Oecologia (1996) 107: 131–140 

Mark Maraun · Stefan Scheu

# Changes in microbial biomass, respiration and nutrient status of beech (*Fagus sylvatica*) leaf litter processed by millipedes (*Glomeris marginata*)

Received: 17 November 1995 / Accepted: 3 January 1996

**Abstract** The effect of processing of beech leaf litter (*Fagus sylvatica* L.) of different ages by the diplopod *Glomeris marginata* (Villers) on status and turnover of microorganisms was investigated in the laboratory. Microbial biomass, basal respiration and metabolic quotient of litter-material from three different beech-wood sites of a basalt hill forming a gradient from basalt (upper part of the hill) to limestone (lower part of the hill) were determined each season (February, May, August and November). The same microbial parameters were also measured after these litter materials had been processed by *G. marginata* (faecal pellets of an average age of 4 days). Short-term changes in microbial biomass and respiration in leaf material and faecal pellets from February and August were investigated after 1, 2, 5, 10, 20 and 40 days of incubation. The ergosterol content of August samples was determined. Processing of beech leaf litter by *G. marginata* increased microbial biomass in February and May but reduced microbial biomass in August and November. It was concluded that processing of litter materials in February and May increased accessibility of carbon resources to microorganisms by fragmentation. In contrast, in litter materials from August and November carbon resources were depleted and fragmentation by diplopods did not increase availability of carbon resources. Addition of carbon (glucose) and nutrients (nitrogen and phosphorus) to litter and faecal pellets indicated that processing of beech litter reduced nutrient deficiency of the microflora. Ergosterol content in faecal pellets was reduced strongly after beech leaf litter processing by *G. marginata*, indicating a decrease in fungal biomass. Presumably, in faecal pellets bacteria flourished at the expense of fungi.

M. Maraun (✉) · S. Scheu
II. Zoologisches Institut, Abteilung Ökologie,
Berliner Strasse 28, D-37073 Göttingen, Germany
FAX (049) 551-395448

**Key words** Decomposition · Diplopods · Ergosterol · Metabolic quotient · Microflora

## Introduction

Macroinvertebrate-microbial interactions play an important role in terrestrial ecosystem processes (Swift et al. 1979; Anderson et al. 1984; Schaefer 1991; Lussenhop 1992). The investigation of animal effects on microorganisms and on decomposition processes dates back to early studies of Gere (1956) and Ghilarov (1963). Since then a variety of methodological and analytical improvements have been made. In recent studies, macroinvertebrate-microbial interactions have been examined in more detail for tipulids (Standen 1978), diplopods (Anderson and Bignell 1980), earthworms (Scheu 1987) and isopods (Van Wensem et al. 1993). Macroinvertebrates modify microbial processes mainly via changes in environmental conditions of the microbial community (e.g. aeration, accessibility of resources, mixing of litter and mineral soil). In contrast, the influence of mesofauna species on soil microorganisms is more specific due to selective grazing on fungi by mites, collembolans and enchytraeids. Fungal activity was increased after collembolan grazing when fungi had grown in high nutrient concentrations but activity declined when fungi were grown in low nutrient concentrations (Hanlon 1981a). In general, fungal biomass appears to increase in response to grazing by Collembola (Visser et al. 1981; Leonard and Anderson 1991) and enchytraeids (Standen 1978; Hedlund and Augustsson 1995).

The functional significance of macrofauna (e.g. earthworms, diplopods, isopods) has mainly been investigated in soil (Anderson 1987; Wolters 1991a) and only few studies have dealt with the relationship between macrofauna and microflora in leaf material. Standen (1978) reported an increase in microbial activity in blanket bog litter as a result of

the feeding activities of tipulid larvae. An increase in microbial respiration due to woodlice and millipede feeding activities was also observed by Anderson and Ineson (1983) and Teuben and Roelofsma (1990) reported an increase in microbial activity in *Pinus nigra* litter after processing by *Philoscia muscorum*. However, when animal density was increased Anderson and Ineson (1983) found that microbial respiration might be reduced by increased feeding intensity of the animals. Hanlon and Anderson (1980) also found microbial respiration in fragmented oak litter to be initially increased in presence of *Glomeris marginata* but respiration rates declined later to rates slightly above the control level. In contrast to these findings Van Wensem et al. (1993) reported a decrease in microbial respiration of poplar leaves processed by isopods. Therefore, despite the several detailed analyses of the functional ecology of litter-feeding macroinvertebrates, their effect on microbial metabolism remains controversial.

We hypothesize that conflicting results are caused by the investigation of litter of different quality (litter of different C/N ratio, different chemical composition and different age) and by the investigation of substrates at different times after processing by macroarthropods. Therefore, in this study we investigated the effect of litter processing by the diplopod *Glomeris marginata* on microbial biomass, basal respiration and metabolic quotient at different seasons (spring, early summer, late summer, autumn) because litter quality changes during the year (Joergensen 1991). We included litter materials from three beechwood sites on a gradient from basalt to limestone because it has been shown that chemical composition of beech litter varies with parent rock of the stand (Nicolai 1988). We chose *G. marginata* because this species is known to be the most important saprophagous macroarthropod in the decomposer subsystem at the study site (A. Nieselt, unpublished work).

In addition to seasonal alterations, we investigated short-term (40 days) changes of microbial parameters due to processing of beech leaf litter by *G. marginata* to obtain a more comprehensive picture on microbial dynamics in the litter layer in presence and absence of *G. marginata*. Generally, we expected a short-term increase in microbial biomass and respiration in faecal pellets after animal processing because litter comminution and digestion increases the surface area for microbial attack (Anderson and Ineson 1984, Hopkin and Read 1992). This short-term increase in microbial activity is expected to decline with depletion of carbon resources (Berg and Staaf 1980).

Recent studies indicate that the gut passage of litter materials through soil invertebrates, e.g. earthworms (Scheu 1993, 1994) or isopods (Teuben 1991; Teuben and Verhoef 1992), releases nutrients which may enable microorganisms in faecal materials to grow. To prove whether nutrients made available in faecal material of saprophagous macroinvertebrates change the nutrient status of microorganisms we investigated the nutrient demand of the microflora in beech leaf litter processed by *G. marginata*. For determination of the microbial nutrient demand we measured the additional microbial respiration (AMR) during growth in intact litter and in faecal pellets after addition of glucose and nutrients (nitrogen, phosphorus).

Previous studies have concluded that invertebrate processing of litter material predominantly affects fungal biomass (Nicholson et al. 1966; Anderson and Bignell 1980; Ineson and Anderson 1985; Hassall et al. 1987). To validate these assumptions we determined the content of ergosterol (cf. Seitz et al. 1977) in leaf material and in faecal pellets of *G. marginata* (August samples only). Ergosterol is an important fungal sterol, which is almost absent in higher plants. It has recently been shown to be a good measure of fungal biomass in plant material (Newell et al. 1988; Gessner et al. 1991; Scheu and Parkinson 1994a), in soil (Davis and Lamar 1992; Djajakirana et al. 1996) and in decomposing wood (Nilsson and Bjurman 1990).

## Materials and methods

### The sites

Beech leaf litter (three replicates) was sampled in February, May, August and November 1993 at three different beechwood (*Fagus sylvatica*) sites on a basalt-limestone gradient at the Kleiner Gudenberg (Hesse, Germany). At the top of the hill parent rock is basalt, whereas at the lower part it is limestone with an intermediate site in between. There is a gradient in soil acidity from the upper part (pH 5.5) to the lower part of the hill (pH 7.6). Freshly fallen leaf material from autumn 1993 was omitted from the analysis because we intended to follow changes in the same litter cohort for 1 year and because it is known that animals refuse to feed on freshly fallen leaf material (Sakwa 1974; Hassall and Rushton 1984).

### The experiments

Individuals of *G. marginata* were sampled at the study site by hand. Litter from each site was hand collected and fed to 15 specimens of *G. marginata* which were kept in vessels of about 200 $cm^3$. Litter pH at the three sites varied parallel to parent rock (5.6, 5.8, 6.2 for basalt, intermediate and limestone site, respectively: means of three replicates, February).

#### *Seasonal variations*

To analyse the seasonal variation in the status of the microflora in leaf material and in faecal pellets, microbial biomass, basal respiration, metabolic quotient and C/N ratio were analysed at each of the sampling dates (February, May, August and November). Beech leaf litter and faecal pellets were investigated after an incubation period of 8 days at room temperature (average age of faecal pellets of 4 days).

*Short-term alterations*

To determine short-term alterations in microbial biomass, basal respiration and metabolic quotient in beech leaf litter processed by *G. marginata* these parameters were investigated 1, 2, 5, 10, 20 and 40 days after litter processing (February and August samples from the basalt site only). For that purpose faecal pellets deposited during 24 h were analysed immediately or incubated for another 1, 4, 9, 19 and 39 days at 20 °C until the analyses were made. Control samples of intact beech litter were treated in the same way. In addition to microbial biomass and respiration, short-term alterations in microbial nutrient status were determined in these materials. Ergosterol content was determined in litter and faecal material of day 1 (August samples from each of the sites).

## Analytical procedures

Microbial biomass, basal respiration and metabolic quotient of leaf material and faecal pellets were determined by the substrate-induced respiration (SIR) method (Anderson and Domsch 1978). Microbial respiration was measured as oxygen consumption with an automated electrolytic $O_2$ microcompensation apparatus (Scheu 1992).

Basal respiration of beech leaf litter was determined in freshly collected litter material (equivalent to 0.1 g dry weight) after fragmentation in a blender for 3–5 s (cf. Beare et al. 1990) to a size of $< 25$ mm$^2$ and adjusted to the water content to 800% of dry weight (Maraun and Scheu 1995). Basal respiration was calculated as mean $O_2$ consumption of hours 15–20 (Insam 1990). Faecal pellets of *G. marginata* were measured in the same way, omitting the blending procedure.

For measurement of microbial biomass carbon ($C_{mic}$) 80000 µg glucose g$^{-1}$ dry weight were added as a solution to field-moist material to increase the water content to 800% of the dry weight (Beare et al. 1990, Maraun and Scheu 1995). Maximum initial respiratory response (MIRR) was converted to $C_{mic}$ according to the formula given by Anderson and Domsch (1978):

$C_{mic}$ (µg C g$^{-1}$ dry weight) = 40 × MIRR (µl $O_2$ h$^{-1}$ g$^{-1}$ dry weight)

Respiratory quotient ($CO_2$ release per $O_2$ consumed) was assumed to be 1.0 (Smith and Brown 1932; Ross 1980).

To estimate the specific respiratory activity of the microflora we calculated the metabolic quotient ($qCO_2$; Pirt 1975; Anderson and Domsch 1990). For calculation of $qCO_2$ (µg $CO_2$-C µg$^{-1}$ $C_{mic}$-C h$^{-1}$) basal respiration measured as oxygen consumption was converted into $CO_2$ production. For conversion we used a respiratory quotient of 0.8 as proposed by Stotzky (1960) and Ross (1980) for unamended soil microorganisms.

Microbial nutrient status in litter materials and faecal pellets was determined by adding glucose (C), nitrogen (N), and phosphorus (P) in the combinations C, CN, CP, and CNP and by measurement of the additional microbial respiration (AMR) during microbial growth (Scheu 1993). AMR was calculated as cumulative $O_2$-consumption during microbial growth (if existing) after reaching the maximum initial respiratory response (Anderson and Domsch 1978). As for SIR measurement, 80000 µg glucose g$^{-1}$ dry weight were added to litter and faecal samples. Nitrogen [as $(NH_4)_2SO_4$] and phosphorus (as $Na_2HPO_4$) were added in mass ratios of C:N:P of 10:2:1. These ratios correspond to the ratio of C:N:P in microbial tissue (Anderson and Domsch 1980).

Ergosterol content was determined as outlined in Djajakirana et al. (1996) with modifications for litter materials (Scholle et al. 1993). In short, fresh leaf and faecal material equivalent to 1 g dry weight was shaken with 100 ml methanol for 30 min (250 rev min$^{-1}$), filtered (Whatman GF/A) and dried at 40 °C. Before HPLC measurement the residue was redissolved by adding 10 ml ethanol (96%) and filtered again (0.45 µm). The extracts were injected and eluted with methanol at a flow rate of 1 ml min$^{-1}$. Column eluant was monitored for absorbance at 282 nm.

C/N ratio in litter materials and faecal pellets was analysed by means of an elemental analyser (Carlo Erba Company, Milano).

## Statistical analysis

Seasonal alterations in microbial biomass, basal respiration, metabolic quotient and C/N ratio were analysed by three-way analysis of variance. The factors were SEASON (February, May, August and November), SITE (basalt, intermediate and limestone) and GLOMERIS (beech leaf litter and faecal pellets of *G. marginata*). Short-term alterations in microbial biomass, basal respiration and metabolic quotient were analysed by two-way analysis of variance with factors GLOMERIS (see above) and TIME (destructive samplings after 1, 2, 5, 10, 20 and 40 days). Data on additional microbial respiration (AMR) were analysed by four-way analysis of variance. Factors were TIME (see above), GLOMERIS (see above), NITROGEN (with and without nitrogen) and PHOSPHORUS (with and without phosphorus). Data on ergosterol content were analysed by two way analysis of variance with factors SITE and GLOMERIS (see above). Data were analysed using Proc ANOVA, a procedure for analysis of variance, in SAS (SAS Institute 1985). Tukey's honestly significant difference was used for comparison of means (Sokal and Rohlf 1995).

# Results

## Seasonal variations

The C/N ratio in beech leaf litter declined later in the year (overall mean of 23.1, 20.2 and 18.8 for February, May and August samples, respectively) but increased slightly in November (overall mean of 23.2). SEASON accounted for 37% of the variation (Table 1). C/N ratio was not significantly affected by processing of litter materials by *G. marginata*. The overall mean C/N ratio in litter and faeces was 21.4 and 21.3, respectively. C/N ratio at the limestone site (22.8) exceeded that at the basalt and the intermediate site (overall mean 20.8 and 20.5, respectively).

Microbial biomass of beech leaf litter remained very constant throughout the year (Fig. 1). Processing of leaf material by *G. marginata* differentially altered microbial biomass. In February and May microbial biomass in leaf material of faecal pellets was higher whereas in August and November it was lower (Fig. 1). The interaction of GLOMERIS and SEASON was the most important factor, accounting for 42% of the variation (Table 1). The effect of the animals was independent of site, even though microbial biomass varied significantly among sites and was lowest at the intermediate site (−21% and −13% in comparison with the basalt and limestone site, respectively).

Basal respiration in litter and faecal pellets declined from February to August but increased in November and SEASON accounted for 30% of the variation (Table 1). Similar to microbial biomass the effect of leaf litter processing on basal respiration also varied with season (Fig. 1). In February and May basal respiration was only slightly affected by *G. marginata* processing but in August and November basal respiration was

**Table 1** Three factorial ANOVA table on the effect of site (basalt, intermediate, limestone), litter processing by *Glomeris marginata* and season (sampling in February, May, August and November) on microbial biomass, basal respiration, metabolic quotient and C/N ratio of beech leaf litter (for details see text)

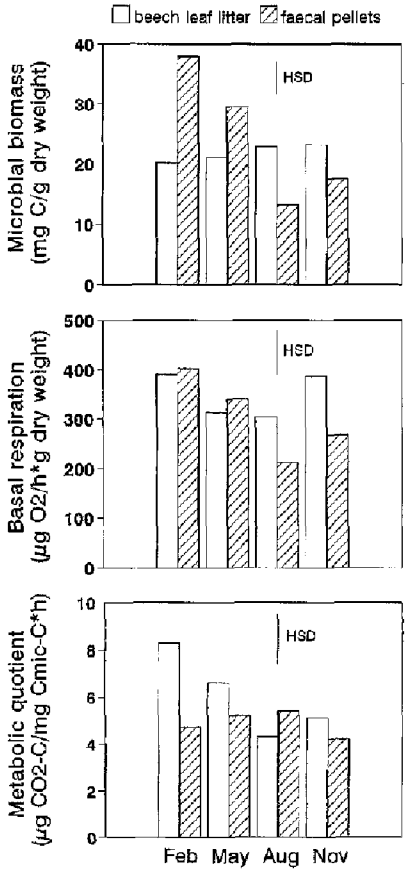
| | | Microbial biomass | | Basal respiration | | Metabolic quotient | | C/N ratio | |
|---|---|---|---|---|---|---|---|---|---|
| | *df* | SS (%) | *F* | SS (%) | *F* | SS (%) | *F* | SS (%) | *F* |
| SITE | 2 | 7.1 | 13.6*** | 7.9 | 6.1** | 24.2 | 27.4*** | 16.7 | 17.8*** |
| GLOMERIS | 1 | 2.7 | 10.5** | 7.3 | 11.3** | 9.0 | 20.3*** | 0.0 | 0.1 ns |
| SEASON | 3 | 26.3 | 33.5*** | 29.9 | 15.5*** | 14.7 | 11.1*** | 36.9 | 26.3*** |
| SITE × GLOMERIS | 2 | 0.3 | 0.6 ns | 0.7 | 0.6 ns | 0.3 | 0.3 ns | 2.0 | 2.1 ns |
| SITE × SEASON | 6 | 6.9 | 4.4*** | 3.6 | 0.9 ns | 8.4 | 3.2 ns | 8.9 | 7.0*** |
| GLOMERIS × SEASON | 3 | 41.7 | 53.2*** | 15.3 | 7.9*** | 17.6 | 13.3*** | 9.8 | 3.2* |
| SITE × GLOMERIS × SEASON | 6 | 2.5 | 1.6 ns | 4.5 | 1.2 ns | 4.5 | 1.7 ns | 3.2 | 1.1 ns |
| MODEL | 23 | 87.5 | 14.6*** | 69.1 | 4.7*** | 78.8 | 7.7*** | 77.5 | 7.2*** |

$^{***}P < 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$, *ns* $P > 0.05$

strongly reduced. The interaction of GLOMERIS and SEASON accounted for 15% of the variation.

Metabolic quotient in leaf litter declined continuously from February to August (Fig. 1) and SEASON accounted for 15% of the variation (Table 1). In contrast to microbial biomass, metabolic quotient was highest at the intermediate site (+ 42% and + 46% in comparison to the basalt and limestone site, respectively). The influence of *G. marginata* on the metabolic quotient of the microflora was opposite to that on microbial biomass. Metabolic quotient was strongly reduced in February and to a lesser extent also in May (not significant). The interaction of GLOMERIS and SEASON accounted for 18% of the variation.

**Fig. 1** Seasonal variations in microbial biomass, basal respiration and metabolic quotient in beech leaf litter and in faecal pellets of *Glomeris marginata* deposited after processing of beech leaf litter. Data were pooled for the three study sites (*HSD*, Tukey's honestly significant difference)




## Short-term alterations

Microbial biomass in leaf litter material of February and August remained very constant during the 40 days of incubation (Fig. 2). In contrast, in February microbial biomass in faecal pellets of *G. marginata* was reduced 1 day after deposition (−46%) but increased strongly to 5 times the level in litter material offered as food substrate. Then, microbial biomass declined strongly to values below the level in leaf litter. In August, microbial biomass in faecal pellets was also reduced 1 day after litter processing, but then, in contrast to February, no significant increase in microbial biomass in faecal pellets occurred during the following 39 days of incubation. The interaction of GLOMERIS and TIME was highly significant and accounted for 48.5% and 23.4% of the variation in February and August, respectively (Table 2).

Basal respiration changed in a similar pattern as microbial biomass during the 40 days of incubation. In February, basal respiration in leaf material was very constant throughout the incubation period whereas basal respiration in faecal pellets was at a maximum after 20 days of incubation (Fig. 2). In August, basal respiration in both, litter and faecal pellets, varied in a similar pattern during the incubation period.

Metabolic quotient in beech leaf litter from February and August varied only slightly during the 40 days of

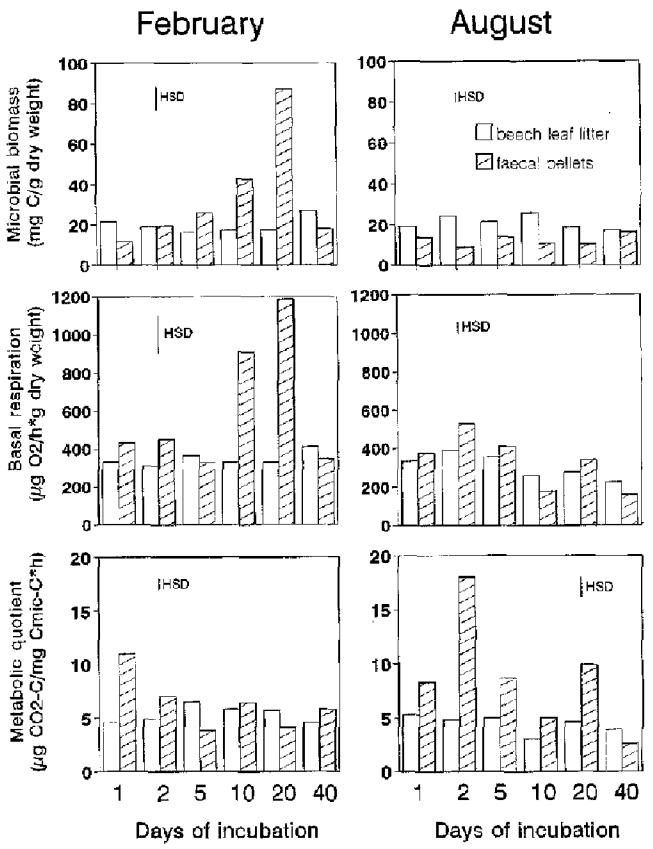



**Fig. 2** Short-term alterations in microbial biomass, basal respiration and metabolic quotient in beech leaf litter and faecal pellets of *G. marginata* deposited after processing of beech leaf litter from the basalt site in February and August (*HSD*, Tukey's honestly significant difference)

incubation (Fig. 2). In contrast, metabolic quotient in faecal pellets from February and August varied strongly during the incubation period. In February it was significantly increased after 1, 2 and 40 days of incubation, but decreased at day 5 and 20. In August it was generally increased except at day 40.

The response of microorganisms to glucose and nutrient amendments was very different in beech leaf litter and faecal pellets of *G. marginata* (Fig. 3, Table 3). In leaf litter microbial growth was low or absent when only glucose was added except at day 40. During the first 20 days of incubation additional amendment with nutrients strongly increased microbial growth. Nitrogen and phosphorus appeared to be of similar importance but their effect varied with time (Table 3). In contrast to intact beech leaf litter, microorganisms in faecal pellets were able to grow when only glucose was added and additional amendment with nutrients generally did not increase microbial growth ability.

Ergosterol content in beech leaf litter (August) varied among the study sites, however, only slightly ($F = 6.4$, $P < 0.05$). It was at a maximum at the intermediate site (496.4 µg $g^{-1}$ dry weight) and similar at the basalt and limestone site (406.3 and 433.9 µg $g^{-1}$

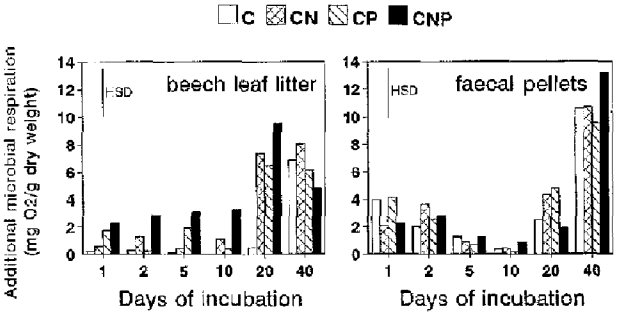



**Fig. 3** Short-term alterations in the additional microbial respiration (AMR) in beech leaf litter and faecal pellets of *G. marginata* from the basalt site after amendment with glucose (C), glucose and nitrogen (CN), glucose and phosphorus (CP) and glucose, nitrogen and phosphorus (CNP) in February (*HSD*, Tukey's honestly significant difference)

**Table 2** Two factorial ANOVA table on the effect of litter processing by *G. marginata* and time of incubation (1, 2, 5, 10, 20 and 40 days) on microbial biomass, basal respiration and metabolic quotient of beech leaf litter from the basalt site sampled in **a** February and **b** August

| | | Microbial biomass | | Basal respiration | | Metabolic quotient | |
|---|---|---|---|---|---|---|---|
| | *df* | SS (%) | *F* | SS (%) | *F* | SS (%) | *F* |
| (a) February | | | | | | | |
| GLOMERIS | 1 | 13.4 | 159.1*** | 24.3 | 167.2*** | 7.8 | 180.9*** |
| TIME | 5 | 37.1 | 88.5*** | 33.6 | 46.3*** | 29.1 | 135.4*** |
| GLOMERIS × TIME | 5 | 48.5 | 115.6*** | 40.3 | 55.5*** | 62.6 | 291.9*** |
| MODEL | 11 | 99.0 | 107.2*** | 98.3 | 61.5*** | 99.5 | 210.7*** |
| (b) August | | | | | | | |
| GLOMERIS | 1 | 69.0 | 374.3*** | 1.6 | 16.6** | 28.3 | 863.2*** |
| TIME | 5 | 5.4 | 5.9** | 83.4 | 178.1*** | 41.7 | 254.5*** |
| GLOMERIS × TIME | 5 | 23.4 | 25.3*** | 13.9 | 29.7*** | 29.6 | 180.6*** |
| MODEL | 11 | 97.8 | 48.2*** | 98.9 | 96.0*** | 99.6 | 276.3*** |

$^{**}P < 0.001$, $^{**}P < 0.01$

**Table 3** Four factorial ANOVA table on the effect of litter processing by *G. marginata* (basalt site, February), nitrogen (with and without nitrogen), phosphorus (with and without phosphorus) and time (sampling after 1, 2, 5, 10, 20 and 40 days of incubation) on the additional microbial respiration (AMR) after glucose and nutrient addition (for details see text)

| | *df* | SS (%) | *F* |
|---|---|---|---|
| GLOMERIS | 1 | 1.2 | 31.9*** |
| NITROGEN (N) | 1 | 1.9 | 49.2*** |
| PHOSPHORUS (P) | 1 | 1.2 | 32.0*** |
| TIME | 5 | 68.4 | 354.9*** |
| GLOMERIS × N | 1 | 1.2 | 32.2*** |
| GLOMERIS × P | 1 | 0.8 | 20.9*** |
| GLOMERIS × TIME | 5 | 11.9 | 61.8*** |
| N × P | 1 | 0.0 | 0.8 ns |
| N × TIME | 5 | 1.8 | 9.3*** |
| P × TIME | 5 | 1.5 | 7.7*** |
| GLOMERIS × N × P | 1 | 0.0 | 0.1 ns |
| GLOMERIS × N × TIME | 5 | 2.8 | 14.4*** |
| GLOMERIS × P × TIME | 5 | 2.4 | 12.4*** |
| N × P × TIME | 5 | 1.9 | 9.9*** |
| GLOMERIS × N × P × TIME | 5 | 1.1 | 5.8*** |
| MODEL | 47 | 98.2 | 54.2*** |

***$P < 0.001$, *ns* $P > 0.05$

dry weight, respectively) indicating that fungal biomass at the intermediate site exceeded that at the basalt and limestone site. Ergosterol content was strongly affected by *G. marginata* processing. On average, ergosterol content in leaf litter (overall mean of basalt, intermediate and limestone site 445.6 μg $g^{-1}$ dry weight) was reduced to 131.2 μg $g^{-1}$ dry weight in faecal pellets. GLOMERIS accounted for 95.5% of the variation ($F = 1086$, $P < 0.001$) and the interaction of GLOMERIS and SITE accounted for additional 2% of the variation ($F = 11$, $P < 0.01$). The corresponding reduction in microbial biomass by processing of leaf litter by *G. marginata* in August was considerably less pronounced (−42%, Fig. 2) than the decline in ergosterol content (−71%). This indicates that digestion and pellet formation of *G. marginata* reduced fungal biomass in beech leaf litter much more than bacterial biomass.

## Discussion

Effects of the feeding activities of *G. marginata* on the microbial biomass of beech leaf litter varied strongly with season. Microbial biomass in faecal pellets was increased strongly in February and to a lesser extent also in May, whereas it was reduced in August and to a lesser extent also in November. Presumably, in February and May carbon was not the primary limiting element for the microflora in beech leaf litter which had been comminuted, as indicated by increase in microbial biomass after mechanical fragmentation (Maraun and Scheu 1995) and animal processing (this study). In August and November microbial populations did not recover from the reduction caused by *G. marginata* processing because carbon resources were lacking. In litter, soluble and more easily available carbon resources are depleted later in the year due to leaching and microbial attack (Nykvist 1962; Berg and Staaf 1980; Bargali et al. 1993).

Basal respiration in faecal pellets of *G. marginata* also changed with season. In February and May basal respiration was hardly affected by *G. marginata* processing but in August and November it was reduced strongly. Reduction in basal respiration in August and November also indicates depletion of carbon resources later in the year.

The greater surface area of beech leaf litter material in faecal pellets of *G. marginata* may increase the accessibility for bacterial and fungal attack and this has been assumed to be the most important factor responsible for the stimulation of the decomposition process in arthropod faeces (Jensen 1974; Hanlon 1981b; Hopkin and Read 1992). The results of this study suggest that the surface area of beech leaf litter is not the primary limiting factor for microbial metabolism and growth throughout the year, because in August and November microbial biomass and respiratory activity in faecal pellets of *G. marginata* declined despite the increase in surface area in faecal pellets. Only early in the year (February and May) was an increase in surface area by litter fragmentation by *G. marginata* accompanied by an increase in microbial biomass and respiratory activity.

Most studies investigating effects of processing of leaf litter by saprophagous macroinvertebrates reported an increase in microbial activity. Hanlon and Anderson (1980) found that microbial respiration in fresh oak litter was increased in presence of *G. marginata* and *Oniscus asellus*. In *Pinus nigra* litter microbial respiration was increased in presence of *Philoscia muscorum* (Teuben and Roelofsma 1990). However, a decrease in microbial activity of litter materials due to macrofauna processing was also found in some investigations. Microbial respiration in poplar leaves (*Populus* × *canadensis*) was reduced by isopod processing (Van Wensem et al. 1993) and microbial biomass in poplar leaf litter (*Populus tremuloides*) was also found to be reduced in the presence of earthworms (Scheu and Parkinson 1994b).

These conflicting results are presumably caused by investigating different litter types for different time periods. Results of the present study indicate that processing of litter material by saprophagous macroarthropods caused an increase in microbial respiration and biomass in litter which is little decomposed (beech leaf litter in February), but decreased microbial respiration and biomass in litter strongly attacked by microorganisms (beech leaf litter in August). This pattern is generally supported by the findings of the above cited authors: The increase in microbial respiration in oak litter processed by *G. marginata* and *Oniscus asellus*

only occurred within the first 10 days and declined thereafter (Hanlon and Anderson 1980); the increase in microbial respiration in *Pinus nigra* litter by isopods (Teuben and Roelofsma 1990) was at a maximum after few weeks of the experiment, and although Van Wensem et al. (1993) generally emphasized that isopod processing decreased microbial respiration they also found an increase in freshly fallen litter material. A decrease in microbial activity in litter due to presence of macroinvertebrates has been reported in experiments lasting for several weeks (Van Wensem et al. 1993) or in experiments using litter material of low C/N ratio, indicating strong microbial attack (Van Wensem et al. 1993; Scheu and Parkinson 1994b). Changes in litter quality with season and duration of storage of litter and faecal material must be considered in more detail in further investigations.

Processing of beech leaf litter by *G. marginata* hardly affected C/N ratio. Most studies reported a slight reduction in C/N ratio after leaf litter processing by macroarthropods (Bocock 1963; Marcuzzi 1970; Bano 1992) but an increase was also found (Jocteur Monrozier and Robin 1988). Generally, a reduction in C/N ratio is more likely to occur, due to carbon assimilation during gut passage. However, assimilation efficiency has been shown to be low (Bocock 1963) but depends on leaf litter material (Anderson and Bignell 1982) and macrofauna species (Striganova 1971; Davidson 1976; Daniel 1991).

Seasonal changes in litter and faecal material in the present study were generally analysed by investigating faecal pellets of an average age of 4 days. To investigate short-term alterations, we also studied changes in microbial biomass, basal respiration and metabolic quotient during 40 days after litter processing. Microbial biomass was strongly reduced in faecal material one day after deposition (−46% in February and −29% in August) indicating that processing of beech leaf litter by *G. marginata* generally results in a strong reduction in microbial biomass. Then, during incubation of faecal pellets, changes in microbial biomass occurred but these changes depended on litter quality (i.e. season). As already mentioned, the increase in microbial biomass which occurred in February presumably was caused by an increase in accessibility of carbon resources in faecal pellets in combination with nutrient release during gut passage (Anderson and Ineson 1983; Anderson et al. 1983). In contrast, carbon resources were depleted in beech leaf litter from August and fragmentation by *G. marginata* did not increase resource availability. The different response of microbial biomass in February and August after processing by *G. marginata* is summarized in Fig. 4. As indicated by the investigation of short-term alterations in microbial biomass after litter processing by *G. marginata*, faecal pellets of an average age of 4 days as used to study seasonal changes in microbial biomass in the present study are of an appropriate age to reflect diplopod mediated changes in microbial growth ability.

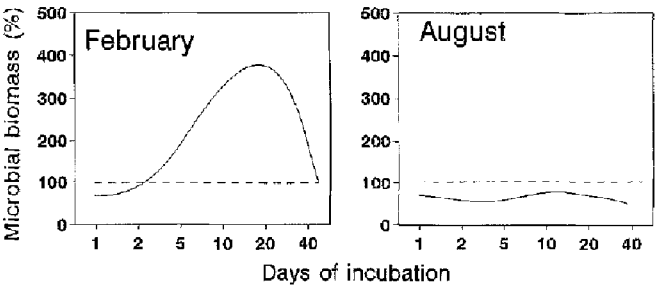



**Fig. 4** Scheme of short-term alterations in microbial biomass in beech leaf litter caused by processing of *G. marginata* in February and August. Data are percentages of the control (intact beech leaf litter offered as food substrate)

Short-term alterations in basal respiration during the 40 days of incubation generally followed the same pattern as microbial biomass (Fig. 2). However, the increase in basal respiration in February preceded that in microbial biomass by about 2 days, indicating that an enhanced basal respiration is a precondition for microbial growth.

The metabolic quotient has been used as an indicator of stress (Wolters 1991a, b; Scholle et al. 1992; Anderson and Domsch 1993), but has also been assumed to indicate the quality of carbon resources (Hund and Schenk 1994) and the efficiency of carbon use by microorganisms (Insam 1990; Scheu and Parkinson 1995). There are several factors that can stress the microbial community, but in the present study carbon availability appeared to be most important, and therefore the latter two interpretations of the metabolic quotient are most useful for our data. Generally, in beech leaf litter the metabolic quotient appeared to decrease from February to August, indicating a decrease in availability of carbon resources in ageing litter materials. In February, during short-term incubation of faecal pellets metabolic quotient declined for 40 days, indicating a decline in availability of carbon resources in litter material which had been fragmented. During incubation the metabolic quotient in faecal pellets approached that in leaf litter, which remained almost constant. In August, the metabolic quotient in faecal pellets exceeded that in beech leaf litter for 20 days. In contrast to February, higher $qCO_2$ in faecal pellets from August mainly resulted from the decline in microbial biomass, not from increased microbial respiration in processed litter material.

Interpretation of changes in $qCO_2$ values is ambiguous because alterations may be caused by changes in microbial biomass or microbial respiration. An increase in $qCO_2$, indicating an increase in availability of carbon resources, may result from a decline in microbial biomass with microbial respiration remaining constant, i.e. constant availability of carbon resources as observed by litter processing by *G. marginata* in August.

Therefore, the interpretation of changes in $qCO_2$ may be misleading without consideration of changes in biomass and respiration.

Microbial growth ability in faecal pellets of *G. marginata* and beech leaf litter was very different as indicated by glucose and nutrient additions (February). Addition of glucose resulted in a strong microbial growth phase in faecal pellets, whereas in leaf litter microbial growth was absent or low when only glucose was added (except at day 40). This indicates that carbon limited microbial growth in faecal pellets but not in leaf litter according to the definition of Odum (1971).

Addition of glucose only induced microbial growth in faecal pellets of *G. marginata* and the additional amendment with N and P was of minor importance. This indicates that the availability of these nutrients was increased by litter processing, a phenomenon which has been observed previously (Teuben 1991). In contrast to the first 20 days of incubation, addition of glucose only to beech leaf litter induced strong microbial growth after 40 days of incubation, which may have been caused by an increase in the cellulolytic activity and an accompanied mobilization of nutrients.

The content of ergosterol was shown to be a good measure for fungal biomass in organic materials (Seitz et al. 1977; Newell et al. 1988; Gessner et al. 1991; Scheu and Parkinson 1994a). In the present study, processing of beech leaf litter by *G. marginata* caused a reduction in ergosterol content by 71% (August, faecal pellets 1 day after deposition) whereas microbial biomass was only reduced by 42%. This indicates that in comparison to bacteria fungi were more intensively digested during gut passage of litter material through *G. marginata*. More intensive digestion of fungi during gut passage through saprophagous macroinvertebrates has been supposed previously by Nicholson et al. (1966), Ineson and Anderson (1985) and Hassall et al. (1987). The importance of fungi as a food resource for saprophagous macroarthropods has also been emphasized by Anderson and Bignell (1982). More intensive digestion of fungi may be related to the fact that fungi are more sensitive to mechanical disturbance. Increase in bacterial contribution to microbial biomass may also be related to the fact that fungi generally grow slower than bacteria resulting in bacterial dominance early after deposition of faecal pellets (Anderson and Bignell 1980).

In conclusion, the gut passage of beech leaf litter by *G. marginata* caused a short-term decrease in microbial biomass, particularly the fungal component, indicating disproportional digestion of fungi. This short-term decrease in microbial biomass was followed by a strong increase. However, this increase depended on season and occurred only early in the year. Digestion and pellet formation generally resulted in an increase in nutrients (phosphorus, nitrogen) available to microorganisms. However, these nutrients could only be used for microbial growth if the fragmented litter in faecal pellets also contained little decomposed carbon resources, which was only the case early in the year. Therefore, the effect of litter processing by macroarthropods on microorganisms depends on the extent to which litter is decomposed (changes in litter quality in time). In contrast, variations in litter quality due to growth of beech trees on different parent rock (variations in litter quality in space) were of minor importance for macroarthropod-microbial interactions.

**Acknowledgements** We thank G. Scholle for ergosterol measurements. This study was supported by the German Ministry of Science and Technology.

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