Incorporation of microbial biomass by *Peltoperla* sp. (Plecoptera) and *Tipula* sp. (Diptera)

**STUART FINDLAY, JUDY L. MEYER, AND PHILLIP J. SMITH**  
Institute of Ecology, University of Georgia, Athens, Georgia 30602 USA

**Abstract.** The quantitative contribution of microbial carbon to the nutrition of aquatic detritivores has been discussed for some years with no direct measurements of assimilation of bacterial or fungal carbon. Using radiotracers to specifically label bacteria and fungi on leaf litter, we have determined the rate of incorporation of bacteria and a hyphomycete by larvae of *Peltoperla* sp. (stonefly) and incorporation of bacteria by larvae of *Tipula* sp. (cranefly). Incorporation of bacterial carbon accounted for less than 1% of the carbon respired by either insect. Incorporation of fungal carbon was greater but still only 25% of the carbon respired by the stonefly larva. These findings suggest that other mechanisms may explain the frequently observed correlation between “microbial conditioning” and food quality.

**Key words:** detritus, bacteria, fungi, nutrition, *Peltoperla*, *Tipula*, food quality.

The quantitative significance of microbial biomass in detritivore nutrition has come into question in the last few years (Baker and Bradnam 1976, Cammen 1980, Cummins and Klug 1979, Findlay et al. 1984b, Iversen 1973, Lawson et al. 1984). The shift away from the opinion that microbial biomass was the only assimilable component of detritus (e.g., Cummins 1974, Levinton and Lopez 1977) began when reliable estimates of microbial biomass on natural detritus showed that less than 5% of total carbon in the detritus-microbial complex was microbial biomass (Cammen 1980, Iversen 1973). In addition, much of the relative increase in nitrogen commonly observed during detritus decomposition (previously ascribed to microbial growth) may be due to processes other than microbial immobilization of exogenous nitrogen (Odum et al. 1979, Rice 1982). We had previously found that neither bacterial nor fungal biomass could meet the respiratory carbon needs of an isopod (Findlay et al. 1984b, 1986). To further test the generality of these conclusions, we undertook direct measurement of incorporation of bacterial carbon by *Tipula* and bacterial and fungal carbon by *Peltoperla*. These two leaf-shredding insects are common in streams of the southern Appalachian mountains.

**Methods**

All insect larvae used in these experiments were collected from a small stream (Ball Creek) at the Coweeta Hydrologic Laboratory in southern North Carolina, USA. Insects were held in the laboratory at 15°C and used within one week.

Oxygen consumption rates of *Peltoperla* and *Tipula* were determined with a YSI Model 53 Biological Oxygen Monitor and converted to CO₂ resired assuming a RQ of 1. This RQ assumes the diet is primarily carbohydrate. Catabolism of stored lipid may also change the RQ, but body reserves cannot serve as a long-term carbon source. Using a very low RQ (e.g., 0.7 on a pure protein diet, see p. 211 in Schmidt-Nielsen 1975) would not change the basic conclusions of this paper (see Results).

Leaf discs (3 cm² total surface area) were punched from air-dried birch (*Betula* spp.) and oak (*Quercus alba*) leaves collected from leaf fall traps. Leaves were aged 14 d in aerated stream water. To label bacteria, birch and oak leaf discs were incubated with ³H-thymidine (15 μCi/disc, 20 Ci/mmole) for 4 hr at 15°C in the dark. Another set of discs was pre-incubated with ¹H-thymidine (15 μCi/disc, 20 Ci/mmole) for 4 hr at 15°C in the dark. After the pre-incubation, ³H-thymidine (15 μCi/disc) was added to these discs as well and incubated for an additional 4 hr. These discs are referred to as +Inhib discs and the first set
as -Inhib discs. Following incubation, both sets were washed five times with filtered stream water to remove unincorporated label. Discs were either frozen for subsequent DNA analysis or used in feeding experiments. DNA extraction, washing and counting was as described in Findlay et al. (1984a). Discs treated as above but without isotope were used for bacterial biomass determination (Findlay et al. 1984b). Bacterial cell volumes were measured on photographs of the epifluorescent slides and converted to carbon per cell using 0.1 g/cm$^3$ of biovolume (Cammen 1982).

Peltoperla larvae (10-15 individuals in separate dishes) were fed on +Inhib or -Inhib discs for 11 hr and allowed to clear their guts for 6 hr while feeding on either oak or birch leaves. Larvae were rinsed in 10% HCl followed by distilled water, blotted dry, wet-weighed and dissolved in 1 ml of a tissue solubilizer (NCS, Amersham). Consumers fed -Inhib discs may obtain label from digestion of $^3$H-thymidine-labelled bacteria and from non-bacterial processes such as fungal-incorporated $^3$H-thymidine. Larvae fed +Inhib discs may obtain label only from non-bacterial sources. By difference, we calculated consumer-incorporated label resulting only from digestion of bacteria (see Findlay et al. 1984b). Similar experiments were carried out with larvae of Tipula sp.

To prepare $^14$C-labelled fungi, sterile leaf discs were inoculated with a spore suspension from a hyphomycete (Articulospora tetradadia Ingold) isolated from a stream at Coweeta. $^14$C-glucose was added daily (0.5 μCi/disc) for the first 8 d. No glucose was added the last 3 d to allow distribution of isotope into components of hyphae, such as cell walls, with a slower turnover time (Findlay et al. 1986). Sterile discs were carried through the entire procedure. Fungal biomass was determined with epifluorescent direct counts of hyphal intersections with a counting grid at 500× (Findlay et al. 1986, Newell and Hicks 1982). Total $^14$C incorporated by fungi was determined by combusting leaf discs in a Harvey sample oxidizer, NaOH-soluble and MeOH-CHCl$_3$-soluble $^14$C was determined to provide at least a crude separation into components of different solubility (Findlay et al. 1986).

Peltoperla larvae (10-15 individuals in separate dishes) were fed inoculated or sterile discs for 12 hr, then allowed to clear their guts for 10 hr while feeding on unlabelled leaves. Larvae were rinsed, frozen and lyophilized. After weighing, larvae were combusted in a Harvey sample oxidizer and radioassayed. Incorporation of fungal biomass was calculated from the radioactivity in the consumer divided by the specific activity of the fungus (Findlay et al. 1986). Similar experiments were not done with Tipula because early trials and published reports indicated that Tipula will not consume pure fungi (Lawson et al. 1984). Samples were counted in a Beckman LS1800 Scintillation Counter. Quench was determined using the relationship between counting efficiency and H number (Horrocks 1977).

Results

Oxygen consumption by Peltoperla was 1.35 μl O$_2$ mg$^{-1}$ hr$^{-1}$ (±0.08 (SE)). This value is in the middle of the range for stonefly larvae reported by Knight and Gaufin (1966). Oxygen consumed was converted to CO$_2$ respired using two different values for the RQ (0.7 and 1.0). These estimates represent the minimum carbon needs of the insects (0.51-0.72 μg C mg dry wt$^{-1}$ hr$^{-1}$). Assuming insect tissue is 50% carbon, these rates are equivalent to respiring 2.4-3.5% of body carbon per day. For Peltoperla, growth rates are ~1.2% per day (O'Hop et al. 1984) and thus respiration represents 66-75% of carbon needed for maintenance plus growth.

Bacteria on birch discs incubated with $^3$H-thymidine in the absence of inhibitors of DNA synthesis incorporated 1.4×10$^6$ DPM into DNA/disc (Fig. 1A). Discs where DNA synthesis was blocked incorporated 8.5% of this amount (1.2×10$^6$ DPM/disc) (Fig. 1A). Peltoperla and Tipula incorporated more label when fed discs containing labelled DNA than when fed discs where non-bacterial processes were the only source of label (Fig. 1B).

Numbers of bacteria per disc (±1 SE) were 1.6×10$^7$ (±0.4×10$^7$) on birch and 1.4×10$^7$ (±0.2×10$^7$) on oak. The average cell volume, weighted by the relative abundance of cocci and rods was 0.33 μm$^3$ corresponding to 3×10$^{-8}$ μg C/cell. Bacterial biomass was 0.48 μg C/disc on birch discs and 0.42 μg C/disc on oak discs. These values for biomass are close to bacterial biomass on birch discs incubated for two weeks.
Birch discs inoculated with *A. tetracladia* spores incorporated $2.63 \times 10^6$ DPM/disc while sterile discs had only 3.6% of this amount $(9.5 \times 10^4$ DPM/disc) (Fig. 2A). Oak discs showed essentially the same pattern (Fig. 2A). Most of the label was soluble in NaOH (77% and 81% for birch and oak respectively) indicating that the label was not completely refractory. *Peltoperla* fed inoculated leaf discs incorporated $3.7 \times 10^5$ DPM mg$^{-1}$ hr$^{-1}$ (Fig. 2B) while *Peltoperla* fed sterile discs incorporated 61.4 DPM mg$^{-1}$ hr$^{-1}$ (Fig. 2B). Larvae fed inoculated oak discs incorporated $7.5 \times 10^5$ DPM mg$^{-1}$ hr$^{-1}$ (Fig. 2B) and those fed sterile discs incorporated 110.2 DPM mg$^{-1}$ hr$^{-1}$.

*Articulospora tetracladia* biomass on 10-day-aged birch discs was 25.1 (±0.5) µg C/disc and on 10-day-old oak 14.4 (±1.0) µg C/disc. Fungal biomass on birch discs was 29.2 (±2.3) and 45.4 (±4.2) µg C/disc on days 21 and 45 respectively.

The rate of incorporation of fungal carbon by *Peltoperla* was calculated from the rate of incorporation of radioactivity by the insect and the specific activity of the fungus (DPM/(µg fungal C)) (Table 1).

**Discussion**

The assumptions inherent in our radiotracer techniques have been discussed in detail previously (Findlay et al. 1984b, 1986). Data from the current experiments do not falsify any of the assumptions.

Fungi used in the radiotracer feeding measurements were grown for 10 d to conform with the period of maximum biomass enzymatic activity and palatability reported by Arsuffi and Suberkropp (1984) and Suberkropp et al. (1983). In our experiments, biomass increased further.

---

**Table 1. Incorporation of bacteria and fungal biomass by consumers feeding on birch or oak detritus.**

<table>
<thead>
<tr>
<th>Consumer</th>
<th>Microbe</th>
<th>Birch Discs</th>
<th>Oak Discs</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Peltoperla</em></td>
<td>Bacteria</td>
<td>0.24 (0.2%)</td>
<td>0.09 (0.06%)</td>
</tr>
<tr>
<td><em>Peltoperla</em></td>
<td><em>Articulospora</em></td>
<td>35 (25%) RQ=1.0</td>
<td>38 (27%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>35 (36%) RQ=0.7</td>
<td>38 (39%)</td>
</tr>
<tr>
<td><em>Tipula</em></td>
<td>Bacteria</td>
<td>0.51 (0.9%)</td>
<td>Not measured</td>
</tr>
</tbody>
</table>
by day 45. This 92% increase in biomass would also increase our incorporation estimates by 92% yet the contribution of fungal carbon to *Peltoperla* would still be only 45% of C respired by the consumer. Carbon respired is clearly a minimum estimate of total C required.

The respiratory carbon needs of *Peltoperla* could be met by direct digestion of non-living leaf carbon, possibly mediated by microbial enzymes. Given the ingestion rate (0.17-0.31 mg DW mg DW⁻¹ d⁻¹; oak and birch, respectively) and respiration rates (0.035 d⁻¹) for *Peltoperla*, an assimilation efficiency of 11-20% for non-living leaf substrate would be sufficient to account for respired carbon. Note that microbial carbon was always less than 2.5% of leaf carbon so even a 100% assimilation of microbes would make only a small contribution to the overall assimilation efficiency. The required 11-20% assimilation efficiency is below estimates for another stonefly (Golladay et al. 1983) and appreciable rates of assimilation of pure cellulose have been reported for three other shredders (Sinsabaugh et al. 1985) as well as microcrustaceans (Schoenberg et al. 1984).

The idea that microbial growth determined the food quality of detritus arose from many studies showing correlation between some measure of microbial biomass or "activity" and the palatability of the leaf material for consumers (see Bärlocher and Kendrick 1981, Cummins and Klug 1979). The concept of "microbial conditioning" of leaf litter is widely accepted but the mechanism has never been precisely determined (Bärlocher 1985). Our previous measures of bacterial production on leaf litter (Findlay and Meyer 1984) in conjunction with the present results suggests that microbial biomass was not the proximal cause of changes in food quality and other reasons may account for this relationship. First, it is becoming apparent that microbial exoenzymes may modify the leaf substrate (e.g., Arsuffi and Suberkropp 1984, Suberkropp et al. 1983) and this modification may make substrate carbon more digestible (Bärlocher 1982, Bärlocher and Kendrick 1975, Sinsabaugh et al. 1985). This process could account for the coincidence of microbial biomass with palatability without requiring that microbial biomass be the major source of carbon for the consumer. Second, a leaf type that supports high microbial biomass may also have intrinsic characteristics that make it palatable. Leaves with, for instance, low lignin content may be a suitable growth substrate for microbes and may also be available for direct digestion by consumers. With these points in mind, factors influencing food quality for stream detritivores should be examined from the standpoint of initial leaf quality or microbial modification of leaf substrate rather than looking for possibly spurious correlations between microbial biomass and food quality.

**Acknowledgements**

This research was supported by NSF Grant DEB 8306440 to SF and JM and BSR 85-14328. Messrs. R. Kelly and H. Lehsau assisted with lab and field work. Dr. K. Suberkropp identified the hyphomycete and Dr. D. Strayer read and improved the manuscript.

**Literature Cited**

ARSUFFI, T. L., AND K. SUBERKROPP. 1984. Leaf processing capabilities of aquatic hyphomycetes: in-

Received: 4 March 1986
Accepted: 8 December 1986