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Sewage Sludge Effects on Mesofauna and Cork Oak (*Quercus suber* L.) Leaves Decomposition in a Mediterranean Forest Firebreak

Céline Pernin,* Jérôme Cortet, Richard Joffre, Jean Le Petit, and Franck Torre

ABSTRACT

Effects of sewage sludge on litter mesofauna communities (Collembola and Acari) and cork oak (*Quercus suber* L.) leaf litter decomposition have been studied during 18 mo using litterbags in an in situ experimental forest firebreak in southeastern France. The sludge (2.74 t DM ha⁻¹ yr⁻¹) was applied to fertilize and maintain a pasture created on the firebreak. Litterbag colonization had similar dynamics on both the control and fertilized plots and followed a typical Mediterranean pattern showing a greater abundance in spring and autumn and a lower abundance in summer. After 9 mo of litter colonization, Collembola and Acari, but mainly Oribatida, were more abundant on the sludge-fertilized plot. Leaf litter decomposition showed a similar pattern on both plots, but it was faster on the control plot. Furthermore, leaves from the fertilized plot were characterized by greater nitrogen content. Both chemical composition of leaves and sludges and the decomposition state of leaves have significantly affected the mesofauna community composition from each plot.

THERE HAS BEEN A PROGRESSIVE REINFORCEMENT of legislation related to sludge utilization in the EU, particularly in France, for approximately 10 yr. This reinforcement aims at improving the quality of sludge and forbids some ways of eliminating it, which are environmentally hazardous, such as sea-dumping and land-filling. Moreover, the quantity of sludge has dramatically increased—800 000 t dry wt. was produced in 2000 and 1.3 Mt dry wt. is expected for 2005. In France, there are three major ways of eliminating sludge—incineration (<20% of the total amount of sludge produced), although not recommended for economic and environmental reasons, or by land-filling (25%), progressively limited to the final waste, and finally, sludge spread on agricultural land (55 to 60%) for soil amendment in organic matter. However, new alternatives of valorization are being developed, such as composting or sludge application on non-agricultural land to revegetate.

Sludge is rich in nutrients, calcic, and organic matter and its application is known to have favorable effects, more or less long-lasting, on the biomass production and chemical and physical soil properties. These effects have been the subject of a number of studies such as Mitchell

et al. (1978), Wei et al. (1985), Logan et al. (1997), Stamatiadis et al. (1999), Aggelides and Londra (2000), and Al-Assiuty et al. (2000). These studies have shown that spreading sludge improved the structural properties and fertility of soil, and increase the permeability, hydric conductivity, and water retention ability of soil. Moreover, sludge decreases the rate of soil erosion. Sludge spreading also has a favorable effect on soil biological characteristics by stimulating microbial activity and biomass (Mitchell et al., 1978; Robert, 1996; Banerjee et al., 1997). Generally, soil amendment in organic matter via sludge application additionally favors soil invertebrates and abundance of Carabidae, earthworms (Stevenson et al., 1984), nematodes, and mesofauna (Andrès, 1999; Bruce et al., 1999; Koehler, 1999; Cole et al., 2001). The responses of organisms to sludge application are specific. Changes in microbial and invertebrate community structure such as a decrease of species and functional diversity was observed by Lübben (1989), Banerjee et al. (1997), and Bruce et al. (1999), especially if sludge is contaminated by heavy metals.

Soil mesofauna has not only a role in the regulation of decomposition (Seastedt, 1984) but it is also an important source of preys for predators (Hopkin, 1997). Moreover, as it lives in close association with the soil microflora and -fauna, the soil mesofauna gives an early indication of ecosystem disturbances. For these reasons, soil mesofauna, especially Collembola and mites, is often used to assess the impact of environmental disturbances (Cortet et al., 1999) such as sludge application (Cole et al., 2001).

In this study, wastewater sludge was applied on a firebreak in southeastern France. The firebreak was sown and grazed by heifers during winter pasture. To perpetuate the pasture and prevent soil erosion due to frequent fire, sludge had been applied since 1995. The sludge spreading allowed the disposal of waste and the correction of the low organic matter content of the soil. Despite the fact that there was an improvement of sludge quality and control procedure, the sludge contained pollutants such as metal trace elements, organic compounds, and pathogens. The purpose of the study was to assess the impact of an experimental and repeated sludge spreading on both mesofauna community (characteristics and composition) and functional (litter decomposition) level over 18 mo by using the litterbag method. This long-term study helped us improve our knowledge of the effect of sludge spreading in the Mediterranean region, characterized by a typical climate.

MATERIALS AND METHODS

Study Area

The experiment was performed in a firebreak situated in the Massif des Maures in southeastern France (6°40'47"—

C. Pernin, J. Le Petit, and F. Torre, Inst. Méditerranéen d'Ecologie et Paléocologie, Univ. Paul Cézanne, case 441, F-13397 Marseille cedex 20, France. J. Cortet, Inst. National Polytechnique de Lorraine (ENSAIA), 2 av. de la Forêt de Haye, BP 172, F-54505 Vandœuvre-lès-nancy, France. R. Joffre, Centre d'Ecologie Fonctionnelle et Evolutive, CNRS, 1919 route de Mende, F-34293 Montpellier cedex 05, France. Work supported by the French Agency for Environment and Energy Management (ADEME). Received 26 Nov. 2005. *Corresponding author (perninceline@yahoo.fr).

6°41'36"E; 43°21'43"-43°22'48"N). This firebreak was characterized by an eroded soil which had low organic matter content (Table 1). According to Duchaufour (1983), the sandy soil is a crystalline Ranker. The site was under the influence of a typical Mediterranean climate characterized by a dry and hot summer between two wet seasons. Mean annual air temperature during the study was 10.28°C (min) and 21.95°C (max). Total annual precipitation was 785 mm in 2000 and 462 mm in 2001.

The firebreak contained a typical maquis vegetation characterized by Mediterranean chamaephytes (*Cistus albidus* L., *Cistus monspeliensis* L.), *Silene gallica* L., *Trifolium subterraneum* L. (sowed for grazing), and Cork oak (*Quercus suber* L.).

Two study plots were selected: a control plot (CP) (200 m²) and a fertilized plot (FP) (300 m²), hereafter denoted CP and FP, respectively. These two chosen plots had the same topographic characteristics such as orientation (north-east), slope (5–10°), and altitude (150 m).

Sludge

The sludge used was processed in a physical–chemical treatment (flocculation, coagulation, and decantation) and stabilized by liming. The sludge was directly collected from the wastewater treatment plant of les Issambres town (Var County, France); its characteristics are summarized in Table 2. The sludge was applied from 1995 through 2002 initially in liquid form but from 1998 through 2002 in past form (water content 33.5%). The quantity spread was 2.7 t ha⁻¹ yr⁻¹. For the purpose of the study, sludge was applied manually every 2 mo on top of the soil, except during the first 5 mo of monitoring. The sludge was applied in January, May, July, September, and November 2000, and in January, March, May, and July 2001.

Sampling Method

Mesofauna was sampled using litterbags (10 × 10 cm) made from nylon gaze. Litterbags had a mesh size of 4 mm and were filled with 5 ± 0.01 g of 1-yr-old Cork oak leaves which represent the surrounding local forest litter. These leaves were sampled in November 1999 on the forest topsoil, cleaned with water, and air-dried. In December 1999, four blocks of 50 litterbags were laid out on top of the soil on each plot and fixed with plastic peaks. Plastic and rigid mesh (1 cm) was added after the storm of December 1999 to protect the litterbags. From January 2000, 10 bags per plot (2 or 3 per block) were

Table 1. Soil characteristics and heavy metal content on fertilized and control plots (Analyses performed by the Laboratoire d'Hygiène Santé Environnement de Lyon, 03/2001).

Parameter	Fertilized plot	Control plot	Threshold values†
Clay %	7.8	9.6	–
Fine silt % (<2 µm)	8.8	13.9	–
Coarse silt % (2–20 µm)	5.5	8.4	–
Fine sand % (20–50 µm)	14.5	19.8	–
Coarse sand % (50–200 µm)	63.4	48.3	–
Organic matter %	7.4	6.4	–
pH (H ₂ O)	8.4	5.6	–
N Kjeldahl g kg ⁻¹	1.7	2.3	–
C/N	13.0	15.0	–
Cd mg kg ⁻¹	0.2	<0.5	2
Cr mg kg ⁻¹	18.1	45.0	150
Cu mg kg ⁻¹	46.9	18.0	100
Ni mg kg ⁻¹	9.3	25.0	50
Pb mg kg ⁻¹	38.4	21.0	100
Se mg kg ⁻¹	–	<4.8	–
Zn mg kg ⁻¹	85.7	78.0	300
Mg mg kg ⁻¹	0.1	<0.1	1

† Council Decision of 8 January 1998, Journal Officiel 31 Jan. 1998.

Table 2. Sludge characteristics and heavy metal content. Mean of 10 values for dry matter, pH, N, CaO, heavy metals, and mean of 5 values for humidity, OM, and C/N (analyses performed by the Laboratoire d'Hygiène Santé Environnement de Lyon).

Parameter	Mean values	Threshold values†
Dry matter %	32.3	–
Humidity %	69.5	–
pH (H ₂ O)	8.8	–
Organic matter g kg ⁻¹	54.2	–
N % dry matter	2.7	–
CaO % dry matter	20.4	–
C/N	11.6	–
Cd mg kg ⁻¹	0.8	10
Cu mg kg ⁻¹	488.5	1000
Pb mg kg ⁻¹	26.4	800
Zn mg kg ⁻¹	421.8	3000
Cr mg kg ⁻¹	26.4	1000
Ni mg kg ⁻¹	9.8	200
Se mg kg ⁻¹	<5	100
Hg mg kg ⁻¹	0.6	10
7 PCB mg kg ⁻¹	0.113	0.8

† Council Decision of 8 January 1998, Journal Officiel 31 Jan. 1998.

randomly and monthly sampled for 18 mo, a minimum of 8 d after sludge application. This method allowed the simultaneous monitoring of the dynamics of both mesofauna colonization and organic matter decomposition.

Mesofauna

Sampled litterbags were placed in plastic bags and transported to the laboratory. Mesofauna was collected using a Berlese extractor for 10 d and stored in 70% ethanol (Berlese, 1905). Mesofauna was then counted under a binocular and identified to the species level for Collembola (Gisin, 1960; Christiansen and Bellinger, 1980a, 1980b, 1980c; Christiansen and Bellinger, 1981) and to the sub-order for Acari (Oribatida, Gamasida, Actinedida, and Acaridida).

Litter Decomposition

After 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. Then, all the samples were 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 Mediterranean species litters 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 characterized and corresponding NIRS calibration equation built. Ash concentration were determined after treatment at 550°C for 3 h. Nitrogen was determined with a PerkinElmer elemental analyzer (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, using tyrosine as a reference. Results were expressed as equivalent tyrosine L⁻¹. 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 (Javillier et al., 1959). Calibration equations between spectral and chemical data were conducted using the ISI software system (Shenk and Westerhaus, 1991).

Data Analyses

The respective abundances of each taxon was calculated for each sampling date and expressed in number of individuals per square meter for the 3 main taxa (Collembola, Acari, Oribatida) and for other arthropods. To monitor their respective contribution, the relative abundance of each taxon was calculated. The mean Collembola species richness and Shannon's diversity index (\log_2) were also calculated for each treatment and sampling date.

The residual ash-free litter mass, N, cellulose, hemicelluloses, lignin, and phenols contents were calculated.

To compare treatment effects on individual species, species richness, and diversity, a partly nested analysis of variance was performed with sampling date and treatment as factors and with error due to block effect including within-factor treatment (R Development Core Team, 2006). The same analysis was also performed to compare sludge effects on each leaf parameter (N, cellulose, hemicelluloses, lignin, and phenols content). These analyses were performed using $\log(x+1)$ -transformed data, apart from Shannon's diversity index.

When the data did not fulfill the requirements for parametric testing at each sampling date, the nonparametric Kruskal-Wallis test was performed to assess the effect of sludge application (Statsoft, 1998).

The effects of sludge application on both mesofauna community and leaf chemical composition were analyzed by using 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 optimally show 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; Pernin et al., 2006), focuses on differences in species or chemical composition between treatments and the control at each particular time point. This method has also been used for field study (Frampton, 1997; Frampton et al., 2000; Frampton, 2001). The 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 presenting the deviations in time of the treatment compared to the control. The species and chemical component weights, which appear on the right side of the figure can be interpreted as the weight of each species or component with 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. The first PRC was performed on the mesofauna data set community using only Collembola species and taxa that had a relative abundance above 5%. The second PRC was done using the percentage of mass of different components remaining in leaves (N, cellulose, hemicelluloses, lignin, and phenols) estimated by the NIRS.

RESULTS

Mesofauna Community

The dynamics of litterbag colonization followed the same pattern on both plots and for all taxa we con-

sidered (Fig. 1a), with peaks of abundance in spring and autumn, apart from April 2001. The colonization was slow and progressive. From January through September 2000, few individuals were sampled on both plots. Peaks of abundance appeared in April and June. During this period, the mesofauna was significantly more abundant on CP ($p < 0.05$). From October, mesofauna increased in number on both plots. Peaks of abundance appeared in October 2000, and March and May 2001. From October 2000 through July 2001, the mesofauna abundance was significantly greater on FP ($p < 0.001$). Few individuals were sampled during the summers of 2000 and 2001. Over the 18-mo monitoring, the sampled mesofauna was significantly more abundant on FP ($p < 0.01$).

Collembola represented respectively 50 and 52% of mesofauna communities sampled on FP and CP. The litterbag colonization by Collembola was low from January through September 2000 on both plots with a peak of abundance in April (Fig. 1b). Over this period more individuals were sampled on CP ($p < 0.01$). From October 2000, the number of Collembola increased on both plots. Peaks of abundance appeared in October 2000, in March 2000 (>25000 ind m^{-2}) and in May 2001. From October 2000 to July 2001, the number of Collembola was significantly greater on FP ($p < 0.001$).

As for Collembola, the number of oribatid mites was low during the first 9 mo of monitoring on both plots (Fig. 1c). In October 2000, the number of Oribatids increased and reached a maximum of 7490 ind m^{-2} . Peaks of abundance appeared in autumn 2000 and spring 2001. Oribatids were significantly more abundant on FP over the 18-mo period ($p < 0.001$). Their proportions on FP (21%) were twice as high as on CP (11%).

The colonization of litterbags by other mites (*Gamasida*, *Acaridida*, *Actinedida*) was also initially low (Fig. 1d). Over the first 9 mo of monitoring, mites were more abundant on CP ($p < 0.05$). Their number increased from October 2000 but this increase was less pronounced than for Collembola and oribatids. Mites were then more abundant in the FP ($p < 0.01$). Peaks of abundance also appeared in spring and autumn. Acari represented 4% of the community on FP and 11% on CP.

During the summer, Oribatid mites and Collembola were not sampled in great numbers. Only other Acari and other arthropods were sampled such as Araneae, Homoptera, and Psocoptera.

Over the 18 mo of monitoring, 22 species of Collembola were identified, 17 on CP and 20 on FP. Fifteen species were found on both plots (Table 3). Dynamics of Collembola species richness was similar on both plots (Fig. 2). Few Collembola species were found during the first months of monitoring. This number increased drastically from October 2000 and became significantly higher on FP ($p < 0.001$). The species diversity index followed the same dynamics (Fig. 2) than the species richness index, without any significant difference between the two plots.

Effects of sludge application on the mesofauna community structure were assessed performing PRC analysis (Fig. 3). The curve representing the evolution of the mesofauna communities sampled on FP showed a large

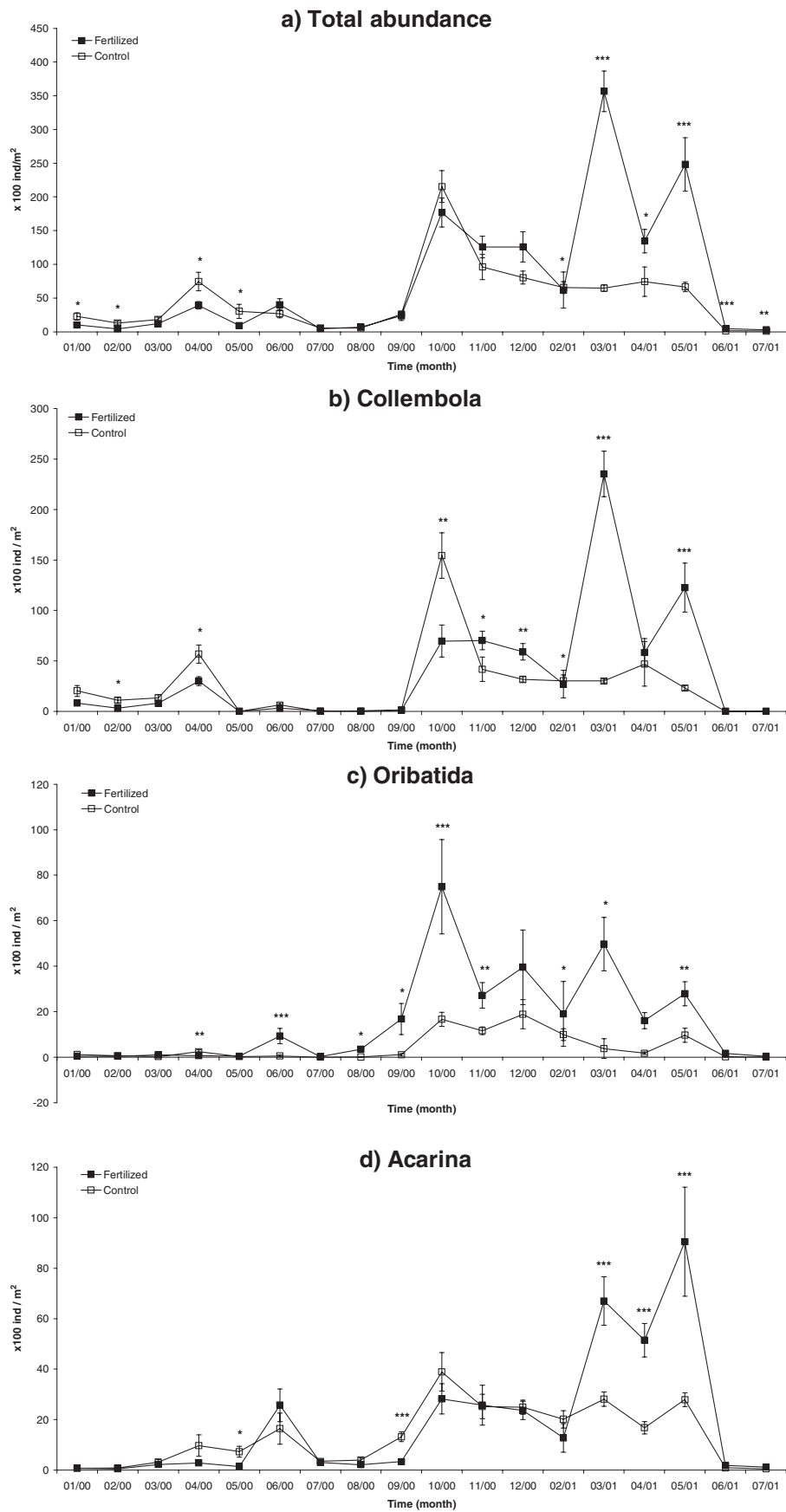


Fig. 1. Mean abundance (mean of 10 samples \pm standard error) of total mesofauna communities: (a) total abundance of mesofauna, (b) Collembola, (c) Oribatida, and (d) Acarina on the fertilized plot (FP) and the control plot (CP). Mann-Whitney test, *: $p \leq 0.05$, **: $p \leq 0.01$, and ***: $p \leq 0.001$.

Table 3. Collembola species sampled on both control and fertilized plots.

Collembola species	Control plot	Fertilized plot
<i>Entomobrya quinquelineata</i> Börner	✓	✓
<i>Entomobrya lanuginosa</i> Nicolet	✓	✓
<i>Entomobrya multifasciata</i> Tullberg	✓	✓
<i>Entomobrya myrmecophila</i> Reuter	✓	✓
<i>Entomobrya</i> sp.	✓	✓
<i>Heteromurus major</i> Moriez	✓	✓
<i>Lepidocyrtus curvicolis</i> Bourlet	✓	✓
<i>Lepidocyrtus cyaneus</i> Tullberg	✓	✓
<i>Lepidocyrtus lanuginosus</i> Gmelin	✓	✓
<i>Lepidocyrtus</i> sp.	✓	✓
<i>Orchesella quinquefasciata</i> Bourlet	✓	✓
<i>Pseudosinella alba</i> Packard	✓	✓
<i>Seira ferrarii</i> Parona	✓	✓
<i>Isotomurus maculatus</i> Schäffer	✓	✓
<i>Parisotoma notabilis</i> Schäffer	✓	✓
<i>Vertagopus abeloosi</i> Poinot	✓	✓
<i>Protaphorura armata</i> Tullberg	✓	✓
<i>Brachystomella parvula</i> Schäffer	✓	✓
<i>Friesea</i> sp. Dalla Torre	✓	✓
<i>Neanura</i> sp. MacGillivray	✓	✓
<i>Xenylla maritima</i> Tullberg	✓	✓
<i>Megalothorax</i> sp. Willem	✓	✓
<i>Sminthurinus elegans</i> Fitch	✓	✓
<i>Sminthurus multifasciatus</i> Reuter	✓	✓
<i>Sminthurus</i> sp.	✓	✓

deviation compared to the curve representing the community sampled on CP, especially from September 2000. The Monte Carlo test showed a significant difference ($p < 0.01$) over the 18 mo of monitoring.

Relative abundance of each taxa (Fig. 4) showed that the composition of communities was similar on both plots during the first months of monitoring. Until April, they were mainly composed of Collembola *Entomobryidae* (*Entomobrya multifasciata* Tullberg and *Entomobrya quinquelineata* Börner) which represented 65 to 80% of the communities and by *Actinedida* in summer (50 to 75%). The communities greatly differed from autumn with Oribatid mites, which became dominant on FP and represented more than 70% of the community

in September. Species weight confirmed that Oribatids were particularly sensitive to sludge application with a weight close to 0.5 (Fig. 3). Other species or taxa included in the PRC analysis had low weights with values between -0.1 and 0.3 , and were sampled on both plots. From October 2000 to May 2001, Collembola species such as *Isotomurus maculatus* Schäffer, *Vertagopus abeloosi* Poinot, *Protaphorura armata* Tullberg, *Lepidocyrtus cyaneus* Tullberg, and *E. multifasciata* represented 40 to 45% of communities on both CP and FP. However, they had positive weights and were more abundant on FP. Only *Entomobrya quinquelineata*, *Parisotoma notabilis* Schäffer, and *Seira ferrarii* Parona had negative weights and were more abundant on CP.

Dynamics of Litter Decomposition

Residual ash-free litter mass (litter mass minus ash mass) decreased with time on both plots (Fig. 5). From January to October the litter mass loss was low on both plots, with 10% on FP and 13% on CP. After 9 mo of monitoring, the decomposition of Cork oak leaf litter accelerated, especially on CP. This mass loss was 33.4% on FP and 43.6% on CP after 18 mo. The litter mass loss was significantly higher on CP ($p < 0.001$) than on FP.

The PRC analysis showed a significant difference between litter sampled on FP and CP ($p < 0.01$) (Fig. 6). A large deviation appeared from September 2000 between the two curves representing the evolution of the chemical composition of litter sampled on each plot. Except N with a weight of 0.65, the weight of the different components was low, between -0.3 and 0.1 . Only N had a high and positive weight showing a higher N content in litter coming from FP. Table 4 includes information on the evolution of the litter chemical compounds estimated by the NIRS. The leaf N content increased over time on both plots. This increase was significantly higher on FP, especially from September ($p < 0.001$). Cellulose and

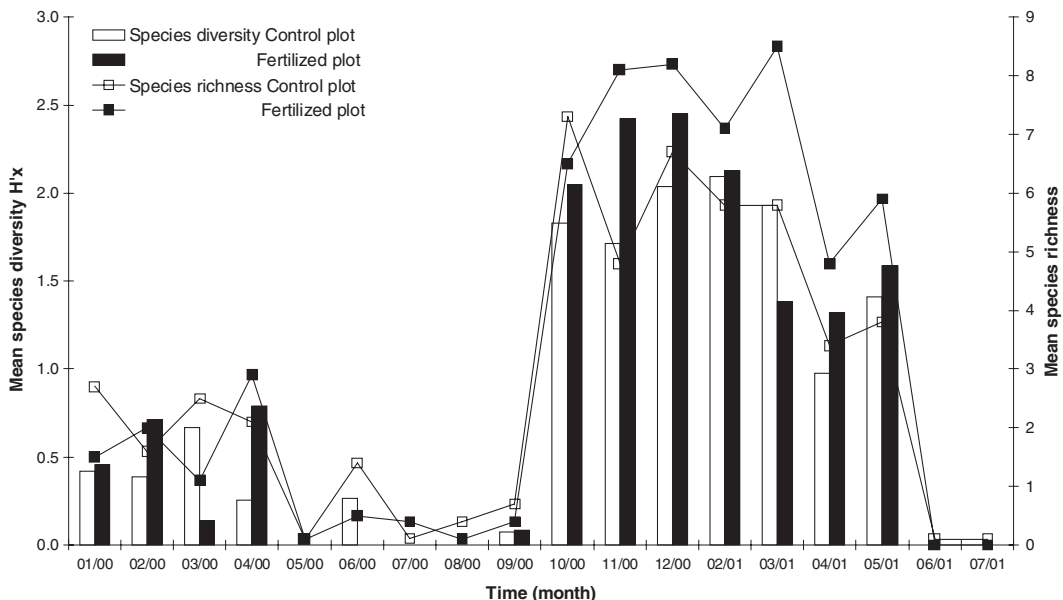


Fig. 2. Evolution of species richness and diversity on the fertilized and control plots during the 18 mo of monitoring. Mann–Whitney test, *: $p \leq 0.05$, **: $p \leq 0.01$, and *: $p \leq 0.001$.**

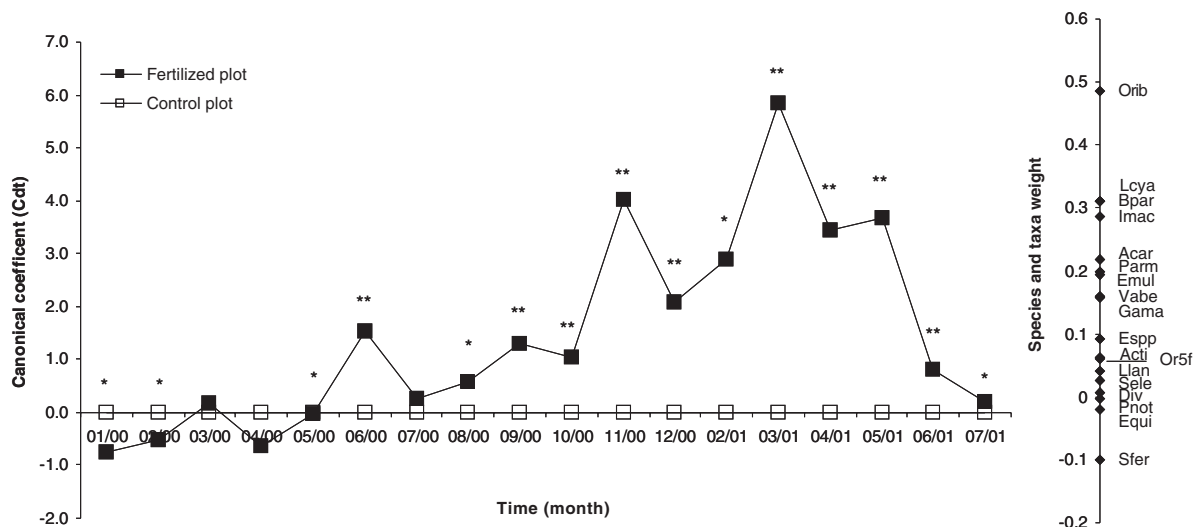


Fig. 3. Principal response curve diagram resulting from the analysis of the mesofauna data set indicating the effect the sludge application on the mesofauna community. Only Collembola species and taxa which had a relative abundance above 5% were used. Of all variance, 61.7% could be attributed to the sampling date displayed on the horizontal axis and 80.7% of all variance could be attributed to treatment; 45.4% of this variance is displayed on the vertical axis. The lines represent the course of the treatment levels in time. The species or taxa weight can be interpreted as the affinity of the taxon with the principal response curves. Monte Carlo test, *: $p \leq 0.05$, **: $p \leq 0.01$. Orib, *Oribatida*; Bpar, *Brachystomella parvula*; Lcya, *Lepidocyrtus cyaneus*; Imac, *Isotomurus maculatus*; Acar, *Acaridida*; Parm, *Protaphorura armata*; Emul, *Entomobrya multifasciata*; Gama, *Gamasida*; Vabe, *Vetagopus abeloosi*; Esp, *Entomobrya* sp.; Acti, *Actinedida*; Or5f, *Orchesella quinquefasciata*; Llan, *Lepidocyrtus lanuginosus*; Sele, *Sminthurinus elegans*; Div, *Divers*; Pnot, *Parisotoma notabilis*; Equi, *Entomobrya quinquefasciata*; Sfer, *Seira ferrarii*.

hemicellulose contents decreased with time on FP and CP from 20.8 to 9.8 and 13.5% for cellulose and from 14.2 to 8.6 and 12.5% for hemicelluloses. The decrease was initially slow but it accelerated after 9 mo. Lignin content remained stable on both plots and then slightly increased from October onward. This increase was significantly higher on FP ($p < 0.001$). Insoluble phenol leaf content increased until March and then decreased on both plots. This decrease was fast in spring 2000 and then slowed down until summer 2001. The maximal losses, with 71.5% on CP and 73.3% on FP, appeared in June. The soluble phenol leaf content was slowly decreasing until September 2000. Over this period, the decrease of soluble phenols was significantly higher on CP ($p < 0.001$). From October 2000, the decrease of soluble phenols accelerated. The decrease was then higher on FP ($p = 0.001$). The maximal loss appeared in July 2001 with 70.2% on CP and 83.4% on FP.

DISCUSSION

Dynamics of litterbag colonization by mesofauna was similar on both plots—slow and progressive. These dynamics were greatly influenced by the typical Mediterranean climate, characterized by great precipitation in spring and autumn and a dry summer. These weather conditions explained the seasonal peaks of abundance observed in spring 2000 and 2001 and in autumn. Results of this study confirm that Collembola are more sensitive to desiccation than mites, which are more resistant due to their cuticle (Ashraf, 1971). Sensitivity to hydric stress also appeared in April 2001. Few individuals were collected during this month characterized by little precipitation.

Evolution of the mesofauna community structure was also similar on both plots. Over the first few months, only epedaphic species colonized litterbags; then from October 2000, species described as hemiepigic and euedaphic also appeared. Among the most numerous species we observed were *Brachystomella parvula* Schäffer on FP and *Isotomurus maculatus*, *Protaphorura armata*, and *Vetagopus abeloosi* on both plots. These changes coincided with the visual subsiding of litterbags into the soil.

Repeated sludge application seemed to have a positive effect on the community abundance sampled on FP, especially after 9 mo of monitoring. Favorable living conditions met by mesofauna on FP may be partly explained by an improvement of some soil properties such as water retention ability (Wei et al., 1985; Stamatiadis et al., 1999), even if the sludge quantity was low and only spread on top of the soil. Moreover, it is well-known that sludge, whether it is contaminated by heavy metals (Lübben, 1989) or not (Pimentel and Warneke, 1989; Andrès, 1999; Cole et al., 2001), represents a source of nutrients and energy that is more easily available than cork oak leaf litter for mesofauna and microorganisms. However, while the overall mesofauna community subject to sludge application 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, in our study, whereas Oribatid mites were more abundant on FP, *Seira ferrarii* found better life conditions on CP. This could explain why, in our study, sludge application had also an effect on the structure of mesofauna communities. On the other hand, species richness and diversity tended to be greater on FP which has been previously reported by

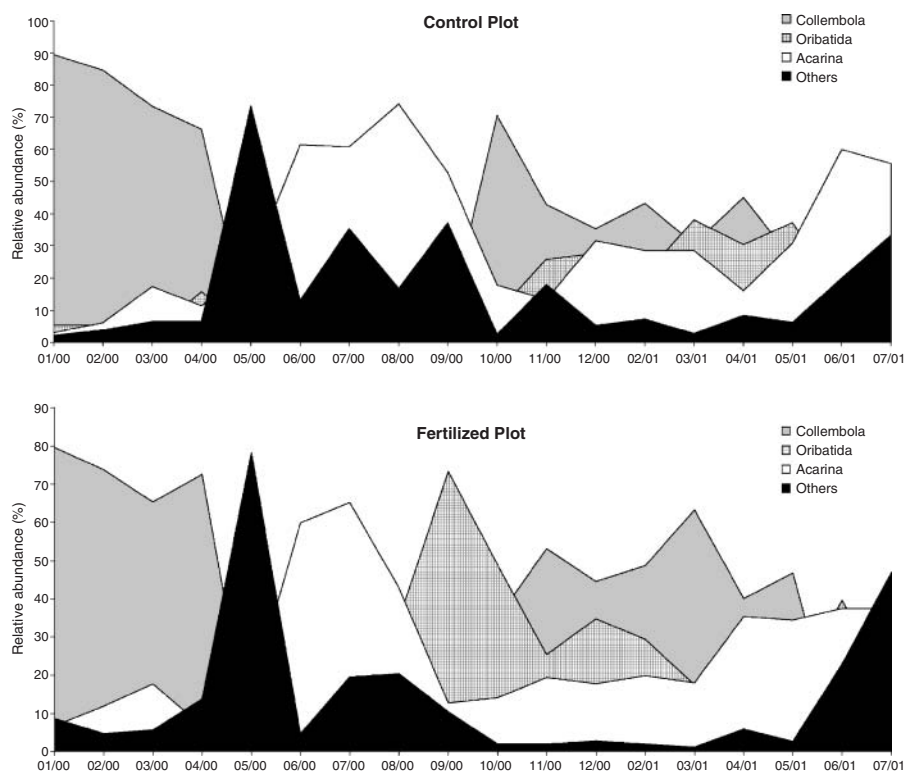


Fig. 4. Relative abundance of Collembola, Acari, Oribatida and other arthropods on the fertilized and the control plot during the 18 mo of monitoring.

Pimentel and Warneke (1989) and Al-Assiuty et al. (2000) showing that species diversity increased proportionally to the sludge quantity applied. Thus, as found by Pernin et al. (2006) in a complementary mesocosm study using the same sludge, no negative effect due to heavy metal content were observed. Heavy metal 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).

The slow colonization of litterbags by mesofauna during the first 9 mo coincided with the low decomposition of litter. Only 10% of litter mass was lost on FP and 13% on CP over this period. This low decomposition rate is explained by the initial high C/N ratio (56.5) characteristic of ligneous leaves (Gobat et al., 1998). In addition, oak leaves are rich in aliphatic and aromatic compounds, which are noncomestible for mesofauna (Gallardo and Merino, 1993; Andrès et al., 1999). To play a role in the decomposition process, mesofauna needs the preliminary action of microorganisms for obtaining a certain level of decomposition. Colonization of litter by these microorganisms was delayed by the initial cleaning of leaves contained in litterbags.

During the same time, the N content of leaves increased. This N immobilization was observed when the leaf C/N ratio was above 20 to 25 (Balesdent, 1998). This immobilization was attributed to a transfer from sludge to biologically inactive leaves through microbes and fungi, which immobilize it in decaying leaves (Seastedt, 1984; Cortez et al., 1990). Ibrahima et al. (1995) suggests

the fixation of atmospheric nitrogen or contamination by rain leaching. We also observed a low degradation of fibers, cellulose, and hemicelluloses, confirming a low enzymatic activity during the first 9 mo.

From October onward, the chemical composition of leaf litter which received sludge became different from the CP litter. Moreover, dynamics of litter decomposition accelerated, as did the colonization of litterbags by mesofauna. Finally, the litter mass loss was faster on CP. Thus, it probably seems that sludge does not only stimulate the microorganism activities (Mitchell et al., 1978; Banerjee et al., 1997; Stamatiadis et al., 1999), but also represents a source of food and energy that is more easily available than cork oak leaves. Indeed, when the N content of litter from CP tended to decrease and hence followed a mineralization process, the N content of litter from FP continued to increase. Consequently, we hypothesize that mineral N contained in sludge under NO_3 and NH_4 forms may contribute to the proteinic biomass synthesis by microorganisms. Cellulolytic activities also became more efficient than during the first 9 mo. On the other hand, the subsiding of litterbags into the soil and the microfragmentation of leaf fragments probably favored the increase of mesofauna, providing better conditions to bacterial and fungal colonization and activity. The weakening cell walls allowed a better degradation of leaf compounds such as cellulose and hemicelluloses.

CONCLUSIONS

This study allowed the assessment of the long-term effects of repeated sludge spreading on a firebreak in

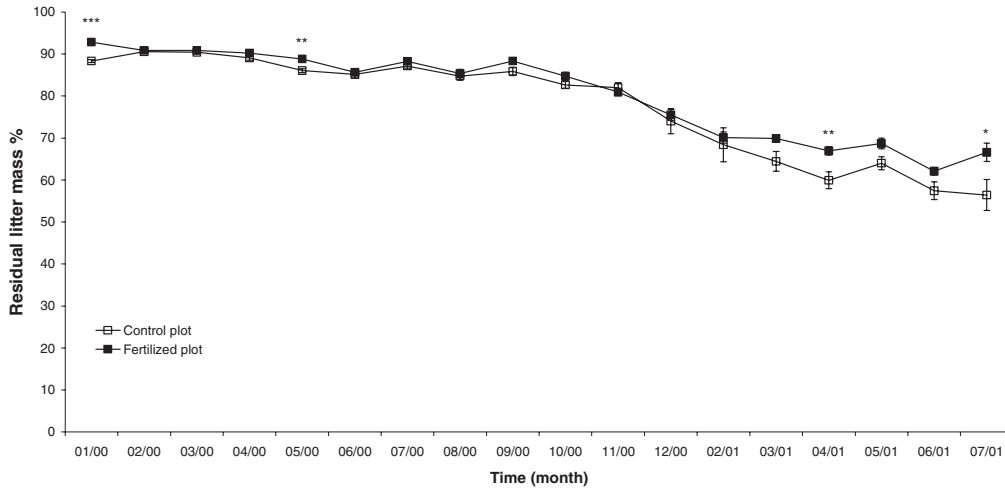


Fig. 5. Evolution of mean residual litter mass corrected with ash weight on the fertilized and the control plot (mean of 10 samples \pm standard error). Mann–Whitney test, *: $p \leq 0.05$, **: $p \leq 0.01$, and ***: $p \leq 0.001$.

the Mediterranean area, which is characterized by its climate, particularly severe over summer, favoring forest fire. As a result, soil layer is thin, poor in organic matter, and eroded. Sludge spreading would then appear to be a way of addressing two distinct issues—the elimination of sludge and an organic matter amendment to soil. Moreover, as the firebreak was sown, it may contribute to prevent soil erosion.

Furthermore, this study underlined the positive effect of sludge spreading on the mesofauna abundance despite the sludge contamination by heavy metals. The increase of the mesofauna community did not occur during the weeks following sludge application but on a longer term. Sludge, as other organic wastes, is known for improving the soil properties and providing nutrients to saprophytes and microorganisms. Species richness and diversity also tended to be higher on the FP. Differences in the mesofauna community structure between both plots were explained by differences in species relative abundance and the predominance of oribatid mites on the fertilized plot. Moreover, as litterbag colonization

was influenced by litter decomposition rate, the difference between communities was more pronounced after 9 mo of monitoring. Indeed, microorganisms preferentially used sludge as a source of nutrients rather than cork oak leaves which are ligneous and rich in compounds such as tannins that are difficult to digest.

The study also showed that the dynamics of litterbag colonization were greatly influenced by the weather conditions. Humidity had a great influence on mesofauna abundance, especially Collembola, and the litter decomposition process. Indeed, litter decomposition and litterbag decomposition accelerated when rain took place in autumn. Humidity was more beneficial to the FP where it may be stored in soil, thanks to a potential improvement of water retention capacity.

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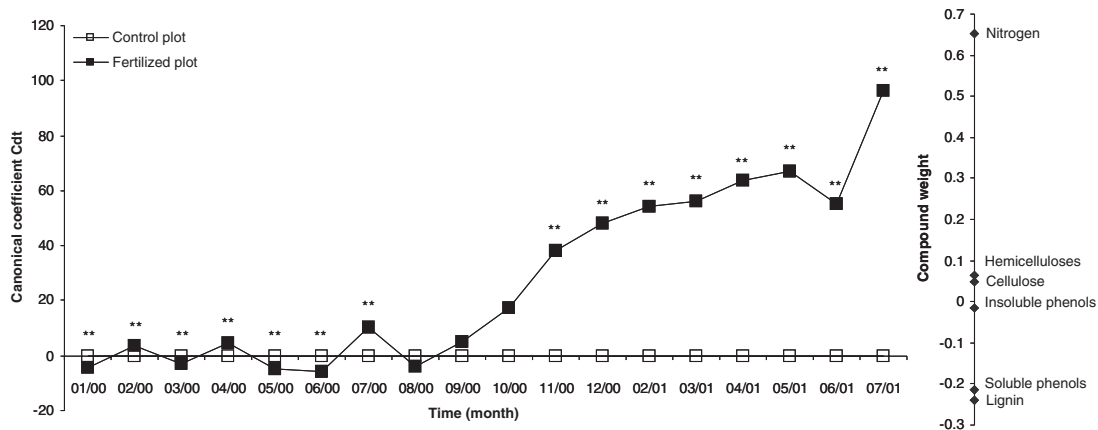


Fig. 6. Principal response curve diagram resulting from the analysis of the litter data set indicating the effect the sludge application on the litter chemical composition. Of all variance, 77.8% could be attributed to the sampling date displayed on the horizontal axis. Only 10.6% of all variance could be attributed to treatment; 84.7% of this variance is displayed on the vertical axis. The lines represent the course of the treatment levels in time. The component weight can be interpreted as the affinity of this component with the principal response curves. Monte Carlo test, *: $p \leq 0.05$, **: $p \leq 0.01$.

Table 4. Main characteristics (means \pm standard errors) of leaf litter in the control plot (CP) and the fertilized plot (FP).

Date	Azote		Cellulose		Hemicelluloses		Lignin		Soluble phenols		Insoluble phenols	
	FP	CP	FP	CP	FP	CP	FP	CP	FP	CP	FP	CP
12/99		0.832 [†]		20.8	14.2	13.9	30.7		1.4		1.3	
01/00	0.863 [‡]	0.83	20.8	19.1	14.5	14.9	29.2	29.2	3.0	2.5	1.7	1.2
02/00	0.89	0.88	20.8	20.9	14.5	14.9	29.8	29.8	2.8	2.9	1.6	1.7
03/00	0.86	0.86	20.8	20.5	15.1	14.7	29.3	29.3	3.0	2.9	1.7	1.6
04/00	0.87	0.85	20.5	20.7	14.3	14.5	30.5	30.3	2.7	2.7	1.5	1.4
05/00	0.92	0.94	20.4	19.1	14.6	13.8	30.5	30.5	2.5	2.3	1.2	1.1
06/00	0.90	0.95	19.6	19.8	14.4	14.7	30.9	30.9	2.5	2.3	1.0	0.9
07/00	0.92	0.92	19.8	20.3	14.0	14.7	30.7	31.1	2.6	2.4	1.2	1.0
08/00	0.94	0.98	20.1	20.2	14.5	14.9	30.5	31.2	2.6	2.8	1.1	1.0
09/00	0.97	0.96	19.5	19.6	14.2	14.6	30.9	31.7	2.2	2.2	1.0	0.9
10/00	1.05	0.97	17.2	18.3	14.2	14.6	32.9	32.4	1.8	2.1	0.7	0.8
11/00	1.13	0.96	17.3	17.3	12.6	13.9	33.2	31.5	1.0	1.6	0.7	0.8
12/00	1.23	1.05	14.8	16.8	12.2	13.6	34.5	33.1	1.1	2.0	0.6	0.7
02/01	1.28	1.02	13.6	15.5	11.1	13.1	34.8	33.1	0.9	1.3	0.6	0.6
03/01	1.34	1.13	13.4	15.0	11.1	13.1	35.9	33.9	0.6	1.2	0.4	0.6
04/01	1.40	1.14	11.9	14.8	10.7	12.9	35.4	33.9	0.6	1.5	0.4	0.6
05/01	1.43	1.11	11.6	14.4	10.2	12.4	35.1	34.8	0.7	1.6	0.5	0.7
06/01	1.44	1.18	11.8	14.5	9.9	12.7	35.6	34.7	0.8	1.1	0.5	0.5
07/01	1.49	1.19	9.8	13.5	8.6	12.5	33.8	34.7	0.7	1.5	0.6	0.6

[†] Analysis of one initial sample.

[‡] From 01/00 to 06/00, one analysis by NIRS was performed for 10 samples.

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