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M. Larchevêque, Virginie Baldy, Nathalie Korboulewsky, Elena Ormeño, Catherine Fernandez. Compost effect on bacterial and fungal colonization of kermes oak leaf litter in a terrestrial Mediterranean ecosystem. *Applied Soil Ecology*, 2005, 30 (2), pp.79 - 89. 10.1016/j.apsoil.2005.02.010 . hal-01769083

HAL Id: hal-01769083

<https://hal.science/hal-01769083>

Submitted on 17 Apr 2018

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Compost effect on bacterial and fungal colonization of kermes oak leaf litter in a terrestrial Mediterranean ecosystem

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Abstract

Mediterranean soils are generally low in organic matter and have a low water holding capacity. Moreover, recurrent fires are common in Mediterranean regions and increase the deterioration of these soils. Soil properties and ecosystem resilience after fire could be improved by amendment with compost, which is a source of organic matter and nutrients. A way to determine ecosystem integrity is to study leaf litter breakdown, which is a vital process in the functioning of terrestrial ecosystems and involves fungi and bacteria.

In this study, we determined fungal and bacterial biomass associated with decomposing oak leaves (*Quercus coccifera* L.) in a burnt shrub amended ecosystem during 1.5 years. Three treatments were studied: control, 50 t ha⁻¹ and 100 t ha⁻¹ of co-composted sewage sludge and green wastes. Bacterial biomass was not affected, although moisture, N and P concentrations in litter were increased by the amendment. However, fungal biomass was depressed on plots amended with 100 t ha⁻¹, and negatively correlated to exchangeable P. For all treatments, both types of microorganisms followed marked seasonal dynamics, with peaks of biomass during the wet periods of the year.

Keywords: Mediterranean ecosystem; Sewage sludge compost; Leaf litter; Ergosterol; Bacterial numbers; Microbial biomass; *Quercus coccifera* L.

1. Introduction

Soils under Mediterranean climate are undergoing degradation due to water erosion and recurrent fires, which affect their fertility (De Luis et al., 2001).

Nitrogen, which is often a limiting plant nutrient in soil, is easily lost by volatilisation during wildfires.

Guerrero et al. (2001) pointed out that organic matter addition is a suitable technique for accelerating the natural recovery process of burned soils. The spreading of biosolids, defined as the nutrient rich product of wastewater treatment, can improve the low fertility of soils and constitutes an alternative to landfill disposal. Biosolids are a source of organic matter and plant nutrients (Brockway, 1983; Martinez

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et al., 2003) and can improve soil physical, chemical and biological properties (McKay and Moffat, 2001; Caravaca et al., 2002). But biosolids present potential environmental risks. Their use can induce heavy metal and organic contaminants accumulation in soils (Brockway, 1983), as well as the discharge of nutrients, especially N and P, to surface and ground waters (Martinez et al., 2003). To decrease risks of heavy metal and salt leaching, the organic matter of biosolids can be stabilized by composting (Garcia et al., 1990; Planquart et al., 1999). In addition, mixing biosolids and other organic wastes with large C/N ratios (such as green wastes) can reduce the rate of nitrogen leaching (McKay and Moffat, 2001). Therefore, compost presents better agronomic potential than biosolids.

Compost amendment has been frequently shown to increase soil fertility (Caravaca et al., 2002; Martinez et al., 2003), plant biomass (Guerrero et al., 2001) and nutrition (Moreno et al., 1996). As a consequence, it could lead to a high nutrient content litter and enhance litter breakdown. Litter breakdown is the principal pathway of nutrients' return to soil in a form available to plants (Kavvadias et al., 2001). This aspect is especially important on Mediterranean nutrient-poor soils where plant communities rely, to a great extent, on the recycling of litter nutrients (Cañellas and San Miguel, 1998). Factors contributing to the litter breakdown are: soil fertility, litter quality and supply and climatic conditions (Kavvadias et al., 2001).

The litter breakdown process involves three types of organisms: invertebrates, fungi and bacteria. The crucial role of microorganisms is clearly established, and consecutive changes in fungal and bacterial biomass dynamics are a useful way to investigate the impact of factors controlling leaf breakdown (Gessner and Chauvet, 1994; Isidorov and Jdanova, 2002). Moreover, the soil microbial biomass is often regarded as an early indicator of changes which may occur in the long term with regard to soil fertility. Likewise, Wardle et al. (1999) showed that microbial biomass responds to addition of fertilizers and of organic residues.

Most studies deal with total microbial biomass (fumigation-incubation, fumigation-extraction, substrate induced respiration and ATP methods; Martens, 1995), i.e. bulked fungi and bacteria (Borken et al., 2002; Khan and Scullion, 2002; Kunito et al., 2001).

However, the respective roles of bacteria and fungi in the litter breakdown process are different. Fungi are able to decompose and assimilate as refractory compounds as lignin or tanins (Criquet, 1999), although bacteria are not thought to assume notable importance before the leaf material has been partially broken down and decomposed by fungi (Jensen, 1974). Thus, it is of great interest to study separately their reactions to compost amendment.

In this study, bacterial and fungal biomass were determined on kermes oak (*Quercus coccifera* L.) leaf litter on a Mediterranean burnt area amended with sewage sludge and greenwaste compost. Kermes oak is one of the most important shrub species in the Mediterranean basin, where it covers more than 2 Mha and accounts generally for 60–70% of the total litter (Cañellas and San Miguel, 1998).

Our objectives were to (i) determine the effects of compost amendment on kermes oak leaf litter colonization by bacteria and fungi, (ii) offset the drastic Mediterranean climatic conditions (e.g. drought) against the potential improvement of soil fertility by compost, (iii) provide comprehensive data on leaf litter breakdown in terrestrial Mediterranean ecosystems by separately quantifying fungal and bacterial biomass.

2. Material and methods

2.1. Study site and experimental design

The experiment was carried over 6000 m² on the plateau of Arbois (Southern Provence, France; 5°18'6"E–43°29'10"N in WSG-84 Geodetic system) at 240 m above sea level and under Mediterranean climatic conditions (Fig. 1). The soil was a silty-clayey chalky rendzina, with a high percentage of stones (77%) and a low average depth (24 cm). The last fire occurred in June 1995 and the site was colonised by typical Mediterranean sclerophyllous vegetation, with 70% total cover; *Q. coccifera* L. and *Brachypodium retusum* Pers. being the two dominant species. This natural vegetation belongs to the holm oak (*Quercus ilex* L.) succession series.

Compost was surface applied in January 2002. The experimental design was a complete randomised block of twelve plots of 500 m². Four plots did not receive any compost (D0 = control), four received 50 t ha⁻¹

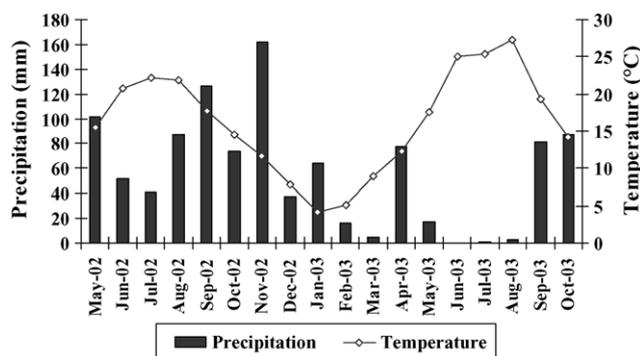


Fig. 1. Mean air temperature and precipitation from June 2002 to May 2003 (Météo France).

(D50), and four 100 t ha⁻¹ (D100). The compost was produced by a local company (Biotechna, Ensues, Southern Provence) and is certified conform to the French norm on composts made with materials of water treatment origin (NF U 44-095, 2002). This compost was made with greenwastes (1/3 volume), pine barks (1/3 volume), and local municipal sewage sludge (1/3 volume). The mixture was composted for 30 days (windrows submitted to forced ventilation, Beltsville method) at 75 °C to kill pathogenic microorganisms and decompose phytotoxic substances, and then sieved (<20-mm mesh) to remove large bark pieces and stored in windrows. The

windrows were mixed several times over the next 6 months to promote organic matter humification. The final compost met the French legal standards for pathogenic microorganisms, organic trace elements and heavy metals (order of the 08/01/1998 on sewage sludge use in agriculture). Soil and compost characteristics are shown in Table 1.

2.2. Field procedures

Humiferous episolium was sampled nine times superficially from June 2002 to October 2003, and 2-mm sieved. The fraction >2 mm was classed as litter

Table 1
Soil (0–24 cm: maximal depth; N = 12) and compost (N = 3) physico-chemical characteristics

Parameter	Soil		Compost	
	Mean (S.E.)	Allowed French limit value before sewage sludge amendment	Mean (S.E.)	Allowed French limit value (08/01/1998)
pH _{H₂O}	7.34 (0.008)		7.7 (0.05)	
Humidity (% FM)			4.8 (0.29)	
CEC (meq. 100 g ⁻¹)	23.12 (0.31)			
Total calcareous (% DM)	4.17 (0.13)			
OM (% DM)	7.58 (0.12)		46.8 (2.74)	
Total N (% DM)	0.36 (0.005)		2.03 (0.03)	
C/N	12.42 (0.09)		13.4 (0.78)	
Total P (% DM)	0.037 (0.001)		3.24 (0.03)	
Exchangeable P (mg kg ⁻¹ DM)	23.3 (0.35)		2514.8 (7.82)	
Copper (mg kg ⁻¹ DM)	19.8 (0.14)	100	144.1 (0.84)	1000
Zinc (mg kg ⁻¹ DM)	78.2 (0.24)	300	265.0 (5.49)	3000
Cadmium (mg kg ⁻¹ DM)	0.31 (0.002)	2	0.8 (0.0)	15
Chrome (mg kg ⁻¹ DM)	67.3 (0.33)	150	27.1 (0.65)	1000
Mercury (mg kg ⁻¹ DM)	0.06 (0.001)	1	0.86 (0.06)	10
Nickel (mg kg ⁻¹ DM)	45.3 (0.17)	50	16.5 (0.23)	200
Lead (mg kg ⁻¹ DM)	43.1 (0.26)	100	57.3 (2.53)	800

DM: dry mass; FM: fresh mass.

fraction ($O-h^{>2}$). Chemical analyses were performed on this coarse mixed litter. Each analysed sample was a mix of three samples randomly collected on each plot. Kermes oak entire leaf litter, on which leaf litter breakdown is the most efficient (Toutain, 1987), was separated from the $O-h^{>2}$ samples to determine microbial biomass. Green leaves were also collected on three bushes per plot to analyse N and P concentrations, at maximum *Q. coccifera* litterfall in late May (2002 and 2003) (Cañellas and San Miguel, 1998).

2.3. Laboratory procedures

Fungal colonization of litter was estimated using the ergosterol concentration, taking advantage of its recent improvement. Ergosterol is a fungal indicator that offers an efficient measure of living fungal biomass (Gessner et al., 1991; Gessner and Schmitt, 1996; Cortet et al., 2003). Fifty milligrams of leaf litter were roughly crushed and lyophilized. Ergosterol was extracted from leaf litter by 30 min refluxing in alcohol base (Gessner et al., 1991) and purified by solid-phase extraction (Gessner and Schmitt, 1996). Final purification and quantification of ergosterol was achieved by high performance liquid chromatography (HPLC; HP series 1050 chromatograph). The system was run with HPLC-grade methanol at a flow rate of 1.5 ml min^{-1} . Ergosterol eluted after 9 min and was detected at 282 nm; peak identity was checked on the basis of retention times of commercial ergosterol (Fluka®; >98% purity).

Bacterial colonization was determined by counting numbers of bacteria following the general protocol of Porter and Feig (1980) modified by Schallenberg et al. (1989). Samples of fresh litter were stored in 2% formalin before analysis. Bacteria were detached from entire leaves by 2 min probe sonication, according to Buesing and Gessner (2002). Then, 4',6-diamidino-2-phenylindole (DAPI) at a concentration of 5 mg l^{-1} (Baldy et al., 1995) was added for DNA staining. Finally, numbers of bacteria were counted by epifluorescence microscopy.

Soil, compost (<4 mm) and $O-h^{>2}$ chemistry (total N, total and exchangeable P, total Cu and Zn) were determined using standard procedures (AFNOR, 1999). Exchangeable P was measured by the Olsen method (Olsen et al., 1954). Humidity of *Q. coccifera*

leaf litter was measured by oven drying samples at 60°C during 3 days.

Green leaves of *Q. coccifera* were washed with demineralised water, oven dried at 40°C and crushed (2 mm mesh) in a trace metal free grinder (FOSS TECATOR Sample Mill 1093 Cyclotec). Foliar N concentration was determined according to Masson and Andrieu (1996), modified. Samples (250 mg) were digested in H_2SO_4 and H_2O_2 at 400°C during 3 h (Bioblock Scientific Digestion Unit 10401). Then, the solutions were diluted 500 times, filtered at $0.45 \mu\text{m}$ and analysed by ion chromatography (Dionex DX120). The eluent used was 26 mmol l^{-1} methan sulfonic acid. Foliar P concentration was measured by atomic absorption spectrometry (VARIAN VISTA Radial) after digestion in aqua regia.

2.4. Statistical analyses

Two-way ANOVAs combined with Tukey tests (Zar, 1984) were used to make comparisons of the different parameters (ergosterol, bacterial numbers, litter and green leaves chemistry) according to the two studied factors (sampling date and compost rate). Previously, normality and homocedasticity were verified by Shapiro-Wilks and Bartlett tests, respectively (Zar, 1984). Data were ln-transformed when test assumptions were not met (i.e. bacterial numbers). To relate microbial biomass and chemical characteristics of leaf litter, linear regressions were performed. Significant level was considered to be 95%. The software Statgraphics plus (version 2.1: Statistical Graphics Corporation©, Copyright 1994–1996) and Minitab (release 13 for Windows, 2000, Minitab Inc., USA) were used.

3. Results

3.1. *Q. coccifera* green leaves at maximal litterfall and coarse mixed litter ($O-h^{>2}$)

N and P concentrations in green leaves increased significantly after compost amendment (Table 2, Fig. 2). There was no difference between the two compost rates for P, whereas N concentration in green leaves was higher in D100 than D50 for both years (2002, 2003). Moreover, N concentration in green

Table 2

Results of two-way ANOVA on N and P foliar concentrations in green leaves of *Quercus coccifera* at maximal litterfall (late May) and on O-h^{>2} parameters (only statistically significant results are reported)

Parameter	Factor	F	p	Tukey's test
<i>Q. coccifera</i> N concentration in green leaves at maximal litterfall	Year	4.88	0.0403	May02 ^a , May03 ^b
	Rate	23.55	<0.001	D0 ^a , D50 ^b , D100 ^c
<i>Q. coccifera</i> P concentration in green leaves at maximal litterfall	Year	8.66	0.0123	May02 ^a , May03 ^b
	Rate	4.13	0.0433	D0 ^a , D50 ^{ab} , D100 ^b
<i>Q. coccifera</i> litter humidity	Date	145.43	<0.001	June02 ^{bc} , July02 ^d , Oct02 ^e , Dec02 ^f , Mar03 ^e , Apr03 ^d , June03 ^{ab} , July03 ^a , Oct03 ^c , D0 ^a , D50 ^b , D100 ^b
	Rate	10.8	<0.001	
O-h ^{>2} exchangeable P	Year	53.77	<0.001	2002 ^b , 2003 ^a
	Rate	115.46	<0.001	D0 ^a , D50 ^b , D100 ^b
O-h ^{>2} total P	Year	0.54	0.464	2002 ^a , 2003 ^a
	Rate	117.6	<0.001	D0 ^a , D50 ^b , D100 ^b
O-h ^{>2} total N	Year	30.06	<0.001	2002 ^a , 2003 ^b
	Rate	202.2	<0.001	D0 ^a , D50 ^b , D100 ^b
O-h ^{>2} total Cu	Year	46.08	<0.001	Mar02 ^a , Mar03 ^b
	Rate	58.52	<0.001	D0 ^a , D50 ^b , D100 ^b
O-h ^{>2} total Zn	Date	31.53	<0.001	Mar02 ^a , Mar03 ^b
	Rate	20.69	<0.001	D0 ^a , D50 ^b , D100 ^b

Results of the comparison are given by an exponent letter: values that do not differ at the 0.05 level are noted with the same letter (a < b < c). D0: control plots; D50: plots amended with 50 t ha⁻¹ of compost and D100: plots amended with 100 t ha⁻¹ of compost.

leaves increased significantly from May 2002 to May 2003, respectively, 5 months and 1.5 years after compost amendment (Table 2, Fig. 2). P concentration in green leaves decreased significantly from 2002 to 2003 (Table 2, Fig. 2).

Compost amendment significantly increased the following physical and chemical parameters of O-h^{>2} (Table 2): O-h^{>2} humidity (Fig. 3), exchangeable P (Fig. 3), total N (0.92, 1.58, and 1.67% DM, for D0, D50 and D100, respectively, means of the nine

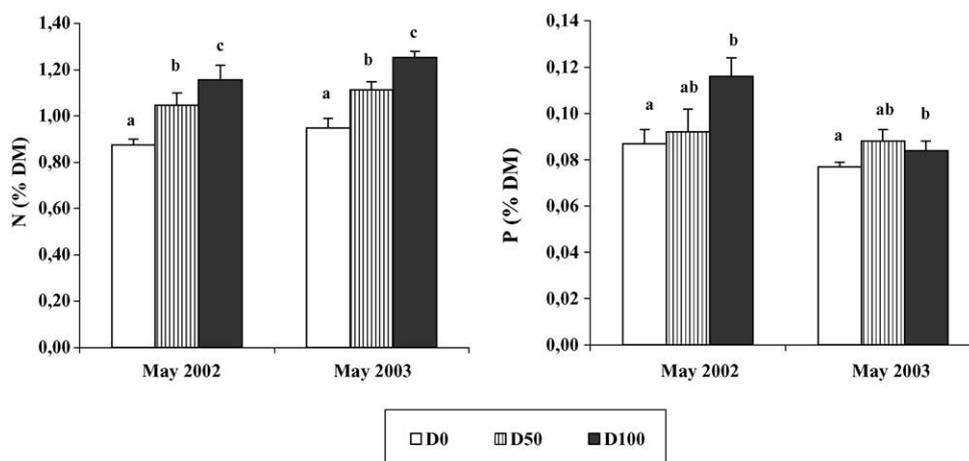


Fig. 2. N and P foliar concentrations in green *Quercus coccifera* leaves at maximum litterfall (late May 2002 and 2003). Bars denote S.E. (N = 4). D0: control plots; D50: plots amended with 50 t ha⁻¹ of compost and D100: plots amended with 100 t ha⁻¹ of compost. Results of the comparison are given by a letter: values that do not differ at the 0.05 level are noted with the same letter (a < b < c).

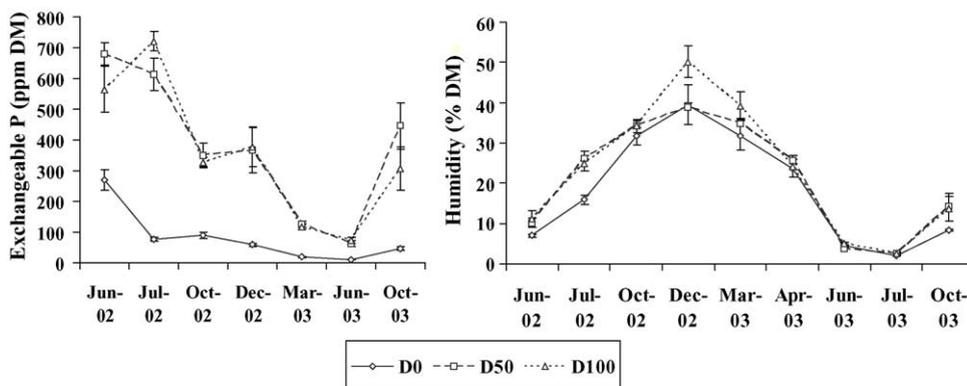


Fig. 3. Exchangeable P concentration (in ppm of dry mass) and humidity (in % of dry mass) in the O-h^{>2} from June 2002 to October 2003. Bars denote S.E. ($N=4$). D0: control plots; D50: plots amended with 50 t ha⁻¹ of compost and D100: plots amended with 100 t ha⁻¹ of compost.

sampling dates), total Cu (8.75, 75.4 and 69.8 ppm DM, means of the 2 sampling years), and total Zn (50.9, 137.6 and 139.4 ppm DM, means of the 2 sampling years). However, there was no significant difference between the two compost rates (D50 and D100) for these parameters.

Sampling year had also a significant effect on O-h^{>2}. Total N and exchangeable P concentrations in O-h^{>2} had similar dynamics to N and P in green leaves from 2002 to 2003 (Table 2). Moreover, N and P concentrations in O-h^{>2} green leaves were positively correlated with concentrations in green leaves ($R = 0.45$, $p = 0.026$ and $R = 0.64$, $p = 0.004$, respectively, for N and P). Total N in O-h^{>2} increased from 1.3% DM in 2002 (mean of the three treatments) to 1.5% DM in 2003, whereas exchangeable P decreased from 859 ppm DM in 2002 (mean of the three treatments) to 309 ppm DM in 2003 (Fig. 3). Total P in

O-h^{>2} remained similar (Table 2) from 1 year (2002, 0.08% DM) to another (2003, 0.09% DM).

Humidity of O-h^{>2} was lowest during the summer 2003 (Fig. 3). Spring, summer, and autumn 2003 were exceptionally dry (Fig. 1), and as a consequence, October 2003 values were found to be similar to summer 2002 values.

3.2. Microbial biomass of kermes oak leaf litter

Compost amendment had a significant effect on leaf litter colonization by fungi, whereas it did not affect bacterial numbers (Fig. 4, Table 3). Compost at highest rate decreased overall the ergosterol concentration in *Q. coccifera* leaf litter compared to control (159 and 194 $\mu\text{g g}^{-1}$ DM in D100 and D0, respectively, mean over the studied period). Ergosterol concentration for D50 rate (189 $\mu\text{g g}^{-1}$ DM, mean

Table 3

Results of two-way ANOVA on ergosterol concentration and bacterial numbers associated with kermes oak leaf litter

Parameter	Factor	F	p	Tukey's test
Ergosterol	Date	10.55	<0.001	June02 ^{bc} , July02 ^b , Oct02 ^{dc} , Dec02 ^f , Mar03 ^{ef}
	Rate	5.54	0.0056	Apr03 ^{bcd} , June03 ^{cde} , July03 ^a , Oct03 ^{dc}
	Date \times rate	1.2	0.2845	D0 ^a , D50 ^a , D100 ^b
Number of bacteria	Date	4.52	0.0002	June02 ^{ab} , July02 ^a , Oct02 ^{bcd} , Dec02 ^{abc} , Mar03 ^{cd}
	Rate	0.9	0.4096	Apr03 ^d , June03 ^{ab} , July03 ^a , Oct03 ^{ab}
	Date \times rate	0.98	0.4852	D0 ^a , D50 ^a , D100 ^a

Results of the comparison are given by an exponent letter: values that do not differ at the 0.05 level are noted with the same letter ($a < b < c$). D0: control plots; D50: plots amended with 50 t ha⁻¹ of compost and D100: plots amended with 100 t ha⁻¹ of compost.

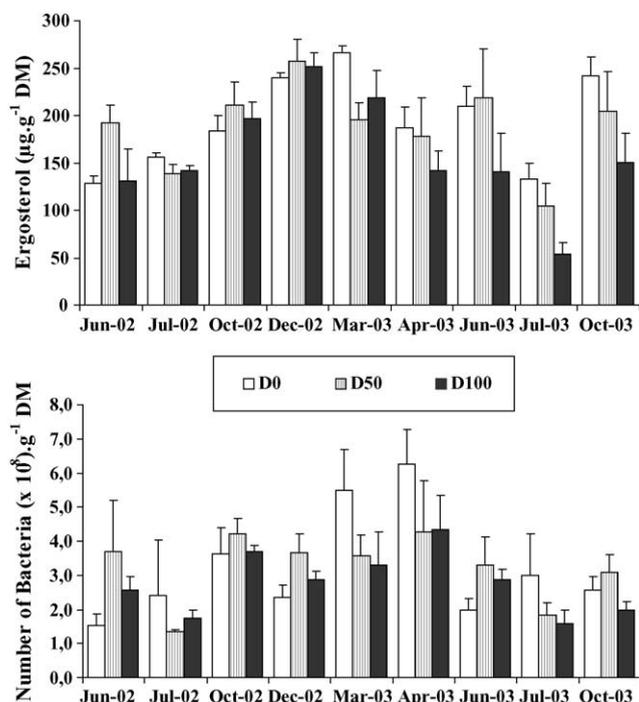


Fig. 4. Dynamics of ergosterol concentrations (in μg of ergosterol per gram of dry mass) and bacterial numbers (per gram of dry mass) associated with leaf litter of *Quercus coccifera* decomposing in control plots (D0), plots amended with 50 t ha^{-1} (D50) and plots amended with 100 t ha^{-1} (D100) of compost. Bars denote S.E. ($N = 4$).

over the studied period) was similar to control. Moreover, ergosterol was negatively correlated to exchangeable phosphorus in $\text{O-h}^{>2}$. However, neither bacterial number nor ergosterol are correlated to total P, N, Cu or Zn concentrations (Table 4).

Both ergosterol concentration and bacterial number of leaf litter varied significantly according to sampling date within a year. The lowest values corresponded to summers (June and July, both years), when drought is

maximum under Mediterranean climatic conditions (Fig. 1). In addition, both bacterial and fungal colonisation were positively correlated with $\text{O-h}^{>2}$ humidity, particularly ergosterol (Table 4). As $\text{O-h}^{>2}$ humidity (month n) is positively correlated ($R = 0.76$, $p = 0.03$) to precipitation (month $n-1$), the highest ergosterol values corresponded to the highest rainfall of the month before.

Table 4

Matrix of Pearson correlation analysis between ergosterol, numbers of bacteria and $\text{O-h}^{>2}$ parameters

	Ergosterol	Bacterial numbers
$\text{O-h}^{>2}$ humidity	$R = 0.44^{***}$	$R = 0.25^*$
$\text{O-h}^{>2}$ exchangeable P	$R = -0.31^*$	$R = -0.13$
$\text{O-h}^{>2}$ total P	$R = -0.21$	$R = -0.02$
$\text{O-h}^{>2}$ total N	$R = -0.16$	$R = 0.04$
$\text{O-h}^{>2}$ total Cu	$R = -0.39$	$R = -0.05$
$\text{O-h}^{>2}$ total Zn	$R = -0.38$	$R = -0.001$

* Significant ($0.05 < p < 0.01$).

*** Highly significant ($p < 0.001$).

4. Discussion

4.1. Microbial biomass of kermes oak leaf litter

Compost amendment significantly decreased kermes oak leaf litter fungal colonization at 100 t ha^{-1} rate whereas it had no effect on bacterial colonization. This shows the importance of studying separately fungi and bacteria in order to determine precisely the response of microbial communities after compost amendment. In our study, the two groups of microorganisms exhibited

different reactions. The decrease in ergosterol concentrations found within D100 amended plots differs from numerous studies which have found enhancing effects of organic amendments on soil microbial biomass (Albiach et al., 2000; Kunito et al., 2001; Ros et al., 2003). However, the ergosterol concentrations observed on *Q. coccifera* leaf litter of D100 plots remained similar to those of the rare studies that have attempted to quantify separately bacterial and fungal colonization of Mediterranean litter or soil (Barajas-Aceves et al., 2002; Cortet et al., 2003).

Conversely, litter humidity highly improved *Q. coccifera* leaf litter colonization. *Q. coccifera* leaf litter moisture was positively correlated both to ergosterol concentrations and bacterial numbers, and fungi were more affected than bacteria. Thus, microbial colonization was lowest during dry months (June and July) in both years, and 2003 values were lower than 2002 values due to exceptional drought. This is in accordance with previous works, such as Papatheodorou et al. (2004) who reported seasonal patterns for bacterial richness in soil. Likewise, Criquet et al. (2000) and Barajas-Aceves et al. (2002) found that fungal biomass reaches maximum values under humidity conditions in Mediterranean areas.

4.2. *Q. coccifera* green leaves at maximal litterfall and coarse mixed litter ($O-h^{>2}$)

The compost significantly increased the moisture of *Q. coccifera* leaf litter in amended plots. This result is in contradiction with the depreciating effect of the compost on fungal colonization. Thus, some changes induced by compost amendment may have had strong enough decreasing effects on fungal colonization of kermes oak leaf litter to annul the increasing moisture effect, even if drought is one of the major limiting factors in Mediterranean areas.

Total N concentration significantly increased in amended plots (D50 and D100) in kermes oak green leaves and in $O-h^{>2}$. These two parameters are positively correlated ($R = 0.45$), which suggests that the N increase in green leaves is involved in the $O-h^{>2}$ total N enrichment. This species generally provides 60–70% of the total litter in garrigue ecosystems (Cañellas and San Miguel, 1998). As nitrogen is frequently a limiting nutrient in Mediterranean ecosystems (Archibold, 1995), an increase in $O-h^{>2}$

amended plots improves microbial biomass (Berg and Söderström, 1979). Moreover, the N enrichment of *Q. coccifera* litter may decrease its C/N ratio, as well as its polyphenol concentration, leading to an easier breakdown (Gosz, 1984). However, in our experiment, no significant positive correlation was found between microbial colonization of kermes oak litter and total N concentration in $O-h^{>2}$. In contrast to the literature, we observed the highest depletion of fungal biomass when total N in $O-h^{>2}$ was the highest: 2 years after amendment (2003), in D100. Therefore, it is very likely that N accumulates in $O-h^{>2}$ consequently to fungal biomass decrease in D100 plots.

The reduction of fungal colonization at D100 could be the result of a decrease in another limiting factor in Mediterranean ecosystems (Archibold, 1995): P is mostly unavailable, because it is associated with calcium in inorganic forms on calcareous soils (Khanna and Ulrich, 1984). Thirukkumaran and Parkinson (2002) reported that microbial variables were unaffected by N addition, whereas an increase of substrate induced respiration (SIR) was found with P fertilization. In addition, Kwabiah et al. (2003) found that phosphorus is the most important quality factor affecting microbial biomass. But, this explanation is not confirmed by our results. Compost amendment led to a marked increase in exchangeable P in $O-h^{>2}$, as a result of an increase in P concentration in *Q. coccifera* green leaves ($R = 0.64$). However, exchangeable P in $O-h^{>2}$ was negatively correlated to fungal colonization ($R = -0.31$).

In our study, compost amendment induced significant increases in $O-h^{>2}$ total copper and zinc concentrations. As heavy metals are known to affect growth, morphology and metabolism of microorganisms in soils (Dai et al., 2004), Cu and Zn increases could have decreased the fungal colonization of leaf litter collected on plots amended with 100 t ha^{-1} . However, neither bacterial numbers, nor ergosterol content of leaf litter were correlated to Cu and Zn total concentrations. On the one hand, total heavy metal concentrations do not reflect exactly their biological effects and available concentrations in the soil have to be taken into account. On the other hand, Cu accumulates more in plant roots (Van Den Driessche, 1984) than in shoots. Thus, the *Q. coccifera* leaf litter may have been slightly contaminated by this element, explaining the absence of Cu effect on microbial

colonization. However, Zn (as Cd, Hg and Ni) is a metal reported to be toxic to microorganisms at lower levels than other metals (Kabata-Pendias and Pendias, 1992). Moreover, this element is considered to be readily soluble relative to the other heavy metals in soils (Kabata-Pendias and Pendias, 1992). Thus, Zn in available form could have reduced fungal colonization of *Q. coccifera* leaf litter in D100. However, bacteria are known to be less resistant to heavy metals than fungi (Dai et al., 2004) and are not affected by compost amendment in our study. Therefore, the hypothetical negative effect of Zn on fungal colonization of kermes oak litter must be put in perspective. Moreover, it is not easy to predict microbial consequences of soil pollution without determining microbial diversity changes under heavy metal contamination, according to species relative sensibility (Dai et al., 2004).

Thus, fungal biomass was decreased at D100 rate despite an increase of humidity, N and P concentrations of O-h^{>2} after compost amendment. We hypothesize that amendment led to a redistribution of fungi from O-h^{>2} to O-h^{<2}. The compost used in this study contained 57% of sewage sludge (<2 mm fraction), which presents a higher decomposability (rich in low-molecular dissolved organic carbon and in salts, Agassi et al., 1998) than O-h^{>2} (containing leaves, rich in inhibiting molecules as polyphenols, especially in the Mediterranean region, Gershenson, 1984). This would induce an accumulation of O-h^{>2} litter and could explain the total N increase that occurred in O-h^{>2} of D100 amended plots from 2002 to 2003. Likewise, Borken et al. (2002) showed that compost could induce some redistribution of microbes from one fraction to another. They noted that after compost application, microbial biomass decreased in the organic horizons (O-h^{>2} and O-h^{<2}), whereas it increased in the mineral soil, the latter being enriched by nutrients released from organic horizon.

5. Conclusion

In conclusion, compost at D100 induced significant decrease in colonization of *Q. coccifera* leaf litter by fungi, especially the second year after amendment. Compost had no significant effect on litter colonization by bacteria. Furthermore, compost at D100

increased O-h^{>2} humidity, as well as N and P concentrations, which should have improved the decomposition of O-h^{>2} OM. Compost Zn enrichment could be responsible for the observed fungal biomass decrease, but it should have affected bacteria as well. Thus, we hypothesize a redistribution of fungi from refractory components of O-h^{>2} to easily decomposable O-h^{<2}. This could be responsible for leaf litter N accumulation in O-h^{<2}. Therefore, it would be of great interest to study the microbial colonization of O-h^{<2} and mineral soil.

Acknowledgements

This research was supported by the Conseil Général des Bouches-du-Rhône, the ADEME (Agence De l'Environnement et de la Maîtrise de l'Energie) and the Agence de l'Eau Rhône-Méditerranée-Corse. We thank the two reviewers for their interesting remarks, and Mr. Michael Paul for English improvement.

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