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## Effect of Geogenic Lead on Fungal and Collembolan Communities in Garden Topsoil



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### ABSTRACT

Geogenic lead (Pb) is considered to be less bioavailable than anthropogenic Pb and exerts less effect on the soil fauna. However, Pb contamination in vegetables has been reported in the case of geogenic anomalies, even at moderate concentrations (around 170 mg kg<sup>-1</sup>). In this study, we investigated collembolan communities using both taxonomic- and trait-based approaches and observed fungal communities to assess the effects of a moderate geogenic Pb anomaly on collembolans and fungi in an urban vegetable garden soil. Results indicated that geogenic Pb indeed modified fungi communities and altered the functional structure of collembolan communities in garden soils. Although geogenic Pb presented low bioavailability, it affected soil fauna and vegetables similar to anthropogenic Pb.

**Key Words:** functional diversity, functional trait, soil fauna, soil food web, vegetable garden, urban soil

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### INTRODUCTION

Urban allotment gardening is currently a growing worldwide phenomenon, especially in industrialized countries (Guitart *et al.*, 2012). The chemical study of topsoil quality along an anthropization gradient has shown that these garden soils may contain metals, especially lead (Pb) (Joimel *et al.*, 2016). Lead is considered to be neurotoxic, even at low concentrations, beginning at 12 µg L<sup>-1</sup> in the blood (European food safety norm). Thus, high Pb concentrations could induce health risks through the consumption of contaminated vegetables from allotment gardens. Lead contamina-

tion results not only from human activities such as industry, car traffic, and refuse (El Khalil *et al.*, 2013; Huot *et al.*, 2013) but also from intensive gardening practices, such as the use of pesticides containing Pb (Belon *et al.*, 2012). The presence of metals, such as Pb, may also be of geogenic origin, resulting from metalliferous mineralization, inducing local naturally high concentrations in soils (Bourennane *et al.*, 2010; Ander *et al.*, 2013). However, urban vegetable garden soils usually also have a basic pH and a high rate of organic matter (Joimel *et al.*, 2016), contributing to reduced Pb mobility. This assumption is especially true for geogenic Pb, which is often considered to present lower

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bioavailability than anthropogenic Pb.

Recent soil analyses, performed in 2011 and 2012 on allotment gardens in Nantes (France) (Bouquet *et al.*, 2017), found elevated Pb concentrations averaging  $170 \text{ mg kg}^{-1}$ , mainly of geogenic origin due to the alteration of a particular parent rock, rich in Pb-bearing minerals (Jean-Soro *et al.*, 2015). Despite Pb being primarily of geogenic origin and presenting moderate anomaly (2.5-times higher than background levels) (Bouquet *et al.*, 2017), health risks have been demonstrated due to the consumption of contaminated vegetables from these allotment gardens, with the Pb concentrations in leeks, cabbage, and carrots exceeding the European regulatory standards by up to 25 times (Jean-Soro *et al.*, 2015).

These results raise questions as to the effects of moderate anomalies in geogenic Pb on the soil biota. In fact, metals, such as Pb, could also affect soil organisms, thereby inducing a decrease both in biodiversity and in soil biological activity (Giller *et al.*, 1998), which are important factors in the provision of ecosystem services, such as food production (Scheu, 2003). Anthropogenic Pb contamination is often accused of impacting the soil fauna by inducing a decrease either in biodiversity or in the biological activity of some soil fauna groups, such as macrofauna (Nahmani *et al.*, 2003), collembolans (Fountain and Hopkin, 2004a), and nematodes (Campos-Herrera *et al.*, 2016; Gutiérrez *et al.*, 2016).

However, to date, numerous studies have only concerned the effects of anthropogenic Pb contamination and/or elevated geogenic Pb concentrations (higher than  $1\,000 \text{ mg kg}^{-1}$  soil) (Russell and Alberti, 1998). In contrast, the effects of moderate geogenic Pb anomalies on soil fauna have as yet been scarcely studied.

The relationships between metal in the soil and fungi communities have been widely explored, but results of the studies trying to establish the effect of metal toxicity to soil microorganisms vary greatly (Giller *et al.*, 1998). The increase in studies of the effects of metals on soil microorganisms is related to an increasing awareness of pollution issues (Bååth, 1989). A shift in a fungi species' composition induced by Pb contamination was found when Pb was present with Cd, Cu, and Zn as well as for Zn and Cd alone (Bååth, 1989). Again, major gaps in knowledge and theory exist as to how the microorganisms are exposed and respond to the metals in soils (Giller *et al.*, 2009).

Among the soil fauna, collembolans are a key component of the food web and play a key role both in litter decomposition (Cortet *et al.*, 2003) and nitrogen and carbon cycling (Filser, 2002) as well as in soil mi-

croaggregation, and, therefore, in soil productivity as a whole. The primary role of collembolan is decomposition and the way the soil functions because they feed on detritus and/or fungi (O'Brien *et al.*, 2005). In addition, soil fungi are partly influenced by collembolan grazing activity.

Relationships between metal in the soil and collembolan communities have mainly been explored using taxonomic approaches (including species richness, abundance, and community structure and composition). A functional trait-based approach would be complementary to these approaches because traits are properties of individuals that govern the responses to their environment (Pey *et al.*, 2014b). For invertebrates, Pey *et al.* (2014b) defined a functional trait as being any morphological, physiological, phenological, or behavioral (MPPB) feature measurable at the individual level, without reference to any other level of organization. Over the last 20 years, functional trait investigations have contributed to highlighting the way in which environmental stressors impact plant selection (Lavorel and Garnier, 2002; Garnier *et al.*, 2007) and more recently have included the effects on soil fauna too (Hedde *et al.*, 2012; Santorufu *et al.*, 2014a). For example, a study of the functional traits of ground beetles has shown their sensitivity to anthropogenic Zn contamination (Skalski *et al.*, 2010).

Our study focuses on an allotment garden, which presents a local geogenic Pb anomaly with concentrations ranging from 97 to  $314 \text{ mg kg}^{-1}$  soil, which consequently induces Pb contamination in the vegetables consumed by gardeners (Jean-Soro *et al.*, 2015). Within this framework, our objective was to evaluate the effect of this geogenic anomaly on collembolan and fungal communities. A single garden on an urban allotment with a pronounced Pb gradient was studied. We hypothesized that the total Pb concentration induced a direct chemical constraint on soil fauna that reduces the relative abundance of taxa exhibiting traits linked to Pb exposure (body length and localization in soil profile), avoidance (motion strategy), population recovery (reproduction type), and/or an indirect effect as a result of the change in the structure of the fungal community.

## MATERIALS AND METHODS

### *Experimental site and soil characteristics at plot scale*

An experiment was carried out on a  $280\text{-m}^2$  individual plot at the Eglantiers allotment garden located in Nantes (Loire-Atlantique, France). The climate is temperate oceanic with a mean temperature of  $12 \text{ }^\circ\text{C}$  and

a mean rainfall of 820 mm per year. At the beginning, the garden plot was divided into two sub-plots. Soil analysis was performed in autumn 2012. Twenty sub-samples of cultivated topsoils (0–20 cm) were collected from each sub-plot and then mixed and homogenized to obtain two representative samples. Few differences were noticed in the main soil characteristics analyzed (Table I). The soil had a sandy loam texture and high nutrient contents. Its mineralogy mainly consisted of quartz (71%); feldspars such as orthoclase (9%) and plagioclase (8%); phyllosilicates such as kaolinite (6%); and traces of chlorite and illite or micas (6%). Cd, Cu, and Zn were not considered as possible contaminants in this garden, conversely to the usually observed levels in urban vegetable gardens (Joimel *et al.*, 2016).

In contrast, Pb was the major concern in this allotment garden, with a median of 171 mg kg<sup>-1</sup>, which was considered as highly anomalous compared to usual Pb concentrations both in French soils in general (Baize *et al.*, 2008) and particularly in the district (Le Guern *et al.*, 2013a, b). Lead in this garden is known to be geogenic, probably linked to mineralized veins rich in Pb-bearing minerals in the parent material, which is constituted of mica schists (Jean-Soro *et al.*, 2015). Lead speciation in the garden was also assessed through sequential extraction (Ure, 1996) following the Community Bureau of Reference (BCR) procedure (Bouquet *et al.*, 2017). Four fractions were obtained: exchangeable Pb (1.2%), Pb linked to the oxy/hydroxides of Mn and Fe (11.9%), Pb linked to sulfides and organic matter (4.6%), and the residual fraction (82%).

TABLE I

Topsoil characteristics of an urban vegetable garden at the garden plot scale (values for two replicates, which were derived from 20 sub-replicates each) and sub-plot scale (32 sub-plots)

Characteristics	Scale	
	Plot	Sub-plot
Sand (%)	57, 58 <sup>a)</sup>	
Silt (%)	28, 28 <sup>a)</sup>	
Clay (%)	13, 14 <sup>a)</sup>	
pH	7.4, 7.4 <sup>a)</sup>	5.75–7.63 <sup>b)</sup>
Organic matter (g kg <sup>-1</sup> )	36, 29 <sup>a)</sup>	
C:N	14, 13 <sup>a)</sup>	
CEC (cmol+ kg <sup>-1</sup> )	8.7, 7.4 <sup>a)</sup>	
Total Pb (mg kg <sup>-1</sup> )	133, 208 <sup>c)</sup>	97–314 <sup>b),c)</sup>
Total Cu (mg kg <sup>-1</sup> )	38, 28 <sup>c)</sup>	
Total Zn (mg kg <sup>-1</sup> )	94, 79 <sup>c)</sup>	
Total Cd (mg kg <sup>-1</sup> )	0.15, 0.12 <sup>c)</sup>	
CaCl <sub>2</sub> -extractable Pb (μg kg <sup>-1</sup> )		34–285 <sup>b)</sup>

<sup>a)</sup>Joimel (2015).

<sup>b)</sup>Jean-Soro *et al.* (2014).

<sup>c)</sup>Béchet *et al.* (2018).

#### Soil characteristics at sub-plot scale

In order to study the variations in Pb concentration at plot scale in greater detail, the garden was divided into 32 sub-plots of 6 m<sup>2</sup> each separated by 50-cm wide grass strips (Fig. 1). Total Pb, CaCl<sub>2</sub>-extractable Pb, and pH were analyzed in each of the 32 sub-plots. Total Pb concentrations ranged from 97 to 314 mg kg<sup>-1</sup>, with a median of 197 mg kg<sup>-1</sup> (Jean-Soro *et al.*, 2015). These values increased along the west-east gradient. Values of CaCl<sub>2</sub>-extractable Pb and pH also

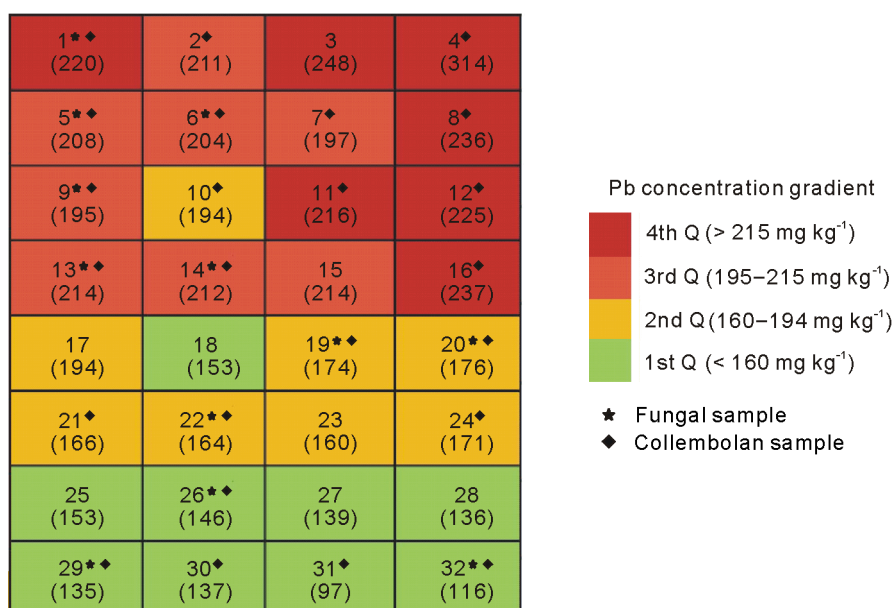


Fig. 1 Distribution of total Pb concentrations (values in the parentheses) in the soil of urban vegetable garden (modified from Béchet *et al.*, 2016). The garden plot was divided into 32 sub-plots of 6 m<sup>2</sup> each separated by 50-cm wide grass strips. Q = quartile.

varied according to this gradient.

Total Pb level of each sub-plot was analyzed using a portable X-ray fluorescence tool (Niton 792). A selection of soil samples set for laboratory analysis by inductively coupled plasma mass spectrometry (ICP/MS) was obtained to calibrate the device. This method provides a semi-quantitative analysis of Pb concentrations, with a detection threshold of 4 mg kg<sup>-1</sup> (in a SiO<sub>2</sub> matrix). Blank tests and internal quality control samples were introduced into each series. The quality of the analyses was assessed by analyzing a reference soil, BCR-7002, using the same procedure. The quantification threshold was 0.1 µg L<sup>-1</sup> for Pb. Soil samples were dried for 5 d at 40 °C and prepared according to the NF ISO 11464 standard (AFNOR, 2006). Soil pH was measured according to the NF ISO 10390 standard (AFNOR, 1994), and mobile Pb was extracted with 0.01 mol L<sup>-1</sup> CaCl<sub>2</sub> for 2 h according to the NEN 5704 standard (1996).

#### *Soil fungi diversity sampling at sub-plot scale*

Fungi were sampled in April 2013. Among the 32 sub-plots, twelve replicates were randomly sampled along the concentration gradient (Fig. 1). Fifty grams of litter-free topsoil were collected from each of the sub-plots selected.

#### *Fungal DNA diversity analysis*

The fungal community structure was studied using polymerase chain reaction-temporal temperature gel electrophoresis (PCR-TTGE). DNA was extracted from soil samples (0.5 g) using the Nucleospin<sup>®</sup> soil kit (Macherey-Nagel GmbH & Co, Düren, Germany). Before DNA analyses, soil samples were ground using a mixer mill (MM 400, Retsch, Haan, Germany) three times for a period of 30 s each at a frequency of 25 Hz. The extracted DNA was quantified, and its quality was controlled using SPECTROstar Nano (BMG LABTECH LVi Plate, Offenburg, Germany). The 5.8S DNA region was amplified using ITS5-gc (forward) and ITS2 (reverse) primers (Joly *et al.*, 2012). The final reaction volume totaling to 50 µL comprised 1.2 ng L<sup>-1</sup> of DNA template, 0.3 mol L<sup>-1</sup> of each primer, 200 mol L<sup>-1</sup> of dNTPs, 0.012 unit (U) L<sup>-1</sup> of *Taq* polymerase, 1× reaction buffer, 2.5 mmol L<sup>-1</sup> of MgCl<sub>2</sub>, and 500 ng L<sup>-1</sup> of bovine serum albumin. The cycling conditions were set as follows: 1 cycle at 95 °C for 15 min, followed by 35 cycles of 95 °C for 30 s, 55 °C for 45 s, and 72 °C for 30 s, and a final extension cycle at 72 °C for 7 min (CFX96 Touch<sup>™</sup>, Thermal Cycler, Bio-Rad, USA). Analyses of TTGE were performed using a DCode<sup>™</sup> system (Bio-Rad, Hercules,

USA). The polyacrylamide gel (9.5%) (volume:volume) was composed of two parts: the upper, urea-free, “concentration” (stacking gel) of about 1 cm upward from the base of the wells, and the lower, “denaturation” part (resolving gel), at 8 mol L<sup>-1</sup> urea. Fifteen microliters of PCR products were deposited in each well. Migration was performed in Tris-acetate-EDTA 1.25× buffer for 750 min at 50 V, with a temperature gradient from 65 to 70 °C, *i.e.*, increasing the temperature at the rate of 0.4 °C h<sup>-1</sup>. The gels were stained using GelRed<sup>™</sup> (Biotium, Hayward, USA) and then imaged under ultraviolet (UV) light (Molecular Imager<sup>®</sup> Gel Doc<sup>™</sup> XR System and Image Lab<sup>™</sup> Software, Bio-Rad, Hercules, USA). Microbial community structure analysis was performed from genetic fingerprints.

Fungal communities were described by composition, richness (mean number of taxa per sample), Shannon diversity index, and evenness index (diversity and distribution index per sample).

#### *Collembolan diversity sampling at sub-plot scale*

Collembolans were sampled in April 2013. Among the garden plots, 24 replicates were randomly sampled along the concentration gradient (Fig. 1). One sample of intact soil core (5 cm depth and 6 cm diameter) was collected per replicate.

#### *Collembolan diversity analysis*

Collembolans were extracted from soil samples using a high-gradient McFadyen extractor for one week (Petersen *et al.*, 2003). Except for *Mesaphorura*, identified only at the genus level, collembolans were identified at the species level using various available dichotomous keys (Gisin, 1943; Zimdars and Dunger, 1995; Dunger, 1999; Potapow, 2001; Thibaud *et al.*, 2004; Hopkin, 2007; Dunger and Schlitt, 2011; Jordana, 2012).

For the taxonomic approach, collembolan communities were described by density (abundance of each taxon per m<sup>2</sup>, richness (mean number of taxa per sample), Shannon diversity index, and evenness index (diversity and distribution index per sample).

For the functional approach, 8 functional traits and ecological performances were selected for their ability to reflect the effect of Pb exposure or avoidance on population recovery (Table II). Body shape, body length, visual organs, and pigmentation are related to the vertical distribution of collembolans in the soil (Ponge *et al.*, 2006; Farská *et al.*, 2014; Salmon *et al.*, 2014). Reproduction type is related to their ability to colonize or survive after disturbances (Chernova *et al.*, 2010). Motion strategy determines the ability of collembolan to

TABLE II

Description and attributes of morphological, behavioral, and physiological traits considered for the trait-based approach of the collembolan community

Trait	Abbreviation	Attributes
Body shape	BSH	Spherical, cylindrical
Body length	BLR	< 1, 1–2, 2–3, > 3 mm
Motion strategy	MS	With, without furcula
Pigmentation	PIG	With, without coloration
Visual organ	VO	With, without ocelli
Reproduction	REP	Sexual, asexual
Habitat	HABI	Forest, agricultural, anthropogenic areas
Microhabitat	MHABI	Organic matter in decomposition, mineral soil, vegetation

jump using its furcula, *e.g.*, to avoid predators. The ability to jump is defined by the presence of a short, reduced, or long furcula. Habitat and microhabitat indicate ecological preference and trophic strategy (Salmon *et al.*, 2014).

Traits were obtained from the BETSI (biological and ecological functional traits of soil invertebrates) database on functional traits (CESAB/FRB) (Pey *et al.*, 2014a). Data on functional traits were primarily adopted from identification keys in the literature (Gisin, 1943; Zimdars and Dunger, 1995; Dunger, 1999; Potapow, 2001; Thibaud *et al.*, 2004; Hopkin, 2007; Dunger and Schlitt, 2011; Jordana, 2012). The trait matrix contained trait attribute scores, encoded using a fuzzy method (Chevene *et al.*, 1994) and adapted to soil organisms (Hedde *et al.*, 2013). Briefly, a species affinity percentage for each attribute of a trait was determined per source and then averaged through the sources to obtain the mean distribution of species affinity across the different attributes of a trait (*i.e.*, the species trait profile).

In order to study the repartition of traits within the communities, we calculated the community weighted mean (CWM) for each trait. Community weighted mean is the sum of species trait profiles weighted by their relative abundance in the community (Lavorel *et al.*, 2008). The multidimensional functional structure of collembolan communities was characterized by three distance-based functional diversity indices (Laliberté and Legendre, 2010), *viz.*, functional richness (FRic), functional evenness (FEve), and functional divergence (FDiv), and Rao's quadratic entropy (RaoQ). These indices quantify different facets of the functional diversity for a community (Villéger *et al.*, 2008); FRic represents the volume of the functional space occupied by a community, FEve corresponds to the regularity of the distribution of species abundance in a given volume, and FDiv is the divergence in the distribution of abundance in this volume (Laliberté and Legendre,

2010). In addition, we used the RaoQ index, which incorporates both the relative abundance of a species and a measure of the pairwise functional differences between the species (Botta-Dukát, 2005). The RaoQ index is a suitable approach to measure functional diversity, where several traits are considered (Pelosi *et al.*, 2014). The maximum functional dissimilarity within the collembolan community was observed at a value of 1.

#### Statistics

The effects of Pb concentrations on fungi and collembolan communities were investigated. Pearson's test was used to compare TTGE profiles between the fungi communities. The unweighted pair group method with an arithmetic mean (UPGMA) was used to construct a dendrogram from similarity coefficients (Ibekwe *et al.*, 2010).

We analyzed the effect of Pb on the specific composition of collembolans by performing a principal coordinate analysis (PCoA) using the Bray-Curtis index to explore dissimilarities between the communities. The CWM values were compiled into a single matrix (26 taxa  $\times$  16 CWM). Then, we performed principal component analysis (PCA) to investigate the functional composition of collembolan communities. For binary attributes, only one attribute per taxa was used in PCA to avoid auto-correlation. Moreover, each variable in PCA was weighted by the number of attributes for each functional trait.

Pearson's test was again used to assess the correlations of the parameters describing fungal and collembolan communities with the Pb concentration gradient.

The significance level for all analyses was fixed at the rejection level of  $P = 0.05$ . Analyses of TTGE profile were performed with FPQuest<sup>TM</sup> software (BioRad, Hercules, USA). Fungal community structure analysis was performed from genetic fingerprints. All statistical analyses for collembolan communities were

performed using R software version 3.2.2 (R Development Core Team, 2015) with vegan packages (Oksanen *et al.*, 2014) for the taxonomic approach and the FD package (Laliberté and Legendre, 2010) for the functional approach. The ade4 package (Thioulouse *et al.*, 1997) was used for multivariate analysis. All data mentioned in the results are mean values.

## RESULTS

### Fungi

Species richness varied between 5 and 10 species per plot, with 8 species on an average. Analysis of the fungal structure community revealed two clusters with 64.5% of similitude (Fig. 2). These two clusters were related to total Pb concentration. The first group included sub-plots with low Pb concentrations ( $< 197 \text{ mg kg}^{-1}$ ), except for Sub-plot 6 ( $204 \text{ mg kg}^{-1}$ ). The second group was composed of sub-plots with higher Pb concentrations ( $> 197 \text{ mg kg}^{-1}$ ), except for Sub-plot

20 ( $176 \text{ mg kg}^{-1}$ ). The species richness, Shannon diversity index, and evenness index of fungal communities were not correlated to total and  $\text{CaCl}_2$ -extractable Pb concentrations or to pH (Table III).

### Taxonomic composition and structural collembolan communities

A total of 26 taxa were found in the garden topsoil, belonging to nine families, Brachystomellidae, Entomobryidae, Hypogastruridae, Isotomidae, Neelidae, Onychiuridae, Sminthuridae, Sminthurididae and Tullbergiidae. Three species were singletons and six species were doubletons. The most abundant species were *Proisotoma minuta* (Tullberg, 1871) (36%), *Parisotoma notabilis* (Schaeffer, 1896) (29%), and *Folsomia similis* (Bagnall, 1939) (18%). Species with lower abundances were *Protaphorura armata* (Tullberg, 1869) and *Entomobrya multifasciata* (Tullberg, 1871). *Mesaphorura* sp. was also a frequent taxon.

Total collembolan density in the soil samples ran-

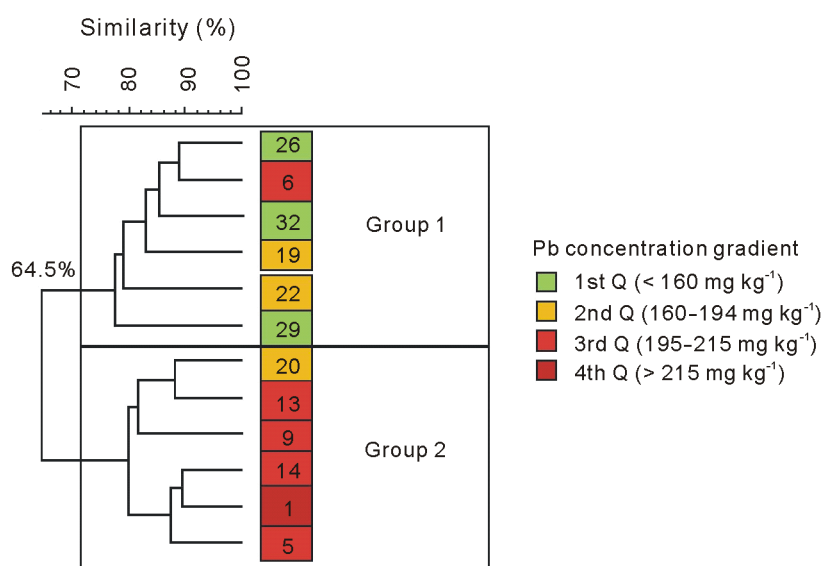


Fig. 2 Similarity between the fungi community of each sub-plot in garden soil along the gradient of total Pb concentration increase in soil. Q = quartile.

TABLE III

Species richness, Shannon diversity index, and evenness index of fungal community and Pearson's correlation coefficients ( $r$ ) with total Pb,  $\text{CaCl}_2$ -extractable Pb, and pH in garden soil

Parameter	Min. <sup>a)</sup>	Max. <sup>b)</sup>	Mean	$r$		
				Total Pb	$\text{CaCl}_2$ -extractable Pb	pH
Species richness	5	10	7.8	$-0.20\text{ns}^{\text{c}}$	$-0.30\text{ns}$	0.48ns
Shannon diversity index	1.1	2.1	1.8	$-0.40\text{ns}$	$-0.31\text{ns}$	0.20ns
Evenness index	0.4	0.6	0.6	$-0.48 \text{ ns}$	$-0.22\text{ns}$	$-0.11\text{ns}$

a) Min. = minimum.

b) Max. = maximum.

c) Not significant.



TABLE IV

Species richness, Shannon diversity index, evenness index, and density of collembolan community and Pearson's correlation coefficients ( $r$ ) with total Pb, CaCl<sub>2</sub>-extractable Pb, and pH in garden soil

Parameter	Min. <sup>a)</sup>	Max. <sup>b)</sup>	Mean	$r$		
				Total Pb	CaCl <sub>2</sub> -extractable Pb	pH
Species richness	6	13	10	-0.38ns <sup>c)</sup>	0.06ns	-0.16ns
Shannon diversity index	0.6	2.1	1.5	-0.35ns	-0.29ns	0.18ns
Evenness index	0.3	0.8	0.6	-0.22ns	-0.32ns	0.25ns
Density ( $\times 10^3$ individuals $m^{-2}$ )	19	505	123	0.03ns	0.21ns	-0.36ns

<sup>a)</sup>Min. = minimum.

<sup>b)</sup>Max. = maximum.

<sup>c)</sup>Not significant.

ged from about  $19 \times 10^3$  to  $505 \times 10^3$  individuals  $m^{-2}$  (Table IV). Other parameters also ranged widely. On an average, there were ten species, with the Shannon diversity index of 1.5 and evenness index of 0.6 per sub-plot. The first two axes of PCoA for collembolan species composition represented 51% of the total inertia (Fig. 3). The collembolan species composition varied depending on the sub-plot considered. No defined response to soil parameters, such as Pb concentration, was observed. The number of species, Shannon diversity index, evenness index, and density of collembolan communities were not correlated to total and CaCl<sub>2</sub>-extractable Pb concentrations and to pH.

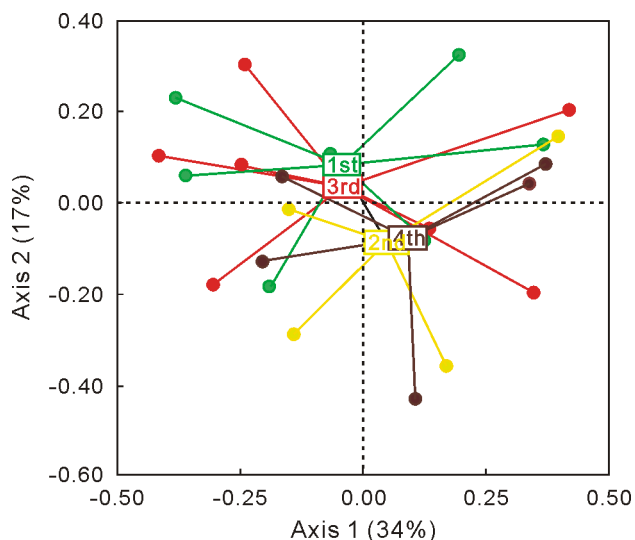


Fig. 3 Principal coordinate analysis (PCoA) of the specific composition of collembolans in garden soil along the gradient of total Pb concentration increase (4th quartile (Q) > 3rd Q > 2nd Q > 1st Q) in soil.

#### Functional parameters of collembolan communities

Based on CWM values, the main characteristics of collembolan communities were small bodied (1–2 mm) (CWM = 76%), cylindrical (CWM = 99%), with ocel-

li (CWM = 85%), with furcula (CWM = 90%), pigmented (CWM = 61%), and with sexual reproduction (CWM = 74%). Concerning the ecological preferences for habitat, CWM values were 52% for forest, 28% for anthropogenic, and 19% for agricultural habitats. For microhabitat, CWM values were 7% for vegetation and 44% and 48% for decomposing organic matter and mineral soil, respectively.

The first two axes of PCA on CWM represented 68% of the total variability (Fig. 4). Axis 1 seemed to be related to species habitat and microhabitat preferences. Axis 2 represented an opposition between the organisms presenting pigmentation, ocelli, furcula, spherical body, and body length > 2 mm living in a vegetation microhabitat. The trait profile for Sub-plot 26 presented a higher differentiation than that of the other sub-plots.

However, two functional parameters of collembolan communities were significantly correlated to total Pb concentrations (Table V), *viz.*, FRic and the RaoQ index, which significantly decreased as total soil Pb concentration increased. Concerning other indices and soil parameters, no significant relationship was observed, even if a tendency existed along the gradient towards a decrease in FEve.

TABLE V

Functional richness (FRic), functional divergence (FDiv), functional evenness (FEve), and Rao's quadratic entropy (RaoQ) of collembolan community and Pearson's correlation coefficients ( $r$ ) with total Pb, CaCl<sub>2</sub>-extractable Pb, and pH in garden soil

Parameter	Mean	$r$		
		Total Pb	CaCl <sub>2</sub> -extractable Pb	pH
FRic	$8.06 \times 10^{-5}$	-0.41*	-0.17ns <sup>a)</sup>	-0.19ns
FDiv	0.78	-0.07ns	0.24ns	-0.25ns
FEve	0.53	-0.31ns	-0.02ns	-0.05ns
RaoQ	0.03	-0.43*	-0.37ns	0.18ns

\*Significant at  $P < 0.05$ .

<sup>a)</sup>Not significant.



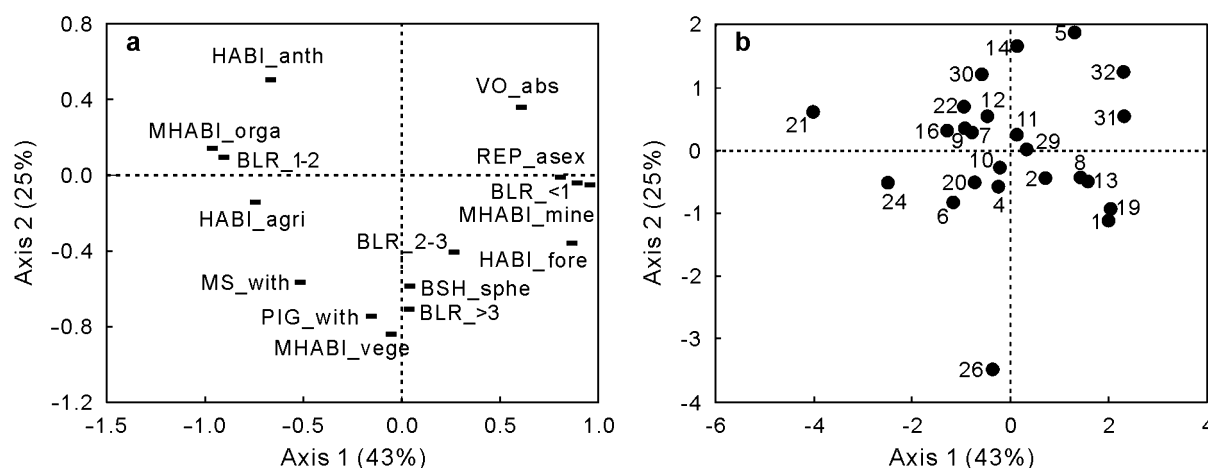


Fig. 4 Principal component analysis (PCA) of functional composition of collembolans based on values of community weighted mean (CMW) for 8 traits (a) in the 26 sub-plots of garden soil (b) (26 taxa, 24 CWM). Labels in Fig. 4b correspond to plot numbers along the decrease in total Pb concentration in soil. VO\_abs = visual organ, without ocelli; BSH\_sphe = body shape, spherical; BLR\_<1, BLR\_1-2, BLR\_2-3, and BLR\_>3 = body length, < 1, 1-2, 2-3 or > 3 mm, respectively; PIG\_with = pigmentation, with coloration; REP\_asex = parthenogenesis reproduction; HABI\_fore, HABI\_agri, and HABI\_anth = forest, agricultural, anthropogenic habitats, respectively; MHABI\_organica, MHABI\_mineral, and MHABI\_vegetation = microhabitat, organic matter in decomposition, mineral soil, and vegetation, respectively; MS\_with = motion strategy, with furcula.

## DISCUSSION

Numerous studies have dealt with the effect of anthropogenic Pb contamination on the microfauna and microorganisms, but only a few relate to geogenic Pb. Yet, environmental and sanitary risks do indeed exist, even with this geogenic Pb. In the present study, we investigated the effect of a moderate geogenic Pb anomaly in an urban vegetable garden on fungi, using a genetic fingerprinting method, as well as on its collembolan communities, using taxonomic and trait-based approaches.

Our results showed that geogenic Pb affected the composition of fungi communities. These results could be related to the potential toxicity of metals, as previously reported for anthropogenic metals on soil microorganisms (Giller *et al.*, 1998), in particular on fungal diversity (Yang *et al.*, 2015). The review of Bååth (1989) regarding the effects of metals on microbial populations noted, in numerous investigations, a reduction in both the abundance and biomass of fungi due to metals, often with a selection of tolerant species, but no clear effect on the number of taxa (or colony forming unit). Other effects on fungal biodiversity could be related to accumulation of metals in the fungal hyphae. However, in the present study, no correlation was demonstrated between the taxonomic parameters of fungi communities and total or extractable Pb. These contrasting results could also be induced by the influence of other factors such as vegetation or cultivation practices. Several factors have been reported that influence the whole vegetable garden (Chenot *et*

*al.*, 2012) and, perhaps, soil biodiversity.

The collembolan community interacts with soil fungi within the soil food web. The metals in soil are largely transferred to and accumulated in collembolans, which are known to feed on fungi. To maximize their fitness, collembolans are able to adjust the proportion of food material they ingest (Scheu and Folger, 2004). In addition, collembolans can discriminate between fungal mycelium grown on a metal-contaminated medium and a medium that is not metal-contaminated (Pfeffer *et al.*, 2010). Therefore, a Pb-related modification to fungal communities may, in turn, have affected collembolan communities.

In the vegetable garden, as 83% of the total collembolan abundance comprised only three species, the evenness index was low. This dominance of a few species in the collembolan community structure is often related to highly disturbed or contaminated sites (Russell and Alberti, 1998). Among the three abundant species, *P. minuta* and *P. notabilis* are widely distributed and frequently dominant in soils, including urban soils (Kuznetsova, 2003; Santorufo *et al.*, 2014a). *P. minuta* predominance may be due both to the preference of this species for manure, which is widely used for managing garden soil fertility, and its high tolerance to stress (Santorufu *et al.*, 2015). Although *F. similis* is rarely observed in Europe, it has been already noticed in garden soils. In this context, its relatively high abundance is not surprising, since the species is associated with composts (Gisin, 1943; Hopkin, 2007), which are widely present in gardens. The abundances of a dominant species and the evenness index were not,

however, related to the soil Pb concentration. The hypothesis that the selection of tolerant taxa is associated to the conservation of more sensitive species at low abundance, involving a decrease in evenness (Fountain and Hopkin, 2004b), was not proven in the present study. However, the evenness index was very low across the whole garden, which could indicate that the index was not sensitive along a gradient but only to marked differences in metal concentration. Thus, in our study, the taxonomic diversity of collembolan species was not altered by higher geogenic Pb concentrations (total and extractable). This result is in accordance with previous studies on anthropogenic metals. For example, Winkler (2014) showed that, in a forest contaminated by various metals, including Pb, collembolan species richness was almost the same in both metal-polluted and control forests. In contrast, Santorufo *et al.* (2012, 2014a) demonstrated a correlation between a decrease in species number and increased concentrations of Cu, Pb, and Zn. However, our garden soil had a lower metal concentration than those of soils from other studies conducted in urban environments on anthropogenic Pb alone (*i.e.*, higher than 200 mg kg<sup>-1</sup> of anthropogenic Pb in Santorufo *et al.*, 2012).

Moreover, even if collembolans feed mostly on fungi (Pollierer *et al.*, 2012), they can also feed on organic matter such as feces (Ponge, 1991). The high organic matter content in garden soil (Joimel *et al.*, 2016), often imported from non-contaminated sites, could provide a variety of food resources for collembolans, potentially allowing collembolan communities to avoid the negative effects of metal.

However, although there were differences between the community compositions of each sub-plot, no clear relationship between taxonomic composition and Pb or pH was observed. This shift in species distribution indicates the divergent responses of the community structure to their soil habitat.

Our results also showed an effect of geogenic Pb concentrations, but no effect of pH, on the functional structure and composition of collembolan communities.

According to the functional trait-based approach, most collembolans in the garden shared a common trait profile, *i.e.*, small-bodied (< 2 mm), cylindrical, pigmented, and with ocelli, furcula, and sexual reproduction. Santorufo *et al.* (2014a) observed that the species most tolerant to urban environmental conditions, often in multi-contaminated soil, had a small body size, jumping motion strategy, sexual reproduction, and pigmentation. Furthermore, in our results, FRic and FDiv indices decreased along the gradient of total Pb con-

centration increase, indicating a clear trend for species to share the same traits when Pb concentrations increase.

Higher pigmentation protects the organisms from UV radiation (Hopkin, 2007), whilst allowing sexual identification (Santorufu *et al.*, 2015), thereby permitting organisms to survive on the surface. These organisms also have a high mobility owing to furcula and ocelli, thereby pointing to the ability of these organisms to avoid contamination. Natal da Luz *et al.* (2004) demonstrated the use of avoidance behavior by *Folsomia candida* in highly contaminated soils. Austruy *et al.* (2016) confirmed these exclusion/avoidance mechanisms for Pb in the case of highly polluted soils or soils with low metal bioavailability. Geogenic contamination often occurs at low bioavailability, which could explain the shifts in functional profiles along the contamination gradient.

Sexual reproduction could also represent an advantage over asexual reproduction in contaminated soil. Gillet and Ponge (2003) noted that *Mesaphorura macrochaeta*, a parthenogenic collembolan, can switch to sexual reproduction in soil polluted by Zn.

In fact, the increased proportion of individuals with pigmentation, furcula, or ocelli is related to the selection of epigeic organisms (Salmon *et al.*, 2014), which are less sensitive to environmental stress (Kærsgaard *et al.*, 2004). These characteristics are more frequently observed in open habitats, such as vineyards and arable soils (Auclerc *et al.*, 2009), and could also occur in vegetable gardens. Furthermore, this selection could be due to the advantages of epidaphic collembolans *vs.* eudaphic collembolans, which more frequently live in contact with more deeply contaminated soils.

The taxonomic approach provided no indication of the degree of Pb concentration, whereas changes in morphological traits and ecological preferences were indeed observed. Moreover, it seemed that the taxonomic indices of the fungal communities were less sensitive than fungal composition. This underlines the idea put forward by Ladygina *et al.* (2008) that it is not possible to detect responses to stress using general methods (*e.g.*, determining the microbial biomass or numbers of collembolans) to determine food web components. In contrast, functional traits provide information on the community responses to Pb concentration effects, even if the concentration is moderate.

Finally, all these results highlight both a direct and perhaps an indirect effect of total geogenic Pb on soil biota. However, no relationship was reported with extractable Pb, which could be explained by its low bioavailability. Similarly, the geogenic Pb transferred

to vegetables (Jean-Soro *et al.*, 2015) was not clearly related to extractable Pb. However, it should be noted that the geogenic origin of Pb does not prevent its accumulation by vegetables or by soil biota. Indeed, the relationship between soil biota and extractable Pb or other metals is often complex. Some studies on collembolan failed to determine its relationship with extractable Pb (Santorufu *et al.*, 2014b), whereas another study demonstrated the opposite (Luo *et al.*, 2014). Beaumelle *et al.* (2014) mentioned this lack of genericity when studying the response of earthworms to moderately and multi-contaminated soils. Many other parameters could be involved and affect the relationship, in particular the divergent effects of different metals and complexity of soil textures (Beaumelle *et al.*, 2014).

There is still no universally acceptable method for assessing soil bioavailable metal concentrations (Giller *et al.*, 2009), and our results further highlight the need for reliable indicators to predict the effects of metals on soil biota, especially in case of moderate or geogenic contamination.

## CONCLUSIONS

The geogenic origin of Pb, even at moderate concentrations, does not prevent it from affecting the soil biota. These effects cause modifications to the fungal community and to the functional traits of collembolan communities. Indices based on functional structure, such as functional traits, are more sensitive than those based on taxonomic structure, when used to study the effect of Pb, even of geogenic origin. These indicators could also be used to better understand the relationship between organisms in soil food web.

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