TM contaminated sites allows to specify TM contamination impacts on communities and to compare them between studies carried out in sites with different vegetation types.

In the present study, the potential effects of plant diversity on diversity and composition of invertebrate communities were assessed in relation to soil TM contamination and soil properties in a smelter-impacted area in northern France. We hypothesized that diversity and composition of invertebrate communities would be more explained by plant diversity than by soil properties and TM concentrations. Variation of taxa at local level (i.e. alpha diversity) and spatial variation in their composition (i.e. beta diversity) were measured in plants and invertebrates. Relationships between soil properties and TM concentrations and plants diversity were first focused on to evaluate their dependence. We then analyzed what variables among plant diversity indices and soil properties and TM concentrations best explain alpha and beta diversity of invertebrates. Proportion of effects was also estimated. We then focused on changes in the diversity and the composition of invertebrate community in relation to both vegetation diversity and soil TM concentrations, hypothesizing a stronger effect of vegetation rather than effects of soil pollution due to cumulative effects of TMs on both vegetation and invertebrates. We finally compared independent effects of vegetation diversity and TM contamination on invertebrate communities under a hypothesis that each of them would importantly modify both the diversity and the composition of invertebrate communities.

Materials and methods:

Study sites

This study was carried out in the surroundings of the former lead (Pb) and zinc (Zn) smelter named ‘Metaleurop Nord’ located in Northern France (Noyelles Godault, Hauts-de-France, formerly Nord Pas-de-Calais, France). This smelter was created in 1894 and closed in 2003. It was the only producer of primary Pb in France and one of the largest in Europe and its pyro-metallurgic process had generated large quantities of dust for more than a century. For example, about 1.0 ton of cadmium (Cd), 16.9 tons of Pb and 31.6 tons of Zn were released from it in 2002, despite the implementation of technical improvements during the 1970s (DRIRE, 2003). With another large Zn smelter which has been working since 1869 in a neighbouring town (Auby), namely ‘Umicore’, the dust emission has affected an area of around 120 km^2 (Douay et al., 2008). Several studies have revealed high contamination by TMs in the surrounding soils. In agricultural top soils, concentrations as high as 21 mg kg^{-1} of dry soil for

Cd, 1132 mg kg^{-1} for Pb, and 2167 mg kg^{-1} for Zn have been measured (Sterckeman et al., 2002), whereas they reached up to 67, 4890 and 2685 mg kg^{-1}, respectively, in the upper organic layers of grasslands around the smelter (Sterckeman et al., 2000). In the study of Fritsch et al. (2010), total concentrations of those three metals reached 236, 7331 and 7264 mg kg^{-1} of dry soil, respectively, in soils sampled in woody habitats such as hedges or woods, largely exceeding the values in a reference site (0.9 - 2.4, 43 - 200, 89-278 mg kg^{-1} of dry soil for and for Cd, Pb, and Zn, respectively). Extreme values of 2402, 41,960 and 38,760 mg kg^{-1} of Cd, Pb and Zn, respectively, have been measured in a dredged material deposit in the area (Fritsch et al., 2010).

Table 1. Description of the seven sites about soil physico-chemical properties and number of buffers used in the present study.

	TE2	103	117	097	171	043	113
Soil contamination level	"Control[a]"	+	++	+++	++	++	++
Landscape feature	Forest	Forest	Forest	Forest	Forest	Arable	Urban
$[Cd]_{soil}$ (mg kg^{-1})[b] Min - Max (Median)	0.9 - 2.4 (1.4)	1.5 - 6.0 (4.3)	3.6 - 17.8 (9.1)	15.3 - 236.5 (48.3)	4.9 - 14.5 (7.5)	1.3 - 42.7 (15.2)	4.4 - 13.0 (11.5)
$[Pb]_{soil}$ (mg kg^{-1})[b] Min - Max (Median)	43.3 - 199.8 (107.4)	237.5 - 333.0 (267.2)	244.7 - 859.8 (512.0)	658.5 - 6809.4 (1295.3)	287.6 - 2063.3 (584.0)	105.0 - 1028.9 (323.1)	266.6 - 806.0 (678.9)
$[Zn]_{soil}$ (mg kg^{-1})[b] Min - Max (Median)	89.3 - 277.7 (168.8)	114.4 - 407.5 (352.7)	302.8 - 958.5 (555.8)	1069.3 - 7263.5 (1874.7)	487.2 - 2451.5 (1362.7)	153.9 - 1549.6 (512.8)	414.7 - 1170.4 (1001.2)
pH [b] Min - Max (Median)	4.5 - 7.2 (5.9)	4.6 - 6.9 (5.6)	7.3 - 8.1 (7.9)	7.9- 8.2 (8.0)	7.7 - 8.3 (8.0)	6.2 - 8.3 (7.0)	7.0 - 7.9 (7.2)
OM (g kg^{-1})[b] Min - Max (Median)	26.1 - 186.0 (52.6)	47.7 - 96.3 (54.8)	34.3 - 77.3 (60.5)	31.5 - 110.9 (50.5)	35.0 - 223.0 (95.8)	28.5 - 125.4 (57.9)	38.8 - 276.8 (97.4)
Number of patches	8	5	4	6	7	7	0
Trees were present	*8*	*4*	*3*	*3*	*5*	*7*	*0*
Shrub were present	*7*	*4*	*3*	*3*	*3*	*2*	*0*
Herbs were present	*7*	*3*	*3*	*6*	*6*	*7*	*0*
All strata were present	*6*	*3*	*1*	*3*	*2*	*2*	*0*
Number of buffers[c] for ground-dwelling invertebrates	14	15	6	9	14	10	0
in spring	*7*	*8*	*2*	*7*	*7*	*5*	*0*
in autumn	*7*	*7*	*4*	*2*	*7*	*5*	*0*
Number of buffers[c] for flying invertebrates	4	5	3	4	4	4	0
in spring	*1*	*3*	*1*	*2*	*3*	*2*	*0*
in autumn	*3*	*2*	*2*	*2*	*1*	*2*	*0*

a: TM concentrations as close as possible to background concentrations.
b: Values taken from Fritsch et al. (2010)
c: Buffers where plants of all strata were present.

The present study was undertaken on seven sites of 25 ha (500m x 500m) in the surroundings of the former smelter. Six sites were located within an area of 40 km^2 (5 x 8 km) including Metaleurop Nord in its center, and the other site, which was considered to be the control site, was located about 10 km northeast of the former smelter. The seven sites in the present study were chosen for representing both a gradient of TM soil concentrations and different landscape feature types, which were measured in previous works (Douay et al., 2008; Fritsch et al., 2010) (Table 1).

Field inventory of plants and invertebrates in the field

Vegetation survey was realized from 4th June to 5th September 2012, once in each square. Vascular plant taxa were described in three different strata defined by their height: tree stratum (woody species > 8 m high), shrub stratum (woody species < 8 m high), and herbaceous stratum, and identified at species level in the field following Dudman and Richards (1997) and Lambinon et al. (2004). Cover-abundance of each taxa was visually estimated as the vertically projected area following Braun-Blanquet et al. (1952). Vegetation habitats were determined by plants' composition of one or more strata and delineated as polygons based on aerial pictures. The polygons were geo-referenced and digitalized using the QGIS software (version 2.18). Two hundreds thirty six different plant taxa were listed in the field: 25 species in tree stratum; 42 species in shrub stratum and 193 species in herbaceous stratum (N.B. Some plant taxa were observed in several strata. For details of the plant inventory, see Ozaki et al., 2018).

Invertebrate fauna was sampled in spring (April) and in autumn (September and October) 2012 by two types of trapping: pitfall traps and yellow pan traps. The pitfall trap is one of the most frequently used methods for sampling epigeic invertebrates such as ground beetles, rove beetles, wandering spiders, and ants (Leather, 2005). Ten lines, each of which was composed of three 800ml polypropylene beakers with neither roof nor preservative fluid placed at 15m intervals, were set in woody habitats, per site and per season (i.e. 70 lines of 210 pitfall traps in total were used in each season). The yellow pan trap is also frequently used for sampling insects that are flying above or between plants within the understory. Trapping color plays a determinant role in the effectiveness with which different insect groups are caught. Yellow color is efficient for catching a wide range of phytophagous insects and predators or parasitoids of them (Kirk, 1984). Four yellow pan traps with soap mixed water were set per site and per season (i.e. 28 plates was used in total in each season). Locations of the two types of traps were geo-referenced and checked every morning for three consecutive days. Captured invertebrates were stored in ethanol or in freezer at -20°C and identified in laboratory at the lowest possible taxonomic levels by morphological characteristics. The main references used for invertebrate determination were Coulon (2003), Forel and Leplat (2001), Jeannel, (1941) and Trautner and Geigenmueller (1987). Springtails (mainly Collembolans) were removed from our inventory. In total, 88 different taxa were captured by pitfall traps. Invertebrates of some families such as

Carabidae or *Coccinellidae* were identified at species level, but other invertebrates were identified at family or higher taxonomic levels (e.g. order or class). We also captured 95 different taxa by pan traps, which were identified at family or higher taxonomic levels. Captured fauna by each type of traps were considered to be "ground-dwelling invertebrates" and "flying invertebrates", respectively. Ground-dwelling invertebrates were principally composed of zoophagous (i.e. chilopods, arachnids, Coleoptera) or saprophagous (i.e. diplopods) trophic groups, whereas flying invertebrates were principally composed of Hymenoptera, Diptera and Coleoptera belonging to phytophagous, mycetophagous or zoophagous trophic groups (for details of the captured invertebrates, see Ozaki et al., 2018).

Measuring biodiversity

In this study, richness (*S*: number of different taxa) and Simpson's diversity index (*D: 1/*$\sum P_i^2$, where P_i is the proportional abundance of taxa *i*) were used for contrasting total number of species to number of abundant taxa at local level (Jost, 2006). Simpson's evenness (*E: D/S*) was calculated and used as another variable with reference to the proportion of dominant taxa among all taxa. When S was 0, D and E were also considered to be 0. Abundance of all taxa (*N*) was also added as another information about the community. It is worth to note that those indices S, D, E and N, cannot be considered as real values, but as comparative values, because of our simplified sampling methods. The four indices at local level were hereinafter referred to as '*alpha diversity indices*'. The indices for plants were measured for each stratum (tree, shrub and herb) based on species identified and cover-abundance (m^2) of each species present in 37 "*woody patches*", where soil properties including soil TM concentrations had been measured in the study of Fritsch et al. (2010) (Table 1). Measurement of diversity indices for invertebrates were based upon individuals captured by 140 trap lines (i.e. 480 pitfall traps) and 48 yellow pan traps, respectively. For homogenizing taxonomic levels, family level (or higher levels for individuals which were identified at higher taxonomic levels than family) was used for the measurement (24 and 95 different taxa for ground-dwelling and flying invertebrates, respectively).

Spatial variation in species composition among communities (i.e. beta diversity) was estimated by using the total variance of the site-by-species community data (Legendre et al., 2005; Legendre and De Cáceres, 2013). Legendre (2014) demonstrated that beta diversity based on dissimilarity matrix among communities can be partitioned into two matrices representing species turnover (N.B. the term "species *replacement*" was used in the publication and used hereinafter in the present study) and richness difference, each of which can be analyzed in relation to explanatory environmental variables. For all types of plant strata and of invertebrates, dissimilarity matrices for beta diversity were built from the species composition data converted to presence-absence because binary dissimilarity coefficients produce more interesting results than quantitative indices when species are largely different among communities (Legendre,

2014). The dissimilarity matrices were partitioned into the two matrices for species replacement and richness difference, hereinafter referred to as '*beta diversity matrices*'. Beta diversity matrices for invertebrates were estimated based upon individuals captured by trap lines or pan traps.

For analyses on relationships between plant invertebrate diversity, plant diversity indices were recalculated based upon abundance of plants present in an area of 1000 m^2 around invertebrate traps. Those areas were hereinafter referred to as '*buffers*'. Plant data collected between June and September were regarded to be similarly representative for their availability in the two seasons, supposing that available plant species and relative cover-abundance would not substantially differ between the two seasons.

Soil properties of woody patches (Fritsch et al., 2010) were linked to buffers. Soil properties of each patch were used for plant diversity indices. For invertebrate diversity indices or matrices, soil properties of the patch corresponding to or nearest to the buffers were used.

Statistical analyses

Correlations among alpha diversity indices within each vegetation stratum or invertebrate type were checked by a correlation test. Effects of soil properties and TM concentrations on plant alpha and beta diversity were assessed using a multivariate analysis, separately performed for each strata. For assessing independent effects of either soil properties or plant alpha diversity on invertebrate alpha and beta diversity, another multivariate analysis was carried out, separately for ground-dwelling and flying invertebrates.

Data used in each analysis

As multivariate analysis on composition data cannot be carried out with empty community (i.e. community where no species was present), we used communities where species richness is not 0. For analyses by each plant strata, number of patches used were 30, 22, 32 for tree, shrub and herbaceous stratum, respectively, among the 37 patches. Correlations among alpha diversity of different strata was assessed by using 17 patches where plants of all strata were present. Analyses about relationships between plant and invertebrates diversity were carried out by using buffers where all plant strata were presents: 36 and 32 buffers in spring and in autumn for ground-dwelling invertebrates and 12 buffers in each season for flying invertebrates.

Data transformation

Concentrations of Cd, Pb and Zn in soils were highly correlated (Pearson's $r > 0.9$, p-value of correlation test < 0.001), and only Pb concentration in soil was used as a proxy of soil pollution in our statistical analyses. Soil pH and organic carbon content (g kg^{-1}) in soil, considered as a proxy of the organic matter (OM) content in soils, were used as soil properties importantly related to bioavailability (Bradham et al., 2006; Giller et al., 1998; Visioli et al., 2013). Soil pH was also positively correlated to soil TM concentrations (Spearman's *rho* > 0.6,

p-value of correlation test < 0.001 for the three TMs). Both soil Pb concentration and OM content were logarithmically transformed because of skewed distributions. The three variables, soil Pb concentrations, soil OM content and soil pH were hereinafter referred to as '*soil properties*'. Abundance N (i.e. total cover-abundance of plants or total number of individual invertebrates) was also logarithmically transformed because of skewed distributions. Dissimilarity matrix for beta diversity was built using Jaccard dissimilarity coefficient and partitioned into matrices for replacement and richness difference, hereinafter referred to as $Repl_J$ and $RichDiff_J$, respectively. For executing multivariate analyses, alpha diversity indices and soil properties were scaled to zero mean and to unit variance for each variables because of their different unit.

Analyses for plant diversity

Correlations between alpha diversity indices of plants are given in Supporting Information Table S1. The redundancy analysis (RDA) was then executed for the alpha diversity indices in relation to the soil properties. A forward selection procedure was carried out for determining significant explanatory variables (Borcard et al., 2011). Proportion of variance explained by the selected explanatory variables was indicated by an adjusted R^2 (R^2_{adj}) (Peres-Neto et al., 2006). Relationships between plant beta diversity matrices and soil properties were assessed by using the distance-based RDA (dbRDA) (Legendre, 2014). Briefly, a principal coordinate analysis (PCoA) was carried out for each dissimilarity matrix after square-root transformation. Their principal coordinates were used as response variables and a forward selection procedure was executed for determining significant explanatory variables. R^2_{adj} was measured based upon the set of the selected explanatory variables. Species presence-absence data were *a posteriori* projected on the ordination plot using weighted averages. Species appeared in extremes of the selected explanatory variables were considered as species whose occurrence in communities were affected by the variables. Ecological indication values and life history traits of some species in those extremes of the explanatory variables were also checked based on Landolt et al. (2010).

Relationships between invertebrate diversity and plant diversity

Correlations between alpha diversity indices of invertebrates are given in Supporting Information Table S2. Relationships between invertebrate alpha diversity indices, soil properties and plant alpha diversity were assessed by the partial RDA (pRDA) and the variation partitioning (Borcard et al., 2011). The pRDA in relation to plant diversity was executed as follows: after RDA for invertebrate alpha diversity data in relation to soil properties, the residual variation of this RDA (i.e. variation of invertebrate diversity data non-explained by soil property data) was handled by another RDA in relation with plant alpha diversity data. This was *vice versa* for the pRDA in relation to soil properties. Variation explained by selected variables of each explanatory matrix, as well as variation explained jointly by them were showed in Venn

diagram. If one of the two explanatory matrices was not significantly related to the response marix, ordinal RDA was carried out. For invertebrate beta diversity matrices, partial dbRDA was applied and invertebrate presence-absence data were *a posteriori* projected on the obtained ordination. Like plant beta diversity analysis, the 10 taxa furthest from the center of the ordination plot and their diet mainly based on Jeannel (1941) and Mora (2002) were checked.

All statistical analyses were computed using the statistical software R (ver. 3.4.4; R Development Core Team). Computing PCA, PCoA and RDA were handled with '*vegan*' package. Function '*forward.sel*' of R package '*Packfor*' (Dray et al., 2012) was used for forward selection. Functions '*beta.div.comp*' and '*dbRDA.D*' from (Legendre, 2014) were used for building dissimilarity matrices BD_J, $Repl_J$ and $RichDiff_J$, and for carrying out accurate significance test for dbRDA, respectively.

Results

Plant alpha and beta diversity in relation with soil properties

Alpha diversity

Alpha diversity indices in plants were not significantly explained by soil properties including soil pollution, whatever plant stratum was.

Beta diversity

57.4% and 42.6% of the variation in beta diversity of tree stratum were explained by replacement ($Repl_J$) and richness difference ($RichDiff_J$) matrices, respectively. Likewise, 62.1% and 37.9% of the variation of beta diversity of shrub stratum were explained by $Repl_J$ and $RichDiff_J$, whereas 64.4% and 35.6% of the variation in beta diversity of herbaceous stratum were explained by $Repl_J$ and $RichDiff_J$, respectively. Although $RichDiff_J$ was not significantly explained by any soil property in all plant strata, $Repl_J$ of tree and herbaceous strata were significantly explained by pH (R^2_{adj} = 0.052 and 0.030, respectively), whereas $Repl_J$ of shrub stratum was significantly explained by soil Pb concentrations (R^2_{adj} = 0.055). The biplots of partial dbRDA on $Repl_J$ of the three plant strata are given in Supporting Information Figure S3. Table 2 shows plant species that appeared in the two extremes of the selected soil properties with their indication value for soil pH and tolerance to soil metal content. The other indication values of the plant species are given in Supporting Information Figure SI4. Almost of all those species showed certain tolerance to soil metal content. However, they did not show any notable indication value for climate or soil conditions. Species appearing both in high and low pH patches indicates from weakly to neutral acidity of soil.

Table 2: Plant species appeared in extremes of soil properties and their three life history treats (metal tolerance, pH tolerance and flowering months) (from Landolt et al., 2010). Reaction characterizes the content of free H-ions in the soils, from 1 (extremely acid) to 5 (alkaline, high pH), and x signifies a very large range of variation. Certain tolerance to heavy metals in the soil, referring predominantly to serpentine tolerance i.e. the tolerance to nickel and chromium, is represented by 'm' and emply cells signify no particular studies for a given species.

Taxa			**Soil properties**		**Soil indicators**	
Stratum	**Species**	**Family**	**$pH_{[soil]}$**	**$Pb_{[soil]}$**	Reaction	Heavy metal tolerance
Tree	*Hedera helix*	*Araliaceae*	Low		3	m
	Tilia platyphyllos	*Tiliaceae*	Low		4	m
	Aesculus hippocastanum	*Hippocastanaceae*	Low		4	m
	Salix caprea	*Salicaceae*	High		3	m
	Betula pendula	*Betulaceae*	High		x	m
	Salix alba	*Salicaceae*	High		4	m
	Populus x canescens	*Salicaceae*	High		4	m
	Ulmus minor	*Ulmaceae*	High		4	
Shrub	*Tilia platyphyllos*	*Tiliaceae*		Low	4	m
	Fraxinus excelsior	*Oleaceae*		Low	4	m
	Salix caprea	*Salicaceae*		Low	3	m
	Populus x canescens	*Salicaceae*		Low	4	m
	Cornus sanguinea	*Cornaceae*		High	4	m
	Frangula alnus	*Rhamnaceae*		High	3	m
	Ligustrum vulgare	*Oleaceae*		High	4	m
	Rubus caesius	*Rosaceae*		High	4	m
	Viburnum opulus	*Caprifoliaceae*		High	3	m
	Corylus avellana	*Betulaceae*		High	3	m
Herb	*Cirsium palustre*	*Asteraceae*	Low		3	m
	Holcus lanatus	*Poaceae*	Low		3	m
	Juncus effusus	*Juncaceae*	Low		2	m
	Carex riparia	*Cyperaceae*	Low		4	
	Convolvulus arvensis	*Convolvulaceae*	Low		4	m
	Dactylis glomerata	*Poaceae*	Low		3	m
	Heracleum sphondylium	*Apiaceae*	Low		3	
	Pastinaca sativa	*Apiaceae*	High		4	m
	Silene vulgaris	*Caryophyllaceae*	High		3	m
	Veronica persica	*Scrophulariaceae*	High		4	m

Alpha and beta diversity of ground-dwelling invertebrates in relation to soil properties and vegetation

Alpha diversity

Alpha diversity indices in ground-dwelling invertebrates in spring were significantly explained by both soil properties, Pb_J concentrations and OM content in soils, and plant diversity indices, herb D and herb N. After removing variance explained by the two herb diversity indices, ground-dwelling invertebrate E and N were positively and negatively correlated with OM content in soils, respectively. Ground-dwelling invertebrate S and D were positively correlated to Pb in soils. (Figure 1a). After removing variance explained by the soil properties, ground-dwelling invertebrate E was positively correlated with herb N was whereas ground-dwelling invertebrate S and N were negatively correlated with herb N. Ground-dwelling invertebrate D was negatively correlated with herb D to (Figure 1b). 26.7% of variance was explained only by herb D and N, 8.0% of variance was explained by Pb and OM contents in soil, and 7.7% were shared by the plant diversity indices and the soil properties (Figure 1c).

In autumn, alpha diversity indices of ground-dwelling invertebrates were significantly explained by tree S ($R^2_{adj} = 0.125$) but not by any soil property. Ground-dwelling invertebrate N was positively correlated with tree S, whereas Ground-dwelling invertebrate S, D and E were negatively correlated with tree S (Figure 1d).

Beta diversity

In spring, 49.1% and 50.9% of variation in beta diversity of ground-dwelling invertebrates were explained by $Repl_J$ and $RichDiff_J$, respectively. $Repl_J$ was significantly explained by both pH and shrub D. 2.3% of variance were explained by Shrub D itself, 2.6% by pH itself and 1.9% were shared by them (Figure 2a). $RichDiff_J$ was also significantly explained by Pb and OM contents in soils, as well as by herb N. 7.2% of variance were explained by herb N itself, 4.0 % were explained by Pb and OM contents in soils , and 8.7% were shared by them (Figure 2b).

In autumn, 52.9 % and 47.1% of variation in beta diversity of ground-dwelling invertebrates were explained by $Repl_J$ and $RichDiff_J$, respectively. $Repl_J$ was significantly explained by OM contents and pH in soils, and shrub N. The two soil properties explained themselves 8.3% of variance, and the rest of variance was shared with shrub N (Figure 2c). $RichDiff_J$ was significantly explained by plant diversity tree S ($R^2_{adj} = 0.112$), but not by any soil property. The biplots of partial dbRDA on beta diversity matrices of ground-dwelling invertebrates in spring and in autumn are given in Supporting Information Figure S5 and S6, respectively. show Ground-dwelling invertebrates appeared in the two extremes of the selected variables are shown Table 3 and Table 4, respectively for spring and in autumn, with their diet.. Diet of ground dwelling invertebrate in spring seemed to be diversified in buffers with low pH or high shrub D in spring: zoophagous invertebrates were rather observed in these buffers.

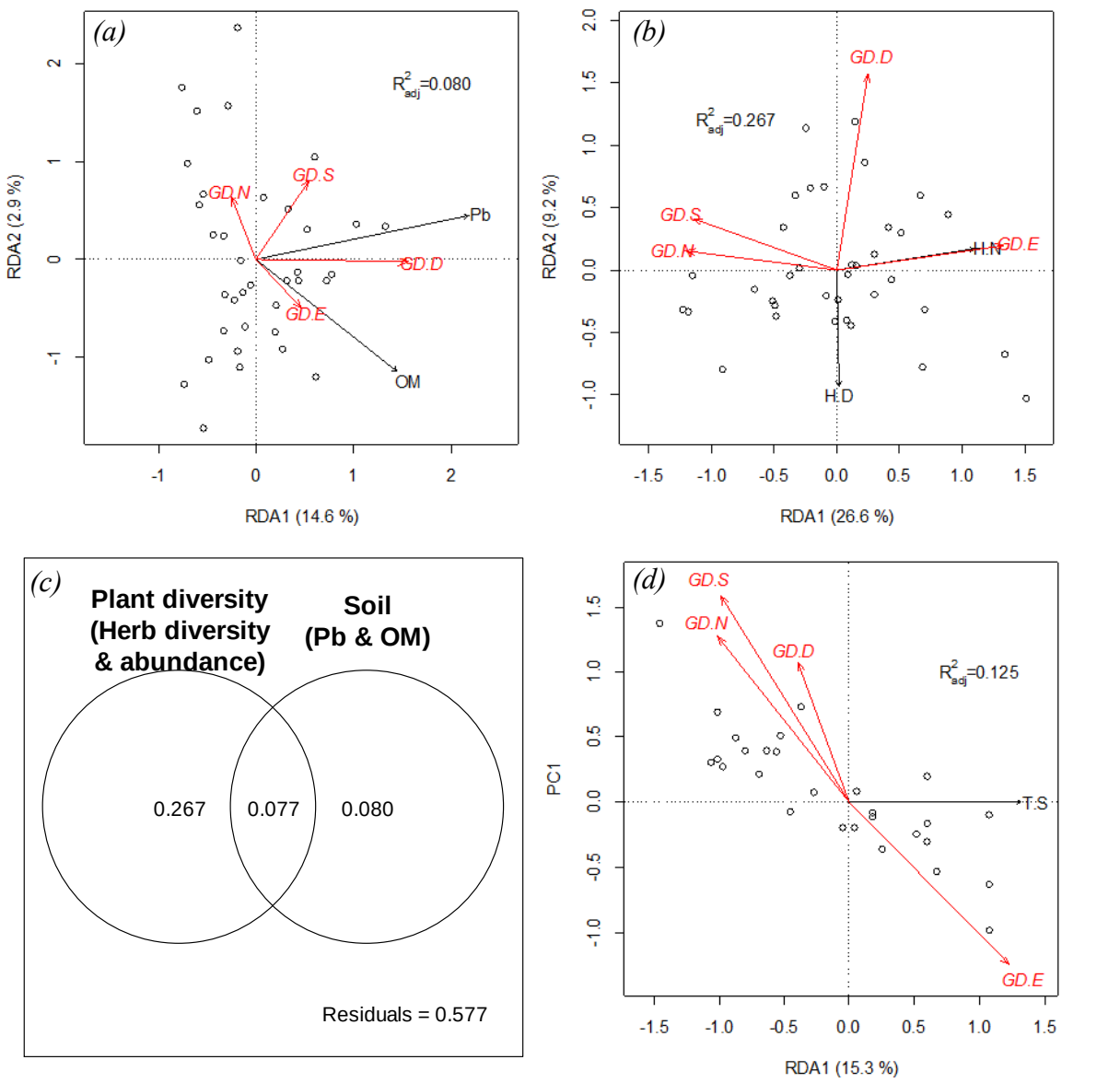

Figure 1: Results of redundancy analysis (RDA) or partial RDA (pRDA) on alpha diversity indices of gound-dwelling invertebrates. (a) Biplot of pRDA on the diversity indices in spring explained by soils properties controlling for plant diversity indices, and (b) explained by plant diversity indices controlling for soil properties. (c) Venn diagram represents variance explained by each of the two sets of variables. (d) Biplot of RDA on the diversity indices in autumn explained by plant diversity indices. The x axis represents the first canonical axis, and the y axis represent the second one (or the first unconstrained axis when only one variables was selected as explanatory variable). Buffers were represented by points and selected variables were represented by arrows. Adjusted R^2 (R^2_{adj}) was mentioned. (For each varaible of diversity indices, the first letters of abbreviation represents plant stratum (T: tree; S: shrub; H; herbaceous stratum) or type of invertebrates (GD: ground-dwelling), and the second letter represents diversity indices (S: richness; D: Simpson's diversity index; E: Simpson's evenness; N: abundance).

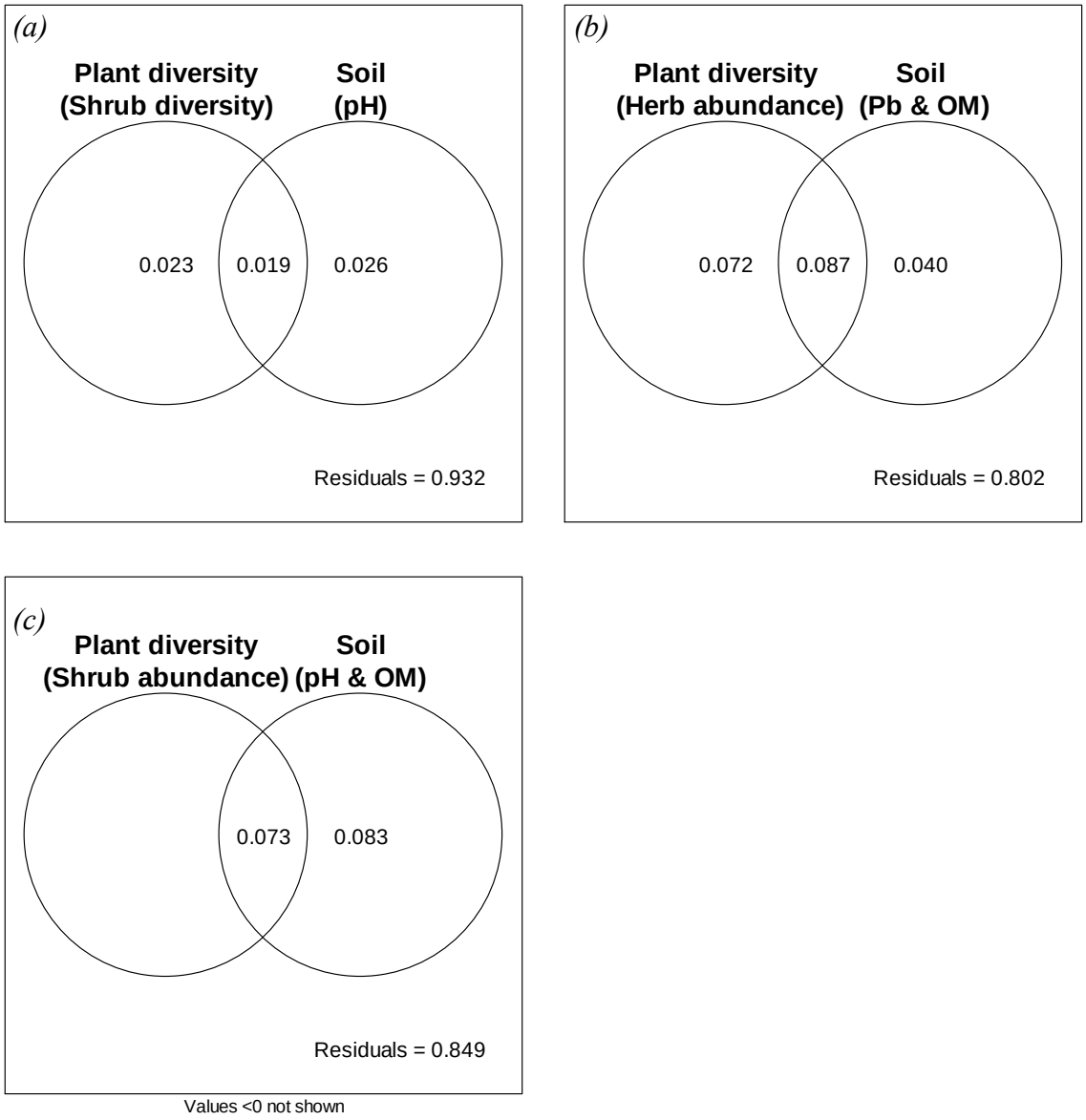

Figure 2: Venn diagram represents variance (Adjusted R^2) explained by each of the two sets of variables (plant alpha diversity and soil properties) for replacement of ground-dwelling invertebrates in spring (a), and richness difference of ground-dwelling invertebrates in spring (b) and in autumn (c).

Table 3: Ground-dwelling invertebrates appeared in extremes of soil properties or plant diversity indices in spring and their diet (mainly from Jeannel (1941) and Mora (2002)).

Beta diversity	Variables influencing beta diversity		Taxa	Diet
Replace-ment	**Soil properties**	High pH	*Chrysomelidae (Coleoptera)*	phytophagous
			Forficulidae (Dermaptera)	saprophagous
		Low pH	*Curculionidae (Coleoptera)*	phytophagous
			Chilopoda (Myriapoda)	zoophagous
			Lampyridae (Coleoptera)	zoophagous
			Silphidae (Coleoptera)	zoophagous
			Opiliones (Spider)	zoosaprophage
			Aphodiinae (Coleoptera)	saprophagous
			Julidae (Diplopoda)	saprophagous
			Glomeridae (Diplopoda)	saprophagous
	Plant diversity	High shrub Simpson's diversiy	*Curculionidae (Coleoptera)*	phytophagous
			Gastropoda	phytophagous
			Silphidae (Coleoptera)	zoophagous
			Lampyridae (Coleoptera)	zoophagous
			Staphylinidae (Coleoptera)	zoophagous
			Araneae (Spider)	zoophagous
			Opiliones (Spider)	zoosaprophagous
			Aphodiidae (Coleoptera)	saprophagous
		Low shrub Simpson's diversiy	*Forficulidae (Dermaptera)*	saprophagous
			Elateridae (Coleoptera)	mixed
Richness Difference	**Soil properties**	High Pb	*Chrysomelidae (Coleoptera)*	phytophagous
			Gastropoda	phytophagous
			Lampyridae (Coleoptera)	zoophagous
			Arachnida (Spider)	zoophagous
			Forficulidae (Dermaptera)	saprophagous
			Glomeridae (Diplopoda)	saprophagous
		High Pb & low OM	*Curculionidae (Coleoptera)*	phytophagous
			Lampyridae (Coleoptera)	zoophagous
		Low OM	*Silphidae (Coleoptera)*	zoophagous
			Leiodidae (Coleoptera)	saprophagous
			Aphodiidae (Coleoptera)	saprophagous
	Plant diversity	Low herb abundance	*Curculionidae (Coleoptera)*	phytophagous
			Gastropoda	phytophagous
			Lampyridae (Coleoptera)	zoophagous
			Silphidae (Coleoptera)	zoophagous
			Staphylinidae (Coleoptera)	zoophagous
			Chilopoda (Myriapoda)	zoophagous
			Arachnida (Spider)	zoophagous
			Glomeridae (Diplopoda)	saprophagous
			Aphodiidae (Coleoptera)	saprophagous
			Polydesmidae (Diplopoda)	saprophagous

Table 4: Ground-dwelling invertebrates appeared in extremes of soil properties or plant diversity indices in autumn and their diet (mainly from Jeannel (1941) and Mora (2002)).

Beta diversity	Variables influencing beta diversity		Taxa	Diet
Replace-ment	**Soil properties**	High pH & High OM	*Gastropoda*	phytophagous
			Coccinellidae (Coleoptera)	zoophagous
			Opiliones (Spider)	zoosaprophage
			Polydesmidae (Diplopoda)	saprophagous
			Julidae (Diplopoda)	saprophagous
		Low pH & low OM	*Arachnida (Spider)*	mixed
		High pH	*Formicidae (Hymenoptera)*	omnivorous
			Forficulidae (Dermaptera)	saprophagous
			Isopoda	saprophagous
	Plant diversity	*(No variable)*		
Richness Difference	**Soil properties**	*(No variable)*		
	Plant diversity	Low tree richness	*Gastropoda*	phytophagous
			Formicidae (Hymenoptera)	omnivorous
			Chilopoda (Myriapoda)	zoophagous
			Araneae (Spider)	zoophagous
			Opiliones (Spider)	zoo-saprophagous
			Geotrupidae (Coleoptera)	saprophagous
			Forficulidae (Dermaptera)	saprophagous
			Glomeridae (Diplopoda)	saprophagous
			Polydesmidae (Diplopoda)	saprophagous
			Julidae (Diplopoda)	saprophagous

Alpha and beta diversity of flying invertebrates in relation to soil properties and vegetation

Alpha diversity

Alpha diversity indices in flying invertebrates were significantly explained neither by soil properties nor by alpha diversity of plants in both spring and autumn.

Beta diversity

69.8% and 30.2% of variation in beta diversity of flying invertebrates were respectively explained by $Repl_J$ and $RichDiff_J$ in spring, whereas 51.0% and 49.0% in autumn. No soil property significantly explained beta diversity in both seasons. In spring, $Repl_J$ was significantly explained by shrub S ($R^2_{adj} = 0.062$). $RichDiff_J$ was not significantly explained by any plant diversity index. In autumn, $Repl_J$ was not significantly explained by plant diversity indices, but $RichDiff_J$ was significantly explained by tree D, shrub E and herb N ($R^2_{adj} = 0.523$). The biplots of partial dbRDA on beta diversity matrices of flying invertebrates are given in Supporting Information Figure S7. Flying invertebrates appeared in the two extremes of the selected variables are shown in Table 5 with their diet. In spring, predator or parasitoid flying invertebrates were observed rather in buffer with low shrub S. In autumn, flying invertebrates of several types of diet (e.g. zoophagous, saprophagous, phytophagous, opophagous) were observed in buffer with high tree D, shrub E and herb N.

Table 5: Flying invertebrates appeared in extremes of soil properties or plant diversity indices and their diet in larvae and in adult (mainly from Mora, (2002)).

Beta diversity	Variables influencing beta diversity		Taxa	Diet (larvae)	Diet (adult)
Replacement in spring	**Soil properties**	*No variable*			
	Plant diversity	High shrub richness	*Apionidae (Coleoptera)* *Cynipidae (Hymenoptera)* *Sepsidae (Diptera)*	phytophagous endophytophagous saprophagous	phytophagous ? saprophagous
		Low shrub richness	*Torymidae (Hymenoptera)* *Tineidae (Lepidoptera)* *Bibionidae (Diptera)* *Formicidae (Hymenoptera)* *Dolichopodidae (Diptera)* *Pompilidae(Hymenoptera)* *Eucoilidae (Hymenoptera)*	phytophagous saprophagous saprophagous omnivorous zoophagous zoophagous parasitoid	mixed nectarivorous none omnivorous zoophagous zoophagous ?
Richness Difference in autumn	**Soil properties**	*No variable*			
	Plant diversity	High tree Simpson's diversity, shrub evenness & herb abundance	*Noctuidae (Lepidoptera)* *Delphacidae (Hemiptera)* *Chloropidae (Diptera)* *Agromyzidae (Diptera)* *Apionidae (Coleoptera)* *Mycetophilidae (Diptera)* *Milichiidae (Diptera)* *Psychodidae (Diptera)* *Nitidulidae (Coleoptera)* *Sepsidae (Diptera)* *Forficulidae (Dermaptera)* *Pompilidae (Hymenoptera)* *Carabidae (Coleoptera)*	phyllophagous opophagous endophytophagous endophytophagous phytophagous mycetophagous zoosaprophagous saprophagous saprophagous saprophagous saprophagous zoophagous zoophagous	nectarivorous opophagous nectarivorous phytophagous phytophagous ? zoosaprophagous none saprophagous saprophagous saprophagous zoophagous zoophagous
		Low shrub evenness	Limoniidae (Diptera)	mixed	none

Discussion

This study demonstrated that vegetation was an important factor shaping invertebrate diversity even in a TM contaminated site. Alpha diversity of ground-dwelling invertebrates was explained by plant diversity rather than by soil properties in spring, and only by tree richness in autumn. Beta diversity was weakly explained by both plant diversity and soil properties in both seasons. Although alpha diversity of flying invertebrates was explained by neither plant diversity nor soil properties, their beta diversity was explained by plant diversity. Our results in general supported our hypothesis: more important influence of vegetation than influence of TMs on invertebrate diversity and composition. However, underlying mechanisms could differ according to seasons, type of invertebrates, and plant strata.

Diversity of plants in relation to soil properties

In our study area, plant alpha diversity indices showed no significant correlation with soil TM concentrations or soil properties affecting metal bioavailability. Some plants were slightly replaced along the gradient of pH or TM concentrations in soils. Actually, almost all plants species in our inventory showed tolerance to metals. Some other species such as *Salix* sp. or *Populus* sp. are considered as TM accumulator plants and often used for phytoremediation (Pulford and Watson, 2003). On the other hand, several woody habitats in our study area often showed high degree of soil anthropization (Douay et al., 2009). This indicates that several patches and buffers used in the present study could be maintained and/or modified more or less by humans. The relationship between vegetation and soil properties in our study site could not be totally comparable with other studies carried out under more natural conditions. However, this can suggest that diversity indices of vegetation can be considered as to be independent of soil properties.

Diversity of ground-dwelling invertebrates in relation to soil properties and vegetation

Herbaceous stratum plants influenced alpha diversity of ground-dwelling invertebrates in spring. Herb abundance was negatively correlated to richness and abundance of ground-dwelling invertebrates. However, in both grassland and woodlands, positive correlations have been widely reported between richness and/or abundance of herb layer plants and both herbivorous and predator invertebrates richness and/or abundance (e.g. Borer et al., 2012; Haddad et al., 2009). Difference between the literature and our observation could be explained by trappability bias due to herb cover. Pitfall traps are effective for capturing high mobile invertebrates (Uetz and Unzicker, 1976). Trappability of pitfall traps depends on movement behavior. However, movement behavior of invertebrates and/or their surface area to move could be affected, when field layer vegetation around pitfall traps shows complex structure (Greenslade, 1964; Melbourne, 1999). On the other hand, the negative correlation between Simpson's diversity indices of herbs and of invertebrates was not explained by such trappability

bias. Simpson's index of ground-dwelling invertebrates was also positively correlated with soil TM concentrations. No or even positive correlations between diversity indices and soil TM contamination levels have been often reported (i.e. Migliorini et al., 2004; Nahmani and Lavelle, 2002). One of common explanations about such correlations is change in species composition due to different sensibility to metal contaminants which acts as a filter shaping communities. For example, some Diplopoda like Glomeridae and Polydesmidae are known for their abundance in polluted site (Read et al., 1998). Positive correlations between soil Zn content and abundance of carabid beetle Staphylinidae was observed in northern France (Nahmani and Lavelle, 2002). Ants are considered to be relatively resistant to metal pollution (Eeva et al., 2004). Some of those taxa were observed in buffers with high TM concentration or low pH in our study site. Influence of vegetation and soil properties on ground-dwelling invertebrates could be based upon different responses of each species to those factors.

Likewise, change in composition of ground-dwelling invertebrates along the gradients of both vegetation and soil properties could also be affected by life histories of given invertebrates. Alpha diversity indices of shrub influenced replacement of ground-dwelling invertebrates, and diet were more diversified in buffer with high shrub diversity. On the other hand, zoophagous ground-dwelling invertebrates were observed rather in low pH buffers. Although Gongalsky et al. (2007) reported that zoophagous carabid beetle species could be more sensible to soil TM contamination levels such as Cd, Pb, Zn, and copper. Soil pH is also one of factors determining composition of ground-dwelling invertebrates even in unpolluted sites (Schuldt et al., 2011). Other soil conditions also determine composition of ground-dwelling invertebrates, such as temperature, soil moisture, and quality of humus (Koivula et al., 1999; Niemelä et al., 1992; Perner and Malt, 2003; Zimmer et al., 2000; Zimmer and Topp, 2000).

In autumn, both richness and Simpson's diversity of ground-dwelling invertebrates decreased along the gradient of tree richness. Schuldt et al. (2011) demonstrated a negative correlation between tree species richness and both spider richness and abundance in forests, due to possible change in some parameters such as litter depth and cover. Indeed, abundance of predatory arthropods such as spiders, ants or carabids depends strongly on presence of specific trees due to mechanisms such as favorable microclimates, prey abundance or foraging efficiency created by those trees (Schuldt et al., 2008; Vehviläinen et al., 2008). Even though our study did not particularly focus on other life histories of invertebrates, those soil properties might more importantly influence alpha and beta diversity than the soil properties used in our study.

Diversity of flying invertebrates in relation to soil properties and vegetation

It is not surprising that diversity of flying invertebrates was not explained by any soil properties. This could be because of their no or only little contact with soils. The meta-analysis of Zvereva and Kozlov (2010) showed a decrease in population density of epigeic and endogeic

arthropods by air pollution (not only by TMs) but an increase in density of other arthropod groups. The same meta-analysis also demonstrated that responses of population density to pollution were negative for spiders and Colleoptera but positive for Lepidoptera and Hemiptera. Despite different responses according to pollution types in that meta-analysis, responses to pollutions could be generally different between ground-dwelling and flying invertebrates.

Replacement of flying invertebrates in spring along the gradient of diversity indices of shrub stratum could be linked to diversified diet. In our study area, spring was a main flowering period of several woody plant of tree and shrub strata (Landolt et al., 2010). Availability of young leaves and flowers of shrub strata might mostly draw attention of phytophagous and/or nectarivorous invertebrates. In autumn, diversity indices of several strata were related to beta diversity. Tree species richness in woodland system conserves richness, diversity and/or abundance of flying invertebrate families like Hymenoptera (Fraser et al., 2007; Sperber et al., 2004) or Diptera (Scherber et al., 2014) due to an increased heterogeneity of structures and availability of resources. Hirao et al. (2009) also showed that lepidopteran community was vertically stratified, which can suggest that more vertically diversified habitats might enclose more diverse flying invertebrates. Seasonal difference of diversity of flying invertebrates could be explained, at least partly, by flowering seasons of plants. Furthermore, trapping efficiency by pan traps could also vary among seasons. Rodriguez-Saona et al. (2012) actually demonstrated seasonal change in color preference coincided with blooming season in blunt-nosed and sharp-nosed leafhoppers (Hemiptera: Cicadellidae). Composition of flying invertebrates where highly explained by diversity of several plant strata. Although some possible explanations were discusses, details about relationship between plant diversity – invertebrates diversity – soils still remain as an issue for further studies.

Conclusions

This study shed light on effects of plant diversity on diversity and composition of aboveground invertebrates in a TMs contaminated site. Our results showed that plant diversity itself was an important factor controlling both the diversity and composition of invertebrates. Although underlying mechanisms of positive and negative correlations between invertebrates diversity and both plant diversity and soil properties still remain as an issue for further studies, those results suggest that soil TM concentrations and certain soil properties controlling bioavailability of TMs cannot sufficiently explain modification of invertebrate communities affected by TMs. TM transfer from soils to higher trophic levels like birds and mammals cannot be sufficiently explained by only soil TM, but also explained, at least partly, by their habitat conditions like diversity and composition of their resources. Taking into account those habitat conditions improves explications of TM transfer in food webs.

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Supporting information

S1: Values of Spearman's correlation *rho* within alpha diversity indices of the three strata and within alpha diversity indices among each plants strata.
S2: Values of Spearman's correlation *rho* within alpha diversity indices of ground-dwelling invertebrates and of flying invertebrates in spring and in autumn.
S3: Biplots of distance-based redundancy analysis on replacement of plants of tree, shrub, and herbaceous strata.
S4: Ecological indication values and life history treats of plants which appeared in two extremes of soil properties influencing their composition.
S5: Biplots of (partial) distance-based redundancy analysis on beta diversity matrices of ground-dwelling invertebrates in spring.
S6: Biplots of (partial) distance-based redundancy analysis on beta diversity matrices of ground-dwelling invertebrates in autumn.
S7: Biplots of distance-based redundancy analysis on beta diversity matrices of flying invertebrates.

Supporting Information Table S1: Values of Spearman's correlation rho within alpha diversity indices of: (a) tree, (b) shrub, and (c) herbaceous stratum and (d) within alpha diversity indices among each plants strata. Significant correlations by the Spearman's correlation test are expressed by asterisk (: p-value < 0.05, **: p-value < 0.01 and ***: p-value < 0.001). Only significant correlations are represented in Table SI (d).*

(a)	**Simpson's diversity**	**Simpson's evenness**	**Abundance**
Richness	0.88***	0.02	-0.11
Simpson's diversity	-	0.32	-0.06
Simpson's evenness		-	0.05
Abundance			-

(b)	**Simpson's diversity**	**Simpson's evenness**	**Abundance**
Richness	0.80***	-0.45*	0.55**
Simpson's diversity	-	0.04	0.34
Simpson's evenness		-	-0.11
Abundance			-

(c)	**Simpson's diversity**	**Simpson's evenness**	**Abundance**
Richness	0.43*	-0.68***	-0.36*
Simpson's diversity	-	0.29	-0.36*
Simpson's evenness		-	0.06
Abundance			-

(d)	**Tree stratum**		
	Richness	**Evenness**	**Abundance**
Shrub stratum abundance	0.49*	-	-0.51*
Herbaceous stratum Simpson's diversity	-	-0.74***	

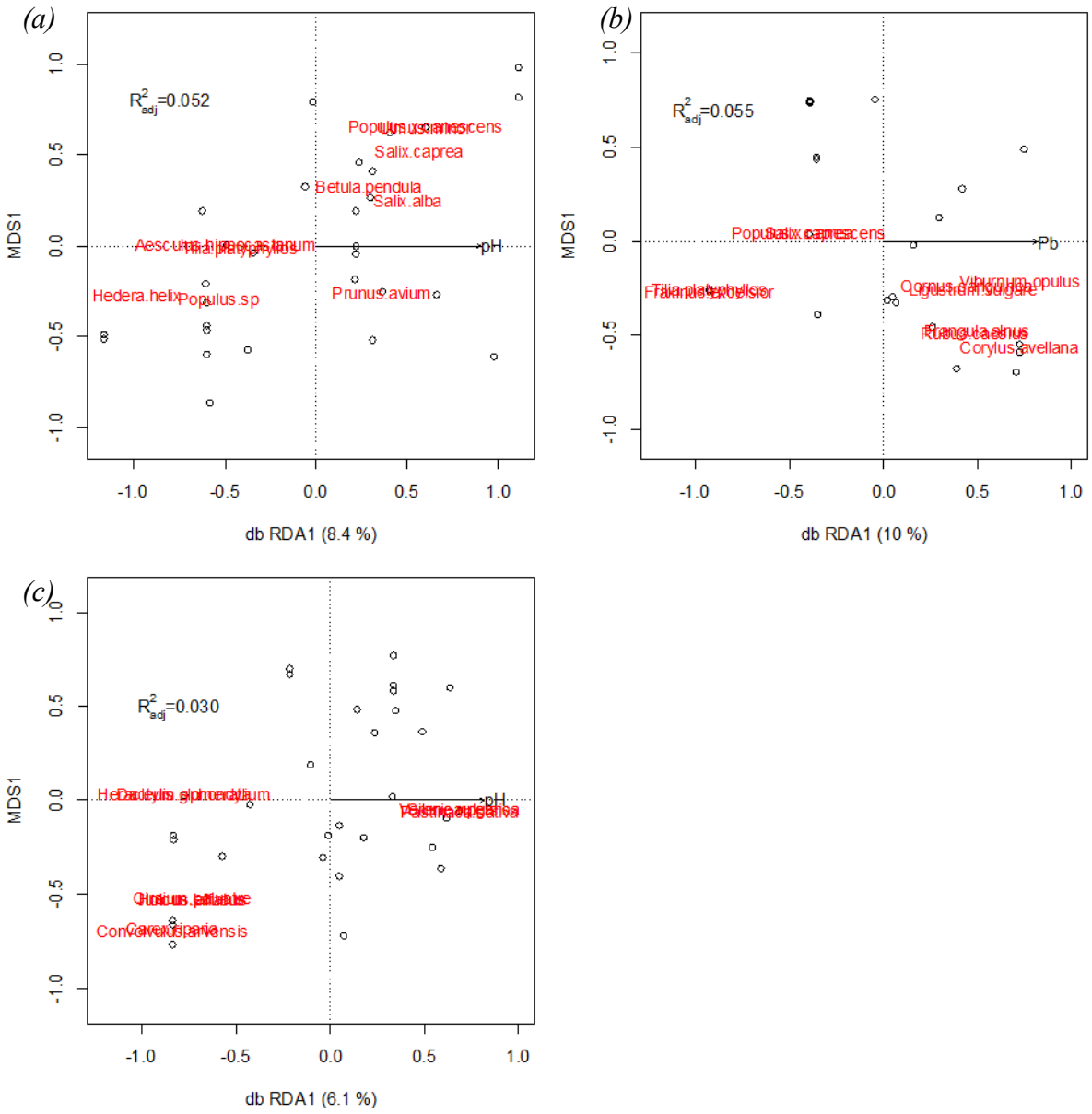
Supporting Information Table S2: Values of Spearman's correlation rho within alpha diversity indices of ground-dwelling invertebrates in spring (a) and in autumn (b) and of flying invertebrates in spring (c) and in autumn (d). Significant correlations by the Spearman's correlation test are expressed by asterisk (: p-value < 0.05, **: p-value < 0.01 and ***: p-value < 0.001).*

(a)	**Simpson's diversity**	**Simpson's evenness**	**Abundance**
Richness	0.17	-0.75***	0.85***
Simpson's diversity	-	0.32	-0.15
Simpson's evenness		-	-0.92***
Abundance			-

(b)	**Simpson's diversity**	**Simpson's evenness**	**Abundance**
Richness	0.74***	-0.81***	0.66***
Simpson's diversity	-	-0.30	0.25
Simpson's evenness		-	-0.80***
Abundance			-

(c)	**Simpson's diversity**	**Simpson's evenness**	**Abundance**
Richness	-0.05	-0.40	0.71**
Simpson's diversity	-	0.87***	-0.58
Simpson's evenness		-	-0.79**
Abundance			-

(d)	**Simpson's diversity**	**Simpson's evenness**	**Abundance**
Richness	0.45	-0.03	0.39
Simpson's diversity	-	0.83**	-0.43
Simpson's evenness		-	-0.73*
Abundance			-

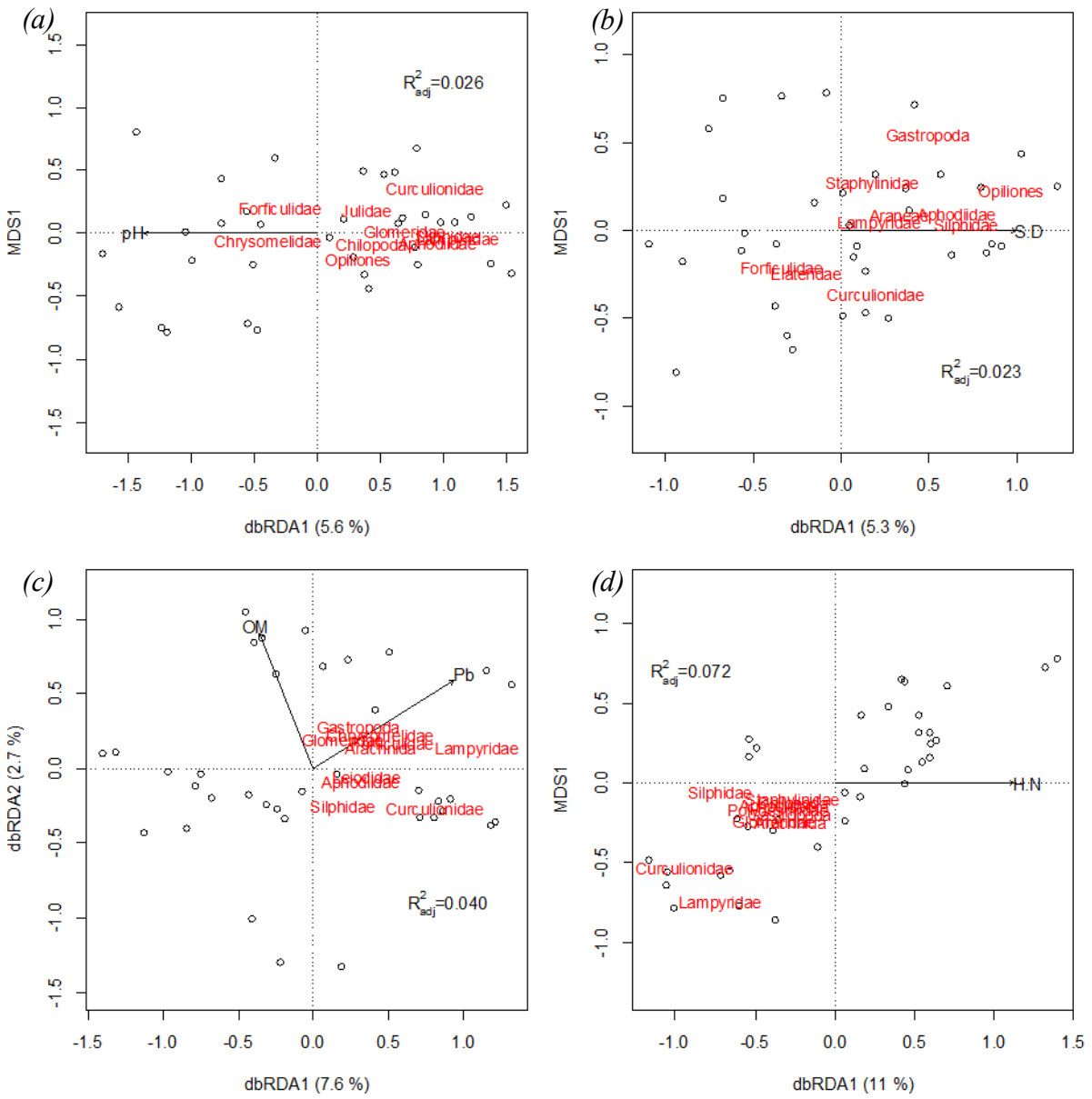


Supporting Information SI3: Biplots of distance-based redundancy analysis on replacement of plants of tree (a), shrub (b) and herbaceous (c) strata. The x axis represents the first (and the only) canonical axis and the y axis represent the first unconstrained axis. Patches were represented by points and selected soil property variable was represented by arrow. The 10 species furthest from the center of the plot were a posteriori projected as weighted averages (in rouge). Adjusted R^2 (R^2_{adj}) was mentioned.

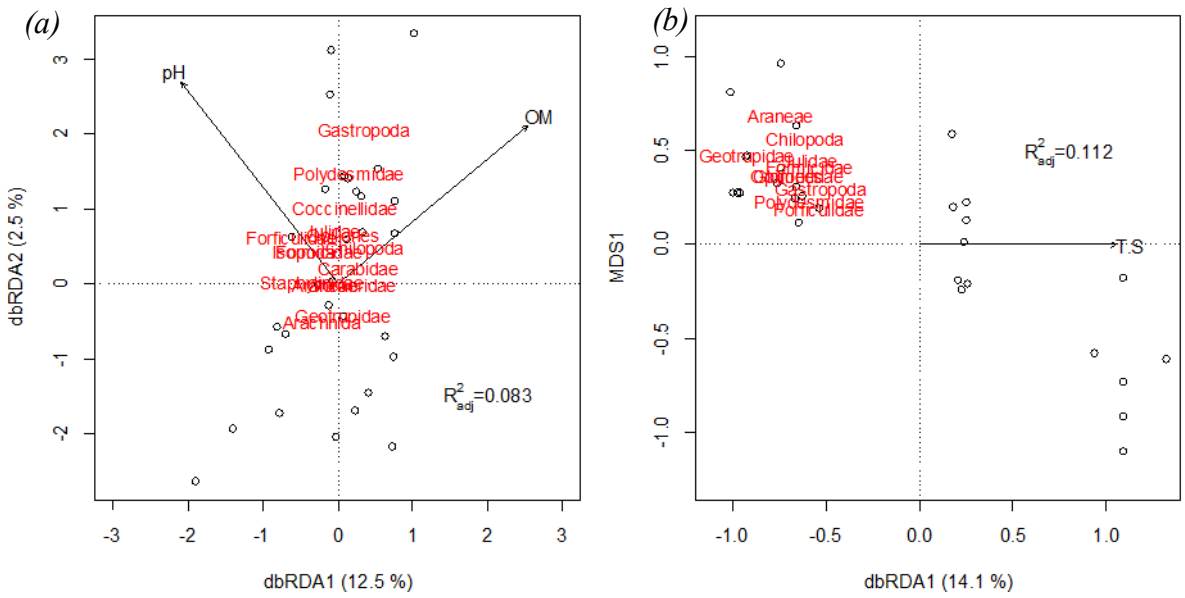
Supporting Information Figure S4: Ecological indication values and life history treats of plants which appeared in two extremes of soil properties influencing their composition (from Landolt et al. (2010)).

Taxa			Soil properties		Climate indicators		Soil indicators						Growth and life strategies		Life history treats		
Stratum	**Species**	**Family**	**pH**	**Pb**	**T**	**L**	**M**	**R**	**N**	**HMt**	**H**	**A**	**Ld**	**Rd**	**D**	**Fp**	**Pa**
Tree	*Hedera helix*	*Araliaceae*	Low		4	2	3	3	3	m	3	3	i	1	En Dy	8-10	ve my hm
	Tilia platyphyllos	*Tiliaceae*	Low		4	2	3	4	3	m	3	3	s	3.5	Me Dy	6	an me my
	Aesculus hippocastanum	*Hippocastanaceae*	Low		4	3	3.5	4	3	m	3	3	s	4	Dy	4-5	me
	Salix caprea	*Salicaceae*	High		3	3	3	3	3	m	3	1	s	4.5	Me	3-5	my me ps (ca) (ve)
	Betula pendula	*Betulaceae*	High		3.5	4	x	x	2	m	x	x	s	5	Me Dy	4-5	an
	Salix alba	*Salicaceae*	High		4	3	4.5	4	4	m	1	1	s	5	Me	4-5	
	Populus x canescens	*Salicaceae*	High		4	3	3	4	3	m	3	1	s		Me	3-4	an
	Ulmus minor	*Ulmaceae*	High		4.5	3	3.5	4	3		3	1	s	5	Me	3	an
Shrub	*Tilia platyphyllos*	*Tiliaceae*		Low	4	2	3	4	3	m	3	3	s	3.5	Me Dy	6	an me my
	Fraxinus excelsior	*Oleaceae*		Low	3.5	3	3.5	4	3	m	3	1	s	4	Me Dy	4-5	
	Salix caprea	*Salicaceae*		Low	3	3	3	3	3	m	3	1	s	4.5	Me	3-5	my me ps (ca) (ve)
	Populus x canescens	*Salicaceae*		Low	4	3	3	4	3	m	3	1	s		Me	3-4	an
	Cornus sanguinea	*Cornaceae*		High	3.5	3	3	4	3	m	3	3	s	3.5	En Dy	5-6	
	Frangula alnus	*Rhamnaceae*		High	3.5	3	3.5	3	2	m	5	1	s	1.5	En Dy	5-6	au me ve my ca
	Ligustrum vulgare	*Oleaceae*		High	4	3	2.5	4	3	m	3	3	t	3	En Dy	5-7	au my me ps sp (ca)
	Rubus caesius	*Rosaceae*		High	3.5	3	3.5	4	4	m	3	1	s	3.5	En Dy	6-9	au ca my me ps hm
	Viburnum opulus	*Caprifoliaceae*		High	3.5	3	3.5	3	3	m	5	1	s	3	En Dy	5-6	au my (me)
	Corylus avellana	*Betulaceae*		High	3	3	3	3	3	m	3	3	s	5	Dy	2-4	an
Herb	*Cirsium palustre*	*Asteraceae*	Low		3.5	3	4	3	3	m	5	1	w	3.5	Me Dy	7-10	ca my sp me ph ps hm
	Holcus lanatus	*Poaceae*	Low		3.5	4	3	3	3	m	3	3	t	3.5	Me En	5-8	an au
	Juncus effusus	*Juncaceae*	Low		3.5	3	4	2	4	m	3	1	t	2.5	Bo Ep	6-8	an
	Carex riparia	*Cyperaceae*	Low		4	4	5	4	4		5	1	w		Me Hy	5-6	an
	Convolvulus arvensis	*Convolvulaceae*	Low		4	4	2.5	4	4	m	3	1	s	4.5	Dy	6-9	au my me ps
	Dactylis glomerata	*Poaceae*	Low		4	4	3	3	4	m	3	3	t	4	En Ep	5-9	
	Heracleum sphondylium	*Apiaceae*	Low		3	3	3	3	4		3	3	s	4	Me En	6-9	ca my me hm ve (ps)
	Pastinaca sativa	*Apiaceae*	High		4	4	2.5	4	4	m	3	1	s		Me At	5-7	
	Silene vulgaris	*Caryophyllaceae*	High		3	4	2.5	3	2	m	3	3	s	3	Bo En	6-9	au? ph me (ps)
	Veronica persica	*Scrophulariaceae*	High		3.5	4	3	4	4	m	3	3	s w	1	My	2-10	

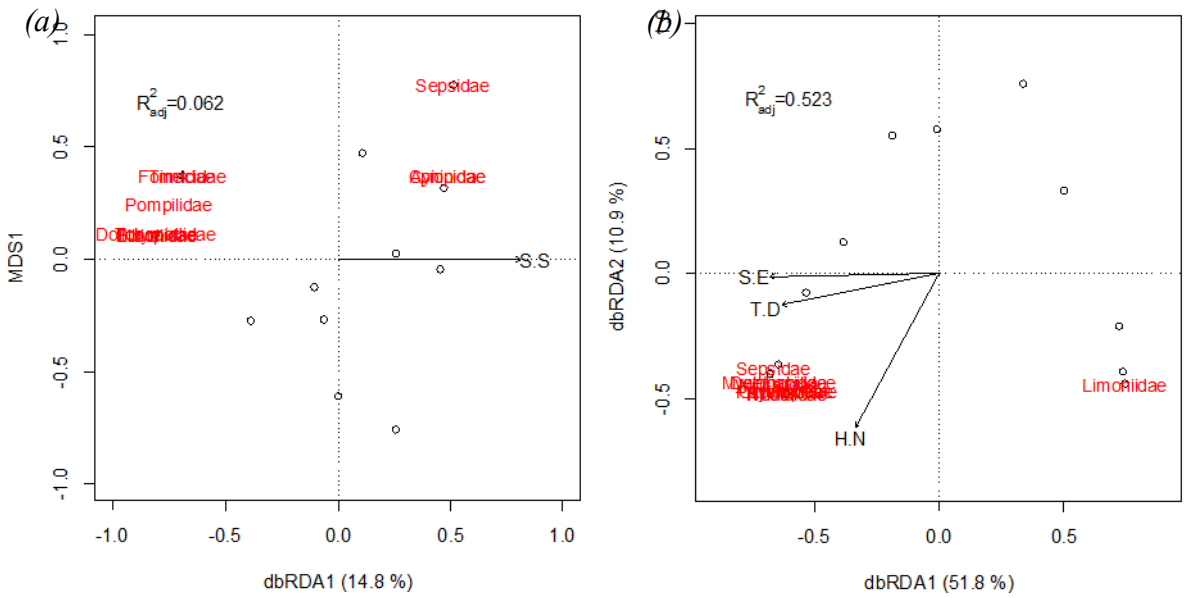
***Taxa**: species and family names of plants and its stratum (tree: tree stratum i.e. woody species > 8 m high; shrub: shrub stratum i.e. woody species < 8 m high; herb: herbaceous stratum). **Soil properties**: soil properties significantly selected by forward selection in partial distance-based redundancy analysis (**pH**: soil pH value; **Pb**: lead concentration in soils). **Temperature (T)**: the temperature value characterizes the average air temperature during the growth period of a plant (1: alpine and nival; 5: warm colline). **Light (L)**: the light value indicates the average light quality received in the respective habitats (1: deep shade; 5: full light). **Moisture (M)**: the moisture value signifies an average soil moisture during the growth period in places where the species most frequently occurs (1: very dry; 5: flooded, submerged). **Reaction (R)**: the reaction value characterizes the content of free H-ions in the soils (1: extremely acid; 5: alkaline, high pH). **Nutrients (N)**: the nutrient value characterized the nutrient content in the soil, referring mostly to nitrogen and often also to phosphorous (1: very infertile; 5: very fertile and over-rich). **Heavy metal tolerance (HMt)**: the attribute indicates a certain tolerance to heavy metals in the soil, referring predominantly to serpentine tolerance i.e. the tolerance to nickel and chromium (m: tolerance to metals in the soil). **Humus (H)**: the humus value characterizes the humus content, i.e. the soil totally of dead organic material in or on the soil (1: little or no humus; 2: moderate humus content, mostly as mull; 3: high humus content, mostly as raw humus, moder or peat). **Aeration (A)**: the aeration value specifies the oxygen supply of the soil (1: bad aeration, compacted or wet soil; 3: good aeration, loose, often rocky or sandy soil). **Leaf duration (Ld)**: the attributes for lead duration notify both the length and the seasons with respect to the ability of the leaves to assimilate (i: evergreen; s: deciduous; t: partly wintergreen; w: wintergreen). **Root depth (Rd)**: The root depth specifies the depth of the root penetration in the soil, (1: < 25 cm; 5: > 200 cm). **Dispersal of diaspores (D)**: diaspores are dispersal units containing seeds, i.e. singular seeds, singular fruits or infructescences. In general, a species can spread in several ways. In such cases, the two most important dispersal types are listed. If a species has no indications on diaspore dispersal, it disperses only by dropping off or by laying diaspores on the ground, by water transport after heavy rainfall or by sticking to animals or mobile parts during wet conditions (At: anthropochory, dispersal by man; Bo: boleochory, dispersal by wind gusts, wind dispersers; Dy: dysochory, dispersal by animals, seeds being cached and forgotten; En: endochory, dispersal by animals, seeds passing through the intestines; Ep: epichory, dispersal by animals, seeds clinging to animals (or means of transport); Hy:hydrochory, dispersal by water transport; Me: meteorochory, dispersal by air currents; My: myrmecochory, dispersal by ants). **Flowering period (Fp)**: the flowering period defines the months during which a species mainly flowers. Note that many flowering plants may flower for a second time in autumn (not listed). **Pollination agents (Pa)**: the attributes for pollination agents indicate the agent by which flowers are pollinated. They are listed in order of their importance for the particular species (an: anemogamous, pollinated by wind; au: autogamous, self-pollinating; ca: cantharophilous, pollinated by beeltes; hm: hymenopterophilous, pollinated by hymenopterans; me: melittophilous, pollinated by bees and bumble-bees; my: myophious, pollinated by flies; ph: phalaenophilous, pollinated by moths; ps: psychophilous, pollinated by butterflies; sp: sphingophilous, pollinated by hawk moths; ve: vespidophilous, pollinated by wasps; ( ): inferior importance; ?:doubtful information). x: Instead of a value, range of variation is very large and cannnot be disinctly characterized by an average value. (N.B. Empty cells signify that the attribute has not yet been carefully studied for the particular species.)*



Supporting Information SI5: The biplot of partial distance-based redundancy analysis on replacement of ground-dwelling invertebrates in spring explained by soils properties controlling for plant diversity indices (a), and explained by plant diversity indices controlling for soil properties (b), as well as on richness difference in spring explained by soils properties controlling for plant diversity indices (c), and explained by plant diversity indices controlling for soil properties (d). The x axis represents the first canonical axis, and the y axis represent the second canonical axis (c) or the first unconstrained axis (a, b and d). Buffers were represented by points and the selected variable was represented by arrow. The 10 species furthest from the center of the plot were a posteriori projected as weighted averages (in rouge). Adjusted R^2 (R^2_{adj}) was mentioned.



Supporting Information SI6: The biplot of partial distance-based redundancy analysis on replacement of ground-dwelling invertebrates in autumn explained by soils properties controlling for plant diversity indices (a), and the biplot of distance-based redundancy analysis on richness difference of ground-dwelling invertebrates in autumn explained by plant diversity indices (b). The x axis represents the first canonical axis, and the y axis represent the second canonical axis (a) or the first unconstrained axis (b). Buffers were represented by points and the selected variable was represented by arrow. The 10 species furthest from the center of the plot were a posteriori projected as weighted averages (in rouge). Adjusted R^2 (R^2_{adj}) was mentioned.



Supporting Information SI7: The biplots of distance-based redundancy analysis on replacement of flying invertebrates in spring (a) and on richness difference in autumn (b) explained by plant diversity indices. The x axis represents the first canonical axis, and the y axis represent the second canonical axis (b) or the first unconstrained axis (a). Buffers were represented by points and the selected variable was represented by arrow. The 10 species furthest from the center of the plot were a posteriori projected as weighted averages (in rouge). Adjusted R^2 (R^2_{adj}) was mentioned.

III.2 Chapter 2

Abstract and keywords in French.

Résumé:

Les mammifères sont considérés exposés aux éléments traces métalliques (ETM) principalement par voie trophique. Plusieurs études ont montré des relations postitives entre les concentrations en ETM dans les items du régime alimentaire et dans les tissus de mammifères. Cependant, les effets potentiels des ETM sur le comportement alimentaire des animaux ont été peu documentés en conditions naturelles, même si quelques études ont montré que la sélection alimentaire peut être influencée par la contamination des ressources par des ETM. Dans cette étude, l'hypothèse est que le régime almimentaire d'un rongeur généraliste, le mulot sylvestre (*Apodemus sylvaticus*), est modifié par la contamination des sols par des ETM sur le terrain. Des mulots sylvestres ont été échantillonnés au printemps et à l'automne le long d'un gradient de contamination des sols autour d'une ancienne fonderie de plomb et de zinc située dans le nord de la France. La disponibilité des ressources en plantes et en ivertébrés sur le terrain a été inventoriée et le régime alimentaire des rongeurs a été analysé par « *metabarcoding* ». Cette étude a montré que (i) la relation entre la richesse des ressources alimentaires et la richesse de ces ressources sur le terrain est modifiée par la contamination des sols par des ETM. Les mulots ont spécialisé leur régime le long du gradient de contamination pour des ressources végétales et invertébrées au printemps. Nous avons également démontré que (ii) la préférence pour les *Salicaceae*, une famille de plantes considérée comme accumulatrice d'ETM, a diminué le long du gradient de contamination. Ces résultats suggèrent que la pollution par les ETM constitue un facteur modulant les interactions trophiques dans les écosystèmes terrestres, ce qui a une incidence sur l'exposition de la faune aux contaminants par voie trophique.

Mots-clés : *Apodemus sylvaticus*, biodiversité, diversité alimentaire, sélection alimentaire, éléments traces metalliques.

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

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Abstract:

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

Keywords:

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

Introduction

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

Trace metals (TM) are environmental pollutants of concern for both human health and wildlife. They occur often as a legacy of past industrial activities. Even if the emissions of many of them have been reduced in the past decades thanks to environmental regulation and technical improvements at least in so-called developed countries, the pollution still persists over vast surfaces due to non-degradability of metals and to their current emissions in many countries (Kabata-Pendias, 2011; EMEP, 2013). TM pollution may have effects on plants and invertebrates at several levels of organisation, from sub-individual physiological effects reducing survival or reproduction to the modification of community composition and structure (Walker et al., 2012). For instance, a recent meta-analysis about the responses of terrestrial biota to TM pollution -dealing with 206 pollution point sources worldwide- showed a decrease of diversity for most of the taxa (producers, secondary consumers and decomposers) (Kozlov & Zvereva, 2011). In addition, Dazy et al. (2009) demonstrated that plant species richness correlated negatively to TM concentrations in soils in eastern France. Metal pollution can also reduce species richness in earthworm communities (Spurgeon & Hopkin, 1999; Nahmani et al., 2003) and impact the structure and composition of soil macro-fauna communities (Nahmani & Lavelle, 2002). Trophic transfer , i.e. the transfer of matter via food consumption, is generally

considered to be the main route for TM from the environment to animals of higher trophic levels (Shore & Rattner, 2001; Smith et al., 2007). For small mammals, several studies have demonstrated relationships between soil pollution by TM, contamination of food, and subsequently, high concentrations of TM in wild animals' tissues (Hunter & Johnson, 1982; Hunter et al., 1987a; Torres & Johnson, 2001; Rogival et al., 2007; van den Brink et al., 2010). Avoidance of TM contaminated food can be a mechanism that mitigates organisms' exposure to contaminants (Gillet & Ponge, 2003). This has been reported for the wood mouse (*Apodemus sylvaticus*) under laboratory settings but not confirmed in natural conditions (Beernaert et al., 2008). To the best of our knowledge, no study has reported variations of richness in the diet and selection as a response to both the variation of resource availability and the contamination of the environment *in natura* in terrestrial ecosystems.

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

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

Materials and methods

Study sites

This study was carried out in the surroundings of the former lead (Pb) and zinc (Zn) smelter named "Metaleurop Nord" located in northern France (Noyelles Godault, Hauts-de-France, France). The surrounding soils were highly contaminated by TM, mainly cadmium (Cd), Pb, and Zn, over a large area of about 120 km^2 because of metal-contaminated dust released for decades from this smelter and another one named "Umicore", located about 4 km east of the first (Sterckeman et al., 2002; Douay et al., 2008; Douay et al., 2009; Waterlot et al., 2016). A study area of 40 km^2 was defined and divided into 160 sites of 25 ha (500m x 500m) (Fritsch et al., 2010), from which seven are used in the present study (see below). Total TM concentrations in the soil of woody patches ranged from 0.1 to 236.5 mg kg^{-1} of dry soil for 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 Zn in an area of 40 km^2 in the surroundings of the former smelter (Fritsch et al., 2010). These values largely exceed background TM concentrations in soils of the region Hauts-de-France (Sterckeman et al., 2007). Those TM concentrations in woody patches were used in our modelling (cf. below). The seven sites are located along a soil pollution gradient and composed of three types of dominant habitats (woodland, urban features, arable lands) to provide a wide range of resource richness in the field (for details see Supporting Information SI1).

Rodent trapping

At each site, we trapped wood mice in spring (April) and in autumn (September and October) 2012 according to Fritsch et al. (2011) and in accordance with current French legislation about ethics and use of animals in research. Briefly, small break-back traps were used with peanut as rodent's bait. Ten lines, composed of 10 traps 3 m spaced each, were used per site and per season. All trap lines were set in woody habitats of the sites and their location was geo-referenced. The trap lines were checked in the morning for three consecutive days and re-set and/or re-baited, if necessary. After being weighed in the field, animals were immediately frozen at -20ºC for further analyses.

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

Stomach content samples

Consumed items were identified by biomolecular analysis of stomach contents (SC). Wood mice were thawed at room temperature. Their SC was extracted from the body with a spatula, which was thoroughly cleaned with disposable tissue, then washed with ultra-pure water (18.2 MΩ/cm^2) and wiped off with other tissue between each extraction. Remaining bait was removed from SC. Each SC was then homogenized and split into two aliquots: one (about 10 mg) was stored in 95% ethanol for metabarcoding analysis (see below), the other was reserved for another study. For 13 mice among the 246 rodents, faeces taken from intestines were used as an alternative material for food identification because their stomach was empty. The 13 mice were equitably distributed according to the explanatory factors tested in this study.

Metabarcoding analysis

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 (Table 1): the P6 loop of the chloroplast *trnL* (UAA) intron *g*/*h* (Taberlet et al., 2007) was used for identifying general plant species; primer targeting a short fragment of mitochondrial 16S gene (16S mtDNA) was used for arthropods and molluscs DNA (*16SMAV-F*/*16SMAV-R*) (De Barba et al., 2013); and primer targeting short region of 16S mtDNA for earthworms (*ewD*/*ewE*) (Bienert et al., 2012). 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*; De Barba et al., 2013) was 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 /cm^2) 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 analysed in parallel in order to monitor potential contamination. After amplification, the samples were titrated using capillary electrophoresis (QIAxcel; Qiagen GmbH) and purified using a MinElute PCR purification kit (Qiagen GmbH). Before sequencing, purified DNA was titrated again using capillary electrophoresis. 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).

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 *(forward)*	GGGCAATCCTGAGCCAA	Taberlet *et al.*, 2007
			h *(reverse)*	CCATTGAGTCTCTGCACCTATC	
Arthropods & molluscs	Mitochon-drial	16S mtDNA	16SMAV-F	CCAACATCGAGGTCRYAA	De Barba *et al.*, 2013
			16SMAV-R	ARTTACYNTAGGGATAACAG	
Earthworms	Mitochon-drial	16S mtDNA	ewD *(forward)*	ATTCGGTTGGGGCGACC	Bienert *et al.*, 2012
			ewE *(reverse)*	CTGTTATCCCTAAGGTAGCTT	

Reads were handled by the software Mothur pipeline (Schloss et al., 2009). Briefly, forward and reverse reads were assembled in contig sequences. Then, 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) and no ambiguous nucleotides. After de-replication (occurrence count of each different sequence), only unique sequences with a minimum count of 10 (sum of all samples) were used for further analysis.

Field inventory of plant and invertebrate resources

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

Invertebrate fauna was sampled at the same time as the rodent trapping (i.e. in the two seasons). Ground-dwelling invertebrates were captured near rodent traps using three pitfall traps per line. Flying invertebrates were captured with yellow pan traps containing a mix of soap and water. Four yellow pan traps were set in each site. Yellow colour attracts especially flower visiting insects. The traps were checked every morning for three consecutive days. All

traps were geo-referenced. Captured invertebrates were conserved in ethanol or in freezer at -20°C and identified to the highest possible taxonomic resolution by morphological characteristics (the main references are Coulon, 2003; Forel & Leplat, 2001; Jeannel, 1941; Trautner & Geigenmueller, 1987). Captured ground-dwelling and flying invertebrates consisted of 88 and 95 taxa, respectively (see SI1 for the taxonomic richness in the field per site and SI2 for the list of the taxa and their abundance per site). Because of the different taxonomic resolution, we used mainly family level (and higher levels) for invertebrate richness in the field (cf below): 24 and 95 families (or higher) in ground-dwelling and flying invertebrates, respectively.

We then estimated available resources for wood mice near their trapping location. Although notion of "availability" is distinct from "abundance" in selection studies (Manly et al., 2002), we considered them equivalent for wood mice given its generalist diet behaviour. Plant resource availability was estimated by the cover-abundance of each plant species found within a buffer of 10 m around trap lines (referred to as "buffer" thereafter). This buffer is equivalent to an area of about 1000 m^2, considered as the average size of wood mouse vital domain (Quéré & Le Louarn, 2011). We considered the data set collected between June and September to be representative of food availability in spring and autumn. Since the traps used to capture invertebrates (pitfall trap and yellow pan trap) were inappropriate for catching earthworms and molluscs, both earthworm and mollusc richness in the field were unknown. Resource availability of ground-dwelling invertebrates in buffers both in spring and in autumn was estimated by the abundance of each taxa found per trap line (sum of the three traps per line), whereas availability of flying invertebrates was based on abundance in the yellow pan trap nearest to the trap line.

Diet diversity analyses

Sequence data for diet diversity analyses

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

Diet richness and modelling

Richness in the diet of each mouse was calculated as the total number of MOTUs per mouse by three types of food: plant, arthropod and mollusc (referred to as "arthropod" thereafter), or earthworm. We assumed that plants potentially consumed by the invertebrates would not artificially increase the number of plant MOTUs consumed by the rodent (i.e. there may be no secondary predation, for detail see Harwood et al., 2001) because the number of plant MOTUs was not significantly different between SCs containing or not containing invertebrate food (arthropod and/or earthworm) (data not shown). The diet richness per mouse was compared among seasons, wood mouse sexes and age-classes, and landscape features using the nonparametric Wilcoxon-Mann-Whitney (for comparison of two categories) or Kruskal-Wallis tests (for more than two categories), due to skewed distribution of MOTUs data. Frequency of occurrence was also compared by chi-square goodness-of-fit test.

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

All candidate models were established as generalized linear models (GLM) with logarithm link function for Poisson distribution. Models were built separately per season, given potential seasonal diet variation of the animal (Butet, 1986b; Abt & Bock, 1998), as well as per TM, considering their different effects on organisms and transfer pathways (Eisler, 2000). We used the diet richness in buffer as response variable and number of analysed mice per trap line as one of the explanatory variables. Soil TM concentrations of woody patch characterised by Fritsch et al. (2010) were applied to buffer set in or near a given woody patch. Mice of age class

I were discarded from modelling because diet of juveniles could differ from adults (Watts, 1968; Montgomery & Montgomery, 1990). The modelling was carried out on 38 buffers in spring and 37 buffers in autumn. Number of mice used in the analysis was 181 and their number per line ranged from 1 to 6.

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

Food selection analyses

Sequence data for selection analysis and association with field survey data

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

Plant food selection and modelling

Frequency of occurrence of each plant food item (i.e. percentage of number of occurrences of a given item compared to the total number of occurrence of all items, referring to Klare et al., 2011) was calculated at family level using Grp. Food selection was assessed, for each plant food item, per season, comparing the frequency of occurrence and relative proportion of resources in the field (i.e. cover-abundance of a given plant food of all strata compared to the total cover-abundance of all plants of all strata). Plant resource taxa in the field were gathered into corresponding Grp and then into the same plant family. Resource availability was estimated based on the abundance of the plant families in each buffer, summed up per family for all mice in each season. Significance of food selection was evaluated per plant family using Bailey's confidence intervals at p-value < 0.05 (Bailey, 1980; Cherry, 1996): when relative availability of a given resource was located below or above the confidence interval of its frequency of occurrence, this resource is considered as preferred or avoided, respectively.

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

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

Results

Resources richness in the field, trace metals and diet richness

Plant items occurred in the diet of 96.3% of wood mice, arthropod items in 65.9% and earthworm items in 29.3%. The occurrence of earthworm items was significantly lower in forest landscape and significantly higher in urban landscape ($\chi^2 = 7.619$, p-value = 0.022; for details see SI5). The variations of the total richness in the diet and of the relative proportion of the

three food item categories (plants, arthropods and earthworms) along the soil contamination gradient are shown in SI6 and SI7, respectively.

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

No single "best" model was evidenced and thus model averaging was applied for all sets of models. Complete results of modelling are shown in SI9. The final model for plant richness in the diet in buffer included mouse number, soil TM concentrations, richness in the field and the interaction between the last two for the three TM in the two seasons. Figure 1a and 1b show plant richness in the diet according to plant richness in the field and Cd concentration in soils in spring, respectively. The pattern was similar for the other metals. Influence of richness in the field on the diet richness was tiny in spring (Figure 1a), whereas diet richness decreased according to TM in soils regardless of the richness in the field (e.g. from 6 to 4 plant MOTUs along the range of TM concentration; Figure 1b). R^2_D was 0.301, 0.313 and 0.307 for Cd, Pb and Zn, respectively. Such tendency changed in autumn (Figure 1c and 1d for Cd, similar for other TM). Diet richness was negatively correlated to plant richness when soil contamination was low, while this correlation disappeared, or even reversed, when TM concentration increased (Figure 1c). A similar tendency was predicted for the relationship between diet richness and TM contamination in soils (Figure 1d): diet richness was negatively correlated to TM concentration when plant richness was low and positively correlated when the plant richness was high. R^2_D was 0.624, 0.492 and 0.601 for Cd, Pb and Zn, respectively.

The model for invertebrate richness in the diet included all proposed explanatory variables in the two seasons, except the interaction between TMs in soils and richness of ground-dwelling invertebrates for the models for Cd and Zn in autumn. In spring, arthropod richness in the diet increased according to richness of both ground-dwelling and flying invertebrates when soil contamination was low. This correlation disappeared, then reversed, when TM concentration in soils increased. For example, our model for Cd predicted that arthropod MOTUs increased from 2 to 5 along the gradient of ground-dwelling invertebrate richness in the field when Cd concentration in soils was low, whereas arthropod MOTUs decreased from 5 to 3 when Cd concentration was high (Figure 2a; the pattern was similar for flying invertebrates). The relationship between arthropod richness in the diet and TM concentration in soils followed the same tendency (Figure 2b). In autumn, arthropod richness in the diet was explained almost only by mouse number. Neither TM concentration in soils nor

richness of both types of invertebrates had sensible influence on diet richness (Figure 2c and 2d). R^2_D for Cd, Pb and Zn was respectively 0.502, 0.472 and 0.495 in spring, and 0.495, 0.504 and 0.497 in autumn.

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

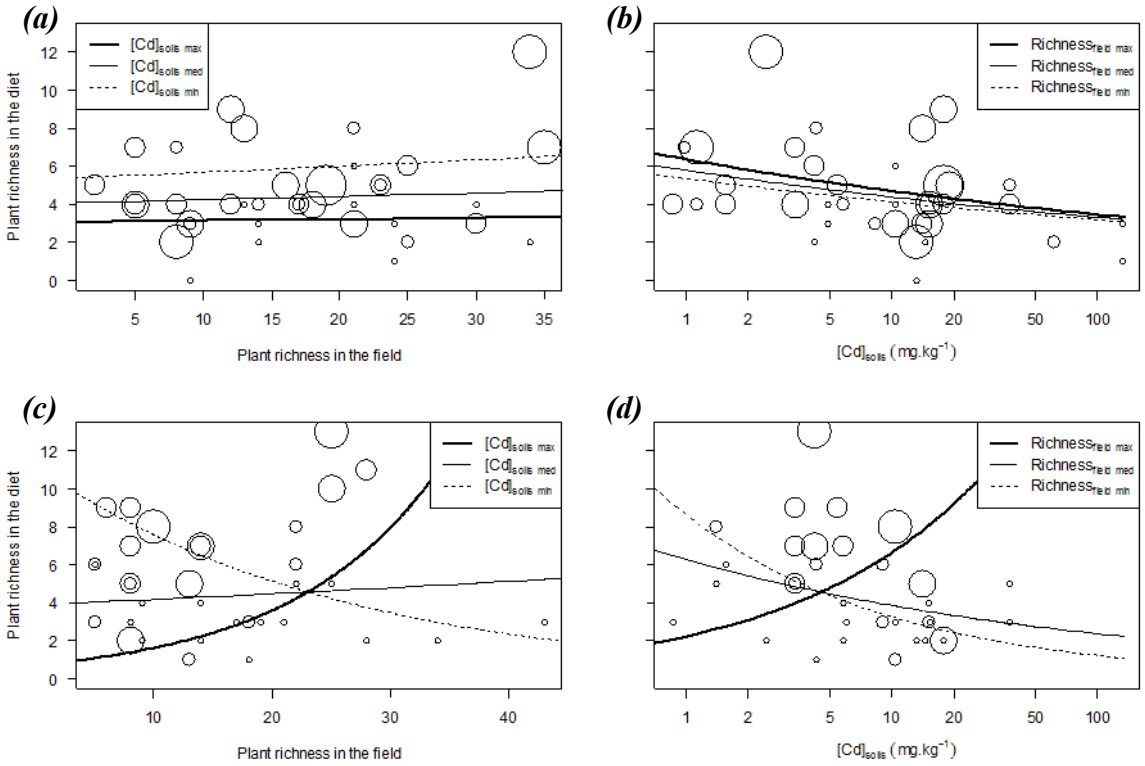
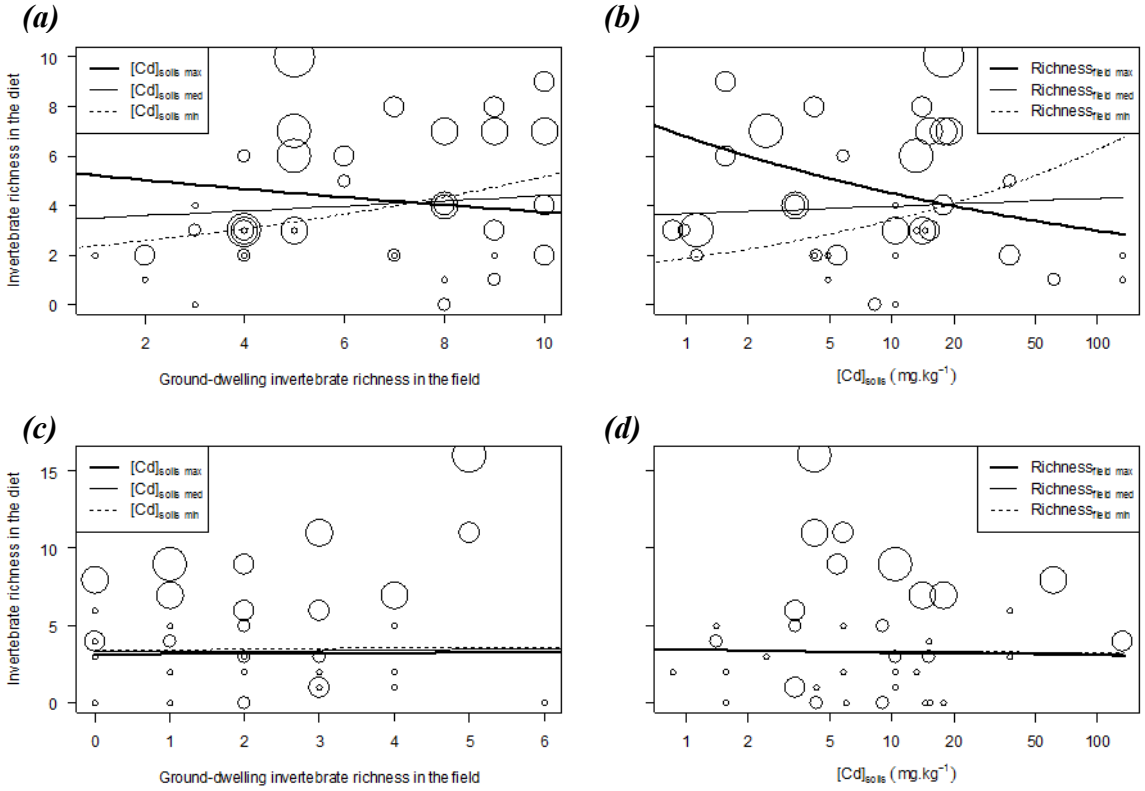
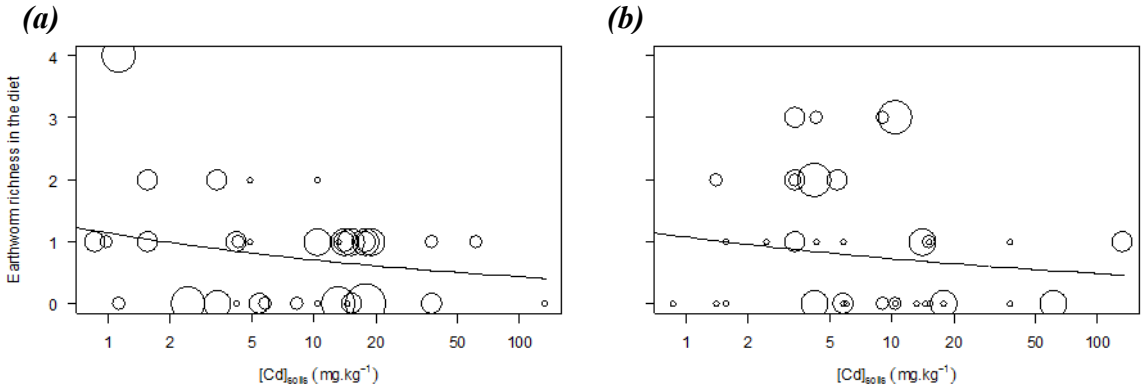


Figure 2: Invertebrate richness in the diet of the wood mouse along the gradient of ground-dwelling invertebrate richness in the field and Cd concentration in soils in spring (a and b) and in autumn (c and d). Each point represents a buffer and point size is proportional to the number of mice captured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



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

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

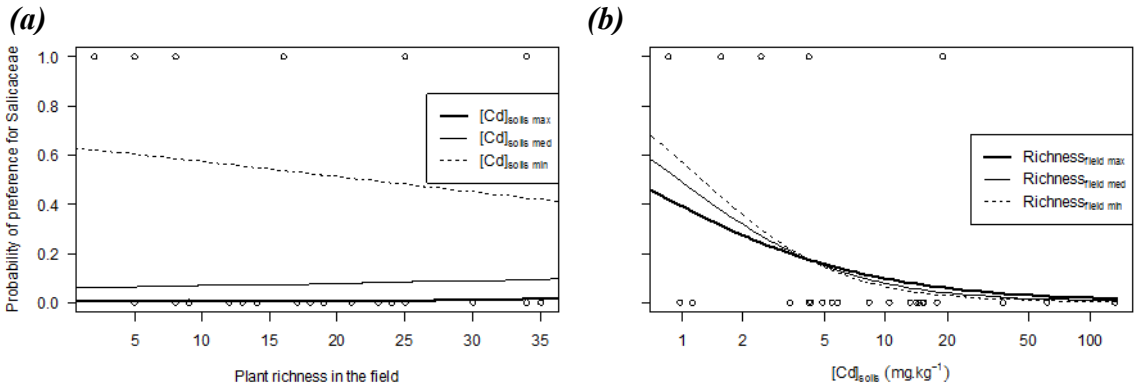


Plant food selection

We found 48 Grps in the food of the 246 mice. Sapindaceae, Salicaceae, Cornaceae, Adoxaceae, Asteraceae, and Rosaceae were plant taxa frequently eaten in our area (see SI10 for details). Selection for some plant families was observed at the scale of all study sites together and at the two seasons: preference for Fagaceae and avoidance of Urticaceae. Furthermore, selected plant resources changed between the two seasons, for example preference for Salicaceae, Sapindaceae and Rosaceae in spring and preference for Poaceae, Adoxaceae, Asteraceae and Cornaceae in autumn (see SI11 for details).

Model averaging was used for the final model for Salicaceae preference in spring (see SI12 for complete results). The model included all variables: mouse number, plant richness in the field, TM concentration in soils and the interaction. Preference for Salicaceae was negatively correlated to plant richness in the field when soil contamination was low (e.g. from 0.6 to 0.4; Figure 4a). On the other hand, preference for Salicaceae disappeared along the gradient of TM concentration in soils, whatever plant richness in the field was (e.g. preference probability for Salicaceae decreased from 0.6 to 0; Figure 4b). R^2_D of the final model was 0.280, 0.165 and 0.271 for Cd, Pb and Zn, respectively.

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



Discussion

We first hypothesised that soil TM contamination would reduce diet richness of wood mouse due to adverse effect of TM on resources' richness in the field. Our study revealed negative correlations between diet richness and TM in soils. This was true for both earthworms and plants in spring and in autumn (although only at low and medium levels of plant richness in autumn), and for arthropods in spring when richness in the field was high. On the contrary, positive correlations were observed, for plants in autumn when plant richness in the field was high, and for arthropods in spring when arthropod richness in the field was low. A negative relationship between diet richness and pollution by TM is expected if resource richness in the field is negatively affected by TM concentrations. Literature about the effects of pollution on various taxonomic groups suggests a global negative effect of pollution on the richness of organisms, and an absence or a positive correlation for a few taxa groups or situations. Although decreasing richness of earthworms with increasing TM concentrations gradients in soils has been widely reported (Spurgeon & Hopkin, 1996, Spurgeon & Hopkin, 1999; Pérès et al., 2011), several studies have reported that relationships between species richness of soil macro-fauna and TM contamination is not straightforward (Nahmani & Lavelle, 2002; Gillet & Ponge, 2003; Migliorini, et al., 2004). Moreover, several studies showed that community composition was affected by soil TM concentrations. Tolerance to, and/or accumulation capacity of metals differ according to taxa, organs, and metals (Das et al., 1997; Broadley et al., 2007; Pourrut et al., 2011). Lower resistance to toxic effects can make metal sensitive plants disappear in

contaminated areas, which may favour metal tolerant ones, sometimes without modification of taxonomic richness. Such modification of community composition has been reported by several authors in plants (Banásová et al., 2006; Strandberg et al., 2006; Dazy et al., 2009) and in invertebrates (Nahmani & Lavelle, 2002; Migliorini et al., 2004; Babin-Fenske & Anand, 2011). In our study area, we found no correlation or even positive trends between TM concentration in soils and both plant and invertebrate richness in the field (unpublished data). However, there were confounding factors in our site like the anthropogenic increase of habitat diversity (see Douay et al., 2009) and possibly of plant richness along the gradient of contamination on the Metaleurop Nord site. Community composition also differed according to soil contamination levels or other soil properties affecting metal bioavailability (unpublished data). Difference in resource composition could alter resource availability and thus modify feeding behaviour and diet composition. Few studies documented the effects of pollution on the diet of terrestrial vertebrates, most of them dealing with diet composition rather than richness or diversity. Clare et al. (2014), describing the diet of insectivorous bats *Myotis lucifugus* in different locations, showed a trend of lower diet species richness in area of lower habitat quality (acidification or organic pollution). Eeva et al. (2005) showed a significant difference in diet composition of nestlings of two insectivorous birds, *Parus major* and *Ficedula hypoleuca*, between unpolluted and TM polluted sites, reflecting differences in resources composition, phenology and feeding ecology among the two bird species: in polluted areas where caterpillars were less abundant, both species ate more beetles and different larvae of flying insects but less moth. The study of Heroldová (2002) demonstrated that the diet of field vole *Microtus agrestis* in forest clearings caused by air pollution was dominated by grasses, reflecting higher abundance of grasses and a poorer diversity of food supply in clearings. Thus, it is suggested that diet richness (as demonstrated in the present study) and/or composition can be affected by pollution induced changes in trophic resources.

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

and predators. Avoidance of contaminated food, leading to shifts in diet, can be one of the mechanisms allowing animals to mitigate their exposure in highly polluted environments (Gillet & Ponge, 2003). Actually, Salicaceae family is considered as TM accumulator and resistant to highly TM contaminated soils (Pulford & Watson, 2003). Migeon et al. (2009) showed that Salicaceae family in the surroundings of Metaleurop Nord, composed of two genera *Salix* and *Populus*, accumulated larger amount of Cd and Zn in their shoot parts than the other families like Fagaceae or Sapindaceae. These two last are also preferred plant families in our study and known as common food of wood mice (i.e. Watts, 1968). However, mechanisms of changes in preference remains unclear. The nutritional quality of metal contaminated plant organs has been shown to be lowered, with for instance a decrease of sugar or protein concentrations (Scheirs et al., 2006; Beernaert et al., 2008), features which play an important role for food choice in rodents (Butet, 1990; Kerley & Erasmus, 1991). It is also possible that animals might identify metal contaminated plants due to metal-induced metabolic compounds (Poschenrieder et al., 2006), largely known as compounds limiting feeding (Foley & Moore, 2005). Dearing et al (2000) reported that plant secondary compounds might play more important role than nutrient level in the diet of the generalist woodrat, *Neotoma albigula*. It is therefore supposed that mice could directly or indirectly detect noxious substances (plant toxins or TM) and avoid ingesting such items. Also, Behmer et al. (2005) demonstrated from their experiments using nutritionally balanced synthetic food that high Zn concentration *per se* altered the feeding behaviour of the desert locust *Schistocerca gregaria*. Whatever the mechanism, the hyperaccumulation of metals in plant tissues contribute to defensive effects of plants against natural enemies, including invertebrate herbivory (Poschenrieder et al., 2006; Boyd, 2007). Such defensive effect has not been reported against vertebrates so far, but the disappearance of the preference for Salicaceae family along TM contamination in soils could be associated to its high metal accumulation capacity. Furthermore, plant richness in the diet in autumn increased along the soil TM concentration gradient when richness in the field was high. Apart from changes of resource composition, this pattern could be explained, at least partly, by the nutrient balance hypothesis or the detoxification limitation hypothesis: if nutritional quality of all plant resources was altered (i.e. if nutritional quality was not clearly contrasted among resources) diet richness of animals could increase for completing nutrient and/or for avoiding loads of similar secondary compounds.

Contrary to plants, selection for invertebrate food could not be analysed in part because of the primer used for arthropods and molluscs: its short size is more reliable for the analysis of degraded DNA in the case of diet (Taberlet et al., 2012) but the precision in taxonomic assignment was low. We also did not sample earthworms in the field. Moreover, it is difficult to determine selection for invertebrate resources because of their mobility. Foraging strategy of predators can be modified according to mobility of preys (Sih & Christensen, 2001), suggesting

that relative quantity in the field is not the only factor to consider when analysing mobile prey selection. Even though we supposed that decreased earthworm diet richness might be linked to their richness in the field, invertebrate food selection in wood mice remains as a further challenge.

An important feature of our results is the strong seasonal difference of patterns, for both food selection and correlations with TM contamination. In temperate ecosystems, seasons have a key influence on the dynamics of ecosystems and on the various aspects of biology and ecology of living organisms. Seasonal difference of patterns might result from the adaptation of wood mice feeding behaviour to phenology of resources. Indeed, wood mice consume mainly seeds and other reproductive organs (Watts, 1968; Butet, 1986a), seeking high energy or protein-rich tissues (Butet, 1990). Although parts of plant resources actually consumed could not be distinguished by using DNA metabarcoding, we supposed that parts of plants preferably consumed have changed between spring and autumn. Moreover, the nutritional composition and quality of plants and invertebrates are likely to vary according to seasons. Also, resources functionally important for wood mice might have a strong phenology, such as mushrooms in autumn. However, the molecular primers we used did not target mushrooms, which prevents further conclusion. Finally, TM accumulation in organisms is also subject to seasonality at contaminated site (i.e. Hunter et al, 1987b; Migeon et al., 2009). Seasonal variation of TM accumulation pattern can be related to growth season or physiological changes of each taxa (Kabata-Pendias, 2011; Hussein et al., 2006). Disentangling the combined effects of these factors on the patterns of interest in this study is beyond our present scope, but it is clear that seasonality is a key feature of the interplay between pollution, resource dynamics and consumer feeding behaviour.

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

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Data Accessibility:

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

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 F.R, R.S. and C.F.

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Supporting Information:

SI1: Information for each study site about TM contamination and dominant landscape feature, as well as richness of plants and invertebrates observed in the field.
SI2: Inventory of plant and invertebrate taxa observed in each site (Appendix A).
SI3: Sequence data of plant, arthropod and earthworm MOTUs for each mouse (in the file "SI3_Ozaki.exe" available in "https://onlinelibrary.wiley.com/doi/abs/10.1111/mec.14823").
SI4: Sequence data of sequence groups for each mouse (in the file "SI4_Ozaki.exe" available in "https://onlinelibrary.wiley.com/doi/abs/10.1111/mec.14823").
SI5: Frequency of occurrence of plant, arthropod and earthworm items in wood mouse food across several environmental and biological factors.
SI6: Variation of the total richness in the diet along the soil contamination gradient.
SI7: Relative proportion of the three food item categories (plants, arthropods and earthworms) along the soil contamination gradient.
SI8: Plant, arthropod and earthworm richness in the diet of wood mouse across several environmental and biological factors.
SI9: Complete data on models of richness in the diet (in the file "SI9_Ozaki.exe" available in "https://onlinelibrary.wiley.com/doi/abs/10.1111/mec.14823").
SI10: Complete data on number and frequency of occurrence of plant food item (sequence group) (Appendix B).
SI11: Information on food selection for plant families corresponding to sequence groups in the wood mouse in spring and in autumn.
SI12: Complete results on plant food selection modelling at buffer scale (in the file "SI12_Ozaki.exe" available in "https://onlinelibrary.wiley.com/doi/abs/10.1111/mec.14823").

Supporting Information SI1: Concentrations of cadmium (Cd), lead (Pb) and zinc (Zn) in soils (mg kg^{-1} of dry soil) and dominant landscape feature of each study site based on data published in Fritsch et al. (2010), as well as resource richness in the field (at species level for plants and at family or higher levels for invertebrates in each site). Distance between each site are indicated.

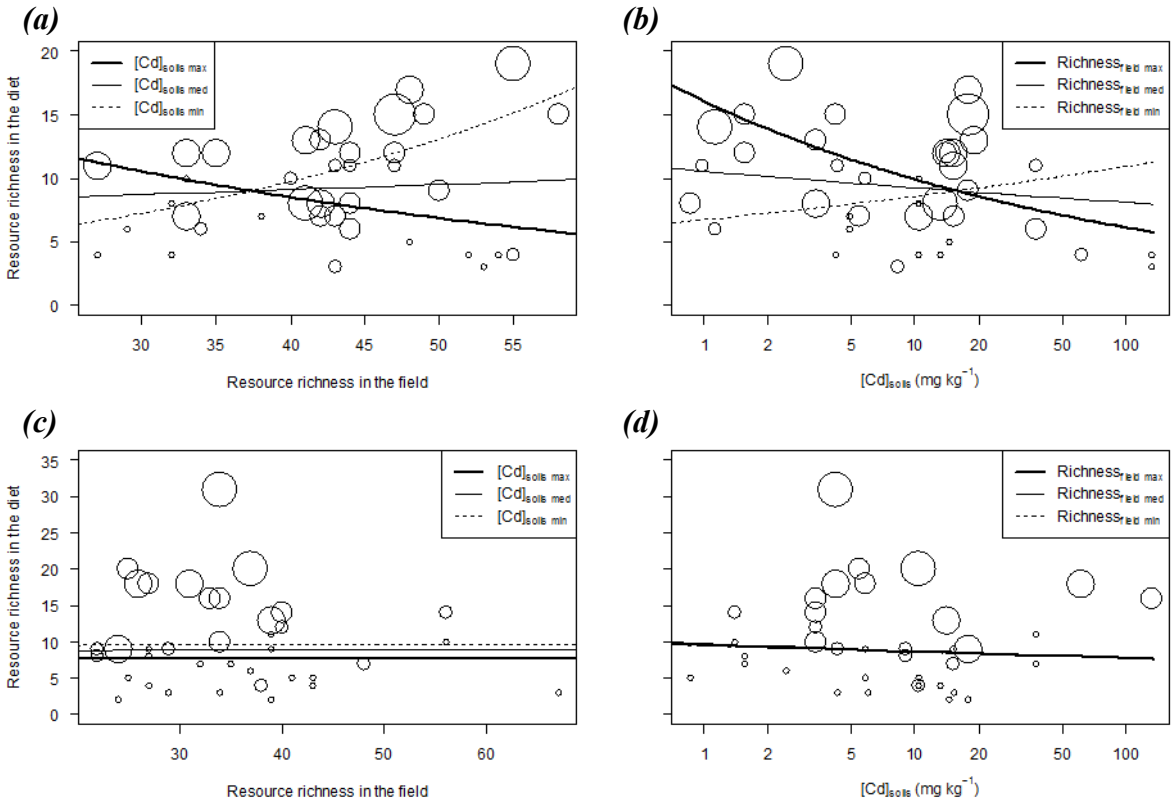
Site name		TE2	103	117	097	171	043	113
Soil contamination level		"Control[a]"	+	++	+++	++	++	++
Landscape feature		Forest	Forest	Forest	Forest	Forest	Arable	Urban
$[Cd]_{soil}$ (mg kg^{-1} dry mass) Min - Max (Median)		0.9-2.4 (1.4)	1.5-6.0 (4.3)	3.6-17.8 (9.1)	15.3-236.5 (48.3)	4.9-14.5 (7.5)	1.3-42.7 (15.2)	4.4-13.0 (11.5)
$[Pb]_{soil}$ (mg kg^{-1} dry mass) Min - Max (Median)		43-200 (107)	238-333 (267)	245-860 (512)	659-6809 (1295)	288-2063 (584)	105-1029 (323)	267-806 (679)
$[Zn]_{soil}$ (mg kg^{-1} dry mass) Min - Max (Median)		89-278 (169)	114-408 (353)	303-959 (556)	1069-7264 (1875)	487-2452 (1363)	154-1550 (513)	415-1170 (1001)
Tree stratum richness		15	7	9	7	14	14	-[b]
Shrub stratum richness		23	14	13	11	16	10	7
Herbaceous stratum richness		70	58	56	68	71	45	33
Ground-dowelling invertebrate richness		13	18	16	18	11	20	10
	Spring	*11*	*15*	*13*	*18*	*11*	*19*	*8*
	Autumn	*8*	*10*	*11*	*4*	*5*	*10*	*8*
Flying invertebrate richness		64	64	57	54	43	66	65
	Spring	*48*	*50*	*44*	*43*	*32*	*44*	*41*
	Autumn	*46*	*51*	*29*	*35*	*31*	*51*	*52*
Distance between each site (km)	TE2		7	9	10	9.5	9.5	11
	103	-		2	3	2.5	3	4
	117	-	-		1	1.5	3	4
	097	-	-	-		2	1.5	1
	171	-	-	-	-		3.5	2.5
	043	-	-	-	-	-		2.2
	113	-	-	-	-	-	-	

[a]: TM concentrations as close as possible to background concentrations.

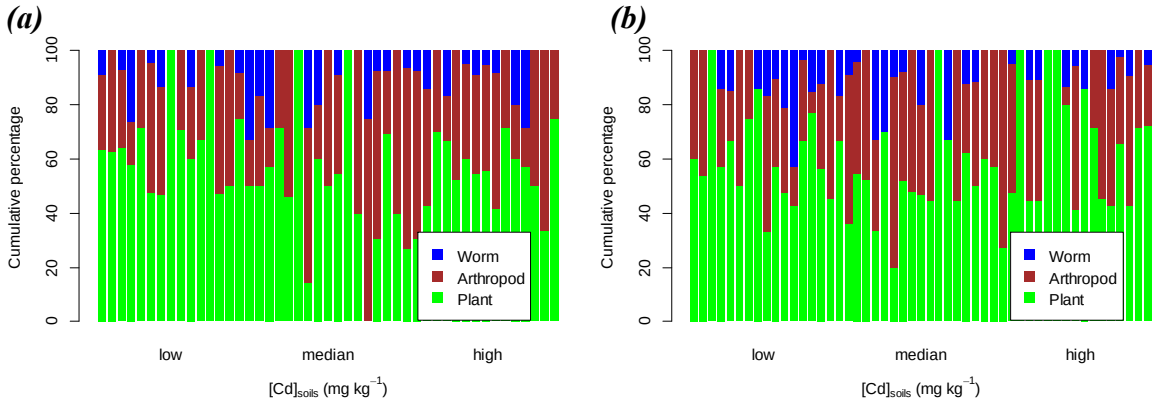
[b]: No survey was carried out because there were few trees.

Supporting Information SI5: Frequency of occurrence of plant, arthropod and mollusc (represented simply as 'arthropod'), and earthworm items in the diet of wood mouse across seasons, sex of mouse, landscape feature, and age class of mouse. Number of mice is indicated in brackets. An asterisk (*) denotes significant difference by the chi-square goodness-of-fit test.

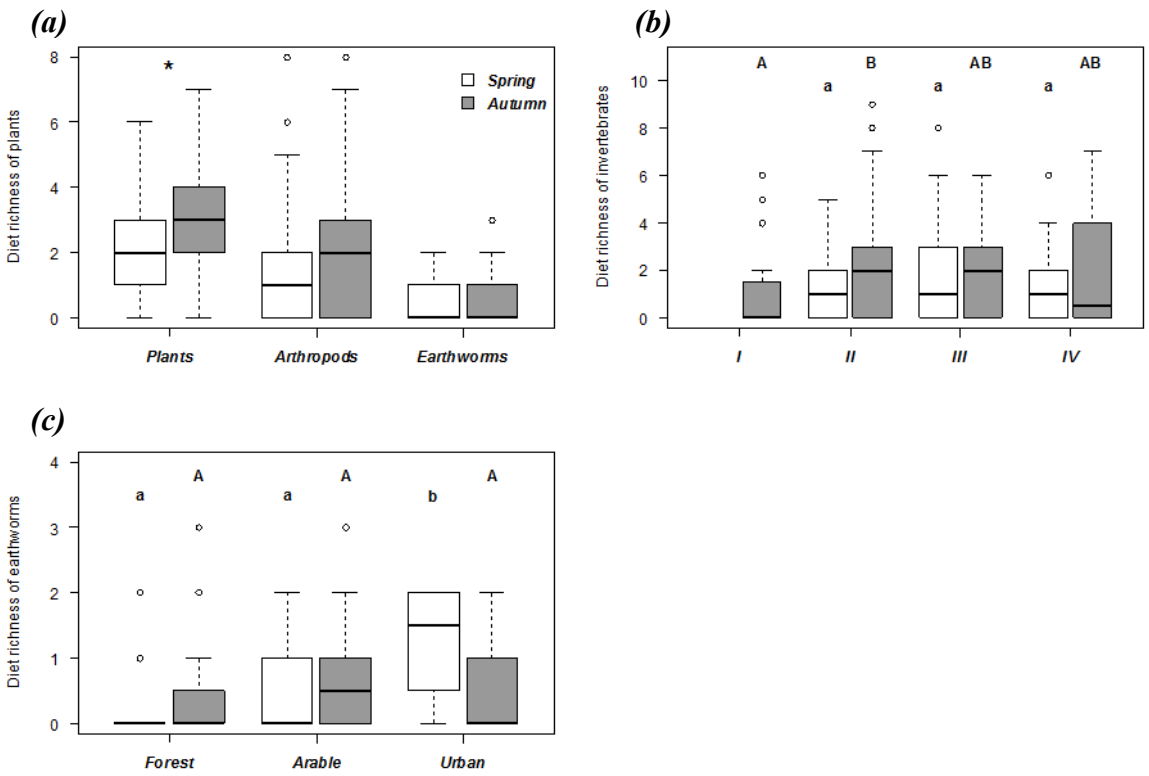
Factor	**General**	**Season**		**Sex**			**Landscape feature**			**Age class**			
		Spring	Autumn	Female	Male	Unknown	Forest	Arable	Urban	I	II	III	IV
(Mouse number)	*(246)*	*(117)*	*(129)*	*(116)*	*(127)*	*(3)*	*(188)*	*(40)*	*(18)*	*(35)*	*(116)*	*(65)*	*(30)*
Plant	0.96	0.94	0.98	0.96	0.97	1.00	0.97	0.95	0.94	0.97	0.98	0.95	0.90
Arthoropod	0.66	0.67	0.65	0.69	0.63	0.67	0.64	0.70	0.72	0.49	0.70	0.71	0.60
Earthworm	0.29	0.27	0.31	0.24	0.34	0.33	0.25*	0.40*	0.50*	0.31	0.28	0.29	0.33



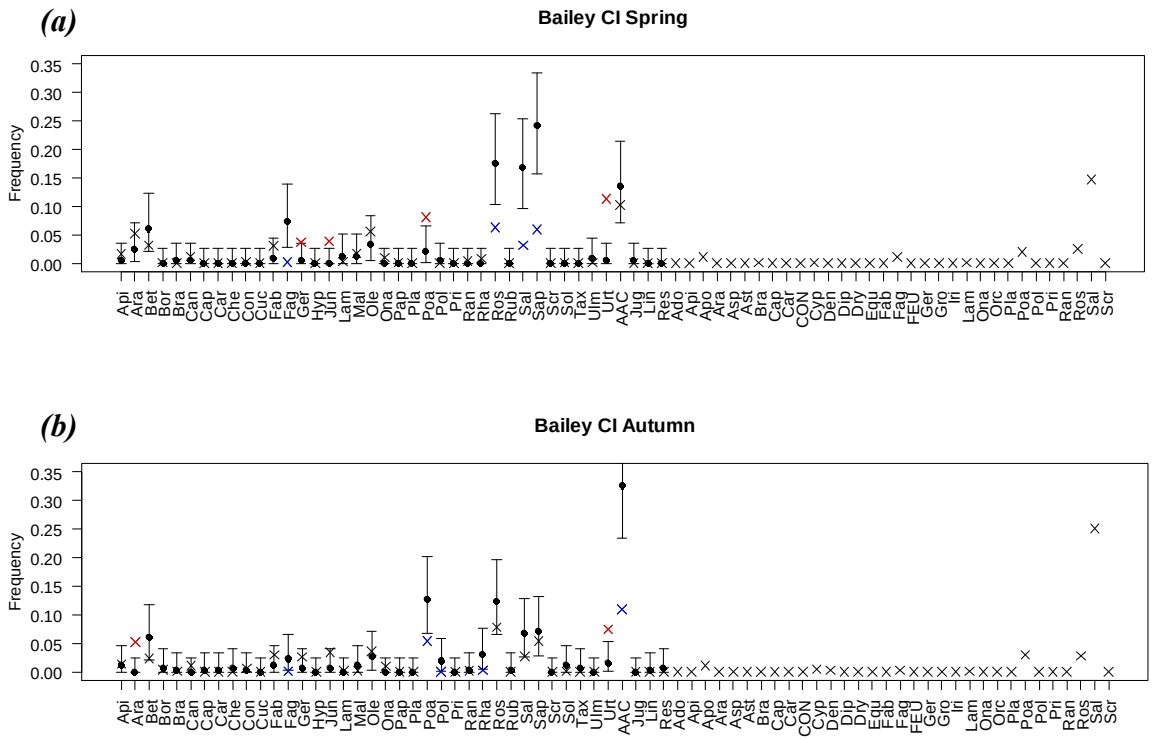
Supporting Information SI6: Resource richness in the diet of the wood mouse (total number of plant, arthropod, and earthworm MOTUs in buffer) along the gradient of resource richness in the field (total number of plant families and of both ground-dwelling and flying invertebrate families) and Cd concentration in soils, in spring (a and b) and in autumn (c and d). Each point represents a buffer and point size is proportional to number of mice captured in a given buffer. Values predicted from models are indicated by three lines: dotted, normal and bold lines correspond to minimal, median and maximum value of Cd concentrations in soils (a and c) or resource richness in the field (b and d) in our data, respectively. Mouse number is fixed to the median value for drawing the lines (three mice in spring and two mice in autumn). The patterns are similar for Pb and Zn concentrations in soils.



Supporting Information SI7: Relative proportion of occurrence of the three food item categories (plant, arthropod, and earthworm MOTUs) in the diet of wood mice along the soil Cd contamination gradient in spring (a) and in autumn (b). Plant, arthropod, and earthworm items were represented in green, brown and blue, respectively.



Supporting Information SI8: (a): Richness of the three food item categories (plants, arthropods and earthworms) in the diet of wood mouse across two seasons. (b): Arthropod richness in the diet of wood mouse across age classes. (c): Earthworm richness in the diet of wood mouse across landscape features. An asterisk (*) denotes significant difference between seasons. Different letters denote significant differences between classes of age or landscape feature, lowercase letters for spring and uppercase for autumn.



Supporting Information SI11: Frequency of occurrence per plant families corresponding to sequence groups in the wood mouse diet with Bailey intervals (represented by a dot and a line) and proportion of plant resources (represented by a cross) in the field in spring (a) and autumn (b). Plant resources whose proportion in abundance in the field is below the intervals mean significantly preferred resources (in blue) and resources above the intervals mean avoided ones (in red). Taxa in the field whose occurrence was not found in food were separately marked (without frequency of occurrence). Api: Apiaceae; Ara: Araliaceae; Bet: Betulaceae; Bor: Boraginaceae; Bra: Brassicaceae; Can: Cannabaceae; Cap: Caprifoliaceae; Car: Caryophyllaceae; Che: Chenopodiaceae; Con: Convolvulaceae; Cuc: Cucurbitaceae; Fab: Fabaceae; Fag: Fagaceae; Ger: Geraniaceae; Hyp: Hypericaceae; Jug: Juglandaceae; Jun: Juncaceae; Lam: Lamiaceae; Lin: Linaceae; Mal: Malvaceae; Ole: Oleaceae; Ona: Onagraceae; Pap: Papaveraceae; Pla: Plantaginaceae; Poa: Poaceae; Pol: Polygonaceae; Pri: Primulaceae; Ran: Ranunculaceae; Res: Resedaceae; Rha: Rhamnaceae; Ros: Rosaceae; Rub: Rubiaceae; Sal: Salicaceae; Sap: Sapindaceae; Scr: Scrophulariaceae; Sol: Solanaceae; Tax: Taxaceae; Ulm: Ulmaceae; Urt: Urticaceae; AAC: Adoxaceae, Asteraceae & Cornaceae; Ado: Adoxaceae; Asp: Asparagaceae; Ast: Asteraceae; CON: conifers; Cyp: Cyperaceae; Den: Dennstaedtiaceae; Dip: Dipsacaceae; Dry: Dryopteridaceae; Equ Equisetaceae: ; FEU: broad-leaved trees; Gro: Grossulariaceae; Iri: Iridaceae; Orc: Orchidaceae.

III.3 Chapter 3

Abstract and keywords in French.

Résumé :

L'exposition des mammifères terrestres aux contaminants chimiques comme les éléments traces metalliques (ETM) est considérée comme étant principalement liée au transfert trophique. Bien que les relations entre le transfert des ETM aux animaux et l'identité des aliments contaminés aient été étudiées, la variation du transfert des ETM en fonction de la diversité alimentaire a été peu documentée. Dans cette étude, l'exposition trophique aux ETM des mulots sylvestres, *Apodemus sylvaticus*, a été étudiée en fonction de la richesse (nombre d'items différents consommés) et de la composition (identité des items déterminés par *métabarcoding* sur les contenus stomacaux) du régime alimentaire. Les résultats montrent que la consommation de plantes de la famille des *Salicaceae*, considérés comme accumulateurs de cadmium, augmente significativement l'exposition au cadmium et au zinc. Cependant, l'augmentation de la richesse du régime alimentaire diminue l'exposition au cadmium lorsque les mulots consomment ces *Salicaceae*. Ces résultats suggèrent que les accumulateurs d'ETM peuvent augmenter l'exposition aux ETM mais que cette exposition élevée due aux accumulateurs peut être « diluée » par une richesse élevée dans le régime alimentaire, grâce à d'autres items moins accumulateurs. Nos résultats indiquent clairement que la présence de certains éléments dans le régime alimentaire et la richesse du régime alimentaire sont des déterminants importants de l'exposition aux ETM chez les animaux généralistes, ce qui correspond à « l'hypothèse de dilution ».

Mots-clés : identification des aliments, *metabarcoding*, transfert trophique, faune sauvage, *Salicaceae*

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

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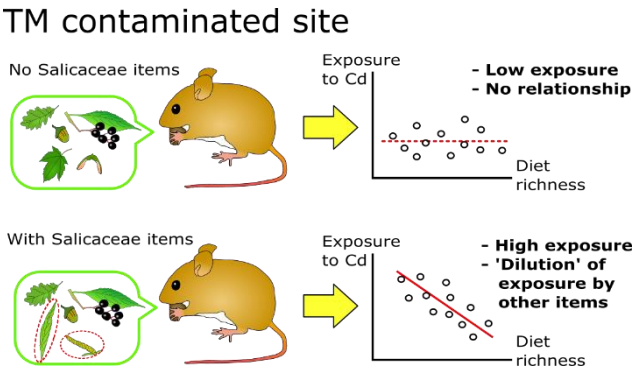
Abstract

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*".

Keywords

Food identification, metabarcoding, trophic transfer; wildlife; *Salicaceae*.

Abstract Art



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

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

Food Composition. For the detail of food identification method, see Ozaki et al.[36] Briefly, each sequence extracted from SCs (i.e., query sequence) was compared with reference sequences of species recorded in the study area,[36] taken from the GenBank sequence database (www.ncbi.nlm.nih.gov/genbank). When query sequence matched with more than one reference sequence, query sequences were gathered into one group composed of the corresponding species, and then gathered at the family level. The sequences were finally converted into presence/absence of each plant family in SCs. Invertebrate resources in the field were identified mainly at family level, which could not allow to identify invertebrate food. We used only the presence/absence of arthropod and mollusk items (referred to as only "*arthropod*" hereinafter) 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 difference are observed. Permutation tests by Strasser and Weber[40] are applied for the splitting tests. In this study, CIT was performed across binary partitioning variables: season (spring or autumn) and the presence/absence of the 10 types of items mentioned above. Sites were considered to be random effects for exposure in the Wilcoxon-Mann-Whitney test and CIT because of potential effects linked to each site. Those tests were executed for the residuals of the random effect model, i.e. the model with only site as random effect. As all items were used as partitioning variables without taking into account the result of each Wilcoxon-Mann-Whitney test, *p-value* of CIT was not adjusted by Bonferroni correction.

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

Relationships between exposure and the proportion of sequences of the given hyper-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 ($p\text{-}value_{node}$). 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).

Diet richness. Plant, arthropod and earthworm diet richness ranged from 0 to 6 MOTUs (median: 2), from 0 to 9 (median: 2), and from 0 to 3 (median: 0), respectively. Total diet richness, as well as both plant and arthropod diet richness, were significantly higher in autumn than in spring (data not shown, W = 3463, *p-value* < 0.001; W = 3646, *p-value* < 0.001; W = 4190.5, *p-value* = 0.04, respectively). No significant correlation between the three diet richness values was observed, either in spring or in autumn (Supporting Information Figure S4a and

S4b). Only plant diet richness in spring significantly decreased along the gradient of TMs in soils (Spearman's ρ from -0.26 to -0.19, *p-value* < 0.05 for the three TMs; Figure S4a).

Exposure to TMs and items consumed. Cd, Pb and Zn concentrations in SCs ranged from 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: 71.1) µg g^{-1} DM, respectively. The TM concentration in SCs and the exposure were significantly higher in spring than in autumn for the three TMs (*p-value* < 0.05; for details see Supporting Information Figure S5). The exposure to Cd was significantly higher when Salicaceae items were present in SCs and significantly lower when AAC plants were present in SCs (W = 2559 and 5986; *p-value* < 0.05, respectively; Supporting Information Figure S6a). The exposure to Zn was significantly higher when Salicaceae items or Oleaceae items were present in SCs (W = 3073 and 524; *p-value* < 0.05, respectively; Figure S6b). The exposure to Pb was not influenced by any item consumed.

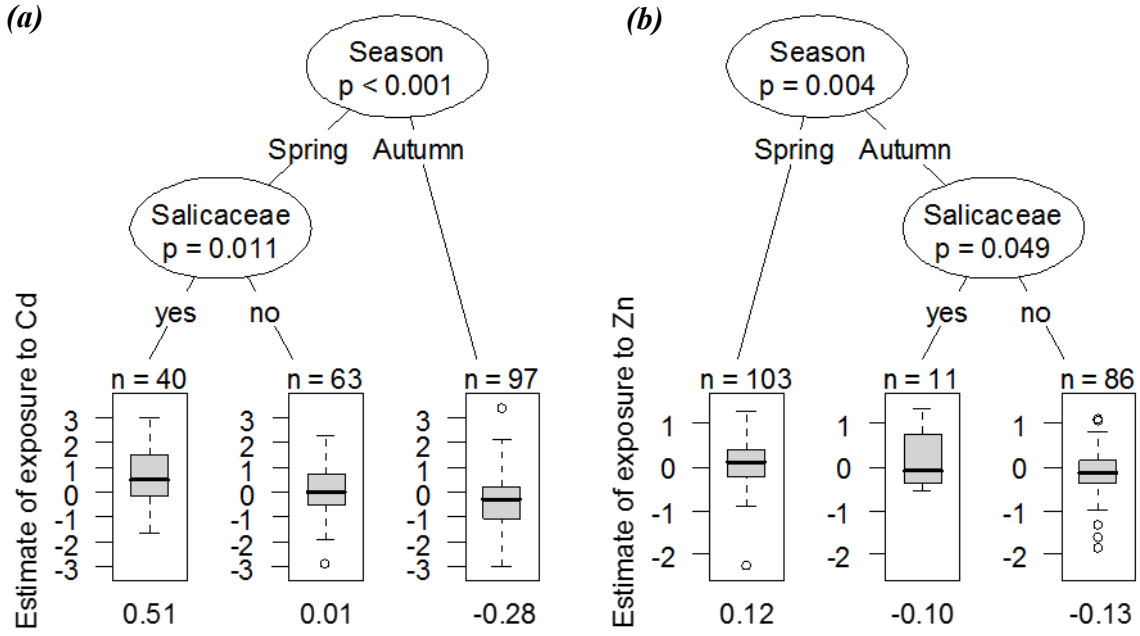


Figure 1. *Conditional inference trees (CIT) for (a) the exposure to Cd and (b) the exposure to Zn, i.e. residuals of linear model between logarithmically-transformed concentrations of TMs in stomach content and in soils (µg g^{-1} DM). Partitioning variables significantly selected for splits (univariate 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.*

CIT analysis revealed that season was the most important variable influencing the exposure of rodents to Cd (*p-value* < 0.001), followed by the presence of Salicaceae items in SC in spring (Figure 1a; *p-value* = 0.01). The exposure to Cd was the highest when Salicaceae

items were present in SC in spring, followed by when Salicaceae item were absent in spring, and the lowest 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; $R^2_m = 0.154$; $R^2_c = 0.280$).

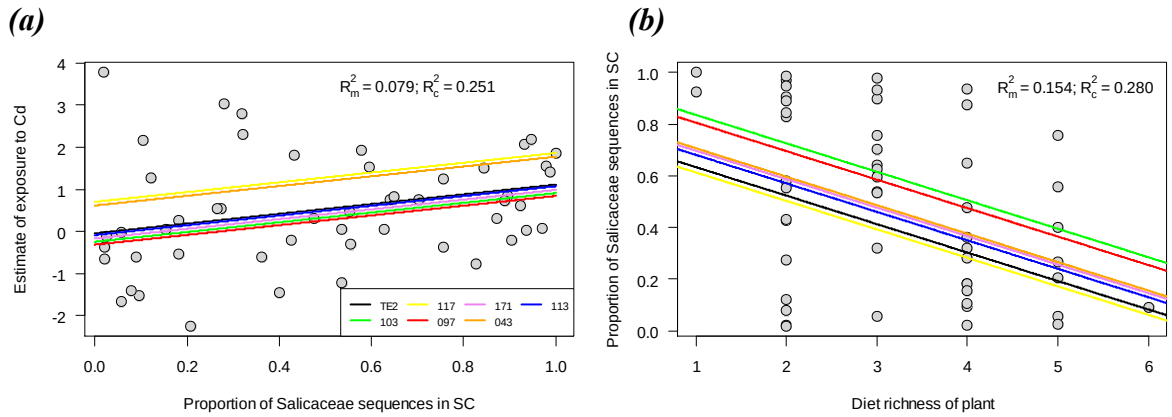


Figure 2. *(a) Exposure of the wood mice to Cd along the gradient of proportion of Salicaceae items' sequence number in stomach content, considered as a proxy of biomass consumed and (b) the proportion of Salicaceae items' sequences along the gradient of diet richness of plant. Plotted points indicate 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.*

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.

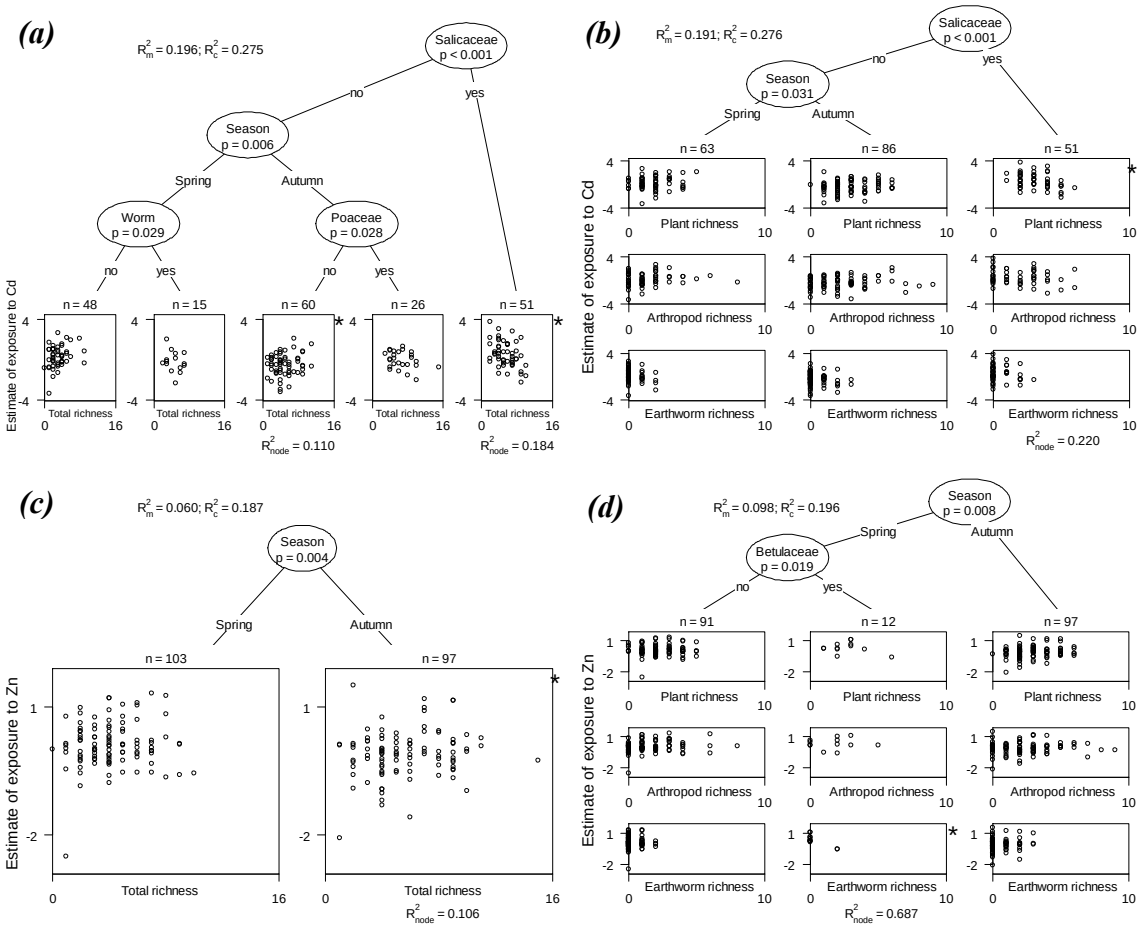


Figure 3. *Model-based recursive partitioning (MOB) on mixed linear models for the exposure to TMs, i.e. residuals of linear model between logarithmically-transformed concentrations of TMs in stomach content and in soils (µg g^{-1}), with respect to either three diet richness of plant, arthropod and 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.*

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

To conclude, the level of exposure of small mammals to TMs is influenced not only by the presence of certain items in the diet but also by the diet richness. The functional role of the diet richness on exposure to TMs observed in the present study concurs with the diet dilution hypothesis. Challenging such hypotheses about the functional role of biodiversity on the trophic transfer of pollutants and investigating trait-based mechanisms are possible if ingested food is identified in details. Although the metabarcoding approach allows identifying the taxonomy of food under field conditions, associating life history traits and chemical composition of each item and clarifying mechanisms of foraging behavior of animals will improve chemical risk assessment in wildlife. Further perspectives also deal with the quantification of trophic links to

address the functional significance of trophic interactions for the transfer of matter, energy, and essential elements versus the transfer of toxicants, and the evolutionary trade-offs between acquiring nutrients but being exposed to toxic substances.

Acknowledgment

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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.

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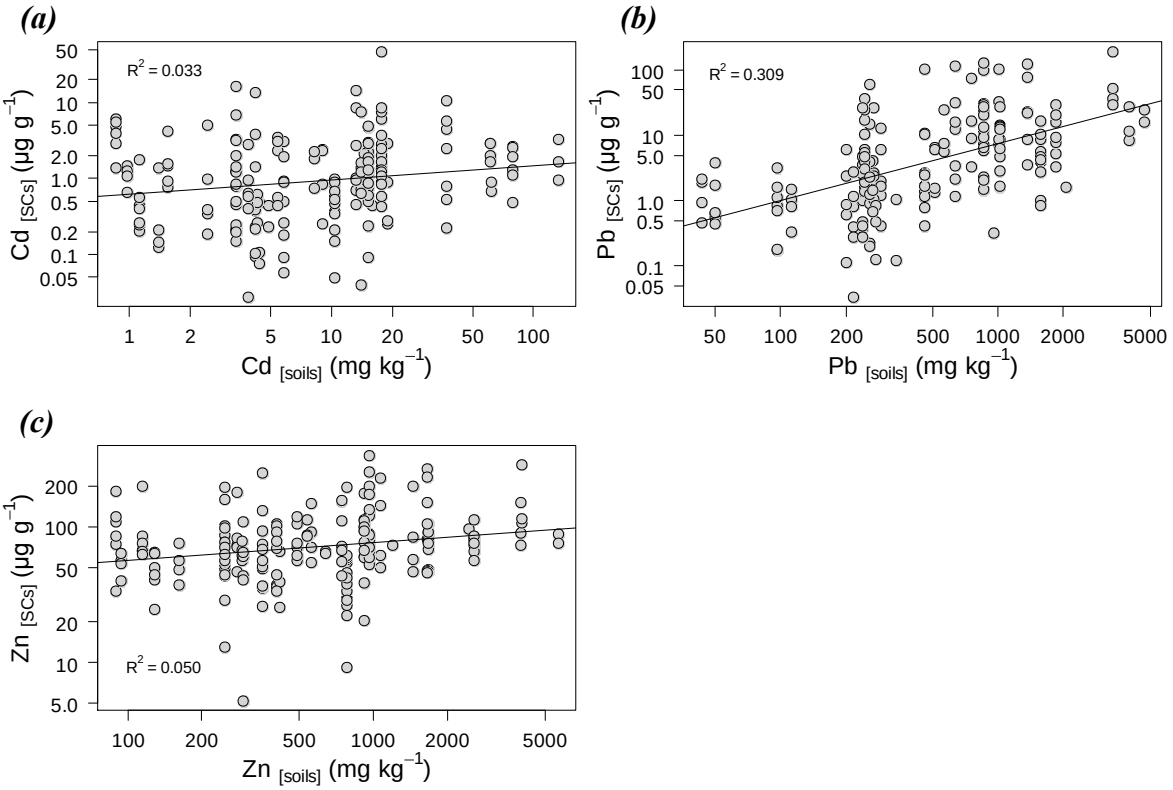
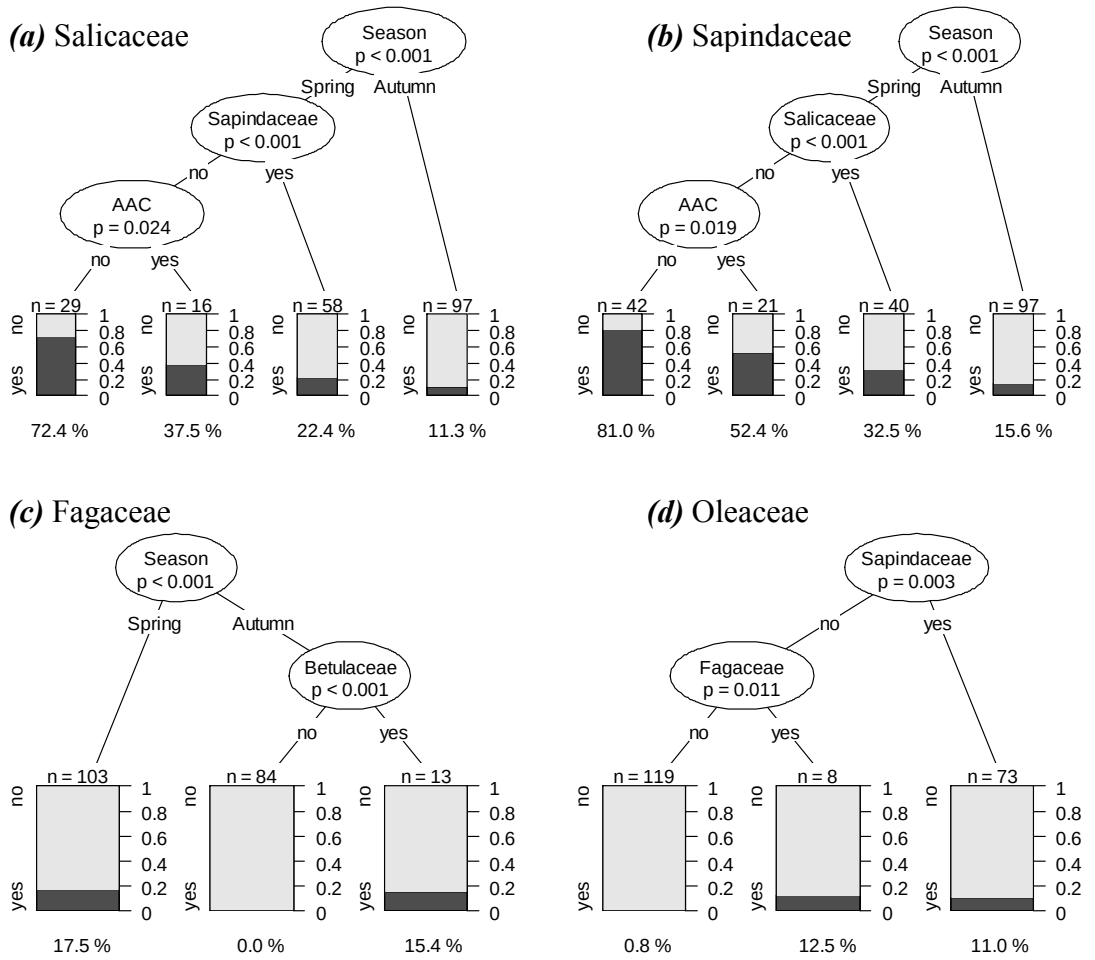
Supporting Information

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.

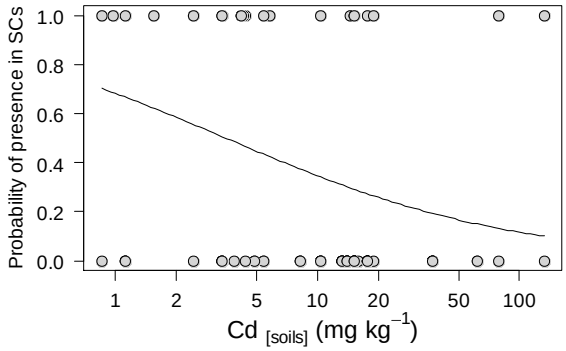


Supporting Information Figure S1: Concentrations of TMs (mg kg^{-1} dry soil), i.e., (a) cadmium (Cd), (b) lead (Pb), and (c) zinc (Zn), in stomach contents (SCs) of wood mice (µg g^{-1} of dry mass) along the gradient of TM concentrations in soils (mg kg^{-1} of dry soil). Linear relationships between logarithmically transformed TM concentrations in SCs and those in SCs are positive and significant (p-value < 0.05). The coefficient of determination of the linear model (R^2) is mentioned in each figure.

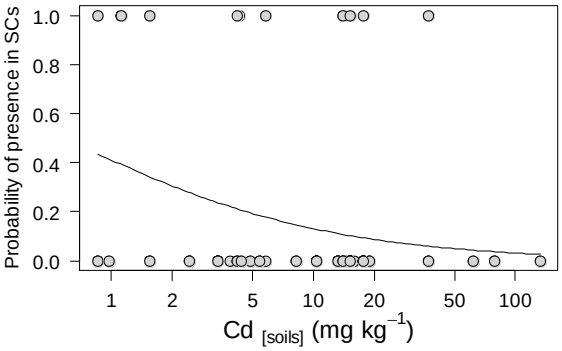


Supporting Information Figure S2: Conditional inference trees for frequency of occurrence of (a) Salicaceae, (b) Sapindaceae, (c) Fagaceae, and (d) Oleaceae items in SCs of wood mouse. Partitioning variables significantly selected for splits (univariate p-value <0.05) are listed in inner nods (represented as ovals) with p-value. 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 occurrence of the given item for each final classification group with number of mice, and the frequency of occurrence of the item in the classification group is mentioned below.

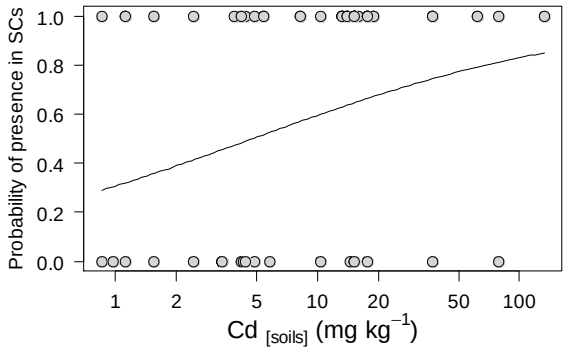
(a) Salicaceae in spring



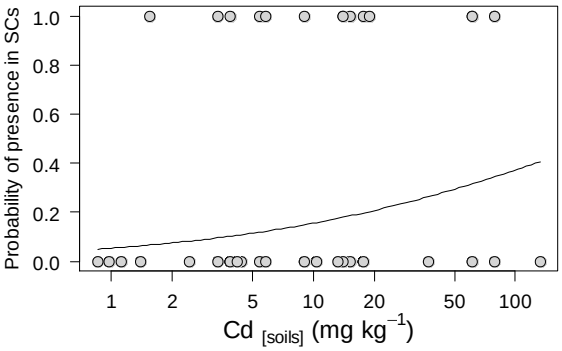
(b) Fagaceae in spring



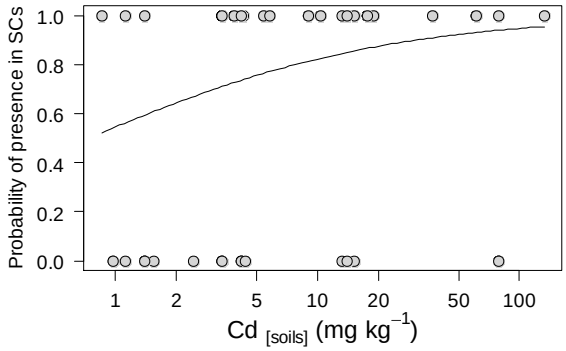
(c) Sapindaceae in spring



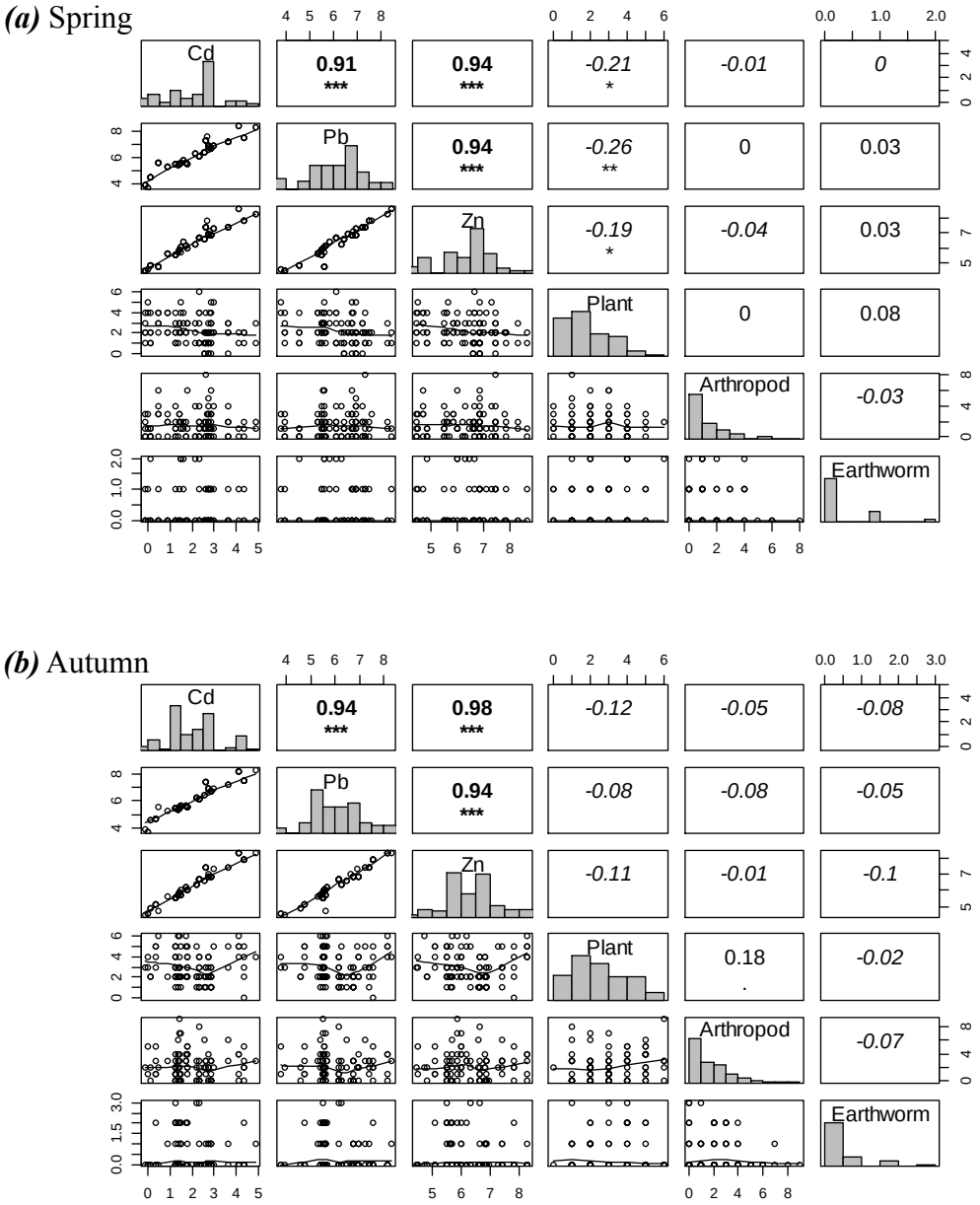
(d) Sapindaceae in autumn



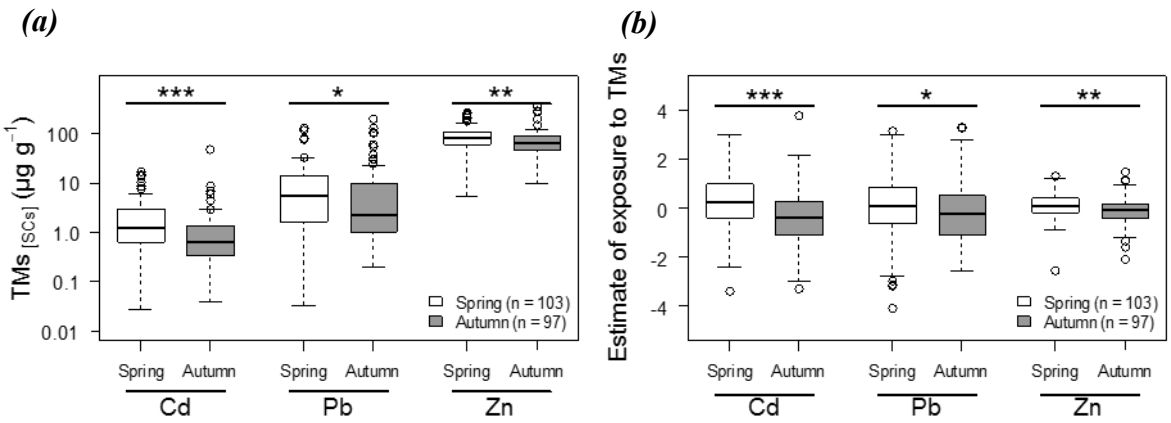
(e) Group AAC in autumn



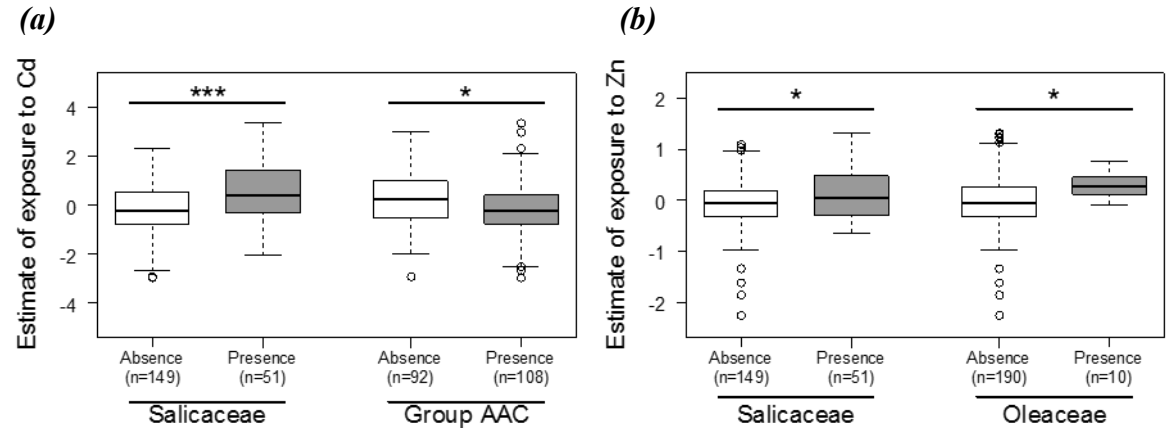
Supporting Information Figure S3: Predicted probabilities for occurrence of items consumed in SCs along a gradient of Cd concentration in soils (mg kg^{-1} dry soil). Plotted points at probability value 1 indicate SCs in which a given item was observed, whereas points at 0 indicate SCs in which the item was not observed. Predicted probability is drawn. (a): Salicaceae items in spring, (b): Fagaceae items in spring, (c): Sapindaceae items in spring, (d): Sapindaceae items in autumn and (e): sequence group composed of Adoxaceae, Asteraceae and Cornaceae items in autumn. The predicted probability patterns are similar for Pb and Zn concentrations in soils.



Supporting Information Figure S4: Correlation matrix for TM concentrations in soils and three diet richness in spring (a) and in autumn (b). The diagonal part describes histograms of each variable: logarithmically transformed concentrations of Cd, Pb and Zn in soils (mg kg^{-1} of dry soil) and numbers of plant, arthropod and earthworm MOTUs in SCs. The lower triangular matrix part describes scatter plots for each pair of the variables with LOWESS smoother line, whereas the upper triangular matrix part shows Spearman's ρ for each pair of the variables with asterisk (*) for significant correlation by the Spearman's correlation test (: p-value <0.05, **: p-value <0.01 and ***: p-value <0.001).*



Supporting Information Figure S5: Concentrations of Cd, Pb and Zn in SCs (µg g^{-1} of dry mass) (a) and estimate of exposure to the three TMs, i.e. residuals of linear model between logarithmically-transformed concentrations of TMs in SCs (µg g^{-1} of dry mass) and in soils (mg kg^{-1} of dry soil) (b), in spring and in autumn. Significant difference between the two seasons by the Wilcoxon-Mann-Whitney test is denoted by asterisk (: p-value <0.05, **: p-value <0.01 and ***: p-value <0.001).*



Supporting Information Figure S6: Significant differences in estimate of exposure to (a) Cd and (b) Zn, i.e. residuals of linear model between logarithmically-transformed concentrations of TMs in SCs (µg g^{-1} of dry mass) and in soils (mg kg^{-1} of dry soil), between the presence/absence of Salicaceae, Oleaceae or the sequence group composed of Adoxaceae, Asteraceae, and Cornaceae (AAC) items in SCs. Significant difference by the Wilcoxon-Mann-Whitney test is denoted by asterisk (: p-value <0.05, **: p-value <0.01 and ***: p-value <0.001).*

Supporting Information Table S1: Concentrations of Cd, Pb and Zn in soils (mg kg^{-1} of dry soil), synthetic soil contamination levels, and landscape dominant habitat of seven study sites based on data published in Fritsch et al.[1]

		TE2	103	117	097	171	043	113
$[Cd]_{soils}$ (mg kg^{-1})	Minimum	0.86	1.5	3.6	15.3	4.9	1.3	4.4
	Median	1.4	4.3	9.1	48.3	7.5	15.2	11.5
	Maximum	2.4	6.0	17.8	236.5	14.5	42.7	13.0
$[Pb]_{soils}$ (mg kg^{-1})	Minimum	43.3	237.5	244.7	658.5	287.6	105.0	266.6
	Median	107.4	267.2	512.0	1295.3	584.0	323.1	678.9
	Maximum	199.8	333.0	859.8	6809.4	2063.3	1028.9	806.0
$[Zn]_{soils}$ (mg kg^{-1})	Minimum	89.3	114.4	302.8	1069.3	487.2	153.9	414.7
	Median	168.8	352.7	555.8	1874.7	1362.7	512.8	1001.2
	Maximum	277.7	407.5	958.5	7263.5	2451.5	1549.6	1170.4
Soil contamination level		"Control[a]"	+	++	+++	++	++	++
Landscape feature		Forest	Forest	Forest	Forest	Forest	Arable	Urban

[a]*: TM concentrations as close as possible to regional background concentrations (Sterckeman et al.*[2]*).*

Supporting Information Table S2: Characteristics of the three primers used to amplify DNA samples obtained from SCs.

Taxonomic group	*DNA type*	*DNA region*	*Primer name*	*Primer sequence 5'-3'*	*Reference*
Plants	*Chloroplast*	*trnL (UAA)*	*g (forward)*	*GGGCAATCCTGAGCCAA*	*Taberlet et al.*[3]
			h (reverse)	*CCATTGAGTCTCTGCACCTATC*	
Arthropods & mollusks	*Mitochondrial*	*16S mtDNA*	*16SMAV-F*	*CCAACATCGAGGTCRYAA*	*De Barba et al.*[4]
			16SMAV-R	*ARTTACYNTAGGGATAACAG*	
Earthworms	*Mitochondrial*	*16S mtDNA*	*ewD (forward)*	*ATTCGGTTGGGGCGACC*	*Bienert et al.*[5]
			ewE (reverse)	*CTGTTATCCCTAAGGTAGCTT*	

Supporting Information Table S3: Frequency of occurrence of different items in the diet of wood mouse both in spring and in autumn, as well as in the two seasons. Number of mice is indicated in brackets. Difference of frequency of occurrence between the two seasons is tested by the chi-square goodness-of-fit test. The statistic and the p-value of the test are mentioned, and an asterisk (*) denotes significant difference by the test.

Taxonomic group	**Frequency of occurrence** *(Number of mice)*			**Chi-squared test within the seasons**	
	General *(200)*	Spring *(103)*	Autumn *(97)*	***Statistic***	***P-value***
Plant	96.5% *(193)*	94.2% *(97)*	99.0% *(96)*	2.13	0.145
Arthropod	72.5% *(145)*	70.9% *(73)*	74.2% *(72)*	0.14	0.710
Earthworms	27.0% *(54)*	24.3% *(25)*	29.9% *(29)*	0.54	0.462
Betulaceae	12.5% *(25)*	11.7% *(12)*	13.4% *(13)*	0.03	0.873
Fagaceae	10.0% *(20)*	17.5% *(18)*	2.1% *(2)*	11.53	< 0.001*
Oleaceae	5.0% *(10)*	6.8% *(7)*	3.1% *(3)*	21.38	< 0.001*
Poaceae	16.0% *(32)*	3.9% *(4)*	28.9% *(28)*	21.38	< 0.001*
Rosaceae	30.5% *(61)*	34.0% *(35)*	26.8% *(26)*	0.90	0.343
Sapindaceae	36.5% *(73)*	56.3% *(58)*	15.5% *(15)*	34.22	< 0.001*
Salicaceae	25.5% *(51)*	38.8% *(40)*	11.3% *(11)*	18.46	< 0.001*
Sequence group Adoxaceae, Asteraceae and Cornaceae	54.0% *(108)*	31.1% *(32)*	78.4% *(76)*	48.48	< 0.001*

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(1) 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.
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(3) 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 trnL (UAA) Intron for Plant DNA Barcoding. *Nucleic Acids Res.* **2007**, *35* (3), e14.
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(5) 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.

III.4 Chapter 4

Abstract and keywords in French.

Résumé :

Certains éléments traces métalliques (ETM) sont dits essentiels car ils possèdent des fonctions biologiques cruciales pour la physiologie des organismes (le Fe dans l'hémoglobine par exemple). Ils peuvent exercer des effets délétères sur les organismes en cas de carence ou d'exposition à des doses extrêmement élevées. Les ETM non essentiels, qui n'ont pas de fonction biologique connue, peuvent exercer des effets toxiques à faible concentration. L'exposition d'organismes à des ETM non essentiels peut, en plus d'effets toxicologiques directs, perturber l'homésostasie des éléments essentiels (l'exposition au cadmium, un analogue du calcium, peut perturber la régulation de ce dernier). La perturbation de la composition en éléments essentiels dans des plantes cultivées due à la contamination par des éléments non-essentiels, tels que le Cd ou le Pb, a été documentée, ainsi que les répercussions éventuelles pour la santé des consommateurs (animaux domestiques, homme). En revanche, l'éventuelle modification de la composition en éléments essentiels de plantes sauvages sur des sites contaminés par des ETM n'a pas fait lobjet d'études à notre connaissance. Dans ce chapitre, la composition biochimique de 3 espèces végétales (*Acer speudoplatanus* (*Sapindaceae*), *Salix caprea* et *Populus* sp. (*Salicaceae*)) a été étudiée le long d'un gradient de contamination en ETM, plus précisément dans les environs de l'ancienne fonderie de plomb et de zinc Metaleurop Nord. Les concentrations en Cd et en Zn sont plus élevées chez les *Salicaceae*, connues comme hyper-accumulatrices de Cd, que chez les *Sapindaceae*. En revanche, les concentrations en Pb ne diffent pas. La concentration en Pb est négativement corrélée avec la concentration en phosphore (P), azote (N) et en soufre (S) dans les trois plantes. Les corrélations entre les concentrations en éléments essentiels et non-essentiels varient d'une espèce à l'autre. Ces résultats suggèrent que la contamination d'un site par les ETM peut modifier la composition biochimique des ressources végétales, et entrainer des effets indirects (carence en éléments nutritionnels) pour leurs concommateurs, ce qui reste à étudier.

Mots-clés : composition biochimique, macroéléments, éléments traces métalliques, hyper accumulateurs

Scientific manuscript in preparation for publication

Change in elementary composition of plants in a polluted area.

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# both authors contributed equally to supervising this work

Abstract

Some trace metals (TMs) are considered as essential because they have biological functions that are crucial for the physiology of organisms (Fe in hemoglobin, for example). They can have deleterious effects on organisms if their concentrations in organisms are insufficient (deficiency) or if organisms are exposed to extremely high doses. Non-essential TMs, which do not have any known biological function, may cause toxic effects at low concentrations. Exposure of organisms to non-essential TMs can, in addition to direct toxicological effects, disrupt the homeostasis of essential elements (e.g. the exposure to cadmium, a calcium analogue, can disrupt the regulation of calcium). The disruption of essential element composition in cultivated plants due to contamination by non-essential elements, such as Cd or Pb, has been documented, as well as the potential health implications for consumers (domestic animals, humans). The possible modification of the composition of wild plants in essential elements on sites contaminated by TMs, however, has not been the subject of studies to our knowledge. In this chapter, the biochemical composition of 3 plant species (*Acer speudoplatanus* (Sapindaceae), *Salix caprea* and *Populus sp.* (Salicaceae)) was studied along a MTE contamination gradient, more precisely in the vicinity of the former Metaleurop Nord lead and zinc smelter. Cd and Zn concentrations are higher in Salicaceae, known as hyperaccumulators of Cd, than in Sapindaceae. The Pb concentrations, however, do not differ. The Pb concentration is negatively correlated with the concentration of phosphorus (P), nitrogen (N) and sulphur (S) in the three plants. Correlations between concentrations of essential and non-essential elements vary from species to species. These results suggest that contamination of a site by TMs can modify the biochemical composition of plant resources, and lead to indirect effects (lack of nutritional elements) for their consumers, which remains to be studied.

Keywords

Biochemical composition, bioconcentration factor, trace metals, macro-elements, hyper accumulators

Introduction

Trace metals (TMs) are considered as widespread chemical pollutants. Although they occur naturally in the environment, human activities such as industries are mainly responsible for a large part of global emissions (Nriagu, 1979, 1989). TMs released in the environment are then partly assimilated by organisms. Although some metals are essential elements and required for various biochemical and physiological functions of organisms (WHO, 1996), biochemical or physiological dysfunctions by metals depends on their range of optimal intakes for maintaining their biological performance (i.e. window of essentiality) (Walker et al., 2012). Both a deficiency and an over-intake cause dysfunctions for essential metals. Non-essential elements such as Cd and Pb show no established biological functions. Threshold of non-essential metals for an over-intake is lower than essential metals. Moreover,, it has been widely reported that uptake, transport, and subsequent distribution of essential elements in vegetation can be affected by non-essential TMs (Nagajyoti et al., 2010; Siedlecka, 1995). For example, Cd can interfere with the uptake, transport, and use of several essential elements such Ca, Mg, Mn, P, S and K in plants (Das et al., 1997; Kabata-Pendias, 2011). Plants contaminated by Cd often show Fe deficiency (di Toppi and Gabbrielli, 1999). High exposure to Pb decreases concentrations of divalent cations such as P, S, Ca, Fe, Mg or Mn in leaves of edible crops such as cabbage *Brassica oleracea* (Sinha et al., 2006), cowpea *Vigna unguiculata* (Kopittke et al., 2007), or radish *Raphanus sativus* (Gopal and Rizvi, 2008). An excess of Zn, one of the essential metals in plants, also induces a photosynthetic dysfunction by replacing Mg in chlorophyll structure (Tsonev and Lidon, 2012). Such effects of TM contamination on elemental composition of edible crops for humans have been widely reported to evaluate impacts of TM contaminations on human consumers.

Terrestrial wildlife is also exposed to TMs mainly via oral routes (Shore and Rattner, 2001). Some ecological works have been investigated for measuring impacts of plant TM levels on their vertebrate consumers (e.g. Hunter et al., 1987a, 1987b; Mertens et al., 2001; Rogival et al., 2007) In most of ecotoxicological works under phytoremediation programs, TM concentrations in plant tissues have been measured under field conditions. For example, Migeon et al. (2009) showed difference in TM concentrations in leaves and stems of several woody plants observed in TM contaminated sites. Deram et al. (2007) measured bioaccumulation of Zn and Cd in a perennial metallicolous grass with a high biomass production, *Arrhenatherum elatius*. Some plants such as Brassicaceae family plants show high metal concentrations in tissues and/or high bioconcentration factor (van der Ent et al., 2013). They are known as “accumulator” or even “hyper-accumulator”. Among woody species, *Salix* sp. or *Populus* sp. (Salicaceae family) are considered as TM accumulators and often used for phytoremediation programs (Pulford and Watson, 2003). Potential impacts of such hyper accumulator plants on wild animals have been discussed on the basis of their high TM concentrations (Brekken and

Steinnes, 2004; Nolet et al., 1994). However, gastrointestinal uptake of ingested TMs is regulated by nutrient quality of food in mammals (Nordberg et al., 2014; Shore and Rattner, 2001). Although high TM concentrations in food increase trophic exposure of wild mammals to TMs, lack of data about composition of other elements in those accumulators could bias the evaluation of their impacts on wildlife. Currently, documentations about elemental composition of TM contaminated woody plants under natural conditions are poorly found in the literature (e.g. Farahat and Linderholm, 2015). However, nutrient uptake, translocation between different organs and strategy for adaptation to nutrient stress widely differs between species, depending on their life histories, growth stages, and/or environmental conditions (Chapin III, 1980). Chemical responses of woody plants to TM contamination are thought to differ between species, especially between species showing different sensibility to TMs.

In this chapter of the thesis, elemental composition of young leaves, organs potentially consumed by small mammals, were measured for two plant families considered as to be hyper TM accumulator (Salicaceae plants) and low TM accumulators (Sapindaceae plants) widely observed in the surrounding of the former Pb and Zn smelter Metaleurop Nord in Northern France. We hypothesized that high and low TM accumulator plant species would show different elemental compositions, not only for non-essential contaminants but also for essential elements. Elemental composition of different organs was compared to estimate their contribution to exposure of small mammals. Accumulation patterns in leaves of three TMs, Cd Pb and Zn, along a gradient of soil TM contamination levels were also computed. Finally, bioconcentration factors were calculated for the three TMs. Their potential change along a gradient of plant diversity indices were analyses to complete information for this present thesis.

Materials and Methods

For details of the study sites, preparation of materials, chemical analyses and statistical analyses, see the General Material and Methods part above. Briefly, sampling was realized in spring 2017, from 10th to the 21st of April, in 38 woody patches within seven sites (25 ha) in the surrounding of Metaleurop Nord. Only leaves of sycamore maples (*Acer pseudoplatanus*, Sapindaceae), goat willows (*Salix caprea*, Salicaceae) and poplar tree (*Populus* sp, Salicaceae) were sampled because of their availability in the field in the sampling period. For each species, young leaves of randomly selected five trees were picked at about 1-2m height. Female catkins of *S. caprea* could be also picked up in the patches where *S. caprea* were presents. The samples were stored at -20 °C and transported to the laboratory.

For each species, samples taken from the same patches were mixed and then freeze-dried and grinded. The freeze-dried powders were used for measuring concentrations of 25 elements (Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, P, Pb, S, Sb, Se, Si, Sn, Sr, Ti and Zn) by ICP-MS and content of C, N and S by elemental analyzer (expressed as μg g^{-1} DM).

Numbers of samples per species were 34, 13 and seven for *A. pseudoplatanus*, *S. caprea* and *Populus* sp., respectively.

Concentrations of each element, C/N ratio and N/P ratio in leaves of the three species were compared by the Kruskal-Wallis test. Concentrations of each element, C/N ratio and N/P ratio between leaves and female catkins of *S. caprea* were compared by the Wilcoxon-Mann-Whitney test. PCA was applied to concentrations of all elements in leaves of the three species to describe correlations between each element. Centroid positions of three species and 95% confidence ellipses for those centroid positions were projected on dimensionally reduced space. Correlations between concentrations of Cd, Pb and Zn in leaves and concentrations of the other elements in leaves were checked by the Pearson's correlation test for each species.

Concentrations of Cd, Pb and Zn in leaves in relation to soil TM concentrations were analysed by LM. Soil TM concentrations, species and their interactions were used as explanatory variables, and their significance were checked by ANOVA. If assumptions of linear regression were not met, the Spearman's correlation test was applied for each species.

Bioconcentration factors for Cd, Pb and Zn in leaves were compared between the three species by the Kruskal-Wallis test. On the other hand, change in bioconcentration factors for the three TMs in leaves of *A. pseudoplatanus* along the gradient of diversity indices of plants in the field was assessed in this chapter of the thesis by LM or the Spearman's correlation test, depending on assumptions of linear regression for the general discussion.

Results and discussion

Concentration of elements in plant tissues.

Concentrations of Cd, Co and Zn in leaves of Salicaceae plant family (*S. caprea* and *Populus* sp.) were significantly higher than *A. pseudoplatanus* (*p-value* < 0.05) (Table 1). In the study of Migeon et al. (2009) showing concentrations of TMs in woody species observed in the surroundings of Metaleurop Nord, concentrations of Cd in leaves of Salicaceae family plants (*Salix alba*, *S. caprea* and*Populus* sp.) were largely higher than Sapindaceae plants (*Acer campestre* and *A. pseudoplatanus*) both in June and October. This indicates that even young leaves could differently contribute to exposure of small mammals to TMs. However, other essential trace and macro-nutrients also differ between the three species. Concentrations of Mo and N/P ratio in leaves of *A. pseudoplatanus* were significantly higher than Salicaceae plant family. Moreover, concentrations of Na in leaves of *A. pseudoplatanus* were significantly higher than *S. caprea* but not significantly different of poplar tree. Concentrations of Ca, K and Mg in leaves of poplar tree were higher than the other species. Concentrations of S in leaves of poplar tree were also the highest. Concentrations of Ni and C were the highest in leaves of *S. caprea*, but concentrations of B in leaves of *S. caprea* were significantly lower than in the other species. Macro-elements such as Ca, P, and Mg are sometimes considered as main antagonistic elements

in the absorption of several micro-cations (Kabata-Pendias, 2004). Absorption of non-essential TMs such as Cd or Pb can be inhibited also by other essential TMs such as Cu, Mn or F (Kabata-Pendias, 2011). Higher concentrations of some macro-elements in Salicaceae plants could serve as inhibitor factor for transfer of TM to small mammals. Concentrations of the other elements, including Pb, were not significantly different between the three species.

Concentrations of Cd, Pb and Zn in catkins were not significantly different from concentrations in leaves (Table 2). This concurs with the results of Madejón et al. (2013). They demonstrated that Cd and Pb concentrations in green capsule of catkins of *P. alba* were as high as in leaves in TM contaminated sites in Spain. Both leaves and catkins could equally contribute to trophic exposure to TMs, if mice ate those organs. However, concentrations of Na and C/N ratio were significantly higher in catkins that in leaves of *S. caprea*, whereas concentrations of Mg, P, Ti, N and C, as well as N/P ratio, were significantly higher in leaves. The nutrient composition of catkins was different from the composition of leaves, which indicates that different organs could different roles as resources of animals.

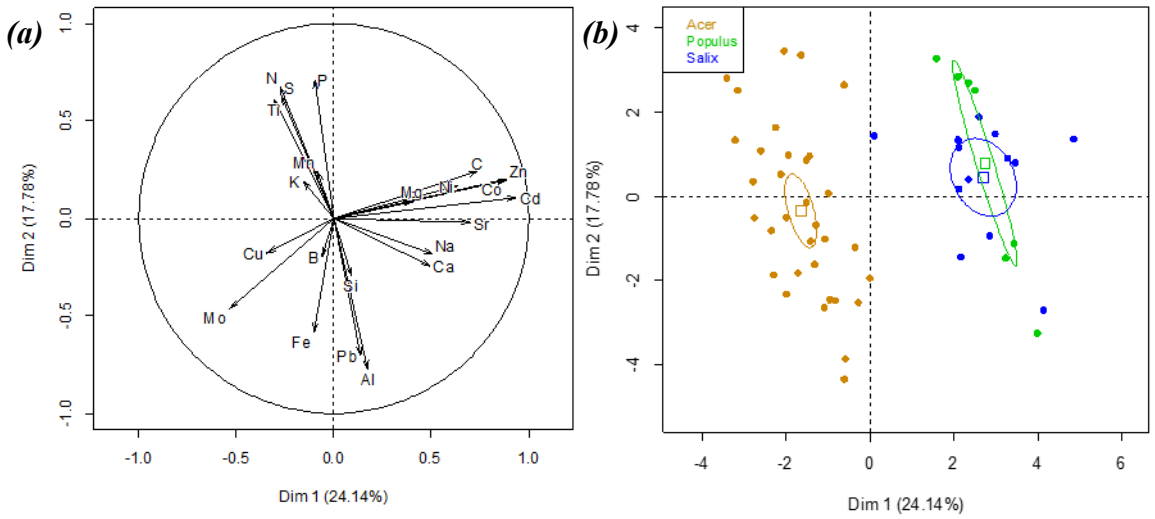

Figure 1. Biplot of PCA on composition of elements in leaves of A. pseudoplatanus, Populus sp. and S. caprea for representing (a) correlations between concentrations of elements and (b) difference of composition between samples with 95% confidence ellipse for the centroid position of each species.

Table 1. Minimum, median and maximum values of concentrations of elements in leaves of A. psuedoplatanus, Popular sp. and S. caprea. Significant differences of concentrations between plant species under the Kruskal-Wallis test (p-value < 0.05) are represented by letters beside median values (in descending order). Mean values sharing the same letter do not significantly differ.

Concentration in leaves (µg g^{-1})		Al	B	Ca	Cd	Co	Cu	Fe	K	Mg	Mn	Mo	Na
Acer	Min.	1.16	12.78	3056	<0.01	<0.01	0.76	5.96	13707	1244	1.41	< 0.01	12.41
pseudoplatanus	Median	2.20	18.53 **a**	6545 **b**	0.03 **b**	0.01 **b**	1.20	9.07	18394 **b**	1796 **b**	2.95	0.04 **a**	35.31 **a**
(N=25)	Max.	12.74	29.55	9557	0.15	0.04	1.67	21.54	23758	2205	28.49	0.19	75.94
Populus sp.	Min.	0.90	18.17	7594	0.36	0.03	0.79	5.30	18487	1820	0.80	0.01	30.88
(N=7)	Median	1.59	20.40 **a**	11413 **a**	0.99 **a**	0.07 **a**	1.07	7.46	22771 **a**	2552 **a**	1.33	0.01 **b**	69.25 **ab**
	Max.	13.26	34.10	13360	4.17	0.11	1.28	15.39	34840	2776	6.14	0.02	209.27
Salix caprea	Min.	1.51	13.23	5045	0.41	0.03	0.73	5.90	14122	1455	1.07	0.01	17.93
(N=12)	Median	2.82	14.18 **b**	7105 **b**	0.75 **a**	0.06 **a**	1.14	8.27	16084 **b**	1820 **b**	4.50	0.02 **b**	70.50 **b**
	Max.	14.56	18.10	7968	6.35	0.30	1.73	18.48	17595	2742	27.71	0.06	140.48

Concentration in leaves (µg g^{-1})		Ni	P	Pb	S	Si	Sr	Ti	Zn	N	C	C/N ratio	N/P ratio
Acer	Min.	0.11	3269	0.03	2070	76.01	0.45	0.52	5.69	28652	439996	8.0	5.4
pseudoplatanus	Median	0.20 **b**	5071	0.06	3086 **ab**	90.97	1.43	0.78	7.48 **b**	40997	455427 **b**	10.9	8.1 **a**
(N=25)	Max.	0.40	8036	2.60	4822	111.58	6.44	1.08	14.50	57297	460234	15.9	10.8
Populus sp.	Min.	0.17	4178	0.03	2881	86.19	1.89	0.58	24.17	30975	456093	10.1	5.7
(N=7)	Median	0.24 **ab**	6021	0.06	3835 **a**	97.88	4.07	0.78	28.13 **a**	40452	462307 **ab**	11.3	6.3 **b**
	Max.	0.38	7748	0.55	4485	113.75	6.39	0.97	48.46	45633	467176	15.1	7.6
Salix caprea	Min.	0.16	4919	0.03	2530	42.61	1.66	0.68	15.12	34601	462012	11.4	5.7
(N=12)	Median	0.32 **a**	5709	0.09	2711 **b**	96.56	3.07	0.80	22.74 **a**	40035	473065 **a**	11.8	7.0 **b**
	Max.	1.06	6824	2.27	3205	115.15	18.58	0.95	42.19	41473	482353	13.9	7.8

Table 2. Minimum, median and maximum values of concentrations of elements in female catkins of S. caprea. Significant differences of concentrations in leaves and catkins under the Wilcoxon-Mann-Whitney test (p-value < 0.05) are represented by capital letters beside median values (L: concentrations in catkins were significantly lower than leaves; H: concentrations in catkins were significantly higher than leaves).

Concentration in catkins (µg g^{-1})		Al	B	Ca	Cd	Co	Cu	Fe	K	Mg	Mn	Mo	Na
Salix caprea	Min.	1.61	10.23	3903	0.14	0.02	0.61	6.19	11553	1059	1.04	0.01	28.22
(N=12)	Median	3.96	16.65	6384	0.73	0.06	1.01	9.98	15677	1458 **L**	4.48	0.02	125.25 **H**
	Max.	25.68	19.18	13400	8.20	0.29	1.85	34.92	16703	2128	18.18	0.04	334.41

Concentration in catkins (µg g^{-1})		Ni	P	Pb	S	Si	Sr	Ti	Zn	N	C	C/N ratio	N/P ratio
Salix caprea	Min.	0.10	3930	0.04	2206	79.07	1.20	0.54	9.11	25852	407024	12.14	5.55
(N=12)	Median	0.27	4505 **L**	0.14	2718	107.17	3.56	0.64 **L**	19.86	28116 **L**	423550 **L**	15.03 **H**	6.24 **L**
	Max.	0.79	5625	3.91	3164	153.68	17.22	0.76	64.34	34759	431745	16.70	6.97

Correlations between concentrations of elements in leaves.

PCA clearly demonstrated difference in elemental composition of the three plants. The first and second principal components explained 24% and 18 % of variation in concentration of elements in leaves of the three species, respectively. Cd, Zn, Co, C, Sr, Ni, Na, Ca and Mg were significantly and positively correlated with the first principal component axis, whereas Mo, Cu and Ti were negatively correlated with the axis (Figure 1a). These elements also distinguished the elemental composition of elements in leaves of *A. pseudoplatanus* and of Salicaceae family plants (Figure 1b). Interactions between Cd and Zn are commonly observed as both antagonism and synergism, whereas Cd-Cu and Zn-Cu antagonistic interactions were often observed in the uptake–transport processes (Kabata-Pendias, 2011). In the study of Assad et al. (2018), poplar planted in TM contaminated substrates showed high accumulation of Cd and Zn but low particular accumulation of Cu and Pb in leaves. Elemental composition of Salicaceae and Sapindaceae plants were clearly distinguished by some elements like C, Sr, Ni, Na, or Ca. On the other hand, P, N, S and Ti were significantly and positively correlated with the second principal component axis, whereas Al, Pb, Mo and Fe were negatively correlated with the axis. This is in accordance with the results of Sinha et al. (2006) demonstrating negative effects of Pb on concentrations of P or S in leaves of *B. oleracea*. Such effects of Pb were observed in the three species.

Correlations between concentrations of elements in leaves differed between species (Table 3). Concentrations of Pb were never correlated with concentrations of N, C or Mg in leaves of the three species. However Concentrations of Pb were negatively correlated with concentrations of P in poplar trees and concentrations S in poplar trees. Likewise, concentrations of Zn were negatively correlated with concentrations of P in *S. caprea*.

Table III.3. Spearman's ρ for correlations between concentrations of Cd, Pb and Zn and other elements in leaves of A. pseudoplatanus, Populus sp. and S. caprea. Significance of correlation by Spearman's correlation test is represented by an asterisk (: p-value <0.05, **: p-value <0.01 and ***: p-value <0.001).*

Concentration in leaves (µg g^{-1})		Al	B	Ca	Cd	Co	Cu	Fe	K	Mg	Mn	Mo
Acer pseudoplatanus (N=25)	**Cd**	**0.36 ***	-0.32	-0.84	-	0.18	0.03	**0.38 ***	0.16	0.16	0.02	-0.01
	Pb	**0.93 *****	0.10	-0.05	**0.37 ***	0.19	0.17	**0.70 *****	0.24	0.25	**-0.42 ***	**0.47 *****
	Zn	0.30	0.05	-0.26	**0.38 ***	**0.44 ***	**0.37 ***	0.22	**0.44 ***	0.10	-0.24	-0.03
Populus sp. (N=7)	**Cd**	0.58	0.38	0.43	-	-0.09	**0.77 ***	0.59	-0.45	0.13	-0.45	0.45
	Pb	**0.95 ****	**0.85 ***	0.56	0.68	0.43	**0.83 ***	0.44	0.23	0.10	-0.43	**0.86 ***
	Zn	0.66	0.43	0.41	**0.94 ****	-0.02	0.72	0.60	-0.45	0.13	-0.22	0.44
Salix caprea (N=12)	**Cd**	-0.07	0.10	-0.26	-	**0.71 ****	-0.45	0.03	0.10	-0.09	0.31	-0.10
	Pb	**0.91 *****	0.36	0.13	0.04	0.46	0.06	**0.86 *****	-0.51	0.54	-0.06	0.31
	Zn	-0.09	-0.13	**-0.56 ***	**0.88 *****	**0.73 ****	-0.40	0.01	-0.05	-0.13	0.32	-0.17

Concentration in leaves (µg g^{-1})		Na	Ni	P	Pb	S	Si	Sr	Ti	Zn	N	C
Acer pseudoplatanus (N=25)	**Cd**	-0.01	0.08	-0.22	**0.37 ***	-0.28	0.22	0.07	-0.12	**0.38 ***	-0.30	-0.06
	Pb	**0.51 ****	-0.08	-0.01	-	**-0.39 ***	**0.35 ***	-0.04	0.05	**0.38 ***	-0.26	-0.27
	Zn	0.03	-0.04	0.23	**0.38 ***	0.05	-0.12	-0.01	0.21	-	0.18	0.18
Populus sp. (N=7)	**Cd**	-0.34	-0.22	-0.71	0.68	-0.34	0.40	0.37	-0.75	**0.94 ****	-0.37	0.17
	Pb	0.34	0.02	**-0.88 ****	-	-0.74	**0.89 ****	0.24	**-0.86 ***	0.73	-0.67	0.18
	Zn	-0.12	-0.40	**-0.76 ***	0.73	-0.47	0.50	0.11	**-0.78 ***	-	-0.55	0.34
Salix caprea (N=12)	**Cd**	0.04	0.46	0.11	0.04	**0.61 ***	0.18	-0.17	0.04	**0.88 *****	0.30	0.05
	Pb	0.43	0.41	-0.33	-	0.45	0.29	0.20	-0.42	0.04	0.05	0.22
	Zn	0.04	0.37	0.08	0.04	**0.65 ***	0.21	-0.06	-0.01	-	0.52	0.29

Concentrations of Cd, Pb and Zn in leaves along the gradient of soil TM concentrations.

Concentrations of Cd and Zn in leaves were significantly explained by concentrations of each TMs in soils, but the statistical interaction between soil TM concentrations and species was not significant (Figure 2a, b). This indicates similar patterns of increase with soil pollution level whatever was the species. Exposure of mammals to TMs increase along a gradient of environmental TM concentrations. The coefficients R^2 were 0.89 and 0.87 for Cd and Zn, respectively. This indicate that animals consuming the plant species could be more importantly exposed to Cd and Zn along the gradient of a soil TM concentrations in the field. On the other hand, homoscedasticity of residuals was not met for a linear regression for Pb. Concentrations of Pb in leaves and in soils were significantly and positively correlated for all of the three species (p-value < 0.01; Spearman's ρ was 0.63, 0.92 and 0.85 for *A. pseudoplatanus*, poplar trees and *S. caprea*, respectively) (Figure 2c).

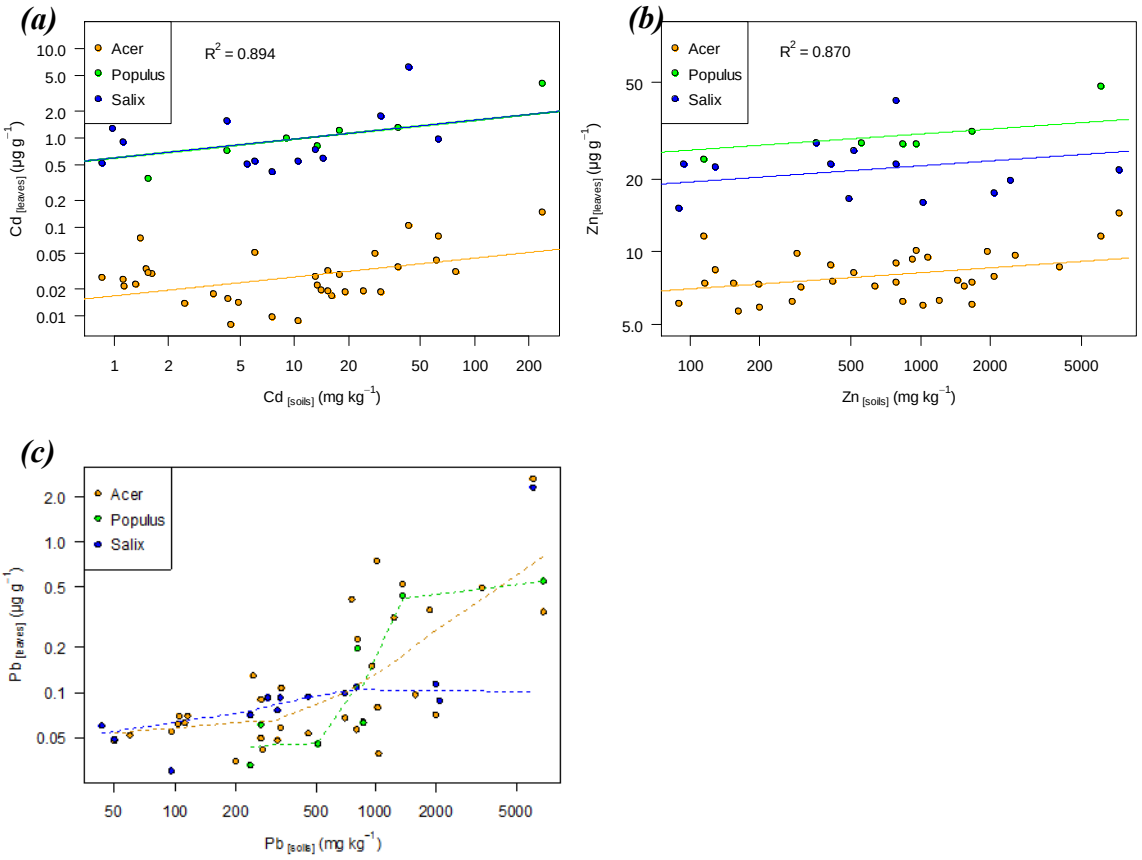

Figure III.2. Concentrations in leaves of A. pseudoplatanus, Populus sp. and S. caprea (µg g^{-1} of dry matter) along the gradient of concentrations in soils (mg kg^{-1} of dry soil) for (a) Cd, (b) Zn and (c) Pb. Plotted points indicate leaves, and species are represented by different colors. Values predicted from linear models are indicated by normal lines for concentration of Cd (a) and Zn (b), whereas a trend of relationship between concentrations of Pb in leaves and soils (c) by LOWESS smoother is represented by dotted lines.

Bioconcentration factors.

Bioconcentration factors for Cd in leaves ranged from 0.00062 to 0.054 (median: 0.0024), from 0.017 to 0.23 (median: 0.068) and from 0.016 to 1.3 (median: 0.092) for *A. pseudoplatanus*, poplar tree and *S. caprea*, respectively. Bioconcentration factors for Zn in leaves ranged from 0.0019 to 0.10 (median: 0.012), from 0.0080 to 0.21 (median: 0.034) and from 0.0030 to 0.25 (median: 0.052), respectively. Bioconcentration factors for Pb in leaves of the three species were < 0.01. Bioconcentration factor for Cd in leaves of Salicaceae family were significantly higher than *A. pseudoplatanus* (*p-value* < 0.001), whereas bioconcentration factors for Pb and Zn between the three species were not different. However, these values were lower than bioconcentration factors measured by Migeon et al. (2009): bioconcentration factors for Cd, Pb and Zn in leaves of *S.caprea* reached 1.42, 0.09 and 0.80, respectively. Bioconcentration factors were not significantly different between leaves and catkins of *S. caprea*.

On the other hand, no change in bioconcentration factors for TMs in leaves of A. pseudoplatanus were observed along the gradient of plant diversity indices.

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III.5 Chapter 5

Abstract and keywords in French.

Résumé :

Le rôle de la biodiversité sur le fonctionnement des écosystèmes est un questionnement majeur en écologie. L'effet de dilution, à savoir le rôle protecteur de la biodiversité vis-à-vis de la transmission d'agents pathogènes et le risque de maladies, a été largement documenté dans la communauté scientifique. En revanche, les effets potentiels de la biodiversité sur le transfert et la contamination des organismes par des polluants ont été peu étudiés jusqu'à présent. L'exposition à certains polluants, comme les éléments traces métalliques (ETM), se fait principalement par transfert trophique chez les mammifères ou les oiseaux. Considérant certains similarités dans les mécansimes de transmission des pathogènes et dans le transfert d'ETM dans les réseaux trophiques, nous posons l'hypothèse que l'exposition et la contamination des mammifères aux ETM pourraient être influencées par la diversité de leurs ressources, créant un effet de dilution. Dans cette étude, l'exposition trophique des mulots sylvestres (Apodemus sylvaticus) aux ETM et la contamination de leurs tissus par ETM ont été étudiées en fonction de la diversité de leurs ressources disponibles sur le terrain (plantes et invertébrés), dans une zone contaminée par les ETM. Les ressources potentielles induisant des effets de dilution ont également été évaluées. Les résultats ont démontré que l'exposition et la contamination des mulots aux ETM diminuaient quand la richesse des plantes augmentait. Cela indiquerait l'existence de l'effet de dilution dans le transfert d'ETM chez les mammifères terrestres. Certaines plantes, en particulier les Adoxaceae (Sambucus sp.) et les Salicaceae (Populus sp. et Salix sp.) sont supposées être des ressources impliquées dans les effets de dilution dans notre zone d'étude. Bien que d'autres études sur les mécanismes et les conditions soient nécessaires, nous proposons une nouvelle fonction écologique potentielle de la biodiversité comme pouvant réduire le transfert de contaminants chimiques dans les réseaux trophiques.

Mots-clés : *Apodemus sylvaticus*, biodiversité, effet de dilution, fonctionnement des écosystèmes, réseaux trophique

Scientific manuscript in preparation for publication

Is biodiversity good for health in a polluted area? A test on trace metal exposure and contamination in a small mammal

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Abstract

The importance of biodiversity on ecosystem functioning has recently attracted much attention. The dilution effect, i.e. a protective effect of high community diversity against transmission of pathogens and disease risk, has been widely documented. In contrast, potential effects of biodiversity on pollutant transfer and contamination have been poorly studied so far. Mammal or bird exposure to pollutants like trace metals (TMs) is performed mainly via trophic transfer. Considering similarities in mechanisms of pathogens transmission and TMs transfer in food webs, we hypothesise that both the trophic exposure of mammals to TM and TM bioaccumulation in their tissues would be influenced by diversity of their resources, as a kind of dilution effect. We tested this hypothesis using wood mice (*Apodemus sylvaticus*) and its resources in the field, plants and invertebrates, in a TM contaminated area. Potential resources inducing dilution effects were assessed. Our results demonstrated that exposure to TMs and TM bioaccumulation of wood mice decreased when plant richness increased. This suggested the existence of a dilution effect in TM transfer and contamination in terrestrial mammals. Plants like Adoxaceae (*Sambucus* sp.) and Salicaceae (*Populus* sp. and *Salix* sp.) were supposed to be resources involved in the dilution effect in our study area. Although further studies on its mechanisms and conditions are required, we propose a new potential ecological function of biodiversity against chemical contamination and thus for welfare of wildlife.

Keywords

Apodemus sylvaticus, Biodiversity; Dilution effect; Ecosystem function; Food webs

Introduction

In their essay, Dobson et al. (2006) highlighted the importance of '*sacred cows and sympathetic squirrels*' for human health. These species served as flagship examples of zooprophylaxis, i.e. the influence of non-reservoir hosts on transmission of vector-borne diseases to human (reviewed in Service, 1991), because they receive bites from infected vectors and can lower the level of pathogen transmission to humans. Interests in potential effects of species diversity on disease risk has recently attracted much attention, in part due to hot debates about the importance of biodiversity on ecosystem functioning (e.g. Loreau, 2001; Cardinale et al., 2012; Mace et al., 2012). During the last decades, a number of studies have identified that changes in biodiversity affect the risk of infectious disease exposure in plants and animals, including humans (Keesing et al., 2010; Ostfeld and Keesing, 2012; Johnson et al., 2015). It is termed as '*dilution effect*' the fact that an increase in species diversity leads to a net decrease in disease risk (Keesing et al., 2006). A dilution effect is expected to occur when (i) "*hosts differ in quality for pathogens or vectors*", (ii) "*higher quality hosts* (i.e. amplification hosts) *tend to occur in species-poor communities, whereas lower quality hosts* (i.e. dilution hosts) *tend to occur in more diverse communities*", and (iii) "*lower quality hosts regulate abundance of high-quality hosts or of vectors, or reduce encounter rates between these (high-quality) hosts and pathogens or vectors*" (Keesing et al., 2006; Ostfeld and Keesing, 2012). Although the opposite pattern, called '*amplification effect*', can be also observed in nature, biodiversity frequently serves as protector against disease transmission (Keesing et al., 2010; Ostfeld and Keesing, 2012).

Trace metals (TMs) are non-degradable chemical contaminants widely spread in the environment. Mammals are considered to be exposed to TMs mainly through oral consumption of contaminated food, and chronic exposure to TMs may cause health problems, e.g. renal dysfunction, hepatocellular necrosis, bone injury (Shore and Rattner, 2001; Eisler, 2000; (Nordberg et al., 2014)). Positive correlations between TM contamination levels in mammals' tissues and in their main food resources available in the field have been demonstrated (e.g. Hunter et al., 1987; Mertens et al., 2001; Rogival et al., 2007). However, the effect of biodiversity on both oral transfer of pollutants and/or accumulation of pollutants in animal tissues has never been considered to the best of our knowledge. Though, there are similarities between pollutant transfers in food webs and pathogen transmissions in animal communities. The first condition of the dilution effect listed above would correspond to a variability in TM accumulation capacities in food resources. Certain plants are indeed known as '*hyper-accumulator*' of TMs because they accumulate high concentrations of TMs in their tissues compared to other species (van der Ent et al., 2013; Pollard et al., 2014),. Similarly, some invertebrate taxa accumulate metals like snails or isopods at higher concentrations than others (e.g. carabid beetles) due to direct contact with soil, a diet containing metal-rich food and/or

physiological abilities to store metals in their bodies (e.g. Heikens et al., 2001). The second condition corresponds to the sensibility to biodiversity loss. Harmful effects of TMs usually trigger a degradation of species diversity and/or a modification of species composition in both plant (e.g. Zvereva et al., 2008; Dazy et al., 2009) and invertebrate communities (e.g. Spurgeon and Hopkin, 1996; Nahmani and Lavelle, 2002; Zvereva and Kozlov, 2010). Tolerance to TMs is often linked to high TM accumulation capacity (Gall et al., 2015), which may explain the presence of TM accumulators in degraded ecological communities of highly contaminated sites. The third condition is linked to the feeding ecology of animals. Avoidance of TM contaminated food has been reported in invertebrates like isopods (Odendaal and Reinecke, 1999; Zidar et al., 2004) or small moths (Scheirs et al., 2006) under laboratory conditions. In mammals, wood mice (*Apodemus sylvaticus*) have been shown to display avoidance against TM contaminated food under laboratory settings (Beernaert et al., 2008) and to lower their preference for TM accumulating plants (Salicacae) along a pollution gradient in natural conditions (Ozaki et al., 2018).

In the present study, we hypothesized that TM exposure and/or concentrations in tissues of the wood mouse would be negatively correlated with the diversity of their resources, because of a dilution effect. In this case, resources less and more competent in TM transfer, i.e. '*dilution*' and '*amplification resources*', respectively, should be resources (i) in which TM concentrations are respectively lower or higher than in others (i.e. difference in TM accumulation capacity), and (ii) which tend to occur respectively in diversity-rich or -poor communities, (i.e. relationship between occurrence and community diversity). When the two types of resources co-exist, (iii) dilution resources should be more abundantly and/or frequently consumed in diversity-rich communities (i.e. important feeding dilution resources in diversity-rich communities). The trophic exposure of wood mice to TMs, as assessed by TM concentration in stomach content and TM contamination levels in their tissues, were compared to diversity of their resources surveyed in a TM contaminated site in northern France. We also assessed candidate dilution or amplification resources in TM transfer and contamination and discussed the three hypothetical mechanisms underlying the dilution effect.

Materials and methods:

Study sites and biological model

Wood mice were chosen as our biological models because of their well-known ecology and diet. They are small rodents widely spread in Europe (Quéré and Le Louarn, 2011) and frequently used for monitoring effects of environmental metal pollution on mammals (e.g. González et al., 2006; Sánchez-Chardi et al., 2007; Tête et al., 2014a). They consume both plant and invertebrate preys, and their diet shows high adaptability according to season and habitats (e.g. Watts, 1968; Butet, 1986; Montgomery and Montgomery, 1990; Rogers and Gorman,

1995; Abt and Bock, 1998). Wood mice have also shown their preference for some resources (e.g. acorn) (Watts, 1968; Ozaki et al., 2018).

This study was carried out in the surroundings of the former lead (Pb) and zinc (Zn) smelter named '*Metaleurop Nord*' located in Northern France (Noyelles Godault, Hauts-de-France, France). Until its cloture in 2003, the pyro-metallurgic process had generated large quantities of dust for more than a century. For example, about 1.0 ton of cadmium (Cd), 17 tons of Pb and 32 tons of Zn were released from it in 2002, despite the implementation of technical improvements during the 1970s (DRIRE, 2003). With another large Zn smelter which has been working since 1869 in a neighboring town (Auby), namely '*Umicore*', the dust emission has affected an area of around 120 km^2 (Douay et al., 2008). In agricultural top soils, contamination has been shown as high as 21, 1132 and 2167 mg kg^{-1} of dry soil for Cd, Pb and Zn, respectively (Sterckeman et al., 2002). In the study of Fritsch et al. (2010), total concentrations of the pollutants respectively reached 236, 7331 and 7264 mg kg^{-1} of dry soil in soils of woody habitats, largely exceeding the values in reference site of the same study: 0.9–2.4, 43–200 and 89–278 mg kg^{-1} of dry soil.

The present study was undertaken on seven sites of 25 ha (500m x 500m) from an area of 40 km^2 around Metaleurop Nord, based on both landscape feature types and a TM soil contamination gradient (Table 1). Soil properties of woody habitats measured in previous works (Douay et al., 2009; Fritsch et al., 2010) were used in this study (cf. below).

Table 1. Concentrations of cadmium (Cd), lead (Pb) and zinc (Zn) in soils (mg kg^{-1} of dry soil) and dominant landscape feature of each study site based on data published in Fritsch et al. (2010), as well as number of buffers (statistical effective in the present study) per site.

		TE2	103	117	097	171	043	113
$[Cd]_{soils}$ (mg kg^{-1})	Minimum	0.86	1.5	3.6	15.3	4.9	1.3	4.4
	Median	1.4	4.3	9.1	48.3	7.5	15.2	11.5
	Maximum	2.4	6.0	17.8	236.5	14.5	42.7	13.0
$[Pb]_{soils}$ (mg kg^{-1})	Minimum	43.3	237.5	244.7	658.5	287.6	105.0	266.6
	Median	107.4	267.2	512.0	1295.3	584.0	323.1	678.9
	Maximum	199.8	333.0	859.8	6809.4	2063.3	1028.9	806.0
$[Zn]_{soils}$ (mg kg^{-1})	Minimum	89.3	114.4	302.8	1069.3	487.2	153.9	414.7
	Median	168.8	352.7	555.8	1874.7	1362.7	512.8	1001.2
	Maximum	277.7	407.5	958.5	7263.5	2451.5	1549.6	1170.4
Soil contamination level		"Control[a]"	+	++	+++	++	++	++
Landscape feature		Forest	Forest	Forest	Forest	Forest	Arable	Urban
Number of buffers[b]	Two seasons	11	14	9	12	16	10	0
	Spring	*5*	*6*	*4*	*7*	*10*	*4*	*0*
	Autumn	*6*	*8*	*5*	*5*	*6*	*6*	*0*

[a]: TM concentrations as close as possible to background concentrations.
[b]: Buffers used in the statistical analyses in the present study.

Exposure to TM

Field sampling

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. All trap lines were set in woody habitats following Fritsch et al. (2011) and their position was geo-referenced. The trap lines were checked in the morning for three consecutive days and re-set and/or re-baited, if necessary. After being weighed in the field, captured animals were immediately frozen and stored at -20 ºC for further analyses.

In this study, 169 wood mice (80 females and 88 males, and one mouse whose sex could not be identified) captured from 72 trap lines were used (Table 1). Number of mice captured in each trap line ranged from 1 to 6 (median = 2). The sex-ratio was not significantly different from 1:1 (chi-squared = 0.38, p-value = 0.54). The mice were composed of 89 and 80 individuals captured in spring and autumn, respectively. The ratio between the seasons was not significantly different from 1:1 (chi-squared = 0.48, p-value = 0.49). Age was estimated by using body weight according to Vandorpe and Verhagen (1980) and Tête et al. (2014b). Mice were significantly older in spring (median: 157 days) than in autumn (median: 77 days) (Wilcoxon-Mann-Whitney W = 5871.5; p-value < 0.001; see Supporting Information Figure S1).

Measure of TM concentrations

Exposure to TMs was estimated using TM concentrations in stomach contents (SCs). For the detail of measuring TM concentrations in SCs and results, see (Ozaki et al., submitted for publication). Briefly, SC was extracted from each rodent's body thawed at room temperature in laboratory. After removing remaining bait, each SC was dried at 50°C in an oven until constant mass, digested in HNO_3 (67-69 %; Fisher Scientific Bioblock, ultratrace quality (gamme Optima)) with H_2O_2 (Fisher Scientific Bioblock) in a Digiprep (SCP Sciences), and diluted by adding ultra-pure water (18.2 MΩ/cm^2 by Millipore Milli-Q Integral 3).The concentrations of Cd, Pb and Zn were measured by inductively coupled plasma mass spectrometry (ICP-MS: X Series II, ThermoFischer Scientific) and expressed as micrograms per grams of dry mass (µg.g^{-1} DM), using certified reference materials (INCT-OBTL-5: Oriental Basma Tobacco Leaves) for checking analysis accuracy.

TM contamination in mice tissues were estimated using TM concentrations in two organs, liver and kidneys. After extraction from rodent's body, the organs were dried at 60 °C in an oven until constant mass and digested in HNO_3 in a Digiprep. The samples were then diluted by adding ultra-pure water. The concentrations of the TMs were measured with an inductively coupled argon plasma atomic emission spectrometry (ICP-AES: ICAP 6500 Radial Thermos) and expressed as µg.g^{-1} DM. Analysis accuracy was checked by using certified reference materials (TORT-3: Lobster Hepatopancreas and DOLT-5: Dogfish Liver; National Research Council, Canada). As Pb concentrations were below the detection limit in more than 60 % of the samples in the two organs, we used only Cd and Zn concentrations in the two organs in the following analyses. TM concentrations in organs were not significantly different among sex (Wilcoxon-Mann-Whitney W = 3442 and 3240 for Cd and Zn in liver; 3421 and 3556 for Cd and Zn in kidneys; p-value > 0.05; see Supporting Information Figure S2 for details).

Diversity of resources

Field inventory of plant and invertebrate resources

For details of field inventory method for both plant and invertebrate resources, see (Ozaki et al., 2018). Briefly, vegetation survey was realized once in each site from 4th June to 5th September 2012. Vascular plant taxa were identified at species level in the field. Cover-abundance of each taxa was visually estimated as the vertically projected area. Vegetation habitats were determined by plants' composition and delineated as polygons, which were geo-referenced using the QGIS software (ver. 2.18). Invertebrate resources were estimated based on ground-dwelling invertebrates sampled in spring (April) and in autumn (September and October) 2012 by pitfall traps. Three polypropylene beakers with neither roof nor preservative fluid were set at 15m intervals near each trap line for rodents. The traps were checked every morning for three consecutive days, and captured invertebrates were conserved in ethanol or in freezer at -20°C. The invertebrates were identified in laboratory at the lowest possible

taxonomic levels by morphological characteristics. Springtails (mainly Collembola) were removed from our inventory because of their too small body size compared to other captured invertebrates for being considered as to be resources of rodents. In total, 236 different plant taxa (principally at species level) and 22 different invertebrate taxa (composed of family or higher taxonomic levels such as order or class) were listed as potential rodents' resources.

Estimation of resource diversity

In this study, species richness was calculated (see details below) and used as a proxy for the number of total available resources of wood mice because of their generalist diet. Simpson's diversity index ($1/\sum P_i^2$, where P_i is the relative abundance, i.e. proportional abundance in relation to total abundance of all species, of species i) was also calculated with reference to the number of abundant resources (Jost, 2006). When richness was 0 or 1, Simpson's diversity index (called simply '*Simpson's index*' hereinafter) was also considered to be 0 or 1, respectively. Both species richness and Simpson' diversity index are hereinafter referred to as '*diversity indices*'.

The diversity indices for plants were based on number and cover-abundance (m^2) of species present in an area of 1000 m^2 around trap lines, considered to be a mean area of vital domain of wood mice (Quéré and Le Louarn, 2011) (hereinafter referred to as '*buffer*'). Plant data collected between June and September were regarded to be similarly representative for their availability in the two seasons, supposing that available plant species and relative cover-abundance would not substantially differ between the two seasons. The indices for invertebrates were calculated based on the animals captured per trap line (i.e. sum of the 3 traps for 3 days per trap line) at family or higher taxonomic levels (e.g. order or class). The total richness of resources was estimated as sum of the plant and invertebrate richness.

Richness and Simpson's index were positively correlated for both plants and invertebrates (Spearman's $\rho = 0.43$ and 0.78, respectively; p-value < 0.001). There was, however, no correlation between plant and invertebrate diversity indices. Plant richness was positively correlated with the concentrations of Cd and Zn in soils (Spearman's $\rho = 0.24$ and 0.34, respectively; p-value < 0.05), whereas Simpson's index of plants was positively correlated with the concentrations of the three TMs in soils (Spearman's ρ from 0.47 to 0.55; p-value < 0.001). Invertebrate richness and their Simpson's index were not correlated with the TM soil concentrations. The total richness was positively correlated with the TM concentrations (Spearman's ρ from 0.23 to 0.37 for the TMs in soils; p-value < 0.05). Details are shown in Supporting Information Figure S3.

Statistical analysis

TM exposure and accumulation

Statistical analysis was carried out on 72 buffers (36 buffers for each season) located in 6 sites, only because the availability of data did not allow to calculate diversity indices at the

buffer level on all 10 sites. The median value of the TM concentrations in SCs, in liver and in kidneys of the wood mice were calculated per trap line. The median values were then linked to diversity indices calculated at buffer level Critical values for exposure to TMs were defined based on the Lowest Observed Adverse Effect Levels (LOAELs) for daily intake of Cd and Pb (Shore and Douben, 1994a, b) and the No Observed Adverse Effect Levels (NOAELs) for daily intake of Zn, assuming the average weight of wood mice is 23 g (Butet and Paillat, 1997) and the average daily food intake is 10 g dry matter day^{-1} (Hunter et al., 1987). The critical values were 8.1, 11.5 and 239.2 µg g^{-1} for Cd, Pb and Zn, respectively. Critical values for Cd accumulation in liver and kidneys of wood mice were taken from Shore and Douben (1994a): 15 and 105 µg g^{-1} DM for liver and kidneys, respectively. Moreover, we estimated exposure to TMs and TM accumulation levels without any potential bias related to environmental TM contamination as follows: The TM concentrations in soils of woody habitat (Fritsch et al., 2010) were applied to buffers corresponded to the given habitat. For each TM, a linear model was built using concentrations in soils, season and their interaction as explanatory variables. The residuals of the significant linear models (or of null model if there was no significant explanatory variable) were considered to be the level of trophic exposure at buffer scale, referred to as '*exposure'* hereinafter. Likewise, a linear model was built for TM concentrations in each organs using TM concentrations in soils, age of mouse per buffer, season and their interactions. The residuals were considered to be the level of TM accumulation at buffer scale, referred to as '*accumulation*' hereinafter. TM concentrations and age were logarithmically transformed because of their skewed distributions.

Dilution effects in TM transfer and contamination

Relationships between richness and both exposure and accumulation were assessed by using a linear mixed model. The exposure or accumulation for each TM was analyzed in relation to (i) total richness, (ii) plant and invertebrate richness, and (iii) Simpson's diversity indices of plants and of invertebrates. The sites were considered as to be random effects in each model. An optimal random effect structure was checked following Zuur (2009), and only the models for exposure to Pb and Zn included sites as random effects. The models for exposure to Cd and acculturation of TMs) contained only a fixed structure. We checked significance of diversity indices by the likelihood ratio test compared to null model for mixed effect models (i.e. exposure to Pb and Zn) or by ANOVA type III for the others. R^2 of the optimal model, or marginal R^2 (R^2_m: variance explained by fixed effects) and conditional R^2 (R^2_c: variance explained by random effects) for the two mixed effect models were then calculated (Nakagawa and Schielzeth, 2013).

Candidate dilution or amplification resources

Candidate dilution or amplification resources were selected on the basis of the three conditions for dilution effects in TM transfer and contamination exposed in the introduction,

i.e. (i) difference in TM accumulation capacity, (ii) relationship between occurrence and community diversity, and (iii) important consumption of dilution resources in diversity-rich communities. Exposure and accumulation were analyzed with respect to presence or absence of each resource in the buffer, given the assumption that presence of dilution or amplification resources would significantly modify exposure and accumulation due to condition (i). However, wood mice consume a very large range of food, and it is not realistic to carry out such analysis for all potential resources present in the field. We thus examined only resources frequently and/or preferentially consumed, supposing that they would importantly influence TM transfer and contamination in relation to the point (iii). Wood mice in our study sites frequently and/or preferentially consumed Adoxaceae (principally composed of genus *Sambucus* in our study sites; for details see Ozaki et al. (2018), Asteraceae (*Cirsium*, *Eupatorium* and *Picris*), Cornaceae (*Cornus*), Fagaceae (*Fagus*), Poaceae (*Arrhenatherum*, *Holcus*, *Lolium*, etc), Polygonaceae (*Persicaria*, *Rumex*, etc), Rosaceae (*Crataegus*), Ramenaceae (*Frangula*), Salicaceae (*Populus*, *Salix*) and Sapindaceae (*Acer*) plant families. Earthworms are often considered as to be staple invertebrate food for wood mice, but our sampling method was not suited to collect them. Moreover, as the study of Ozaki et al. (2018) failed to identify arthropod and mollusk items consumed by mice, we could not know what invertebrates were frequently and/or preferentially consumed in our study sites. Significant candidate resource families were assessed by the non-parametric Wilcoxon-Mann-Whitney test. Because of potential change in feeding behavior of wood mice in relation to soil TM contamination (Ozaki et al., 2018), the analysis was carried out separately in highly and lowly TM contaminated buffers, and in high and low richness buffers. The intervention values of Pb (530 mg kg^{-1} dry soil; VROM, 2000), and the median value of the plant richness were used as thresholds. Probability of occurrence of significant candidate resource family was then modeled against diversity indices in order to verify the condition (ii), by using generalized linear models with logit function for binary data. Deviance R^2 (R^2_D) was calculated when the logistic model was significant by the likelihood ratio test (Zuur, 2009).

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 using '*lme*' function in '*nlme*' package, and the logistic models were carried out using '*glm* function in '*stas* package.

Results

TM concentrations in SCs and in organs

TM concentrations in SCs were significantly and positively explained by concentrations in soils for the three TMs. Cd concentrations in SCs was higher in spring than in autumn, whereas concentrations of Pb and Zn in SCs were not significantly different between the two seasons. Interaction between TM concentrations in soils and season was not significant for the

models (for details see Supporting Information Figure S4). Concentrations of Pb and Zn in SCs were superiors to the critical values in 12 and 1 buffers, respectively.

Cd concentrations in liver increased with respect to both age and Cd concentrations in soils, but Cd concentrations in liver were more strongly correlated with Cd concentrations in soils in autumn (Supporting Information Figure S5a, b). Cd concentrations in kidneys increased with respect to both Cd concentrations in soils and age, but age was more strongly correlated with Cd concentrations in kidneys in autumn (Figure S5c, d). Only Cd concentrations in liver were superiors to the critical values in 4 buffers, but not in kidneys. Zn concentrations in liver increased with respect to only age and was higher in spring (Figure S5c). No variable explained Zn concentration in kidneys. As indicated in Materials and methods part (2.2.2. Measure of TM concentrations), Pb concentrations in the organs were frequently under detection limits and could not have been analyzed here.

Dilution effects in TM exposure and contamination

Exposure to Cd and Zn were not significantly explained by diversity indices. Exposure to Pb was significantly and negatively explained only by plant richness ($R^2_m = 0.062$ and $R^2_c = 0.228$; p-value = 0.03; Figure 1). Accumulation of Cd in the liver was significantly and negatively explained by total richness ($R^2 = 0.116$; p-value < 0.01), plant richness ($R^2 = 0.134$; p-value < 0.01, Figure 2.a), or Simpson's index of plants ($R^2 = 0.060$; p-value = 0.04). Accumulation of Cd in kidneys was also significantly and negatively explained by total richness ($R^2 = 0.113$; p-value < 0.01), plant richness ($R^2 = 0.118$ p-value < 0.01, Figure 2.b), or Simpson's index of plants ($R^2 = 0.100$; p-value = 0.01). Accumulations of Zn in the liver and in kidneys were not significantly explained by diversity indices.

Potential dilution or amplification resources

Table 2 summarizes candidate dilution or amplification resources. In buffers with low plant richness and low TM contamination, exposure to Cd was significantly lower when Adoxaceae family was present in buffers (W = 104; p-value = 0.04), and exposure to Pb was significantly lower when Sapindaceae family was present (W = 135; p-value < 0.001). Moreover, accumulation of Zn in the liver was significantly lower when Asteraceae family was present (W = 114; and p-value = 0.03). In buffers with low richness and high TM contamination, however, accumulation of Cd in both liver and kidneys was significantly higher when Asteraceae family was present (W = 0; p-value < 0.001 for both liver and kidneys).

In buffers with high richness and low TM contamination, exposure to Zn was significantly lower when Cornaceae family was present (W = 76; p-value = 0.01). In buffers with high richness and high TM contamination, we found no significant candidate plant family.

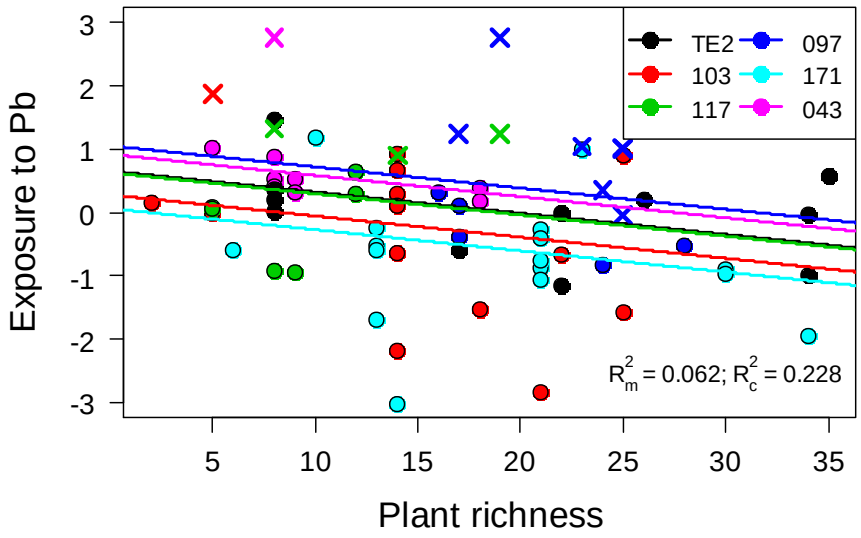

Figure 1. Exposure to Pb of the wood mice along the gradient of plant richness in the field. Plotted points indicate buffers, and values predicted from our models are indicated by line. Buffers where concentration in SCs was above the LOAEL are protted by cross. Different colors of points and lines represent different sites. Variation explained by fixed and random effects are respectively explained by marginal and conditional R2 (R2m and R2c) in the figure.

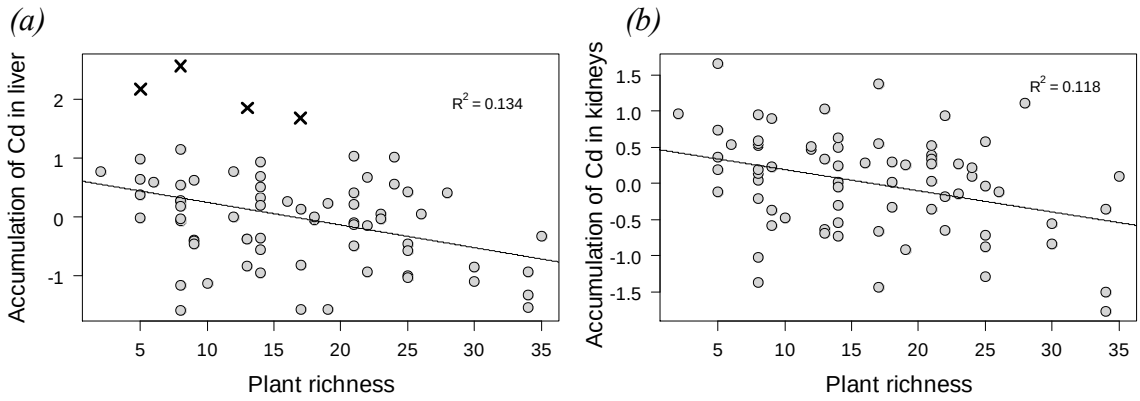

Figure 2. Accumulation of Cd in liver (a) and in kidneys (a) along the gradient of plant richness in the field. Plotted points indicate buffers, and values predicted from our models are indicated by line. Buffers where concentration in SCs was the above LOAEL are protted by cross. R^2 of the models are indicated in the figures.

Table 2. Summary of plant families whose presence on buffer significantly decreased ('L' in green) or increased ('H'; in orange) exposure to TMs and/or accumulation of TMs in wood mice under the Wilcoxon-Mann-Whitney test (p-value < 0.05) in four categories of buffers defined by total richness in the field (median of the richness) and the soil TM contamination level (the intervention value of Pb: 530 mg kg^{-1} of Pb; VROM,2000): Table 2a shows buffers with high richness and low (left) or high soil TM contamination (right), and Table 2b shows buffers with low richness and low (left) or high soil TM contamination (right). Non-significant results are marked by 'N'. Number of buffers analized (n) and number of buffers where a given family was present (P) and absent (A) are in Tables. The analysis was carried out only when both P and A are ≥ 5. The sign '-' means that the analysis was not carried out due to few number of buffers.

(a)

Family	Principal component genera of family in the sites	Low soil contamination level								High soil contamination level							
		n = 19 (P/A)	Exposure			Accumulation				n = 17 (P/A)	Exposure			Accumulation			
						Liver		Kidneys						Liver		Kidneys	
			Cd	Pb	Zn	Cd	Zn	Cd	Zn		Cd	Pb	Zn	Cd	Zn	Cd	Zn
Adoxaceae	*Sambucus*	(18/1)	-	-	-	-	-	-	-	(17/0)	-	-	-	-	-	-	-
Asteraceae	*Cirsium, Eupatorium and Picris*	(19/0)	-	-	-	-	-	-	-	(14/3)	-	-	-	-	-	-	-
Cornaceae	*Cornus*	(10/9)	N	N	L	N	N	N	N	(13/4)	-	-	-	-	-	-	-
Fagaceae	*Fagus*	(5/14)	N	N	N	N	N	N	N	(2/15)	-	-	-	-	-	-	-
Poaceae	*Arrhenatherum, Holcus, etc*	(19/0)	-	-	-	-	-	-	-	(12/5)	N	N	N	N	N	N	N
Polygonaceae	*Persicaria, Rumex, etc*	(5/14)	N	N	N	N	N	N	N	(3/14)	-	-	-	-	-	-	-
Rosaceae	*Crataegus*	(19/0)	-	-	-	-	-	-	-	(16/1)	-	-	-	-	-	-	-
Ramenaceae	*Frangula*	(4/15)	-	-	-	-	-	-	-	(6/11)	N	N	N	N	N	N	N
Salicaceae	*Populus & Salix*	(16/3)	-	-	-	-	-	-	-	(14/3)	-	-	-	-	-	-	-
Sapindaceae	*Acer*	(16/3)	-	-	-	-	-	-	-	(9/8)	N	N	N	N	N	N	N

(b)

Family	Principal component genera of family in the sites	Low soil contamination level								High soil contamination level							
		n = 25 (P/A)	Exposure			Accumulation				n = 11 (P/A)	Exposure			Accumulation			
						Liver		Kidneys						Liver		Kidneys	
			Cd	Pb	Zn	Cd	Zn	Cd	Zn		Cd	Pb	Zn	Cd	Zn	Cd	Zn
Adoxaceae	*Sambucus*	(8/17)	L	N	N	N	N	N	N	(5/6)	N	N	N	N	N	N	N
Asteraceae	*Cirsium, Eupatorium and Picris*	(10/15)	N	N	N	N	L	N	N	(6/5)	N	N	N	H	N	H	N
Cornaceae	*Cornus*	(9/16)	N	N	N	N	N	N	N	(2/9)	-	-	-	-	-	-	-
Fagaceae	*Fagus*	(0/25)	-	-	-	-	-	-	-	(3/8)	-	-	-	-	-	-	-
Poaceae	*Arrhenatherum, Holcus, etc*	(14/11)	N	N	N	N	N	N	N	(3/8)	-	-	-	-	-	-	-
Polygonaceae	*Persicaria, Rumex, etc*	(0/25)	-	-	-	-	-	-	-	(2/9)	-	-	-	-	-	-	-
Rosaceae	*Crataegus*	(22/3)	-	-	-	-	-	-	-	(11/0)	-	-	-	-	-	-	-
Ramenaceae	*Frangula*	(0/25)	-	-	-	-	-	-	-	(0/11)	-	-	-	-	-	-	-
Salicaceae	*Populus & Salix*	(24/1)	-	-	-	-	-	-	-	(7/4)	-	-	-	-	-	-	-
Sapindaceae	*Acer*	(14/11)	N	L	N	N	N	N	N	(9/2)	-	-	-	-	-	-	-

Occurrence of candidate dilution or amplification plant families in relation to the diversity of communities

Occurrence of Adoxaceae family was significantly positively correlated to total and plant richness, as well as to Simpson's diversity of plants (R^2_D = 0.458, 0.554 and 0.113; p-value < 0.001, respectively). Occurrence of Asteraceae family was significantly positively correlated to total and plant richness (R^2_D = 0.353 and 0.418, respectively; p-value < 0.001). Occurrence of Cornaceae family was significantly positively correlated to plant richness and Simpson's diversity of plants (R^2_D = 0.039 and 0.289; p-value = 0.05 and < 0.001, respectively). Occurrence of Sapindaceae family was significantly positively correlated to total and plant richness (R^2_D = 0.063 and 0.062; p-value = 0.02 respectively), but not to Simpson's diversity of plants. Figure 3 shows occurrence of the plant families along the gradient of the diversity index the most representative, i.e. showing the highest R^2_D.

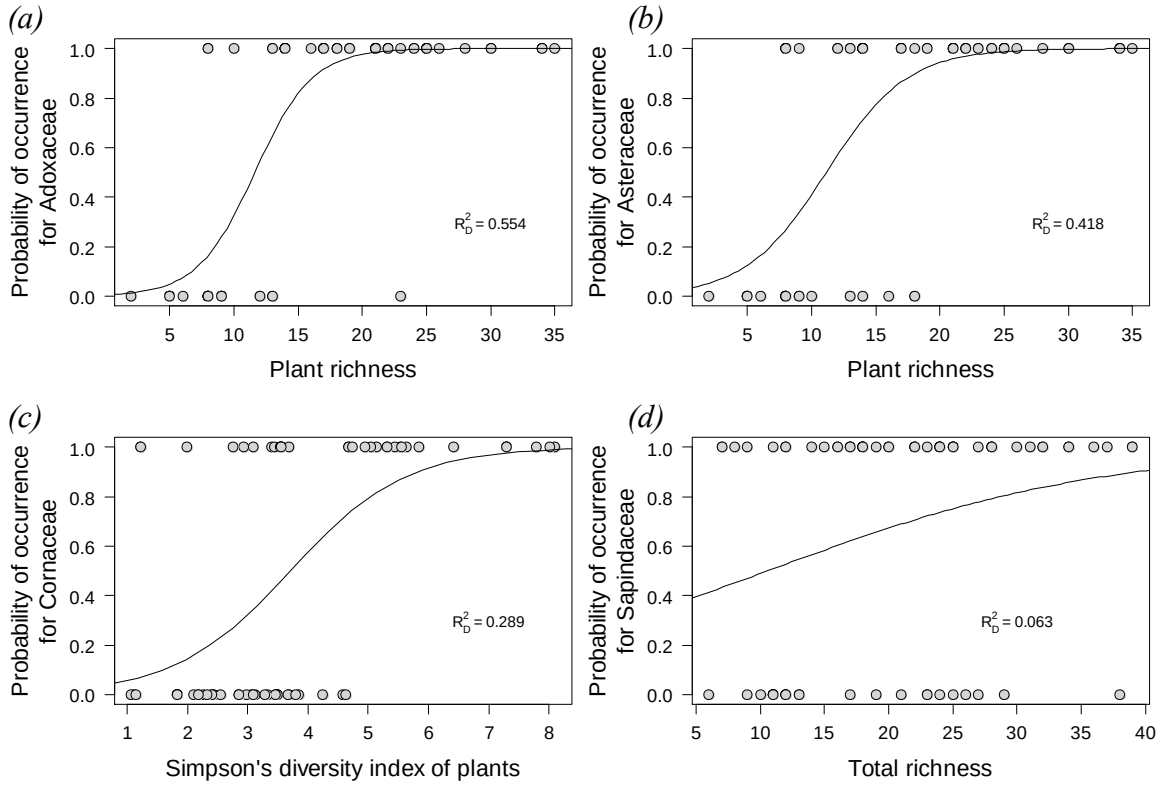

Figure 3: Predicted probabilities of occurrence in the field for Adoxaceae (a), Asteraceae (b), Cornaceae (c) and Sapindaceae (d) along a gradient of plant richness (a, b)Simpson's diversity index of plants (c), or total richness (d) in the field. Plotted points at probability value 1 indicate buffers where the given plant family was observed, and points at 0 indicate buffers where the given plant family was not observed. Predicted probability form the model is indicated by line. Deviance R2 (R2D) of the model is indicated in the figures.

Discussion

Our results support the hypothesis that diversity of resources on the field can exert a dilution effect in decreasing the exposure of small mammal to TMs and their TM concentrations in internal tissues. Both exposure to Pb and accumulation of Cd decreased with plant richness and with all diversity indices, respectively. However, the three diversity indices were positively correlated, and it is therefore difficult to know whether plant richness or biodiversity is responsible for the dilution effect observed in the present study. Because all of the significant relationships between exposure/accumulation and diversity indices showed the highest R^2 with plant richness, number of plant resources could be the most important factor to trigger dilution effects in TM transfer and contamination in wood mice.

Some plants were also supposed as dilution and amplification resources: Adoxaceae, Cornaceae and Sapindaceae could be the former and Asteraceae could be the latter. However, dilution effect in TM transfer and contamination, as well as dilution/amplification resources depended on metals and organs. That could be due to different mechanisms of dilution effects among metals or organs.

As the first condition for dilution effects, difference in TM accumulation capacities has been shown in both plant and invertebrate species, on our study site among others. Migeon et al. (2009) studied concentrations of TMs in several woody species observed around Metaleurop Nord and reported higher concentrations of Cd and Zn in leaves or stems of Salicaceae family plants, *Populus* and *Salix*, than in other plants belonging to Sapindaceae, Adoxaceae and Cornaceae families. Concentrations of Cd and Zn in leaves of *Acer pseudoplatanus* (Sapindaceae) were significantly higher than in the leaves of *Salix caprea* and *Populus* sp. (Salicaceae) in our study sites (unpublished data). *Populus* and *Salix* plants are generally considered as to be TM accumulator plants and often used for phytoremediation projects (Pulford and Watson, 2003), and Salicaceae family plants have been suggested as an important contributor for exposure of mammals to Cd as beavers (*Castor fiber*) (Nolet et al., 1994) or moose (*Alces alces*) (Brekken and Steinnes, 2004). Ozaki et al. (submitted for publication) showed that wood mice consuming Salicaceae items were more exposed to Cd and Zn than mice not consuming those items. Asteraceae family is considered as one of the plant families having a greater number of highly Cd accumulating species (Abe et al., 2008; Kuboi et al., 1986). Presence of Asteraceae family plants, composed of several herbaceous genura genera like *Cirsium*, *Eupatorium*, *Matricaria*, or *Picris*, increased accumulation of Cd. On the contrary, Migeon et al. (2009) reported that bioconcentration factor for Pb was significantly different among species, though low in woody species around Metaleurop Nord. Pb is indeed a less mobile metal and slightly available for plants (Kabata-Pendias, 2011). Pb concentrations in leaves of Salicaceae and Sapindaceae were actually not significantly different (unpublished data). Because of high contrast in concentrations of Cd and Zn between Salicaceae and other

families, Adoxaceae, Cornaceae and Sapindaceae, the first condition for dilution effects could be satisfied for Cd and Zn. We however could not totally suppose those resources as dilution or amplification resources for Pb due to less contrasted difference in concentrations of the metal.

The second condition for dilution effects, i.e. a relationship between occurrence of dilution/amplification resources and community diversity, was shown in our results. Occurrence of Adoxaceae, Cornaceae, and Sapindaceae families indeed increased with respect to plant richness. This result could indicate those families as dilution resources. However, Asteraceae family also increased with respect to plant richness. Several resources of wood mice coexisted in a buffer, and the third condition, i.e. important feeding dilution resources in diversity-rich communities, would more importantly influence on dilution effects. Seasonal change in diet of wood mice has been widely indicated (e.g. Watts, 1968; Butet, 1986). Ozaki et al. (2018) reported details of diet of wood mice in spring and autumn in our study area by applying a DNA based identification on their stomach contents: wood mice frequently consumed Sapindaceae, Salicaceae and a sequence group composed of Adoxaceae, Asteraceae and Cornaceae items in spring and the sequence group, Sapindaceae and Poaceae in autumn. Furthermore, food preference of wood mice could seasonally vary: preference for Sapindaceae and Salicaceae in spring and for the sequence group and Poaceae in autumn (Ozaki et al., 2018). In autumn, Salicaceae plants, considered as to be amplification resource due to the first and the second conditions, were thus not preferentially consumed, whereas Adoxaceae, Sapindaceae and Poaceae, considered as to be dilution resources, were preferentially consumed. On the other hand, Ozaki et al. (submitted for publication) demonstrated that Salicaceae, Sapindaceae and the sequence group were alternative resources in diet of wood mice in spring. Feeding Salicaceae plants of wood mice could be conditioned by potential dilution resources, Sapindaceae and Adoxaceae plants in spring. In both season, Adoxaceae and Sapindaceae plants could serve for wood mice as dilution resources, whereas Salicaceae plants as and amplification resources, in relation to the third condition. As a consequence, we supposed that at least the dilution effect in Cd contamination in liver of wood mice in this study could be due to Adoxaceae and Salicaceae families.

Mechanisms for dilution effects in other exposure or accumulation are still not clear. Exposure to Cd differed among presence/absence of Adoxaceae on buffer but showed no significant correlation with diversity indices. Because of slow excretion (e.g. the half-lives of Cd in mice and rats are approximately 200-700 days (Nordberg et al., 2014), Cd concentrations in its target organs reflects long-term exposure and increases with age (Shore and Rattner, 2001). Indeed, concentrations of Cd in both liver and kidneys showed complex relationships with age and season in our study. Effects of biodiversity on Cd might be expressed rather in long-term (i.e. accumulation) than in short-term (i.e. exposure). Exposure to Pb was negatively correlated with plant richness but, unfortunately, we failed to measure Pb concentrations in organs.

Relationships between Pb concentrations in SCs and in organs have been poorly documented, but Godwin et al. (2016) demonstrated positive but year depending correlation between Pb concentrations in SCs and kidneys, but not between SCs and liver of tree swallow (*Tachycineta bicolor*) nestlings of the same age. Moreover, Tête et al. (2014a) reported that Pb concentrations were positively correlated with Cd concentrations in both liver and kidneys of wood mice. Although protective effects of biodiversity on the body burden of Pb can be supposed due to cumulative effects of biodiversity in short-term, this point should be exploited in further studies. On the other hand, no effect of biodiversity was observed for Zn. Zn in liver and kidneys also showed no correlation with soil Zn concentrations. As Zn is an essential element for organism, and its uptake and metabolism can be regulated in homeostatic ways (Shore and Rattner, 2001). As Zn concentrations in SCs increased with soil Zn concentrations, we thought that such homeostatic regulation could function rather in wood mice than in resources.

One polarizing debate about dilution effect in transmission of pathogens is its generality. Ostfeld and Keesing (2012) argued on the basis of literature that the dilution effect was far more often observed than its opposite phenomenon: the amplification effect. The meta-analysis conducted by Salkeld et al. (2013) indicated that influence of diversity of disease risk was idiosyncratic, whereas the meta-analysis of Civitello et al. (2015) showed consistent evidence for dilution effect in diverse host communities, independent of host or parasite type. We cannot extrapolate our results because our finding about dilution effects in TM transfer and contamination was only one case study. The question for its generality is whether the three conditions are always satisfied in TM contaminated sites. For generalist feeding animal like wood mice, diet reflects available resources and thus varies among study areas (e.g. Montgomery and Montgomery, 1990; Rogers and Gorman, 1995). However, feeding behavior of wood mice might be controlled by the environmental TM contamination itself. Under the laboratory stetting of Beernaert et al. (2008), wood mice consumed rather acorns taken from a non-contaminated site rather than acorns form a TM contaminated site. Within different resources, the study of (Ozaki et al., 2018) showed preference of mice for Salicaceae items decreased with respect to TM contamination levels, which could also correspond with an increase in occurrence of Sapindaceae in their diet with TM contamination levels (Ozaki et al., submitted for publication). These results suggest that difference in food preference could be due to difference in resource TM contamination. Moreover, (Ozaki et al., submitted for publication) demonstrated a result concurring with our dilution effects of plant richness: a high exposure of wood mice to Cd due to consuming Salicaceae items significantly reduced with respect to number of other plant items (namely '*dilution diet hypothesis*'; Boyd, 1998). High plant richness could provide not only a wide choice for low contaminated sources but also various compositions of food, both of which can lead to avoid a single resource highly contaminated. We thus supposed that, whatever resource composition is, feeding behaviors of animals in

relation to the environmental TM contamination might be one of the most important matters for general generality of dilution effects in TM transfer/contamination.

Furthermore, several other factors could influence on feeding behaviors and/or TM transfer. (1) *Role of invertebrate food*: Despite no significant observation in this study, invertebrates could play some functions in TM transfer. Earthworms are widely considered to be important contributors of TMs for their predators (e.g. Rogival et al., 2007; van den Brink et al., 2010; Gall et al., 2015). However, wood mice in our study area did not frequently consume earthworms (about 30 %; Ozaki et al., 2018), and showed no significant increase of exposure to TMs by consuming earthworm items(Ozaki et al., submitted for publication). On the other hand, other invertebrates also show high metal accumulation capacity, e.g. snails, isopods, and spiders (Hopkin and Martin, 1982; Janssen et al., 1991; Heikens et al., 2001; Hendrickx et al., 2003), and their composition in diet of wood mice, as well as importance of each taxa in TM transfer was unclear. In most studies, wood mice consumed animal matter all seasons but more frequently and/or abundantly in spring and late summer than the other seasons (e.g. Watts, 1968; Butet, 1986; Montgomery and Montgomery, 1990). Even if invertebrates could serve as amplification resources in short-term (i.e. exposure), effects of their diversity in long-term (accumulation) might be limited. Verifying this point will nonetheless require further studies. (2) *Competitors and predators*: Presence of other mammals could complicate feeding behavior of wood mice. In the study on dietary overlap within wood mice, yellow-necked mice (*Apodemus flavicollis*) and bank voles (*Clethrionomys glareolus*) in a mixed farmland, Abt and Bock (1998) supposed that divergence of food might be explained in part by competitive interaction within them. Shortage in resources of one competitor could cause a high dietary overlap and thus provoke a shortage of resources of others. Predators could also control behavior of mice. Actually, wood mice in Mediterranean forest habitats changed foraging behavior in response to both presence and activity of their predator genets (*Genetta genetta*) (Díaz et al., 2005). Feeding behavior of wood mice could be influenced not only by availability of their own resources but also by intra-and inter-trophic interactions. (3) *Bioavailability of TMs*: We finally cannot exclude possible changes in bioavailability of TMs in relation to biodiversity. Abbas et al. (2013) demonstrated that community-wide plant stoichiometry varied with plant diversity under a grassland biodiversity experiment. In TM contaminated sites, plant diversity positively enhances soil microbial community structure and its activity (Gao et al., 2010; Gao et al., 2012; Stefanowicz et al., 2012). As soil microbes are important regulators in nutrient cycle for plants (van der Heijden et al., 2008), plant stoichiometry might vary with plant diversity also in TM contaminated sites. However, no significant change in bioconcentration factor of leaves of *A. pseudoplatanus* was observed with respect to plant richness (unpublished data), and TM transfers form soils to plants could not differ with plant richness. Nonetheless, gastrointestinal uptake of TMs depends on other nutrients. For instance,

uptake of Cd and Pb increases when iron and calcium are deficient in diet (Shore and Rattner, 2001; Nordberg et al., 2014). Chemical composition of essential elements reducing bioavailability of TMs in resources should be also taken into further studies on functions of biodiversity on TM transfer and contamination.

Conclusion

In this study, functional roles of biodiversity on TM transfer in wildlife was investigated in reference to the dilution effect in disease transmission. Diversity of resources, especially richness of plant resources, serves as protector of wood mice against TM transfer from environmental TM contamination and thus as factor reducing TM burden in their body. Some hypothetical mechanisms were also studies on the basis of the dilution effect in disease transmission. Although some plants are considered as to be resources reducing (e.g. genus *Sambucus*; Adoxaceae) or increasing (e.g. genura *Populus* & *Salix*; Salicaceae) TM transfer, environmental TM contamination itself can function as sub-mechanisms for occurrence of dilution effect in TM transfer and contamination. Although elucidating details of mechanisms and conditions still remains in question, our study proposes the dilution effects in TM transfer and contamination as one functional role of biodiversity controlling chemical pollutants in food webs and thus promoting welfare in wildlife.

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Supporting information

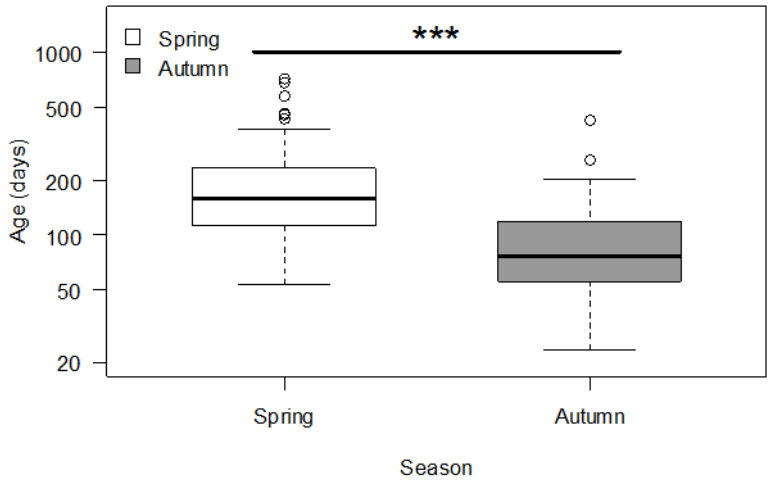
S1: Age of wood mice among season.

S2: Concentrations of Cd and Zn in liver and kidneys of wood mice among sex.

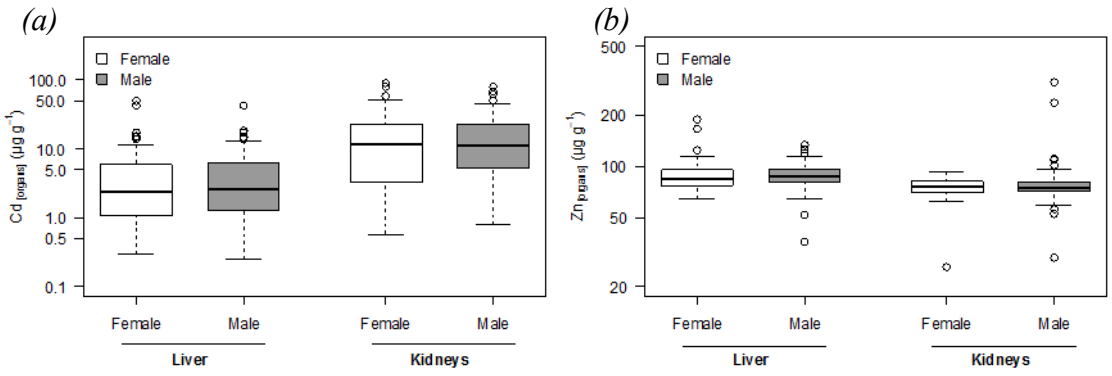
S3: Correlation matrix for diversity indices (total, plant and invertebrate richness, as well as Simpson's diversity indices of plants and invertebrates) and Cd, Pb and Zn concentrations in soils.

S4: Concentrations of TMs (Cd, Pb and Zn) in SCs of wood mice along the gradient of concentrations of TMs in soils.

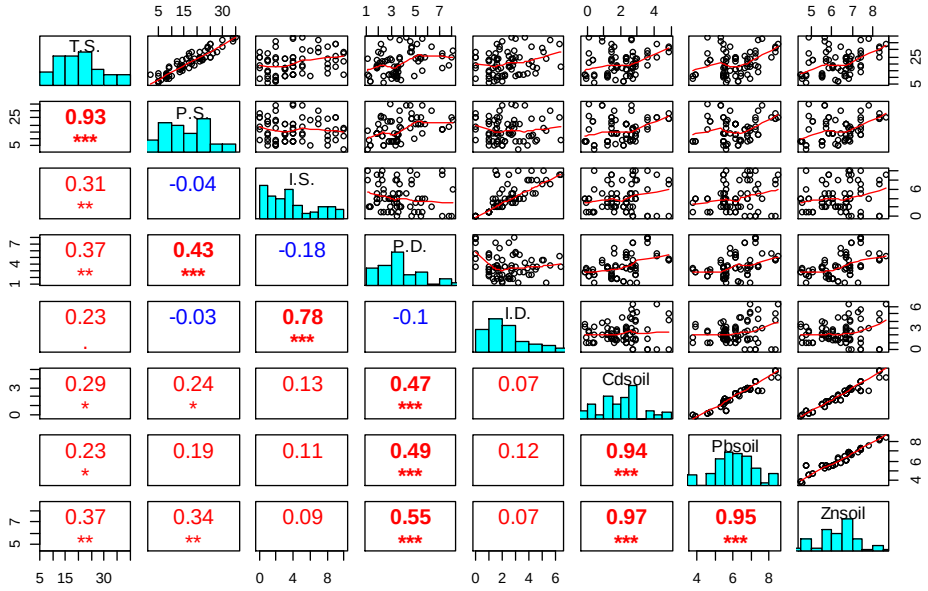
S5: Concentrations of TMs (Cd and Zn) in liver and kidneys of wood mice along the gradient of concentrations of TMs in soils and according to age of wood mice.



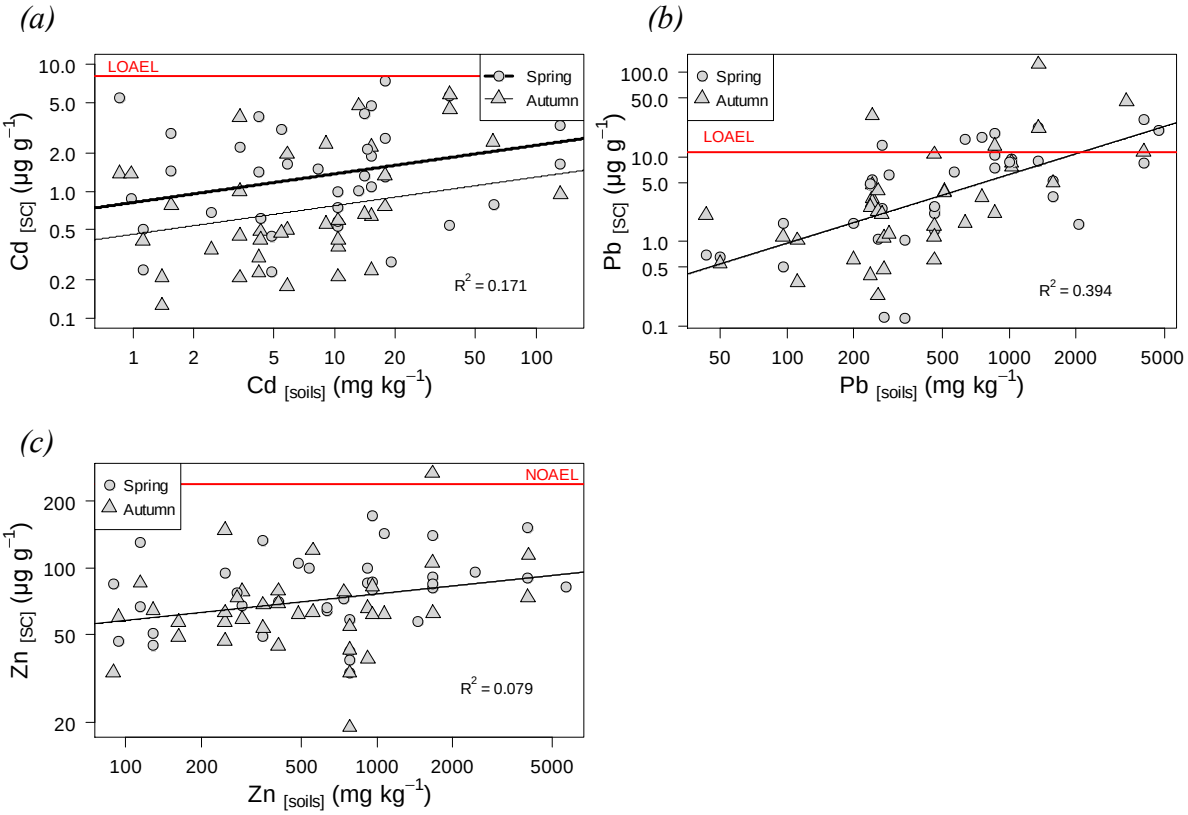
Supporting Information Figure S1: Age of wood mice between spring (n = 89) and autumn (n = 80). Significant differences by the Wilcoxon-Mann-Whitney test are expressed by asterisk (: p-value < 0.05, **: p-value < 0.01 and ***: p-value < 0.001).*



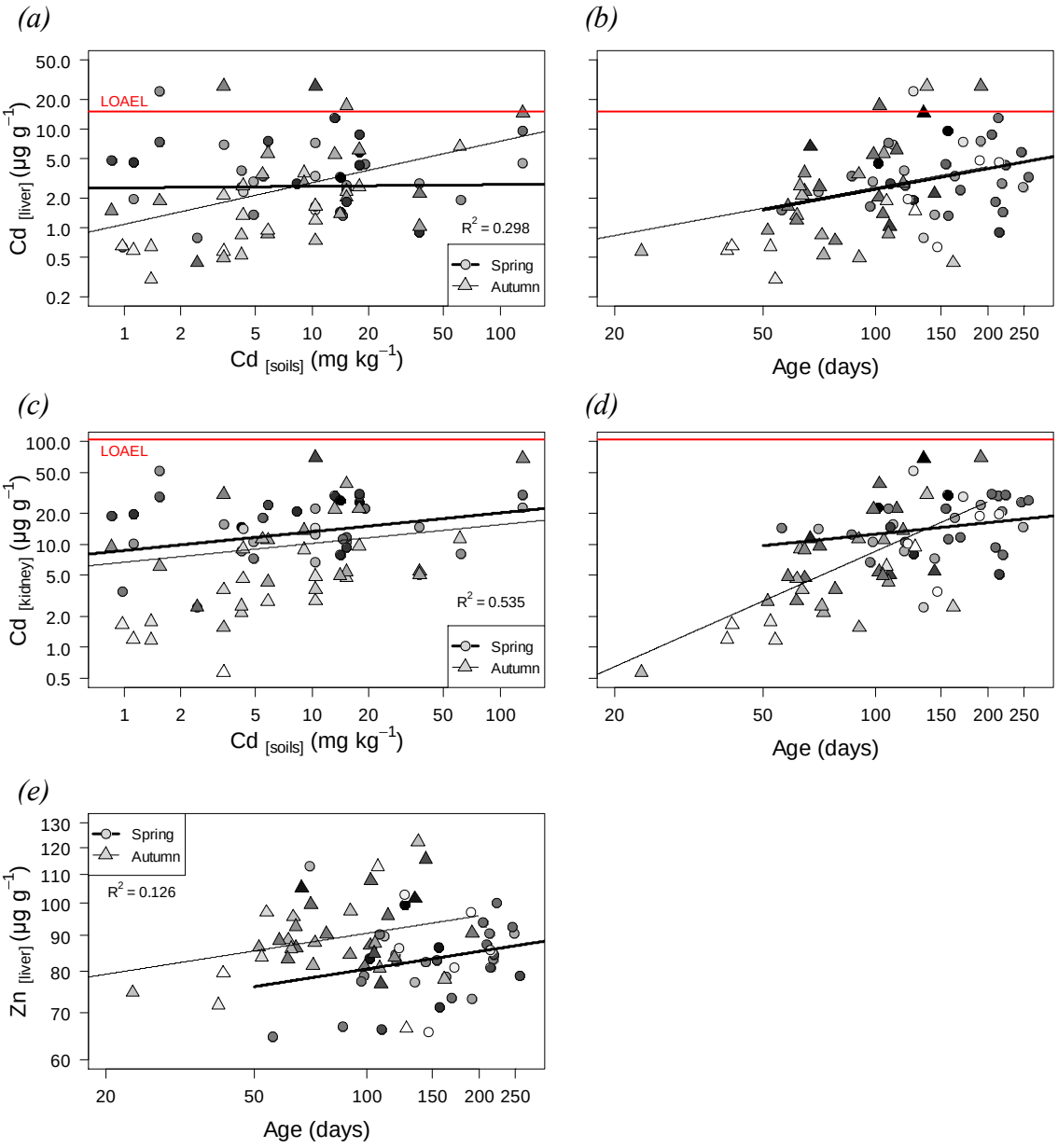
Supporting Information Figure S2: Concentrations of Cd (a) and Zn (b) in liver and kidneys of wood mice among sex. One mouse whose sex could not be identified is removed from analyses. Significant differences between (n=80) and males (n=88) by the Wilcoxon-Mann-Whitney test are expressed by asterisk (: p-value < 0.05, **: p-value < 0.01 and ***: p-value < 0.001).*



Supporting Information Figure S3: Correlation matrix for diversity indices: total, plant and invertebrate richness ('T.S.', 'R.S.' and 'I.S.', respectively), as well as Simpson's diversity indices of plants and invertebrates ('R.D.' and 'I.D.', respectively) and logarithmically transformed TM concentrations in soils (mg kg^{-1} of dry soil): Cd (Cdsoil), Pb (Pbsiol) and Zn (Znsoil). Histogram of each variable is shown on the diagonal. The upper triangular matrix part describes scatter plots for each pair of the variables with LOWESS smoother line. Points in scatter plots indicate buffers. The lower triangular matrix part shows Spearman's ρ for each pair of the variables. Positive correlation values are expressed in red, whereas negative correlation values are in blue. Significant correlations by the Spearman's correlation test are expressed by asterisk (: p-value < 0.05, **: p-value < 0.01 and ***: p-value < 0.001).*



Supporting Information Figure S4: Concentrations of TMs in SCs of wood mice (µg g^{-1} of dry matter) along the gradient of concentrations of TMs in soils (mg kg^{-1} of dry soil) for Cd (a), Pb (b) and Zn (c). Plotted points indicate buffers (circles for spring and triangles for autumn), and values predicted from models are indicated by line(s) (when predicted values differ between the two seasons, bold and normal lines correspond spring and autumn, respectively). The Lowest Observed Adverse Effect Levels (LOAELs) for exposure to Cd and Pb were estimated assuming the daily intake LOAELs (respectively 3.5 and 5 µg g^{-1} body weight day^{-1}; (Shore and Douben, 1994a; b), the average weight of wood mice (23g; Butet and Paillat, 1997) and the average daily food intake (10 g dry matter day^{-1}; Hunter et al., 1987). R^2 of each models is indicated in the figures.



Supporting Information Figure S5: Concentrations of TMs in organs of wood mice (µg g^{-1} of dry matter) along the gradient of concentrations of TMs in soils (mg kg^{-1} of dry soil) (a, c) or according to age (days) (b, d, e) for Cd in liver (a, b), Cd in kidneys (b, c) and Zn in liver (e). Plotted points indicate buffers (circles for spring and triangles for autumn), and grayscale shades correspond the gradient of mice age (white–black: young–old; a, c) or the gradient of concentrations of TMs in soils (white–black: low–high concentration; b, d, e). Values predicted from our models with the median value of age (a, c) or soil TM concentrations (b, d, e) are indicated by lines (bold and normal lines correspond spring and autumn, respectively). The Lowest Observed Adverse Effect Levels (LOAELs) for Cd in liver and kidneys (respectively 15 and 105 µg g^{-1} dry matter; Shore and Douben, 1994a) are represented by a red line. R^2 of each model is indicated in the figures.

Part IV. General Discussion

IV.1 Synthesis of the results of each chapter

The present thesis proposes functional roles of resource diversity on the transfer of TMs as the global hypothesis and explores its underlying mechanisms. Principal results obtained from each chapter are presented below:

– Chapter 1 –

(*1A*): Physico-chemical soil properties, including soil TM concentrations, did not affect diversity indices of plants but weekly modified species composition of plant communities.

(*1B*): Diversity indices and composition of ground-dwelling invertebrate communities were weekly affected by physico-chemical soil properties and soil TM concentrations. Diversity indices and composition of flying invertebrates were not affected by physico-chemical soil properties.

(*1C*): Diversity indices and composition of both ground-dwelling and flying invertebrates were more explained by vegetation than by physico-chemical soil properties or soil TM concentrations.

– Chapter 2 –

(*2A*): Some plants families such as Sapindaceae or Salicaceae were preferentially consumed by wood mice in spring, whereas other families such as Adoxaceae, Asteraceae and Cornaceae were preferred in autumn.

(*2B*): Diet richness was not influenced by plant richness in the field in spring, whereas diet richness was modified along the gradient of plant richness in the field in autumn.

(*2C*): Soil TM contaminations affected the relationships between diet richness and resource richness in the field and made disappear preference for Salicaceae plants in spring

– Chapter 3 –

(*3A*): Exposure to Cd was higher when mice consumed Salicaceae items in spring, whereas exposure to Zn was higher when mice consumed Salicaceae items in autumn.

(*3B*): Exposure of wood mice to Cd was negatively correlated with diet richness of plants when mice consumed Salicaceae items.

– Chapter 4 –

(*4A*): Concentrations of Cd and Zn in leaves of *Salix caprea* and *Populus* sp. (Salicaceae) were higher than in leaves of *Acer pseudoplatanus* (Sapindaceae), whereas concentrations of Pb in leaves were not different among the three taxa.

(*4B*): Concentrations of Pb in leaves were negatively correlated with concentrations of P, S, and N in the three plants.

(*4C*): Concentrations of Cd, Pb and Zn increased along the gradient of soil TM concentrations, whereas bioconcentration factors for the three elements in leaves were not correlated with plant diversity indices.

– Chapter 5 –

(*5A*): Accumulations of Cd in the liver and the kidney were negatively correlated with richness of plants and Simpson's index of plants in the field, but not with diversity of invertebrates.

(*5B*): Only exposure to Pb was negatively correlated with richness of plants in the field.

(*5C*): When Adoxaceae, Sapindaceae and Cornaceae family plants were present in the field, exposure to Cd, Pb and Zn were lower, respectively. When Asteraceae family plants were present in the field accumulation of Zn in liver was lower but accumulation of Cd in both liver and kidneys was higher.

(*5D*): Occurrences of Adoxaceae, Asteraceae, Cornaceae and Sapindaceae families in the field increased along the gradient of the diversity indices of plant resources in the field, especially richness of plants.

To summarize, the global hypothesis of the present thesis, a dilution effect of the diversity of resources on the transfer of TMs in food webs, was demonstrated in the last chapter: dilution effects of the plant diversity, especially plant richness, on oral exposure to Pb (described in (*5A*)) and on accumulation of Cd in the critical organs (*5B*) were observed (Figure IV.1a). The other results are related to potential underlying mechanisms (Figure IV.1b). In the case of plant resources, accumulation of TMs in tissues were different between low and hyper accumulator resources (*4A*). Exposure of mice to TMs depended on consumption of such hyper accumulators (*3A*), but diet richness served as a filter reducing exposure to TMs (*3B*). Both composition and richness in the field were not totally reflected in diet composition and richness which were indeed controlled through feeding behavior of mice (*2A* and *2B*). Exposure to TMs and accumulation of TMs differed according to presence/absence of some preferred resources in the field (*5C*). Occurrence of these preferred resources in the field was in most of the cases positively correlated with plant diversity (*5D*). On the other hand, potential correlation between plant diversity and bioconcentration factor in plants was not observed (*4C*). Soil TM contamination not or weakly influenced diversity and composition of resources (*1A* and *1B*). However, soil TM contamination affected composition of macro-elements in resources (*4B*) and feeding behavior of mice (*2C*). In the case of invertebrate resources, effects of their consumption on oral exposure were not observed (*2B*). Accumulation of TMs in invertebrates and preference for invertebrates were not measured in the present thesis. However, their distribution in the field was more governed by vegetation than by physico-chemical soil properties (*1B* and *1C*).

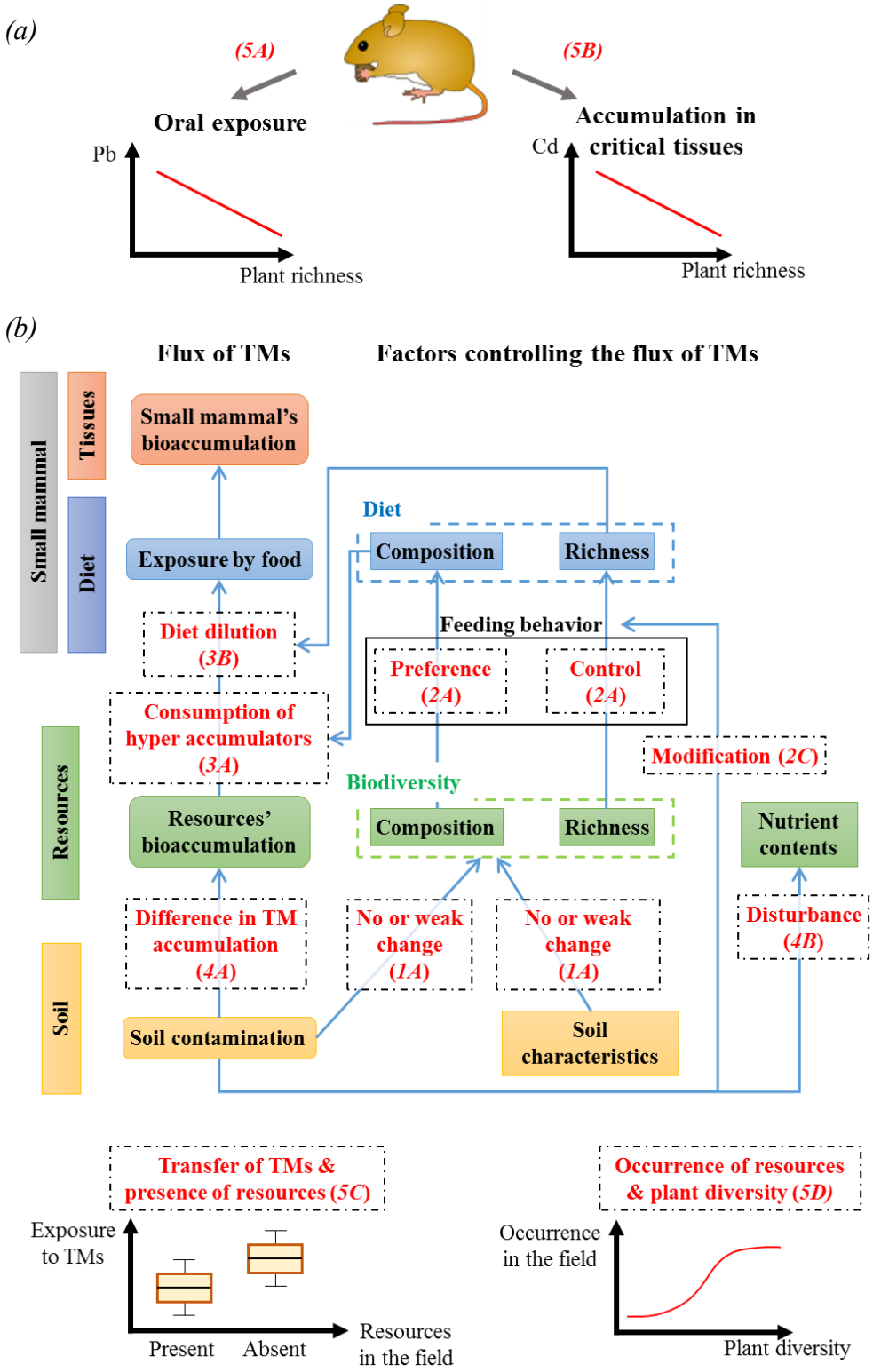


Figure IV.1. (a) Schema summarizing the results about the global hypothesis of the present thesis. (b) Diagrams representing the results about underlying mechanisms in the flux of TMs in the terrestrial ecosystem from the soil to the small mammal through the trophic route on the basis of Figure I.9. Number and capital letter in brackets correspond to each results described in the part IV.1.

IV.2 Functional roles of diversity of resources in the field and their underlying mechanisms

The present thesis demonstrated dilution effects of diversity of plant resources on both exposure to TMs and accumulation of TMs. Diversity indices of plants in the field showed no correlations with soil TM concentrations. This do not explain the negative correlations between exposure or accumulation and diversity indices. Similarly, bioavailability of TMs did not change along the gradient of the diversity indices. These results indicate that the observed dilution effects can be an effect related to biodiversity. However, dilution effects clearly differed between elements, and plant resources rather than invertebrate resources were involved in. In the introduction part, the three conditions were hypothesized as the factors controlling occurrence of the dilution effect in the transfer TMs: (i) the difference in TM accumulation capacity among the resources available in the field, (ii) the difference in the sensibility of the resources to biodiversity loss, and (iii) the difference in the food preference of the wood mouse between hyper and low accumulator resources. If the conditions are not totally met, change in resource diversity would lead to different consequence (e.g. amplification or no relationships) (Figure I.8). The underlying mechanisms observed in the present thesis are compared with three hypothetical conditions.

IV.2.1 Difference in TM accumulation capacity among the resources available in the field

The condition firstly controlling occurrence of the dilution effect is the difference in TM accumulation capacity. Difference in TM accumulation capacity of plant resources present in the surroundings of Metaleurop Nord has been documented in the literature (e.g. Deram et al., 2006, 2007; Migeon et al., 2009). However, the result (*4A*) directly demonstrated such difference in resources frequently and preferentially consumed by mice (i.e. Sapindaceae and Salicaceae family plants). Moreover, the results (*3A*) and (*3B*) indirectly suggested higher concentrations of Cd and Zn in Salicaceae than in most other plant resources. The results indicate that the first condition of the dilution effect was met for the two TMs. However, Pb hyper accumulator resource was not observed in the present thesis.

IV.2.2 Differences in the food preference of the wood mouse between hyper and low accumulator resources and in the sensibility of the resources to biodiversity loss

The condition secondly controlling occurrence of the dilution effect is the difference in the food preference. This condition can be subdivided into two sub-questions: existence of food

preference and the relationship between preferred resources and their accumulation capacity (Figure I.8). The result (*2A*) showed that some plant resources were preferentially consumed. The first sub-question of the second condition was met. However, the response to the second sub-question is complex because both low and hyper accumulators were preferentially consumed by wood mice. This could lead to several consequences (e.g. dilution amplification effects or no relationship) according to the other condition: the difference in the sensibility of the resources to biodiversity loss. Furthermore, preferred food depends on seasons and also on soil TM contamination levels (*2C*). This indicates that underlying mechanisms can also differ according to seasons and soil TM contamination levels (Figure IV.2a).

In spring, both low accumulator plants (Sapindaceae) and hyper accumulator plants (Salicaceae) of Cd and Zn were preferentially consumed when soil TM contamination level was low. However, pattern of their occurrence in the field and along the gradient of plant diversity was different (*5D*): Occurrence of Sapindaceae plants in the field increased along the gradient of plant diversity, whereas occurrence of Salicaceae plants in the field did not change along the gradient of plant diversity. According to the three conditions of the dilution effect, an increase in occurrence of preferred and low accumulator resources along the diversity gradient leads to a dilution effect, whereas contribution of preferred and hyper accumulator resources to transfer of TMs depends on other resources really consumed when their occurrence does not change along the diversity gradient. (Figure IV.2b). Actually, the results (*3B*) showed that high diet richness served as a reducing filter for exposure to Cd by hyper accumulator plants (i.e. the diet dilution). Functional effects of plant diversity on transfer of Cd depends on the relationship between individual diet richness and richness in the field. When soil contamination level was high, preference for Salicaceae plants disappeared (*2C*). As occurrence of preferred and low accumulator plants in the field increased along the gradient of plant diversity, a dilution effects is expected to occur (Figure IV.2c). In autumn, the two families were neither frequently nor preferentially consumed by wood mice. Although not directly measured, Adoxaceae and Asteraceae could be low and hyper accumulator plants, respectively (*5C*). In this case, however, occurrence of the two family plants increased along the gradient of the plant diversity (*5D*), which can lead to the opposite results, dilution and amplification effects (Figure IV.2d).

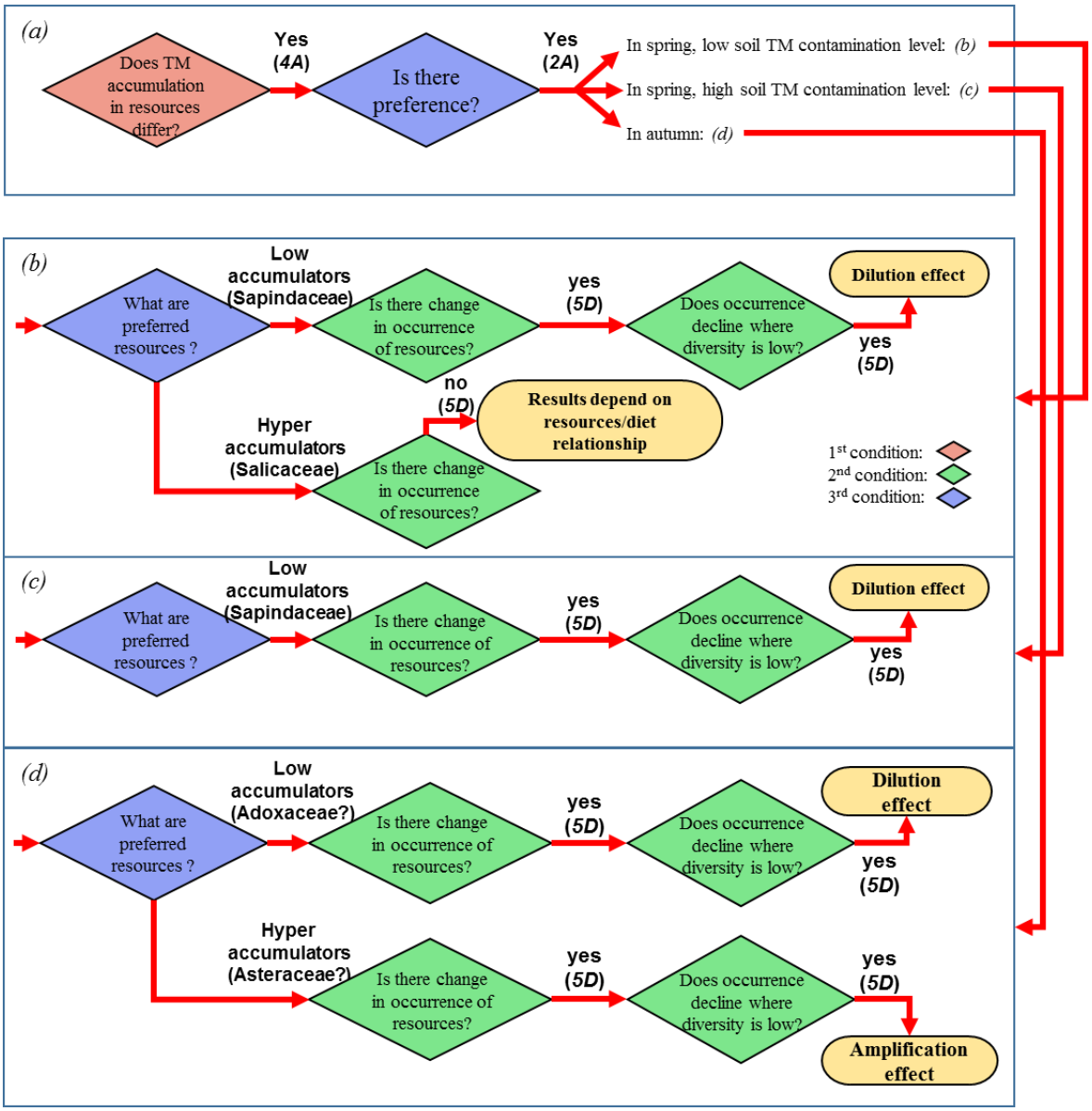


Figure IV.2. Flowchart illustrating the three conditions of the dilution effect of plant resource diversity and the theoretical consequences from the results obtained in the present thesis. (a): The conditions which were met in the present thesis. (a): The conditions which were met in the present thesis. (b)-(d): the conditions which depended on seasons or soil TM contamination levels. Number and capital letter in brackets correspond to each results described in the part IV.1.

IV.2.3Theoretical conditions of the dilution effect and the results observed in the thesis

According to the conceptual schema, a dilution effect of plant diversity on exposure to Cd and Zn is expected to occur in spring where soil TM contamination level was high. A dilution effect on exposure can occur in spring also where soil TM contamination level was low, depending on the relationship between diet richness and richness in the field. Both dilution and amplification effects can occur in autumn. The conditions for the dilution effect are not always met. Actually, only a dilution effect on exposure to Cd or Zn was not observed in the present thesis. Dilution effect on exposure to Cd and Zn could be conditioned by other factors than the three conditions, such as seasons or soil TM contamination levels. Exposure to Pb reduced along the gradient of plant diversity. Sapindaceae plants could be considered as low Pb accumulator plants (*5C*).

On the other hand, dilution effects of plant diversity on accumulation of Cd in both the liver and the kidneys of wood mice were clearly demonstrated. Accumulation of TMs in the critical tissues results from the toxicokinetics of TMs during the animal life (Nordberg et al., 2014). Mice used in the present thesis had lived over several months, during which resources really consumed could vary seasonally (e.g. (Butet, 1986a). Exposure of TMs was certainly not similar among seasons. However, there is no clear data about what mice consumed in other seasons (i.e. winter and summer). Details about food of mice in other seasons remain as an issue for further studies. There was no effect of diversity on accumulation of Zn. Zn is an essential element for organism, and its uptake and metabolism can be regulated in homeostatic ways (Shore and Rattner, 2001). Effects of resource diversity on accumulation of Zn cannot be discussed. In the present thesis, we failed to quantify accumulation of Pb in the organs. Effects of diversity on accumulation of Pb in organs of mice also cannot be verified. This remains as an issue further studies.

IV.2.4Potential role of invertebrate resources on exposure and accumulation

The present thesis failed to demonstrate potential role of invertebrates on transfer of TMs to wood mice because of limitation in identification of ingested invertebrates. Arthropod and earthworm resources did not play an important roles on trophic exposure to TMs (*3a*). In the literature, some invertebrates, such as Formicidae (Eeva et al., 2004), Isopoda (Hopkin and Martin, 1982) or spiders (Rabitsch, 1995), were reported in TM contaminated sites as TM accumulators. On the contrary, carabid beetles are in general considered not to accumulate TMs in the body (Hopkin, 1989). Our category “arthropod items” did not allow to distinguishing functional roles of accumulator invertebrates in the transfer of TM in food webs. On the other hand, earthworms were not sampled in the field. However, the proportion of the ecological

groups of earthworms varied between sites of different TM contamination levels in the surrounding of Metaleurop Nord (Pérès et al., 2011). Although invertebrate resources are considered to be occasionally consumed by wood mice (Butet and Paillat, 1997), it should be examined whether preference for certain invertebrate taxa exists in wood mice. Identification of ingested invertebrate resources followed by for a food preference analysis should be carried out in further studies.

IV.3 Research perspectives: other environmental and biological factors potentially involved in the dilution effect as underlying mechanisms

Some underlying mechanisms of the dilution effect were revealed in this present thesis. However, several other environmental and biological factors can interfere in those relationships. Those factors can be classed into four categories: factors potentially controlling (i) bioavailability of TMs for resources, (ii) foraging behavior of wood mice, (iii) food preference of wood mice, and (iv) toxicokinetics of ingested TMs in wood mice (Figure IV.3). In addition to identification more detailed of food consumed by mice and to measuring TM concentrations in more various resources potentially involved in the dilution effect, those factors can be considered as research perspectives of the present thesis.

IV.3.1 Factors potentially controlling bioavailability of TMs

The thesis focused on both diversity and composition of resources in relation to soil TM concentrations. Several factors are involved in the bioavailability of TMs to plants. For instance, the chemical composition of other elements in soils may shape the pattern of transfer to plants. Plants must acquire several essential elements to grow and complete the life cycle, and interactions between chemical elements influence the availability of those elements. Diversity of plants could be involved in such relationship. Actually, several studies have demonstrated positive effects of plant species richness on soil microbial biomass and functioning (e.g. Eisenhauer et al., 2010). Positive effects of plant diversity or richness on maintaining diverse microbial communities, soil enzyme activities, microbial biomass and soil basal respiration have been demonstrated even in in TM contaminated sites (Gao et al., 2012; Stefanowicz et al., 2012). On the other hand, significant roles of soil invertebrates for stability of organic matter and thus nutrient quality in soils have been well documented for a long time (e.g. De Deyn et al., 2003; Eisenhauer et al., 2011; Stork and Eggleton, 1992; Wolters, 2000). Further studies on multiple systems, such as soil nutrient cycles, soil microbes, plants and above- and underground invertebrates' communities, will be required for elucidating reciprocal reactions between biodiversity and transfer of TMs.

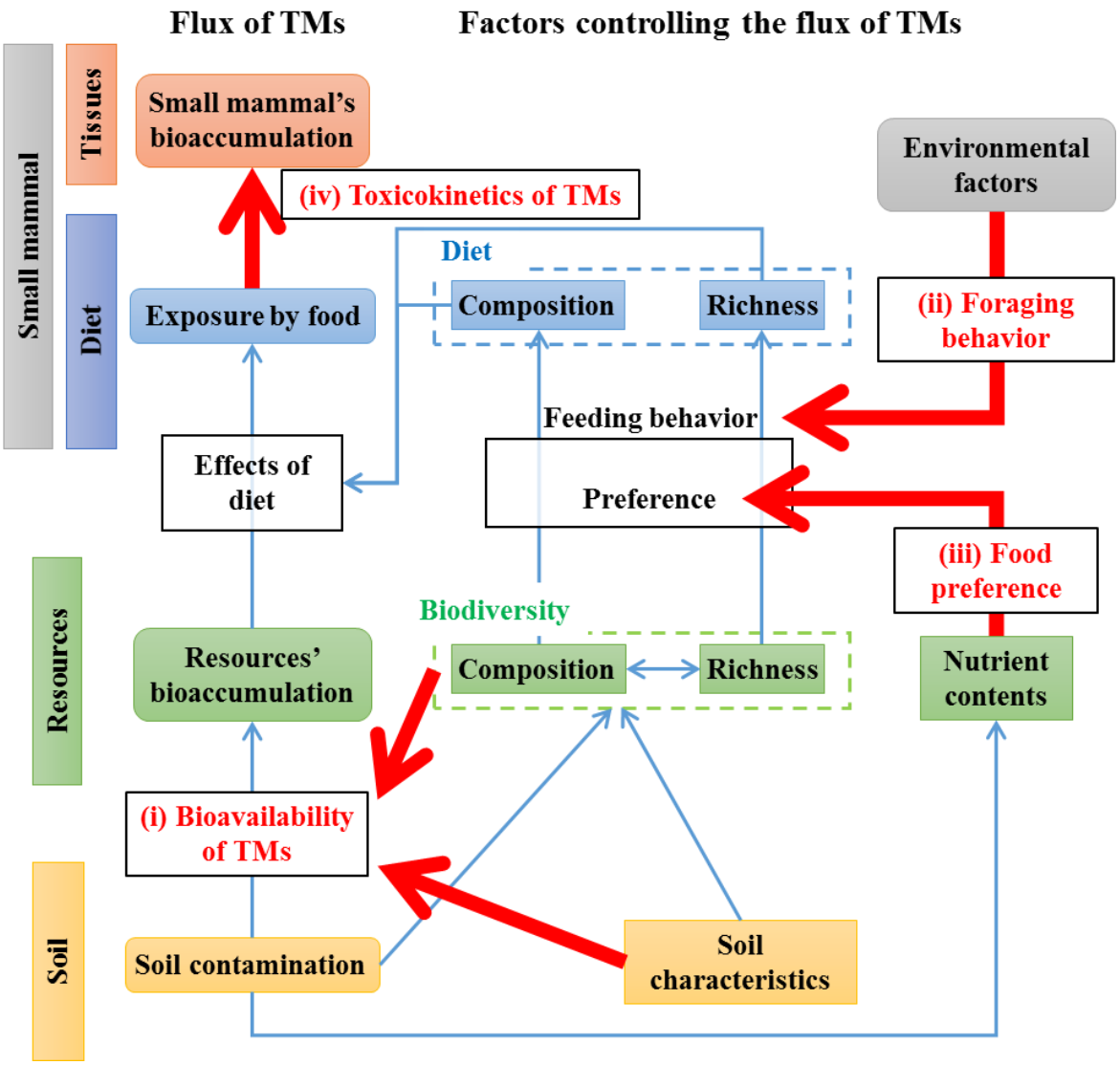


Figure IV.3. Diagram representing four other factors potentially interfering in the flux of TMs in the terrestrial ecosystem from the soil to the small mammal through the trophic route (on the basis of Figure I.9): factors controlling (i) bioavailiability of TMs, factors controlling (i) bioavailiability of TMs, (ii) feeding behavior, (iii) food preference and (iv) toxicokinetics of TMs.

IV.3.2Ecological factors controlling foraging behaviors of wood mice

The relationship between food supply and requirement is not the only factor controlling feeding behaviors of wood mice. Animals often balance demands for food and safety form their predator. Reduce of foraging by predatory risk has been widely documented in rodents, such as Arizona pocket mouse *Perognathus amplus*, Bailey's pocket mouse *P. baileyi* and Merriam's kangaroo rat *Dipodomys merriami* (Brown et al., 1988), two species of gerbils, *Gerbillus allenbyi* and *G. pyramidum* (Abramsky et al., 1996), or the Central American agouti Dasyprocta punctate (Suselbeek et al., 2014). Díaz et al. (2005) observed in Mediterranean forest habitats

that wood mice changed times and frequency of their foraging activities in response to foraging activity of their predator, smalls-potted genets *Genetta genetta*. Moreover, anti-predator strategies have been developed. For example, rodents preferentially forage in sheltered microhabitats, e.g. under vegetative cover, under precipitation or under the situation with lack of moonlight (Kotler, 1984; Orrock et al., 2004). On the other hand, feeding behavior of wood mice can be also influenced by intra-and inter-trophic overlaps. For example, foraging activity *per capita* of each of two gerbils, *Gerbillus allenbyi* and *G. pyramidum* were negatively correlated with thier own population density, whereas interspecific competition between the two species were asymmetric (Abramsky and Pinshow, 1989). In the study of Abt and Bock (1998) carried out in a mixed farmland, seasonal variation in dietary overlap within wood mice, yellow-necked mice *A. flavicollis* and bank voles *Clethrionomys glareolus* could be explained by change in competitive interaction over seasons. The authors supposed that shortage in resources of one competitor could cause a high dietary overlap and provoke a shortage of resources of others. In the present thesis, food and feeding behaviors were assessed only for wood mice. However, interaction with presence and/or behaviors of other vertebrates, especially competitive rodents and predator vertebrates, should be taken into account for further studies to describe accurate feeding behaviors.

Furthermore, correlations between home range sizes and either food availability or population density have been shown in several mammals (Agrell, 1995; Erlinge et al., 1990; Ims, 1987; Ostfeld, 1985, 1986; Ostfeld et al., 1985). Home range size is related to fitness of animals, and supply of sufficient energy to survive and reproduce in a space is related to density of food and dimension of the space (Burt, 1943). The field experiment of Schoepf et al. (2015) showed that home range sizes of female African striped mice *Rhabdomys pumilio* was negatively affected by population density and positively affected by food availability in the field.

IV.3.3 Factors controlling food preference of wood mice

Although preference for certain food is considered as one of the conditions for the dilution effect, underlying mechanisms of change in preference was not explained in the present thesis. An important factor controlling food preference is food quality. For example, energy-rich but protein-poor food is preferred by white-footed mice *Peromyscus leucopus* (Lewis et al., 2001) or by deer mice *Peromyscus maniculatus* (Vickery et al., 1994). In the case of wood mice, their main trophic range cover high energetic resources in their diet (Butet and Delettre, 2011; Hansson, 1971). Furthermore, wood mice preferred fatty resources to starchy resources among high energetic resources (Butet, 1986a). Jennings (1976) supposed that wood mice could detect resources by olfactory detection particularly developed for fatty acid. Preference for food can be based on content of chemical compounds. On the other hand, nutrient quality of plant

resources can be deteriorated by TM contamination. TMs cause oxidative damage to plants, either directly or indirectly through reactive oxygen species (ROS) formation, resulting in lipid peroxidation, protein peroxidation, and DNA damage (Mourato et al., 2012; Nagajyoti et al., 2010). These harmful effects of TMs cause deterioration of both carbohydrate and protein content. A significant modification of glucose, fructose and soluble protein concentration in lettuce *Lactuca sativa* by Cd contamination was actually observed in the experiment study of Dias et al. (2013). Contamination by Pb also resulted in a decrease in the sucrose content of vegetable crop species such as lettuce, spinach *Spinacia oleracea*, radish *Raphanus sativus*, carrot *Daucus carota*, red beet *Beta vulgaris* and onion *Allium cepa* (Gawęda, 2007). Change in preference for food could be related to change in nutrient content due to high exposure of plants to TMs.

However, food quality is determined through the balance between the levels of nutrients (e.g. energy and protein) and anti-predator defensive chemicals (e.g. toxin) (Manteca et al., 2008; Provenza et al., 2003). For example, plant secondary metabolites (PSMs) act in many ways against herbivory of mammals as toxins or digestibility reducers, which can lead to modify both quality of food and trophic responses of mammals (Iason, 2005). Meadow voles *Microtus pennsylvanicus* for example chose food rich in protein but poor in total phenolic compounds (Bergeron and Jodoin, 1987). Tannins are especially one of the most abundant PSMs produced in many woody plants, commonly ranging from 5% to 10% of dry weight of tree leaves, and one of substances affecting food choice. Choice and preference for low tannin consent food have been observed in several mammals, such as grey squirrels *Sciurus carolinensis* (Barthelmess, 2001), Sichuan field mice *Apodemus latronum* and Chevrier's field mice *A. chevrieri* (Wang and Chen, 2008) and also degus *Octodon degus* and leaf-eared mice *Phyllotis darwini* (Bozinovic, 1997). Actually, mammals can recognize and respond to a diverse repertoire of chemical entities, including sugars, salts, acids and a wide range of plant toxic substances including tannins which taste bitter (Barbehenn and Constabel, 2011; Lindemann, 1996). In relation to TMs, phenolic compounds function as antioxidant substances due to their high tendency to chelate metals (Michalak, 2006). ROS in plants due to exposure to TMs results in biosynthesis of diverse biomolecules such as glutathione, phytochelatins and metallothionein, but also enzymatic and non-enzymatic antioxidants, including phenolic compounds such as flavonoids and tannins (Emamverdian et al., 2015; Sharma and Dietz, 2006; Viehweger, 2014). Actually, the experiment of André et al. (2006) demonstrated that condensed tannin content in leaves of *A. pseudoplatanus* tended to be more important in samples with an artificial exposure to TMs (Cu, Zn, Cd and Pb) during three years than in samples without the TM treatment. Although the present thesis measured concentrations of some major elements in leaves of Salicaceae and Sapindaceae family plants, determining biochemical composition of organic

compounds, including plant toxic substances, provides relevant interpretation on the control of trophic responses of mice in the field.

IV.3.4Factors controlling toxicokinetics of ingested TMs in wood mice

Although not analyzed in the present thesis, toxicokinetic profiles of TMs can be another factor considered as to be potential underlying mechanisms for the dilution effects. Protecting role of high diet richness against high exposure to TMs (i.e. the dilution diet; Boyd, 1998) was demonstrated in the present thesis. However, variety of food consumed could also play further functional roles on assimilation and accumulation of TMs. For example, food quality could control gastrointestinal absorption of micro-elements in body. Inhibitor effects of essential elements such as Ca, Mg, P or Fe on uptake of non-essential elements, e.g. Cd and Pb, have been widely documented (e.g. Nordberg et al., 2014; Shore and Rattner, 2001). Deficiency of macronutrients in food increases uptake of TMs. On the other hand, it has been also reported that absorption of TMs is controlled by macro-nutrient store as in human body (e.g. Flanagan et al., 1978). Deficiency of some macronutrients in daily food could impact on toxicokinetics of TMs. The present thesis demonstrated that elemental composition was correlated with non-essential elements, and such responses to TM contamination were species dependent (*4b*). These results can support the nutrient balance hypothesis (Westoby, 1978): the nutrient balance can hardly be maintained from a single resources for wildlife. In addition to the relationship between nutrient balance in food and trophic responses of wood mice mentioned above, potential effects of nutrient balance on toxicokinetics of TMs remains as an issue for further studies.

Part V. Conclusion

The present thesis has dealt with potential functional roles of biodiversity on the transfer of TMs in food webs and some underlying mechanisms. As a parallel to the dilution effect in the transmission of pathogens, which is also still debated in scientific community, it was hypothesized that diversity of resources in the field would function as a protecting filter against exposure to TMs and accumulation of TMs of wood mice in a TM contaminated area. The results demonstrated reducing effects of resource diversity on exposure to TMs and accumulation of TMs, which however depends on elements and types of food. After examining underlying mechanisms, it was shown that trophic responses of wood mice to each resource were key factors controlling the dilution effect. However, such underlying mechanisms still remain as complex issues because trophic responses themselves could be controlled by the environmental TM contamination. Further studies for elucidating the hypothetical underlying mechanisms and also for completing other possible mechanisms are required to understand functional roles of biodiversity on the transfer of TMs.

The most important question of the present thesis is whether the dilution effect in the transfer of TMs is expected to generally occur in nature. The present thesis demonstrated one case of relationships between soil TM contaminations and transfer of TM in food webs using only one biological model, the wood mouse. However, some underlying mechanisms for the dilution effects require certain specific properties of biological models (e.g. preference) or resources of the given model (e.g. accumulation capacity). Furthermore, some properties of biological models or resources required as important aspects for underlying mechanisms could differ between ecosystems or types of pollutants. This means that each combination among biological models, available resources, and pollutants can lead to different results about effects of biodiversity on the transfer of pollutants in food webs. Given no other study has been investigated for this topic so far, no one can predict what happens in other sites, using other biological model, or for other pollutants, until other works are investigated in the similar theme.

Even if certain technical approaches in the present thesis were original or even unusual, ecological concepts providing the basis for each hypothetical underlying mechanism of the dilution effect, such as food preference or TM accumulation capacity, have been studied and discussed for a long times in scientific communities. The dilution effect in the transmission of pathogens was built on the basis of several theoretical, experimental and observational studies cumulated during more than a centuries, on ecology of pathogens, invertebrate vectors and mammals involved in. Nowadays, several hundreds of examples demonstrating the dilution effect in transmission of pathogens have been reported. If the present thesis successes to stimulate interest of other scientists in different domains, this thesis could serve as a first step for further investigation about this topic and thus for responding in future to the last question: Can the dilution effect in the transfer of pollutants be considered as an ecosystem service?

Part VI. Bibliography

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Part VII. Appendices

VII.1 Appendix A

Inventory of plant and invertebrate taxa observed in each site

(Supporting Information SI2 of the Chapter 2)

Names	Explanation
Class	Identified class name. Unidentified class was marked by "-".
Order	Identified order name. Unidentified order was marked by "-".
Family	Identified family name. Unidentified family was marked by "-".
Genus	Identified genus name. Unidentified genus was marked by "-".
Species	Identified species name. Unidentified species was marked by "-".
Cover-abundance	Cover-abundance of vascular plants estimated by vertically projected area (m^2).
Number of captures	Number of animals captured.
TE2	Site "TE2" (contamination level: 0 (control); dominant landscape feature: forest).
103	Site "103" (contamination level: +; dominant landscape feature: forest).
117	Site "117" (contamination level: ++; dominant landscape feature: forest).
097	Site "097" (contamination level: +++; dominant landscape feature: forest).
171	Site "171" (contamination level: ++; dominant landscape feature: forest).
043	Site "043" (contamination level: ++; dominant landscape feature: arable).
113	Site "113" (contamination level: ++; dominant landscape feature: urban).

a: Both unidentified coniferous and deciduous plants were considered as one taxon for each stratum.
b: No survey was carried out for the tree stratum in the site 113 because there were few trees.
c: Individuals identified at other taxonomic level (e.g. sub-family; sub-species) were classified to higher taxonomic level (e.g. family; species).
d: Different life stages (e.g. larvae/adult) were ignored for counting.

Trees

Class	Order	Family	Genus	Species	Estimated cover-abundance (m^2)[c]						
					TE2	**103**	**117**	**097**	**171**	**043**	**113**[b]
Magnoliopsida	Sapindales	Sapindaceae	*Acer*	*Acer campestre*	0.00	0.00	0.00	0.00	35.42	0.00	-
Magnoliopsida	Sapindales	Sapindaceae	*Acer*	*Acer pseudoplatanus*	2258.46	0.00	27481.66	0.00	29706.14	3090.71	-
Magnoliopsida	Sapindales	Sapindaceae	*Aesculus*	*Aesculus hippocastanum*	634.74	0.00	0.00	0.00	216.31	0.00	-
Magnoliopsida	Fagales	Betulaceae	*Alnus*	*Alnus glutinosa*	15.77	22.67	699.98	0.00	0.00	1384.06	-
Magnoliopsida	Fagales	Betulaceae	*Betula*	*Betula pendula*	0.00	27.93	18.36	1727.82	821.42	9801.21	-
Magnoliopsida	Fagales	Betulaceae	*Carpinus*	*Carpinus betulus*	544.38	0.00	0.00	0.00	0.00	3186.05	-
Magnoliopsida	Fagales	Fagaceae	*Castanea*	*Castanea sativa*	0.00	0.00	0.00	0.00	35.42	0.00	-
Magnoliopsida	Cornales	Cornaceae	*Cornus*	*Cornus sanguinea*	0.00	0.00	27.23	0.00	0.00	0.00	-
Magnoliopsida	Fagales	Fagaceae	*Fagus*	*Fagus sylvatica*	1919.99	0.00	0.00	0.00	0.00	3186.05	-
Magnoliopsida	Lamiales	Oleaceae	*Fraxinus*	*Fraxinus excelsior*	13407.89	4565.76	11107.84	263.24	10058.54	7192.22	-
Magnoliopsida	Apiales	Araliaceae	*Hedera*	*Hedera helix*	1661.05	0.00	0.00	0.00	0.00	0.00	-
Magnoliopsida	Proteales	Platanaceae	*Platanus*	-	0.00	0.00	0.00	3289.67	0.00	0.00	-
Magnoliopsida	Malpighiales	Salicaceae	*Populus*	*Populus tremula x Populus alba*	15.77	0.00	0.00	5326.49	68.65	0.00	-
Magnoliopsida	Malpighiales	Salicaceae	*Populus*	*Populus balsamifera*	0.00	0.00	2898.53	0.00	0.00	546.87	-
Magnoliopsida	Malpighiales	Salicaceae	*Populus*	-	15275.35	58861.78	17600.56	0.00	46.25	2833.91	-
Magnoliopsida	Rosales	Rosaceae	*Prunus*	*Prunus avium*	0.00	0.00	78.17	0.00	42.62	0.00	-
Magnoliopsida	Fagales	Fagaceae	*Quercus*	*Quercus robur*	1483.69	27.93	35.96	0.00	7.70	146.03	-
Magnoliopsida	Fagales	Fagaceae	*Quercus*	*Quercus rubra*	0.00	0.00	0.00	0.00	0.00	20.47	-
Magnoliopsida	Fabales	Fabaceae	*Robinia*	*Robinia pseudoacacia*	0.00	2.79	0.00	5138.26	77.01	3257.31	-
Magnoliopsida	Malpighiales	Salicaceae	*Salix*	*Salix alba*	300.50	1.20	0.00	0.00	8285.93	1070.30	-
Magnoliopsida	Malpighiales	Salicaceae	*Salix*	*Salix caprea*	316.28	0.00	0.00	263.24	35.42	4453.44	-
Pinopsida	Pinales	Taxaceae	*Taxus*	*Taxus baccata*	840.72	0.00	0.00	0.00	0.00	0.00	-
Magnoliopsida	Malvales	Malvaceae	*Tilia*	*Tilia platyphyllos*	714.50	0.00	0.00	0.00	571.43	0.00	-
Magnoliopsida	Rosales	Ulmaceae	*Ulmus*	*Ulmus minor*	0.00	0.00	0.00	49.94	0.00	0.00	-
[Various coniferous trees][a]					524.47	0.00	0.00	0.00	0.00	392.75	-
				Taxonomic richness	**15**	**7**	**9**	**7**	**14**	**14**	**-**

Shrubs

Class	Order	Family	Genus	Species	Estimated cover-abundance (m^2)[c]						
					TE2	103	117	097	171	043	113
Magnoliopsida	Sapindales	Sapindaceae	*Acer*	*Acer campestre*	0.00	90.49	196.12	0.00	77.01	0.00	0.00
Magnoliopsida	Sapindales	Sapindaceae	*Acer*	*Acer platanoides*	0.00	0.00	107.04	0.00	0.00	42.16	0.00
Magnoliopsida	Sapindales	Sapindaceae	*Acer*	*Acer pseudoplatanus*	575.02	187.29	18.36	35.89	145.55	207.92	0.00
Magnoliopsida	Fagales	Betulaceae	*Alnus*	*Alnus glutinosa*	223.09	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Lamiales	Scrophulariaceae	*Buddleja*	*Buddleja davidii*	69.42	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Fagales	Betulaceae	*Carpinus*	*Carpinus betulus*	311.93	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Ranunculales	Ranunculaceae	*Clematis*	*Clematis vitalba*	0.00	0.00	0.00	0.00	0.00	607.27	127.91
Magnoliopsida	Cornales	Cornaceae	*Cornus*	*Cornus sanguinea*	2.90	268.75	1106.53	2593.58	12997.64	8590.97	127.91
Magnoliopsida	Fagales	Betulaceae	*Corylus*	*Corylus avellana*	0.00	904.88	0.00	499.36	821.42	0.00	0.00
Magnoliopsida	Rosales	Rosaceae	*Crataegus*	*Crataegus monogyna*	1498.66	182.31	398.08	26.32	460.54	6642.03	0.00
Magnoliopsida	Rosales	Rosaceae	*Crataegus*	*Crataegus laevigata*	0.00	178.26	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Rosales	Rhamnaceae	*Rhamnus*	*Frangula alnus*	2.90	27.93	0.00	2330.34	0.00	0.00	0.00
Magnoliopsida	Lamiales	Oleaceae	*Fraxinus*	*Fraxinus excelsior*	309.48	0.00	0.00	0.00	14.36	641.19	0.00
Magnoliopsida	Apiales	Araliaceae	*Hedera*	*Hedera helix*	309.48	0.00	0.00	0.00	6521.58	0.00	0.00
Magnoliopsida	Rosales	Cannabaceae	*Humulus*	*Humulus lupulus*	0.00	1184.17	0.00	0.00	6149.05	0.00	0.00
Magnoliopsida	Fagales	Juglandaceae	*Juglans*	*Juglans regia*	12.57	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Lamiales	Oleaceae	*Ligustrum*	*Ligustrum vulgare*	0.00	0.00	0.00	2330.34	617.38	0.00	0.00
Magnoliopsida	Rosales	Rosaceae	*Malus*	*Malus sylvestris*	12.57	0.00	6.94	0.00	0.00	0.00	0.00
Magnoliopsida	Malpighiales	Salicaceae	*Populus*	-	0.00	0.00	650.09	0.00	0.00	0.00	0.00
Magnoliopsida	Malpighiales	Salicaceae	*Populus*	*Populus tremula x Populus alba*	411.94	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Rosales	Rosaceae	*Prunus*	*Prunus avium*	12.57	0.00	1.84	0.00	439.20	0.00	0.00
Magnoliopsida	Rosales	Rosaceae	*Prunus*	*Prunus cerasifera*	12.57	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Rosales	Rosaceae	*Prunus*	*Prunus spinosa*	0.00	6177.93	0.00	0.00	0.00	428.68	27.41
Magnoliopsida	Rosales	Rosaceae	*Pyrus*	*Pyrus communis*	12.57	0.00	6.94	0.00	0.00	0.00	0.00
Magnoliopsida	Fagales	Fagaceae	*Quercus*	*Quercus robur*	0.00	0.44	0.00	0.00	0.00	207.92	0.00
Magnoliopsida	Fagales	Fagaceae	*Quercus*	*Quercus rubra*	0.00	0.00	0.00	0.00	0.00	42.16	0.00
Magnoliopsida	Fabales	Fabaceae	*Robinia*	*Robinia pseudoacacia*	0.00	0.00	0.00	0.00	42.62	0.00	27.41
Magnoliopsida	Rosales	Rosaceae	*Rubus*	*Rubus caesius*	0.00	44.40	0.00	5326.49	0.00	0.00	0.00
Magnoliopsida	Rosales	Rosaceae	*Rubus*	*Rubus sp.*	0.00	4267.19	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Malpighiales	Salicaceae	*Salix*	*Salix x rubra*	0.00	0.00	0.00	95.63	77.01	0.00	0.00
Magnoliopsida	Malpighiales	Salicaceae	*Salix*	*Salix alba*	0.00	0.00	421.60	0.00	34.35	0.00	2.74
Magnoliopsida	Malpighiales	Salicaceae	*Salix*	*Salix caprea*	223.09	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Malpighiales	Salicaceae	*Salix*	*Salix cinerea*	0.00	0.00	184.45	1816.91	14.36	0.00	0.00
Magnoliopsida	Dipsacales	Adoxaceae	*Sambucus*	*Sambucus ebulus*	0.00	0.00	0.00	0.00	0.00	16.45	0.00
Magnoliopsida	Dipsacales	Adoxaceae	*Sambucus*	*Sambucus nigra*	306.46	2780.57	449.94	263.24	491.53	0.00	127.91
Magnoliopsida	Dipsacales	Caprifoliaceae	*Symphoricarpos*	*Symphoricarpos albus*	2987.54	0.00	0.00	0.00	0.00	0.00	0.00
Pinopsida	Pinales	Taxaceae	*Taxus*	*Taxus baccata*	2.77	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Malvales	Malvaceae	*Tilia*	*Tilia platyphyllos*	3.09	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Rosales	Ulmaceae	*Ulmus*	*Ulmus minor*	544.07	178.26	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Dipsacales	Adoxaceae	*Viburnum*	*Viburnum lantana*	0.00	0.00	4.20	0.00	7.70	0.00	2.74
Magnoliopsida	Dipsacales	Adoxaceae	*Viburnum*	*Viburnum opulus*	0.00	0.00	0.00	2356.66	0.00	0.00	0.00
[Various coniferous shrubs][a]					694.24	0.00	0.00	0.00	0.00	0.00	0.00
[Various deciduous shrubs][a]					69.42	0.00	0.00	0.00	0.00	0.00	0.00
				Taxonomic richness	**23**	**14**	**13**	**11**	**16**	**10**	**7**

Herbs

Class	Order	Family	Genus	Species	Estimated cover-abundance (m^2)[c]						
					TE2	103	117	097	171	043	113
Magnoliopsida	Ericales	Primulaceae	*Anagallis*	*Anagallis arvensis*	0.00	0.00	0.00	1.39	0.00	0.00	0.00
Liliopsida	Poales	Poaceae	*Agrostis*	*Agrostis capillaris*	0.00	0.00	0.00	30.72	0.00	0.00	0.00
Liliopsida	Poales	Poaceae	*Arrhenatherum*	*Arrhenatherum elatius*	15153.64	15853.34	27723.97	6940.32	1438.09	5587.86	8.71
Liliopsida	Poales	Poaceae	*Avena*	*Avena fatua*	0.00	0.00	0.00	0.00	0.00	0.00	19.90
Magnoliopsida	Fabales	Fabaceae	*Astragalus*	*Astragalus glycyphyllos*	0.00	0.00	0.00	0.00	0.00	934.19	0.00
Magnoliopsida	Asterales	Asteraceae	*Arctium*	*Arctium lappa*	0.00	0.00	94.32	0.00	0.00	0.00	0.00
Liliopsida	Alismatales	Araceae	*Arum*	*Arum maculatum*	5.44	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Asterales	Asteraceae	*Achillea*	*Achillea millefolium*	0.00	0.00	0.00	0.00	0.00	0.00	149.44
Magnoliopsida	Caryophyllales	Plumbaginaceae	*Armeria*	*Armeria maritima*	0.00	0.00	0.00	275.79	0.00	0.00	0.00
Liliopsida	Poales	Poaceae	*Alopecurus*	*Alopecurus myosuroides*	25.44	0.00	0.00	0.00	0.00	9.36	0.00
Magnoliopsida	Ranunculales	Ranunculaceae	*Anemone*	*Anemone nemorosa*	5.44	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Asterales	Asteraceae	*Anthemis*	-	0.00	0.00	0.00	8.91	0.00	0.00	0.00
Magnoliopsida	Rosales	Rosaceae	*Aphanes*	*Aphanes arvensis*	0.00	0.00	0.00	124.06	0.00	0.00	0.00
Magnoliopsida	Brassicales	Brassicaceae	*Alliaria*	*Alliaria petiolata*	0.00	0.00	0.00	0.00	740.23	0.00	0.00
Liliopsida	Alismatales	Alismataceae	*Alisma*	*Alisma plantago aquatica*	1.77	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Apiales	Apiaceae	*Aegopodium*	*Aegopodium podagraria*	848.03	0.00	0.00	0.00	2461.22	0.00	0.00
Liliopsida	Poales	Poaceae	*Alopecurus*	*Alopecurus pratensis*	607.25	147.45	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Sapindales	Sapindaceae	*Acer*	*Acer pseudoplatanus*	0.00	0.00	0.00	0.00	8369.83	0.00	0.00
Liliopsida	Poales	Poaceae	*Agrostis*	*Agrostis stolonifera*	292.51	0.00	358.86	790.34	0.00	0.00	0.00
Magnoliopsida	Asterales	Asteraceae	*Aster*	-	0.00	0.00	0.00	4.99	0.00	0.00	0.00
Liliopsida	Poales	Poaceae	*Apera*	*Apera spica venti*	0.00	0.00	0.00	0.00	0.00	0.00	8.71
Magnoliopsida	Apiales	Apiaceae	*Angelica*	*Angelica sylvestris*	0.00	10.63	0.00	0.00	72.98	0.00	0.00
Magnoliopsida	Caryophyllales	Chenopodiaceae	*Atriplex*	*Atriplex patula*	0.00	0.00	0.00	0.00	0.00	4.47	0.00
Magnoliopsida	Asterales	Asteraceae	*Artemisia*	*Artemisia vulgaris*	0.00	0.00	0.00	0.00	13.91	0.00	0.00
Magnoliopsida	Cucurbitales	Cucurbitaceae	*Bryonia*	*Bryonia dioica*	0.00	0.00	2.02	0.00	0.00	0.00	0.00
Magnoliopsida	Asterales	Asteraceae	*Bellis*	*Bellis perennis*	0.00	0.00	0.00	16.94	490.13	0.00	447.52
Liliopsida	Poales	Poaceae	*Bromus*	*Bromus sterilis*	69.42	0.00	0.00	28.88	0.00	0.00	0.00
Liliopsida	Poales	Poaceae	*Brachypodium*	*Brachypodium sylvaticum*	30.40	0.00	0.00	0.00	3591.90	0.00	0.00
Liliopsida	Poales	Cyperaceae	*Carex*	*Carex acutiformis*	0.00	2019.09	994.84	0.00	340.57	0.00	0.00
Magnoliopsida	Caryophyllales	Chenopodiaceae	*Chenopodium*	*Chenopodium album*	0.00	0.00	0.00	0.00	0.00	0.00	19.90
Magnoliopsida	Asterales	Asteraceae	*Cirsium*	*Cirsium arvense*	1722.78	14.75	1138.95	0.05	1908.48	0.00	207.67
Magnoliopsida	Brassicales	Brassicaceae	*Capsella*	*Capsella bursa-pastoris*	0.00	0.00	0.00	0.98	0.00	0.00	0.00
Magnoliopsida	Asterales	Asteraceae	*Crepis*	*Crepis capillaris*	125.68	0.00	69.39	0.00	0.00	0.00	18.89
Magnoliopsida	Asterales	Asteraceae	*Conyza*	*Conyza canadensis*	0.00	0.00	0.00	0.00	7.70	0.00	0.00
Liliopsida	Poales	Cyperaceae	*Carex*	*Carex cuprina*	9.77	11.75	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Asterales	Asteraceae	*Centaurea*	*Centaurea cyanus*	0.00	0.00	0.00	8.91	0.00	0.00	0.00
Magnoliopsida	Brassicales	Brassicaceae	*Lepidium*	*Lepidium didymus*	0.00	0.00	0.00	124.06	0.00	0.00	0.00
Liliopsida	Poales	Poaceae	*Calamagrostis*	*Calamagrostis epigeios*	0.00	138.29	1318.62	2451.08	1432.12	15941.95	0.00
Magnoliopsida	Asterales	Asteraceae	*Cirsium*	*Cirsium eriophorum*	3.04	0.00	0.00	0.00	0.00	15.65	0.00
Magnoliopsida	Gentianales	Gentianaceae	*Centaurium*	*Centaurium erythraea*	0.00	0.00	0.00	0.00	0.00	62.62	0.00
Magnoliopsida	Caryophyllales	Chenopodiaceae	*Chenopodium*	*Chenopodium ficifolium*	0.00	0.00	0.00	124.06	0.00	0.00	0.00

Herbs (continuous)

Class	Order	Family	Genus	Species	Estimated cover-abundance (m^2)[c]						
					TE2	103	117	097	171	043	113
Magnoliopsida	Caryophyllales	Caryophyllaceae	*Cerastium*	*Cerastium fontanum*	0.00	1474.50	9.37	804.23	0.00	0.00	43.26
Liliopsida	Poales	Cyperaceae	*Carex*	*Carex spicata*	0.00	0.00	0.00	1.39	0.00	0.00	0.00
Magnoliopsida	Brassicales	Brassicaceae	*Arabidopsis*	*Arabidopsis halleri*	0.00	0.00	0.00	65.76	0.00	0.00	0.00
Liliopsida	Poales	Cyperaceae	*Carex*	*Carex hirta*	0.00	54.82	0.00	790.34	40.81	14.60	0.00
Magnoliopsida	Lamiales	Plantaginaceae	*Callitriche*	*Callitriche hamulata*	0.00	12.04	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Myrtales	Onagraceae	*Circaea*	*Circaea lutetiana*	0.00	0.00	2420.42	0.00	7629.60	0.00	0.00
Magnoliopsida	Ranunculales	Papaveraceae	*Chelidonium*	*Chelidonium majus*	0.00	0.00	0.00	0.00	740.23	0.00	0.00
Magnoliopsida	Solanales	Convolvulaceae	*Convolvulus*	*Convolvulus arvensis*	0.00	2350.25	0.00	1.44	0.00	2649.02	89.21
Magnoliopsida	Asterales	Asteraceae	*Cirsium*	*Cirsium oleraceum*	3.04	0.00	0.00	0.00	7.30	0.00	0.00
Magnoliopsida	Ranunculales	Ranunculaceae	*Caltha*	*Caltha palustris*	0.00	1.20	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Lamiales	Plantaginaceae	*Callitriche*	*Callitriche platycarpa*	0.00	0.00	0.00	1.31	0.00	0.00	0.00
Magnoliopsida	Asterales	Asteraceae	*Cirsium*	*Cirsium palustre*	238.86	2997.69	27.23	0.00	0.00	20.79	0.00
Liliopsida	Poales	Cyperaceae	*Carex*	*Carex riparia*	0.00	4963.93	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Solanales	Convolvulaceae	*Calystegia*	*Calystegia sepium*	796.72	78.70	961.44	1.30	912.98	0.00	198.96
Magnoliopsida	Cornales	Cornaceae	*Cornus*	*Cornus sanguinea*	0.00	0.00	196.12	0.00	1988.90	0.00	0.00
Magnoliopsida	Lamiales	Plantaginaceae	*Callitriche*	*Callitriche stagnalis*	1.77	12.04	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Ranunculales	Ranunculaceae	*Clematis*	*Clematis vitalba*	0.00	0.00	0.00	0.00	0.00	14.60	0.00
Magnoliopsida	Asterales	Asteraceae	*Cirsium*	*Cirsium vulgare*	70.40	14.75	90.36	124.06	0.00	20.47	0.00
Magnoliopsida	Apiales	Apiaceae	*Daucus*	*Daucus carota*	0.00	147.45	1909.84	30.32	0.00	0.00	0.00
Liliopsida	Poales	Poaceae	*Deschampsia*	*Deschampsia cespitosa*	2.90	1303.33	0.00	0.00	0.00	0.00	0.00
Polypodiopsida	Polypodiales	Dryopteridaceae	*Dryopteris*	*Dryopteris dilatata*	2.77	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Ranunculales	Ranunculaceae	*Delphinium*	-	0.00	0.00	0.00	8.91	0.00	0.00	0.00
Polypodiopsida	Polypodiales	Dryopteridaceae	*Dryopteris*	*Dryopteris filix mas*	36.39	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Dipsacales	Dipsacaceae	*Dipsacus*	*Dipsacus fullonum*	0.00	0.00	89.42	0.00	0.00	0.00	0.00
Liliopsida	Poales	Poaceae	*Dactylis*	*Dactylis glomerata*	1511.38	6935.84	286.74	0.00	624.37	204.68	149.44
Magnoliopsida	Myrtales	Onagraceae	*Chamerion*	*Chamerion angustifolium*	0.00	0.00	0.00	0.00	0.00	923.72	0.00
Equisetopsida	Equisetales	Equisetaceae	*Equisetum*	*Equisetum arvense*	0.00	0.00	15.00	0.00	40.81	17.05	0.00
Liliopsida	Poales	Poaceae	*Echinochloa*	*Echinochloa crus galli*	2.54	0.00	0.00	0.00	0.00	0.00	0.00
Liliopsida	Asparagales	Orchidaceae	*Epipactis*	*Epipactis helleborine*	0.00	0.00	0.00	4.99	46.35	0.00	0.00
Magnoliopsida	Myrtales	Onagraceae	*Epilobium*	*Epilobium hirsutum*	0.00	0.00	669.53	0.00	62.24	0.00	0.00
Magnoliopsida	Myrtales	Onagraceae	*Epilobium*	*Epilobium parviflorum*	0.00	0.00	0.00	0.00	476.45	0.00	0.00
Liliopsida	Poales	Poaceae	*Elymus*	*Elymus repens*	0.00	0.00	0.00	0.00	1904.27	0.00	30.69
Magnoliopsida	Ranunculales	Papaveraceae	*Eschscholzia*	*Eschscholzia californica*	0.00	0.00	0.00	8.91	0.00	0.00	0.00
Magnoliopsida	Asterales	Asteraceae	*Eupatorium*	*Eupatorium cannabinum*	0.00	12.01	12827.94	13.01	3299.80	759.22	0.00
Magnoliopsida	Boraginales	Boraginaceae	*Echium*	*Echium vulgare*	0.00	0.00	0.00	124.06	0.00	0.00	0.00
Liliopsida	Poales	Poaceae	*Festuca*	*Festuca arundinacea*	8932.94	0.00	660.71	0.00	4352.62	0.00	43.26
Liliopsida	Poales	Poaceae	*Festuca*	*Festuca brevipila*	0.00	0.00	0.00	9.35	0.00	0.00	0.00
Magnoliopsida	Caryophyllales	Polygonaceae	*Fagopyrum*	*Fagopyrum esculentum*	25.44	0.00	0.00	0.00	0.00	0.00	0.00
Liliopsida	Poales	Poaceae	*Festuca*	*Festuca rubra*	1074.34	0.00	1005.33	7770.83	0.00	0.00	2522.39
Magnoliopsida	Rosales	Rosaceae	*Filipendula*	*Filipendula ulmaria*	0.00	258.10	0.00	0.00	462.54	0.00	0.00
Magnoliopsida	Rosales	Rosaceae	*Fragaria*	*Fragaria vesca*	0.00	0.00	130.04	0.00	0.00	0.00	0.00

Herbs (continuous)

Class	Order	Family	Genus	Species	Estimated cover-abundance (m^2)[c]						
					TE2	103	117	097	171	043	113
Magnoliopsida	Gentianales	Rubiaceae	Galium	Galium aparine	833.26	303.07	0.00	0.00	0.00	297.03	0.00
Magnoliopsida	Geraniales	Geraniaceae	Geranium	Geranium dissectum	0.00	0.00	0.00	0.00	0.00	935.91	0.00
Liliopsida	Poales	Poaceae	Glyceria	Glyceria fluitans	3.04	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Lamiales	Lamiaceae	Glechoma	Glechoma hederacea	0.00	0.00	1309.97	0.00	1437.60	734.79	0.00
Magnoliopsida	Gentianales	Rubiaceae	Galium	Galium mollugo	0.00	0.00	0.00	0.00	408.06	15.65	0.00
Magnoliopsida	Fabales	Fabaceae	Galega	Galega officinalis	0.00	0.00	42.03	0.00	0.00	0.00	0.00
Magnoliopsida	Gentianales	Rubiaceae	Galium	Galium palustre	11.54	22.67	62.77	0.00	72.98	0.00	0.00
Magnoliopsida	Geraniales	Geraniaceae	Geranium	Geranium robertianum	84.80	0.00	0.00	0.00	18518.66	0.00	0.00
Magnoliopsida	Lamiales	Lamiaceae	Galeopsis	Galeopsis tetrahit	0.00	0.00	0.00	0.00	74.02	0.00	0.00
Magnoliopsida	Rosales	Rosaceae	Geum	Geum urbanum	74.86	0.00	0.00	0.00	740.23	0.00	0.00
Magnoliopsida	Apiales	Araliaceae	Hedera	Hedera helix	10636.07	0.00	0.00	0.00	19445.06	0.00	0.00
Magnoliopsida	Asterales	Asteraceae	Hieracium	Hieracium lachenalii	0.00	0.00	0.00	0.00	0.00	15.65	0.00
Liliopsida	Poales	Poaceae	Holcus	Holcus lanatus	8570.14	31254.80	271.74	1.44	216.31	29611.42	0.00
Magnoliopsida	Rosales	Cannabaceae	Humulus	Humulus lupulus	0.00	303.07	2.02	0.00	72.98	0.00	0.00
Liliopsida	Poales	Poaceae	Holcus	Holcus mollis	0.00	1303.33	0.00	0.00	0.00	0.00	0.00
Liliopsida	Poales	Poaceae	Hordeum	Hordeum murinum	0.00	0.00	0.00	0.00	0.00	0.00	17.41
Magnoliopsida	Malpighiales	Hypericaceae	Hypericum	Hypericum perforatum	0.00	0.00	4.20	0.00	146.85	0.00	0.00
Magnoliopsida	Asterales	Asteraceae	Hypochaeris	Hypochaeris radicata	0.00	0.00	0.00	0.00	0.00	0.00	107.94
Magnoliopsida	Apiales	Apiaceae	Heracleum	Heracleum sphondylium	646.18	1.17	92.06	0.00	2329.06	0.00	0.00
Magnoliopsida	Brassicales	Brassicaceae	Iberis	-	0.00	0.00	0.00	8.91	0.00	0.00	0.00
Liliopsida	Asparagales	Iridaceae	Iris	Iris pseudacorus	0.98	1.20	0.00	0.00	5.28	0.00	0.00
Liliopsida	Poales	Juncaceae	Juncus	Juncus effusus	7459.25	247.47	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Lamiales	Plantaginaceae	Kickxia	Kickxia elatine	0.00	0.00	0.00	0.00	0.00	179.82	0.00
Magnoliopsida	Fabales	Fabaceae	Lotus	Lotus corniculatus	0.00	0.00	5.68	0.00	0.00	0.00	0.00
Magnoliopsida	Lamiales	Lamiaceae	Lycopus	Lycopus europaeus	1.77	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Asterales	Asteraceae	Leucanthemum	Leucanthemum vulgare	0.00	202.27	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Lamiales	Plantaginaceae	Linaria	-	0.00	0.00	0.00	8.91	0.00	0.00	0.00
Liliopsida	Poales	Poaceae	Lolium	Lolium multiflorum	0.00	1474.50	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Ericales	Primulaceae	Lysimachia	Lysimachia nummularia	0.00	1.38	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Fabales	Fabaceae	Lotus	-	0.00	0.00	0.00	8.91	0.00	0.00	0.00
Magnoliopsida	Dipsacales	Caprifoliaceae	Lonicera	Lonicera periclymenum	0.00	27.93	0.00	0.00	0.00	0.00	0.00
Liliopsida	Poales	Poaceae	Lolium	Lolium perenne	6764.55	15426.99	2294.40	3573.24	9353.35	0.00	8755.60
Magnoliopsida	Fabales	Fabaceae	Lathyrus	Lathyrus pratensis	0.00	11.75	0.00	0.00	139.15	0.00	0.00
Magnoliopsida	Myrtales	Lythraceae	Lythrum	Lythrum salicaria	0.00	178.26	62.77	0.00	340.57	0.00	0.00
Magnoliopsida	Malpighiales	Linaceae	Linum	Linum usitatissimum	0.00	0.00	0.00	8.91	0.00	0.00	0.00
Magnoliopsida	Lamiales	Plantaginaceae	Linaria	Linaria vulgaris	0.00	0.00	0.00	30.32	0.00	0.00	0.00
Magnoliopsida	Ericales	Primulaceae	Lysimachia	Lysimachia vulgaris	0.00	182.31	6.28	0.00	7.30	0.00	0.00
Magnoliopsida	Fabales	Fabaceae	Melilotus	Melilotus albus	0.00	0.00	4.20	952.99	0.00	0.00	0.00
Magnoliopsida	Malpighiales	Euphorbiaceae	Mercurialis	Mercurialis annua	0.00	0.00	0.00	0.00	0.00	0.00	36.30
Magnoliopsida	Lamiales	Lamiaceae	Mentha	Mentha aquatica	1.77	56.20	292.92	0.00	535.52	0.00	0.00
Magnoliopsida	Boraginales	Boraginaceae	Myosotis	Myosotis arvensis	8.14	0.00	0.00	0.00	0.00	0.00	0.00

Herbs (continuous)

Class	Order	Family	Genus	Species	Estimated cover-abundance (m^2)[c]						
					TE2	103	117	097	171	043	113
Liliopsida	Poales	Poaceae	Milium	Milium effusum	2.77	303.07	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Fabales	Fabaceae	Medicago	Medicago lupulina	0.00	0.00	56.82	0.00	7.70	156.50	14.94
Magnoliopsida	Asterales	Asteraceae	Matricaria	Matricaria maritima	0.00	0.00	0.00	6414.46	0.00	0.00	0.00
Magnoliopsida	Brassicales	Brassicaceae	Nasturtium	Nasturtium officinale	82.37	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Asterales	Asteraceae	Matricaria	Matricaria recutita	0.00	0.00	0.00	13828.90	0.00	0.00	0.00
Magnoliopsida	Ranunculales	Ranunculaceae	Nigella	-	0.00	0.00	0.00	8.91	0.00	0.00	0.00
Magnoliopsida	Myrtales	Onagraceae	Oenothera	Oenothera glazioviana	0.00	0.00	0.00	0.00	13.91	0.00	0.00
Magnoliopsida	Lamiales	Lamiaceae	Origanum	Origanum vulgare	0.00	0.00	0.00	0.00	0.00	15.65	0.00
Magnoliopsida	Caryophyllales	Polygonaceae	Polygonum	Polygonum aviculare	0.00	0.00	0.00	0.00	0.00	0.00	8.71
Liliopsida	Poales	Poaceae	Poa	Poa annua	25.44	0.00	93.71	0.00	2287.29	0.00	902.01
Polypodiopsida	Polypodiales	Dennstaedtiaceae	Pteridium	Pteridium aquilinum	0.00	2979.04	0.00	0.00	0.00	0.00	0.00
Liliopsida	Poales	Poaceae	Phalaris	Phalaris arundinacea	83.69	1198.74	79.28	0.00	0.00	0.00	0.00
Magnoliopsida	Rosales	Rosaceae	Potentilla	Potentilla anserina	60.72	0.00	65.01	0.00	0.00	0.00	0.00
Liliopsida	Poales	Poaceae	Phragmites	Phragmites australis	104.24	259.22	79.76	4907.95	1738.25	0.00	0.00
Liliopsida	Poales	Poaceae	Panicum	Panicum capillare	25.44	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Asterales	Asteraceae	Picris	Helminthotheca echioides	0.00	0.00	56.82	0.00	0.00	0.00	0.00
Magnoliopsida	Asterales	Asteraceae	Picris	Picris hieracioides	0.00	0.00	1565.99	1350.62	13.91	20.47	18.89
Magnoliopsida	Lamiales	Plantaginaceae	Plantago	Plantago lanceolata	0.00	0.00	56.82	1303.07	40.81	0.00	1151.32
Magnoliopsida	Caryophyllales	Polygonaceae	Persicaria	Persicaria lapathifolia	118.73	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Lamiales	Plantaginaceae	Plantago	Plantago major	12.57	0.00	93.71	0.00	0.00	0.00	36.30
Magnoliopsida	Caryophyllales	Polygonaceae	Persicaria	Persicaria maculosa	0.00	0.00	159.78	0.00	0.00	935.91	0.00
Liliopsida	Asparagales	Asparagaceae	Polygonatum	Polygonatum multiflorum	2.77	0.00	0.00	0.00	0.00	0.00	0.00
Liliopsida	Poales	Poaceae	Poa	Poa nemoralis	0.00	128.45	0.00	0.00	0.00	0.00	0.00
Liliopsida	Poales	Poaceae	Poa	Poa pratensis	230.21	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Rosales	Rosaceae	Potentilla	Potentilla reptans	0.00	1.17	331.65	499.36	2691.80	85.04	0.00
Magnoliopsida	Apiales	Apiaceae	Pastinaca	Pastinaca sativa	0.00	0.00	1105.98	9.91	35.03	85.04	0.00
Liliopsida	Poales	Poaceae	Poa	Poa trivialis	3213.48	0.00	0.00	1300.19	0.00	268.15	0.00
Magnoliopsida	Lamiales	Lamiaceae	Prunella	Prunella vulgaris	50.85	0.00	74.38	0.00	15.08	0.00	0.00
Magnoliopsida	Ranunculales	Ranunculaceae	Ranunculus	Ranunculus acris	508.50	0.00	0.00	0.00	787.53	0.00	0.00
Magnoliopsida	Rosales	Rosaceae	Rosa	Rosa arvensis	3.04	13.83	0.00	0.00	0.00	20.47	0.00
Magnoliopsida	Rosales	Rosaceae	Rosa	Rosa canina	0.00	13.83	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Caryophyllales	Polygonaceae	Rumex	Rumex conglomeratus	30.40	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Rosales	Rosaceae	Rubus	Rubus caesius	0.00	0.00	0.00	0.00	10037.86	3949.90	0.00
Magnoliopsida	Brassicales	Resedaceae	Reseda	Reseda luteola	0.00	0.00	0.00	5848.88	0.00	0.00	0.00
Magnoliopsida	Brassicales	Resedaceae	Reseda	Reseda lutea	0.00	0.00	0.00	5849.03	0.00	0.00	0.00
Magnoliopsida	Caryophyllales	Polygonaceae	Rumex	Rumex obtusifolius	74.68	147.45	0.00	0.00	13.91	0.00	0.00
Magnoliopsida	Saxifragales	Grossulariaceae	Ribes	Ribes rubrum	54.41	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Ranunculales	Ranunculaceae	Ranunculus	Ranunculus repens	6087.44	147.45	1668.31	294.34	139.15	225.48	43.26
Magnoliopsida	Brassicales	Brassicaceae	Rapistrum	Rapistrum rugosum	0.00	0.00	0.00	3868.26	0.00	20.12	0.00
Magnoliopsida	Rosales	Rosaceae	Rubus	-	10726.05	1307.38	28.03	0.00	13759.85	4015.38	0.00
Magnoliopsida	Asterales	Asteraceae	Sonchus	Sonchus arvensis	0.00	0.00	0.00	0.00	46.25	0.00	0.00

Herbs (continuous)

Class	Order	Family	Genus	Species	Estimated cover-abundance (m^2)[c]						
					TE2	103	117	097	171	043	113
Magnoliopsida	Asterales	Asteraceae	Sonchus	Sonchus asper	50.85	0.00	0.00	0.00	0.00	0.00	0.00
Liliopsida	Poales	Poaceae	Setaria	-	118.73	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Solanales	Solanaceae	Solanum	Solanum dulcamara	224.86	13.83	62.77	1.30	0.00	0.00	0.00
Magnoliopsida	Dipsacales	Adoxaceae	Sambucus	Sambucus ebulus	0.00	0.00	0.00	499.36	1047.99	3658.90	0.00
Magnoliopsida	Caryophyllales	Caryophyllaceae	Stellaria	Stellaria graminea	0.00	147.45	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Caryophyllales	Caryophyllaceae	Silene	-	0.00	0.00	0.00	8.91	0.00	0.00	0.00
Magnoliopsida	Asterales	Asteraceae	Jacobaea	Jacobaea vulgaris	0.00	0.00	63.18	153.08	926.68	28.97	0.00
Magnoliopsida	Caryophyllales	Caryophyllaceae	Silene	Silene latifolia	0.00	0.00	0.00	59.24	13.91	156.50	8.71
Magnoliopsida	Caryophyllales	Caryophyllaceae	Silene	Silene vulgaris	0.00	0.00	0.00	72.84	0.00	0.00	0.00
Magnoliopsida	Dipsacales	Adoxaceae	Sambucus	Sambucus nigra	0.00	0.00	0.00	2.23	0.00	0.00	0.00
Magnoliopsida	Boraginales	Boraginaceae	Symphytum	Symphytum officinale	0.00	2633.91	42.23	0.00	386.92	77.77	0.00
Magnoliopsida	Asterales	Asteraceae	Sonchus	Sonchus oleraceus	0.00	0.00	0.00	0.00	0.00	0.00	198.96
Magnoliopsida	Lamiales	Lamiaceae	Stachys	Stachys palustris	0.00	0.00	0.00	0.00	3.49	77.77	0.00
Magnoliopsida	Apiales	Apiaceae	Silaum	Silaum silaus	0.00	54.82	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Lamiales	Lamiaceae	Stachys	Stachys sylvatica	202.55	0.00	0.00	4.99	74.02	0.00	0.00
Magnoliopsida	Asterales	Asteraceae	Senecio	Senecio vulgaris	0.00	0.00	0.00	2.89	0.00	0.00	0.00
Magnoliopsida	Fabales	Fabaceae	Trifolium	Trifolium dubium	0.00	0.00	0.00	0.00	0.00	0.00	18.89
Magnoliopsida	Fabales	Fabaceae	Trifolium	Trifolium pratense	238.35	0.00	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Fabales	Fabaceae	Trifolium	Trifolium repens	2423.05	811.95	4644.98	814.78	5628.44	331.87	1192.54
Magnoliopsida	Asterales	Asteraceae	Tanacetum	Tanacetum vulgare	0.00	0.00	0.00	0.00	13.91	0.00	0.00
Magnoliopsida	Asterales	Asteraceae	Taraxacum	-	12.57	0.00	0.00	0.00	49.01	85.04	697.41
Magnoliopsida	Rosales	Urticaceae	Urtica	Urtica dioica	18644.41	9262.25	13771.26	500.29	9794.71	14311.36	226.68
Magnoliopsida	Fabales	Fabaceae	Vicia	Vicia cracca	0.00	247.47	0.00	2.89	408.06	0.00	0.00
Magnoliopsida	Gentianales	Apocynaceae	Vinca	Vinca minor	7291.64	10.63	0.00	0.00	0.00	0.00	0.00
Magnoliopsida	Lamiales	Plantaginaceae	Veronica	Veronica persica	0.00	0.00	0.00	1.39	0.00	0.00	19.90
Magnoliopsida	Fabales	Fabaceae	Vicia	Vicia sativa	0.00	0.00	0.00	124.06	0.00	0.00	0.00
Magnoliopsida	Lamiales	Plantaginaceae	Veronica	Veronica serpyllifolia	0.00	0.00	0.00	0.14	0.00	0.00	0.00
Magnoliopsida	Fabales	Fabaceae	Vicia	Vicia tetrasperma	0.00	0.00	0.00	0.00	0.00	3046.95	0.00
Magnoliopsida	Lamiales	Scrophulariaceae	Verbascum	Verbascum thapsus	0.00	0.00	0.00	0.00	13.91	156.50	0.00
				Taxonomic richness	**70**	**58**	**56**	**68**	**71**	**45**	**33**

Ground-dwelling invertebrates

Class	Order	Family	Genus	Species	Number of captures[c d]													
					Spring							Autumn						
					TE2	103	117	097	171	043	113	TE2	103	117	097	171	043	113
Gastropoda	-	-	-	-	3	0	4	7	2	2	1	1	2	2	1	7	2	2
Arachnida	-	-	-	-	0	6	7	2	1	10	0	2	0	2	0	1	1	0
Arachnida	Araneae	-	-	-	10	17	21	22	4	29	4	0	3	0	0	0	0	0
Arachnida	Opiliones	-	-	-	0	0	6	7	2	2	0	0	1	1	0	0	2	0
Diplopoda	Glomerida	Glomeridae	-	-	0	550	0	0	1	16	2	0	139	1	0	0	6	4
Diplopoda	Julida	Julidae	-	-	63	61	4	13	17	22	3	1	2	0	0	4	28	3
Diplopoda	Polydesmida	Polydesmidae	-	-	3	5	36	49	2	17	0	1	0	3	5	0	0	1
Chilopoda	-	-	-	-	3	5	0	4	0	1	1	3	2	1	0	0	0	0
Malacostraca	Isopoda	-	-	-	4	76	20	257	22	85	9	1	6	5	2	4	18	20
Insecta	Dermaptera	Forficulidae	-	-	0	0	3	12	1	59	0	0	0	0	0	0	0	0
Insecta	Dermaptera	Forficulidae	Forficula	Forficula auricularia	0	0	0	1	1	1	0	0	0	0	0	0	1	0
Insecta	Coleoptera	Carabidae	-	-	6	3	46	18	2	9	2	7	0	0	0	1	0	0
Insecta	Coleoptera	Carabidae	Abax	Abax parallelepipedus	0	8	0	0	0	0	0	1	3	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Abax	Abax parallelus	0	21	0	0	2	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Amara	Amara aenea	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Amara	Amara communis	0	2	1	1	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Amara	Amara familiaris	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Amara	Amara nitida	0	8	0	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Amara	Amara ovata	0	0	0	3	0	1	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Amara	Amara similata	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Asaphidion	Asaphidion curtum	1	0	0	0	0	0	1	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Badister	Badister bipustulatus	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Badister	Badister lacertosus	0	1	0	1	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Bembidion	Bembidion sp.	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Bembidion	Bembidion dentellum	0	0	0	0	0	0	0	0	0	0	0	0	11	0
Insecta	Coleoptera	Carabidae	Bembidion	Bembidion lampros	0	1	3	0	0	22	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Bembidion	Bembidion quadrimaculatum	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Bradycellus	Bradycellus verbasci	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Carabus	Carabus nemoralis	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Carabus	Carabus problematicus	0	0	0	0	0	0	0	0	2	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Clivina	Clivina fossor	2	1	1	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Harpalus	Harpalus latus	5	0	0	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Leistus	Leistus ferrugineus	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Leistus	Leistus fulvibarbis	0	0	0	0	0	1	1	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Loricera	Loricera pilicornis	0	0	0	0	2	1	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Bembidion	Metallina properans	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Microlestes	Microlestes maurus	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Nebria	Nebria brevicollis	0	0	7	3	4	0	0	0	6	64	1	43	3	17
Insecta	Coleoptera	Carabidae	Notiophilus	Notiophilus biguttatus	1	6	36	12	0	4	0	0	0	2	0	0	0	0
Insecta	Coleoptera	Carabidae	Notiophilus	Notiophilus palustris	0	4	5	1	0	3	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Notiophilus	Notiophilus quadripunctatus	0	0	0	0	0	1	0	0	0	1	0	0	0	0
Insecta	Coleoptera	Carabidae	Notiophilus	Notiophilus rufipes	0	5	2	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Bembidion	Ocydromus tetracolus	0	0	1	0	0	1	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Harpalus	Ophonus rufibarbis	0	0	0	1	0	0	0	0	0	0	0	0	0	0

Ground-dwelling invertebrates (continuous)

Class	Order	Family	Genus	Species	Number of captures[c d]													
					Spring							Autumn						
					TE2	103	117	097	171	043	113	TE2	103	117	097	171	043	113
Insecta	Coleoptera	Carabidae	Parophonus	Parophonus maculicornis	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Bembidion	Philochthus biguttatum	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Bembidion	Philochthus guttula	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Insecta	Coleoptera	Carabidae	Bembidion	Philochthus lunulatus	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Bembidion	Philochthus mannerheimii	1	0	1	0	0	1	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Bembidion	Phyla obtusa	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Poecilus	Poecilus cupreus	0	1	0	1	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Poecilus	Poecilus versicolor	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Carabus	Procrustes coriaceus	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Insecta	Coleoptera	Carabidae	Harpalus	Pseudoophonus rufipes	0	0	4	0	0	2	0	0	1	0	0	0	0	1
Insecta	Coleoptera	Carabidae	Pterostichus	Pterostichus diligens	0	1	0	0	0	0	0	2	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Pterostichus	Pterostichus madidus	3	6	1	0	0	0	0	4	8	6	0	2	3	0
Insecta	Coleoptera	Carabidae	Pterostichus	Pterostichus melanarius	5	0	0	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Pterostichus	Pterostichus niger	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Pterostichus	Pterostichus oblongopunctatus	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Pterostichus	Pterostichus strenuus	1	0	0	1	0	2	0	1	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Stomis	Stomis pumicatus	1	3	1	0	1	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Trechoblemus	Trechoblemus micros	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Carabidae	Trechus	Trechus obtusus	0	0	0	0	0	5	0	1	0	0	0	0	0	0
Insecta	Coleoptera	Silphidae	Phosphuga	Phosphuga atrata	0	1	0	3	0	2	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Leiodidae	Choleva	Choleva sp.	0	0	0	4	0	2	0	0	0	1	0	0	0	1
Insecta	Coleoptera	Carabidae	Bembidion	Ocydromus sp.	2	1	0	1	0	1	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Staphylinidae	Ocypus	Ocypus olens	0	0	0	0	0	0	1	0	0	0	0	0	1	1
Insecta	Coleoptera	Staphylinidae	Philonthus	Philonthus sp.	0	2	2	0	0	0	0	0	0	0	0	0	1	0
Insecta	Coleoptera	Staphylinidae	Xantholinus	Xantholinus sp.	0	0	0	0	4	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Staphylinidae	-	-	2	11	11	9	1	10	0	0	0	1	0	0	0	1
Insecta	Coleoptera	Geotrupidae	Anoplotrupes	Anoplotrupes stercorosus	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Insecta	Coleoptera	Aphodiidae	-	-	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Aphodiidae	Aphodius	Aphodius sp.	1	2	0	6	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Byrrhidae	Byrrhus	Byrrhus pilula	0	0	3	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Byrrhidae	Cytilus	Cytilus sericeus	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Byrrhidae	Lamprobyrrhulus	Lamprobyrrhulus nitidus	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Byrrhidae	Simplocaria	Simplocaria semistriata	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Elateridae	-	-	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Elateridae	Agriotes	Agriotes sputator	1	1	11	4	0	1	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Lampyridae	Lampyris	Lampyris noctiluca	0	2	0	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Chrysomelidae	-	-	0	0	0	8	0	5	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Coccinellidae	-	-	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Insecta	Coleoptera	Coccinellidae	Brumus	Brumus quadripustulatus	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Coccinellidae	Harmonia	Harmonia axyridis	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Insecta	Coleoptera	Curculionidae	Otiorhynchus	Otiorhynchus sp.	0	3	0	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Curculionidae	Otiorhynchus	Otiorhynchus ligustici	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Salpingidae	Vincenzellus	Vincenzellus ruficollis	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Insecta	Hymenoptera	Formicidae	-	-	1	4	32	55	0	57	0	0	30	5	0	0	0	0
				Taxonomic richness	**27**	**33**	**32**	**32**	**17**	**36**	**11**	**13**	**16**	**14**	**4**	**8**	**14**	**10**

Flying invertebrates

Class	Order	Family	Genus	Species	Number of captures[c d]													
					Spring							Autumn						
					TE2	103	117	097	171	043	113	TE2	103	117	097	171	043	113
Arachnida	-	-	-	-	6	13	10	3	0	3	5	1	0	1	0	1	0	0
Arachnida	Araneae	-	-	-	2	5	2	7	3	0	5	6	2	3	8	3	9	8
Arachnida	Opiliones	-	-	-	0	0	0	0	3	0	0	1	0	0	0	1	1	0
Insecta	Orthoptera	Tettigoniidae	-	-	0	0	0	0	0	0	0	0	1	0	1	0	0	5
Insecta	Dermaptera	Forficulidae	-	-	0	0	0	1	1	0	0	0	0	0	1	0	3	1
Insecta	Psocoptera	Psocidae	-	-	0	1	0	0	1	0	2	5	0	3	6	0	10	2
Insecta	Thysanoptera	Thripidae	-	-	33	45	137	9	53	1	9	6	6	0	2	1	3	0
Insecta	Hemiptera	Cicadellidae	-	-	22	9	9	9	5	3	14	50	21	6	4	3	50	21
Insecta	Hemiptera	Delphacidae	-	-	0	0	0	0	0	0	0	4	3	0	0	0	1	6
Insecta	Hemiptera	-	-	-	6	6	3	10	0	0	14	15	229	86	404	76	25	714
Insecta	Hemiptera	Psyllidae	-	-	0	2	0	1	0	1	0	3	0	5	1	4	1	1
Insecta	Hemiptera	Miridae	-	-	0	2	1	0	0	0	0	0	1	0	0	1	1	1
Insecta	Hemiptera	Tingidae	-	-	0	0	0	0	0	0	0	0	1	0	1	0	1	0
Insecta	Coleoptera	Carabidae	-	-	0	0	1	0	0	0	0	0	0	0	0	0	1	0
Insecta	Coleoptera	Leiodidae	-	-	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Insecta	Coleoptera	Ptiliidae	-	-	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Insecta	Coleoptera	Pselaphidae	-	-	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Insecta	Coleoptera	Staphylinidae	-	-	1	16	43	12	2	1	2	1	3	0	0	4	3	1
Insecta	Coleoptera	Apionidae	-	-	1	2	1	0	0	0	0	1	0	0	0	0	0	0
Insecta	Coleoptera	Chrysomelidae	-	-	12	7	58	4	7	3	19	10	1	2	10	1	6	12
Insecta	Coleoptera	Coccinellidae	-	-	0	0	1	0	0	0	1	1	0	0	1	1	0	1
Insecta	Coleoptera	Curculionidae	-	-	1	0	0	1	0	0	0	0	0	0	0	0	0	1
Insecta	Coleoptera	Lathridiidae	-	-	1	0	1	1	1	0	0	0	0	1	0	1	2	3
Insecta	Coleoptera	Nitidulidae	-	-	102	129	167	20	6	4	8	1	0	0	0	0	0	0
Insecta	Neuroptera	Chrysopidae	-	-	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Insecta	Hymenoptera	Cimbicidae	-	-	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Insecta	Hymenoptera	Tenthredinidae	-	-	13	16	9	16	0	2	0	0	1	0	0	1	1	0
Insecta	Hymenoptera	Braconidae	-	-	4	6	0	19	3	2	81	39	14	12	50	5	15	22
Insecta	Hymenoptera	Chalcididae	-	-	0	0	0	0	0	0	0	2	0	0	0	0	0	1
Insecta	Hymenoptera	Cynipidae	-	-	2	2	0	0	0	1	0	6	2	0	1	1	8	2
Insecta	Hymenoptera	Eucoilidae	-	-	1	0	0	0	0	1	0	0	0	0	0	0	0	0
Insecta	Hymenoptera	Eupelmidae	-	-	8	15	4	7	1	4	10	34	13	6	1	2	9	7
Insecta	Hymenoptera	Eurytomidae	-	-	1	1	1	0	0	0	1	0	1	0	0	0	2	0
Insecta	Hymenoptera	Figitidae	-	-	0	0	0	0	0	0	0	8	8	1	1	0	2	2
Insecta	Hymenoptera	Diapriidae	-	-	0	0	0	3	0	1	0	32	8	8	17	7	17	17
Insecta	Hymenoptera	Encyrtidae	-	-	0	0	0	2	1	0	0	0	5	0	0	1	0	15
Insecta	Hymenoptera	Ichneumonidae	-	-	6	8	13	26	12	12	16	37	11	14	24	6	24	57
Insecta	Hymenoptera	Mymaridae	-	-	5	1	3	1	0	1	1	7	2	2	1	2	3	1
Insecta	Hymenoptera	Pteromalidae	-	-	3	1	0	3	0	0	3	14	8	0	4	0	7	2
Insecta	Hymenoptera	Scelionidae	-	-	0	0	0	1	0	0	0	40	10	1	23	5	11	60
Insecta	Hymenoptera	Serphidae	-	-	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Insecta	Hymenoptera	Torymidae	-	-	0	0	0	0	0	1	0	0	0	0	0	0	0	0

Flying invertebrates (continuous)

Class	Order	Family	Genus	Species	Number of captures[c d]													
					Spring							Autumn						
					TE2	103	117	097	171	043	113	TE2	103	117	097	171	043	113
Insecta	Hymenoptera	Trichogrammatidae	-	-	2	1	3	2	0	1	0	7	7	1	0	2	2	2
Insecta	Hymenoptera	Andrenidae	-	-	54	126	482	62	1	43	16	0	1	0	0	0	0	1
Insecta	Hymenoptera	Anthophoridae	-	-	6	7	49	13	0	39	6	0	0	0	0	0	0	0
Insecta	Hymenoptera	Apidae	-	-	5	4	8	0	0	6	1	0	2	0	0	0	0	0
Insecta	Hymenoptera	Halictidae	-	-	23	2	11	11	0	19	4	0	3	0	0	0	0	7
Insecta	Hymenoptera	Megachilidae	-	-	0	2	14	0	0	1	0	0	0	0	0	0	0	0
Insecta	Hymenoptera	Formicidae	-	-	0	0	0	0	0	1	0	1	2	1	6	1	1	2
Insecta	Hymenoptera	Pompilidae	-	-	0	1	0	0	0	2	0	0	0	0	0	0	4	0
Insecta	Hymenoptera	Sphecidae	-	-	0	0	0	0	0	0	0	0	0	2	0	0	1	0
Insecta	Hymenoptera	Vespidae	-	-	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Insecta	Lepidoptera	-	-	-	0	0	0	0	0	0	0	1	0	0	1	0	2	0
Insecta	Lepidoptera	Geometridae	-	-	0	0	0	0	0	0	0	1	0	0	0	0	3	0
Insecta	Lepidoptera	Noctuidae	-	-	0	0	0	0	0	0	0	1	0	0	0	0	0	2
Insecta	Lepidoptera	Nymphalidae	-	-	1	1	0	0	0	0	0	0	1	0	0	0	0	0
Insecta	Lepidoptera	Tortricidae	-	-	0	0	0	0	0	0	0	0	0	0	1	0	0	0
Insecta	Lepidoptera	Tineidae	-	-	0	0	0	0	0	1	0	0	0	0	0	0	0	0
Insecta	Mecoptera	Panorpidae	-	-	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Insecta	Diptera	Anisopodidae	-	-	0	0	0	1	0	1	1	0	0	0	0	0	0	1
Insecta	Diptera	Bibionidae	-	-	0	1	2	1	0	4	1	0	0	0	0	0	0	0
Insecta	Diptera	Cecidomyiidae	-	-	4	3	1	6	2	13	13	9	4	2	7	5	3	11
Insecta	Diptera	Ceratopogonidae	-	-	0	1	2	0	0	0	4	0	1	0	0	0	0	0
Insecta	Diptera	Chironomidae	-	-	29	54	58	25	63	272	9	4	2	5	15	4	13	21
Insecta	Diptera	Culicidae	-	-	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Insecta	Diptera	Limoniidae	-	-	11	13	4	0	2	1	4	0	2	0	0	0	0	0
Insecta	Diptera	Mycetophilidae	-	-	3	2	2	0	1	0	5	9	0	0	0	0	0	2
Insecta	Diptera	Psychodidae	-	-	1	2	1	4	1	7	1	3	0	0	0	0	1	1
Insecta	Diptera	Sciaridae	-	-	32	21	19	47	12	58	136	9	13	1	4	2	4	11
Insecta	Diptera	Simuliidae	-	-	0	1	0	0	0	0	0	0	0	0	0	0	5	0
Insecta	Diptera	Tipulidae	-	-	0	2	0	4	3	1	5	2	2	0	2	0	4	0
Insecta	Diptera	Asilidae	-	-	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Insecta	Diptera	Bombyliidae	-	-	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Insecta	Diptera	Dolichopodidae	-	-	1	0	0	0	0	2	0	4	7	2	0	0	4	3
Insecta	Diptera	Empididae	-	-	1	3	0	0	2	2	0	3	13	3	0	1	8	10
Insecta	Diptera	Phoridae	-	-	14	8	15	9	7	4	14	40	63	22	28	4	27	56
Insecta	Diptera	Lonchopteridae	-	-	0	0	0	1	5	0	0	3	0	1	3	2	3	8
Insecta	Diptera	Syrphidae	-	-	12	78	13	10	8	24	23	9	14	10	1	1	17	7
Insecta	Diptera	Agromyzidae	-	-	6	5	6	4	5	10	8	2	5	0	0	0	1	9
Insecta	Diptera	Chloropidae	-	-	7	2	1	1	0	0	6	1	12	0	0	0	0	0
Insecta	Diptera	Conopidae	-	-	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Insecta	Diptera	Drosophilidae	-	-	2	0	0	0	0	0	1	0	1	0	1	0	1	1
Insecta	Diptera	Heleomyzidae	-	-	0	0	0	0	3	0	1	8	4	0	4	1	2	4
Insecta	Diptera	Milichiidae	-	-	14	1	2	2	4	12	7	3	7	0	0	0	0	2

Flying invertebrates (continuous)

Class	Order	Family	Genus	Species	Number of captures[c d]													
					Spring							Autumn						
					TE2	103	117	097	171	043	113	TE2	103	117	097	171	043	113
Insecta	Diptera	Psilidae	-	-	0	0	0	0	0	0	0	0	2	1	0	0	0	0
Insecta	Diptera	Sciomyzidae	-	-	1	0	0	1	0	0	0	0	0	0	0	0	0	0
Insecta	Diptera	Sepsidae	-	-	1	1	1	0	0	0	0	1	1	0	0	0	1	6
Insecta	Diptera	Sphaeroceridae	-	-	4	5	1	3	1	2	3	8	5	0	2	1	3	4
Insecta	Diptera	Tephritidae	-	-	0	0	0	0	0	0	0	0	0	0	0	0	4	1
Insecta	Diptera	Anthomyiidae	-	-	67	13	3	1	7	35	26	4	9	1	2	1	1	2
Insecta	Diptera	Calliphoridae	-	-	0	4	1	2	1	4	1	5	7	6	1	0	4	0
Insecta	Diptera	Muscidae	-	-	272	280	53	124	109	128	297	90	110	46	29	2	123	39
Insecta	Diptera	Sarcophagidae	-	-	2	0	0	0	0	2	1	0	1	0	0	0	0	0
Insecta	Diptera	Scathophagidae	-	-	1	5	1	0	2	0	1	0	0	0	0	0	0	0
Insecta	Diptera	Tachinidae	-	-	2	3	4	3	0	3	0	0	5	0	0	0	0	0
		Taxonomic richness			**48**	**50**	**44**	**43**	**32**	**44**	**41**	**46**	**51**	**29**	**36**	**31**	**51**	**52**

VII.2 Appendix B

Complete data on number and frequency of occurrence of plant food item (sequence group)

(Supporting Information SI10 of the Chapter 2)

Names	Explication
Sequence group (Grp)	Group of clustered sequences extracted from stomach contents of wood mouse.
Number of occurrence per Grp	Total number of occurrences (for spring, autumn or both).
Frequency of occurrence	Percentage of the number of occurrences of a given Grp compared to the total number of occurrence of all Grp.
Corresponding families	Family names of reference sequences corresponding to sequences of given Grp.
Component Sp. number	Number of species corresponding to sequences of given Grp.
Component species	Species corresponding to sequences of given Grp.

In the two seasons (mouse number: 246)

Sequence group (Grp)	Number of occurrence per Grp	Frequency of occurrence	Corresponding families	Component Sp. number:	Component species
Grp02	128	21.9%	*Cornaceae, Adoxaceae & Asteraceae*	20	*Achillea millefolium; Arctium lappa; Artemisia vulgaris; Bellis perennis; Centaurea cyanus; Conyza canadensis; Cornus sanguinea; Eupatorium cannabinum; Helminthotheca echioides; Hieracium lachenalii; Hypochaeris radicata; Jacobaea vulgaris; Leucanthemum vulgare; Picris hieracioides; Sambucus ebulus; Sambucus nigra; Senecio vulgaris; Sonchus asper; Sonchus oleraceus; Tanacetum vulgare*
Grp01	96	16.4%	*Sapindaceae*	4	*Acer campestre; Acer platanoides; Acer pseudoplatanus; Aesculus hippocastanum*
Grp23	45	7.7%	*Rosaceae*	6	*Fragaria vesca; Geum urbanum; Potentilla reptans; Rosa arvensis; Rosa canina; Rubus caesius*
Grp48	43	7.4%	*Salicaceae*	1	*Populus tremula x Populus alba*
Grp49	26	4.4%	*Rosaceae*	3	*Prunus avium; Prunus cerasifera; Prunus spinosa*
Grp09	25	4.3%	*Poaceae*	13	*Arrhenatherum elatius; Brachypodium sylvaticum; Elymus repens; Festuca arundinacea; Festuca rubra; Lolium multiflorum; Lolium perenne; Milium effusum; Phalaris arundinacea; Poa annua; Poa nemoralis; Poa pratensis; Poa trivialis*
Grp15	25	4.3%	*Fagaceae*	3	*Castanea sativa; Quercus robur; Quercus rubra*
Grp47	23	3.9%	*Salicaceae*	3	*Populus balsamifera; Salix caprea; Salix cinerea*
Grp11	21	3.6%	*Betulaceae*	3	*Betula pendula; Carpinus betulus; Corylus avellana*
Grp21	15	2.6%	*Rosaceae*	4	*Crataegus laevigata; Crataegus monogyna; Malus sylvestris; Pyrus communis*
Grp22	15	2.6%	*Poaceae*	2	*Echinochloa crus galli; Panicum capillare*
Grp25	15	2.6%	*Oleaceae*	2	*Fraxinus excelsior; Ligustrum vulgare*
Grp05	11	1.9%	*Betulaceae*	1	*Alnus glutinosa*
Grp24	8	1.4%	*Rhamnaceae*	1	*Frangula alnus*
Grp03	7	1.2%	*Apiaceae*	5	*Aegopodium podagraria; Angelica sylvestris; Daucus carota; Heracleum sphondylium; Pastinaca sativa*
Grp30	6	1.0%	*Araliaceae*	1	*Hedera helix*
Grp53	6	1.0%	*Fabaceae*	1	*Robinia pseudoacacia*
Grp59	6	1.0%	*Malvaceae*	1	*Tilia platyphyllos*
Grp63	6	1.0%	*Urticaceae*	1	*Urtica dioica*
Grp20	5	0.9%	*Asteraceae*	3	*Cirsium arvense; Cirsium palustre; Cirsium vulgare*
Grp55	5	0.9%	*Solanaceae*	1	*Solanum dulcamara*
Grp10	4	0.7%	*Chenopodiaceae*	3	*Atriplex patula; Chenopodium album; Chenopodium ficifolium*
Grp28	4	0.7%	*Geraniaceae*	1	*Geranium robertianum*
Grp44	4	0.7%	*Polygonaceae*	2	*Persicaria lapathifolia; Persicaria maculosa*
Grp56	4	0.7%	*Caprifoliaceae*	1	*Symphoricarpos albus*
Grp29	3	0.5%	*Lamiaceae*	1	*Glechoma hederacea*
Grp31	3	0.5%	*Poaceae*	1	*Hordeum murinum*
Grp35	2	0.3%	*Juncaceae*	1	*Juncus effusus*
Grp46	2	0.3%	*Polygonaceae*	1	*Polygonum aviculare*
Grp52	2	0.3%	*Resedaceae*	1	*Reseda lutea*
Grp58	2	0.3%	*Taxaceae*	1	*Taxus baccata*
Grp62	2	0.3%	*Ulmaceae*	1	*Ulmus minor*
Grp06	1	0.2%	*Poaceae*	1	*Alopecurus pratensis*
Grp07	1	0.2%	*Primulaceae*	2	*Anagallis arvensis; Lysimachia vulgaris*
Grp08	1	0.2%	*Brassicaceae*	2	*Arabidopsis halleri; Capsella bursa pastoris*
Grp12	1	0.2%	*Poaceae*	1	*Bromus sterilis*
Grp14	1	0.2%	*Convolvulaceae*	2	*Calystegia sepium; Convolvulus arvensis*
Grp27	1	0.2%	*Rubiaceae*	2	*Galium aparine; Galium mollugo*

In the two seasons (mouse number: 246) (continuous)

Sequence group (Grp)	Number of occurrence per Grp	Frequency of occurrence	Corresponding families	Component Sp. number:	Component species
Grp32	1	0.2%	*Cannabaceae*	1	*Humulus lupulus*
Grp34	1	0.2%	*Juglandaceae*	1	*Juglans regia*
Grp38	1	0.2%	*Linaceae*	1	*Linum usitatissimum*
Grp42	1	0.2%	*Boraginaceae*	1	*Myosotis arvensis*
Grp50	1	0.2%	*Ranunculaceae*	1	*Ranunculus repens*
Grp51	1	0.2%	*Brassicaceae*	1	*Rapistrum rugosum*
Grp54	1	0.2%	*Caryophyllaceae*	2	*Silene latifolia; Silene vulgaris*
Grp57	1	0.2%	*Boraginaceae*	1	*Symphytum officinale*
Grp65	1	0.2%	*Adoxaceae*	1	*Viburnum opulus*
Grp66	1	0.2%	*Fabaceae*	1	*Vicia cracca*
Total: 48	**585**			**114**	

In spring (mouse number: 117)

Sequence group (Grp)	Number of occurrence per Grp	Frequency of occurrence	Corresponding families	component Sp. number:	Composant species
Grp01	66	25.0%	*Sapindaceae*	4	*Acer campestre; Acer platanoides; Acer pseudoplatanus; Aesculus hippocastanum*
Grp02	31	11.7%	*Cornaceae, Adoxaceae & Asteraceae*	20	*Achillea millefolium; Arctium lappa; Artemisia vulgaris; Bellis perennis; Centaurea cyanus; Conyza canadensis; Cornus sanguinea; Eupatorium cannabinum; Helminthotheca echioides; Hieracium lachenalii; Hypochaeris radicata; Jacobaea vulgaris; Leucanthemum vulgare; Picris hieracioides; Sambucus ebulus; Sambucus nigra; Senecio vulgaris; Sonchus asper; Sonchus oleraceus; Tanacetum vulgare*
Grp48	31	11.7%	*Salicaceae*	1	*Populus tremula x Populus alba*
Grp15	19	7.2%	*Fagaceae*	3	*Castanea sativa; Quercus robur; Quercus rubra*
Grp23	18	6.8%	*Rosaceae*	6	*Fragaria vesca; Geum urbanum; Potentilla reptans; Rosa arvensis; Rosa canina; Rubus caesius*
Grp49	18	6.8%	*Rosaceae*	3	*Prunus avium; Prunus cerasifera; Prunus spinosa*
Grp47	16	6.1%	*Salicaceae*	3	*Populus balsamifera; Salix caprea; Salix cinerea*
Grp21	10	3.8%	*Rosaceae*	4	*Crataegus laevigata; Crataegus monogyna; Malus sylvestris; Pyrus communis*
Grp05	8	3.0%	*Betulaceae*	1	*Alnus glutinosa*
Grp25	8	3.0%	*Oleaceae*	2	*Fraxinus excelsior; Ligustrum vulgare*
Grp11	7	2.7%	*Betulaceae*	3	*Betula pendula; Carpinus betulus; Corylus avellana*
Grp30	6	2.3%	*Araliaceae*	1	*Hedera helix*
Grp22	3	1.1%	*Poaceae*	2	*Echinochloa crus galli; Panicum capillare*
Grp29	3	1.1%	*Lamiaceae*	1	*Glechoma hederacea*
Grp59	3	1.1%	*Malvaceae*	1	*Tilia platyphyllos*
Grp09	2	0.8%	*Poaceae*	13	*Arrhenatherum elatius; Brachypodium sylvaticum; Elymus repens; Festuca arundinacea; Festuca rubra; Lolium multiflorum; Lolium perenne; Milium effusum; Phalaris arundinacea; Poa annua; Poa nemoralis; Poa pratensis; Poa trivialis*
Grp20	2	0.8%	*Asteraceae*	3	*Cirsium arvense; Cirsium palustre; Cirsium vulgare*
Grp53	2	0.8%	*Fabaceae*	1	*Robinia pseudoacacia*
Grp62	2	0.8%	*Ulmaceae*	1	*Ulmus minor*
Grp03	1	0.4%	*Apiaceae*	5	*Aegopodium podagraria; Angelica sylvestris; Daucus carota; Heracleum sphondylium; Pastinaca sativa*
Grp08	1	0.4%	*Brassicaceae*	2	*Arabidopsis halleri; Capsella bursa pastoris*
Grp28	1	0.4%	*Geraniaceae*	1	*Geranium robertianum*
Grp31	1	0.4%	*Poaceae*	1	*Hordeum murinum*
Grp32	1	0.4%	*Cannabaceae*	1	*Humulus lupulus*
Grp34	1	0.4%	*Juglandaceae*	1	*Juglans regia*
Grp46	1	0.4%	*Polygonaceae*	1	*Polygonum aviculare*
Grp56	1	0.4%	*Caprifoliaceae*	1	*Symphoricarpos albus*
Grp63	1	0.4%	*Urticaceae*	1	*Urtica dioica*
Total: 28	**264**			**87**	

In autumn 2012 (mouse number: 129)

Sequence group (Grp)	Number of occurrence per Grp	Frequency of occurrence	Corresponding families	component Sp. number:	Composant species
Grp02	97	30.2%	*Cornaceae, Adoxaceae & Asteraceae*	20	*Achillea millefolium; Arctium lappa; Artemisia vulgaris; Bellis perennis; Centaurea cyanus; Conyza canadensis; Cornus sanguinea; Eupatorium cannabinum; Helminthotheca echioides; Hieracium lachenalii; Hypochaeris radicata; Jacobaea vulgaris; Leucanthemum vulgare; Picris hieracioides; Sambucus ebulus; Sambucus nigra; Senecio vulgaris; Sonchus asper; Sonchus oleraceus; Tanacetum vulgare*
Grp01	30	9.3%	*Sapindaceae*	4	*Acer campestre; Acer platanoides; Acer pseudoplatanus; Aesculus hippocastanum*
Grp23	27	8.4%	*Rosaceae*	6	*Fragaria vesca; Geum urbanum; Potentilla reptans; Rosa arvensis; Rosa canina; Rubus caesius*
Grp09	23	7.2%	*Poaceae*	13	*Arrhenatherum elatius; Brachypodium sylvaticum; Elymus repens; Festuca arundinacea; Festuca rubra; Lolium multiflorum; Lolium perenne; Milium effusum; Phalaris arundinacea; Poa annua; Poa nemoralis; Poa pratensis; Poa trivialis*
Grp11	14	4.4%	*Betulaceae*	3	*Betula pendula; Carpinus betulus; Corylus avellana*
Grp22	12	3.7%	*Poaceae*	2	*Echinochloa crus galli; Panicum capillare*
Grp48	12	3.7%	*Salicaceae*	1	*Populus tremula x Populus alba*
Grp24	8	2.5%	*Rhamnaceae*	1	*Frangula alnus*
Grp49	8	2.5%	*Rosaceae*	3	*Prunus avium; Prunus cerasifera; Prunus spinosa*
Grp25	7	2.2%	*Oleaceae*	2	*Fraxinus excelsior; Ligustrum vulgare*
Grp47	7	2.2%	*Salicaceae*	3	*Populus balsamifera; Salix caprea; Salix cinerea*
Grp03	6	1.9%	*Apiaceae*	5	*Aegopodium podagraria; Angelica sylvestris; Daucus carota; Heracleum sphondylium; Pastinaca sativa*
Grp15	6	1.9%	*Fagaceae*	3	*Castanea sativa; Quercus robur; Quercus rubra*
Grp21	5	1.6%	*Rosaceae*	4	*Crataegus laevigata; Crataegus monogyna; Malus sylvestris; Pyrus communis*
Grp55	5	1.6%	*Solanaceae*	1	*Solanum dulcamara*
Grp63	5	1.6%	*Urticaceae*	1	*Urtica dioica*
Grp10	4	1.2%	*Chenopodiaceae*	3	*Atriplex patula; Chenopodium album; Chenopodium ficifolium*
Grp44	4	1.2%	*Polygonaceae*	2	*Persicaria lapathifolia; Persicaria maculosa*
Grp53	4	1.2%	*Fabaceae*	1	*Robinia pseudoacacia*
Grp05	3	0.9%	*Betulaceae*	1	*Alnus glutinosa*
Grp20	3	0.9%	*Asteraceae*	3	*Cirsium arvense; Cirsium palustre; Cirsium vulgare*
Grp28	3	0.9%	*Geraniaceae*	1	*Geranium robertianum*
Grp56	3	0.9%	*Caprifoliaceae*	1	*Symphoricarpos albus*
Grp59	3	0.9%	*Malvaceae*	1	*Tilia platyphyllos*
Grp31	2	0.6%	*Poaceae*	1	*Hordeum murinum*
Grp35	2	0.6%	*Juncaceae*	1	*Juncus effusus*
Grp52	2	0.6%	*Resedaceae*	1	*Reseda lutea*
Grp58	2	0.6%	*Taxaceae*	1	*Taxus baccata*
Grp06	1	0.3%	*Poaceae*	1	*Alopecurus pratensis*
Grp07	1	0.3%	*Primulaceae*	2	*Anagallis arvensis; Lysimachia vulgaris*
Grp12	1	0.3%	*Poaceae*	1	*Bromus sterilis*
Grp14	1	0.3%	*Convolvulaceae*	2	*Calystegia sepium; Convolvulus arvensis*
Grp27	1	0.3%	*Rubiaceae*	2	*Galium aparine; Galium mollugo*
Grp38	1	0.3%	*Linaceae*	1	*Linum usitatissimum*
Grp42	1	0.3%	*Boraginaceae*	1	*Myosotis arvensis*
Grp46	1	0.3%	*Polygonaceae*	1	*Polygonum aviculare*
Grp50	1	0.3%	*Ranunculaceae*	1	*Ranunculus repens*
Grp51	1	0.3%	*Brassicaceae*	1	*Rapistrum rugosum*

In autumn 2012 (mouse number: 129) (continuous)

Sequence group (Grp)	Number of occurrence per Grp	Frequency of occurrence	Corresponding families	component Sp. number:	Composant species
Grp54	1	0.3%	*Caryophyllaceae*	2	*Silene latifolia; Silene vulgaris*
Grp57	1	0.3%	*Boraginaceae*	1	*Symphytum officinale*
Grp65	1	0.3%	*Adoxaceae*	1	*Viburnum opulus*
Grp66	1	0.3%	*Fabaceae*	1	*Vicia cracca*
Total: 42	**321**			**107**	

Titre : Biodiversité et fonctionnement des réseaux trophiques terrestres : application aux transferts d'éléments traces métalliques

Mots clés : Biodiversité, fonctionnement des écosystèmes, écotoxicologie, effet de dilution, éléments traces métalliques, *Apodemus sylvaticus*.

Résumé : Les effets de la biodiversité sur le risque de transmission de maladies zoonotiques est l'un des sujets scientifiques débattus sur les rôles fonctionnels de la biodiversité sur le fonctionnement des écosystèmes. Une grande diversité de la communauté d'hôtes peut réduire la transmission des agents pathogènes en raison de différences contexte-dépendentes des trais d'histoire de vie entre les hôtes (« *Dilution effect hypothesis* »). Les éléments traces métalliques (ETM) circulent des ressources alimentaires aux consommateurs par les liens trophiques, et il est supposé que la diversité des ressources joue un rôle fonctionnel dans le transfert des ETM au sein des réseaux trophiques *via* à la variabilité de leurs traits d'histoire de vie.
Le but de cette thèse est de tester cette hypothèse et de déterminer les mécanismes sous-jacents en utilisant le mulot sylvestre (*Apodemus sylvaticus*) comme modèle biologique. L'étude a été effectuée autour de l'ancienne fonderie Metaleurop Nord dans le nord de la France où les sols sont contaminés par du cadmium, du plomb et du zinc.
Nous montrons que le transfert des ETM des sols aux mulots est contrôlé par une combinaison complexe de facteurs environnementaux et biologiques, comme la contamination des sols par les ETM, les traits d'histoire de vie des ressources et le comportement alimentaire des mulots. Dans la plupart des cas, ces facteurs sont liés à la biodiversité des ressources. Nous démontrons ainsi la dilution du transfert des ETM par la biodiversité des ressources. Ces travaux permettent d'envisager des approches écologiques pour le contrôle des impacts de la pollution par les métaux sur la faune.

Title: Biodiversity and functioning of terrestrial food webs: modelling of transfers of trace metals.

Keywords: Biodiversity, ecosystem functioning, dilution effect, ecotoxicology, trace metals, *Apodemus sylvaticus*.

Abstract: Effects of biodiversity on zoonotic disease transmission is one of the currently hot scientific topics about the functional roles of biodiversity on ecosystem functioning. High diversity in host community can reduce the transmission of pathogens, namely "dilution effect hypothesis", due to context-dependent differences in life history traits between hosts. Trace metals (TMs) circulate from resources to consumers via trophic links, and it is hypothesized that their diversity would play functional roles on the transfer of TMs to consumers given the variety of life history traits within resources.
The aim of this work was to test this hypothesis and to determine the underlying mechanisms, using the wood mouse (*Apodemus sylvaticus*) as a model consumer. This work was undertaken around the former smelter Metaleurop Nord in Northern France where soils were contaminated by cadmium, lead and zinc.
This work showed that transfer of TMs from soils to mice was controlled by a complex combination of environmental and biological factors, including soil TM contamination, life history traits of resources, and feeding behavior of wood mice. In most cases, these factors were related to biodiversity. A dilution of TMs transfer to wood mice by a high resource diversity has been shown. This work paves the way for nature-based solutions for the control of metal pollution impacts on wildlife.

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