Compost amended on a degraded Mediterranean soil: effect on microbial colonization of kermes oak leaf litter

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Abstract

Terrestrial Mediterranean ecosystems are characterized by low water and organic matter soil content, which become worse with recurrent fires. Biosolids amendment could be a way to facilitate ecosystem resilience. Leaf litter decomposition is a vital process in the functioning of terrestrial ecosystems and can be studied through fungi and bacteria. We determined fungal and bacterial biomass associated with decomposing kermes oak leaves in a burnt shrub ecosystem for every season over 1.5 years. Three treatments were studied: control, 50t.ha⁻¹ and 100t.ha⁻¹ of co-composted sewage sludge and green wastes. The results showed that bacterial numbers were not affected by organic amendment, although fungal biomass was depressed on plots amended with 100t.ha⁻¹. However, both types of micro-organisms followed a marked seasonal dynamics, with peaks of biomass during the wet periods of the year.

Keywords: Mediterranean ecosystem, sewage sludge compost, leaf litter, decomposition, ergosterol, bacterial numbers, Quercus coccifera L.

1 Introduction

Soils under Mediterranean climate are undergoing degradations due to water erosion and recurrent fires, which affect their fertility [1]. Guerrero et al [2] pointed out that compost addition is a suitable technique for accelerating the natural recovery process of burned soils. Indeed, biosolids can improve soil physical, chemical and biological properties [3]. Composts with large C/N ratio
are preferentially used for their better organic matter stability compared to sewage sludge.

Litter decomposition is the principal pathway of the nutrients return to the soil in an available form to plants. In Mediterranean ecosystems, the role of litter decomposition in nutrient cycling becomes more important as vegetation and soil are degraded by fires [4].

The litter decomposition process involves three types of organisms: invertebrates, fungi and bacteria. The critical role of microorganisms is clearly established, and consecutive changes in fungal and bacterial biomass dynamics are a useful way to approach the impact of factors controlling leaf decomposition [5]. Microbial biomass responds to addition of fertilizers manipulation and of organic residues [6].

In this study, bacterial and fungal biomasses were determined on *Quercus coccifera* L. leaf litter. Indeed kermes oak is one of the most important shrub species in Mediterranean basin, where it covers more than two million hectares and accounts generally for 60-70% of the total litter [7].

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

## 2 Material and methods

### 2.1 Study site and experimental design

The experiment was carried out on 6000 m$^2$ in the plateau of Arbois (South Provence, France), at 240 m above sea level and under Mediterranean climatic conditions (Figure 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 a 70% total cover, *Quercus coccifera* L. and *Brachypodium ramosum* L. being the two dominant species. This natural vegetation belongs to the hoalm oak (*Quercus ilex*) succession series.

Compost was surface applied in January 2002. The experimental design was a complete randomised block of twelve 500 m$^2$ plots. Four plots had not received any compost (D0=control), four received 50 t.ha$^{-1}$ (D50) and four received 100 t.ha$^{-1}$ (D100). The compost was produced by Biotechna (Ensuès, South Provence, France) and is certified conform to the NF U 44-095 norm [8] on composts made with materials of water treatment origin. 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 d at 75°C to kill pathogenic microorganisms and decompose phytotoxic substances, and then sieved (<20-mm mesh). The swathes were turned (mixed) several times in the next 6 mo to promote organic matter humification.

Soil and compost characteristics are shown in Table 1.
Figure 1: Mean air temperature and precipitations from June 2002 to May 2003 (Météo France).

Table 1: Soil (N=12) and compost (N=3) physico-chemical characteristics. Maximum depth=25cm. DM: dry matter. FM: fresh mass.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Soil characteristics</th>
<th>Compost characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH&lt;sub&gt;H2O&lt;/sub&gt;</td>
<td>7.34 (0.008)</td>
<td>7.7 (0.05)</td>
</tr>
<tr>
<td>Humidity (% FM)</td>
<td>4.8 (0.29)</td>
<td>4.8 (0.29)</td>
</tr>
<tr>
<td>CEC (meq.100 g&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>23.12 (0.31)</td>
<td></td>
</tr>
<tr>
<td>OM (% DM)</td>
<td>7.58 (0.12)</td>
<td>46.8 (2.74)</td>
</tr>
<tr>
<td>total N (% DM)</td>
<td>0.36 (0.005)</td>
<td>2.03 (0.03)</td>
</tr>
<tr>
<td>C/N</td>
<td>12.42 (0.09)</td>
<td>13.4 (0.78)</td>
</tr>
<tr>
<td>total P (% DM)</td>
<td>0.037 (0.001)</td>
<td>3.24 (0.03)</td>
</tr>
<tr>
<td>Exchangeable P (ppm)</td>
<td>23.3 (0.35)</td>
<td>2514.8 (7.82)</td>
</tr>
<tr>
<td>Copper (mg.kg&lt;sup&gt;-1&lt;/sup&gt;DM)</td>
<td>19.8 (0.14)</td>
<td>100</td>
</tr>
<tr>
<td>Zinc (mg.kg&lt;sup&gt;-1&lt;/sup&gt;DM)</td>
<td>78.2 (0.24)</td>
<td>300</td>
</tr>
<tr>
<td>Cadmium (mg.kg&lt;sup&gt;-1&lt;/sup&gt;DM)</td>
<td>0.31 (0.002)</td>
<td>2</td>
</tr>
<tr>
<td>Chrome (mg.kg&lt;sup&gt;-1&lt;/sup&gt;DM)</td>
<td>67.3 (0.33)</td>
<td>150</td>
</tr>
<tr>
<td>Mercury (mg.kg&lt;sup&gt;-1&lt;/sup&gt;DM)</td>
<td>0.06 (0.001)</td>
<td>1</td>
</tr>
<tr>
<td>Nickel (mg.kg&lt;sup&gt;-1&lt;/sup&gt;DM)</td>
<td>45.3 (0.17)</td>
<td>50</td>
</tr>
<tr>
<td>Lead (mg.kg&lt;sup&gt;-1&lt;/sup&gt;DM)</td>
<td>43.1 (0.26)</td>
<td>100</td>
</tr>
</tbody>
</table>

* Allowed French limit values in soil and in sewage sludges before amendment (08/01/1998).

2.2 Field procedures

Leaf litter was sampled superficially from June 2002 to October 2003, as in the superficial layer leaf litter decomposition is the most efficient [9]. Analysis were performed on this coarse mixed litter (O horizon > 2mm = O-h<sup>2</sup>). Each analysed
sample was a mix of three samples randomly collected on each plot. Kermes oak entire leaf litter 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 content, at maximal Quercus coccifera litterfall in late may (2002 and 2003) [7].

2.3 Laboratory procedures

Fungal colonization of litter has been detected using ergosterol, taking advantage of its recent development. Ergosterol is a fungal indicator which offers an efficient measure of living fungal biomass [10]. Analysis were performed with 50 mg of roughly crushed and lyophilized leaf litter. Ergosterol was extracted from leaf litter by 30 min refluxing in alcoholic base [10] and purified by solid-phase extraction [11]. Final purification and quantification of ergosterol was achieved by high performance liquid chromatography (HPLC, at 282 nm).

Bacterial colonization was determined by counting numbers of detached bacteria by epifluorescence microscopy after staining with 4',6-diamidino-2-phenylindole (DAPI) following the general protocol of Porter and Feig [12], but using DAPI at a concentration of 5 mg l⁻¹ [13]. Bacteria were detached from entire leaves by 2 min probe sonication, according to Buesing and Gessner [14]. Samples were stored in 2% formalin before analysis.

Standard procedures were employed to analyse soil, compost (4 mm screened) and O-h⁻² chemistry: pH H₂O, total C and organic matter, total N by Dumas’ method, total P, cations (Mg, Ca, K, Na), oligoelements (B, Fe, Cu, Zn, Mn), trace metals (Cd, Cr, Ni, Pb, Hg), and exchangeable P by Olsen method. In O-h⁻², humidity was performed by drying samples at 60°C for 3 days.

Foliar N content in green Quercus coccifera leaves was determined according to Masson and Andrieu [15] modified. The foliar material was washed with demineralised water, oven dried at 40°C and 2mm mesh crushed. Samples (250 mg) were digested in H₂SO₄ and H₂O₂ at 400°C during 3 hours and N foliar content was measured by ion chromatography (Dionex DX120).

3 Results

3.1 Effects of compost amendment and season on microbial biomass associated with kermes leaf litter

Compost amendment has a significant effect on leaf litter colonization by fungi, but bacterial numbers were not affected (Figure 2, Table 2). Indeed, compost at maximal rate (D100, 159 µg.g⁻¹ DM) decreases ergosterol content in Quercus coccifera leaf litter in comparison with control (D0, 194 µg.g⁻¹ DM) which was similar to medium rate (D50, 189 µg.g⁻¹ DM).

In addition, both ergosterol content and bacterial numbers of leaf litter change significantly according to sampling date. The lowest values correspond to summer (June and July, both years), when drought is maximal under Mediterranean climate (Figure 1). Ergosterol content and numbers of bacteria do not change significantly from one year to another (Tukey’s test, p=0.4478 and p=0.1794 respectively).
Figure 2: Dynamics of ergosterol concentrations and bacterial numbers associated with leaf litter of Quercus coccifera decomposing in control plots (D0), plots amended with 50 t.ha\(^{-1}\) (D50) and with 100 t.ha\(^{-1}\) (D100) of compost. Bars denote SD (N=4).

Table 2: Results of two way Anova on ergosterol concentration and bacterial numbers colonising kermes oak leaf litter. 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. D0: control plots; D50: plots amended with 50 t.ha\(^{-1}\) of compost and D100: plots amended with 100 t.ha\(^{-1}\) of compost.

<table>
<thead>
<tr>
<th>parameter</th>
<th>factor</th>
<th>F</th>
<th>p</th>
<th>Tukey's test</th>
</tr>
</thead>
<tbody>
<tr>
<td>ergosterol</td>
<td>date</td>
<td>10.55</td>
<td>&lt;0.001</td>
<td>June02(^{bc}) July02(^{b}) Oct02(^{ab}) Dec02(^{cd}) Mar03(^{c}) Apr03(^{bc}) June03(^{bd}) July03(^{a}) Oct03(^{cd})</td>
</tr>
<tr>
<td>rate</td>
<td>date</td>
<td>5.54</td>
<td>0.0056</td>
<td>D0(^{a}) D50(^{a}) D100(^{b})</td>
</tr>
<tr>
<td></td>
<td>date x rate</td>
<td>1.2</td>
<td>0.2845</td>
<td></td>
</tr>
<tr>
<td>number of bacteria</td>
<td>date</td>
<td>4.52</td>
<td>0.0002</td>
<td>June02(^{ab}) July02(^{b}) Oct02(^{bcd}) Dec02(^{abcd}) Mar03(^{cd}) Apr03(^{b}) June03(^{ab}) July03(^{a}) Oct03(^{ab})</td>
</tr>
<tr>
<td></td>
<td>rate</td>
<td>0.9</td>
<td>0.4096</td>
<td>D0(^{a}) D50(^{a}) D100(^{a})</td>
</tr>
<tr>
<td></td>
<td>date x rate</td>
<td>0.98</td>
<td>0.4852</td>
<td></td>
</tr>
</tbody>
</table>
3.2 Compost effects on chemical composition of O-h\textsuperscript{>2} and on N content of *Quercus coccifera* green leaves at maximal litterfall

Compost amendment increases significantly the following physico-chemical parameters (Tukey’s test, p<0.001 for all parameters): N content in green leaves (0.9, 1.1 and 1.2 %DM respectively for D0, D50 and D100), O-h\textsuperscript{>2} humidity (18.3, 21.2 and 22.8 %DM), exchangeable P (188, 866 and 815 ppm DM), total N (4.8, 8.3 and 8.7 %DM), total Cu (8.75, 75.4 and 69.8 ppm DM), and total Zn (50.9, 137.6 and 139.4 ppm DM). However, there is no significant difference between the two compost rates (D50 and D100), except for N green leaves content for which D100 is higher than D50.

O-h\textsuperscript{>2} humidity is the lowest in summer 2003 (p<0.001). October 2003 values are similar to summer 2002 values. Indeed spring, summer and autumn 2003 were exceptionally dry (Figure 1).

### Table 3: Matrix of Pearson correlation analysis between ergosterol, numbers of bacteria and foliar N and O-h\textsuperscript{>2} parameters. *Significant (0.05<p<0.01); ***Highly significant (p < 0.001).

<table>
<thead>
<tr>
<th></th>
<th>Ergosterol</th>
<th>Bacterial numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>O horizon &gt; 2mm humidity</td>
<td>R= 0.44***</td>
<td>R= 0.25*</td>
</tr>
<tr>
<td>O horizon &gt; 2mm exchangeable P</td>
<td>R= -0.31*</td>
<td>R= -0.13</td>
</tr>
<tr>
<td>O horizon &gt; 2mm total N</td>
<td>R= -0.16</td>
<td>R= 0.04</td>
</tr>
<tr>
<td>O horizon &gt; 2mm total Cu</td>
<td>R= -0.39</td>
<td>R= -5</td>
</tr>
<tr>
<td>O horizon &gt; 2mm total Zn</td>
<td>R= -0.38</td>
<td>R= -0.001</td>
</tr>
</tbody>
</table>

3.3 Correlation between microbial colonization of kermes oak leaf litter and O-h\textsuperscript{>2} parameters

Both compartments, bacterial and fungal, are positively correlated with O-h\textsuperscript{>2} humidity, particularly ergosterol. The highest ergosterol values correspond to the highest values of registered rainfall, as O-h\textsuperscript{>2} humidity (month n) is strongly positively correlated (R= 0.76, p=0.03) to precipitations (month n-1). Ergosterol is also positively correlated to exchangeable phosphorus in O-h\textsuperscript{>2}. However, neither bacterial numbers nor ergosterol are correlated to total N, Cu and Zn contents (Table 3).

4 Discussion

Biotechna compost amendment decreased significantly kermes oak leaf litter fungal colonization at 100t.ha\textsuperscript{-1} rate although it had no effect on bacterial colonization. This result differs from numerous studies, which have found enhancing effects of organic amendments on microbial populations and activities [16, 17]. This shows the importance of studying separately fungi and bacteria in
order to precise microbial communities response after compost amendment, as in our study the two groups of microorganisms exhibited different reactions.

Total N content in mixed coarse litter (O-h⁻² = studied kermes and other garrigue species leaf litter, compost pine barks and green wastes) significantly increased in plots amended at D50 and D100. The N increase in kermes oak’s green leaves may have largely participated to this total N O-h⁻² enrichment, as this species litter generally provides 60-70% of the total litter in garrigue ecosystems [7]. As nitrogen is frequently a limiting nutrient in Mediterranean ecosystems [18], its increased concentration on amended plots should have improved microbial populations. But no significant correlation was found between microbial colonization of kermes oak litter and total N content in O-h⁻².

We could have explained the reduction of fungal colonization at D100 by a reduction of another limiting factor in Mediterranean ecosystems: P [18], which is mostly associated to calcium in inorganic forms on calcareous soils, and then unavailable. Indeed, Thirukkumaran and Parkinson [19] reported that microbial variables were unaffected by N addition, whereas substrate induced respiration (SIR) was increased with P fertilization. In addition, Kwabiah et al [20] found that phosphorus is the most important quality factor affecting microbial biomass carbon. But the results of the present study show that this explanation is untenable. Indeed, exchangeable phosphorus in O-h⁻² was negatively correlated to fungal colonization of Quercus coccifera leaf litter, although compost amendment increased exchangeable phosphorus contents in O-h⁻². In our study, compost amendment induced significant increase in O-h⁻² total copper and zinc contents, and could have implied the depreciation of fungal colonization of leaf litter collected on plots amended with 100 t.ha⁻¹. Although heavy metals are known to affect growth, morphology and metabolism of micro-organisms in soils [21], neither bacterial numbers, nor ergosterol concentrations were correlated to these elements contents. Besides, on one hand, Cu and Zn accumulate more in plant root [22] than in shoot. Thus, the Quercus coccifera leaf litter may have poorly been contaminated by these elements, explaining the absence of Cu and Zn effect on microbial colonization. On the other hand, heavy metal total concentrations do not reflect their biological effects and available concentrations in the soil have to be taken into account.

Organic amendment in a semi-arid site can modify heavy metals mobility and bioavailability in soil, because they can have a high affinity for organic matter [23]. The experimental site was already contaminated by Ni before amendment, probably due to atmospheric deposition [24], and Ni remobilization after amendment could have led to a toxic effect of compost on fungi.

Our results showed the importance of humidity on microbial colonization. Indeed, compost amendment significantly increased kermes oak leaf litter moisture, and this parameter was positively correlated both to fungal and bacterial colonization, though fungi were more affected than bacteria. Microbial colonization was the lowest during dry summer months (June and July) for both years, and 2003 values were lower than 2002 values due to exceptional drought. This is in accordance with Fioretto et al [25] work. Indeed, they reported
seasonal patterns for microbial activity, the lowest values being recorded in summer, when the litter water content was around 10 % DM.

In conclusion, the compost increasing moisture effect on *Quercus coccifera* leaf litter is in opposition with the depreciating effect of the compost on fungal colonization. Some changes induced by compost amendment (e.g. heavy metal mobilization) may have had strong enough decreasing effects on fungal colonization to annul the increasing kermes oak leaf litter moisture effect, even if this parameter is strongly linked to one of the major limiting factor in Mediterranean areas (e.g. drought). But this compost decreasing effect was only significant at D100 amendment (159 µg.g⁻¹ DM on D100, in comparison with 194 µg.g⁻¹ DM on D0), and the ergosterol kermes oak leaf litter contents reported remain in the same order values as reported in literature [26]. Moreover, Borken *et al* [27] noted that microbial biomass decreased in the O horizon whereas it increased in the mineral soil. Therefore, in our study, even if we found a compost decreasing effect on fungal colonization of kermes oak leaf litter from the O horizon > 2mm, this result does not imply that compost has a general negative effect on soil microbial colonization, whatever the fractions considered.

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**References**


