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# Macroarthropod-microorganism interactions during the decomposition of Mediterranean shrub litter at different moisture levels

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## ABSTRACT

Saprophagous macroarthropods reach high population densities in many Mediterranean terrestrial ecosystems of southern Europe but their impact on decomposition processes remains unclear. We studied the direct and indirect effects of the millipede *Ommatoiulus sabulosus* on the decomposition of litter from four shrub species (*Cistus albidus*, *Quercus coccifera*, *Rosmarinus officinalis* and *Ulex parviflorus*) in a 1-month laboratory experiment. Litter mass loss, substrate-induced respiration (SIR) in litter and faecal pellets, and microbial community structure and biomass in litter (using PLFA profiles) were determined under three sets of conditions: (1) litter and microorganisms; (2) litter, microorganisms and millipedes; (3) litter, microorganisms and millipede faecal pellets regularly added to litter. Two watering frequencies were used to study the effects of simulated drought on the measured variables. Millipedes consumed large amounts of shrub litter with low assimilation efficiency. The largest effect was observed for *Cistus* that showed also the highest rate of microbial-driven decomposition. Conversion of litter into faecal pellets by millipedes did not increase organic matter decomposition and there was no evidence for a stimulation of microbial activity in faecal pellets compared to uningested litter. Faecal pellet deposition on litter, which was maximal in *Cistus*, did not change mass loss and SIR rates of litter but significantly changed microbial community structure. Bacterial biomass was lower and fungal:bacterial ratio was higher in moist, but not in dry *Cistus* litter, after 1 month of regular faeces addition. Simulated drought strongly decreased microbial-driven decomposition but not millipede feeding. The resulting sustained production of faecal pellets, however, did not offset the slower microbial-driven decomposition under dry conditions. We conclude that the transformation of large amounts of shrub litter into faeces by macroarthropods and the resultant interactions with microorganisms do not enhance carbon mineralization in the short term, regardless of litter humidity. We however expect that faeces could be incorporated into the soil more easily than intact plant litter, with important consequences for carbon and nutrient cycling in Mediterranean ecosystems under increasing drought.

## 1. Introduction

Litter decomposition, i.e. the gradual breakdown of dead organic material that is ultimately mineralized into CO<sub>2</sub> and mineral nutrients, is a key process in terrestrial ecosystems (Chapin et al., 2011). The activity of microbial decomposers (bacteria and fungi) depends primarily on litter quality and climatic conditions (Aerts, 1997; Heal et al., 1997; Lavelle and Spain, 2001) and, in this respect, Mediterranean ecosystems have several distinctive features. First, in spite of large differences in decomposition rates among plant species, the common and abundant evergreen

sclerophyllous, coniferous and aphyllous species generally decompose slowly due to the toughness and low nutrient contents of their leaves, needles or thorns (Gallardo and Merino, 1993; Gillon et al., 1999; Pérez-Harguindeguy et al., 2000; Cornwell et al., 2008). Second, the summer is characterized by an extended dry period, and drought affecting the litter and upper soil layers exerts a strong climatic control on decomposition rates (Aerts, 1997; Bottner et al., 2000; Saura-Mas et al., 2012). This influence of drought is predicted to become more pronounced with climate change, due to reduced frequencies and amounts of rainfall in the Mediterranean region (IPCC, 2007; Giorgi and Lionello, 2008). Another feature of Mediterranean ecosystems is that saprophagous macroarthropods can reach very high abundance and biomass locally, especially in southern Europe (Iatrou and Stamou, 1991; David, 1999; David et al., 1999). These detritivores ingest large amounts of leaf litter

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with a typically low assimilation efficiency, resulting in the production of large amounts of faecal pellets, clearly visible in the litter and topsoil layers (García-Pausas et al., 2004; Tagger et al., 2008). In a holm oak (*Quercus ilex*) forest of southern France, for example, the millipede *Glomeris marginata* was reported to consume about 109 g (dry mass) m<sup>-2</sup> of leaf litter and egest 103 g (dry mass) m<sup>-2</sup> of faecal pellets per year (David and Gillon, 2002). Such high feeding activities have potentially important consequences for decomposition processes in these systems.

Soil invertebrates can affect decomposition directly and indirectly. Macroarthropods, as litter fragmenters, generally have little direct effect on organic matter decomposition through assimilation and respiration (Wolters, 2000; Lavelle and Spain, 2001). Large amounts of undecomposed plant material, especially cell wall structural constituents, are egested in their faeces (Gillon and David, 2001). Therefore, a clear distinction must be made between the impact of macroarthropods on litter mass loss, which can be substantial in many types of litter, and their direct impact on organic matter decomposition, which can actually remain marginal.

Indirect effects of macroarthropods on decomposition through interactions with microorganisms are thought to be important (Visser, 1985; Wolters, 2000; Lavelle and Spain, 2001). Such interactions were mainly investigated in macroarthropod faeces, and large increases in bacterial counts were observed in freshly egested material compared to uningested leaf litter (Ineson and Anderson, 1985; Hassall et al., 1987; Byzov et al., 1998). This has led to the widely accepted view that microbial activity is stimulated and organic matter decomposition enhanced in macroarthropod faeces (Lavelle and Spain, 2001; Coleman et al., 2004; Bardgett and Wardle, 2010). However, other studies found that microbial respiration was not greater in faecal pellets than in uningested leaf litter (Scheu and Wolters, 1991; Suzuki et al., 2013) and, similarly, that mass loss rates were not higher in macroarthropod faeces than in leaf litter (Nicholson et al., 1966; Webb, 1977; Frouz and Simek, 2009). This may result from the compact structure of faecal pellets that inhibits microbial decomposition (Webb, 1977; Suzuki et al., 2013) and from the depletion of readily assimilable carbon compounds associated with increased concentrations of recalcitrant compounds such as lignin, which is not digested by most macroarthropods (Gillon and David, 2001; Rawlins et al., 2006).

Interactions between macroarthropods and microorganisms also occur in leaf litter (Visser, 1985; Crowther et al., 2012). Some are linked to the grazing behaviour of invertebrates, which inevitably results in damage to fungal tissues. However, such interactions are complex and may have negative or positive effects on decomposition (Crowther et al., 2011). For example, Hanlon and Anderson (1980) showed that macroarthropod feeding activities stimulated carbon mineralization at low but not at high population densities of the woodlouse *Oniscus asellus*, which may depend on the grazing pressure on litter fungi. Macroarthropods are also good dispersers of propagules, both on their body surface and in their faeces (Lilleskov and Bruns, 2005), which may accelerate microbial colonization of litter substrates. Finally, the deposition of fresh faeces, supposed to be hotspots of bacterial activity, on leaf litter may enhance decomposition (Visser, 1985; Frouz and Simek, 2009). This latter type of interaction, however, is only poorly studied and understood.

In this study, we examined the direct and indirect effects of saprophagous macroarthropods on the decomposition of plant litter from Mediterranean species, with special attention to interactions with litter microorganisms and the influence of increased drought. We investigated the effects of the millipede *Ommatoiulus sabulosus* on the decomposition of litter from four

woody plant species (*Cistus albidus*, *Quercus coccifera*, *Rosmarinus officinalis* and *Ulex parviflorus*) in a 1-month laboratory experiment. These four shrub species are typically dominating the garigue ecosystem of southern France on calcareous bedrock. Due to its remarkably high abundance in this type of ecosystem, *Ommatoiulus* produces large amounts of faecal pellets in spring and autumn, which potentially leads to strong interactions with microbial decomposers. As more frequent dry spells and droughts are expected in the Mediterranean region, we carried out the experiment at two moisture levels, to study the effects of intermittent drying of litter on microbial and faunal activity. Microbial activity is known to decrease steadily with decreasing litter moisture (Manzoni et al., 2012). Leaf litter consumption by macroarthropods is also generally reduced by low moisture contents (Bertrand et al., 1987; David and Gillon, 2002; Collisson et al., 2013), but Tian et al. (1997) suggested that fauna-mediated decomposition could be less susceptible to drought than microbial decomposition.

More specifically we assessed (1) the effects of millipedes on litter mass loss and organic matter decomposition, and their effects on microbial activity in faeces and uningested litter. For the first time, we studied the consequences of faecal pellet addition to leaf litter for decomposition and microbial biomass and activity. (2) We evaluated the consequences of low litter moisture contents for both millipede feeding activities and microbial activity in litter and faeces, and tested whether macroarthropods can mitigate the negative effects of drought on decomposition, i.e. have a relatively more important role when moisture conditions are less favourable (Verhoef and Brussaard, 1990; Tian et al., 1997). (3) Finally, we took advantage of the co-occurrence of four plant species producing litter of contrasting quality to test the hypothesis that macroarthropods can mitigate the negative effects of litter recalcitrance on microbial decomposition, i.e. have a relatively more important role in the decomposition of low-quality litter (Tian et al., 1995, 1997; Yang and Chen, 2009; Riutta et al., 2012).

## 2. Materials and methods

### 2.1. Biological material

Leaf litter was collected in a shrubland located in the Massif de l'Etoile near Marseille, France (43°22' N; 5°25' E). A detailed description of the site was given by Montes et al. (2008). Litter from the four dominant plant species, *C. albidus* (Cistaceae), *Q. coccifera* (Fagaceae), *R. officinalis* (Lamiaceae) and *U. parviflorus* (Fabaceae), was collected on the ground in March–April 2011. Freshly fallen litter was excluded because soil macrofauna generally prefer litter with microbial conditioning (Lavelle and Spain, 2001). Litter was air-dried in the laboratory, sorted into species and cleaned of adhering soil particles, fruit and flower parts or twigs. Leaves are woolly in *Cistus*, sclerophyllous in *Quercus*, tough and narrow (needle-like) in *Rosmarinus*, whereas *Ulex* litter consists of thorny stems, which are the photosynthetic organs in this aphyllous species. Litter chemical and physical characteristics were determined (Table 1). Total C and N concentrations were measured using a flash elemental analyzer (EA1112 Series; Thermo Finnigan, Milan, Italy), P concentration was measured colourimetrically using an Evolution II autoanalyzer (Alliance Instruments, Cergy, France), and lignin concentration was determined using a Fibersac 24 fibre analyzer (Ankom, Macedon, USA) (Hättenschwiler et al., 2008). Condensed tannin concentration was measured spectrophotometrically using the butanol–HCl method (Coulis et al., 2009). To determine water holding capacity, litter was soaked in distilled water for 24 h, drained, weighed moist and reweighed after drying at 60 °C for 48 h.

**Table 1**

Characteristics of the four species of shrub litter used in this study (means  $\pm$  SE). All percentages are on a dry mass basis (CT: condensed tannins; WHC: water holding capacity).

| Litter characteristics | Shrub species         |                          |                               |                         |
|------------------------|-----------------------|--------------------------|-------------------------------|-------------------------|
|                        | <i>Cistus albidus</i> | <i>Quercus coccifera</i> | <i>Rosmarinus officinalis</i> | <i>Ulex parviflorus</i> |
| C (%)                  | 43 $\pm$ 0.2          | 45 $\pm$ 0.3             | 50 $\pm$ 0.3                  | 49 $\pm$ 0.2            |
| N (%)                  | 0.64 $\pm$ 0.01       | 1.03 $\pm$ 0.02          | 0.65 $\pm$ 0.02               | 1.08 $\pm$ 0.02         |
| C:N ratio              | 67 $\pm$ 1            | 44 $\pm$ 1               | 76 $\pm$ 1                    | 45 $\pm$ 1              |
| P (‰)                  | 0.56 $\pm$ 0.01       | 0.50 $\pm$ 0.003         | 0.37 $\pm$ 0.01               | 0.20 $\pm$ 0.01         |
| CT (%)                 | 0.6 $\pm$ 0.11        | 0.6 $\pm$ 0.02           | 0.1 $\pm$ 0.003               | 0.1 $\pm$ 0.01          |
| Lignin (%)             | 25 $\pm$ 0.5          | 15 $\pm$ 0.4             | 16 $\pm$ 0.3                  | 23 $\pm$ 1.0            |
| WHC (%)                | 178 $\pm$ 5           | 132 $\pm$ 2              | 146 $\pm$ 6                   | 97 $\pm$ 2              |

The julid millipede *O. sabulosus aimatopodus* — the Mediterranean sub-species of *O. sabulosus* with no dorsal orange–yellow bands — was collected from the same site in April 2011. It is a very abundant species at the Massif de l'Etoile, whose population density was estimated at 164  $\pm$  37 individuals  $m^{-2}$  in the spring of 2010, which represents a live biomass of 9.2  $\pm$  2 g  $m^{-2}$  (J.F. David, unpublished data). Individuals in the range of 100–200 mg in live mass, mostly sub-adults, were selected and kept at 18 °C in a site-specific mixture of moist leaf litter before the experiment.

## 2.2. Decomposition experiment in the laboratory

Leaf litter was put in large (40  $\times$  33  $\times$  8.5 cm), lidded transparent plastic boxes, the inside of which was divided into four equal parts by four plastic dividers (13  $\times$  1  $\times$  1 cm) laid crosswise, which prevented litter mixing while allowing fauna to move freely through small gaps between the dividers. Six grams of air-dried litter from each of the four species were moistened by submersion in distilled water for 10 min, drained and put separately in each box. Millipedes could thus feed on four monospecific litter patches randomly disposed in each box. Boxes were assigned to three faunal treatments: (1) control boxes without millipedes ( $n = 10$ ); (2) boxes with six individuals of *Ommatoiulus* of similar size, representing a mean live biomass of 863  $\pm$  12 mg per box, which was equivalent to about 70% of the field estimate of *Ommatoiulus* biomass ( $n = 10$ ); (3) boxes in which only faecal pellets of millipedes were progressively added to leaf litter ( $n = 10$ ). For this purpose, fresh pellets were collected every second day in an additional set of 10 boxes with the same millipede biomass as in treatment (2) (862  $\pm$  9 mg per box), and put on the corresponding litter patches (i.e. all faecal pellets collected on a specific litter type were added to the same litter type in boxes of treatment (3)). Each litter patch was watered with 1 ml of distilled water either twice a week (moist conditions in five boxes) or only once a week (dry conditions in five boxes). The resulting differences in litter water content were repeatedly assessed in two additional boxes per moisture treatment over the course of the experiment (12 times in total, at various intervals after watering). All the boxes were kept for 30 days in an open shed under Mediterranean conditions (CEFE, Montpellier, France), in which the mean air temperature over the course of the experiment was 19 °C. There was no millipede mortality during the 30-day period and, at the end of the experiment, they were removed and weighed. Leaf litter and faecal pellets were carefully sorted and weighed. One gram of fresh litter was freeze-dried for phospholipid fatty acid (PLFA) analysis and the remaining material was air-dried and reweighed. Some air-dried subsamples were used to determine substrate-induced respiration (SIR) and others were oven-dried at 60 °C for 2 days to estimate the water content and final dry mass of air-dried materials. Initial oven-

dry litter mass was determined using a conversion factor of air-dried to oven-dried mass for each sample.

Initial and final dry masses allowed calculations of the following variables under moist and dry conditions: (1) the percentage mass loss due to microbial decomposition in control boxes without animals. (2) The net effect of millipedes on litter mass loss (g per box), i.e. the difference between the total mass loss of litter observed in their presence and that in their absence in control boxes; the net effect reflects both litter consumption and millipede–microorganisms interactions in uningested litter. (3) The total amount of organic matter that decomposed in the presence of millipedes, i.e. the difference between the total mass loss of litter and the mass of faeces collected at the end of the experiment.

Substrate-induced respiration (SIR) was measured on remaining litter and faecal pellets in order to compare the potential activity of microbial communities in the different treatments. This method assesses the physiological capacity of microorganisms to catabolize glucose under optimal temperature and moisture conditions, and variations in respiration rates reflect changes in both the biomass and composition of microbial communities (Fanin et al., 2011). The procedure of Beare et al. (1990) was used and the rate of CO<sub>2</sub> release ( $\mu\text{g CO}_2\text{-C g}^{-1} \text{h}^{-1}$ ) was measured using a Varian 4900 gas chromatograph with a thermal conductivity detector (Varian, Walnut Creek, USA). To compare SIR in litter and faecal pellets, it was assumed that each litter species was consumed in proportion to the net effect of millipedes on its mass loss. Hence, litter SIR was calculated as the weighted mean (based on relative consumption of the four litter types) of litter-type specific SIR.

As our experiment showed that *Ommatoiulus* fed mainly on *Cistus* and thus also egested much more faecal pellets on this litter species, PLFA analysis was used to compare bacterial and fungal biomass in the different samples of *Cistus* litter at the end of the experiment. We considered faecal pellet densities on the other litter species too low for a detectable effect on microbial community structure. The ratio of fungal to bacterial PLFAs was used as an index of the relative abundance of these two groups of microorganisms (Leckie, 2005). Procedures described by Zelles (1999) were used for lipid extraction and separation. After alkaline methanolysis, tridecanoic acid methyl ester (CAS number: 1731-88-0) was added as internal standard and the concentrations of ester-linked fatty acid methyl esters (EL-FAMES) were determined using a Hewlett Packard 6890 gas chromatograph. Bacterial biomass was estimated by summing the following PLFAs: i15:0, a15:0, i16:0, 16:1 $\omega$ 7, i17:0, a17:0, 18:1 $\omega$ 7 and cy19:0 (Wilkinson et al., 2002; Ruess and Chamberlain, 2010). Fungal biomass was estimated from the concentration of 18:2 $\omega$ 6,9 (Frostegård et al., 2011).

## 2.3. Statistical analyses

All statistical analyses were performed using R software version 2.12.1 (R Development Core Team, 2010). Two types of ANOVA were used: (1) in each faunal treatment, the main effects and interaction of litter species and moisture treatment on litter mass loss, SIR in litter and SIR in faecal pellets, were tested using partly hierarchical ANOVA (Logan, 2010). In this design, data from moist and dry boxes were nested within moisture treatments, and litter species were crossed with moisture treatments and boxes. (2) The main effects and interaction of faunal treatment and moisture treatment on the mass loss of each litter species, SIR in each litter species, and microbial biomass data in *Cistus* litter, were tested using two-way ANOVA (3  $\times$  2 factorial design). Homogeneity of variances was checked prior to analyses and data were log- or power-transformed where required. Tukey's HSD test was used for multiple comparisons among pairs of means. Some

differences between two sample means were tested using paired *t*-tests, as indicated in the text.

### 3. Results

#### 3.1. Litter moisture conditions

The two watering frequencies significantly affected litter moisture in all four species. Measurements on 12 occasions during the experiment showed that mean water contents in *Cistus* leaf litter were  $55 \pm 11\%$  and  $33 \pm 12\%$  (on a litter dry mass basis) in moist and dry treatments, respectively (paired *t*-test,  $P < 0.01$ ). Similarly, *Quercus* leaf litter had mean water contents of  $29 \pm 4\%$  and  $13 \pm 3\%$  in moist and dry treatments, respectively ( $P < 0.001$ ). *Rosmarinus* leaf litter had mean water contents of  $38 \pm 6\%$  and  $15 \pm 3\%$  ( $P < 0.001$ ), and *Ulex* litter had mean water contents of  $28 \pm 4\%$  and  $17 \pm 4\%$  ( $P < 0.001$ ) in moist and dry treatments, respectively.

#### 3.2. Decomposition in control boxes

Litter mass loss in animal- and faeces-free control boxes differed significantly among species ( $P < 0.001$ ), *Cistus* decomposing much faster than the other three species under both moist and dry conditions (Fig. 1a). Although correlations between litter mass loss and the measured litter quality parameters were not significant, litter mass loss in moist control boxes decreased in the same order as the water holding capacity of litter. Litter mass loss was significantly affected by the moisture treatment ( $P < 0.001$ ). On average across all litter species, total mass loss was  $5.50 \pm 0.16\%$  under moist conditions compared to  $1.91 \pm 0.31\%$  under dry conditions. Although there was no significant interaction between litter species and moisture conditions, the four species tended to respond differently to the moisture treatment. Mass loss in dry conditions was reduced by a factor of 9.6 in *Rosmarinus*, 4.6 in *Quercus*, 2.7 in *Ulex* and 1.8 in *Cistus* (Fig. 1a).

Litter SIR rates in control boxes also differed significantly among species ( $P < 0.001$ ) (Fig. 1b). The highest respiration rates were recorded in *Cistus* under both moist and dry conditions, but the rank order of other species was not the same as for litter mass loss, with notably significantly higher SIR in *Quercus* than in *Rosmarinus* and *Ulex* that showed the lowest SIR of all litter types (Fig. 1b). There was a marginally significant correlation between SIR rates and initial P concentrations in the four litter species under moist conditions ( $P = 0.05$ ), a relationship that however disappeared under dry conditions ( $P = 0.16$ ). None of the other initial litter quality characteristics was significantly correlated with litter SIR. In contrast to litter mass loss, SIR was not significantly affected by the moisture treatment. There was however a significant interaction between litter species and moisture conditions ( $P < 0.05$ ): under dry conditions, SIR increased in *Ulex* but did not change significantly in the other species (Fig. 1b).

#### 3.3. Decomposition in boxes with millipedes

The net effect of millipedes on litter mass loss differed significantly among litter species ( $P < 0.001$ ), with a stronger effect on *Cistus* than on any of the other species under both moist and dry conditions (Fig. 2). Millipede effects on litter mass loss were not significantly correlated with the litter quality parameters reported in Table 1, but they were positively correlated with the mass loss of each litter species measured in moist control boxes ( $P < 0.05$ ). For all species combined, the activity of millipedes led to a loss of  $2.30 \pm 0.17$  and  $2.11 \pm 0.18$  g litter per box under moist and dry conditions, respectively. Litter mass loss induced by millipedes was not significantly different from the mass of faecal pellets collected

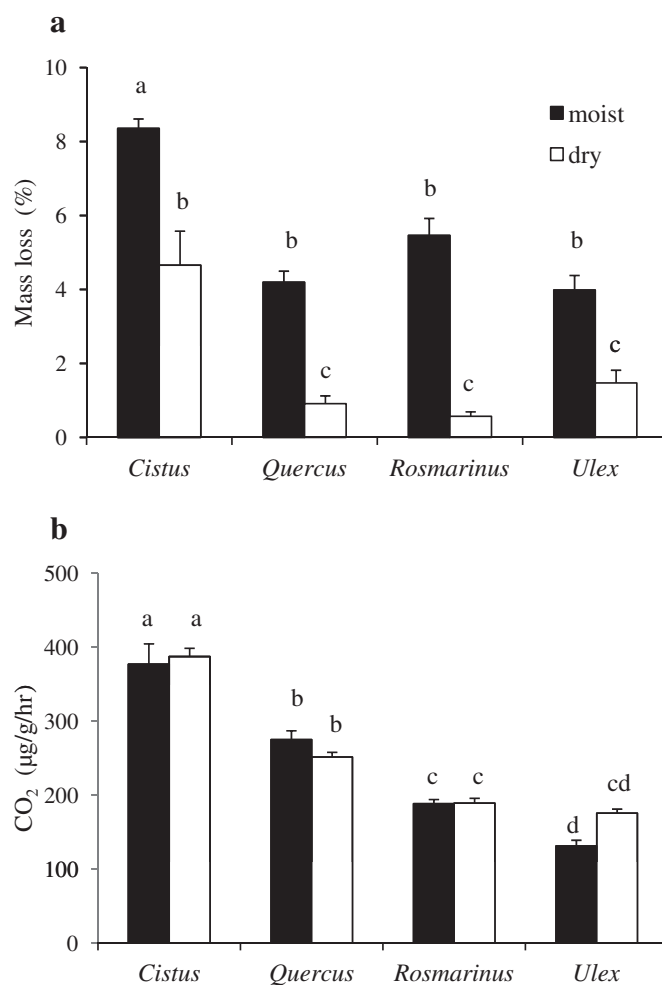


Fig. 1. Mass loss as a percentage of initial dry mass (a) and substrate-induced respiration (b) of four species of shrub litter maintained for 1 month at two levels of litter moisture (means  $\pm$  SE,  $n = 5$ ).

at the end of the experiment ( $2.43 \pm 0.09$  and  $1.99 \pm 0.15$  g per box under moist and dry conditions, respectively; non-significant paired *t*-tests), indicating that most of the consumed litter was not assimilated. The impact of millipedes on litter mass loss was not

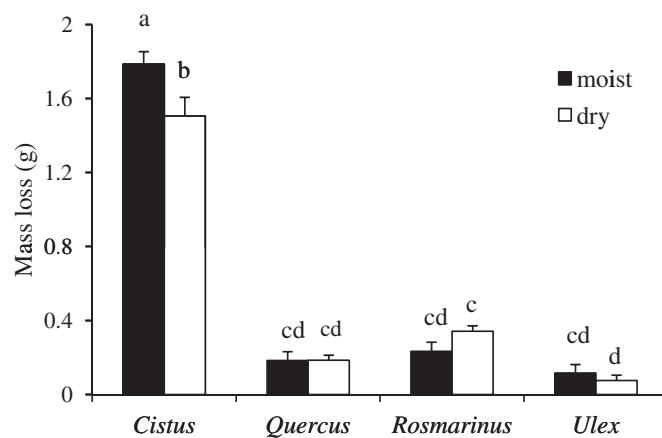


Fig. 2. Net effect of millipedes on the mass loss of four species of shrub litter after incubation for 1 month at two levels of litter moisture (means  $\pm$  SE,  $n = 5$ ). The net effect of millipedes is the difference between the total mass loss of litter measured in their presence and that measured in their absence in control boxes.

significantly affected by litter moisture ( $P = 0.46$ ). There was however a significant interaction between litter species and moisture conditions ( $P < 0.01$ ). Under dry conditions, the millipede effect on *Cistus* was relatively smaller while that on *Rosmarinus* was relatively greater, indicating a shift in food preference when litter was drier.

Organic matter decomposition in the presence of millipedes, i.e. the difference between the total mass loss of litter and the mass of faeces collected at the end of the experiment, was on average  $1.11 \pm 0.11$  g per box under moist conditions vs.  $0.54 \pm 0.07$  g per box under dry conditions. These amounts of mineralized litter were not significantly different from those expected from microbial decomposition alone, i.e.  $1.24 \pm 0.01$  and  $0.43 \pm 0.01$  g per box under moist and dry conditions, respectively (non-significant paired *t*-tests), indicating that millipede feeding activities had very little impact on organic matter decomposition over the experimental duration of one month.

SIR rates in the litter remaining in boxes with millipedes at the end of the experiment did not differ significantly from those measured in control boxes (data not shown). SIR rates in faecal pellets varied significantly depending on the litter species on which faecal pellets were egested by millipedes ( $P < 0.001$ ) (Fig. 3). The highest respiration rates were measured in pellets from *Cistus* patches and the lowest respiration rates were measured in pellets from *Quercus* patches. SIR rates in faecal pellets ranged from 204 to  $310 \mu\text{g CO}_2 \text{g}^{-1} \text{h}^{-1}$  in the moist treatment and from 147 to  $298 \mu\text{g CO}_2 \text{g}^{-1} \text{h}^{-1}$  in the dry treatment (Fig. 3). These rates were consistently lower than those estimated for litter mixtures containing the four species in proportion to the amounts consumed by millipedes, i.e. 340 and  $336 \mu\text{g CO}_2 \text{g}^{-1} \text{h}^{-1}$  under moist and dry conditions, respectively. In contrast to SIR rates in litter, SIR rates in faecal pellets were significantly affected by the moisture treatment ( $P < 0.01$ ), with lower respiration rates under dry conditions (Fig. 3), indicating that microorganisms present in faeces were more sensitive to drought than those present in uningested litter.

#### 3.4. Decomposition in boxes with faeces addition

The collection of fresh faecal pellets in the set of boxes intended for faeces production showed that millipedes deposited much more faecal pellets on *Cistus* litter patches than on the other three species, in accordance with their apparent preference for *Cistus* litter. The dry weight of faecal pellets added to litter in the absence of

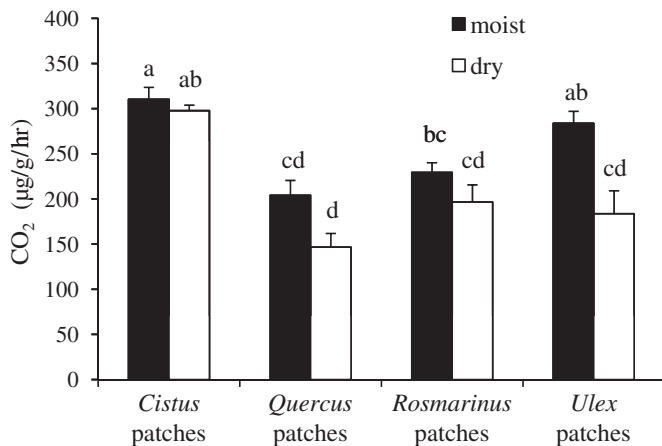


Fig. 3. Substrate-induced respiration of faecal pellets egested by millipedes on four species of shrub litter under moist and dry conditions (means  $\pm$  SE,  $n = 5$ ). The pellets were 2-day to 1-month old in each litter patch.

Table 2

Concentrations of characteristic PLFAs ( $\mu\text{g g}^{-1}$ ) and fungal:bacterial ratios in *Cistus* litter subjected for 1 month to three faunal treatments under moist and dry conditions (means  $\pm$  SE,  $n = 4$ ). Different letters indicate significant differences within a row (see text for *P* values); asterisks indicate significant differences between moist and dry conditions ( $P < 0.05$ ).

| PLFAs         |       | Control boxes              | Boxes with millipedes      | Boxes with faecal pellets  |
|---------------|-------|----------------------------|----------------------------|----------------------------|
| Fungal (F)    | Moist | 263 $\pm$ 27               | 234 $\pm$ 6                | 262 $\pm$ 2                |
|               | Dry   | 247 $\pm$ 20               | 220 $\pm$ 13               | 234 $\pm$ 17               |
| Bacterial (B) | Moist | 101 $\pm$ 2 <sup>a</sup>   | 66 $\pm$ 4 <sup>b</sup>    | 69 $\pm$ 2 <sup>b</sup>    |
|               | Dry   | 79 $\pm$ 7*                | 69 $\pm$ 4                 | 74 $\pm$ 3                 |
| Total (F + B) | Moist | 364 $\pm$ 26 <sup>a</sup>  | 300 $\pm$ 10 <sup>b</sup>  | 331 $\pm$ 2 <sup>ab</sup>  |
|               | Dry   | 325 $\pm$ 27 <sup>a</sup>  | 289 $\pm$ 16 <sup>b</sup>  | 308 $\pm$ 19 <sup>a</sup>  |
| F:B ratio     | Moist | 2.6 $\pm$ 0.3 <sup>a</sup> | 3.6 $\pm$ 0.2 <sup>b</sup> | 3.8 $\pm$ 0.1 <sup>b</sup> |
|               | Dry   | 3.2 $\pm$ 0.1              | 3.2 $\pm$ 0.1              | 3.2 $\pm$ 0.1*             |

millipedes, as estimated at the end of the experiment, was on average  $1.13 \pm 0.06$  g in *Cistus* patches and ranged between  $0.14 \pm 0.01$  and  $0.25 \pm 0.03$  g in the other litter patches. Faecal pellet addition in the absence of millipedes did not significantly affect litter mass loss and SIR in any of the four species, the results being very similar to those recorded in control boxes under either moisture conditions (data not shown).

#### 3.5. Microbial community structure in *Cistus* leaf litter

In control boxes, total microbial biomass in *Cistus* litter determined with PLFAs was not significantly different between the moist and dry treatments (Table 2). However, the effects of these two treatments differed between bacterial and fungal markers. While there was no moisture effect on fungal biomass, bacterial biomass was significantly lower under dry than under moist conditions ( $P < 0.05$ ).

In boxes with millipedes under moist conditions, bacterial biomass on *Cistus* litter was significantly lower than in control boxes ( $P < 0.001$ ) (Table 2). Fungal biomass did not decrease to the same extent, which resulted in a significantly higher fungal:bacterial ratio in the presence of millipedes ( $P < 0.05$ ). Such changes did not occur under dry conditions. Total microbial biomass, however, tended to be lower in boxes with millipedes than in control boxes under both moist and dry conditions ( $P = 0.05$ ).

Similar changes in microbial community structure were observed in boxes with faeces addition under moist conditions. There was a significant decrease in bacterial biomass ( $P < 0.001$ ) and a significant increase in fungal:bacterial ratio ( $P < 0.05$ ) in comparison with control boxes (Table 2). As in boxes with millipedes, such changes did not occur under dry conditions. This indicates that, in boxes with millipedes, bacterial communities on moist *Cistus* litter were mainly affected by faecal pellet deposition and not by other millipede activities.

## 4. Discussion

#### 4.1. Faunal contribution to litter mass loss

In our study system of a Mediterranean garrigue of southern France, the four woody shrub species *C. albidus*, *Q. coccifera*, *R. officinalis* and *U. parviflorus* dominate the vegetation cover (Montes et al., 2008), and thus the litter input to the soil. Our results have shown that the most abundant species of saprophagous macroarthropods, the millipede *O. sabulosus*, consumes large amounts of aboveground litter produced by the four dominant species. Based on our study, the rate of litter consumption by *Ommatoiulus* in spring is about 2.6 g (dry mass) per g animal live mass and per

month. This corresponds to an estimated litter mass loss of 24 g per m<sup>2</sup> per month due to consumption, for an *Ommatoiulus* biomass estimate of 9.2 g (live mass) m<sup>-2</sup> in the field, suggesting a considerable impact of this millipede species on the annual litter turnover in this system.

*Ommatoiulus* shows a marked preference for *Cistus* litter and consumes comparatively small amounts of the other three litter types. Other *Ommatoiulus* species have been reported to feed on *Cistus* spp. in southern Europe (Bailey and De Mendonça, 1990) but litter traits underpinning this apparent preference for *Cistus* remain unknown. Our analyses did not show particularly high nutrient concentrations in *Cistus* litter and it is not known whether this food is selected on the basis of other nutritional characteristics, the presence of specific microorganisms, and/or its high water holding capacity. Clearly, however, the preferred food in our study was also the litter with the highest decomposition rate in the absence of macroarthropods. The relative contribution of *Ommatoiulus* to litter mass loss, calculated using the same formula as Tian et al. (1995), was larger in *Cistus* than in other species. This result does not support the view that saprophagous macrofauna, when offered litter materials of contrasting quality, have a greater relative impact on those that show less microbial decomposition (Tian et al., 1995, 1997). Other studies have shown that litter species preferred by saprophagous macroarthropods tend to have high activity of microbial decomposers (Köhler et al., 1991; Zimmer, 2002; Van Geffen et al., 2011; Collisson et al., 2013), which is consistent with our data. Collectively, our data suggest that the combined high microbial-driven decomposition and the preference by detritivores for *Cistus* leaf litter support a rapid cycling of *Cistus*-derived nutrients, while nutrients derived from the litter of the other three dominant plant species at our study site may cycle more slowly.

#### 4.2. Direct and indirect effects of *Ommatoiulus* on decomposition

Our study confirms that the direct effects of saprophagous macroarthropods on organic matter decomposition, i.e. the amount of organic material they assimilate during gut passage and transform into CO<sub>2</sub> through their own metabolism, are negligible. Similar to previous studies (Cárcamo et al., 2000; David and Gillon, 2002; Ashwini and Sridhar, 2005) we also found that the mass of faecal pellets egested by millipedes was almost identical to that lost from litter due to their feeding, indicating very low assimilation efficiency. In such cases, indirect effects of macroarthropods on decomposition through interactions with microorganisms are assumed to be much more important (Visser, 1985; Wolters, 2000; Lavelle and Spain, 2001).

The most widely mentioned interaction is that the transformation of leaf litter into faeces by macroarthropods stimulates microbial activity, with a higher activity in faeces compared to intact leaf litter, which ultimately should accelerate decomposition (Lavelle and Spain, 2001; Coleman et al., 2004; Bardgett and Wardle, 2010). Our results do not support this mechanism for fauna-driven increased decomposition. First, substrate-induced respiration, which gives an estimate of the catabolic capacity of the physiologically active microbial community, was lower in faecal pellets of *Ommatoiulus* than in uningested litter. Faecal pellets consisted of mixtures of *Cistus* and the other litter species consumed at lower rates, and it can be hypothesized that variation in respiration rates in faecal pellets largely reflected differences in composition (Suzuki et al., 2013). However, even the faecal pellets deposited in *Cistus* litter patches, presumably the richest in this litter species, had respiration rates significantly lower than those we estimated for litter mixtures fed upon by millipedes. Accordingly, there is no evidence for a stimulation of microbial activity in faeces compared to leaf litter. Although we cannot exclude the

possibility that microbial respiration was stimulated in the first few days after egestion, no stimulation was detected in the faeces aged between 2 days and 1 month collected at the end of our experiment. Moreover, the insignificant difference between the litter mass loss attributed to millipede feeding and the mass of faecal pellets collected at the end of the experiment suggests that mass loss was not greater in pellets than in leaf litter over the duration of our study. Although our results were obtained over a relatively short period of time (1 month), they are consistent with previous findings showing that decomposition was not enhanced in faeces produced by other macroarthropod species (Nicholson et al., 1966; Webb, 1977; Frouz and Simek, 2009; Suzuki et al., 2013).

An additional potential indirect fauna effect on litter decomposition is the inoculation of leaf litter with faeces-derived microorganisms. Assuming that freshly produced faeces are enriched in bacteria, at least temporarily (Ineson and Anderson, 1985; Hassall et al., 1987; Byzov et al., 1998), their presence on leaf litter could promote microbial colonization and influence decomposition (Visser, 1985; Frouz and Simek, 2009). We tested this potential interaction by repeatedly adding faecal pellets to all litter patches, thus simulating the effects of *Ommatoiulus* via faeces production without the presence of the animals. Faecal pellet addition did not change the rates of litter mass loss and microbial respiration in any of the four litter species, not even in *Cistus* that received the largest amounts of faecal pellets. Even though the effects of faeces deposition are assumed to be particularly strong in the first few weeks after egestion (Maraun and Scheu, 1996), it would be interesting to follow any potential faeces effects over a longer period than our 1-month study to confirm this lack of effect.

Faecal pellet addition, however, significantly modified microbial community structure in *Cistus* litter. In the moist treatment, bacterial biomass in *Cistus* litter was reduced when faecal pellets were added, compared to control litter. No faeces effect was observed for fungal biomass, which consequently resulted in a higher fungal:bacterial ratio in *Cistus* litter when faecal pellets were present. A reduction in bacterial biomass following faeces addition is opposite to our initial expectation. A possible explanation for this result is that the deposition of fresh faecal pellets may have led to cascading effects on the micro-food web in leaf litter. For example, bacterivorous microfauna (protozoa, nematodes) may have been stimulated by the addition of fresh pellets, as shown by Bastow (2011) using woodlouse faeces, with possible negative effects on bacterial populations in moist leaf litter. Whatever the process involved, our results show that macroarthropod faeces deposition has an impact on the structure of microbial communities in leaf litter. However, the effect of faecal pellet addition on litter microbial community structure depended on litter humidity. In the dry treatment, neither the biomass of bacteria nor that of fungi differed between litter with added faecal pellets and control litter.

The overall impact of *Ommatoiulus*, including all its direct and indirect effects on decomposition over a 1-month period in our simplified systems (litter, microorganisms, detritivores), did not increase the mass loss of organic matter. This leads to the conclusion that even high-density populations of this species that consumes substantial amounts of leaf litter do not enhance carbon mineralization in the litter layers of Mediterranean shrublands. Further studies are needed to clarify other possible effects of these macroarthropods on litter decomposition, such as (1) increased nitrogen mineralization, which is generally uncorrelated with the faunal impact on carbon mineralization (Anderson and Ineson, 1984; Verhoef and Brussaard, 1990; Frouz et al., 2008); and (2) a faster incorporation of dead plant material into the soil (Anderson, 1988), which may be important for decomposition in Mediterranean soils subjected to drought, because the translocation of faecal pellets to deeper soil layers may provide a more favourable

microclimate for decomposition compared to intact leaf litter that remains at the soil surface (Rovira and Vallejo, 1997).

#### 4.3. Drought effects

The simulated drought effect by reducing watering frequency from twice to once per week, was sufficient to strongly reduce microbial-driven litter mass loss (assuming that the effects of leaching were insignificant in both treatments given the very low absolute quantities of water added). The lower activity of microbial decomposers under dry conditions coincided with a decrease in bacterial biomass in litter, at least for *Cistus* leaf litter. Although the response of fungal biomass was less clear because of the high variability in the PLFA data, there was no apparent drought effect on fungal biomass. This is consistent with previous studies showing that fungi, which appear to be dominant in Mediterranean leaf litter (Wilkinson et al., 2002), are less strongly affected by drought than bacteria (Wilkinson et al., 2002; Yuste et al., 2011). In addition, our SIR measurements showed that the potential respiration of microbial communities was not altered in drier leaf litter and was at least equal to that measured under moist conditions. This also suggests that microbial communities dominated by fungi are able to resume their activity after a temporary decrease in response to water shortage.

Feeding activities of millipedes were much less sensitive to simulated drought than microbial activity. In contrast to previous studies that showed that leaf litter consumption by macroarthropods was reduced by low moisture contents (Bertrand et al., 1987; David and Gillon, 2002; Collisson et al., 2013), *Ommatoiulus* continued to convert shrub litter into faecal pellets at the same rate at two levels of litter moisture. Although these contrasting results may be due to different experimental conditions, they may also indicate that typical Mediterranean macroarthropod species are better adapted to feeding on dry litter. *Ommatoiulus* slightly changed its diet in the dry treatment, with higher consumption of *Rosmarinus* litter at the expense of the preferred litter from *Cistus*, but maintained its overall feeding rate. This supports the notion that, under dry conditions, some species of soil fauna play an increasingly important role in decomposition processes and can, to some extent, compensate for the low microbial activity (Verhoef and Brussaard, 1990; Tian et al., 1997). On the other hand, even though litter mass loss remained high under dry conditions due to sustained consumption by *Ommatoiulus*, there was no indication that the material converted into faecal pellets decomposed at a higher rate than dry leaf litter in control boxes. Therefore any positive effect on overall decomposition by maintained millipede consumption and litter transformation into faecal pellets under dry conditions, could only be expressed in the field if these faecal pellets moved to deeper soil layers with more favourable moisture conditions.

It may seem surprising that faecal pellets of macroarthropods, which generally have a high water holding capacity (Webb, 1977; Tajovsky et al., 1992), do not promote decomposition under dry conditions. However, our SIR data have shown that once pellets have dried after egestion in a dry environment, the microorganisms present in faecal pellets are more affected by desiccation than those present in litter, and do not return to the same activity level when replaced in optimal moisture conditions.

#### 4.4. Conclusions

We showed that in the studied Mediterranean garrigue ecosystem, the dominant macroarthropod species transformed large amounts of above-ground litter from the dominant shrub species into faeces. These feeding activities, however, did not

increase C mineralization. No direct litter consumption effects and no indirect feeding- or faeces-induced microbial stimulation effects on C mineralization were apparent, questioning the commonly assumed positive macroarthropod effects on decomposition. Moreover, the studied millipede species does not mitigate the negative effects of litter recalcitrance on microbial decomposition since it consumes primarily leaf litter that decomposes rapidly. However, we cannot exclude potential consequences on nutrient cycling or on the fate and distribution of C within the soil resulting from the different physical structure of faecal pellets compared to intact plant litter.

Under drier conditions, the Mediterranean millipede species studied here, apparently did not reduce its consumption of shrub litter although there was a shift in the preference for certain litter types. A continued conversion of shrub litter into faecal pellets while microbial decomposition is drastically reduced in dry litter could potentially mitigate negative drought effects on C and nutrient cycling in Mediterranean ecosystems. However, such macroarthropod-driven compensation for reduced microbial activity could only result from facilitated spatial translocation of faecal pellets to deeper soil horizons compared to plant litter, because we did not observe an increased decomposition of organic matter despite unchanged fauna activity.

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