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Bacterial colonization and microbial decomposition of limnetic organic aggregates (lake snow)

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ABSTRACT: We studied the composition, microbial colonization and organic matter fluxes of lake snow in Lake Constance, Germany, in 1993. Aggregates were collected between 6 and 25 m by SCUBA or made in the lab in rolling cylinders from samples from the epilimnion. Aggregates were composed of living and senescent phytoplankton, zooplankton molts and carcasses, and other unidentifiable debris. Dry weight and particulate organic carbon (POC) were 3 to 20 µg agg.$^{-1}$ and 1.5 to 33 µg C agg.$^{-1}$, respectively. Carbon in particulate combined amino acids (PCAA) was 8 to 51% of POC on aggregates but only <10% in bulk POC. Aggregates were densely colonized, with 5 to 80 × 10^6 bacteria agg.$^{-1}$, and numbers usually increased with depth. Production and growth rates of bacteria on aggregates (aggregate bacteria) were low compared to free-living bacteria. However, the organic matter turnover within the aggregates and release into the surrounding water were high due to intense activities of the ectohydrolases (aminopeptidase, alkaline phosphatase, α- and β-glucosidase, and chitinase) of aggregate bacteria. Activities of the aminopeptidase were consistently higher than those of the other enzymes. In laboratory experiments dissolved amino acids, mainly DCAA, were released from aggregates into the surrounding water; turnover times of PCAA on aggregates due to hydrolysate release usually ranged from <5 to 25 h. Lake snow thus was a potential source of amino acids for free-living bacteria in the epi- and hypolimnion of Lake Constance. The colonization of lab-made aggregates showed a transition from small to large rods within the first 2 to 5 d after aggregation to filamentous, grazing-resistant bacteria. This community was largely composed of microbes of the domain *Bacteria*, determined by *in situ* hybridization with rRNA-targeted fluorescent oligonucleotide probes. β-*Proteobacteria* were usually dominant, particularly in aged aggregates. At earlier stages α-*Proteobacteria* also comprised substantial fractions of the community. This community structure is similar to that of activated sludge flocs, suggesting that lake snow has a function in lacustrine ecosystems comparable to that of activated sludge flocs in sewage treatment plants.

KEY WORDS: Lake snow · Bacteria · POM · Amino acids · *In situ* hybridization · Ectoenzyme activities · Lake Constance

INTRODUCTION

Macroscopic organic aggregates, ≥3 mm in diameter, both in marine and limnetic environments are known as colonization sites of a rich and diverse community of autotrophic and heterotrophic microorganisms. In general, algae, bacteria, flagellates, and ciliates inhabit marine snow as well as lake snow at concentrations much higher than those found in the surrounding water (Shanks & Trent 1979, Caron et al. 1986, Alldredge & Silver 1988, Grossart & Simon 1993). Enhanced concentrations of organic matter and nutrients on marine snow (Shanks & Trent 1979, Gotschalk & Alldredge 1989, Herndl 1992) indicate that it is an important microhabitat for organic matter decomposition and nutrient recycling in the water column.

The role of marine snow in the rapid transformation of particulate organic matter (POM) in the pelagic zone has been shown by several studies (Alldredge &

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Gotschalk 1990, Kaltenböck & Herndl 1992, Smith et al. 1992). Cho & Azam (1988) were first to argue that particle-attached bacteria must transform the particulate into the dissolved phase to support their measured production rates of free-living bacteria. Measurements of hydrolytic ectoenzyme activities of particle-associated and free-living bacteria have shown that bacteria on particles hydrolyze more organic matter than they take up (Hoppe 1991, Smith et al. 1992); thus, organic aggregates are hotspots of POM solubilization to DOM (dissolved organic matter) in the pelagic zone.

High metabolic activity and respiration of aggregate-associated microorganisms (Paerl & Prufert 1987) may lead to anoxic microzones (Alldredge & Cohen 1987, Shanks & Reeder 1993). As a result, large aggregates may support anaerobic processes, e.g. methanogenesis, in the aerobic bulk water (Bianchi et al. 1992). The significance of aggregates as microenvironments distinct from the surrounding water is reflected by changes of their microbial community in hours to days (Alldredge & Silver 1988), and in community structures different from the surrounding water (DeLong et al. 1993, Weiss et al. 1996).

We investigated bacterial colonization and decomposition of lake snow in Lake Constance, Germany, to test the hypothesis that lake snow, like marine snow, acts as hotspots of enhanced microbial decomposition of POM and thus provides an important source of DOM in the pelagic zone. In addition, we followed the bacterial community structure on lake snow in time to test if it changes during aggregate aging.

MATERIAL AND METHODS

This study was conducted in the Überlinger See, northwestern Lake Constance (47° 40' N, 9° 15' E) at a central station (max. depth = 147 m). Water samples were collected at 6 depths (3, 6, 10, 15, 25, and 50 m) from late March to mid-November 1993. Lake snow was enumerated and collected *in situ* at 6, 15, and 25 m depth, as in Grossart et al. (1997). The morphology and composition of lake snow were studied microscopically. Aggregates were prepared for scanning electron microscopy (SEM; Zeiss DSM 950) using the protocol of Paerl (1974). Dry weight (DW), particulate organic carbon (POC), and CHN of total seston and aggregates were measured after filtration through glass microfibre filters (1 µm pore size; Schleicher & Schüll, Germany). The filters were dried at 110°C for 1 h to determine DW POC and CHN content were measured by CHN analysis (Carlo Erba, Italy). For further details see Grossart & Simon (1998, in this issue).

Dissolved free (DFAA) and combined (DCAA) amino acids were determined by high performance liquid chromatography (HPLC) after precolumn derivatization with o-phthaldialdehyde according to Simon & Rosenstock (1992). Samples were prefiltered through 0.2 µm tuffrin filters (Gelman Acrodisc; low protein binding capacity). DFAA were measured directly. Samples of DCAA and particulate combined amino acids (PCAA) were hydrolyzed prior to analysis in double-distilled 6 N HCl for 20 h at 110°C. Amino acid oxidation due to high nitrate concentrations was prevented by adding 20 µl of ascorbic acid (2 mg ml^{-1}) prior to hydrolysis. Concentrations of DCAA and PCAA in moles were calculated as amino acid equivalents.

Free-living and aggregate bacteria were counted after DAPI staining by epifluorescence microscopy (Porter & Feig 1980, Grossart & Simon 1998). Bacterial biomass was calculated from cell numbers and an annual mean cell volume of 0.053 μm^3 for free-living and 0.153 μm^3 for aggregate bacteria (Simon 1987). Bacterial protein was calculated according to Simon & Azam (1989) and resulted in 19 fg protein per free-living cell and 53 fg protein per cell on aggregates.

Bacterial colonization was examined on lab-made aggregates formed in rolling plexiglass cylinders (1.4 l) at 2.5 rpm (Shanks & Edmondson 1989) during the course of 12 d. Water for these experiments was collected at 6 m depth and incubated in the cylinders at *in situ* temperature in a 12:12 h light:dark cycle. Five experiments were performed: Expts 1 and 2 on 17 August and 16 October 1992, respectively; Expts 3, 4, and 5 on 9 February, 4 May, and 13 August 1993, respectively. Expt 3 was run at 15°C and not at the ambient temperature of 4°C. Numbers, form, and size of bacteria were monitored by epifluorescence and SEM.

Colonization and community structure of aggregate bacteria were examined by *in situ* hybridization with rRNA-targeted fluorescent oligonucleotide probes according to Weiss et al. (1996). Aliquots (29 µl) of sonicated aggregates were pipetted onto gelatin-coated teflon microslides (P. Marienfeld KG, Bad Mergentheim, Germany) and dried at 46°C. The samples were fixed in 40 µl of freshly prepared 4% paraformaldehyde for 4 h at 4°C. Oligonucleotide probes specific for *Bacteria* (EUB 338; Amann et al. 1990) and for the α-, β-, and γ-subclasses of *Proteobacteria* (ALF 1b, BET 42 a, and GAM 42a; Manz et al. 1992) were used.

Anaerobic microzones in the aggregates were characterized by the reduction of 2,3,5-triphenyl-3-tetrazoliumchloride (TTC; Sigma) to microscopically visible crystals of formazan (Paerl & Prufert 1987). TTC was added to the samples at 0.01% (wt/vol) and incubated for 3 h in the dark. Samples were fixed with neutralized borate-buffered glutaraldehyde (3% final conc.) and stored at 4°C until the microscopic analysis. Respiring bacteria were determined after incubation with

5-cyano-2,3-ditolyl-tetrazoliumchloride (CTC; Polyscience-Inc.) according to Schaule et al. (1993) but modified in that we did not add peptone prior to incubation. Samples were incubated with CTC at 5 mM for 1 h in the dark, fixed with 2% formalin, and stored at 4°C until further analysis. Using the dual-staining approach with DAPI the total numbers and respiring bacteria were determined simultaneously by epifluorescence microscopy.

Hydrolytic ectoenzyme activities of free-living and aggregate bacteria were measured with fluorogenic substrate analogs (Hoppe 1993). The substrates used were L-leucine-methyl coumarinylamide (MCA, aminopeptidase), methyl umbelliferyl-α- and β-D-glucoside (MUF, α- and β-glucosidase), MUF-N-acetyl β-glucoside (N-acetyl β-glucosidase), and MUF-phosphate (alkaline phosphatase). To measure hydrolysis by free-living bacteria, samples of 5 ml were incubated with substrates at 50 µM (final conc.) for 1 h at *in situ* temperature in the dark. Hydrolysis rates of aggregate bacteria were measured by pooling 10 aggregates in 5 ml of surrounding water and adding substrates at 250 to 500 µM (final conc.). Incubation was as for free-living bacteria. Added substrate concentrations assured maximum hydrolysis rates as determined by concentration kinetics. All samples were run in triplicate with 1 heat-killed control (80°C for 20 min). Fluorescence was read at 380 nm excitation and 440 nm emission for MUF substrates or 365 nm excitation and 455 nm for MCA substrates using a Kontron SFM 25 fluorometer. The activity of aggregate bacteria was calculated as total activity minus that of free-living bacteria.

Bacterial production was measured by using a dual-label approach (Chin-Leo & Kirchman 1988) of [^{3}H]thymidine (TdR; Fuhrman & Azam 1980) and [^{14}C]leucine incorporation (Leu; Kirchman et al. 1985, Simon & Azam 1989) into the ice-cold trichloroacetic acid precipitate. TdR (75 Ci mmol^{-1}) and Leu (312 mCi mmol^{-1}, both from Amersham) were added to triplicate samples and a formalin-killed control and incubated for 1 h. For free-living bacteria the protocol of Simon & Rosenstock (1992) was applied using 30 nM of each label (final conc.), whereas for aggregate bacteria 10 aggregates were pooled in 5 ml of surrounding water and incubated at a final concentration of 60 nM of TdR and Leu. For further details see Grossart & Simon (1993).

The release of DFAA and DCAA from the aggregates into the surrounding water was measured by incubating 10 natural or 2 lab-made aggregates in 30 ml glass syringes filled with 0.2 µm prefiltered lake water. Three experiments with natural aggregates were performed (Expt 1 on 16 October 1992, Expts 2 and 3 on 3 and 10 April 1993, respectively) and 1 with lab-made aggregates (Expt 4 on 23 January 1991). Each experiment was run in triplicate such that the syringes were rotated vertically at 2.5 rpm and incubated under the same conditions as the plexiglass cylinders (see above). Subsamples of surrounding water were withdrawn periodically through a 3-way valve at the tip of the syringes for subsequent DFAA and DCAA analysis by HPLC (see above). The net release of dissolved amino acids was calculated as the increase of their concentration in the surrounding water over time.

RESULTS

Abundance, type, and composition of lake snow

Abundances of lake snow in 1993 were <1 to 50 l^{-1}. Maximum numbers occurred in the phytoplankton spring bloom, during the clear water phase, the phytoplankton bloom in summer and fall. Aggregates were <3 to 20 mm in diameter and on average 5.5 mm, equivalent to a spherical volume of 87 µm^3 agg.$^{-1}$. Small comet-shaped aggregates were found at the end of the phytoplankton spring bloom when small diatoms were present. The largest aggregates were formed by large diatoms and filamentous green algae after strong wind events at the beginning of the summer bloom. For further details see Grossart et al. (1997) and Grossart & Simon (1998). A special type of aggregated material occurred at the end of March as a dense surface film of diatoms and detritus.

The dry weight of lake snow was 3 to 120 µg agg.$^{-1}$ and the POC content 1.5 to 33 µg agg.$^{-1}$ (Table 1). The

Table 1. Type, dry weight, and POC per aggregate collected at various depths. Mean values of 10 aggregates pooled for the measurements are given

Date	Type of aggregate	Depth (m)	Dry weight (µg agg^{-1})	POC (µg agg^{-1})
24 Apr	Small diatoms	15	3	1.5
		25	3.8	1.9
10 May	Molts of daphnids	15	10	2.8
26 May	Molts and carcasses of daphnids	15	20	3.5
		25	17	3.0
31 May	Large diatoms	15	3	1.7
		25	30	8.5
22 Jul	Large diatoms	6	120	33.0
		15	25	8.5
		25	22	6.2
16 Aug	Miscellaneous	25	9	3.0
23 Aug	Miscellaneous	15	23	6.5
		25	9	3.2
11 Oct	Cyanobacteria	6	3	2.0

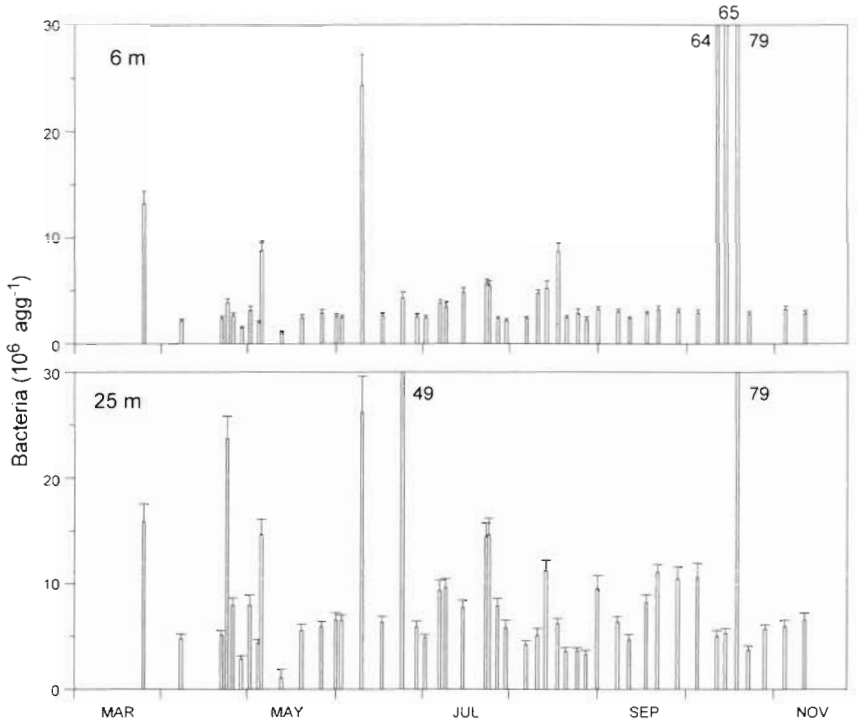


Fig. 1 Bacterial numbers on lake snow collected at 6 and 25 m depth from March until November 1993 in Lake Constance

highest values were found on aggregates composed of large diatoms and low values on those composed of small diatoms, single crustacean molts, or cyanobacteria. Large aggregates which consisted of diatoms and filamentous green algae at all depths often exhibited anaerobic microzones, indicating high respiratory activities of their microbial community. Anaerobic microzones were less frequent on lake snow of small diatoms in spring and on molts of zooplankton in the clear water phase.

Numbers and activities of lake-snow-associated bacteria

Bacterial abundance on aggregates ranged from 5 to 80 × 10^6 cells agg.$^{-1}$ (Fig. 1). In general, it increased with depth, as reflected by the mean values (4.04 ± 3.88 × 10^6 cells agg.$^{-1}$ at 6 m and 8.77 ± 7.83 × 10^6 cells agg^{-1} at 25 m, excluding cyanobacterial aggregates in October). The highest numbers occurred on zooplankton-derived aggregates at the end of June at 25 m and on cyanobacterial aggregates at the end of the bloom in October at 6 and 25 m. Higher numbers on lake snow were also recorded towards the end of the spring bloom, whereas in summer large diatom aggregates were sparsely colonized. In spite of their high numbers, aggregate bacteria comprised only a small fraction of total planktonic bacteria (≤4.3%). However, because aggregate bacteria were, on average, 3× larger than the free-living bacteria, they represented a larger fraction of the total bacterial biomass (≤13%).

More than 60% of aggregate bacteria were respiring, in dense accumulations even 90%, irrespective of the type of aggregate. In contrast, only 6 to 10% of the free-living bacteria were detected by CTC as respiring cells.

Bacterial protein production expressed as carbon (BPP-C) was <0.1 to 1.08 ng C agg.$^{-1}$ h^{-1}, excluding the surface film at the end of March. It usually increased with depth (Fig. 2). Highest BPP-C rates were measured on molts of zooplankton at the end of May and on large diatom aggregates in early August. Rates in the surrounding water were <50 to 3200 ng C l^{-1} h^{-1} and highest during the spring bloom (data not shown). In contrast to aggregates, BPP-C

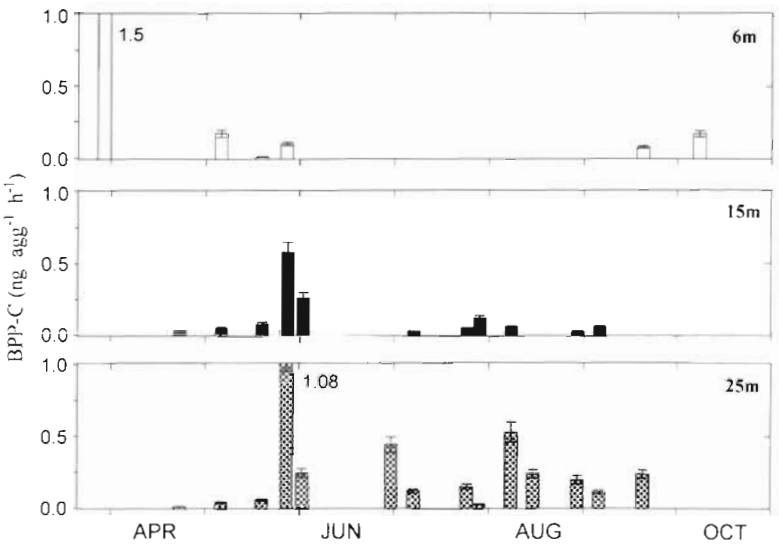


Fig. 2. Bacterial protein production (BPP-C) determined by ^{14}C-leucine incorporation on lake snow collected at 6, 15, and 25 m depth in Lake Constance in 1993

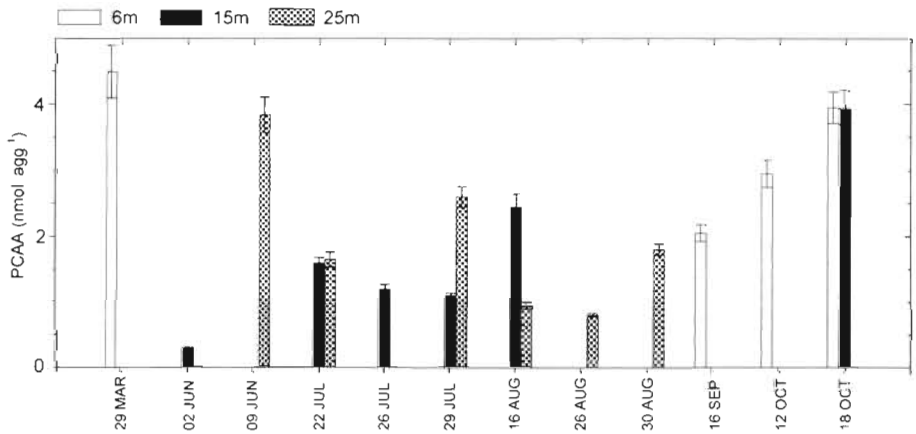


Fig. 3. Concentration of particulate combined amino acids (PCAA) on lake snow collected at 6, 15, and 25 m depth in Lake Constance in 1993

rates in the surrounding water decreased with depth. The direct comparison of BPP-C rates on aggregates with those in the surrounding water showed that <1 to 13.9% of total BPP-C was on lake snow. Highest percentages occurred when BPP-C rates in the surrounding water were lowest. Percentages of BPP-C did not covary with those of bacterial numbers both on aggregates and in the surrounding water.

Growth rates of aggregate bacteria calculated on the basis of TdR incorporation and bacterial numbers ranged between 0.01 and 0.09 d^{-1}. These rates were up to 10 times lower than those of free-living bacteria and only at the end of May and in early August were they similar to or higher than in the surrounding water. In general, the seasonal pattern followed that of BPP-C.

Concentrations of particulate combined amino acids (PCAA)

Concentrations of PCAA were 1.2 to 3.9 nmol $agg.^{-1}$, excluding the surface film at the end of March. Highest values were found on large aggregates at the beginning of June (filamentous green algae) and on aggregates in mid-October (cyanobacteria; Fig. 3). The C:N ratio of PCAA on lake snow was 3.4 to 5.5 (Grossart & Simon 1998) with a mean of 4.5. In contrast, the C:N ratio of PCAA of suspended POM ≤100 µm was lower, with a mean of 3.5. This difference in amino acid composition is due to high percentages of aspartate, glutamate, and serine and low percentages of valine, isoleucine, and leucine on lake snow as compared to suspended POM.

Total concentrations of aggregate-associated PCAA were 0.02 to 0.16 µM and concentrations of bulk PCAA ≤0.042 to 3.16 µM. In spring and during the clear water phase, PCAA on lake snow as percent of bulk PCAA was low (<10%), whereas it comprised 40 to 60% at the beginning of the summer bloom when aggregates were highly abundant (>10 agg. l^{-1}). PCAA on cyanobacterial aggregates in fall comprised ≥30% of total PCAA.

PCAA on aggregates comprised 18 to 270 ng C $agg.^{-1}$, assuming an average formula weight of amino acids of 120 and C as 50% of the formula. PCAA-C was 8 to 51% of lake-snow-associated POC (Grossart & Simon 1998) and only ≤10% on suspended POC ≤100 µm. The bacterial biomass on aggregates was ≤57 to 2175 ng C $agg.^{-1}$, which is generally higher than PCAA-C $agg.^{-1}$. Only on the surface film of diatoms did bacterial biomass comprise 10.6% of PCAA-C. In contrast, on cyanobacterial lake snow it was up to 6× higher than PCAA-C. Taking into account the error in the measurements of PCAA and bacterial biomass on aggregates, this calculation indicates that bacteria are a major component of proteins on aggregates.

Concentrations of dissolved free (DFAA) and combined (DCAA) amino acids

Concentrations of DFAA and DCAA in the matrix water of lake snow were 0.15 to 0.72 µM and 2.3 to 49.9 µM, respectively (Table 2). Extremely high concentrations of DFAA (1.79 µM) and DCAA (46 µM) were found in the diatom surface film in early spring. DFAA and DCAA concentrations in the matrix water were substantially higher than in the surrounding water (Table 2). DCAA/DFAA ratios in the matrix water, however, were 5 to 9× lower than in the surrounding water except for aggregates composed of green algae of benthic origin on 9 June and of cyanobacteria in fall. Aggregates composed of molts of daphnids, large diatoms, and miscellaneous components were more enriched in DFAA than in DCAA (Table 2). In contrast, the aggregates composed of benthic green algae and of cyanobacteria were more enriched in DCAA (except on 12 October).

Table 2. Type of aggregate, depth of sampling, DFAA and DCAA in the matrix water of aggregates and in the surrounding water. Enrichment factor is the ratio of concentrations of DFAA and DCAA in the matrix water of aggregates over those in the surrounding water

Date	Aggregate type	Depth	Aggregate			Surrounding water			Enrichment factor	
		(m)	DFAA (µM)	DCAA (µM)	DCAA/DFAA	DFAA (µM)	DCAA (µM)	DCAA/DFAA	DFAA	DCAA
2 Jun	Molts of daphnids	15	0.52	9.8	18.9	0.05	5.6	112.0	10.4	1.8
9 Jun	Green algae	25	0.52	49.9	95.7	0.18	4.5	25.0	2.9	11.1
22 Jul	Large diatoms	15	0.25	8.2	32.8	0.01	9.5	95.0	25.0	0.9
		25	0.22	18.1	82.3	0.01	5.2	520.0	22.0	3.5
26 Jul	Large diatoms	15	0.35	10.3	29.4	0.02	5.1	255.0	17.5	2.0
29 Jul	Large diatoms	15	0.29	10.0	34.5	0.02	2.5	125.0	14.5	4.0
		25	0.72	5.2	7.2	0.03	2.1	70.0	24.0	2.5
16 Aug	Large diatoms	15	0.32	12.2	38.1	0.09	5.6	62.2	3.6	2.2
		25	0.23	6.8	29.6	0.11	4.5	40.9	2.1	1.5
26 Aug	Miscellaneous	25	0.15	5.1	34.0	0.01	2.7	270.0	15.0	1.9
4 Sep	Miscellaneous	25	0.25	13.1	52.4	0.01	2.7	270.0	25.0	4.9
16 Sep	Cyanobacteria	6	0.38	6.2	16.3	0.15	2.4	16.0	2.5	2.6
12 Oct	Cyanobacteria	6	0.31	4.2	13.5	0.19	3.1	16.3	1.6	1.4
13 Oct	Cyanobacteria	6	0.61	16.5	27.0	0.21	2.2	10.5	2.9	7.5
		15	0.48	2.3	25.6	0.18	2.0	11.1	2.7	6.2

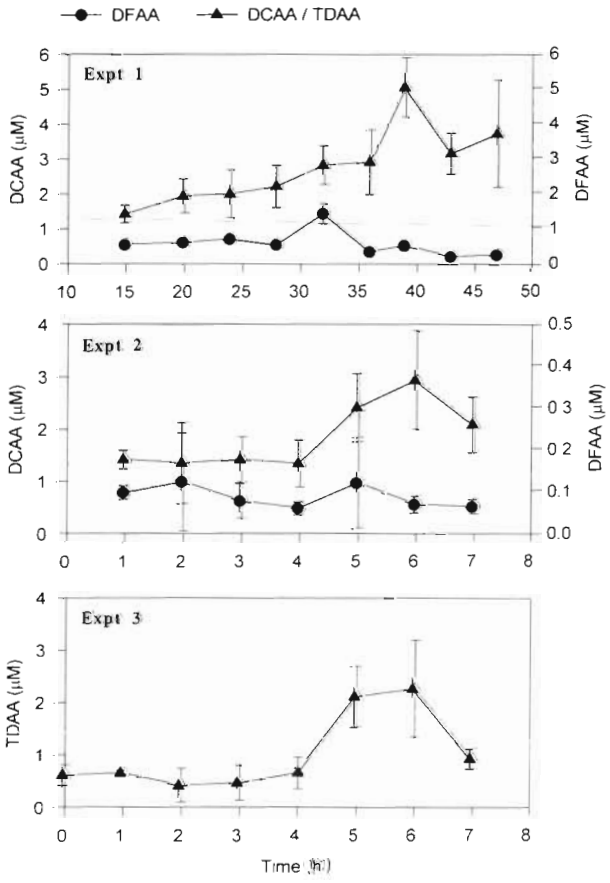


Fig. 4. Release of dissolved free (DFAA) and combined (DCAA) amino acids from lake snow (n = 10) collected at 15 m depth. Aggregates in Expts 1 to 3 were incubated at *in situ* temperature in rolling cylinders on 16 October 1992, 3 April and 10 April 1993, respectively. In Expt 3 only total dissolved amino acids (TDAA) were measured. Note the different time scales

Release of dissolved amino acids into the surrounding water

Microbial processes on aggregates released dissolved amino acids into the surrounding water in all experiments (Fig. 4). Most release occurred as DCAA and little, if any, as DFAA. In Expt 1, TDAA (DFAA + DCAA) release was 3.6 µmol in 24 h, or 15 nmol TDAA agg.$^{-1}$ h^{-1}. Release rates in Expts 2 and 3 were 28 nmol TDAA agg.$^{-1}$ h^{-1}. In Expt 4 using lab-made aggregates, which were ~10× bigger than natural aggregates, the release rate was 82 nmol TDAA agg.$^{-1}$ h^{-1}.

Hydrolytic enzyme activities

The enhanced concentrations of dissolved amino acids in the matrix water and the high release rates of TDAA are consistent with high rates of aminopeptidase activity on lake snow. This activity was <0.5 to 21.8 µmol agg.$^{-1}$ h^{-1} (Fig. 5). Values >2 µmol agg.$^{-1}$ h^{-1} occurred in June when aggregates derived from benthic algae were abundant and in October during the cyanobacterial bloom. In general, activities were highest at 15 m except in the cases mentioned. Excluding these numbers, which were not representative of the plankton-derived aggregates, seasonal means were 12 ± 2 nmol agg.$^{-1}$ h^{-1} at 6 m, 21 ± 3 nmol agg.$^{-1}$ h^{-1} at 15 m, and 8 ± 2 nmol agg.$^{-1}$ h^{-1} at 25 m.

Aminopeptidase activity of free-living bacteria was 20 to 500 nmol l^{-1} h^{-1}. In contrast to aggregate-associated aminopeptidase activity these values decreased slightly with depth. Except for the surface film and aggregates of benthic origin in June, aminopeptidase

activities in the surrounding water were only up to 4.75× higher than on aggregates in the same volume of water, indicating that a large fraction of the potential aminopeptidase activity was due to aggregate bacteria. Cell-specific aminopeptidase activity was 0.8 to 218 fmol cell^{-1} h^{-1} on lake snow and 4 to 214 fmol cell^{-1} h^{-1} in the surrounding water (Table 3). Cell-specific aminopeptidase activities on aggregates were higher than in the surrounding water whenever aggregates were highly abundant and mainly composed of phytoplankton-derived material, e.g. in late April, early August, and in October.

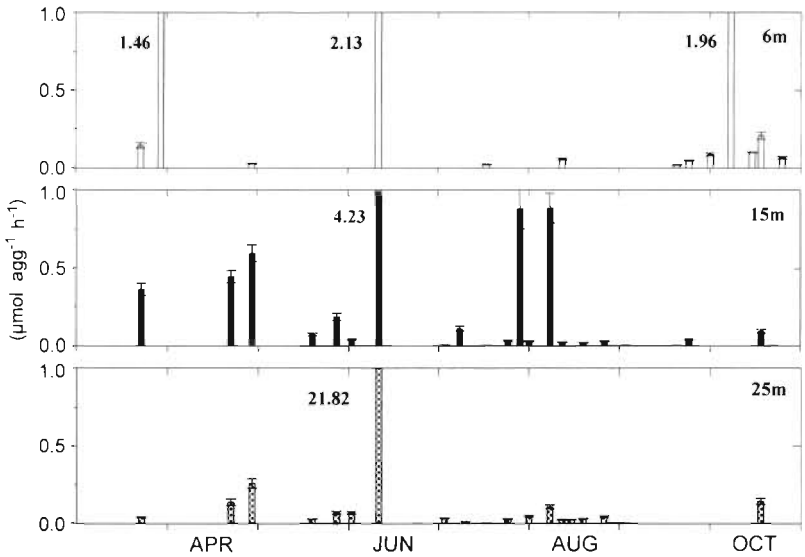


Fig. 5. Activities of aminopeptidase on lake snow at 6, 15, and 25 m depth in Lake Constance in 1993

Alkaline phosphatase (APase) activity was highly variable (8 to 850 nmol agg.$^{-1}$ h^{-1}) throughout the season. As with aminopeptidase, the highest values occurred on the surface layer at the end of March and in June on aggregates derived from benthic algae (Fig. 6). Usually there was no systematic difference with depth. This is also shown by the similar seasonal means at 6, 15, and 25 m of 28 ± 4, 27 ± 3, and 21 ± 3 nmol agg.$^{-1}$ h^{-1}, respectively. APase activities in 1 l of surrounding water ranged between 15 and 115 nmol l^{-1} h^{-1}, with a pronounced maximum during the spring bloom. Thereafter rates never exceeded 50 nmol l^{-1} h^{-1}. Only during the spring was there a systematic decrease of APase activities with depth, whereas at other times rates from 6 to 25 m were fairly similar. In contrast to total activities of APase, the cell-specific activities of aggregate bacteria were up to 14.8× higher than those of free-living ones.

β-Glucosidase activity on lake snow was 2 to 72 nmol agg.$^{-1}$ h^{-1}, excluding the surface film at the end of March (Fig. 7). Excluding the highest rates on benthic aggregates in June, rates did not exceed 50 nmol agg.$^{-1}$ h^{-1}. There was no systematic difference of rates between 6 and 25 m. Rates of β-glucosidase in the surrounding water were 7 to 21 nmol l^{-1} h^{-1} without any pronounced differences in space or time (not shown). Cell-specific activities of β-glucosidase of lake-snow-associated bacteria were ≤13× higher than of free-living ones.

α-Glucosidase activity was in general lower but exhibited similar spatio-seasonal pattern (not shown). Rates and spatio-seasonal pattern of N-acetyl β-glucosidase, which cleaves the dimeric hydrolysis product of the chitinase, were also nearly similar to those of β-glucosidase (not shown).

Activities of aminopeptidase, APase, α- and β-glucosidase on the diatom surface film in late March were in the highest range measured on aggregates throughout the season. Rates of α- and β-glucosidase were even 3 to 4× higher than on any other type of aggregate.

Turnover of particulate combined amino acids (PCAA)

PCCA turnover times on aggregates were calculated from the PCAA content and the aminopeptidase

Table 3. Hydrolytic ectoenzyme activities on lake snow aggregates and in the surrounding water

Enzyme	Hydrolytic activities		Cell-specific activities		
	Aggregate	Surrounding water	Aggregate	Surrounding water	Aggregate/surrounding water
	(µmol l^{-1} h^{-1})		(fmol cell^{-1} h^{-1})		
Aminopeptidase	0.002–218[a]	0.02–0.5	0.8–218	4–214	0.2–46.1
Phosphatase	0.005–8.2	0.015–0.115	0.3–57	0.6–35	0.5–14.8
β-Glucosidase	0.001–0.7[a]	0.007–0.021	0.08–2.21	0.12–2.04	0.2–13.3

[a]Without surface film of diatoms in spring

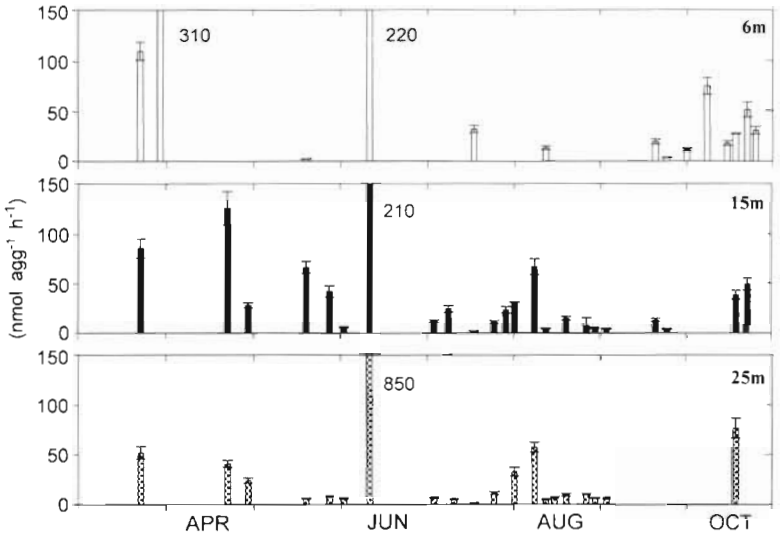


Fig. 6. Activities of alkaline phosphatase (APase) on lake snow at 6, 15, and 25 m depth in Lake Constance in 1993

activity on lake snow. Since the incubated saturating concentration of the artificial substrate was much higher than the natural concentration of PCAA on aggregates, we used the ratio between the concentration of the artifical and natural substrate to correct for the measured maximum rates. Turnover times of PCAA on aggregates due to their aminopeptidase activity were mostly fairly short (0.04 and 381 h; Fig. 8). Very high aminopeptidase activity enhanced the PCAA turnover of the diatom surface film in spring and that of large green algae in early June. In contrast, aminopeptidase activity of resuspended sediment material in September was low and resulted in prolonged PCAA turnover times. During the whole season the PCAA turnover on organic aggregates due to their aminopeptidase activity was <20 h at all depths.

PCAA turnover times calculated from the net release of dissolved amino acids into the surrounding water were probably underestimates because the utilization of released amino acids by aggregates and also free-living bacteria is neglected. However, this method gave PCAA turnover rates somewhat higher than calculated by the aminopeptidase activity. Interestingly, PCAA turnover times on aggregates in Expts 2 and 3 (3 and 10 April 1993) were very similar (89.3 and 79.2 h), whereas aggregates in Expt 1 (October 1992) showed a reduced PCAA turnover (154.8 h) and also a lower aminopeptidase activity.

PCAA turnover on lake snow was further calculated from the BPP-C on aggregates, assuming a bacterial growth efficiency of 30%. The PCAA turnover times thus obtained were significantly higher than those calculated from aminopeptidase activity and net release of amino acids and showed high seasonal fluctuations (5 to 227 d). Like for the PCAA turnover calculated by aminopeptidase activities, those by BPP-C were extremely slow on resuspended aggregates (728 d). The fact that PCAA turnover times on lake snow calculated on the basis of carbon consumption were far higher than those calculated on the basis of amino acid release indicates that the latter process was of much higher significance for the amino acid turnover on aggregates.

Bacterial colonization of aggregates

Colonization of lab-made aggregates incubated in rolling cylinders showed a recurrent pattern in all experiments. During the first 2 d, <1 µm cocci were dominant (Fig. 9). Bacterial community structure shifted to rods when larger aggregates were formed. Bacteria were enlarged and formed large colonies after 2 to 3 d when *Spumella*-type flagellates occurred. Filamentous or flagellated bacteria dominated after 5 to 7 d when *Spumella*-type flagellates reached highest abundances. After 9 d *Kinetoplastid*-type flagellates

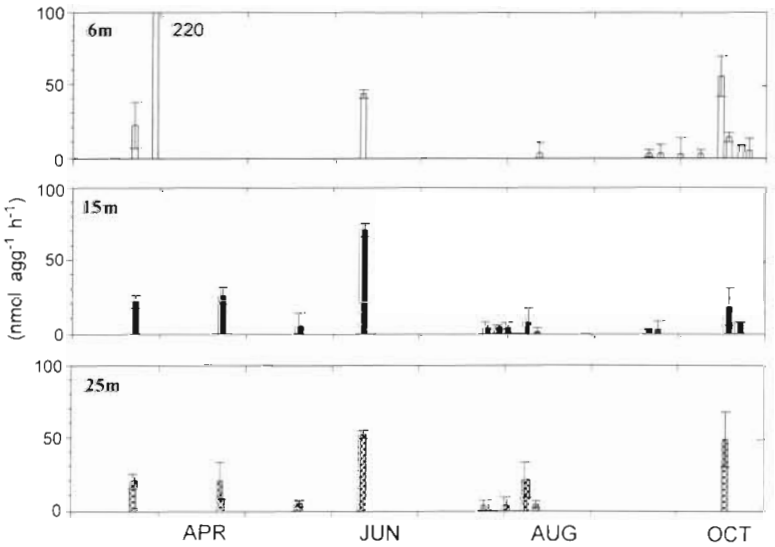


Fig. 7. Activities of β-glucosidase on lake snow at 6, 15, and 25 m depth in Lake Constance in 1993

appeared which later completely outcompeted *Spumella*-type flagellates.

We analyzed the structure of the microbial assemblage on aggregates by *in situ* hybridization with rRNA-targeted oligonucleotide probes. Bacterial counts by DAPI staining were 1 to 13 × 10^6 agg.$^{-1}$ (Fig. 10). *Bacteria* detected with the probe EUB 338 yielded 55 to 100% of total cell numbers. α-, β-, and γ-subclass *Proteobacteria* were usually the dominant bacterial subclasses (>30%; generally >70% of total). β-*Proteobacteria* dominated all experiments except Expt 3 and α- and γ-*Proteobacteria* were less abundant. In contrast, γ-*Proteobacteria* dominated during Expt 3 and comprised >75% of total cells after 6 d. This experiment was run at 15°C instead of the *in situ* temperature (4°C).

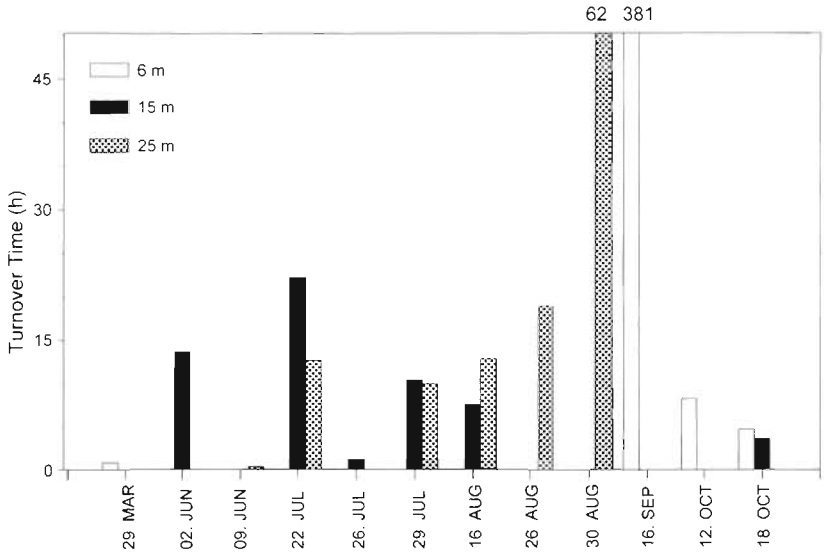
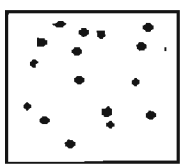
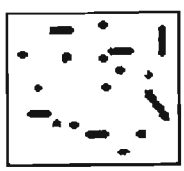


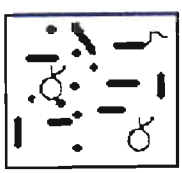
Fig. 8. Turnover time of particulate combined amino acids (PCAA) on lake snow on the basis of aminopeptidase activity. Values were calculated from PCAA content and total aminopeptidase activity on aggregates at 6, 15, and 25 m depth



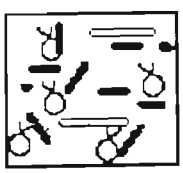
Day 1: microaggregates (<3 mm ∅): fragments of diatoms, bacteria: small cocci (<1 µm ∅)



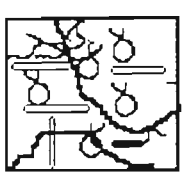
Day 2: microaggregates (<3 mm ∅): detritus and living algae, bacteria: mostly small cocci (<1 µm ∅) and short but thick rods



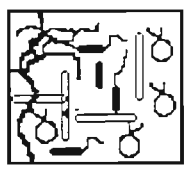
Day 3: aggregates (<5 mm ∅): detritus and living algae, bacteria: mostly short but thick rods, few flagellated forms, few flagellates of the *Spumella* type



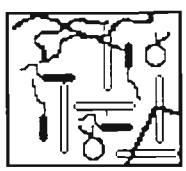
Day 4: aggregates (<5 mm ∅): detritus and living algae, bacteria: mostly short but thick rods, few long rods, increasing number of flagellates of the *Spumella* type



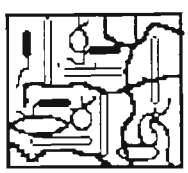
Day 5: aggregates (<9 mm ∅): detritus and living algae, bacteria: mostly long rods, few flagellated and colony-forming rods, increasing number of flagellates of the *Spumella* type



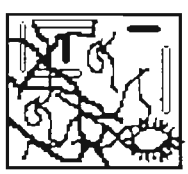
Days 6 + 7: aggregates (= 9 mm ∅): detritus and living algae, bacteria: mostly long rods, few flagellated and colony-forming rods, high numbers of flagellates of the *Spumella* type



Day 8: aggregates (>9 mm ∅): detritus and living algae, only filamentous and flagellated bacteria (*Caulobacter*), maximum of flagellates of the *Spumella* type



Days 9–11. aggregates (>9 mm ∅): detritus and living algae, bacteria: long and thick rods, mostly flagellated, *Spumella*- and few of the *Kinetoplastid*-type flagellates



Day 12: aggregates (>9 mm ∅): detritus and living algae, dense colonization by colony-forming bacteria, few small rods, mostly flagellates of the *Kinetoplastid* type

Fig. 9. Sequence of formation of aggregates and their microbial colonization during incubation of natural lake water in rolling cylinders

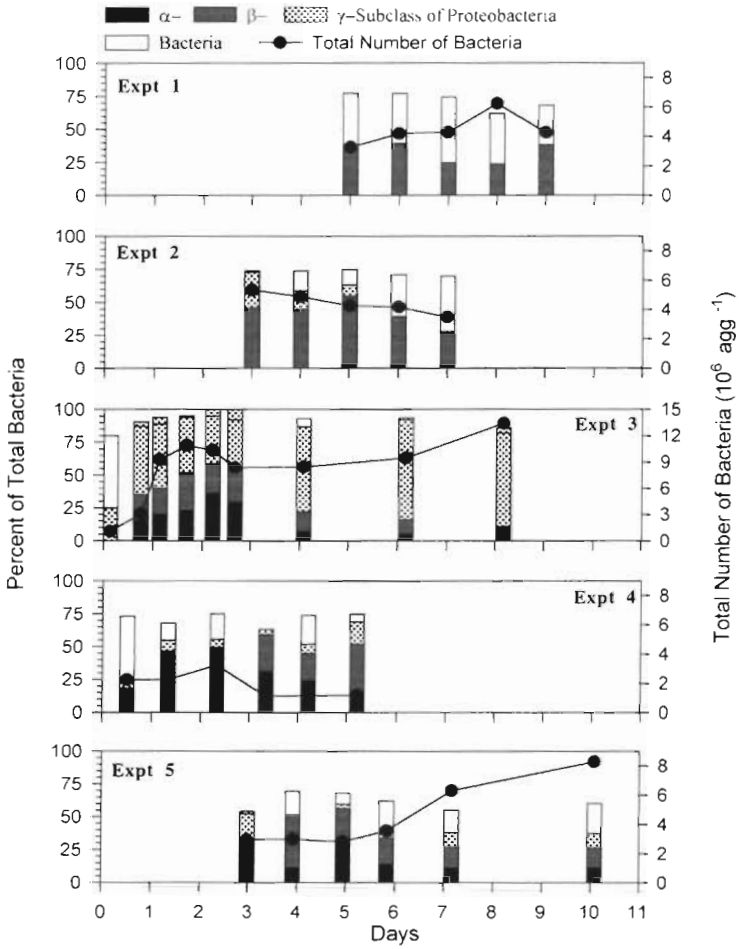


Fig. 10. Succession of *Bacteria* and *Proteobacteria* of the α-, β-, and γ-subclasses as percentage of total bacteria on natural lake snow incubated in rolling cylinders at various times

DISCUSSION

Bacterial production and growth rates

Bacterial production and growth rates on aggregates were quite low despite high bacterial numbers and rapid POM solubilization, indicating loose hydrolysis-uptake coupling. However, the bacterial production and growth rates could have been underestimated due to extracellular and intracellular isotope dilution (ID) of ^{14}C-leucine. DFAA concentrations in the matrix water were much higher than in the surrounding water (Table 2). Since we achieved maximum rate of incorporation at 60 nM added ^{14}C-leucine, the extracellular leucine ID may have been overcome by this high addition. We could not determine the intracellular ID, so we cannot rule out that the ID of 2 we used may have been too low or too high. Azam et al. (1993), using *in vitro* experimental systems, showed that bacterial growth rates in the initial phase of colonization were high but slowed in later stages during the establishment of an aggregate-associated bacterial community. Further, a cross-system overview (Alldredge & Gotschalk 1990) showed that thymidine incorporation per bacterium decreased significantly with increasing abundance of bacteria per µg dry weight on aggregates. We assume, however, that the low values of BPP-C and bacterial growth rates we found were not biased substantially but reflect the fact that bacterial growth on aggregates at least 2 to 3 d old is indeed low.

Release of dissolved amino acids

The release of amino acids from lake snow into the surrounding water was high whereas bacterial production was low. This suggests that protein hydrolysis and hydrolysate utilization by aggregate bacteria are loosely coupled, leading to release of dissolved amino acids into the surrounding water (Smith et al. 1992). Further, in our experiments, this release was not always continuous but occurred in pulses. DFAA and DCAA concentrations in the matrix water were much higher than in the surrounding water. The enrichment factor for DFAA was generally higher than for DCAA (Table 2). Only for the very large benthic aggregates in June and cyanobacterial aggregates in the fall was the enrichment factor for DCAA at least as high as for DFAA. The high DFAA enrichment in the matrix water and their fairly low net release into the surrounding water (see below) suggest that the recycling of amino acids within the aggregates occurred predominantly via DFAA. There is no other information on the relative utilization of DFAA and DCAA within aggregates. Müller-Niklas et al. (1994), however, also showed that marine snow is highly enriched in DFAA relative to the surrounding water.

Hydrolytic enzyme activities

Ectoenzyme activities also suggested rapid POM and dissolved polymer hydrolysis within the aggregates. Aminopeptidase activity on lake snow was always higher than APase, α- and β-glucosidase, and chitinase activities. Müller-Niklas et al. (1994) and Smith et al. (1992, 1995) also found that aminopeptidase on marine

snow was generally much higher than α- and β-glucosidase. However, measuring hydrolytic enzyme activities with substrate analogs yields maximum hydrolysis potential. Rosenstock & Simon (1993) showed that potential rates of DCAA utilization by bacteria are 3 to 10× higher than the actual rates. Taking into account the fact that proteins comprise <10% of DCAA (Keil & Kirchman 1993, B. Rosenstock unpubl.), ambient utilization rates of proteins per cell are even lower. Hence, we hypothesize that at the ambient DCAA and protein concentrations in the bulk water, their hydrolysis rates are not saturated, whereas they are saturated at higher substrate concentrations such as in PCAA rich microenvironments of lysing algae and lake snow. In contrast, cell-specific utilization of dissolved polysaccharides at ambient concentrations have been shown to be in the same range as potential rates of β-glucosidase measured at saturating concentrations of the fluorogenic substrate (Hanisch et al. 1996). Thus, higher concentrations of dissolved polysaccharides on aggregates cannot yield enhanced rates of β-glucosidase activity. Differences in potential and ambient hydrolysis rates may explain why potential rates of aminopeptidase are always higher than that of glucosidases, not only on aggregates but also in the bulk water (Chróst 1991). Thus, bacteria are able to readily hydrolyze and utilize DCAA and dissolved proteins at enhanced concentrations such as in the microenvironment of aggregates.

PCAA turnover

PCAA turnover calculated by aminopeptidase hydrolysis or net release of dissolved amino acids on lake snow was rapid. These calculations are based on the assumption that the DCAA concentration in the matrix water is equal to the ambient aminopeptidase substrate concentration. However, a certain fraction of the DCAA pool can be resistant to bacterial hydrolysis and utilization. Labile proteins as well as peptides comprise only a minor fraction of total DCAA which is in the bulk water, usually 10 to 20% (Keil & Kirchman 1993, Rosenstock & Simon 1993). On the other hand, DCAA in the matrix water of lake snow are of relatively fresh origin and may have higher concentrations of labile proteins and peptides than bulk DCAA. Although we may have underestimated the PCAA turnover on aggregates, we yielded much shorter and more realistic turnover times than calculated by bacterial production and a growth efficiency of 30%. PCAA turnover times calculated by aminopeptidase acitivity were further confirmed by the fact that PCAA turnover times calculated from the net release of dissolved amino acids were similar.

In addition to amino acid turnover it is also important to estimate the significance of the DCAA release on aggregates for the turnover of bulk DCAA. For this we assumed an abundance of 2 to 10 agg. l^{-1}, a release rate of 15 to 28 nmol DCAA $agg.^{-1}$ h^{-1}, and a concentration of bulk DCAA of 2 to 5 µM (Rosenstock & Simon 1993). On this basis the turnover time of bulk DCAA is 40 to 160 h and similar to measured turnover times of bulk DCAA in Lake Constance (Rosenstock & Simon 1993). Turnover times of dissolved proteins in Lake Constance are even faster (B. Rosenstock unpubl.). Our calculation shows that DCAA release from lake snow into the surrounding water could be important for supplying free-living bacteria with DCAA in the epi- and hypolimnion. However, DCAA release from aggregates is not always a continuous process and sinking aggregates may even scavenge DCAA in the hypolimnion (Grossart & Simon 1998).

Changes in aggregate composition during aging and sinking

Release of dissolved amino acids leads to a depletion of particulate organic nitrogen (PON) from aging aggregates. This is reflected by the decreasing PCAA fraction relative to POC from lake snow to POM collected in 50 m sediment traps (Grossart & Simon 1998) and by the higher depletion of PON relative to P and dry weight of sediment trap material (Gries 1995). In addition, the PCAA turnover of sediment material from 50 m calculated by DCAA net release was 6.9 to 54.5 d (Grossart & Simon 1998) and thus much longer than that of lake snow. Even though sinking aggregates become depleted of PCAA and PON in lakes, they still comprise fairly high amounts of PON when reaching the lake sediment. The short sinking distance in most lakes (≤100 to 200 m) results in an aggregate age of 5 to 15 d. In contrast, sinking aggregates in the ocean become much more depleted in PON and show increased C:N ratios with depth due to the longer sinking distance (often >1000 m; Wakeham et al. 1984, Lee 1988, Haake et al. 1993, Wakeham & Lee 1993).

Bacterial colonization during aging and sinking of lake snow

During aging and sinking of lake snow, its microbial community showed changes in morphology and taxonomy which presumably reflect the biochemical composition and the DOM release of the aggregates. Characteristic changes in the microbial community structures were observed during the incubation of lab-made aggregates and also by comparing lake snow with

sediment trap material which consisted of older aggregated material. Bacterial community structure after 1 to 4 d incubation of aggregates presumably reflected more the composition of lake snow in the epilimnion and upper hypolimnion. In later stages the co-occurrence of filamentous bacteria and flagellates presumably reflected structural adaptations of aggregate bacteria to higher grazing pressure by flagellates (Güde 1979, Jürgens & Güde 1994). These authors observed that mixed bacterial assemblages in various environments adapted to flagellate grazing by forming filamentous and thus grazing-resistant forms. Even though lab-made aggregates became larger after 6 to 7 d than natural lake snow, Weiss et al. (1996) showed that the bacterial community structure of these aggregates was not different after 13 d from that on lake snow and sediment trap material. However, when aggregates reach the sediment with different environmental conditions than the water column their community structure may undergo pronounced changes.

rRNA-targeted oligonucleotide probes indicated that the majority of the bacteria on lake snow belonged to the domain *Bacteria*. The remaining fraction not accounted for presumably also consisted of *Bacteria* with numbers of ribosomes too low to be detected with this probe. Neither we nor Weiss et al. (1996) ever detected *Archaea*. β-*Proteobacteria* were dominant whereas γ-*Proteobacteria* were of minor importance except in Expt 3. α-*Proteobacteria* constituted sometimes substantial proportions of the bacterial community, mainly in the earlier stages of the incubation. These results agree well with those of Weiss et al. (1996), who found a similar dominance of β-*Proteobacteria* on lake snow in Lake Constance. They also found that, on younger lab-made and natural aggregates, α-*Proteobacteria* often comprise high proportions, whereas on older aggregates and on sediment trap material β-*Proteobacteria* become more dominant. Interestingly, on activated sludge flocs, β-*Proteobacteria* dominate the bacterial community (Wagner et al. 1994), and microbes closely related to bacteria such as *Zoogloea ramigera* and *Sphaerotilus natans* are often filamentous and most frequent. Hence these observations suggest that lake snow harbors a microbial community closely related to that of activated sludge flocs and that it is characterized by a rapid recycling and release of labile DOM.

Pernthaler et al. (1997) observed in a 2-stage chemostat that flagellate grazing is the driving force forming filamentous bacteria and a community dominated by β-*Proteobacteria*. Flagellates selectively fed on α-*Proteobacteria* which had high growth rates and were always present as a minor fraction of total bacteria. If this is also true for microbial communities on lake snow, α-*Proteobacteria* would mediate the recycling of DOM and substrates within the aggregates. Their growth rates would be more important in early aggregate history. In contrast, filamentous β-*Proteobacteria* on lake snow would have lower growth rates but higher hydrolytic enzyme activities and the dominance of β-*Proteobacteria* could be regarded as an indication of the presence of bacterivorous flagellates and possibly ciliates in later stages.

In Expt 3, run at 15°C instead of 4°C, γ-*Proteobacteria* became dominant which mainly utilize readily available organic compounds (Wagner et al. 1993). Thus, temperature may be an additional important factor controlling bacterial community structure. However, further experiments need to examine more carefully the influence of temperature on bacterial community structure on aggregates and in the surrounding water.

Conclusions

Our results show that lake snow aggregates act as hotspots of enhanced microbial POM decomposition and thus constitute an important source of dissolved organic matter in the epi- and hypolimnion of Lake Constance. The microbial community on lake snow was dominated by β-*Proteobacteria*, especially during aggregate aging when filamentous and thus grazing-resistant bacteria dominated. This community structure is similar to that of activated sludge flocs. These observations suggest that lake snow and activated sludge flocs have similar functions in their environment.

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