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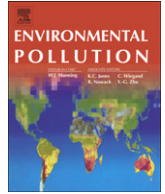
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Functional traits of soil invertebrates as indicators for exposure to soil disturbance

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ABSTRACT

We tested a trait-based approach to link a soil disturbance to changes in invertebrate communities. Soils and macro-invertebrates were sampled in sandy soils contaminated by long-term wastewater irrigation, adding notably organic matter and trace metals (TM). We hypothesized that functional traits of invertebrates depict ways of exposure and that exposure routes relate to specific TM pools. Geophages and soft-body invertebrates were chosen to inform on exposure by ingestion or contact, respectively. Trait-based indices depicted more accurately effects of pollution than community density and diversity did. Exposure by ingestion had more deleterious effects than by contact. Both types of exposed invertebrates were influenced by TM, but geophages mainly responded to changes in soil organic matter contents. The trait-based approach requires to be applied in various conditions to uncorrelate specific TM impacts from those of other environmental factors.

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1. Introduction

Over the last decades growing evidence was given for negative effects of anthropogenic disturbances on the ability of soils to provide goods and services to human well-being (COM179 final, 2002). For a pertinent assessment of such negative effects, but also of restoration efficiency, executive authorities need reliable, accurate, and easy-to-use indicators. Ideally, such indicators must not solely be descriptive, but should also take account of the mechanisms by which the disturbance/restoration acts. Soil disturbance is currently assessed by different wide-used chemical and physico-chemical soil parameters (Hooda, 2010), but measurement of the available concentrations is becoming a general tool in environmental research (ISO, 2008). Among disturbances, trace metal (TM) pollution represents a problem of major interest (van Gestel, 2008). Regarding TM, different chemical methods (isotopic dilution, neutral salt extraction or chelation with organic reagents) were proposed to define different TM pools (e.g. bioavailable, non-available, exchangeable, bound to organic matter) potentially impacting biota (Scheiffler et al., 2003; van Gestel, 2008). The use of biological parameters has also been proposed. Ecotoxicological effects are currently measured by biomarkers, bioindicators or a combination of both (Brulle et al., 2010; Capowiez et al., 2010; van Gestel et al., 2009). However, these indicators depend on different factors like soil texture or microclimate which appear as confounding factors,

hampering the calibration of these tools to large scales (Hobbelen et al., 2006; Lukkari et al., 2004).

To overcome these drawbacks, functional trait-based concepts have emerged as a promising way for understanding the mechanisms that drive invertebrate responses to environmental disturbances (e.g. the habitat templet theory; Southwood, 1977). Functional traits of species are diversely defined but mainly concern species' properties that both affect individual fitness and govern species' impacts and responses to their environment (Violle et al., 2007). Trait-based approach is currently used in different fields of environmental studies, like ecology of plants (Lavorel and Garnier, 2002), stream invertebrates (Archaimbault et al., 2010) or fishes (Mims et al., 2010). Conversely, this approach was little applied on soil ecology, although functional trait profile has been stressed by several authors to be an important parameter for revealing invertebrate responses to contamination (Ribera et al., 2001; Skalski et al., 2010). Its development has been hampered by different factors such as the wide diversity of soil invertebrates and a lack of standardized sampling strategies, as well as low taxonomic expertise (Decaëns, 2010; Giller, 1996). Perhaps one of the most limiting factors may be the absence of a unified, shared database containing multiple information on a great number of taxa.

The present work aimed at demonstrating that functional traits of soil invertebrates provide new insights and better understanding of soil pollution effects than currently used indices of community structure. We applied a trait-based approach to depict disturbance impacts on soil invertebrate communities in a series of agricultural soils showing increasing amounts of organic matter and TM, resulting from long-term irrigation with urban wastewater. Another

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(mT), as the average of category affinity values weighted by the relative abundance of species carrying each affinity (Garnier et al., 2004) with the following equation:

$$mT = \sum_{i=1} p_i x_i$$

where x_i is the trait category affinity of the i -th species, and p_i its relative abundance.

2.6. Statistical analyses

Mean differences in structural indices and proportion in trait categories within communities were tested using Tukey (HSD) post-hoc test after ANOVA. Prior to analysis, data normality and homoscedasticity were tested using Wilk–Shapiro and Bartlett tests. A logarithmic transformation was used to homogenize variances if necessary. Linear models of soft-body and geophage proportions with soil parameters as independent variables were tested. All statistical analyses were performed at the significant level of $\alpha = 0.05$, using the 'base' library for R Software (R Development Core Team 2011).

3. Results and discussion

3.1. Soil pollution gradient

Soil texture, an intrinsic pedogenetic characteristic of the soils showed little variation, with clay contents ranging from 7.4 to 10.5% and total sand contents from 74 to 84%. Characteristic physico-chemical soil data are presented in Table 1. The soil pH was consistently higher in the limed unpolluted top soil compared to the contaminated surface horizons, ascribed to the buffering action of large rates of organic matter of urban origin and despite the presence of 2–4% of secondary carbonates added by wastewater irrigation (van Oort et al., 2008).

The soil organic C content ranged from 11.0 to 64.9 g kg⁻¹, i.e. a six-fold variation. In Pierrelaye topsoil, the filtering of large amounts of wastewater led to the accumulation of exogenous (fine, amorphous) organic matter distributed as coatings around the quartz grains explaining the outstanding dark color of the surface horizon (van Oort et al., 2008). The C/N ratios of irrigated soils were comparable (≈ 23 – 25 , except for the low-intermediate polluted plot), about two-fold higher than for the unpolluted reference soil.

According to the plot selection, the total TM concentrations showed a fairly regular increase, with maximum values for the ratio between the uncontaminated and the most contaminated soil of about 1:10 for Cd, 1:12 for Pb, 1:16 for Zn, and 1:20 for Cu (Table 1). The concentration of NH₄NO₃-extracted metals increased significantly in irrigated plots when compared with unpolluted one. For Zn and Cu, the gradient was similar to the total content one. However, the low-intermediate polluted plot presented significant lower Pb, and higher Cd values than those of other irrigated plots. The concentration of DTPA-extractable metals increased with increasing total metal contents, the ratio between the uncontaminated and the most contaminated soil was about 1:12 for Cd, 1:9 for Pb, 1:39 for Zn, and 1:33 for Cu. In irrigated plots, DTPA-extracted metals represented 18–24%, 11–17%, 18–35% and 36–48% of total concentration, for Zn, Pb, Cu and Cd respectively. These proportions were within the ranges found in literature, rather low for Zn and Pb and high for Cu and Cd (Dai et al., 2004; Kashem et al., 2007).

Physico-chemical parameters reflected a strong influence of the long-term irrigation practices. Metal pollutant concentrations were shown strongly correlated with organic C, due to their concomitant input by wastewater irrigation (Bourenane et al., 2006). Such co-variation of multiple parameters along soil pollution gradient is common in many field studies on pollution. Delineating specific effects of multiple pollution is usually a challenging task, since changes in relative proportion of pollutants, their speciation and/or their bioavailability frequently occur concomitantly with soil characteristics (van Gestel, 2008).

3.2. Impacts of soil contamination on the structure of invertebrate communities

A total of 586 individuals were collected, 428 by pitfall-trapping and 158 by hand sorting. Forty-three taxa were identified, depicted in Table 2. About 85% of trapped invertebrates belonged to four taxonomic groups, i.e. Araneae (41%), Carabidae (24%), Staphylinidae (10%) and Julidae (10%). Similarly, four groups represented about 90% of hand-sorted invertebrates, i.e. Lumbricidae (63%), Carabidae (6%), Julidae (5%) and various insect larva (e.g. campodeiform, melolonthoïde, eruciform, elateriform; 20%). Results showed a decreasing abundance as soil pollution increased (Fig. 1A). The unpolluted plot hosted 80 ind. m⁻². A density of 53 ind. m⁻² was determined in the low polluted plot whereas the three most contaminated plots contained much smaller values, ranging from 1 to 27 ind. m⁻². By contrast, the activity of invertebrates at the surface was only slightly affected by soil pollution (Fig. 1B). Only the intermediate-high polluted plot differed significantly from the others. A mean value of 52 ind. trap⁻¹ was observed while, in average, less than 25 individuals were trapped in the four other plots. Regarding the overall diversity of invertebrates (Fig. 1C), the intermediate-high polluted plot hosted the most diverse assemblages (15.0 OTU). Recorded value was about 2.2-fold greater than the lowest value (low-intermediate pollution, 7 OTU), but did not significantly differ from the other plots (Fig. 1C).

Consequently, in these field conditions, the activity or taxonomic diversity of soil invertebrates were not revealed as suitable indicators for the impacts of soil disturbance by wastewater pollution. In addition, the abundance of invertebrates was found only partially linked to the gradient in soil pollution. These findings were found consistent with a large part of literature. Many authors have stressed the lack of relationships between soil TM contamination and parameters of community structure they measured (Lock et al., 2001; Pizl et al., 2009; Tischer, 2008; van Gestel et al., 2009). Such absence of correlation may be ascribed to the wide range of environmental characteristics covered by the respective studies (Hobbelen et al., 2006; Lukkari et al., 2004) or to substitution of sensitive by tolerant species without changes neither in abundance nor in diversity (Eeva et al., 2004). In most studies on this topic, taxonomic groups were often investigated independently one to each other e.g. earthworms (Spurgeon and Hopkin, 1999), millipedes or isopoda (Grelle et al., 2000), ground beetles (Lock et al., 2001) or ants (Grzes, 2009). Such a group-specific approach limits the study of the range of biological strategies that can be developed by soil invertebrates in response to contamination. Yet, significant relationships between parameters of invertebrate communities and soil metal pollution were reported by some authors, but in fact restricted to two main cases. The first case of good correlation was recorded when the range of contamination of the study situations was very large, i.e. the most contaminated site exhibiting values more than 100-fold higher than those recorded in the reference or the less contaminated one (Grelle et al., 2000; Nahmani and Lavelle, 2002; Spurgeon and Hopkin, 1999). The maximum gradient of pollution then corresponded to extreme soil contaminations, i.e. metalliferous grassland hosting a very low number, if any, of soil invertebrates. The second case of good correlation arising from literature concerns studies conducted in boreal coniferous forest environments, also reporting indices of community structure being influenced by soil metal contamination (Eeva et al., 2004; Haimi and Mätäsniemi, 2002; Koponen and Koneva, 2005). The low diverse communities characterizing these soils are probably more impacted via a greater exposure to pollutants, since trace metals are generally more solubilized in acid soil conditions.

Table 2
Affinity percentage of recorded taxon to selected trait categories. Food has been split in 7 categories (f1: roots, f2: vegetative aerial tissues of plants, f3: fruit or seed, f4: dead animals; f5: living animals; f6: plant detritus; f7: soil), the body sclerotization in two categories (b1 soft-body; b2 hard body).

Phylum	Classes	Order	Taxon	f1	f2	f3	f4	f5	f6	f7	b1	b2
Arthropoda	Chelicerata	Arachnida	Araneae	0	0	0	0	100	0	0	50	50
Arthropoda	Chelicerata	Arachnida	Opiliones	0	0	0	0	100	0	0	50	50
Arthropoda	Diplopoda	Diplopoda	Julidae	0	0	0	0	0	95	5	0	100
Arthropoda	Diplopoda	Diplopoda	Polydesmidae	0	0	0	0	0	95	5	0	100
Arthropoda	Malacostracea	Isopoda	<i>Porcellio scaber</i>	0	0	0	0	0	95	5	0	100
Arthropoda	Insecta	Coleoptera Adephaga	<i>Amara aenea</i>	0	44	41	0	15	0	0	0	100
Arthropoda	Insecta	Coleoptera Adephaga	<i>Amara eurynota</i>	0	26	62	0	12	0	0	0	100
Arthropoda	Insecta	Coleoptera Adephaga	<i>Amara familiaris</i>	0	58	15	0	27	0	0	0	100
Arthropoda	Insecta	Coleoptera Adephaga	<i>Amara sp</i>	0	75	0	0	25	0	0	0	100
Arthropoda	Insecta	Coleoptera Adephaga	<i>Asaphidion stierlini</i>	0	100	0	0	0	0	0	0	100
Arthropoda	Insecta	Coleoptera Adephaga	<i>Harpalus affinis</i>	0	32	48	0	20	0	0	0	100
Arthropoda	Insecta	Coleoptera Adephaga	<i>Harpalus distinguendus</i>	0	0	50	0	50	0	0	0	100
Arthropoda	Insecta	Coleoptera Adephaga	<i>Harpalus froelichi</i>	0	32	68	0	0	0	0	0	100
Arthropoda	Insecta	Coleoptera Adephaga	<i>Harpalus tardus</i>	0	100	0	0	0	0	0	0	100
Arthropoda	Insecta	Coleoptera Adephaga	<i>Leistus sp</i>	0	100	0	0	0	0	0	0	100
Arthropoda	Insecta	Coleoptera Adephaga	<i>Loricera pilicornis</i>	0	6	0	0	94	0	0	0	100
Arthropoda	Insecta	Coleoptera Adephaga	<i>Metallina lampros</i>	0	28	17	6	50	0	0	0	100
Arthropoda	Insecta	Coleoptera Adephaga	<i>Nebria brevicollis</i>	0	0	11	0	89	0	0	0	100
Arthropoda	Insecta	Coleoptera Adephaga	<i>Notiophilus substriatus</i>	0	14	0	0	86	0	0	0	100
Arthropoda	Insecta	Coleoptera Adephaga	<i>Poecilus cupreus</i>	0	28	16	0	57	0	0	0	100
Arthropoda	Insecta	Coleoptera Adephaga	<i>Poecilus lepidus</i>	0	0	50	0	50	0	0	0	100
Arthropoda	Insecta	Coleoptera Adephaga	<i>Pseudoophonus rufipes</i>	0	23	8	0	69	0	0	0	100
Arthropoda	Insecta	Coleoptera Adephaga	<i>Pterostichus niger</i>	14	0	14	0	71	0	0	0	100
Arthropoda	Insecta	Coleoptera Polyphaga	Curculionidae	0	100	0	0	0	0	0	0	100
Arthropoda	Insecta	Coleoptera Polyphaga	Histeridae	0	0	0	0	100	0	0	0	100
Arthropoda	Insecta	Coleoptera Polyphaga	Sylphidae	0	0	0	0	100	0	0	0	100
Arthropoda	Insecta	Coleoptera Polyphaga	Staphylinidae	0	0	0	0	0	0	0	0	100
Arthropoda	Insecta	Coleoptera Polyphaga	Other polyphaga	0	0	0	0	0	0	0	0	100
Arthropoda	Insecta	Coleoptera Polyphaga	Campodeiform larvae	0	0	0	0	100	0	0	75	25
Arthropoda	Insecta	Coleoptera Polyphaga	Elatерiform larvae	50	0	0	0	50	0	0	0	100
Arthropoda	Insecta	Coleoptera Polyphaga	Melolonthoid larvae	95	0	0	0	0	0	5	100	0
Arthropoda	Insecta	Diptera	Other larvae	0	0	0	0	0	0	0	100	0
Arthropoda	Insecta	Diptera	Tipulidae larvae	50	25	0	0	0	25	0	100	0
Arthropoda	Insecta	Hemiptera	Hemiptera	0	50	0	0	50	0	0	0	100
Arthropoda	Insecta	Heteroptera	Heteroptera	0	100	0	0	0	0	0	0	100
Arthropoda	Insecta	Hymenoptera	Hymenoptera	0	0	0	0	0	0	0	0	100
Arthropoda	Insecta	Lepidoptera	Larvae	0	100	0	0	0	0	0	100	0
Mollusca	Gastropoda	Pulmonata	Agriolimacidae	0	100	0	0	0	0	0	100	0
Mollusca	Gastropoda	Pulmonata	Arionidae	0	100	0	0	0	0	0	100	0
Annelida	Oligochaeta	Opisthophora	<i>Allolobophora chlorotica</i>	0	0	0	0	0	0	100	100	0
Annelida	Oligochaeta	Opisthophora	<i>Aporrectodea caliginosa</i>	0	0	0	0	0	0	100	100	0
Annelida	Oligochaeta	Opisthophora	<i>Aporrectodea icterica</i>	0	0	0	0	0	0	100	100	0
Annelida	Oligochaeta	Opisthophora	<i>Lumbricus castaneus</i>	0	0	0	0	0	90	10	100	0

3.3. Functional traits: a footprint of soil contamination impacting invertebrate communities

Results showed a decrease in the proportion of geophages from 49% (unpolluted) to 29% (low polluted) and less than 6% in the three most contaminated plots (Fig. 2). The soft-body proportion decreased regularly. In the unpolluted plots, the proportion was 60% and decreased from 49% to 29% (low and high polluted plots, respectively). This type of expression of the results demonstrated a clear decrease of the proportion of the most exposed invertebrates with increasing contamination. In the present case, effects (−20%) on geophages were encountered even at low pollution level. Conversely, significant effects on soft-body proportion were recorded from low-intermediate plot (−15%). The different trends of relationships between geophagous or soft-body proportions and pollution level suggest that exposure by ingestion was more harmful than by contact. Such approach associates information on species distribution to their biological characteristics providing a biological trait profile of communities. Trait-based indices complement structural measures using taxonomic composition by providing indirect information on soil ecological functioning.

Since geophage or soft-body proportions were negatively impacted by pollution level, a next step was to test whether

observed responses can be attributed to specific TM pools. Geophage proportions were significantly influenced by CEC, organic C, total N, total TM, NH₄NO₃-extracted Pb and DTPA-extracted TM contents (Table 3). Linear models using CEC, total N and organic C content as independent variables expressed 49%, 47% and 36% of variance, respectively. Analyses of covariance showed that adding any TM variable as co-variate in models to pedological parameters did not improve the expressed variance. Thus, we did not verify the hypothesis that more strongly bound metals, rather than total contents, would provide a good indicator of TM damage through ingestion. Nevertheless, we can state that NH₄NO₃-extracted TM had no influence on geophage proportions, except for lead. Alternatively, the decline in geophage proportion illustrated changes in soil organic matter (content and reactivity) added by long-term wastewater irrigation. As discussed above, the filtering of large amounts of wastewater in these soils led to the accumulation of exogenous, amorphous organic matter (van Oort et al., 2008). Consequently, relationships with CEC, organic C and total N contents reflect an effect of variable amounts of soil organic matter, with likely low palatability for soil invertebrates. The disappearance or the significant decrease in geophages likely induces major consequences from a point of view of soil behavior, since these animals are primarily involved in soil dynamics

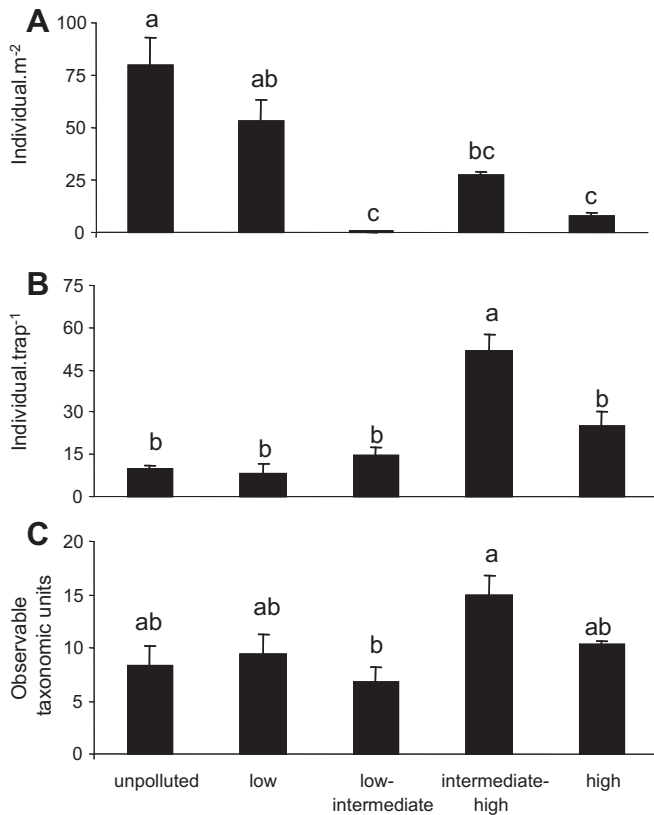


Fig. 1. Relationships (mean \pm SEM; $n = 4$) between indices of macro-invertebrate community structure and soil contamination level in agricultural plots of the Pierrelaye wastewater irrigation area (France). A, density of soil and litter dwelling invertebrates; B, abundance-activity of invertebrates at soil surface; C overall diversity of invertebrates. Different letters indicated statistical differences ($p < 0.05$).

such as nutrient turn-over and soil aggregation (Lavelle and Spain, 2001).

On the other hand, soft-body proportions were significantly influenced by soil pH, CEC, C/N ratio, total N, total TM, DTPA-extracted TM and NH_4NO_3 -extracted TM (except for Pb) contents (Table 3). F-values indicated that models built with Zn extracted by NH_4NO_3 and Cd and Cu extracted by DTPA, as independent variables, expressed a great part of variance. Additionally, analyses of covariance showed that adding any pedological variable as co-variate in the models involving either DTPA-extracted, NH_4NO_3 -extracted (but Pb) or total Zn and Cd contents, did not improve the expressed

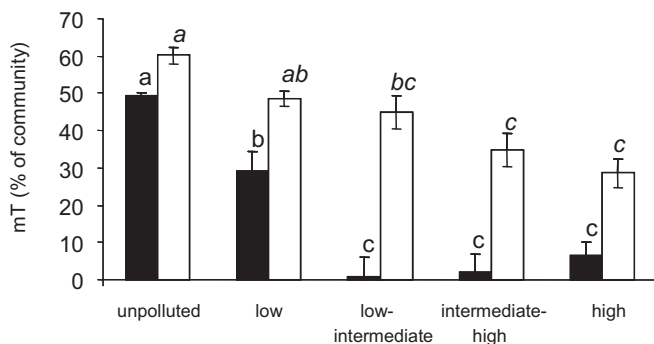


Fig. 2. Relationships (mean \pm SEM; $n = 4$) between mean proportion of soft-body (open bars) and geophage (black bars) individuals in macro-invertebrate community (mT) and soil contamination level in agricultural plots of the Pierrelaye wastewater irrigation area (France). For each data series, different letters indicated statistical differences ($p < 0.05$).

Table 3

Parameters of linear models between mean trait category affinities (geophage and soft-body mT) and soil parameters. All slopes were negative. Bold numbers indicated significant linear model.

		Geophage mT		Soft-body mT	
		F value	p value	F value	p value
Pedological parameters	pH	3.06	0.097	12.41	0.002
	CEC	17.49	0.001	4.58	0.046
	C org	10.19	0.005	3.02	0.099
	N total	16.08	0.001	6.51	0.020
	C/N	0.46	0.505	5.07	0.037
Total metal content	Cu	6.34	0.021	5.87	0.026
	Pb	8.16	0.010	5.54	0.030
	Zn	9.90	0.006	11.98	0.003
	Cd	7.81	0.012	14.96	0.001
NH_4NO_3 -extracted metal content	Cu	2.82	0.110	11.95	0.003
	Pb	5.92	0.026	2.29	0.148
	Zn	4.07	0.059	25.09	<0.001
DTPA-extracted metal content	Cd	0.49	0.493	9.71	0.006
	Cu	8.13	0.011	18.51	<0.001
	Pb	7.58	0.013	9.15	0.007
	Zn	5.17	0.035	12.33	0.002
	Cd	7.98	0.011	24.18	<0.001

variance. For total Cu and Pb contents, a linear model involving pH ameliorated the part of variance expressed but did not differ from the model with pH alone. TM pollution thus appeared as the main factor explaining soft-body animal proportion in macro-invertebrate communities. Nevertheless, interactions with pH cannot be totally excluded. Again, in our conditions the hypothesis of predominant effect of weakly bound TM on soft-body invertebrates was not verified. While linear model with NH_4NO_3 -extracted Zn content explained 58% of soft-body proportion variance, DTPA-extracted Cd and Cu content also modeled 57% and 41% of it.

In a more general context, and as observed for studies on soil invertebrate community structure, most of the literature on trait-based approach using soil fauna focused on only one single taxonomic group, e.g. springtails (Makkonen et al., 2011), carabids (Ribera et al., 2001), or earthworms (Decaens et al., 2008). Studying the entire community, and not taxonomically *a priori* defined groups, permits to encompass most of biological strategies and to infer the mechanisms by which soil contamination alters soil communities. However, it can be argued that taxa allocation for food trait in the initial phase of the analysis is quite limited and reflects the influence of phylogeny on soil invertebrate biology. We assume however that it also results from a failure to the description of soil invertebrate feeding habits in current literature. For instance, the ingestion of soil particles is particularly well-documented for the different ecomorphological groups of earthworms (epigeic, anecic and endogeic), but many soil- and surface-dwelling macroarthropods inevitably take up more or less important amounts of soil (Wolters, 2000), either by hasard or to achieve their requirements in some mineral nutrients like calcium or magnesium. Unfortunately, information on soil ingestion by rhizo- and saprophages are scarce, and very little quantitative data is available. Literature on woodlice (Vijver et al., 2006), millipedes (Crawford, 1976; Kaneko, 1999; Tajovsky, 1992), scarabeiform larvae (McQuillan and Webb, 1994) suggested zero to low proportion of soil in animal food, but high proportions have also been recorded (26–43% for millipedes in Dangerfield, 1993). Consequently, in the absence of large and reliable datasets on 'neglected groups of decomposers' (Wolters, 2001), we attributed a 5% proportion of soil in diet for woodlice, millipedes and scarabeiform larvae. Isotope techniques seem to be particularly useful to overcome this drawback (Curry and Schmidt, 2007).

Finally, to be generally applicable, soil invertebrate trait-based approaches require to interface conceptual, and respective methodological improvements, from soil chemistry and ecology. There is

a need to confront the pools of pollutants identified by different extractions with a wider palette of biological traits implicated in invertebrate fitness (e.g. life span, reproductive strategy). Hence, rapid progress demands an available multi-taxa multi-trait database. Then, it will be possible to uncorrelate specific pollutant impacts from those of other environmental factors.

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Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.envpol.2012.01.017.

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