

Bacterial communities associated with benthic organic matter in headwater stream microhabitats

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Summary

Bacterial communities associated with a variety of benthic detritus types were studied in three streams in the context of the chemical characteristics of the sediment material and the stream water. A cell purification assay was developed for a quantitative microscopic evaluation of bacterial community structure in detritus samples by fluorescence *in situ* hybridization (FISH). The efficiency of FISH with fluorescently monolabelled probes was compared with FISH with signal amplification by catalysed reporter deposition (CARD-FISH). In detritus types poor in organic carbon and nitrogen, the numbers of prokaryotes were related to the chemical characteristics of the stream water column, whereas no such relationship was found for detritus types rich in organic carbon and nitrogen. These results might help to provide criteria for the selection of detritus types for river ecosystem assessment and monitoring. The percentage of bacteria detected by FISH with monolabelled probes was correlated with the detritus total organic matter (OM). This is likely attributed to a higher ribosome content of microbial cells on substrates rich in OM. Cell detection by CARD-FISH did not show any correlation with OM content, indicating that this technique renders the results more independent from the activity state of cells. Fluorescence *in situ* hybridization with four group-specific probes suggested a relationship between substrate quality and the composition of the microbial assemblages on the various types of detritus. The improved protocol for cell purification and CARD-FISH may facilitate future investigations on the relationship between the riverine benthic detritus quality and microbial community composition.

Introduction

Headwater stream systems are composed of a mosaic of patchy aquatic microhabitat characterized by benthic organic matter (OM) of different origin (i.e. fine OM, biofilms, leaf litter, debris). The patches tend to be very small, and their extension strongly depends on hydromorphological and catchment characteristics. In these systems primary production is often negligible (Fleituch *et al.*, 2001; Findlay *et al.*, 2002; Logue *et al.*, 2004). In contrast, processes associated with the detritus compartment, mediated by heterotrophic microbes (bacteria and fungi), are crucial to headwater functioning and affect the overall ecology of the lowest stream reaches. Benthic microbial abundance and metabolic activities highly vary among detritus types: fungi prevail on leaf litter, whereas bacteria dominate heterotrophic biofilms and fine particulate OM (Findlay *et al.*, 2002; Gulis and Suberkropp, 2003). Microbial growth, being affected by the supply of nutrients (mainly nitrogen and phosphorous) and organic carbon (Puddu *et al.*, 2003), may be very sensitive to human impact (Lemke *et al.*, 1997), e.g. to land use differences in the catchment. Despite their potential relevance on multiple ecological scales, microbial communities in streams and in rivers have long been neglected. Only a few studies have reported bacterial *in situ* abundances, or have described the composition of the microbial assemblages in different types of OM (Spring *et al.*, 2000; Logue *et al.*, 2004).

Fluorescence *in situ* hybridization (FISH) has become a powerful tool for ecological investigation of microbes in various aquatic environments (Amann *et al.*, 1995; Pernthaler *et al.*, 2001) including freshwater sediments (Wobus *et al.*, 2003). Sometimes high background fluorescence and non-specific binding to non-bacterial particles can hamper the enumeration of hybridized cells because of low ribosome content of inactive cells (Amann and Ludwig, 2000). Recently, FISH in combination with signal amplification (catalysed reporter deposition, CARD) has been proposed as a more sensitive technique for microbial community analysis (Schönhuber *et al.*, 1997; Pernthaler *et al.*, 2002). To date, few studies have applied this improved technique to environmental samples and none to freshwater sediments.

We analysed benthic microbial communities associated with substrates with different OM content. Six types of

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detritus originating from three streams were studied: (i) leaves; (ii) root debris; (iii) fine benthic OM (<0.5 mm) from bottom sediment patches dominated by large gravel >2 mm (hereinafter named FBOM-L); (iv) fine benthic OM from sediment patches dominated by small gravel <2 mm (hereinafter named FBOM-S); (v) biofilm on stones in pools (hereinafter named Bio-brown); (vi) biofilm in riffles, rocky stretches below running water, dominated by benthic microalgae (hereinafter named Bio-green).

Results and discussion

Differences between sampling sites and detritus types

The upper reaches of three streams (Albegna, Ente and Fiora), located in the same geographical area (Amiata Mountain declivity, Tuscany, Italy) and with similar hydro-morphological characteristics, were selected based on the different levels of anthropogenic influence in the catchments. The Albegna catchment, upstream of the sampling area, is covered by natural woods; the upper reach of Ente flows through an area with small villages and small-scale agricultural activities; the Fiora site is situated immediately downstream from the Santa Fiora village and receives treated wastewater from a small meat-processing factory. Sampling was carried out in mid winter 2003, when in the Mediterranean streams the short-term hydrological variability is minimal. Three replicates of each detritus type were collected in each river; five detritus types (FBOM-L, FBOM-S, Bio-brown, leaves and root debris) were sampled from stream depositional areas (pools) and one (Bio-green) in transport areas (riffles). Sediment samples (about 50 ml) were collected from the top layer (5 cm thickness); biofilms were scraped off of 100 cm² of stone surface with a toothbrush and leaves-roots debris was gathered using pliers, both rinsed in sterilized water. Samples for chemical characterization were kept frozen until processing. Sediments for grain-size characterization were sampled from a standard surface of 150 cm² (5 cm thickness) and sieved 2–0.5 mm. The grain size distribution of the various detrital samples showed consistent differences between the two bottom sediment patches in all the three rivers, one being dominated by large gravel (average over the three rivers: >2 mm = 81.7 ± 8.7%), the other by fine gravel (<2 mm = 85.5 ± 22.4%).

Each detritus type and each sediment grain size class were dried for 72 h at 60°C, weighed, ashed for 3 h at 550°C, and re-weighed to determine the ash-free dry weight (AFDW) content. The percentages of total carbon (C_{tot}) and nitrogen (N_{tot}) were determined in triplicates using a NA-1500 CHN Analyser (Carlo Erba Instruments, Milano). Organic carbon (C_{org}) was determined after acidification with 2 N HCl. The finest detrital component (<0.5 mm), used for microbial community analysis,

showed a relatively low AFDW content significantly (two-way ANOVA, $P < 0.05$) different either between FBOM-L (average over the three rivers: 4.1 ± 0.8%) and FBOM-S (5.1 ± 0.7%) or among rivers. The organic carbon represented 42.4 ± 9.5% and 48.1 ± 10.5% of the total carbon in FBOM-L and FBOM-S respectively (Table 1).

Biofilms characteristics highly differed among the rivers. In Bio-brown the AFDW content significantly increased from Albegna (3.2 ± 0.1%) and Ente (5.1 ± 0.2%) to Fiora (7.1 ± 0.2%). The ratio of organic carbon to total carbon (C_{org} : C_{tot}) rose from 17% in Albegna and 50% in Ente to about 100% in Fiora. In Bio-green the AFDW reached even higher values ranging from 16.6 ± 1.8% (Albegna) to 40.3 ± 4.0% (Ente) with a very high C_{org} : C_{tot} ratio (79% Albegna; ≈100% Ente).

Root debris and leaves showed the highest AFDW content (average over the three rivers: roots 44.7 ± 4.2%, leaves 74.0 ± 3.3%), with an unvarying C_{org} : C_{tot} ratio of about 90–100% in the three rivers (Table 1).

Prokaryote abundances on different types of detritus

Detritus samples were fixed in buffered formaldehyde solution (3.7% final concentration) and analysed for bacterial abundance and community composition. Fine benthic OM subsamples were sieved on 0.5 mm diameter sifter (pre-rinsed with particle-free distilled water) to remove large gravel. Subsamples of FBOM and biofilm slurry (~3 ml), three discs of leaves (10 mm diameter) and small pieces of roots were placed in a chemical detachment solution consisting of distilled water, NaCl (0.85% final concentration), tetrasodium pyrophosphate (0.1 M final concentration) and Tween 20 (0.5% final concentration) (Velji and Albright, 1993). Samples were then incubated for 30 min under mild agitation on a variable-speed vibration shaker at room temperature. Physical detachment was subsequently performed by mild sonication on ice (20–35 W for 60 s, Microson XL2000 ultrasonic liquid processor with 1.6 mm diameter microtip probe – Misonix, New York). The resulting slurry was left at 4°C overnight allowing for sedimentation of coarse particles. Two subsamples (10–100 µl depending on cell abundance) of the supernatant were stained with 4,6-diamidino-2-phenylindole (DAPI) according to Porter and Feig (1980).

A two-way ANOVA for three levels of rivers and five levels of detritus type showed a statistically significant difference both among rivers ($F = 8.73$; $P < 0.001$) and detritus types ($F = 41.13$; $P < 0.001$), as well as significant interaction between these two sources of variance ($F = 10.13$; $P < 0.001$). Pairwise multiple comparisons (Student-Newman-Keuls method) of prokaryote cell numbers showed no significant differences between Albegna and Ente, while both of them significantly differed from Fiora. Two-way ANOVA also showed that in Albegna and Ente

Table 1. Ash-free dry weight, total carbon, total organic carbon, total nitrogen of six types of organic matter across the three streams.

	Ash-free dry weight (%)			Total carbon (%)			Organic carbon (%)			Total nitrogen (%)		
	Albegna	Ente	Fiora	Albegna	Ente	Fiora	Albegna	Ente	Fiora	Albegna	Ente	Fiora
FBOM-L	4.8 ± 0.3	3.2 ± 0.2	4.2 ± 0.0	2.5 ± 0.1	2.9 ± 0.0	2.1 ± 0.1	1.0 ± 0.6	1.5 ± 0.3	0.7 ± 0.1	0.06 ± 0.01	0.04 ± 0.00	0.06 ± 0.00
FBOM-S	4.8 ± 0.2	5.9 ± 0.2	4.5 ± 0.2	2.8 ± 0.2	4.3 ± 0.2	2.7 ± 0.0	1.4 ± 0.3	2.4 ± 0.4	1.0 ± 0.0	0.06 ± 0.00	0.16 ± 0.02	0.09 ± 0.00
Bio-brown	3.2 ± 0.1	5.1 ± 0.2	7.1 ± 0.2	2.9 ± 0.0	4.5 ± 0.1	7.0 ± 1.0	0.5 ± 0.0	2.3 ± 0.4	7.0 ± 0.3	0.05 ± 0.01	0.19 ± 0.01	0.37 ± 0.20
Bio-green	16.6 ± 1.8	40.3 ± 4.0	ND	11.0 ± 0.5	21.9 ± 0.2	ND	8.8 ± 0.9	21.8 ± 2.2	ND	1.38 ± 0.15	3.75 ± 0.36	ND
Roots	49.9 ± 3.6	40.7 ± 7.0	44.6 ± 4.3	44.9 ± 1.9	27.6 ± 3.1	ND	38.6 ± 4.5	24.4 ± 2.7	ND	0.73 ± 0.37	0.85 ± 0.23	ND
Leaves	77.3 ± 4.1	70.7 ± 7.0	74.0 ± 5.3	41.0 ± 2.2	39.8 ± 1.4	40.4 ± 1.3	46.2 ± 3.5	42.3 ± 2.6	43.1 ± 2.5	1.31 ± 0.87	1.63 ± 0.09	1.42 ± 0.11

FBOM-L, fine benthic organic matter (<0.5 mm) from patches dominated by large gravel; FBOM-S, fine benthic matter (<0.5 mm) from patches dominated by fine gravel; Bio-brown, biofilm on stones in pools; Bio-green, biofilm on stones in riffles dominated by benthic algae; ND, not detected. Data are expressed as average of three replicated measurements ± one standard deviation.

prokaryote abundance was significantly higher in Bio-green when compared with Bio-brown.

Cell counts in the detritus types with low OM content (FBOM-L, FBOM-S and Bio-brown) ranging from 3×10^{10} to 2×10^{11} cells per gram of AFDW (in line with findings by Hudson *et al.*, 1992; Findlay *et al.*, 2002), were 10-fold higher in Fiora than in Albegna and Ente. Considering that AFDW slightly differed among rivers (Table 1), prokaryote abundance in Fiora was even higher if expressed in number of cells per unit of dry weight. Moreover in Fiora, FBOM and biofilms supported significantly higher prokaryote abundance than debris from leaves and roots (Fig. 1). This implies that the microbial densities in riverine benthic surface layers with low OM content may be related to water nutrient concentration, as suggested by Claret and Fontvielle (1997). In Fiora sampling site, downstream of a meat factory, water showed substantially higher concentrations of total phosphorous and nitrogen in comparison with the more pristine sites at Albegna and Ente (total phosphorus: Albegna 0.008 mg l^{-1} , Ente 0.038 mg l^{-1} , Fiora 0.237 mg l^{-1} ; total nitrogen: Albegna 0.8 mg l^{-1} , Ente 1.2 mg l^{-1} , Fiora 1.5 mg l^{-1} – Erba *et al.*, 2004). Prokaryote abundance in OM-rich detritus types (roots and leaves), varying between 2×10^{10} and 6×10^{10} cells per gram of AFDW, was also in line with literature values (Findlay *et al.*, 2002; Rier *et al.*, 2002). For root debris we could not find literature data for comparison. As in OM-rich detritus types no differences in prokaryote abundance were observed between rivers, microbial community might be less dependent on water chemistry when growing on substrates with high organic carbon and nitrogen content (Fig. 1).

These results might contribute to the definition of the types of benthic detritus to be mainly considered in the assessment of river ecosystems. To date the standardized river monitoring systems take into consideration macroorganisms and cultivable bacteria; wider analyses on the natural microbial assemblages associated to low OM substrates, and therefore highly responsive to water quality, might open new perspectives for river assessment and for a better understanding of human impact on stream ecosystem functioning.

Cell purification for FISH by density gradient centrifugation

A high background fluorescence and non-specific binding of probes to non-bacterial particles can hamper the enumeration of cells in sediments after FISH (Amann and Ludwig, 2000). Therefore, we performed additional cell purification by Nycodenz purification, as proposed for soil samples (Whiteley *et al.*, 2003). Griffiths and colleagues (2003) argued that Nycodenz purification would permit comparison between equally treated samples even if the total cell numbers were slightly underestimated, and that

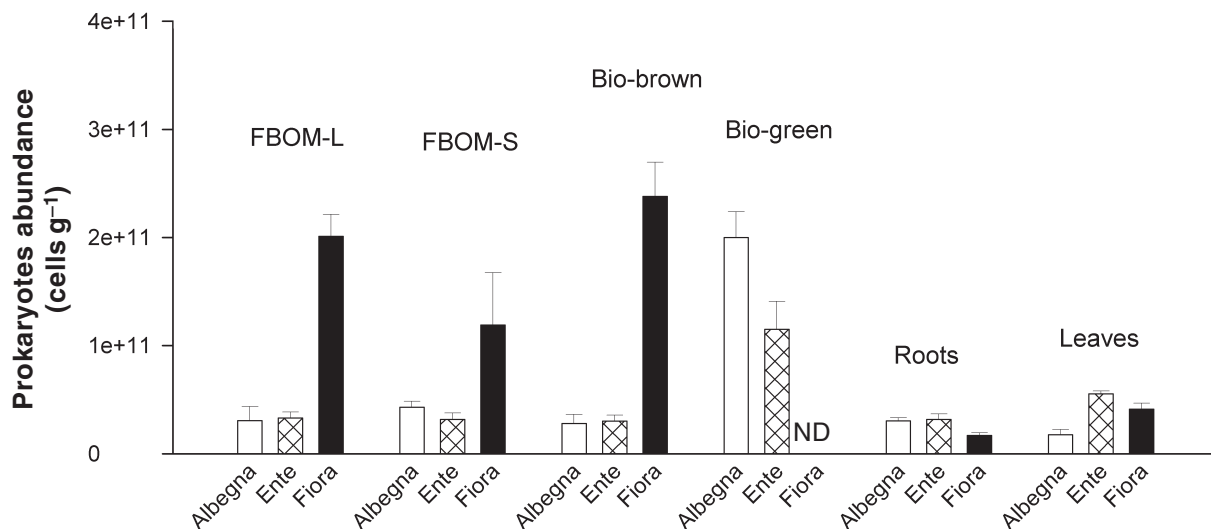


Fig. 1. Prokaryote abundance, expressed as number of prokaryote cells per gram of AFDW, for the six types of organic matter collected from the three streams. Data are means of three independent samples each analysed in two replicates ($n = 6$) \pm standard error. ND, not detected.

this approach might even allow the use of flow cytometry. Moreover, Nycodenz-purified cell suspensions appear to be representative of the original community (Whiteley *et al.*, 2003).

Portions (1 ml) of sonicated slurry prepared for total cell counts (see above) were subsampled after vortex agitation in 2 ml Eppendorf tubes. Density gradient centrifugation was carried out with the non-ionic medium Nycodenz [Nycomed, Oslo, Norway; density $1.310 \pm 0.002 \text{ g ml}^{-1}$ (Lindahl, 1996; Courtois *et al.*, 2001)]. First, a 1-ml Nycodenz cushion was placed underneath 1 ml of sonicated slurry, using a syringe needle in order to avoid mixing of Nycodenz and slurry (for soil samples: Lindahl, 1996; Hesselsøe *et al.*, 2001). Centrifugation was performed at 14.000 g for 90 min at 4°C in a swing-rotor Eppendorf centrifuge. Next, the supernatant above the Nycodenz cushion was carefully collected and vortexed. Subsamples (0.5–1 ml) were filtered on $0.2 \mu\text{m}$ polycarbonate membranes (47 mm diameter, Nuclepore) by gentle vacuum ($<0.2 \text{ bar}$) and washed with 10–20 ml of sterile ultra-pure water. The filters were stored in Petri dishes at -20°C until further processing. The efficiency of Nycodenz extraction was tested by comparing the total cell numbers (cell ml^{-1}) in the supernatant above the Nycodenz cushion, inside the Nycodenz cushion and in the pellets beneath the cushion (re-suspended in 2 ml of ultra-pure water). In five samples from various detritus types, $90.5 \pm 7.4\%$ of the total cells were present within the supernatant above the Nycodenz cushion, about 9.0% within the cushion and merely 0.3% in the pellets (Fig. 2A). Thus, the cell loss during the preparations was comparatively low.

Our results show that Nycodenz density gradient centrifugation highly improved the purity of prokaryote cell

suspensions that originate from freshwater detritus communities, thus making the counting both faster and more reliable.

Cell detection after FISH staining

Fluorescence *in situ* hybridization with fluorescently monolabelled (5'-Cy3) oligonucleotide probes, counterstaining with DAPI, and microscopic evaluation was performed according to the protocol by Pernthaler and colleagues (2001). The percentages of cells in Nycodenz-purified cell suspensions visualized by FISH with the fluorescently monolabelled probes EUB338, EUB338-II and EUB338-III, targeted to most bacteria (Daims *et al.*, 1999), ranged between 19% and 60%. This is similar to values reported for freshwater sediments by other authors (Araya *et al.*, 2003; Bouvier and del Giorgio, 2003). Bacteria hybridized by probe EUB338 represented on average 34.8% of the DAPI-stained objects, while cells hybridized by probes EUB338-II and EUB338-III, which are targeted to the *Planctomycetes* and *Verrucomicrobiales* lineages, represented only 2.4%. The highest values were found in OM-rich detritus – leaves and Bio-green – (Fig. 2C). The fraction of bacteria visualized by FISH in FBOM and Bio-brown was consistently higher in Fiora (range 38–41%) than in Ente and Albegna (range 19–36%); this trend was not observed for bacteria from leaf detritus. The percentage of cells detected by FISH with fluorescently monolabelled EUB338, EUB338-II and EUB338-III was significantly correlated to the detritus AFDW ($P < 0.05$; $r = 0.67$), C_{tot} ($P < 0.05$; $r = 0.66$) and C_{org} ($P < 0.05$; $r = 0.67$). These correlations were not observed for the cells detected by CARD-FISH (see below). This suggests that the sensitivity of FISH with monolabelled probes

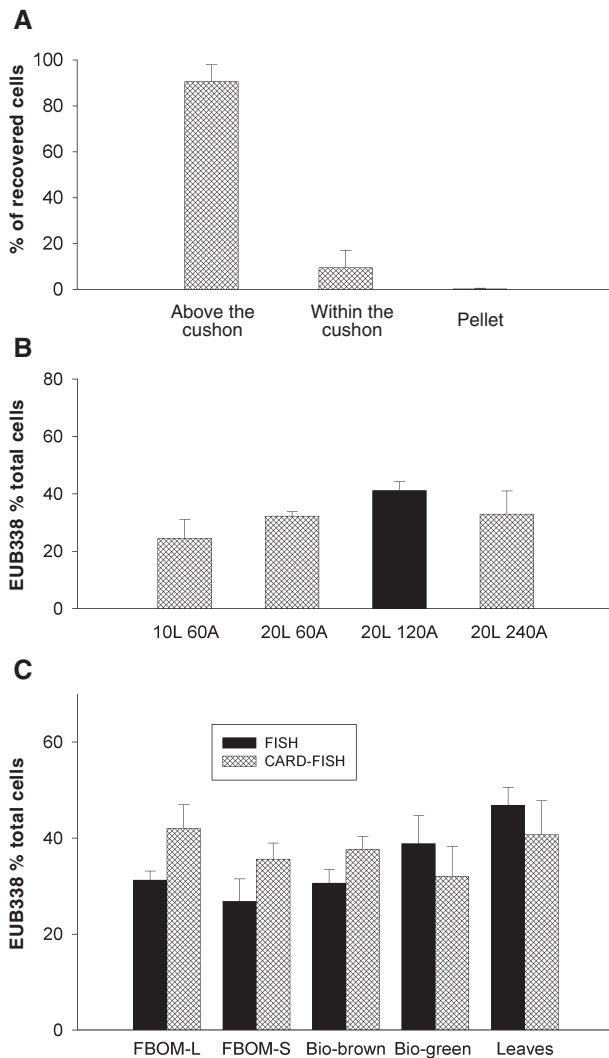


Fig. 2. A. Efficiency of the Nycodenz purification step for the recovery of sediment bacteria. Data are expressed as percentage of total cells in the supernatant above the Nycodenz cushion, within the cushion and in the pellets.

B. CARD-FISH detection rate of bacteria with the general bacterial probe EUB338 after incubation at different dilutions of lysozyme ($L = 10$ and 20 mg ml^{-1}) and achromopeptidase ($A = 60, 120, 240 \text{ unit ml}^{-1}$). Best results were achieved by lysozyme digestion for 60 min [20 mg ml^{-1} , in $0.05 \text{ M EDTA (pH 8)}$ and $0.1 \text{ M Tris-HCl (pH 7.4)}$, 37°C] followed by incubation with achromopeptidase for 30 min [120 U ml^{-1} , in 0.01 M NaCl and $0.01 \text{ M Tris-HCl (pH 8.0)}$, 37°C]. Higher achromopeptidase concentration (240 U ml^{-1} for 15 min) did not further increase the fraction of hybridized cells, but rather caused a disintegration of cells. The darker bar represents the enzyme concentration selected for the whole set of samples evaluation.

C. Detection of bacteria by EUB338 probe with fluorescently monolabelled probes (black bars) or by CARD-FISH (shaded bars) in the different detritus types. The detritus types are ordered following the increasing percentage of AFDW. Data are expressed as percentage of DAPI-stained cells and are means of duplicates over the three rivers ($n = 6$) \pm standard error.

depends, in part, on the activity state of the target cells (Fig. 2C). The detection efficiency of bacterial cells by this FISH approach is related to the cell-specific rRNA content (Oda *et al.*, 2000), which itself has been suggested as an index of cell activity (Williams *et al.*, 1998). Rapidly growing bacteria contain higher ribosome densities (Kerkhof and Kemp, 1999; Luna *et al.*, 2004) binding proportionally more probe molecules per cell (Oda *et al.*, 2000). This might explain why the highest percentage of hybridized bacteria on OM-poor detritus was found in samples from the Fiora River, which also featured the highest water nutrient concentrations.

Fluorescence *in situ* hybridization with signal amplification by catalysed reporter deposition, with probe EUB338 and subsequent signal amplification with fluorescein-labelled tyramides was performed basically following the protocols of Sekar and colleagues (2003) and Pernthaler and colleagues (2004). However, we optimized the permeabilization treatment for our samples from freshwater detritus (Fig. 2B). It is likely that the higher enzyme concentrations (as compared with Sekar *et al.*, 2003) were required because the concentration of fixative in our samples was relatively high (3.7%). The percentage of bacteria hybridized by EUB338 after CARD-FISH ranged between 23% and 58%. There were no significant differences in the percentage of cells visualized by FISH with the fluorescently monolabelled probe or by CARD-FISH with respect to the whole data set (Mann-Whitney Rank Sum Test: $n = 14$, $P = 0.39$). However, the percentage of cells hybridized by CARD-FISH was significantly higher in samples from detritus types with low OM content – FBOM-S, FBOM-L and Bio-brown – (Mann-Whitney Rank Sum Test: $n = 9$, $P < 0.05$) (Fig. 2C). In addition, cells stained by CARD-FISH showed a more intense fluorescence signal. We furthermore observed a lower overall variability of the fractions of cells that could be hybridized in the different rivers and detritus types (coefficients of variation: CARD-FISH, 23%; FISH, 31%). The percentage of cells visualized by CARD-FISH did not show any significant correlation with detritus AFDW, C_{tot} , or C_{org} . Thus, CARD-FISH results were not dependent on substrate quality. This suggests that bacterial cells could be visualized even if their activity (ribosome content) was low. Therefore, CARD-FISH with our optimized protocol likely represents a superior alternative to FISH with monolabelled probes for an accurate analysis of microbial community structure in riverine detritus. This is in agreement with reports from marine sediments (Pernthaler *et al.*, 2002; Ishii *et al.*, 2004).

Microbial community composition

A tentative community analysis was performed using the fluorescently monolabelled probes ALF1b, BET42a,

GAM42a for the α -, β -, γ -subclasses of *Proteobacteria*, respectively, and CF319a, targeted to many groups from the *Cytophaga-Flavobacterium* (CF) cluster of the *Bacteroidetes* (Amann *et al.*, 1995) synthesized by Biomers.net, Ulm Germany. For the probes BET42a, GAM42a unlabelled competitors were added. The description of microbial assemblages by probes for large phylogenetic lineages can only provide limited information about the complexity of the microbial community structure, as there might be different physiological types of bacteria within these broad categories. However, it can be helpful to gain first insight about the affiliation of ecologically relevant bacteria and to follow their spatial-temporal dynamics and activity in natural environments (Bouvier and del Giorgio, 2002; Kirchman *et al.*, 2004).

In our study between 9% and 40% of the total DAPI cell counts could be identified by these four probes (Fig. 3). On average 40% of the cells targeted by EUB338 remained unaffiliated, which is in line with data from marine sediments (Llobet-Brossa *et al.*, 1998). Thus, the

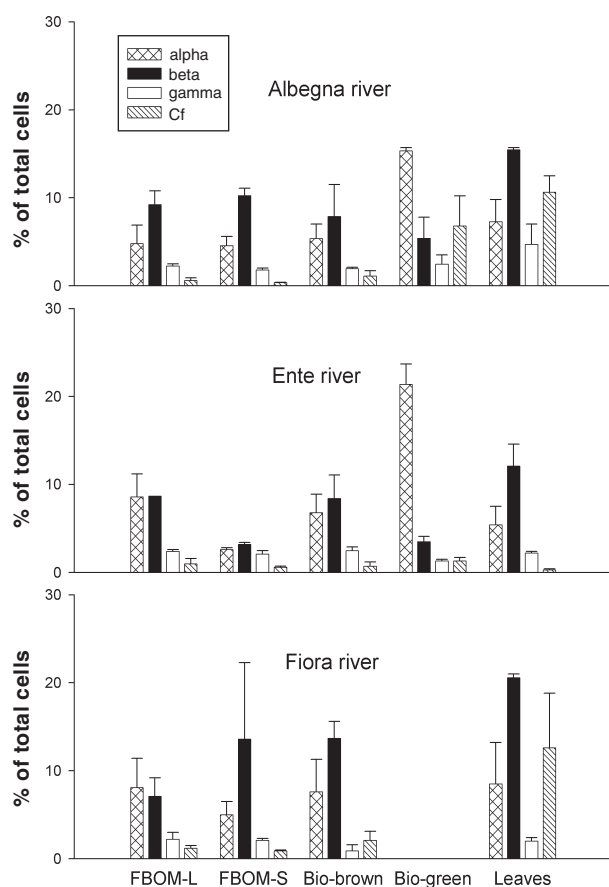


Fig. 3. Taxonomic composition of the bacterial communities as analysed by FISH. Values are expressed as percentages of hybridized cell counts of total counts of DAPI-stained cells. Error bars indicate the range of duplicates. Alpha, beta, gamma: α -, β -, and γ -*Proteobacteria*, respectively; Cf: *Cytophaga-Flavobacterium*.

majority of the FISH detectable bacteria could be assigned to known groups. *Proteobacteria* belonging to the α - and β -subclasses formed the largest fraction of hybridized cells in the microbial communities on FBOM and Bio-brown (5.6% and 9.0% of total DAPI-stained cells respectively). Bio-green showed a higher relative abundance of α -*Proteobacteria* (15.4 ± 0.5 in Albegna and 21.4 ± 3.3 in Ente) and of members of CF (up to 6% in Albegna). In leaf detritus β -*Proteobacteria* and bacteria related to CF reached the highest densities, ranging around 16.0% and 10.4%, respectively. Members of the CF cluster were not found in leaves from the Ente River. The γ -*Proteobacteria* did not constitute a numerically important phylogenetic group, forming less than 2% of DAPI-stained cells in samples from all detritus types.

Bacterial community composition seemed to be related to detritus quality (Fig. 3). There was a significant positive correlation between the percentages of cells detected by probes for the β -*Proteobacteria* and for CF cluster and AFDW (β -*Proteobacteria*, $r = 0.55$; CF, $r = 0.67$; $P < 0.05$), C_{tot} (β -*Proteobacteria*, $r = 0.56$; CF, $r = 0.67$; $P < 0.05$) and C_{org} (β -*Proteobacteria*, $r = 0.58$; CF, $r = 0.67$; $P < 0.05$). In contrast, α -*Proteobacteria* were significantly correlated with detrital nitrogen content ($r = 0.82$, $P < 0.05$).

Our results largely agree with the known distribution patterns of the four phylogenetic groups in oxic freshwater sediments and on suspended particles (Spring *et al.*, 2000). The prevalence of bacteria from the β -subclass of *Proteobacteria* and the CF cluster in substrates highly loaded with organic carbon might be a general feature of microbial communities in such habitats. β -*Proteobacteria* are important in the plankton of lakes, rivers and reservoirs (Glöckner *et al.*, 1999; Kirchman *et al.*, 2004), on lake snow (Simon *et al.*, 2002) and in biofilms of drinking water systems, streams and rivers (Brümmer *et al.*, 2003). Such bacteria are believed to be of particular importance during early biofilms succession (Davey and O'Toole, 2000; Jackson *et al.*, 2001). The presence of a large fraction of β -*Proteobacteria* could be also attributed to their ability to oxidize ammonia or degrade organic pollutants (Araya *et al.*, 2003). CF phylotypes have been found in freshwaters, and in pelagic and benthic marine habitats (Llobet-Brossa *et al.*, 1998; Glöckner *et al.*, 1999; Kirchman, 2002). They constitute a significant proportion of the microbial communities on suspended organic particles (Simon *et al.*, 2002) and in stream and river biofilms (O'Sullivan *et al.*, 2004). Currently, their role in OM processing is poorly understood. Some members of the CF cluster are specialized for the degradation of complex macromolecules (Kirchman, 2002).

Our findings highlight that the composition of microbial assemblages appears to be related to the quality of the detritus. Future studies might thus be aimed at determin-

ing which physiological properties favour the occurrence of specific bacterial taxa in benthic detritus, e.g. by combining *in situ* cell identification with activity measurements.

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