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Effects of sewage sludge and copper enrichment on both soil mesofauna community and decomposition of oak leaves (*Quercus suber*) in a mesocosm

Abstract A laboratory mesocosm experiment was performed to study the effects of copper-enriched sewage sludge application on a mesofauna community. For 12 weeks, characteristics and changes in this defined and artificial mesofauna community structure were monitored as well as the dynamics of leaf litter decomposition. The mesofauna community comprised six species of Collembola (*Folsomia*

fimataria, *Isotomurus prasinus*, *Lepidocyrtus cyaneus*, *Mesaphorura macrochaeta*, *Parisotoma notabilis*, *Protaphorura armata*), two species of acari Oribatida (*Achipteria coleoptrata*, *Adoristes* sp.), one species of acari Gamasida (*Hypoaspis aculeifer*) and one species of enchytraeid (*Enchytraeus crypticus*). Three treatments included the addition of 22 g dry weight (DW) sludge spiked with 0, 200 and 1,000 mg Cu kg⁻¹ DW sludge in each mesocosm, and one treatment had 66 g DW sludge spiked with 1,000 mg Cu kg⁻¹ DW sludge added in each mesocosm. Copper, complexed with sludge due to a favourable pH, had no effect on community and litter parameters when added to low amount of sludge. In contrast, tripling the sludge dose in addition to a high dose of Cu changed in time the sludge and leaf chemical composition as well as mesofauna community structure. Responses of the mesofauna to this treatment differed between species. The abundance of species such as *I. prasinus*, *L. cyaneus*, *M. macrochaeta* and *P. notabilis* decreased, whereas the abundance of *H. aculeifer* increased and became dominant.

This work was carried out at the Department of Terrestrial Ecology, National Environmental Research Institute, Vejlssøvej 25, P.O. Box 314, 8600 Silkeborg, Denmark.

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Introduction

It is well known that the increased nutrient levels accompanying sludge application favour invertebrates, increasing the abundance of Collembola (Lübben 1989; Pimentel and Warneke 1989), carabids, earthworms, soil nematodes and soil mites. However, the positive effect of recycling nutrients may be counteracted by the risk of contamination by its pollutant content, especially heavy metals (Mitchell et al. 1978). These latter are particularly problematic as they are persistent and tend to accumulate in the upper layer of the soil (Bruce et al. 1997; 1999 Berti and Jacobs 1998). They can reach levels that are toxic to invertebrates and thus may disturb the soil ecosystem (Denneman and van Straalen 1991). Copper is a trace element that is an essential element for invertebrates and

plants. It is one of the most widely studied metals and is known to be toxic to soil fauna if biologically available (Streit 1984). Copper effects have been extensively studied in recent years by monitoring responses of soil microarthropod populations (Bruus Pedersen et al. 1999) or soil nematode populations (Korthals et al. 1996a,b) to a contaminated soil exposure on field. Moreover, many laboratory studies have also been performed (Filser and Hölscher 1997; Sandifer and Hopkin 1997; Filser et al. 2000).

Many of the laboratory methods used to assess the toxicity of chemicals on soil fauna in terrestrial ecosystems are single-species tests. These tests use mainly Collembola species such as *Folsomia candida*, *Folsomia fimetaria* or *Protaphorura armata* (Bengtsson et al. 1985; Bruus Pedersen et al. 2000; Scott-Fordsmann et al. 2000; Fountain and Hopkin 2001), oribatid mites, *Platynothrus peltifer* (Denneman and van Straalen 1991) or Oligochaeta such as the earthworm *Eisenia fetida* and enchytraeids *Enchytraeus crypticus* (Streit 1984; Posthuma et al. 1997; Achazi et al. 1999; Achazi 2002) contaminated through soil, water or food exposure. Some of these tests have been internationally standardized. However, according to Parmelee et al. (1997), most are not adequate to assess the effects of chemicals on exceedingly complex soil decomposer food webs. They are not realistic and the results are difficult to extrapolate to field conditions. Moreover, many species used in these tests are not common in soils (Bogomolov et al. 1996). Thus, other authors have performed tests based on a multi-species approach, monitoring microbial, earthworm, nematode and microarthropod communities exposed to a contaminated soil (Parmelee et al. 1993, 1997; Bogomolov et al. 1996; Filser and Hölscher 1997; Parmelee et al. 1993, 1997; Filser et al. 2000). These mesocosms allow us to perform experiments difficult to carry out in the field because of practical, technical and logistical reasons or legislation. They represent a terrestrial model ecosystem where several ecological factors can be controlled such as light, temperature, moisture, species diversity and abundance of the community introduced. This approach allows effects of various disturbances to be monitored on a large range of trophic and functional groups and to link them to ecosystem process changes such as nutrient dynamics or organic matter decomposition (Bogomolov et al. 1996; Filser and Krogh 2002; Cortet et al. 2003).

The objectives of this study were to test the effects of sludge amendment and Cu addition on the dynamics of oak leaf litter decomposition and on an artificial mesofauna community structure. Although this community was completely artificial, our purpose was to form a typical soil community with species living in various habitats and having different life strategies. We tested three treatments that included addition of 22 g dry weight (DW) sludge spiked with 0, 200 and 1,000 mg Cu kg⁻¹ DW sludge in each mesocosm and one treatment with 66 g DW sludge spiked with 1,000 mg Cu kg⁻¹ DW sludge added in each mesocosm.

Materials and methods

Experimental design

Each mesocosm container consisted of a 20-cm diameter × 10-cm high plastic cylinder, which was filled with 1.1 kg of soil collected from Les Issambres (Massif des Maures, Var, France). The sandy soil had the following characteristics: pH 5.4, coarse sand 50%, fine sand 17%, silt 16%, clay 11%, organic matter 6%. It was defaunated by alternately freezing (−15°C) and thawing (20°C), following sieving through a 2-mm sieve. Before the experiment initiation, the soil moisture was determined, water was added to 25.0% (±1.0%) and 4.5 g DW of air-dried cork oak leaves, *Quercus suber*, was spread uniformly on top of the soil to obtain a spatial distribution similar to the field. A mesh of 5 mm was placed between soil and litter in order to separate more easily these two compartments. The leaves, 1 year of age, were collected on the ground on Les Issambres, Southern France, in December 2001. Then, lumps of sludge (0.5–1 cm) were spread uniformly on top of the leaves to obtain a spatial distribution similar to the field. The sludge (pH 9.2) used in this experiment was subjected to a physico-chemical treatment (flocculation, coagulation and decantation) and stabilized by liming. It came directly from the wastewater treatment plant of the town Les Issambres and had an initial concentration of 426.5 mg Cu kg⁻¹ (average of two independent measurements, 383 and 470 mg Cu kg⁻¹) and a dry matter content of 38.6% (105°C, 24 h). The sludge was stored at 5°C, before being spiked with copper as a CuCl₂, 2 H₂O aqueous solution (0.04 mol l⁻¹) and mixed to obtain homogeneity. To improve the contact between sludge and leaves, water (5 g) was sprayed on top of each container before animals were added. After the addition of animals, the mesocosms were closed with a plastic film with holes for ventilation, weighed and incubated at 20°C with a 12-h:12-h light/dark photoperiod. The water content of the mesocosms was checked at weekly intervals, and evaporated water was replenished by spraying water on the surface. For practical reasons, the mesocosms were initiated weekly in five series, each series corresponding to a block including all the treatments, for a total of 80 samples.

The studied treatments were: S1C0, untreated sludge, where S1 is 22 g fresh weight (FW)/mesocosm and C0 is 0 mg Cu kg⁻¹ DW sludge; S1C1, where C1 is 200 mg Cu kg⁻¹ DW sludge; S1C2, where C2 is 1,000 mg Cu kg⁻¹ DW sludge; S3C2, where S3 is 66 g FW/mesocosm and C2 is 1,000 mg Cu kg⁻¹ DW sludge.

A mesofauna community was added to each mesocosm (Table 1) the day after the mesocosm preparation. Only the predator *Hypoaspis aculeifer* was added 1 week after the other species in order to allow them to reproduce. *P. armata*, *F. fimetaria*, *Mesaphorura macrochaeta*, *E. crypticus* and *H. aculeifer* were collected directly from existing permanent laboratory cultures. *Parisotoma notabilis*, *Isotomurus prasinus* and *Lepidocyrtus cyaneus* and oribatids were extracted

Table 1 Characteristics of the mesofauna community added to each mesocosm

| Name | Initial animal number/ mesocosm | Size (mm) | Distribution |
|--------------------------------|------------------------------------|-----------|----------------|
| Collembola | | | |
| <i>Folsomia fimetaria</i> | 20 | 1.4 | Euedaphic |
| <i>Isotomurus prasinus</i> | 10 | 4.0 | Epedaphic |
| <i>Lepidocyrtus cyaneus</i> | 20 | 105 | Epedaphic |
| <i>Mesaphorura macrochaeta</i> | 20 | 0.6 | Euedaphic |
| <i>Parisotoma notabilis</i> | 20 | 1.0 | Edaphic |
| <i>Protaphorura armata</i> | 20 | 1.8 | Euedaphic |
| Acari Gamasida | | | |
| <i>Hypoaspis aculeifer</i> | 20 | 0.5–0.8 | Euedaphic |
| Oribatida | | | |
| <i>Achipteria coleoptrata</i> | 10 | | Epedaphic |
| <i>Adoristes</i> sp. | 10 | | Epedaphic |
| Enchytraeid | | | |
| <i>Enchytraeus crypticus</i> | 20 | 7.1 | Hemi-euedaphic |

from forest and grass litter under light for 2 days. The natural variability between replicates was removed by adding the same number of individuals per species to each replicate.

Analyses

For each of the five series, four containers, one for each treatment, were sampled after 3, 6, 9 and 12 weeks. Both mesofauna and enchytraeids were extracted from soil and from litter (leaves and sludge separately). Collembola and acari were extracted from the litter (taking half of the area of the litter) and from a soil sample (130 g) using a high-gradient extractor for 8 days. Afterwards, they were conserved in glycerol and counted under a binocular microscope. Enchytraeids were extracted from the other half of the litter and from a soil sample (70 g) using a modified O'Connor wet extraction method (O'Connor 1962). Enchytraeids collected were conserved in water in the fridge for a night before being counted under binocular microscope.

After the mesofauna extraction, the remaining leaves were collected, cleaned to remove as much sludge and soil as possible, dried (60°C, 24 h) and milled with a Cyclotec 1093 with a mesh of 1 mm. All the samples were then scanned with a spectrophotometer (NIRS system 6500) as described by Joffre et al. (1992) and Cortet et al. (2003).

Chemical composition of litter was predicted using equation calibrations previously determined with a spectral and chemical data set of litter of Mediterranean species including *Q. suber* (Joffre et al. 1992; Gillon et al. 1999). The following chemical compounds (ash, N, soluble and insoluble phenols, lignin, cellulose and hemicelluloses) were chemically analysed and corresponding near infrared reflectance spectroscopy (NIRS) calibration equation built. Ash concentration was determined after treatment at 550°C

for 3 h. Nitrogen was determined with a Perkin Elmer elemental analyser (PE 2400 CHN) and C fraction (lignin, cellulose, hemicelluloses) by the Fibertec procedure (Goering and Van Soest 1970). Total phenolic and polyphenolic compounds were extracted according to Anderson and Ingram (1993) by heating 0.5 g litter in 50 ml methanol/water (1:1) at 80°C for 1 h. Soluble phenolic compounds were extracted by mixing 1 g foliage in 60 ml cold water for 2 h. Concentrations of phenolic and polyphenolic compounds in extracts were measured using the Folin–Ciocalteu reagent (Kloster 1974), which reacts with hydroxylated aromatic compounds. The absorption due to the product of the reaction was measured with a spectrophotometer at 750 nm, with tyrosine being used as reference. Results were expressed as equivalent tyrosine per litre. The C/N ratio was calculated on C estimated content admitting that organic matter represents about 50% of plant dry matter after deducting the ash weight. Calibration equations between spectral and chemical data were conducted using the ISI software system (Shenk and Westerhaus 1991).

After the mesofauna extraction, the remaining sludge was collected, dried and milled (Cyclotec 1093 with a mesh of 1 mm) before being analysed by the NIRS. As for leaves, this method allowed us to obtain and to monitor spectral information corresponding to the biochemical composition of each sludge sample.

Copper was extracted according to Tessier et al. (1979). Milled sludge (1 g) was shaken with 10 ml of bi-distilled water for 2 h, and then the mixture was centrifuged at 13,000×g for 10 min. The extracts were filtered through a cellulose acetate filter (porosity 0.2 µm) and analysed by an inductively coupled plasma optic emission spectrometry (ICP-OES; JobinYvon, Ultima C).

The soil pH(H₂O) was measured (pH-meter Metrohm E350B, Herisau, Switzerland) after shaking (2 h) 25 g fresh thawed soil in 50 ml demineralised water (Table 2).

Data analysis

Abundances were calculated and expressed in number of individuals per square metre for each treatment and for each sampling date. The mean number of species (species richness) and Shannon's diversity index were also calculated for each treatment and each sampling date.

The ash-free litter mass remaining and the N, cellulose, hemicelluloses, lignin and phenols contents were calculated for each mesocosm. To compare treatment effects on individual species, species richness and diversity, an ANOVA including the four treatments was performed with dates and treatments as factors and using log (x+1) transformed data, except for Shannon's diversity index. The same analysis was also performed to compare treatment effects on each leaf parameter (N, cellulose, hemicelluloses, lignin and phenols content). A Scheffé test was performed for the post hoc comparison of means.

At each sampling date, the non-parametric Kruskal–Wallis test was performed to compare the four treatments

Table 2 Mean species richness and Shannon's diversity index for each treatment and each sampling date (\pm SE)

| | Week | S1C0 | S1C1 | S1C2 | S3C2 |
|------------------|------|-------------------|--------------------|---------------------|-------------------|
| Species richness | 0 | 10 | 10 | 10 | 10 |
| | 3 | 9.0 \pm 0.0 a | 8.4 \pm 0.9 a | 8.4 \pm 1.1 a | 8.0 \pm 1.6 a |
| | 6 | 8.2 \pm 0.8 a | 7.8 \pm 0.8 ab | 7.4 \pm 0.9 ab | 6.6 \pm 1.1 b |
| | 9 | 7.2 \pm 0.4 a | 7.0 \pm 0.7 a | 7.8 \pm 0.4 a | 5.0 \pm 1.6 b |
| | 12 | 7.4 \pm 0.5 a | 6.4 \pm 1.1 a | 7.4 \pm 0.5 a | 5.4 \pm 1.1 b |
| Diversity | 0 | 3.26 | 3.26 | 3.26 | 3.26 |
| | 3 | 2.52 \pm 0.12 a | 2.08 \pm 0.40 ab | 2.36 \pm 0.22 ab | 2.11 \pm 0.22 b |
| | 6 | 2.47 \pm 0.22 a | 2.35 \pm 0.20 a | 2.35 \pm 0.19 a | 1.30 \pm 0.13 b |
| | 9 | 2.12 \pm 0.40 a | 2.01 \pm 0.19 a | 2.20 \pm 0.38 a | 1.14 \pm 0.61 b |
| | 12 | 1.99 \pm 0.17 a | 1.49 \pm 0.39 c | 1.60 \pm 0.41 abc | 1.52 \pm 0.31 b |

Within a sampling date, means (five replicates) followed by a common letter do not differ significantly according to the Kruskal-Wallis test at $p < 0.05$, where S1C0: S1 is 22 g DW sludge/mesocosm and C0 is 0 mg kg⁻¹ DW sludge; S1C1: S1 is 22 g DW sludge/mesocosm and C2 is 200 mg kg⁻¹ DW sludge; S1C2: S1 is 22 g DW sludge/mesocosm and C2 is 1,000 mg kg⁻¹ DW sludge; S3C2: S3 is 66 g DW sludge/mesocosm and C2 is 1,000 mg kg⁻¹ DW sludge

(Statsoft 1998), as the data did not fulfil the requirements for parametric testing.

The effects of copper and sludge dose on both mesofauna community and leaf chemical composition were analysed by the principal response curves method (Van den Brink 1999) or PRC using CANOCO software (Ter Braak and Smilauer 2002). The PRC method is a multivariate method specially designed by Van den Brink and Ter Braak (Van den Brink and Ter Braak 1998) for the analysis of data from microcosm or mesocosm experiments and to show optimally the major changes in treatment effects over time. The method, used by several authors in ecotoxicology (Cuppen et al. 2000; Leonard et al. 2000; Van den Brink et al. 2000), zooms in at differences in species or chemical composition between treatments and control at each particular time point. PRC results in a diagram showing the sampling weeks on the *x*-axis and the first principal

component of the treatment effects on the *y*-axis. This yields a diagram showing the deviations in time of the treatments compared to the control (S1C0 in this study). The species and chemical component weights can be interpreted as the weight of each species or component within the response given in the diagram. The statistical significance of treatment effects at the community level was also tested using Monte Carlo permutation tests (Ter Braak and Smilauer 2002) with 499 permutations. PRC analysis was performed between the four treatments.

A principal components analysis (PCA) was performed to analyse the spectral information from sludge given by the NIRS analysis (CANOCO). For each treatment, we averaged the absorbance corresponding to each wavelength. For each date and each treatment, an average spectrum was calculated and was used as additional variables.

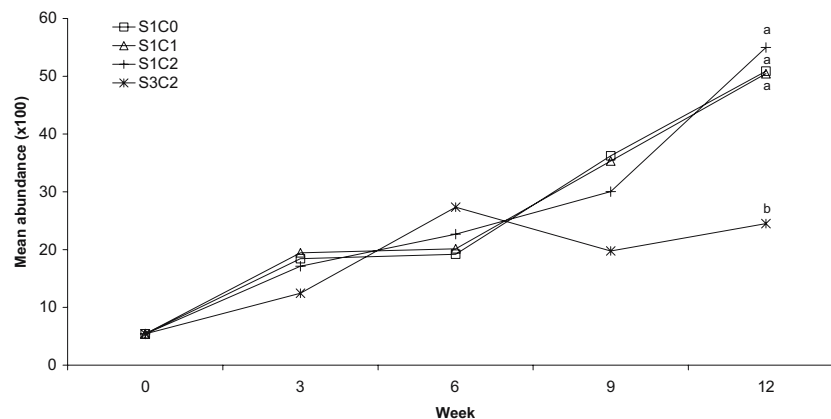


Fig. 1 Mean abundance per square metre of the mesofauna community with time (80 samples). Within a sampling date, significant differences between means (five replicates) are showed by a letter. Means followed by a common letter do not differ significantly according to the Kruskal-Wallis test at $p < 0.05$. S1C0

S1 is 22 g DW sludge/mesocosm and C0 is 0 mg kg⁻¹ DW sludge; S1C1 S1 is 22 g DW sludge/mesocosm and C2 is 200 mg kg⁻¹ DW sludge; S1C2 S1 is 22 g DW sludge/mesocosm and C2 is 1,000 mg kg⁻¹ DW sludge; S3C2: S3 is 66 g DW sludge/mesocosm and C2 is 1,000 mg kg⁻¹ DW sludge

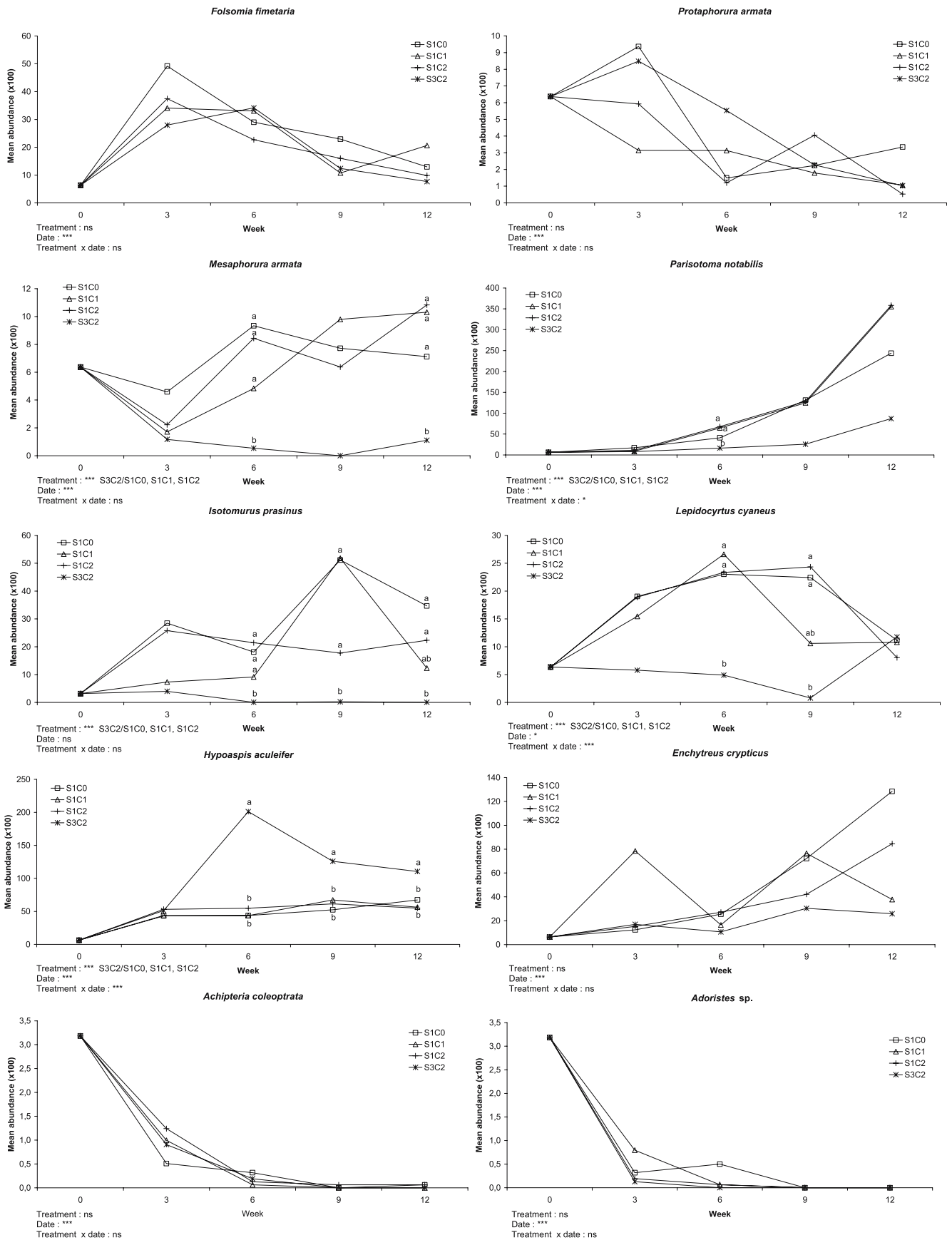


Fig. 2 Mean abundance per square metre of each species (80 samples). Within a sampling date, significant differences between means (five replicates) are shown by a letter. Means followed by a common

letter do not differ significantly according to the Kruskal–Wallis test at $p < 0.05$. For treatment labels, see legend of Fig. 1

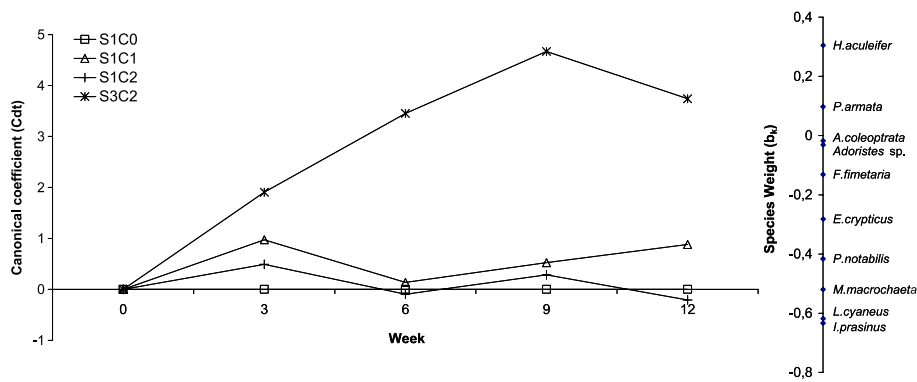


Fig. 3 Principal response curve diagram resulting from the analysis of the mesofauna data set, indicating the effect the different treatments on the mesofauna community (80 samples). Of all variance, 33.5% could be attributed to sampling date; this is displayed on the *horizontal axis*. Twenty-two per cent of all variance

could be attributed to treatment. Of this variance, 80.3% is displayed on the vertical axis. The *lines* represent the course of the treatment levels in time. The species weight (b_k) can be interpreted as the affinity of the taxon with the PRCs. For treatment labels, see legend of Fig. 1

Results

Soil characteristics

Soil pH(H₂O) in S3C2 decreased significantly ($p < 0.001$) from 5.57 to 5.28 after 6 weeks, whereas differences in the other treatments were not significant, from 5.85, 5.72, 5.60 to 5.59, 5.51 and 5.51 in S1C0, S1C1 and S1C2 treatments, respectively.

Effects of treatments on mesofauna

Mean abundance of the mesofauna community increased regularly over the 12 weeks of incubation whatever the treatment applied (Fig. 1). This increase was slower in S3C2.

The reactions of the mesofauna to the different treatments differed between species (Fig. 2). For certain species, neither the dose of sludge nor the dose of Cu added to sludge had significant effect, and the same response patterns were measured to the four treatments. Thus, *F. fimetaria*, except for the first 3 weeks, *P. armata* and oribatids decreased with time when *E. crypticus* bred successfully whatever the treatment applied. Other species were significantly affected by the dose of sludge applied. The abundance of *I. prasinus* ($p < 0.001$), *L. cyaneus* ($p < 0.001$) and *M. macrochaeta* ($p < 0.001$) decreased in

S3C2 compared to S1C2. S3C2 also slowed down significantly the increase in *P. notabilis* ($p < 0.001$). Only the predatory mites *H. aculeifer* seemed to find suitable life conditions in S3C2 ($p = 0.003$).

The PRC analysis performed between the four treatments (Fig. 3) showed a large deviation between the curves representing the dynamics of the communities submitted to the S3C2 treatment and those of the communities submitted to S1C0, S1C1 and S1C2 treatments. The Monte Carlo test showed a significant difference ($p < 0.01$) over the 12 weeks of experiment. Species weight confirmed that four species were particularly sensitive to treatments: *I. prasinus*, *M. macrochaeta* and *L. cyaneus*, which had negative scores, decreased in abundance over

Table 3 Mean water extractable Cu (mg kg⁻¹ sludge) from sludge (±SE)

| Week | S1C0 | S1C1 | S1C2 | S3C2 |
|------|-------------|--------------|---------------|--------------|
| 3 | 3.86±1.21 b | 3.53±0.79 b | 8.79±1.89 a | 11.66±3.03 a |
| 6 | 3.20±0.51 c | 6.07±0.93 b | 14.69±2.21 a | 15.00±1.00 a |
| 9 | 4.03±0.32 b | 10.40±3.32 a | 15.28±4.35 ab | 22.68±1.47 a |
| 12 | 2.98±0.29 b | 4.29±0.92 b | 22.29±0.69 a | 24.22±2.08 a |

Within a sampling date, means (five replicates) followed by the same letter are not significantly different according to the Kruskal-Wallis test at $p < 0.05$. For treatments, see Table 2

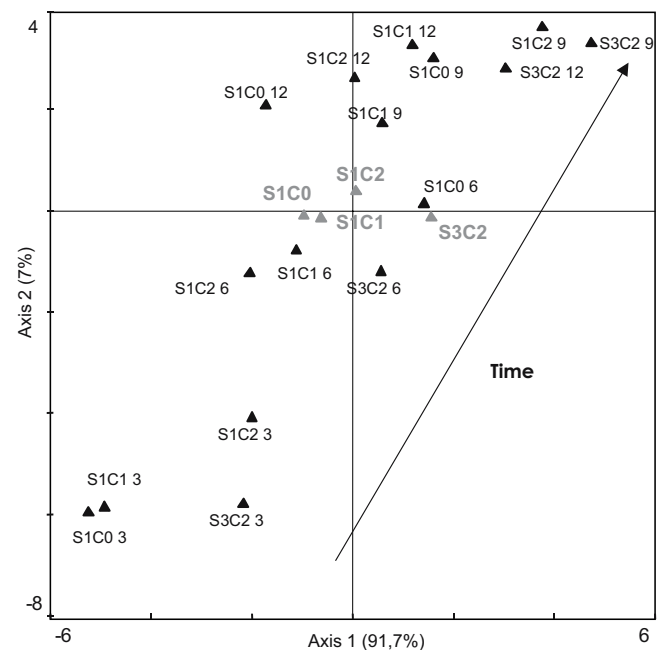


Fig. 4 PCA diagram (axis 1×2) of the analysis of the sludge spectral data. *Number* following the treatment name correspond to the sampling date. For treatment labels, see legend of Fig. 1

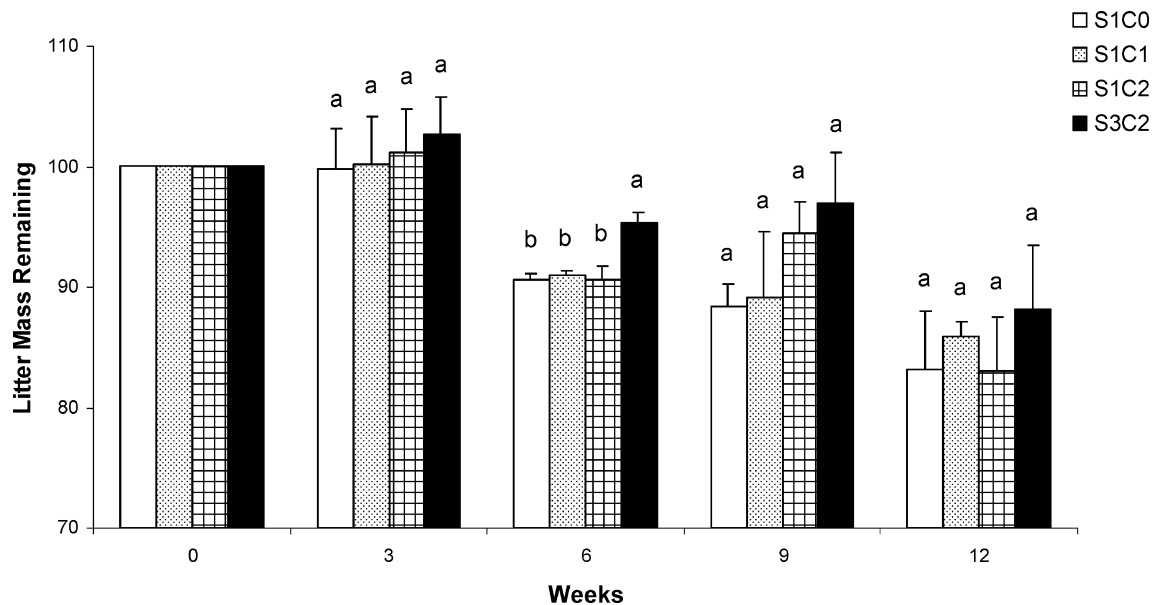


Fig. 5 Mean residual litter mass at each sampling date and for each treatment and standard errors (80 samples). Within a sampling date, means (five replicates) followed by the same letter are not

significantly different according to the Kruskal-Wallis test at $p < 0.05$. For treatment labels, see legend of Fig. 1

time when they were submitted to S3C2, and *H. aculeifer*, which had a positive score, increased in abundance and became dominant. The proportion of *H. aculeifer* increased from 11.8 to a maximum of 73.5% after 6 weeks and then decreased to 45.0% after 12 weeks.

The species richness decreased with time for all the treatments (Table 2), but differences were significant only in the S3C2 treatment ($p < 0.001$). Thus, after 12 weeks, the species number living in the mesocosms submitted to S3C2 treatment was twice lower than the initial richness. The species diversity followed the same pattern than the species richness and decreased progressively for all the treatments over the experiment, but only the application of S3C2 induced a significant decrease ($p < 0.001$) compared to S1C2.

Effects of treatments on copper extractable from sludge

Copper concentration extracted from sludge increased with time in S1C1, S1C2 and S3C2 (Table 3), and it was proportional to the Cu dose initially added to sludge. Only 1.0 to 2.3% for S1C2 and S3C2 (8.79 to 24.22 5 mg Cu kg⁻¹ sludge DW) and 0.35 to 1.00% for S1C1 (3.53 to 10.40 mg Cu kg⁻¹ sludge DW) of Cu dose spiked to sludge were soluble in water and, thus, likely to be taken up by mesofauna and microorganisms growing in mesocosms. This concentration was almost stable in the S1C0 treatment, varying from 2.98 to 4.03 mg Cu kg⁻¹ sludge DW.

The PCA performed with the spectral information data from sludge (Fig. 4) showed that the chemical composition

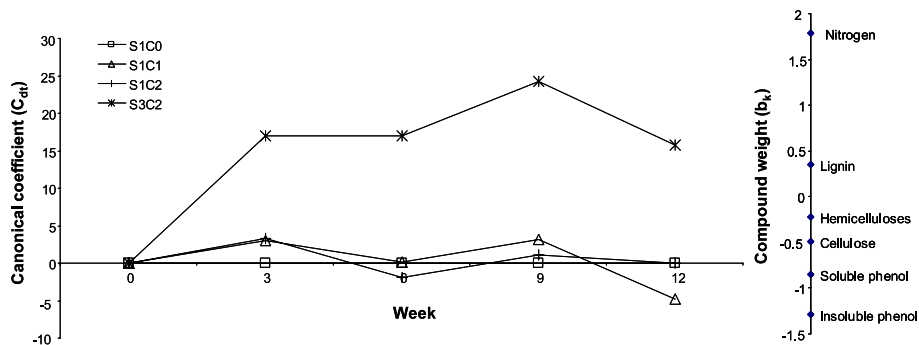


Fig. 6 Principal response curve diagram resulting from the analysis of the leaf composition data set, indicating the effect the sludge doses had on the leaf composition (80 samples). Of all variance, 75.6% could be attributed to sampling date displayed on the horizontal axis, and 8.6% of all variance could be attributed to

treatment; 90.5% of this variance is displayed on the vertical axis. The lines represent the course of the treatment levels in time. The species weight (b_k) can be interpreted as the affinity of the taxon with the PRCs. For treatment labels, see legend of Fig. 1

of sludge changed significantly ($p<0.001$) with time. The chemical composition of sludge also tended to change significantly according to the sludge dose applied ($p<0.06$).

Treatment effects on leaf litter chemical composition

Residual ash-free litter mass (litter mass minus ash mass) decreased with time for all treatments (Fig. 5). The maximum dry matter loss of cork oak leaves was reached after 12 weeks and corresponded to 17% of DW loss in the S1C0 and S1C2 treatments; the minimum loss mass was observed in S3C2 (12% loss). However, only a slight significant effect of treatments occurred after 6 weeks when tripling the sludge dose.

Tripling the dose of sludge resulted in a significant difference ($p<0.01$) of the chemical composition of leaves with particularly a greater N content in the S3C2 than that in the S1C2 (Fig. 6). The leaf N content increased with time for all treatments (Table 4), but the only significant effect occurred in the S3C2 treatment ($p<0.01$). The C/N ratio was high and decreased with time. ANOVA indicated that only the sludge dose had a significant effect on C/N dynamics ($p<0.01$). Cellulose and hemicellulose contents decreased with time for all treatments (Table 4), and the decrease rate was significantly affected by the sludge dose ($p<0.05$). Lignin content in litter increased with time for all treatments, with only a sludge dose effect appearing after 12 weeks (Table 4). Phenol content dropped greatly during the first 3 weeks. Values of soluble phenol content

Table 4 Main characteristics (means±SE) of leaf litter for each treatment

| | Week | S1C0 | S1C1 | S1C2 | S3C2 |
|----------------------|------|-------------------------|--------------------|--------------------|--------------------|
| Nitrogen (%) | 0 | 0.88^a | 0.88 | 0.88 | 0.88 |
| | 3 | 1.33±0.06 a | 1.37±0.06 a | 1.37±0.05 a | 1.47±0.06 a |
| | 6 | 1.48±0.03 a | 1.45±0.11 a | 1.50±0.12 a | 1.64±0.06 a |
| | 9 | 1.65±0.07 a | 1.65±0.14 b | 1.67±0.10 b | 1.93±0.23 a |
| | 12 | 1.80±0.15 a | 1.76±0.16 a | 1.72±0.08 a | 1.99±0.14 a |
| C/N | 0 | 56.6 | 56.6 | 56.6 | 56.6 |
| | 3 | 37.5±1.4 a | 36.7±3.2 a | 37.0±2.6 a | 34.9±1.3 a |
| | 6 | 30.7±1.0 a | 31.6±1.9 a | 30.4±2.2 a | 29.0±1.6 a |
| | 9 | 26.7±1.0 a | 27.1±2.1 a | 28.3±2.3 a | 25.2±1.1 b |
| | 12 | 23.1±2.2 a | 24.3±0.7 a | 24.1±0.7 a | 22.0±1.3 b |
| Soluble phenol (‰) | 0 | 13.4 | 13.4 | 13.4 | 13.4 |
| | 3 | 8.6±0.9 a | 8.5±0.9 a | 8.6±0.6 a | 6.6±0.4 a |
| | 6 | 8.3±0.6 a | 8.3±1.4 a | 7.8±1.3 a | 5.2±0.6 b |
| | 9 | 6.8±0.5 a | 7.1±1.0 a | 6.8±0.8 a | 4.3±0.7 b |
| | 12 | 5.7±0.9 a | 5.7±0.7 a | 5.9±0.5 a | 3.4±0.3 b |
| Insoluble phenol (‰) | 0 | 15.4 | 15.4 | 15.4 | 15.4 |
| | 3 | 5.8±2.2 a | 5.1±2.6 a | 5.2±1.2 a | 1.0±1.6 b |
| | 6 | 5.3±0.9 a | 6.0±2.0 a | 6.2±1.6 a | 1.6±1.0 b |
| | 9 | 5.4±1.4 a | 4.9±0.7 a | 5.5±1.6 a | 1.2±0.7 b |
| | 12 | 4.0±1.3 a | 4.5±1.7 a | 5.2±1.0 a | 1.4±1.0 b |
| Cellulose (%) | 0 | 21.5 | 21.5 | 21.5 | 21.5 |
| | 3 | 17.4±1.0 a | 16.2±2.0 a | 16.3±1.6 a | 14.1±0.7 b |
| | 6 | 14.5±0.5 a | 15.1±1.3 a | 14.6±0.6 a | 13.2±0.4 b |
| | 9 | 14.2±1.0 a | 13.1±1.3 a | 13.8±0.9 a | 10.5±2.2 b |
| | 12 | 12.4±1.4 a | 12.9±1.3 a | 13.6±0.7 a | 10.2±1.1 b |
| Hemicelluloses (%) | 0 | 14.4 | 14.4 | 14.4 | 14.4 |
| | 3 | 14.5±0.96 a | 13.9±1.2 a | 14.5±1.0 a | 14.0±1.1 a |
| | 6 | 13.5±0.50 a | 13.6±0.7 a | 13.3±0.5 a | 12.5±0.4 b |
| | 9 | 12.3±0.68 a | 12.0±1.0 a | 12.2±0.6 a | 10.8±1.6 a |
| | 12 | 10.9±1.20 a | 10.8±1.2 a | 11.6±0.6 a | 9.4±1.5 b |
| Lignin (%) | 0 | 31.9 | 31.9 | 31.9 | 31.9 |
| | 3 | 37.1±1.5 a | 37.1±1.5 a | 36.3±1.5 a | 36.8±1.7 a |
| | 6 | 37.5±1.8 a | 38.2±1.9 a | 38.9±1.6 a | 39.8±1.3 a |
| | 9 | 41.2±0.9 a | 39.8±2.0 a | 40.9±1.9 a | 40.4±1.4 a |
| | 12 | 42.7±2.3 a | 43.3±0.7 a | 43.1±0.5 a | 42.1±1.1 b |

Within a sampling date (row), means (five replicates, in bold-type) followed by the same letter are not significantly different according to the Kruskal-Wallis test at $p<0.05$. For treatments, see Table 2

^aAnalysis of one sample

reached in S3C2 after 3 weeks were comparable to values measured after 12 weeks of incubation with other treatments (Table 4). Soluble phenols continued to decrease with time, whereas insoluble phenols stabilized after 3 weeks. Only the sludge dose had a significant effect ($p < 0.01$), with less phenolic compounds in S3C2 compared to the other treatments.

The PRC analysis done on all chemical parameters confirmed the specific response of the S3C2 compared to the three other treatments (Fig. 6).

Discussion

Dynamics of the community in mesocosms

Abundance of the mesofauna community increased both in litter and soil with all treatments, although the increase was slower in the S3C2 treatment than those in the S1C0, S1C1 and S1C2 treatments. This trend has been found in previous studies (Pimentel and Warneke 1989), and it indicates that addition of sludge usually favours invertebrates and microorganisms mainly as the result of an increase in nutrients. Moreover, fungi may also be a source of nutrients easily available for fungivorous species. However, although the overall mesofauna community subject to the four treatments increased in number, this was not true for each individual species as it has been observed by (Lübben 1989), Filser and Hölscher 1997 and Filser et al. (2000). Thus, the abundance of *F. fimetaria*, *P. armata* and oribatids decreased with time. Although these species appeared to be sensitive to sludge application, they remained almost indifferent to tripling of the sludge dose. The decrease in abundance of these species may be caused by an indirect effect of sludge due to the change in living conditions in mesocosms promoted by changes in the organic matter content, composition of the microbial community, rate of predation (Hopkin 1997), soil moisture or pH. Indeed, in this experiment, the soil pH decreased from 6.02 to 5.28. The conditions in mesocosms were thus relatively alkaline for oribatids that were originally sampled in acid litter.

Effects of copper dose on the mesofauna community

The addition of various doses of Cu to low sludge amendment (treatment S1) had no significant effect on mesofauna community abundance. It is likely that Cu toxicity was masked by the increase in organic matter. Indeed, in soils with large amounts of organic matter, it has previously been observed that Collembola are not affected by heavy metal contamination (Crouau et al. 2002). In sludge, Cu is linked to sulphur, carbonates and organic matter, and the proportion of these associations can be affected by sludge type and application rate (Banerjee et al. 1997). In this experiment, the sludge was limed (pH 9.2), and this amendment can increase binding of Cu to organic

matter (Bourrelier and Berthelin 1998; ADEME 2001). Moreover, throughout the experiment, concentrations of extractable Cu ranged from 3.53 to 23.00 mg kg⁻¹, which are low values compared to the initial Cu concentrations added to sludge. Therefore, Cu may have been complexed with sludge, thus being less available to mesofauna. This may explain why *L. cyaneus* was not affected by Cu doses, although it has been reported to be sensitive to Cu exposure in concentrations equivalent to those used in our experiment (Bruce et al. 1997; Cole et al. 2001). Other species and taxa such as *F. fimetaria*, *P. notabilis*, *E. crypticus* and Oribatida are known to be heavy-metal tolerant (Lübben 1989; Parmelee et al. 1993; Scott-Fordsmann et al. 1997; Bruus Pedersen et al. 1999; Salminen and Haimi 1999). However, the degradation of organic matter can occur during the incubation with release of Cu and increase in the extractable Cu concentration; thus, Cu toxicity can increase at longer incubation times.

Effects of sludge dose on the mesofauna community

The dose of sludge had a significant effect on abundance of certain species and, thus, on community composition. Consequently, *M. macrochaeta*, *I. prasinus* and *L. cyaneus* grew on the lowest sludge dose applied in the mesocosms. However, their abundance decreased with time when they were submitted to treatment S3C2. Abundance of *P. notabilis* was also influenced by sludge application. Moreover, tripling the dose of sludge changed the chemical evolution of the leaves and sludge. These chemical changes may be attributed to a quantitative and/or a qualitative modification of the microbial community degrading litter. In our study, *P. notabilis* tended to become dominant in treatment S1C2, confirming what was reported by (Lübben 1989), Bruce et al. (1999) and Potapow (2001), who have described that this species is quite tolerant, with a preference for large amounts of organic matter and responding positively to liming. However, a too large amount of sludge (S3C2) slowed down the increase in abundance of *P. notabilis*. *E. crypticus* also seemed to grow on conditions in the mesocosms and especially in sludge (Gobat et al. 1998). However, its abundance was reduced after tripling the dose of sludge, especially in soil. All these species might be sensitive to an excessive quantity of organic matter and/or extensive fungal growth; Collembola may be trapped by fungal mycelium (Cragg and Bardgett 2001).

Koehler (1999) has shown that mineral fertilizers in combination with nutritive poor sludge has an indirect and a positive effect on the abundance of Gamasida by stimulating prey organisms. In this study, the predatory mite *H. aculeifer* may have taken advantage of the trapped or sludge-dose-sensitive Collembola, which became an easily available source of food. The abundance of this mite increased in all treatments, with the highest increase in the S3C2 treatment, where it reached 73.55% of the total fauna after 6 weeks. Thus, the mesofauna community is modified in its specific composition as well as in its trophic structure.

Effects of treatments on leaf litter decomposition

After 12 weeks, the residual ash-free litter mass (litter mass minus ash mass) varied from 12 to 17%, respectively, for the S3C2 and S1C0 treatments, and similar values have been reported by Van Wesemael (1993). Furthermore, the leaf N content increased with time in all treatments, confirming the findings of Aber and Melillo (1980) and Gallardo and Merino (1999). This change is usually attributed to microbial immobilization of sludge N into decaying leaves (Seastedt 1984; Cortez et al. 1990). The absolute increase in the leaf lignin concentration during the leaf litter decomposition is due to the low degradation rate of lignin (Gobat et al. 1998; Fioretto et al. 2005). However, the conventional method used to analyse lignin does not distinguish lignin and partially humified products (Coûteau et al. 1995). According to its heterogeneous chemical structure, hemicelluloses disappeared more slowly than cellulose. The initial strong decrease in phenol content might be due to a mechanic effect due to the sudden moistening of leaves by spraying water and the subsequent cell bursting.

It is well known that decomposition processes are strongly influenced by bacteria, fungi and animals living in soil and leaf litter habitats (Kurcheva 1960; Edwards et al. 1970; Gobat et al. 1998). Particularly, mesofauna consumes dead vegetation and excretes partially decomposed faecal pellets, increases the surface area and makes more available organic residues to bacteria and fungi (Van Amelsvoort et al. 1988). Thus, all changes in the community composition may have a great influence on decomposition processes (Edwards and Bohlen 1995; Cortez et al. 2003). In our study, the mesofauna community changed not only in its specific composition but also in its trophic structure in the S3C2 treatment. Probably, Collembola number changed with the advantage of predatory mites, and the result was a change in "grazing pressure" of microorganisms. These modifications in mesofauna/microorganisms interactions may have affected the chemical composition of sludge and leaves. However, unlike Bogomolov et al. (1996), who found that inhibition of decomposition of wheat straw litter occurred at $100 \mu\text{g Cu g}^{-1}$, we found that the concentration of Cu had no effect either on leaf decomposition parameters or on their chemical composition. The Cu adsorption by organic matter in sludge seemed to be confirmed.

Conclusion

Probably, the Cu spiked to sludge was bound to the organic matter of sludge, and for this reason, there was no effect on the mesofauna community and on the litter decomposition, at least over the 12 weeks of monitoring. Thus, species known to be Cu-sensitive such as *L. cynaeus* were not affected by the increased Cu doses.

The increase in the sludge dose greatly affected the community structure at both species and trophic level, with a decrease in diversity and an increase in predator abun-

dance. It is suggested that these changes modified the chemical composition of the litter (leaves and sludge) through a likely change in the microbial community. In spite of the fact that mesocosms of this study were artificially composed, they can reproduce and simulate the complexity of forest soil, characterised by the presence of several species. Moreover, this approach allowed us to relate the community changes with ecosystem processes, such as litter decomposition and changes in the chemical composition of sludge.

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