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CO_2 efflux from a Mediterranean semi-arid forest soil. II. Effects of soil fauna and surface stoniness

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Abstract. Many forest soils in the Mediterranean basin are shallow and contain high amounts of gravel in the organic layers. Recent studies on soil organic matter accumulation have shown high amounts of organic matter occurring mainly in soils with high levels of stoniness at the soil surface. The gravel layer may affect the microclimatic conditions of the soil surface and probably the distribution and activity of soil fauna.

In order to quantify the combined effects soil fauna (epigeic macrofauna and earthworms) and stoniness on the release of soil CO_2, we performed a three factor field experiment by using a series of reconstructed soil profiles. Factors 1 and 2 consisted of the exclusion/presence of soil epigeic macrofauna and earthworms, and factor 3 of the presence/absence of a gravel layer intermingled with the H horizon. We incubated ^{14}C straw in the H horizon and carried out three 40 mm rainfall simulations.

Soil respiration primarily depended on the season. The effects of soil fauna were generally small and did not coincide with periods of high faunal activity. The largest effects of both earthworms and soil epigeic fauna were found after wetting the soil in summer. The effects of the earthworms were concentrated in the mineral soil while the effects of the epigeic fauna were concentrated in the H horizon and mainly arose towards the end of the experiment. This suggests that the effects of epigeic fauna may have been underestimated due to the length of the experiment. The gravel layer increased the effect of fauna probably by creating more favorable microclimatic conditions. The accumulation of organic matter in soils with high levels of stoniness cannot be explained by the effect of gravel on soil microclimate nor by its effect on the activity of soil fauna.

Introduction

The role of soil fauna in the genesis of different humus forms and in forest productivity has been recognized for a long time (Kubiena 1955). In temperate and Mediterranean forest soils, several humus forms are composed largely of faunal faecal material (Bal 1982; Fons 1995). Soil organisms may cause physical changes in biotic or abiotic materials that may modulate the availability of resources to soil biota and affect ecosystem processes (Coûteaux & Bottner 1994; Lawton 1994).

The biomass of soil invertebrates reaches its maximum in Mediterranean and Subtropical soils (Shaw et al. 1991). In Boreal and Atlantic forests, climate factors may account for 95% of the variability in decomposition rates (Jansson & Berg 1985), but this relationship breaks down for more southern latitudes. In Mediterranean ecosystems, the effects of climate on soil organic matter dynamics may be regulated by soil fauna. Soil fauna affect the physical environment of ecosystems by removing and comminuting litter, by transporting soil, and by burrowing (Anderson 1988).

Some groups of soil fauna (i.e. earthworms) are good examples of soil ecosystem engineers (Lawton 1994). Earthworms modify the physical structure of soil by ingestion; their short-term effects may favor the activity of microorganisms in a mutuallistic relationship (Trigo & Lavelle 1993). In forest soils, earthworms can favor the incorporation of soil organic matter (SOM) into the mineral soil and increase the organic matter decomposition rate (Dietz & Bottner 1981). Soil respiration is typically increased by earthworm activity (Wolters & Ekschmitt 1995; Cortez et al. 1989), probably as a consequence of mixing and increased N availability (Wolters & Schaefer 1993). However, the intimate mixing of SOM and soil mineral particles may stabilize clay-bound SOM (Shaw & Pawluk 1986 in Anderson 1988).

Epigeic macrofauna affects the physical properties indirectly by comminuting and transporting litter down the profile (Anderson 1988). The effects of epigeic macrofauna activity on C mineralization can have positive and negative effects depending upon the phase of faunal activity (Scheu & Wolters 1991) and upon the quality of the organic substrate and the complexity of the food web (Coûteaux et al. 1991).

The abundance and activity of soil fauna may explain the occurrence of mull-like forest floor that characterizes many Mediterranean forests. However, moder and mor humus types are not uncommon (Sevink et al. 1989), even in forests where the presence of soil fauna is evident (Fons & Vallejo 1999). The latter authors attributed the existence of an unusually thick forest floor layer to the presence of a layer of stones on the soil surface. Such large amounts of undecomposed organic matter intermingled with gravel may become disconnected from the underlying horizons and thus cannot be incorporated into the mineral soil. The result of these processes is the formation of specific types of humus (Duchaufour 1984).

Among the numerous factors that may affect faunal activity, soil stoniness has received little attention. As summarized by Poesen and Lavee (1994) rock fragments can modify the microclimate of the forest floor by intercepting water and by altering the infiltration rate. Changes in microclimate conditions may affect the distribution and activity of soil organisms. In addition, rock fragments may affect the activity of soil fauna by changing the characteristics of the soil habitat and by limiting the circulation of soil fauna through the soil profile. This layer of gravel can reach up to 30 cm in depth in calcareous soils (Ferran 1997).

Because a large accumulation of litter layers (ectohumus) in Mediterranean forest soils is mainly observed on stony surfaces, it appears that stony surfaces may slow down soil organic matter decomposition by affecting the regulatory processes associated with the activity of soil macroarthropoda and earthworms. In this study we have evaluated the effects of soil fauna and the presence of a surface layer of rock fragments on soil CO_2 efflux in a semiarid Mediterranean forest. The results of a parallel experiment assessing the effect of soil climate on the seasonality of soil CO_2 release are presented in Casals et al. (1999).

Material and methods

Study site

The experiment was conducted in a 40-year-old semiarid *Pinus halepensis* Mill. stand located in the Ebro Basin at Maials (NE Spain) (41°22′ N, 0°22′ E). This site is at the drier and warmer end of the European VAMOS transect (Bottner et al. 1998). Before planting the pines, the site was used as pasture. Nowadays trees are about 8.5 m height and the stand has 2100 stems ha^{-1} with a basal area of 18 m^2 ha^{-1}. The understorey is dominated by the shrubs *Quercus coccifera* L. and *Pistacia lentiscus* L. According to the phytosociological classification the site belongs to the association *Rhamno-Quercetum cocciferae pistacietosum* Br.-Bl et O. de Bolòs (1954–1957). Soils are *Calcaric regosols* (FAO-UNESCO 1988) developed from calcarenite and marl colluvium. The soil profile is 70 cm deep and rock fragments with sharp edges are found throughout the soil profile and are most abundant at or near the soil surface. Organic layers are well developed and consist of a L horizon with undecomposed needles, a poorly structured F horizon and an H horizon made up of faecal pellets. Humus forms of the site range from Vermimull to Leptomoder (according to the classification proposed in Green et al., 1993).

Table 1. Physico-chemical characteristics of soils used for repacking the cylinders.

Cylinder layer	Thickness (cm)	Bulk density (g·cm^{-3})	Organic carbon (g·kg^{-1})	Nitrogen (g kg^{-1})	$CaCO_3$ (%)	pH (1:2.5)	CEC cmol(+)kg^{-1}	Texture
L	2	–	–	–	–	–	–	–
F	1	–	–	–	–	–	–	–
H	2	0.36	372	12	–	–	110.9	–
Gravel	5	0.55	–	–	–	–	–	–
A1	1	0.77	49	3	41.2	8.1	48.6	Loam
A2	3	0.77	49	3	41.2	8.1	48.6	Loam
A3	to 25	0.96	39	–	43.6	8.4	–	Clay loam

Characteristics of soils are presented in Table 1. In the study area mean annual precipitation is 388 mm and mean annual temperature is 15.2 °C.

Experimental design

In the study area we fenced out a 25×30 m plot to avoid disturbances from wild boars. To study the contribution of soil arthropods and earthworms to soil CO_2 effluxes and their interaction with a surface stone layer we measured soil respiration in 96 soil profiles repacked in 12 cm diameter and 25 cm depth PVC cylinders. The cylinders contained mineral (A1) and organic (L, F & H) soil horizons that were collected from the same site. We divided the mineral soil into three subhorizons that were separated by a 1×1.5 cm fiberglass net. The A_1 horizon ranged from 0 to 1 cm, A_2 from 2 to 4 cm and A_3 from 4 to 25 cm. To reconstruct the organic horizons in each cylinder we incubated 1.3 g of L horizon, 12.2 g of F horizon and 60 g of H horizon. The quantity of litter used corresponded to the amount that is commonly found in *Pinus halepensis* forest (Fons 1995). Prior to the organic layers reconstruction we thoroughly mixed 1.32 g of ground ^{14}C labeled wheat straw (%C 41.5; specific activity: 1.10 MBq g^{-1}) with the H horizon. To obtain an homogeneous mixture of labeled straw and H horizon we shook each sample (1.32 g of straw + 60 of H horizon) for 24 h by using a rotational shaker.

Each cylinder was buried so that its upper rim coincided with the upper limit of the H horizon. The L and F horizons were enclosed in a cylindrical net whose walls continued those of the PVC cylinder. In order to achieve lateral continuity, the height of the organic horizons in the cylinders coincided with that of the horizons outside them. Before repacking the cylinders all soils were defaunated by remoistening the air dried samples and by two subsequent deep freezing and thawing episodes (Huhta et al. 1991). In each cylinder we measured soil CO_2 efflux from fresh organic matter (^{14}C labeled wheat straw incubated in the H horizon) and from native SOM. The cylinders were arranged in a full factorial design with 3 factors (presence/absence of epigeic fauna, earthworms and gravel), giving 8 different experimental conditions that were randomly distributed within a 60×180 cm plot. Each treatment was replicated 12 times within the stand.

The factors considered were:

Factor F consisted of the presence (F+) or absence (F−) of soil epigeic fauna (meso and macrofauna). In F− treatments epigeic fauna (macrofauna and partially mesofauna) were excluded from the cylinders by extending the top of the cylinders with a stainless steel net (0.2 mm mesh size). This net surrounded the L and F horizons. In F+ treatments the cylinders were extended with a fiberglass net of 10 mm mesh size. To avoid inputs into the cylinders from litterfall and to minimize microclimatic differences between treatments, the top of the cylinders was closed with a 0.2 mm mesh stainless steel net. We did not add fauna to the cylinders during the incubation but the presence of faecal pellets in the L and F horizons of the F+ cylinders at the end of the experiment indicated that they were colonized by soil epigeic fauna.

Factor E consisted of the presence (E+) or absence (E−) of earthworms. Earthworms were excluded from the E− treatments by covering the bottom of the cylinders with a 0.2 mm mesh size stainless steel net. To allow the earthworms of the site to go into the cylinders, E+ cylinders were covered at the bottom with a 10 mm mesh fiberglass net. To ensure the presence of earthworms in the cylinders, two months after incubating the soil we added two earthworms collected from a nearby area to the A_1 horizon of the E+ cylinders. Towards the end of the experiment (day 516) we added another set of earthworms to all E+ cylinders. Two earthworms per cylinder make a density of 175 earthworms m^{-2} which is rather high for dry areas (Valle et al. 1997). However these earthworms could easily go out the cylinders so that during most of the study period earthworm density in the cylinders is expected to be similar to the density of the site. Earthworm activity was directly observed inside the cylinders during the incubation period (cast production on the soil surface). At the end of the experiment, when we sampled destructively the cylinders, we could find earthworms alive as well as burrows throughout the cylinders in the E+ treatments. *Allolobophora rosea* is the most common earthworm in the area of study. Because most earthworms we collected were not mature we cannot be sure that we introduced the same species of earthworm in all cylinders.

Factor G consisted of the presence (G+) or absence (G−) of a stone layer. The stone layer consisted of a 5 cm layer of gravel under the H horizon.

We used gravel with sharp edges of about 2–4 cm size. In the G+ treatments a significant part of the H horizon (from 40 to 50% of its weight) was intermingled with gravel.

Soil CO_2 efflux

Soil CO_2 release was measured in all cylinders 23 times in periods of 24 h, from November 1994 to February 1996 by using the alkali (NaOH 0.25 N) absorption technique (Anderson 1982). Total C-CO_2 as well as ^{14}C-CO_2 release were determined by titration with HCl 0.5 M, and scintillation counting, respectively. Casals et al. (1999) estimated that the CO_2 resulting from $CaCO_3$ cannot be greater than 1 g C-CO_2 m^{-2}. This release would mainly occur during soil drying episodes that normally take several days. Hence, in our experiment the order of magnitude of C-CO_2 released by lime was well bellow (always less than 1%) of the total effluxes measured. For that reason, we do not consider this source of CO_2 in the discussion.

Soil water potential (ψ)

Soil water potential (ψ) was measured with screen shield Wescor psychrometric thermocouple hygrometers (PST-55-15-SF). Fourteen psychrometric probes were installed in the lower part of the H horizon (3 cm depth) and A1 horizon (8 cm in G− and 11 cm in G+) in 7 PVC cylinders with the same characteristics as those used for the incubations. Four of these cylinders contained a gravel layer (G+) and three did not (G−). G− cylinders were placed alternatively with G+ in one plot. Soil water potential was recorded the days of soil CO_2 efflux measurements using a datalogger (PC208 Campbell Scientific, Inc., Logan) installed in the study area. The data were collected at 6 a.m. and 6 p.m. when soil temperature is expected to be more stable.

Rainfall simulation

To study the interactions between water availability, wetting and drying events, and soil fauna, we simulated rainfall on three occasions (March, July and August 1995). In March and July we sprayed 40 mm of water in 6 out of the 12 plots (R+ plots). To test whether the sequence of wetting and drying events had an effect on soil CO_2 effluxes, in August all 12 plots were sprayed in the same way.

Data analysis

Cumulative CO_2 efflux was calculated by integrating the 23 measurements over time. Except for the rainfall simulation days, the periods of time between soil CO_2 efflux measurements were assigned the value of the previous measurement for the first half of the period and to the latter for the second half. For the rainfall simulation days, CO_2 effluxes measured before rain simulation accounted for the whole period between measurements. Significant differences between treatments and their interactions were tested by a factorial analysis of variance (General linear models; SAS Institute 1988). To test for the effects of seasonality we used repeated measures analysis of variance.

Results

Effects of earthworms on total soil CO_2 efflux

Cumulative soil CO_2 efflux was higher in the presence (E+) than in the absence of earthworms (E−). This increase was especially large when the presence of earthworms coincided with gravel (E+G+ treatments), and represented a 7.4% increase in CO_2 release (see cumulative data; Figure 1). In the day by day analysis the E+G+ effect occurred only on three occasions: in the summer, prior to the first summer rainfall simulation (day 315), in winter and in autumn (days 91 and 219; Table 2). The synergistic effect of gravel and earthworms disappeared after natural and simulated rainfall events in spring and summer (days 272, 338, 342, 365 and 367; Table 2). That is, on those days when soil moisture was high the effect of earthworms was independent of the presence of gravels.

At our site, field observations indicated that earthworm burrowing activity occurred in late autumn and in winter (end of 1994 and beginning of 1995) and coincided with a period of relatively high soil moisture (between −1.5 and −0.3 MPa; see Casals et al. 1999). Except for the 10% increase in the E+ treatments four days after the second inoculation of earthworms, the effect of earthworms on soil CO_2 was mainly observed during the earthworm dormancy (spring and summer 1995 during rainfall simulation events) (Table 2 and, Figures 2 and 3). The largest effect of earthworms on CO_2 efflux occurred in summer after the first rainfall simulation, (15% increase on day 338). Afterwards the magnitude of this effect decreased but it was significant for at least four days (day 342; Figure 3).

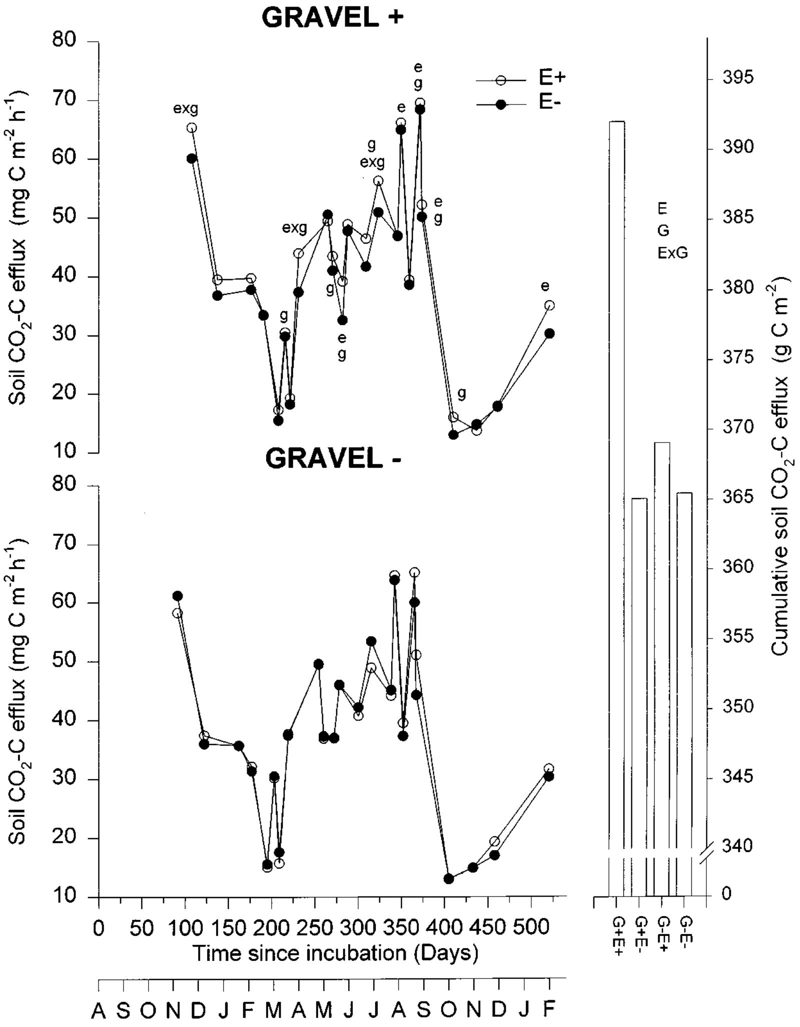

Figure 1. Hourly and cumulative soil CO_2 efflux as affected by a gravel layer (g) between the H and mineral horizons, and earthworms (e). Hourly rate corresponds to the average rate for each 24 h period of measurement. Significant effects and interactions at the 5% significance level are indicated with letters. Cumulative efflux integrates 450 days of nonirrigated plots (from November 1994 to February 1996). Points are means of 12 replicates except for the first two rainfall simulation periods in which the points are means of the 6 nonirrigated replicates. The only rainfall simulation shown is the day 365 (n = 12) when all cylinders where irrigated.

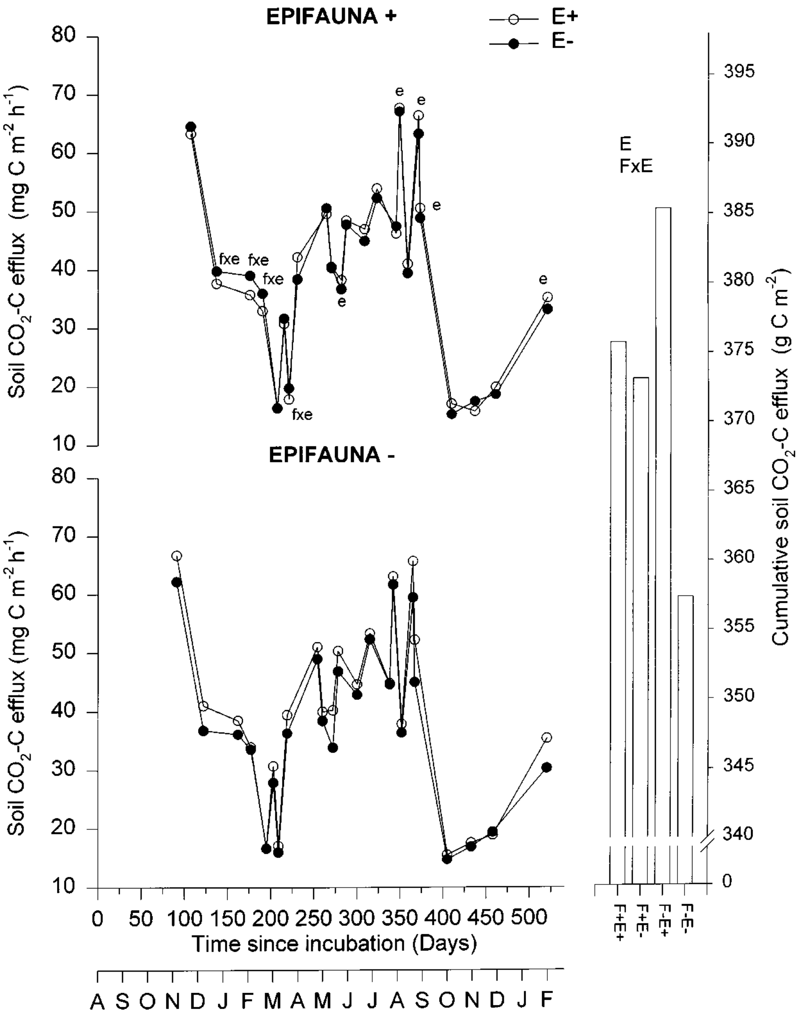

Figure 2. Hourly and cumulative soil CO_2 efflux as affected by epigeic fauna (f) and earthworms (e). Hourly rate corresponds to the average rate for each 24 h period of measurement. Significant effects and interactions at the 5% significance level are indicated with letters. Cumulative efflux integrates 450 days of nonirrigated plots (from November 1994 to February 1996). Points are means of 12 replicates except for the first two rainfall simulation periods in which the points are means of the 6 nonirrigated replicates. The only rainfall simulation shown is the day 365 (*n* = 12) when all cylinders where irrigated.

Table 2. Significant effects of treatments on daily total soil CO_2 efflux and its interactions (significance level <0.05). ns refers to nonsignificant effects ($p > 0.05$). Interactions not shown were not significant. R refers to rainfall simulation; F refers to the presence of soil epigeic fauna; E refers to the presence of earthworms and G refers to the presence of a gravel layer between the organic layers and the mineral soil.

Days of incubation	Rain simulation (mm)	Total C-CO_2 efflux Treatments and interactions									Season
		R	F	E	G	F×E	F×G	E×G	E×R	G×R	
91		ns	ns	ns	ns	ns	0.002	0.03	ns	ns	
122		ns	ns	ns	ns	0.01	ns	ns	ns	ns	Autumn
162		ns	ns	ns	ns	0.02	ns	ns	ns	ns	
177		ns	ns	ns	ns	0.03	ns	ns	ns	ns	
195		ns	ns	ns	ns	ns	ns	ns	ns	ns	Winter
203	40	ns	ns	ns	0.03	ns	ns	ns	ns	0.05	
209		0.0001	ns	ns	ns	0.03 ns	ns	ns	0.04		
219		ns	ns	ns	ns	ns	ns	0.04	ns	ns	
254		ns	ns	ns	ns	ns		ns ns	ns	ns	
260		ns	ns	ns	0.02	ns	ns	ns	ns	ns	
272		ns	ns	0.009	0.05	ns	ns	ns	ns	ns	Spring
278		ns	ns	ns	ns	ns	ns	ns	ns	ns	
300		ns	ns	ns	ns	ns	ns	ns	ns	ns	
315		ns	ns	ns	0.002	ns	0.02	0.003	ns	ns	
338	40	0.001	ns	0.003	ns	ns	ns	ns	0.002	ns	
342		0.001	ns	0.004	ns	ns	ns	ns	0.03	ns	Summer
352		0.001	ns	ns	ns	ns	ns	ns	ns	ns	
365	40 (all)	0.043	ns	0.01	0.009	ns	0.04	ns	ns	ns	
367		ns	ns	0.0006	0.04	ns	ns	ns	ns	ns	
405		ns	ns	ns	0.05	ns	ns	ns	ns	ns	
433		ns	ns	ns	ns	ns	ns	ns	ns	ns	Autumn
458		ns	ns	ns	ns	ns	ns	ns	ns	ns	
520	Worm add.	ns	ns	0.007	ns	ns	ns	ns	ns	ns	Winter
Cumulative		0.0001	ns	0.003	0.03	0.01	ns	0.02	ns	ns	

Effects of epigeic fauna on total soil CO_2 efflux

In the absence of earthworms, soil epigeic fauna increased cumulative soil CO_2 effluxes. In contrast, in E+ treatments soil fauna decreased soil CO_2 effluxes (Figure 2; Table 2). Day by day analysis of the data showed that these effects (F×E) occurred during the first year of incubation and especially during late autumn and early winter. These effects coincided with the period of earthworm activity (end of 1994 and beginning of 1995) and were detect-

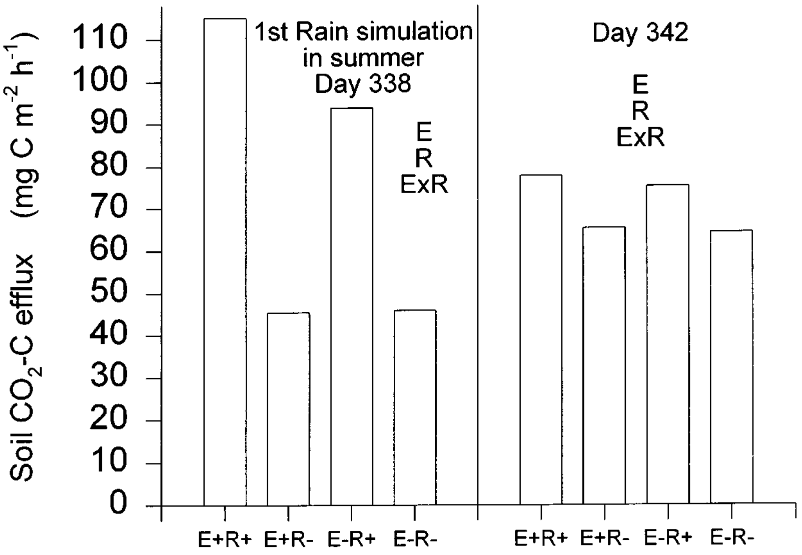

Figure 3. Effects of earthworms (E) and rainfall (R) after the first rainfall simulation in summer. Significant effects and interactions at the 5% significance level are indicated with letters.

able again in R+ plots 10 days after the winter rainfall simulation. During the earthworm dormancy period (summer) these effects (F×E) were observed only in the R+ plots 4 days after the first summer rainfall simulation.

Day by day analysis of the data showed that the effects of soil epigeic macrofauna on soil CO_2 efflux were small and depended on the time of the year (Figure 4 and Table 2). In summer (days 315 and 365), the presence of epigeic macrofauna increased soil CO_2 efflux on two occasions only in the G+ treatments (F×G) (Figure 4). During the rest of the year, this effect was only observed once during autumn 1994 (day 91) and was not detectable in the cumulative data.

Effect of earthworms on labeled-C release

The effect of earthworms on labeled-C release occurred in summer following rainfall simulations (Figure 5(a) and Table 3). Increased labeled-C release in E+ treatments did not depend on the presence of gravels and was not detected in the R− treatments. Four days after the first rain simulation in summer (day 342) the earthworm effect was still highly detectable in the R+ plots (Figure 5(a)). Although the positive effect of earthworms on labeled-C release was again observed after the second rainfall simulation in summer, no effect of

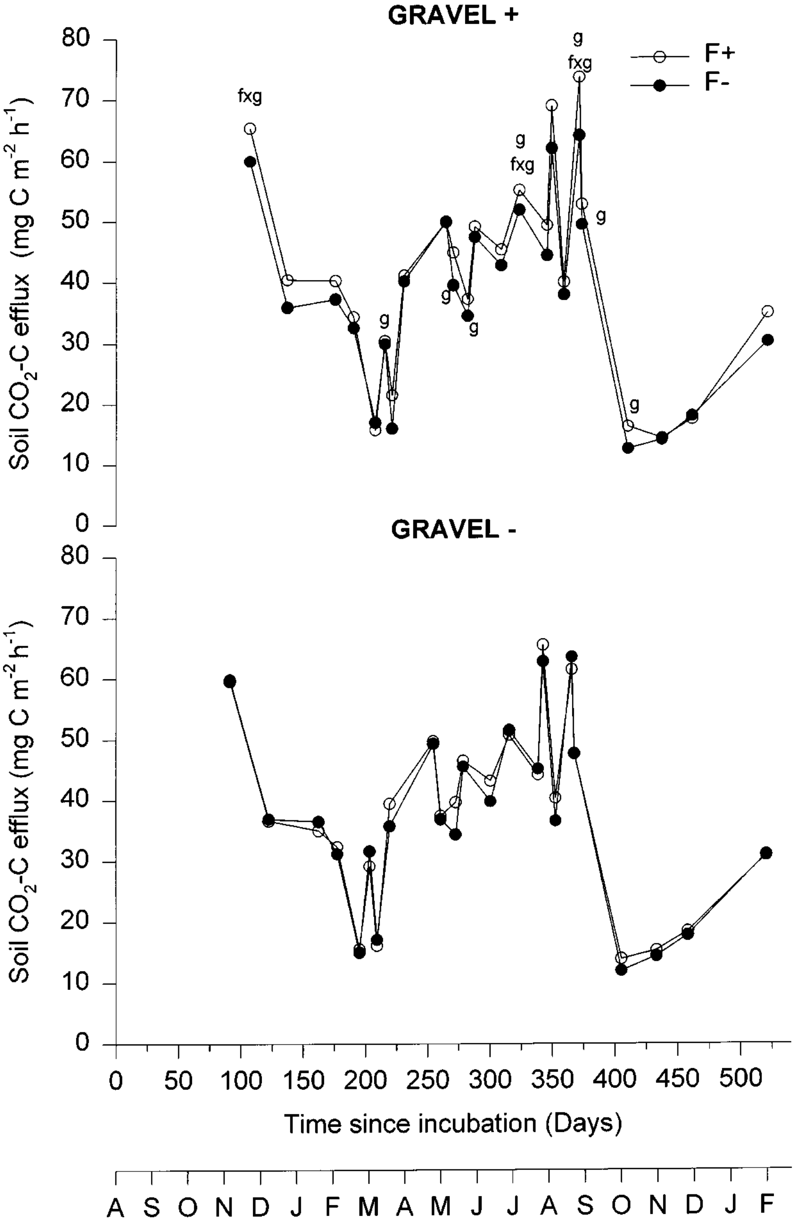

Figure 4. Hourly soil CO_2 efflux as affected by a gravel layer (g) between the H and mineral horizons and by epigeic fauna (f). Hourly rate corresponds to the average rate for each 24 h period of measurement. Significant effects and interactions at a 5% significance level are indicated with letters. Points are means of 12 replicates except for the first two rainfall simulation periods in which the points are means of the 6 nonirrigated replicates. The only rainfall simulation shown is the day 365 (n = 12) when all cylinders where irrigated.

Table 3. Significant effects of treatments on daily labeled-C efflux and its interactions (significance level <0.05). ns refers to non significant effects ($p > 0.05$). Interactions not shown were not significant. R refers to rainfall simulation; F refers to the presence of soil epigeic fauna; E refers to the presence of earthworms and G refers to the presence of a gravel layer between the organic layers and the mineral soil.

Days of incubation	Rain simulation (mm)	Labelled-C efflux Treatments and interactions									
		R	F	E	G	F×E	F×G	E×R	G×R	F×G×R	Season
91		ns	ns	ns	0.0004	ns	ns	ns	ns	ns	Autumn
122		ns	ns	ns	0.009	ns	ns	ns	ns	ns	
162		ns	ns	ns	0.02	ns	ns	ns	ns	ns	Winter
177		ns	ns	0.001	0.0001	ns	ns	ns	ns	ns	
195		ns	0.008	ns	0.03	ns	ns	ns	ns	ns	
203	40	0.0001	ns	ns	ns	ns	ns	ns	ns	ns	
209		0.0001	ns	ns	ns	ns	ns	ns	ns	ns	
219		0.0001	0.0001	ns	ns	ns	ns	ns	ns	ns	
254		ns	ns	ns	0.0001	ns	ns	ns	ns	ns	Spring
260		ns	ns	ns	0.0001	ns	ns	ns	ns	ns	
272		ns	ns	ns	0.005	ns	ns	ns	ns	ns	
278		ns	ns	ns	0.002	ns	ns	ns	ns	ns	
300		ns	ns	ns	ns	ns	ns	ns	ns	ns	
315		ns	0.03	ns	0.0001	ns	ns	ns	ns	ns	Summer
338	40	0.0001	ns	0.02	0.02	ns	0.03	0.01	0.04	0.03	
342		0.0001	ns	0.0008	ns	0.03	ns	0.0007	ns	ns	
352		ns	ns	ns	0.002	ns	ns	ns	ns	ns	
365	40 (all)	0.03	ns	0.01	ns	ns	ns	ns	ns	ns	
367		ns	ns	ns	0.0008	ns	ns	ns	ns	ns	
405		0.03	ns	ns	0.0001	ns	ns	ns	ns	ns	Autumn
433		ns	ns	ns	ns	ns	ns	ns	ns	ns	
458		ns	ns	ns	ns	0.06	ns	ns	ns	ns	
520	Worm inoc.	ns	0.002	ns	ns	ns	0.03	ns	ns	ns	Winter
Cumulative		0.0001	ns	ns	0.0001	ns	ns	ns	ns	ns	

the earthworms was observed two days later (day 367). Earthworms did not affect the cumulative labeled CO$_2$ efflux.

Effect of epigeic fauna on labeled-C release

As with earthworms, the largest increases in labeled-C release associated with epigeic fauna were recorded after the first rainfall simulations in summer (day 338) (Figure 5). Soil epigeic fauna increased the efflux of labeled-C in the

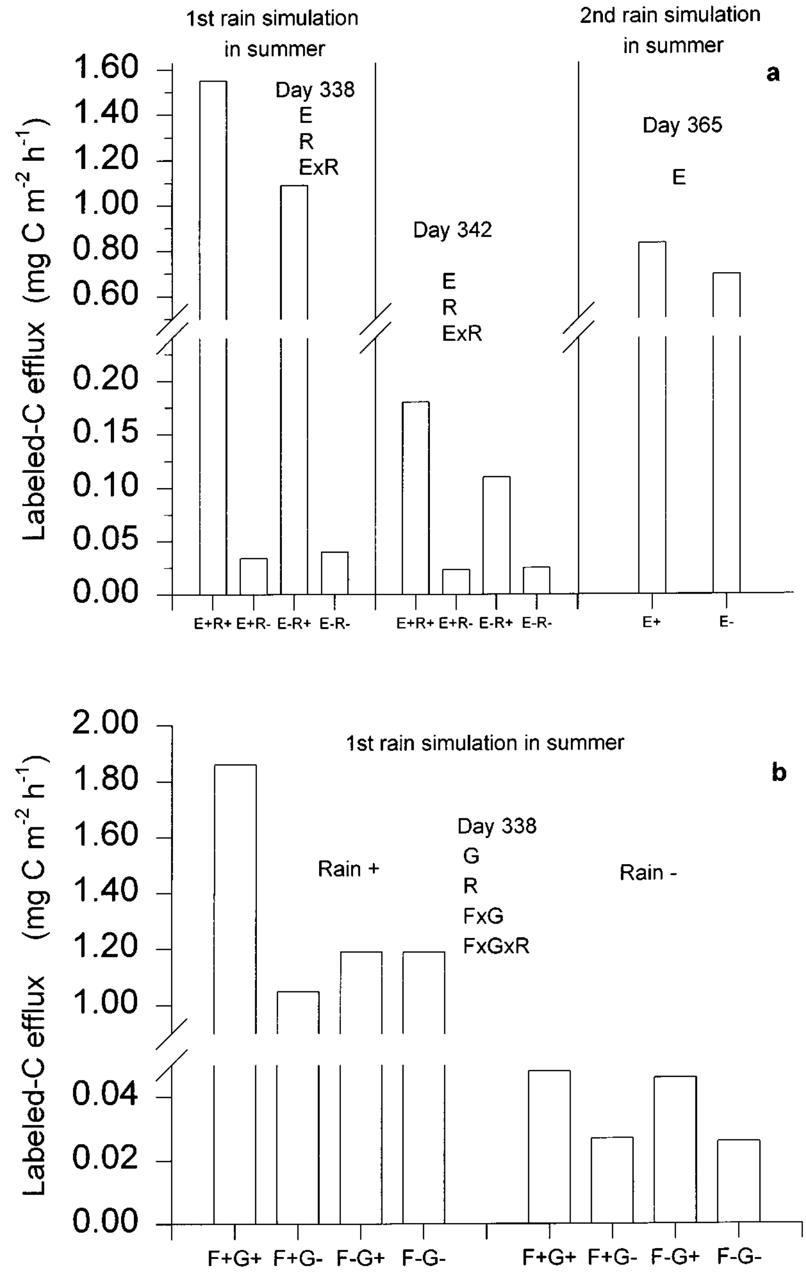

Figure 5. (a) Labeled-C efflux after the first (day 338) and the second (day 342) rainfall simulation in summer as affected by rain (R) and by earthworms (E). (b) Labeled-C efflux after the first rainfall simulation in summer as affected by a gravel layer (G) between the H and mineral horizons, rainfall simulation (R), soil epigeic fauna (F) and earthworms (E). Significant effects and interactions at the 5% significance level are indicated with letters.

G+ treatments and in the R+ plots (Figure 5(b)). This effect represented an increase of 61% for the F+G+ treatments after rainfall simulation compared to the other treatments. This large effect was ephemeral, as no significant differences were observed four days after the rain simulation, and it was not observed after the second rainfall simulation in summer. However, increased labeled-C efflux in F+G+ was again observed during autumn 1995 and winter 1996 (from day 367 to 520), and it represented a 46% increase in labeled-C efflux for F+G+ treatments compared to the other treatments (see repeated measures analysis in Figure 6). During the first winter (winter 1995) the presence of epigeic fauna resulted in a decrease in labeled-C release on two occasions (days 195 and 219).

The gravel layer consistently increased labeled-C release throughout the year (Casals et al. 1999) and provided a cumulative increase of 16%. As stated above, the gravel layer favored labeled-C release in the F+ treatments on several occasions. However, cumulative values of labeled-C showed increases for all G+ treatments irrespective of the presence of epigeic fauna and/or earthworms.

Discussion

Effect of earthworms

E+ cylinders were open. Because of this and because of the addition of earthworms on two occasions, we are sure on the access of earthworms to these cylinders. In this experiment, however, there was no control on the species or functional groups colonizing the soil, and thus all discussion is focused on the potential exclusion or not of earthworms of the site. At the study site earthwroms accumulate large amounts of casts on the soil surface during periods of high water availability. The period of earthworm activity was rather short, lasting form late autumn, through winter to early spring. During this period, the effects of earthworms on total CO_2 efflux was small, and no increase in labeled-C release was observed as a result of earthworm activity. The earthworm active period coincided with the coldest months at our site. At that time soil microbial activity was low probably as a result of low temperatures especially in the organic layers (Casals et al. 1999). Low microbial activity may have kept the effect of earthworms in winter low. In contrast, in summer, despite earthworm dormancy, large effects of earthworms were observed on both total CO_2 and on labeled-C release after wetting. Other authors working in wet temperate areas have found that earthworms increase soil respiration mainly in summer and suggest that the seasonal pattern of the earthworms effect is, in part, due to temporal patterns of fresh organic matter

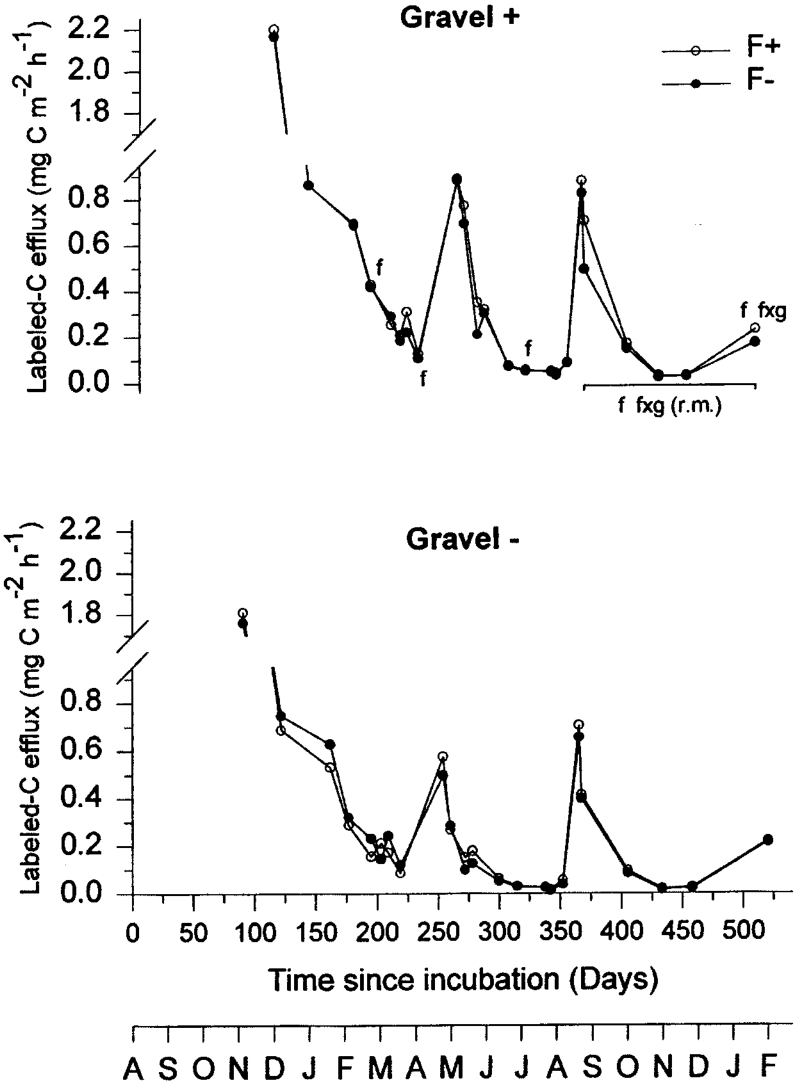

Figure 6. Daily labeled-C efflux as affected by a gravel layer (g) between the H and mineral horizons and epigeic fauna (f). Hourly rate corresponds to the average rate for each 24 h period of measurement. Significant effects and interactions at the 5% significance level are indicated with letters. *r.m.* refers to the significance of the repeated measurements analysis of variance. Points are means of 12 replicates except for the first two rainfall simulation periods in which the points are means of the 6 nonirrigated replicates. The only rainfall simulation shown is the day 365 ($n = 12$) when all cylinders where irrigated.

availability (Schindler-Wessells et al. 1997). In our experiment in a semiarid forest, the availability of resources to soil microbiota may have increased as a result of winter earthworm activity. In summer, drying and wetting cycles may provide the appropriate climate for mineralizing these resources. Thus, the magnitude of earthworm effects was especially large during wetting and drying cycles and lasted as long as the wetting and drying cycle (at least 4 days). Earthworms can stabilize SOM over the medium term (Scheu & Wolters 1991; Scheu 1993; Marinissen 1995). Martin (1991) found that, one year after egestion, earthworm casts had an 11% higher content of coarse organic debris than nonegested soil. This increase in organic matter may eventually induce an increase in soil biological activity. Indeed, Hendriksen (1997) found increased respiration rates in soils incubated in the lab with earthworms compared to others with no earthworms.

Earthworms increased soil respiration under the influence of rainfall simulation in summer. Unlike the effects associated with soil epigeic fauna, the effects of earthworms were synchronized for organic layers (labeled-C) and for the mineral soil surface (total soil CO_2 efflux) and coincided with the drying and wetting cycle. The relative magnitude of the effects of earthworms was larger for total soil CO_2 effluxes than for labeled-C, suggesting that earthworms burrowed mainly in the mineral soil where no labeled-C was added. In warm Atlantic climates earthworm activity in arable lands promotes an increase in straw decomposition ranging from 24 to 47% within a period of 8–10 months (Curry & Byrne 1992). In contrast, the cumulative respiration of the straw incubated in our experiment did not show any effect of earthworms.

Effects of soil epigeic fauna

Mediterranean forest soils normally shelter large populations of soil macroarthropoda (Shaw et al. 1991). As suggested by Zlotin (1971) the activity of epigeic fauna of larger sizes (macrofauna) has a greater importance in enhancing litter decomposition than epigeic fauna of smaller sizes (mesofauna and microfauna). In forest ecosystems, these large organisms live mainly in the organic layers where soil pores are largest (Lavelle 1994). Hence, the effects of soil epigeic fauna on soil CO_2 effluxes may be more easily detected when the effluxes coming from the organic layers can be isolated from those coming from the mineral soil. In our experiment the largest differences attributed to soil epigeic fauna were actually observed in labeled-C effluxes in the G+ treatments, for short periods of time after the first rainfall simulation in summer and during the second autumn and winter. Hassal et al. (1986) observed that, in a temperate forest of *Populus tremuloides*, although soil fauna moved down into the deeper horizons of the litter during the dry summer months, a significant proportion of fauna returned to the surface layers after summer rainstorms. This is unlikely in our Mediterranean system because the high intensity of summer droughts may prevent the activity of soil fauna throughout most of the season. Indeed, in a Mediterranean holm oak (*Quercus ilex*) forest located close to our site, Mateos (1992) found that the abundance of soil fauna was minimum during the summer months. On the other hand, Gallardo and Schlesinger (1995) suggested that, in desert soils during dry periods, soil carbohydrates may accumulate as a result of soil decomposition and that the rapid flush of decomposition after wetting the soils may be due to a persistent pool of enzymes capable of tolerating extended periods of desiccation. In our experiment, because the increases in soil CO_2 associated with the soil fauna were mainly detected for labeled-C effluxes and occurred during the season of low abundance of epigeic fauna after wetting the soil, we suggest that these increases may have resulted from the indirect effects of soil fauna on the physico-chemical properties of litter that can enhance the activity of H layer microbia.

Large increases in labeled-C release in the F+G+ treatments after summer rainfall simulation did not last as long as the rainfall effects during the wetting and drying process. We suggest that the soil fauna transformed the labeled organic matter into relatively labile compounds that decomposed during the wetting and drying cycles. The low microbial activity that occurred under dry conditions in summer was not positively affected by soil fauna (see day 315; Table 3).

Although at the end of the experiment we observed evidences of activity of epigeic fauna in F+ cylinders, we do not know how long did the fauna take to recolonise these cylinders. In the F− treatments we did not observe any sign of faunal activity throughout the experiment. In the cumulative data, we did not detect any overall effect of soil fauna on soil respiration suggesting that the effects of soil fauna were low. However, we did observe large effects (up to a 46% increase) in the day by day analysis, mainly for labeled-C release from the H layer and mostly towards the end of the experiment associated with the G+ treatments (after 370 days of incubation; Figure 6). This fact suggests that the effect of soil epigeic fauna observed under field conditions at our semiarid site was especially relevant in the organic layers and it was probably underestimated as a result of the limited length of the period of study. As one of the mechanisms by which soil fauna accelerates decomposition is by comminuting fresh organic matter (Wachendorf et al. 1997), the fact that the straw we incubated was ground may have delayed the positive effect of soil fauna on soil C mineralization. In a microcosm study, Sulkava et al. (1996) incubated faunal communities of different complexities with fresh humus under different moisture regimes. In dry conditions they found that

N-mineralization did not increase during the first 18 weeks despite the presence of faunal community but that increases did occur by week 24.

The reduction in respiration rates that occurred in the E+ treatments only in the presence of soil epigeic fauna took place mainly during the period of maximum earthworm activity (from late autumn to winter 1994–95; Figure 2). Therefore it appears that soil epigeic fauna may have reduced the effects of earthworm on soil CO_2 effluxes. Lavelle et al. (1994) indicated that litter arthropods seem to be predominant in ecosystems where sufficient litter is available and which consequently have low earthworm activity. Other authors (Coleman 1986) have reported that earthworms may ingest soil microfauna (protozoa and nematodes) or that the activity of earthworm may favor the activity of other soil fauna by providing channels and passageways for them (Rusek 1986; Hamilton & Sillman 1989; Wickenbrock & Heisler 1997). However, to our knowledge, no direct inhibitory effects of earthworms on soil macroarthropoda have been described. In the absence of earthworms, the presence of soil epigeic fauna enhanced total soil CO_2 effluxes. This effect was mainly detected during the first autumn and early winter. Somewhat surprisingly, no effects of epigeic fauna on labeled-C release were detected at that time.

Gravel/fauna interactions

During most of the year, the gravel layer increased the availability of water of both H and A1 horizons (Table 4). In summer, coinciding with the period of low faunal activity, we did not detect any increase in water availability in G+ treatments at the H horizon level but we detected it at the A1 horizon. Increases in total CO_2 effluxes associated with earthworms, were greatest in the G+ treatments (Figure 1). Because no cumulative effects of earthworms on labeled-C were observed, it appears that earthworm activity mainly took place in the mineral soil and did not selectively affect the mineralization of the labeled material.

Increases in cumulative labeled-C release were observed in all G+ treatments and were independent of the presence of fauna (epigeic fauna and earthworms). Thus, it appears that these changes mainly resulted from the more favorable microclimate microbial decomposers that the stone layer created in the G+ treatments during periods of faunal activity (Table 4; see Casals et al. 1999). However, towards the end of the incubation, high labeled-C evolution rates in F+G+ treatments suggested that the effects of soil epigeic fauna were concentrated in the organic layers of the G+ cylinders. Since soil arthropods move on the surface of soil macropores and have a limited ability to dig, soil morphological features can influence the body size distribution of soil arthropods (Deharveng & Bedos 1993; Morse et al. 1985). The

Table 4. Seasonal average of soil water potential (ψ) in H and A1 horizons with and without a gravel layer on the A1 horizon surface. G+ refers to treatments with gravel and G− without gravel. Standard error is shown in brackets ($n = 4$ for G+ and $n = 3$ for G−).

Season	H horizon (ψ)		A1 horizon (ψ)		Factor significance ($p < 0.05$)		
	G+	G−	G+	G−	Gravel	Horizon	Gravel∗Hor.
Autumn	−1.42 (0.37)	−2.77 (0.91)	−2.73 (0.48)	−4.15 (0.09)	0.03	0.04	ns
Winter	−2.48 (0.48)	−3.18 (0.47)	−0.46 (0.09)	−2.68 (0.91)	0.02	0.03	ns
Spring	−5.57 (0.30)	−7.24 (0.59)	−1.68 (0.27)	−3.48 (0.61)	0.003	0.001	ns
Summer	−4.50 (0.41)	−2.23 (1.16)	−1.10 (0.65)	−3.40 (0.46)	ns	ns	0.02

ns: non significant ($p < 0.05$)

different soil morphological features of the G+ treatments compared to G− may have influenced the activity of macroarthropods. Hassink et al. (1993) studied soils of different textures and showed positive relationships between habitable pore space and the biomass of bacteria and soil fauna. The size of pores that is related to the biomass of the soil biota is in relation to the size of the organisms. On the other hand, as the activity of soil fauna under water limiting conditions has been found to directly relate to soil water availability (Harte et al. 1996; Dangerfield & Telford 1991), higher water potentials in the G+ treatments (Casals et al. 1999) may have enhanced faunal activity. The microclimatic changes associated with the stone layer (Table 4) may have also stimulated earthworm activity and may have enhanced soil CO_2 efflux.

Both the enhanced effects of soil fauna on CO_2 efflux in the presence of gravels, and the microclimatic conditions created make it difficult to explain the high forest floor accumulation that occurs in Mediterranean soils with stones at the soil surface. In a study of humus forms of *Pinus halepensis* Mill. forests from a nearby area, Fons (1995) suggested that the high accumulation of soil organic matter in soils with a high gravel content in the organic layer level may be attributed to a decrease in the transfer of particulate material induced by the presence of gravel. An alternative explanation could be related to increased litterfall input resulting from improved microclimatic conditions in stony soils.

Conclusions

Changes in soil C effluxes induced by the interaction between fauna and a gravel layer indicate that gravels had a larger effect than soil fauna. Organic matter mineralization in the organic layers increased as much as 16% for G+ treatments. The largest increase attributed to soil fauna was associated to earthworms, and accounted for 7.4% increase in total soil CO_2 efflux.

These increases occurred in the G+ treatments and were mainly the result of earthworm burrowing activity that primarily took place in the mineral soil. The effects of earthworms and soil epigeic fauna were maximum in the presence of gravels for short periods of time associated with wetting and drying cycles. This increase in CO_2 release was as much as 61% and was mainly observed during periods of adverse conditions for faunal activity. The presence of gravel in the H horizon enhanced soil CO_2 efflux by creating a more favorable microclimate for microbial activity which, at the same time, enhanced the effects of epigeic fauna and earthworms. Thus, we conclude that the presence of gravel favors decomposition. The possible effects of the gravel layer on inhibiting the transfer of particulate material into the mineral soil or on the litterfall input are possible alternative explanations for such high forest floor accumulations.

Observed changes in soil CO_2 efflux overtime suggest that in this Mediterranean semiarid soil the first factor regulating soil C mineralization is the seasonal change in the soil climate. Secondly, CO_2 efflux may be greatly modified at the forest floor level by the presence of a surface layer of rock fragments. When considering total CO_2 efflux, soil fauna, particularly earthworms, may represent the third factor, the effects of which are mainly concentrated in the mineral soil. We must consider however, that organic matter packed in the cylinders, especially the H horizon, was exposed to soil fauna for a long period of time in natural conditions before the incubation. The effects of soil fauna and gravel that we have detected are the result of a period of burrowing and comminuting no longer than a year and a half. Hence, the effect of soil fauna interacting with gravel may be larger than observed if we had considered faunal activity over the longer term.

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