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Are acid-tolerant collembolan communities able to colonise metal-polluted soil?

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Abstract

A short-term microcosm experiment was carried out to determine whether acid-tolerant collembolan communities are able to colonise metal-polluted soil. Polystyrene boxes were divided in two compartments by a perforated wall allowing free passage of soil fauna and preventing physical contact between soil substrates. All compartments were filled either with an acid dysmoder (pH 4.4) collected in a beech forest from Belgian Ardennes (Willerzie, Belgium) or with one of three neutral polluted soils (P1, P2, P3) collected in the Bois des Asturies, along a metal-pollution gradient downwind of a zinc smelter (Auby, France). Different combinations were established, with five replicates each, and were incubated during three weeks at 15°C, in darkness. Afterwards, Collembola were extracted and determined to the species level. It appeared that populations from the acid soil colonised the neutral soil polluted by heavy metals. Within three weeks, the number of species increased in compartments filled with the most heavily polluted soils (P2, P3) when they were in contact with the acid soil. However, colonisation was effected by only a few individuals. At the species level, the onychiurid *Protaphorura eichhorni* and the isotomid *Folsomia quadrioculata* colonised the polluted soil. For *Protaphorura eichhorni* the migration rate was highest when the soil was the least polluted (P1), while *Folsomia quadrioculata* showed a higher rate of dispersion to the medium polluted soil (P2) compared with the least polluted (P1). This behaviour could be explained by the humus form which was a mull in P1, more compact than the mor observed in P2 and P3, the physical structure of which approaches that of the

dysmoder. Our observations point out that both species are more affected by soil structure than by pollution.

Keywords: Soil fauna, Acidophily, Colonisation, Pollution, Heavy metals, Microcosms.

1. Introduction

Under acid conditions, availability and mobility of metal ions are high due to the chemical form in which they are present in the soil solution (Berggren et al., 1990; Reddy et al, 1995). At low pH heavy metals are dominated by mobile ionic forms (Zn^{2+}, Pb^{2+}) followed by soluble salts ($ZnSO_4$, $PbSO_4$) and most aluminium exists as Al^{3+} (Nair and Prenzel, 1978). The accumulation of humified organic matter which characterises acid soils (Bernier et Ponge, 1994 ; Bernier, 1996 ; Ponge et al., 1997) and the presence of high amounts of undissociated phenolic and aliphatic acids in the organic matter also increase the solubility of aluminium in the soil solution (Duchaufour, 1983). Plant secondary metabolites like phenolics play also a role in chemical interference (Ponge et al., 1998). The toxicity of tannins to soil fauna is well documented, even at low concentrations (Poinsot-Balaguer et al., 1993). Verdier (1975) and Sextone and Mains (1990) also noted in the atmosphere of acid soils an increase in carbon dioxide and toxic gases such as methane.

Several parallels can be traced between conditions prevailing in acid and polluted soils. Like acid soils, polluted soils are characterised by an accumulation of organic matter in the topsoil, but in the latter case organic matter is poorly humified (Bengtsson and Rundgren, 1988; Coughtrey et al., 1979; Gillet and Ponge, 2002). In polluted soils, the high mobility of metals can be explained by (1) an excess of metal load as it was observed near petroleum (Adeniyi and Afolabi, 2002) and smelting complexes (Denaix et al., 2002), and (2) soil acidification by nitrogen and sulphur deposition (Tomlinson, 1983). The last parallel lies in the amount of phenolics in the litter since polluted sites are often reclaimed by planting

poplar (Gillet and Ponge, 2002), a tree which is well-known for the high tannin content of its foliage (Lindroth et al., 2002).

In spite of the toxicity of acid soils to a lot of invertebrates (Ponge et al., 1997), no decrease in density and local diversity was observed in collembolan communities (Loranger et al., 2001). This observation can be attributed to physiological adaptation to acid conditions which could be inherited from Palaeozoic times (Ponge, 2000b). Indeed, to live in an acid environment, animals and plants have developed some resistance mechanisms like, for instance, cytoplasmic sites where aluminium may be harmlessly accumulated in the case of plants (Clarkson, 1969), or excreted by periodically renewing the midgut epithelium in the case of Collembola (Joosse and Buker, 1979; Hopkin, 1995). Some authors have described adaptation and resistance of collembolan communities submitted to long-term contamination by heavy-metals (Joosse and Verhoef, 1983; Posthuma et al., 1993; Tranvik et al. 1993). However, a decrease in biodiversity (Hågvar and Abrahamsen, 1990; Filser and Hölscher, 1997) and a change in feeding habits (Gillet and Ponge, 2003) have also been observed under the infuence of metal-pollution. Those observations allow us to hypothesise that acid-tolerant fauna could also be tolerant to conditions prevailing in polluted soils (Chauvat and Ponge, 2002).

This work follows a preliminary study by Chauvat and Ponge (2002) which showed erratic colonisation of lead acetate treated mull humus by acid-tolerant species like *Willemia anophthalma*, *Proisotoma minima* and *Xenylla tullbergi*. The aim of the present work was to simulate in the laboratory, under controlled light and temperature conditions, the inoculation of an acid soil, with its complete acid-tolerant fauna, into a metal-contaminated site. We asked whether fauna accustomed to the presence of free metals like iron and aluminium ions in the soil solution of acid soils was able to colonise a neutral soil polluted by heavy metals (Cd, Zn, Pb) in spite of a pH increase. To follow colonisation of polluted soils by acid-tolerant soil fauna, we selected Collembola as an abundant and diversified faunal group, present in

polluted as well as acid and neutral soils (Hågvar and Abrahamsen, 1990; Bengtsson and Rundgren, 1988).

2. Materials and methods

2.1. Microcosm experiment

In order to study attraction/repulsion behaviour rather than reproductive success of Collembola and to avoid food shortage, a short-term experiment in the laboratory was done.

The polluted soil used in our experiment was collected in June 2001 from the Bois des Asturies at Auby (Nord, France). This poplar plantation, located near to and downwind of a zinc smelter, suffers from heavy pollution by zinc, lead and cadmium. The topsoil was collected from plots P1, P2 and P3, which have been already described by Gillet and Ponge (2002). Plot P1, 490 m from the smelter, is characterised by a mull humus form and the presence of macro-invertebrates (earthworms, millipedes). Plots P2 and P3, 340 m and 235 m from the smelter, respectively, were characterised by a mor humus form (Ponge et al., 2000) with a well-developed organic horizon and a low faunal activity, with micro-invertebrates only. The total amount of zinc decreases from P3 to P1 whereas the total amounts of Pb and Cd are similar at P2 and P3 and lower at P1. On plot P1 we used the top 10 cm of the carbon-rich organo-mineral A horizon and on plots P2 and P3 we used the top 10 cm of the organic OM horizon (Ponge et al., 2000).

The acid humus was collected at the same time in a mature beech forest (*Fagus sylvatica*) at Willerzie (western Ardennes, Belgium), at 445 m altitude. This forested site, which is located 130 km from the polluted site, has been described and studied by Ponge et al. (1997) and Ponge (1999, 2000a) as Site N°16. The humus form is a dysmoder (Brêthes et

al., 1995), with an OH horizon more than 1 cm depth. We used a mixture of holorganic OF and OH horizons from the dysmoder after the OL horizon had been discarded.

The experiment was conducted in 65 mm high polystyrene boxes measuring 175x115 mm divided in two compartments by a wall pierced with 2 mm diameter holes at a rate of 400 holes per wall. The division allowed free passage by micro-invertebrates but prevented physical contact between soils in adjacent compartments. On the same day the two compartments of each box were filled with intact topsoil horizons with their original fauna, one coming from the acid beech forest and the other from one of the three polluted plots. Different combinations were established with five replicates each (Table 1). Microcosms were incubated at 15°C in darkness during three weeks. At the end of the experimental period, Collembola were extracted by the dry-funnel method (Edwards and Fletcher, 1971) and identified to the species level under a light microscope at 400x magnification using Gisin (1960), Stach (1960, 1963), Deharveng (1982), Fjellberg (1992) and Hopkin (in prep.).

2.2. Chemical analyses

Five soil cores 5 cm diameter and 10 cm depth, litter included, were collected with a core sampler in May 2002 at each of the three polluted plots (P1, P2, P3). They were air-dried at 25°C in an air-forced chamber then stored in plastic bags before analysis. Previous to extraction of heavy metals, polluted soils were sieved through a 2 mm mesh. To estimate forms of zinc which can be extracted from the soil by living organisms, also called bio-available zinc (Suter et al., 2000), 50 ml of an equal mixture of 0.1 M $Na_2EDTA.2H_2O$ and 1 M NH_4Ac were added to 5 g of soil, shaken for two hours, filtered and diluted ten times to avoid particulate metal deposition. All filtrates were kept at 4°C until analysis. Zinc was detected by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) at λ=213.856 nm according to the method by Weissenhorn et al. (1995). For analysis of exchangeable zinc 50 ml of a 1 M NH_4NO_3 solution was added to 5 g of soil, shaken for one

hour, filtered and diluted to 1/10. Then the filtrate was analysed for zinc by the above mentioned method. Zinc extracted by the EDTA-acetate method includes exchangeable zinc since in both procedures the ammonium ion displaces the metal from sorption sites (Tan, 1982). The amount of zinc bound to organic matter and metal hydroxides was determined by subtracting exchangeable zinc from EDTA-acetate zinc. The water-soluble zinc was analysed after shaking 50 ml of deionised water with 5 g of soil for one hour and filtered through 0.45μm. The water-soluble fraction was subtracted from exchangeable zinc measured as above, in order to give a more reliable estimate of zinc reversibly sorbed to mineral and organic matter.

The pH of the dysmoder was measured in water and in potassium chloride according to ISO 10390 (AFNOR, 1999). The soil was suspended in deionized H_2O and 1 M KCl (1:5 soil:water in volume) for pH H_2O and pH KCl, respectively. Each suspension was shaken for five minutes, then pH was measured in the supernatant after sedimentation for 2 h with a glass electrode.

2.3. Statistical analyses

Data were analysed by correspondence analysis, a multivariate method using the chi-square distance (Greenacre, 1984). Active variables (species, represented by the number of individuals in microcosm compartments) and passive variables (combinations, coded as 1 or 0) were projected simultaneously in a space formed by the first factorial axes (those explaining the highest global variation). The introduction of passive variables is helpful in interpreting factorial axes when they are well-correlated to these axes. Passive variables were not used in the calculation of eigenvalues, thus they did not influence the formation of factorial axes. The projection of passive variables (combinations) is a point in the vicinity of the species (active variables) which characterise this variable the best. To give the same weight to all variables (discrete as well as continuous), they were transformed into: X = (x-

m)/s + 20, where x is the original value, m the average value of the variable and s its standard deviation. The addition to each standardised variable of a constant 20 allows all values to remain positive. After such a transformation, factorial coordinates of variables can be interpreted directly in terms of their contribution to factorial axes: the further a variable is projected from the origin along a given axis the more it contributes to this axis (Loranger et al., 2001). Each active variable (species) was doubled with a conjugated X' = 40-X since some variables may have a dual nature if their absence from experimental compartments is as significant as their presence (Greenacre, 1984).

To test for the significance of correlations and observed effects, Spearman correlation coefficients and one-way analyses of variance (ANOVAs) followed by Newman-Keuls tests were used at the 0.05 level of probablility for rejection of null hypothesis. Non-parametric Mann-Whitney tests were also used for comparisons between groups.

3. Results

3.1. Chemical analyses

The dysmoder was characterised by a pH H_2O of 4.4 and a pH KCl of 3.3 (Table 2). The difference (delta pH) of 1.1, compared to 0.5 only in the polluted Auby soils, points out that the acidity linked to soil particles was more important in the acid soil than in the polluted soil. The pH H_2O (6.8) and the pH KCl (6.3) were far higher in the polluted soil than in the dysmoder, thus potential colonists from the acid soil have to struggle against a pH increase.

Zinc was by far the most abundant heavy metal in the Bois des Asturies, which explains why measurements of heavy metals were done only on this metal. The maximum amount of bio-available zinc found in our samples was 22 600 mg kg^{-1}. The amount of EDTA-acetate zinc was higher at P2 and P3 than at P1 (Table 3). Zinc linked to organic matter and

oxides was the dominant form of EDTA-acetate zinc, amounting 94% at P1, 84% at P2 and 91% at P3, followed by exchangeable zinc (5.7% at P1, 15.1% at P2 and 8.1% at P3) and a little content of water-soluble zinc (0.5% at P1, 0.6% at P2 and 0.5% at P3).

3.2. Microcosm experiment

Table 4 lists the collembolan species found at the end of the experiment, classified according to the sites (Auby and Willerzie) from which they are issued. Eleven species were common to both sites, 21 were only present in the Ardennes and 10 were only present in the Bois des Asturies. The total species richness in the compartments with polluted soils (22 species) compared well with that of the field community (21 species) as described by Gillet and Ponge (2003) on the same number of replicates.

Correspondence analysis showed that after three weeks the species composition of collembolan communities in compartments filled with polluted soils was influenced by the presence of an adjacent compartment filled with an acid soil, as ascertained by the addition of new species (Fig. 1). Axis 1 (11.9% of the total variance) was correlated with the number of individuals ($r = 0.71$; $p<0.05$). It indicated an increase in the number of individuals in compartments filled with P2 and P3, when adjacent to the acid soil. However this increase was not significant (Fig. 2). Axis 2 (10.8% of the total variance) was correlated with the number of species ($r = 0.48$; $p<0.05$). It indicated an increase in the number of species in compartments filled with P1, P2 and P3, when adjacent to the acid soil (P1/A, P2/A and P3/A). However, this addition was significant only for P2 and P3 (Fig. 3). Axis 1 was correlated with the density of species from Willerzie (acid soil, unpolluted), like *Folsomia quadrioculata* ($r = 0.377$; $p<0.05$), *Mesaphorura tenuisensillata* ($r = 0.32$; $p<0.05$), *Lipothrix lubbocki* ($r = 0.41$; $p<0.05$) and *Pseudisotoma sensibilis* ($r = 0.36$; $p<0.05$). Axis 2 was correlated with the density of Willerzie species such as *Protaphorura eichhorni* ($r = 0.59$;

p<0.05), *Folsomia quadrioculata* (r = 0.61; p<0.05), *Lipothrix lubbocki* (r = 0.32; p<0.05) and *Pseudisotoma sensibilis* (r = 0.35; p<0.05). This indicated that acid-tolerant species penetrated in P1, P2 and P3. However, this colonisation concerned only few individuals (Fig. 2).

Multivariate analysis (Fig. 1) depicted differences in the fate of collembolan communities from polluted soils when faced to an adjacent acid soil. The addition of new (acid-tolerant) species differed according to the distance to the smelter. *Protaphorura eichhorni* characterises the acid-tolerant collembolan community which colonised the least polluted soil P1 while *Folsomia quadrioculata*, *Lipothrix lubbocki*, *Pseudisotoma sensibilis* and *Schaefferia emucronata* characterised the community which colonised the most polluted soils P2 and P3.

In the acid compartment the number of species and individuals was not affected by the presence of a heavy metal polluted soil in the adjacent compartment (Figs. 2 and 3, Table 5).

At the species level, *Folsomia quadrioculata* from the Ardennes colonised compartments filled with polluted soils (Fig. 4). When in contact with the three polluted soils collected in the Bois des Asturies, it colonised P2 to a greater extent than P1. The behaviour of *Protaphorura eichhorni* was somewhat different (Fig 5). The number of animals passing to the polluted compartment decreased from P1 to P3. Other acid-tolerant species showed a significant passage from the unpolluted acid soil to the polluted soils, namely *Lipothrix lubbocki*, *Pseudisotoma sensibilis* and *Mesaphorura tenuisensillata*, while others, such as *Pseudosinella mauli* and *Willemia anophthalma* showed only a weak, insignificant, dispersal rate to the polluted compartments (Table 5).

4. Discussion

In the microcosm experiment, the absence of a negative impact of a compartment heavily contaminated with metals on the population of the adjacent acid soil could be explained by i) the absence of transfer of pollutants through the perforated plastic wall, ii) in the case some transfer occurred, most probably through animal activity, by the tolerance of acid-tolerant fauna to pollution.

Like in the study by Chauvat and Ponge (2002), who used lead acetate as a contaminant, we observed colonisation of soils heavily polluted with heavy metals by acid-tolerant Collembola. Chauvat and Ponge (2002) found that *Folsomia manolachei*, a closely related species (Deharveng, 1982) coming from a coniferous forest, colonised the polluted soil. Filser and Hölscher (1997) demonstrated that *Folsomia manolachei* and *Folsomia quadrioculata* (although insignificantly in the latter case), colonised a copper-polluted soil more than an unpolluted neutral control. In a parallel experiment, they showed a greater abundance of the strongly acidophilic species *Willemia anophthalma* in a soil directly treated by copper salts than in an untreated (and unpolluted) control. In a field study, Hågvar and Abrahamsen (1990) classified *Folsomia quadrioculata* among species the most tolerant to lead pollution, although Sjögren (1997) observed that this species was affected by the presence of heavy metals. The bulk of these observations allow to think that acid-tolerant Collembola are more tolerant than other species to life in metal-polluted soils. However, the absence of *Folsomia quadrioculata* in the most polluted site studied by Bengtsson and Rundgren (1988) and the fact that in our experiment the number of migrants found in polluted compartments was low point out that we need more knowledge on the genetic variation within species such as *Folsomia quadrioculata* sensu lato. This group of species is known to live in acid as well as in neutral soils (Ponge, 1993), thus we can speculate that it exhibits a wide variation in its adaptation to metal pollution as this has been demonstrated in *Orchesella cincta* (Posthuma et al., 1993).

Our study showed a great variability between collembolan species in their rate of colonisation of polluted soils. According to Johnson and Wellington (1983) the colonisation of a new habitat is performed by “dispersers”, which are individuals feeling obliged to disperse. They explained this behaviour and the variability in dispersion rates by the age of individuals. Younger Collembola tend to stay in the vicinity of the oviposition site, while older individuals tend to go away, spreading their eggs over a wide area. Consequently, it was probable that, at the time we collected our experimental soil, some species were represented by a majority of adults (dispersers) and some others by a majority of juveniles with a poor colonisation capability. Moreover, it exists a large between-species variation in the dispersion rate, which depends on both biological and environmental conditions (Joosse and Groen, 1970; Hertzberg, 1997).

Multivariate analysis indicated that the colonisation of the polluted soil by acid-tolerant Collembola differed for the soils taken at different distances to the smelter, and that *Protaphorura eichhorni* better colonised the less polluted site P1, while the contrary was observed in *Pseudisotoma sensibilis*, *Lipothrix lubbocki* and *Folsomia quadrioculata*. Other site factors than the direct effect of heavy metal concentration would probably explain such discrepancies. Differences in humus form correlated with the degree of pollution (Gillet and Ponge, 2002) explain the observed variation better. The humus form at P1 was a fine, carbon-rich mull more compact than the mor present at P2 and P3, the structure of which approaches the dysmoder from the beech forest. The influence of pore size distribution on the recolonisation of defaunated soils by Collembola has been demonstrated by Vannier (1975). In our experiment *Protaphorura eichhorni*, an onychiurid species, is well-adapted to life in deep soil, even when compact, compared to other congeneric species (Didden, 1987; Ponge, 1993). In contrast, species such as *Pseudisotoma sensibilis*, *Lipothrix lubbocki* and *Folsomia quadrioculata*, live in well-aerated environments such as litter and mosses (Ponge, 1993), and could thus be poorly adapted to the compact P1 fine mull. Thus, strongly acidophilic (*Protaphorura eichhorni*, *Pseudisotoma sensibilis*, *Lipothrix lubbocki*) or acid-

tolerant (*Folsomia quadrioculata*) species were all able to colonise metal-polluted soils but they also seemed affected by the physical structure of the topsoil thus by the humus form.

The influence of other soil fauna groups, like predator-prey interactions, on the dispersion of Collembola cannot be discarded in our experiments. For instance, some collembolan species could escape the acid dysmoder towards the polluted soil, because of a reduced impact of predation in an adjacent polluted compartment. However, this kind of interaction is unlikely, because another study on the same polluted site (Gillet and Ponge 2003) revealed that predators of Collembola were abundant in the three plots P1, P2 and P3.

Our study did not demonstrate any attraction of acido-tolerant or acidophilic species to the polluted soil. Species from the Belgian Ardennes were found in much lower densities in adjacent comparments filled with polluted soils (Table 5), which showed that they preferred the acid soil from which they originated. However, at the end of the three-week experiment, these species increased the species richness of the polluted soils, which more than doubled at the medium level of contamination P2 (Fig. 3). Thus the answer to the title question is yes, at least in the short-term.

Another question is whether tolerance to heavy metal toxicity and tolerance to acidity are physiologically related. We did not demonstrate such relationship, since acidophilic species such as *Protaphorura eichhorni*, *Pseudisotoma sensibilis* and *Lipothrix lubbocki* did not disperse better than ubiquitous species such as *Folsomia quadrioculata*. Some tiny, poorly motile acidophilic species such as *Mesaphorura tenuisensillata*, *M. jevanica* and *Willemia anophthalma* dispersed poorly if not at all in polluted compartments. Thus we cannot rule out that random movements of animals from the Ardennes (unpolluted acid soil) in adjacent compartments with neutral polluted soil could result in similar patterns. Other experiments, allowing separation between dispersion and tolerance to heavy metals, are therefore needed before reaching a straighforward conclusion.

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Figure legends

Fig 1. Correspondence analysis. Projection of collembolan species (three-letter codes, see Table 4) and passive variables (compartments, number of species and number of individuals) in the plane formed by the first two axes. Ardennes compartments were excluded from the analysis. Species coming definitely from the acid soil are indicated in grey.

Fig 2. Total numbers of collembolan individuals in compartments A in boxes A/Px (grey bars), Px in boxes Px/A (black bars) and in control boxes Px/Px (white bars). P represents the polluted soils (three sites) from the Bois des Asturies and A the unpolluted acid soil from the Ardennes. Significant differences within grey, black or white series are indicated by different letters (Mann-Whitney U test). Significant differences between series are indicated by stars (Mann-Whitney U test). ns = not significant.

Fig 3. Total numbers of collembolan species in microcosm compartments. Otherwise as for Fig. 2.

Fig 4. Total numbers of *Folsomia quadrioculata* in microcosm compartments. Otherwise as for Fig. 2.

Fig 5. Total numbers of *Protaphorura eichhorni* in microcosm compartments. Otherwise as for Fig. 2.

Table 1
Combinations realised in microcosms (A = acid soil, Willerzie; P1, P2, P3 = polluted soil, Auby)

N° combination	Compartment 1	Compartment 2
1	P1	P1
2	P2	P2
3	P3	P3
4	A	P1
5	A	P2
6	A	P3
7	A	A

Table 2
pH values of the topsoil from the acid beech forest and the three polluted plots. Data are means of five replicates (fifteen for average P1+P2+P3) followed by standard errors

	Ardennes	Auby (average of P1+P2+P3)	P1	P2	P3
pH H_2O	4.39 ± 0.08	6.82 ± 0.07	6.70 ± 0.21	6.88 ± 0.04	6.88 ± 0.07
pH KCl	3.29 ± 0.06	6.29 ± 0.10	5.98 ± 0.25	6.42 ± 0.06	6.46 ± 0.05
delta pH	1.10 ± 0.08	0.53± 0.04	0.72 ± 0.05	0.46 ± 0.04	0.42 ± 0.02

Data from Gillet & Ponge (2003)

Table 3
Concentration of different forms of zinc in the polluted soils (mg/kg). Data are means of five replicates followed by standard errors. Note that forms linked to organic matter and oxides, as well as exchangeable and water-soluble forms are included in EDTA-acetate zinc

	P1	P2	P3
EDTA-acetate extracted	$6200 \pm 1100_b$	$20000 \pm 1200_a$	$19000 \pm 1100_a$
linked to OM and oxides	$5800 \pm 980_b$	$16900 \pm 940_a$	$17000 \pm 1400_a$
exchangeable	$350 \pm 88_c$	$3000 \pm 270_a$	$1500 \pm 330_b$
water-soluble	$32 \pm 3.5_b$	$110 \pm 15_a$	$92 \pm 7.2_a$

1 Significant differences between sites ($P < 0.05$) are indicated by different letters (ANOVA).

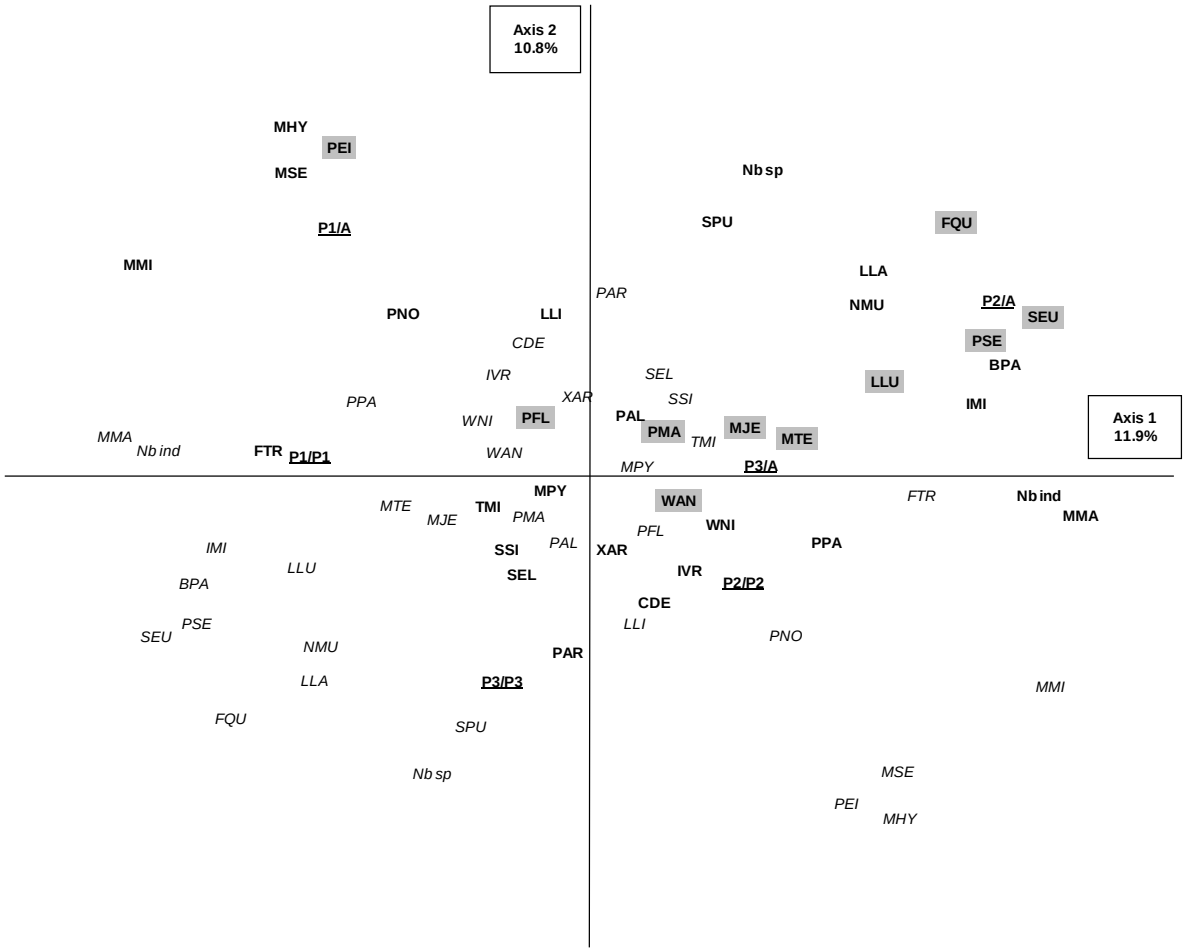
2

Table 4
List of collembolan species inhabiting the Bois des Asturies (polluted soil) and the beech forest in the Belgian Ardennes (acid soil). Data come from control microcosms (same soil in both compartments). Presence is indicated by a cross, absence by a dash

		Bois des Asturies	Ardennes
Name	**Code**	**Boxes P1/P1+ P2/P2+P3/P3**	**Boxes A/A**
Arrhopalites spinosus Rusek, 1967	ASP	-	X
Brachystomella parvula (Schäffer, 1896)	BPA	X	-
Ceratophysella denticulata (Bagnall, 1941)	CDE	X	X
Dicyrtomina minuta (O. Fabricius, 1783)	DMI	-	X
Folsomia quadrioculata (Tullberg, 1871)	FQA	-	X
Friesea truncata Cassagnau, 1958	FTR	X	X
Isotoma violacea Tullberg, 1876	IVI	-	X
Isotoma viridis Bourlet, 1839	IVR	X	-
Isotomiella minor (Schäffer, 1896)	IMI	X	X
Lepidocurtus lanuginosus (Gmelin, 1788)	LLA	X	X
Lepidocyrtus lignorum (Fabricius, 1781)	LLI	X	X
Lipothrix lubbocki (Tullberg, 1872)	LLU	-	X
Megalothorax minimus Willem, 1900	MMI	X	X
Mesaphorura jevanica Rusek, 1996	MJE	-	X
Mesaphorura leitzaensis Jordana, 1993	MLE	-	X
Mesaphorura macrochaeta Rusek, 1976	MMA	X	X
Mesaphorura tenuisensillata Rusek, 1971	MTE	-	X
Mesaphorura yosii (Rusek, 1967)	MYO	-	X
Micranurida granulata (Agrell, 1943)	MGR	-	X
Micranurida pygmaea Börner, 1901	MPY	X	X
Micranurida sensillata (Gisin, 1953)	MSE	X	-
Neanura muscorum (Templeton, 1835)	NMU	X	X
Paratullbergia callipygos (Börner, 1902)	PCA	-	X
Paratullbergia macdougalli Bagnall, 1936	PMC	-	-
Parisotoma notabilis Schäffer, 1896	PNO	X	X
Pogonognathellus flavescens (Tullberg, 1871)	PFL	-	X
Proisotoma minima (Absolon, 1901)	PMI	-	X
Protaphorura armata (Gisin, 1952)	PAR	X	-
Protaphorura eichhorni Gisin, 1954	PEI	-	X
Pseudachorutes parvulus Börner, 1901	PPA	X	-
Pseudanurophorus binoculatus Kseneman, 1934	PBI	-	X
Pseudisotoma sensibilis (Tullberg, 1876)	PSE	-	X
Pseudosinella alba (Packard, 1873)	PAL	X	X
Pseudosinella mauli Stomp, 1972	PMA	-	X
Schaefferia emucronata Absolon, 1900	SEM	-	X
Sminthurinus elegans (Fitch, 1863)	SEL	X	-
Sminthurinus lawrencei Gisin, 1963	SLA	-	X
Sminthurinus signatus (Krausbauer, 1898)	SSI	X	-
Sphaeridia pumilis (Krausbauer, 1898)	SPU	X	X
Tomocerus minor (Lubbock, 1862)	TMI	X	-
Willemia anophthalma Börner, 1901	WAN	-	X
Willemia denisi Mills, 1932	WDE	-	X
Willowsia nigromaculata (Lubbock, 1835)	WNI	X	-
Xenyllodes armatus Axelson, 1903	XAR	X	-
Total species richness		22	33

Table 5
Mean numbers of Collembola from the acid soil (Ardennes) in microcosm compartments. > or = means significant difference (P<0.05, Mann-Whitney U test) or absence of difference between means, respectively

	A in box		A in box		P1 in box		P1 in box	A in box		A in box		P2 in box		P2 in box	A in box		A in box		P3 in box		P3 in box
	A/A		A/P1		A/P1		P1/P1	A/A		A/P2		A/P2		P2/P2	A/A		A/P3		A/P3		P3/P3
Arrhopalites spinosus	0.5	=	0.4	=	0	=	0	0.5	=	1.2	=	0	=	0	0.5	=	0.8	=	0	=	0
Dicyrtomina minuta	0.2	=	0.4	=	0	=	0	0.2	=	0	=	0	=	0	0.2	=	0.2	=	0	=	0
Folsomia quadrioculata	137.9	=	176.4	>	5.6	>	0	137.9	=	198.4	>	12.6	>	0	137.9	=	151	>	13.6	>	0
Isotoma viridis	0.1	=	0	=	0	=	0	0.1	=	0	=	0	=	0	0.1	=	0	=	0	=	0
Lipothrix lubbocki	0.7	=	0.8	=	0	=	0	0.7	=	1.6	=	0.2	=	0	0.7	=	1.2	=	0.4	>	0
Mesaphorura jevanica	5.7	=	3.2	>	0	=	0	5.7	=	3.4	>	0	=	0	5.7	=	5.8	>	0.2	=	0
Mesaphorura tenuisensillata	6.4	=	5.8	>	0	=	0	6.4	=	8	>	0.4	>	0	6.4	=	5.6	>	0.2	=	0
Micranurida granulata	0.1	=	0	=	0	=	0	0.1	=	0	=	0	=	0	0.1	=	0	=	0	=	0
Paratullbergia callipygos	0.1	=	0.2	=	0	=	0	0.1	=	0.2	=	0	=	0	0.1	=	0	=	0	=	0
Pogonognathellus flavescens	1	=	1.6	=	0.2	=	0	1	=	1.2	>	0	=	0	1	=	0.4	=	0	=	0
Protaphorura eichhorni	40.6	=	49.8	>	6	>	0	40.6	=	37	>	1.8	>	0	40.6	=	41	>	0.6	>	0
Pseudanurophorus binoculatus	0.5	=	1	=	0	=	0	0.5	=	0.2	=	0	=	0	0.5	=	0.2	=	0	=	0
Pseudisotoma sensibilis	8.5	=	8.2	=	0	=	0	8.5	=	4	=	0.6	>	0	8.5	=	6.6	>	0.6	>	0
Pseudosinella mauli	1.3	=	2.2	>	0.2	=	0	1.3	=	1	=	0.8	=	0	1.3	=	1.4	>	0	=	0
Schaefferia emucronata	1.7	=	1.8	=	0	=	0	1.7	=	0.6	=	0.2	=	0	1.7	=	5.6	=	0	=	0
Sminthurinus lawrencei	0.1	=	0	=	0	=	0	0.1	=	0	=	0	=	0	0.1	=	0	=	0	=	0
Willemia anophthalma	4.5	=	3	>	0	=	0	4.5	=	2	=	0.2	=	0	4.5	=	5.4	=	0.2	=	0
Willemia denisi	0.4	=	1.2	=	0	=	0	0.4	=	1.6	>	0	=	0	0.4	=	0.2	=	0	=	0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FTR
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WAN
MPY
MTE
TMI
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SSI
PAL
LLU
SEL
BPA
PSE
SEU
NMU
PAR
LLA
P3/P3
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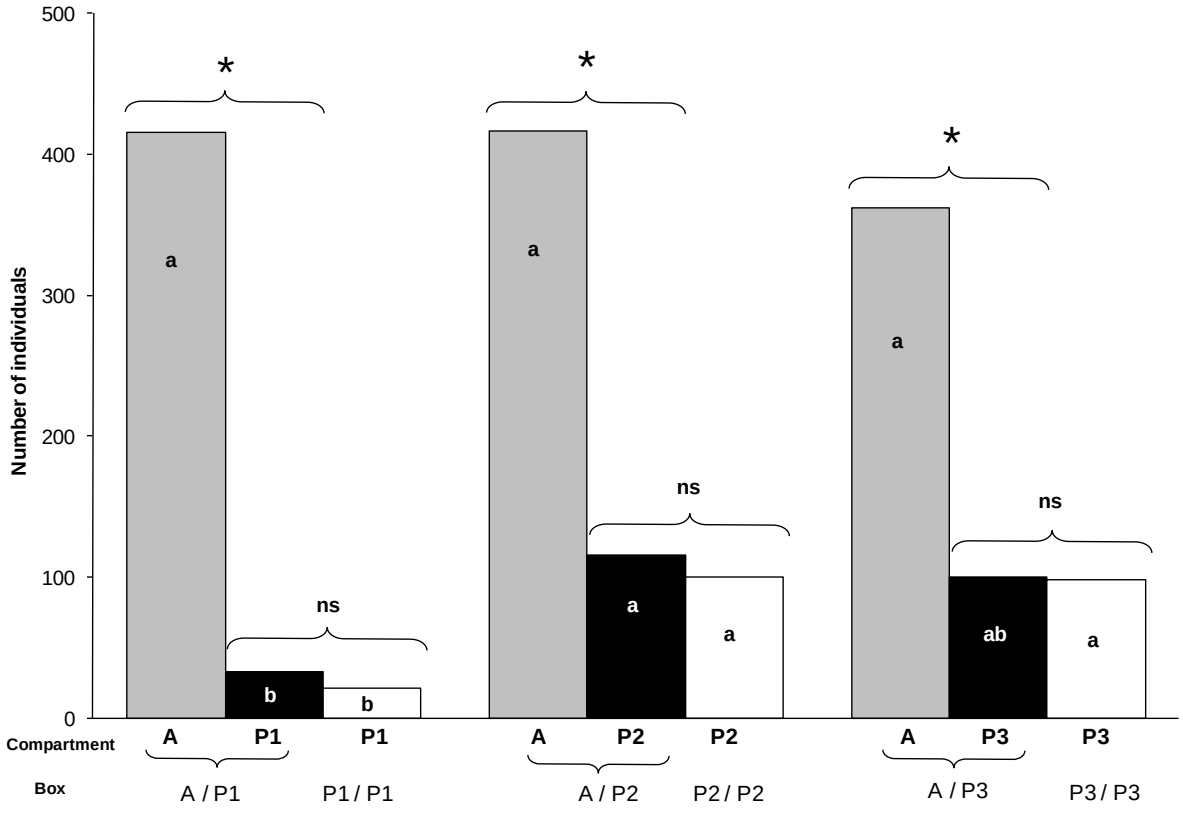
1

2 Fig. 1

3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ab
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Compartment
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P1
P1
A
P2
P2
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Box
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P2 / P2
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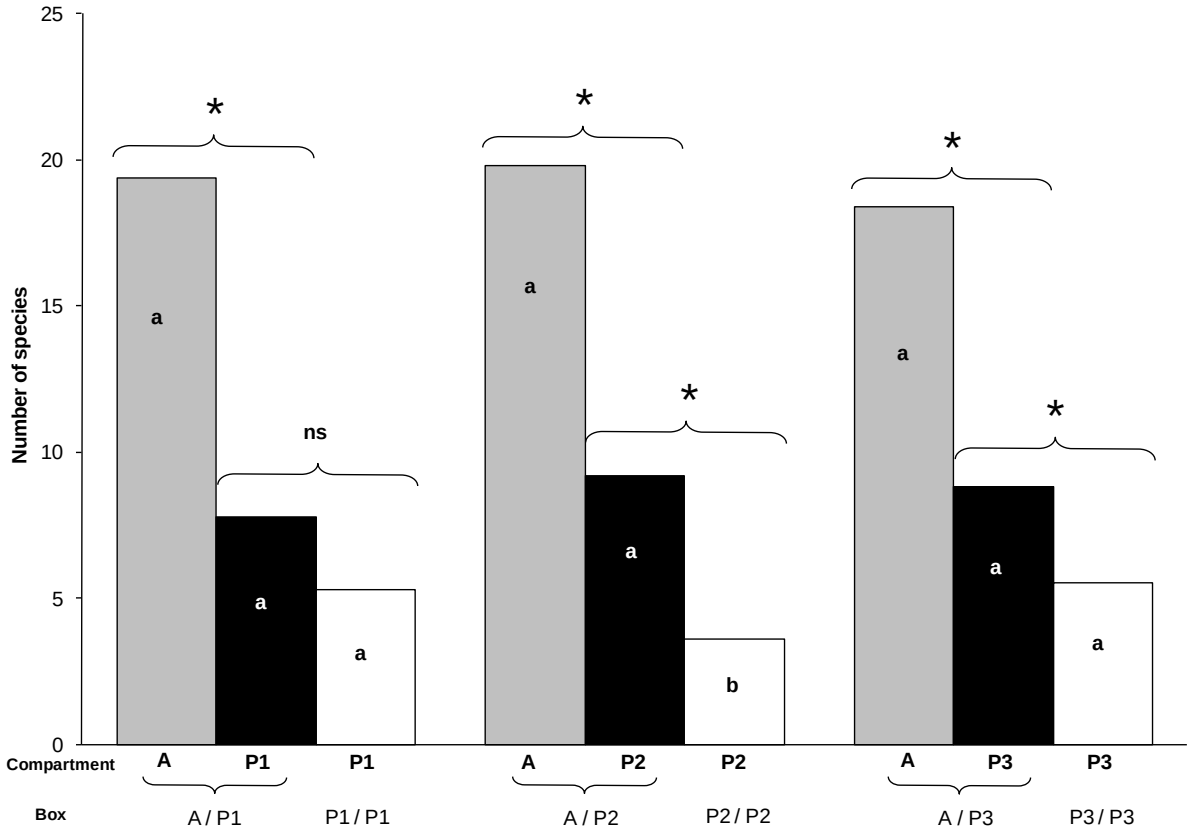
Fig. 2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a
Compartment
A
P1
P1
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P2
P2
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P3
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Box
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P1 / P1
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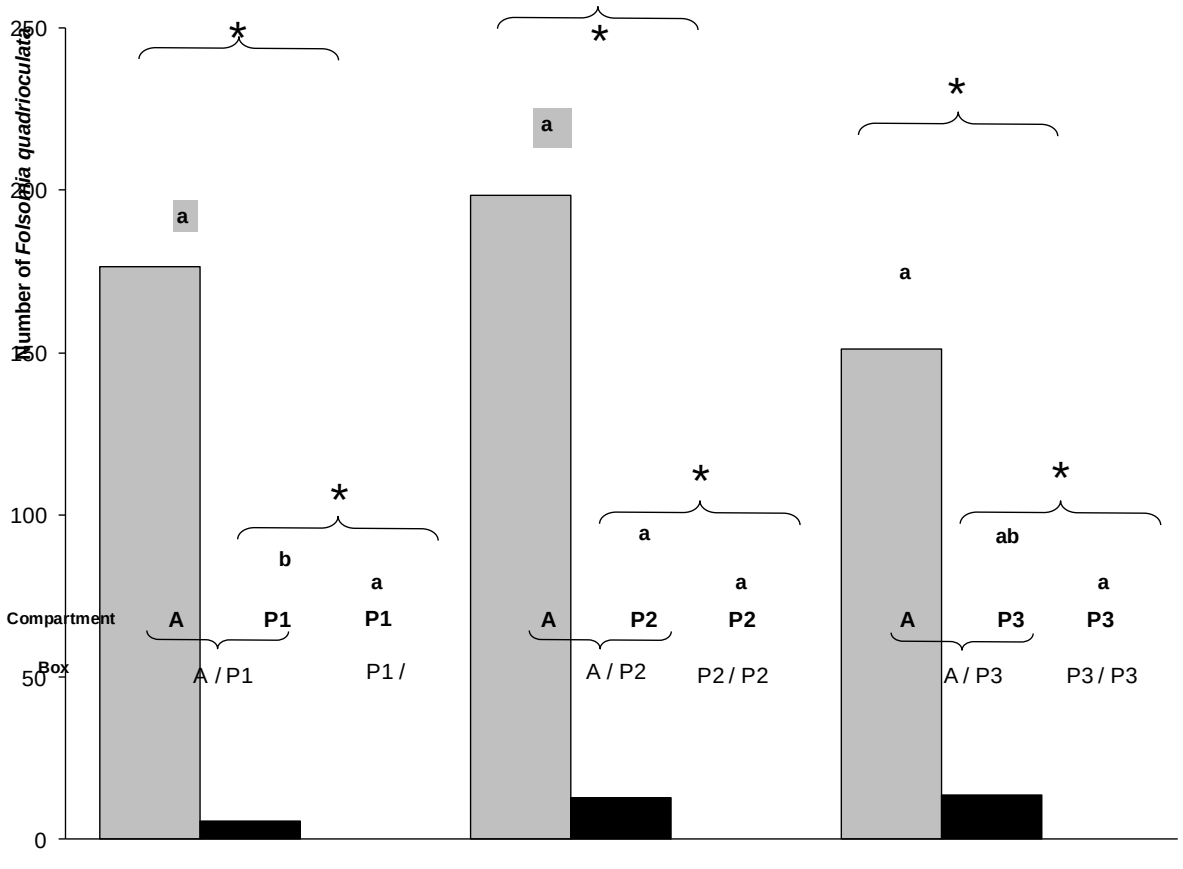
A / P3
P3 / P3

Fig. 3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Compartment
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Box
A / P1
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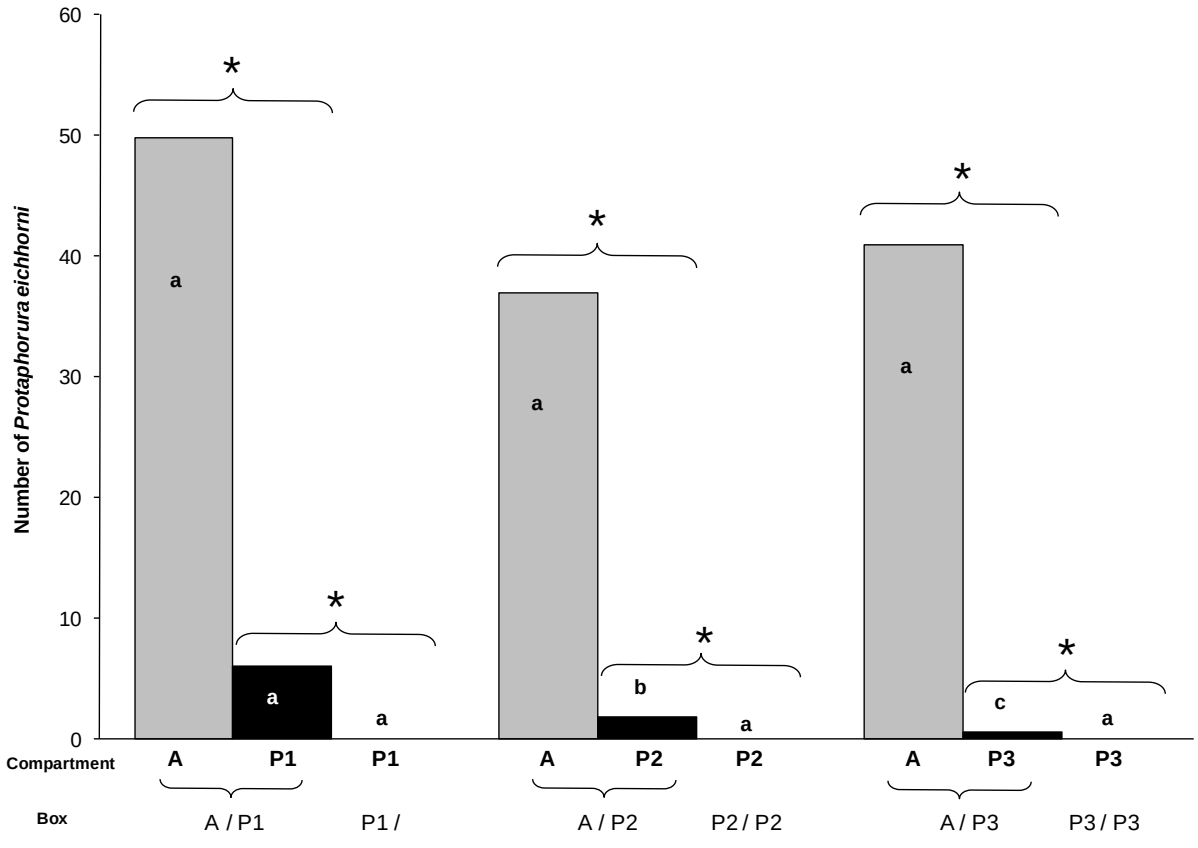
A / P3
P3 / P3

Fig. 4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Compartment
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P1
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P2
P2
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Fig. 5