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Temporal changes in the dominance of major planktonic bacterial groups in an alpine lake: discrepancy with their contribution to bacterial production

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ABSTRACT: We tested the idea that temporal changes in the numerical dominance of major bacterial groups are not necessarily coupled to those in bacterial production. The seasonal dynamics of the bacterial community in the water column of an alpine lake were followed from late spring until the autumn at either bi-weekly or monthly intervals. Changes in community structure were tracked by fluorescence *in situ* hybridization combined with catalyzed reporter deposition (CARD-FISH); the contribution of the dominant bacterial groups to leucine incorporation was assessed by microautoradiography (MAR). Before ice-out, the bacterial assemblage was dominated by *Actinobacteria*. Immediately afterwards, their relative abundance, and that of *Cytophaga*-like bacteria, increased in the water column, suggesting that they had taken advantage of the input of dissolved organic matter (DOM) during the melting of the ice. During summer, *Betaproteobacteria* and *Actinobacteria* together accounted for >50% of the cell counts; however, their maxima were separated temporally and vertically. Water temperature, DOM, and grazing influenced their antagonistic behavior. *Alphaproteobacteria* were consistently present, although their abundance was generally low. Regardless of their relative abundance, *Betaproteobacteria* dominated the assemblage of leucine-positive cells, and the relative abundance of their R-BT subgroup closely matched that of active *Betaproteobacteria*. Of the cells positive for leucine incorporation, the contribution of *Actinobacteria* was lower than expected from their relative abundance. Our results show that bacterial abundance and production are not necessarily dominated by the same phylogenetic groups.

KEY WORDS: Seasonality · Leucine incorporation · MAR-FISH · *Actinobacteria* · *Betaproteobacteria*

INTRODUCTION

How the microbial community structure relates to ecosystem functioning is one of the major challenges of ecological research (Loreau et al. 2001). In the last decade, several protocols (Lee et al. 1999, Ouverney & Fuhrman 1999, Cottrell & Kirchman 2000) that combine fluorescence *in situ* hybridization (FISH) with microautoradiography (MAR) have made it possible to relate *in situ* taxonomic identification (Amann et al. 1995) to single-cell activity, thus bridging the gap between bacterial community structure and function (Ouverney & Furhman 1999). Bacterial utilization of dissolved organic matter (DOM) and the subsequent production of bacterial biomass are crucial steps in the biogeochemical carbon cycle. Nevertheless, the contribution of major phylogenetic groups to total bacterial biomass production has been studied in few ecosystems (Cottrell & Kirchman 2003, Hornák et al. 2006, Zhang et al. 2006). The results of these studies point to a preponderant contribution of *Betaproteobacteria* to bacterial production in freshwater habitats. However, none of these reports included the abundant freshwater group *Actinobacteria* (Glöckner et al. 2000, War-

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necke et al. 2005). Yet, some studies analyzing the contribution of the *Actinobacteria* to leucine incorporation have shown that it was proportional to their representation in the community structure (Pérez et al. 2010), whereas others did not provide such information (Buck et al. 2009, Hornák et al. 2010). Furthermore, the activity of different bacterial groups has been shown to change over time as a consequence, for example, of resource fluctuations (Alonso-Sáez & Gasol 2007, Hornák et al. 2010) or seasonal changes in other environmental parameters (Salcher et al. 2010).

Alpine lakes (i.e. those situated above the tree-line) are extreme environments subject to strong seasonal changes in nutrient availability, temperature, and light conditions between the ice-covered and the ice-free seasons (Sommaruga 2001). The ice-covered season lasts typically for 7 to 9 mo each year. The ice-out is followed by a spring overturn (June) and a distinct thermal stratification until the autumn overturn (Pechlaner 1971). These fluctuations are considered as key factors for understanding the dynamics of the plankton community in these lakes. Thus, it is important to assess whether the contribution of the main freshwater groups to bacterial production is always proportional to their relative abundance — despite the strong seasonal patterns in both bacterial abundance and production rates (Wille et al. 1999).

Here, we present the results of a study of an alpine lake where we followed, in detail, the seasonal dynamics of the bacterioplankton in the water column. Previous seasonal studies did not include all relevant groups (Pernthaler et al. 1998) or were conducted at a single depth (Glöckner et al. 2000). Concomitantly, we followed the contribution of the dominant bacterial groups to leucine incorporation using MAR-FISH to test whether changes in their relative contribution to bacterial production were coupled to changes in their abundance.

MATERIALS AND METHODS

Study site and sampling procedure. Gossenköllesee (GKS) is a small lake (area: 0.017 km^2) located at 2417 m above sea level in the Austrian Central Alps (47° 13′ N, 11° 01′ E). GKS is a dimictic and holomictic lake, with a maximum depth of 9.9 m. Its catchment area is composed of crystalline bedrock covered with a layer of poor soil and sparse patches of alpine rankers. The lake is usually covered by ice for 6 to 7 mo each year. Background information on DOM dynamics, chemical composition and other variables can be found elsewhere (Sommaruga & Augustin 2006).

Sampling started in late spring (20 May 2005), when the lake was still ice-covered, and ended in the late autumn (9 November 2005), shortly before the lake froze. Sampling was done from a boat in the middle of the lake at bi-weekly or monthly intervals. Water samples were collected at 5 different depths (every 2 m, from the surface to a depth of 8 m) with a 5 l Schindler-Patalas sampler equipped with a thermometer. During the ice-covered period, water samples were collected through a hole opened in the ice cover in the central area of the lake. Before ice-out, samples for assessing bacterial composition and single-cell activity were collected only at depths of 2 and 4 m. On every sampling date, we collected single samples for chlorophyll a (chl *a*), dissolved organic carbon (DOC), total dissolved nitrogen (TDN) and inorganic nutrients. Triplicate water samples were used for bacterial abundance, and duplicates (plus a negative control) were used for leucine bulk incorporation rates. Single samples for MAR-FISH were incubated for 1 h, immediately after collection, at *in situ* temperature with L-[^{3}H] leucine (specific activity: 65 Ci $mmol^{-1}$; 20 nmol l^{-1} final conc.). The incubation was stopped by adding formaldehyde at a final concentration of 2%. Samples were left at 4°C overnight; on the next day they were filtered onto white polycarbonate filters (Millipore, GTTP). The filters were stored at –20°C until further processing.

Chlorophyll *a*. For chl *a* analysis, 2 l of lake water were filtered onto Whatman GF/F filters at low vacuum pressure. Filters were then stored frozen until extraction (within 4 mo). Extraction and chl *a* determination were done as described in Tartarotti & Sommaruga (2006). The equation of Lorenzen (1967) was used to calculate the concentration of chl *a*.

DOC and TDN concentrations. Immediately after sampling, sub-samples for DOC and TDN analysis were filtered through a precombusted (4 h at 450°C) GF/F filter (Whatman) placed on a stainless steel syringe holder. Filters were rinsed first with Milli-Q water and then with the sample. The filtrate was collected in combusted glass bottles (Schott), acidified with HCl to pH 2, and stored in the dark at 4°C until further analysis (within 48 h). DOC and TDN concentrations were measured with a total organic carbon analyzer (Shimadzu TOC-Vc series) equipped with a TNM-1 module. Both parameters were detected simultaneously after combustion and catalytic oxidation of the injected sample.

Analysis of nutrients. Nitrate concentration (NO_3^- N, mg l^{-1}) was assessed using spectrophotometry after reduction with sodium salicylate and Seignette salt, as described by Taras (1971). Total dissolved phosphorus (TDP) was determined with a spectrophotometer using the molybdate blue method after digestion with sulphuric acid and hydrogen peroxide (Schmid & Ambühl 1965).

Bulk bacterial production. Leucine bulk incorporation rates were measured in duplicate samples and in a formaldehyde-killed control. Samples (5 to 10 ml) were incubated with 20 nmol l^{-1} (final conc.) of L-[^{3}H] leucine (of the same specific activity as that used for microautoradiography) at *in situ* temperature in the dark for 1 h; the incubations were terminated by adding formaldehyde (2% final conc.). Subsequently, the samples were filtered through Millipore GTTP filters (pore size 0.22 µm) and rinsed twice with 5 ml of 5% trichloroacetic acid (TCA) for 5 min. The filters were dissolved in 5 ml of scintillation cocktail (Ready safe, Beckman-Coulter) and the radioactivity of the filters assessed after 15 h.

Bacterial abundance. Triplicate samples (10 ml), fixed with formaldehyde (2% final conc.), were stained with 4′,6-diamidino-2-phenylindole (DAPI, 1 µg ml^{-1} final conc.) and filtered onto black polycarbonate membranes (Millipore, GTBP, 0.22 µm). Bacterial abundance was determined by epifluorescence microscopy. At least 400 cells per filter were counted.

Hybridization and tyramide signal amplification. FISH with horseradish peroxidase-labeled probes was done on filter sections according to Pernthaler et al. (2002), using a modified permeabilization protocol for freshwater bacteria (Sekar et al. 2003). We used oligonucleotide probes (ThermoHybaid) targeting the domain *Bacteria* (EUB I-III; Daims et al. 1999), *Alphaproteobacteria* (ALF968; Neef 1997), *Betaproteobacteria* (BET42a; Manz et al. 1992) and its subgroup R-BT (R-BT065; Šimek et al. 2001), *Cytophaga*-like bacteria within the phylum Bacteroidetes (CF319a; Manz et al. 1996), and *Actinobacteria* (HGC69a; Roller et al. 1994). The formamide concentration in the hybridization buffer was always 55%, except for probe HGC69a (35%). To quantify *Archaea*, FISH was performed with the Cy3-labeled probe ARCH915 as previously described (Glöckner et al. 1996, Pernthaler et al. 1998). In total, ~300 individual preparations were analyzed as described in the following section.

Microautoradiography. Samples were subjected to MAR as described by Tabor & Neihof (1982) after the hybridization step. Briefly, cells were transferred from filters to glass coverslips (Cottrell & Kirchman 2000, 2003) using 0.2% gelatin for improved transfer efficiency (~90% of cells). Glass slides, with coverslips attached, were coated with a molten Kodak NTB autoradiography emulsion diluted 2:1 with distilled water. Slides were placed on an ice-cold aluminum block for 5 min, to harden the emulsion, before being transferred to light-tight boxes for exposure at 4°C. Different times (24, 48, 72 and 96 h) were tested to determine the optimum exposure time for maximizing the detection of labeled cells. Samples collected under the ice cover were exposed for 72 h, whereas the rest of the samples were exposed for 48 h. Development and fixation were done according to the specifications of the manufacturer (Kodak). Afterwards, cells were stained with an anti-fading solution containing DAPI (final conc.: 1 µg ml^{-1}), and the slides were examined with a Zeiss Axioplan microscope equipped with a 100 W Hg lamp. Silver grains around bacterial cells were observed using the transmission mode of the instrument. Cells were counted in at least 20 randomly selected fields and, for every field, 4 different counts were recorded: (1) DAPI+ cells; (2) probe-specific-positive cells; (3) DAPI+, MAR+ cells; and (4) probe-specific and MAR+ cells. Routinely, at least 350 DAPI-stained cells were counted per sample, or 1000 DAPI-stained cells when the calculated relative abundance was <1%.

Data analysis. Spearman rank correlation coefficients were calculated (whole data set n = 44) to relate changes in bacterial community composition and activity to environmental parameters. Spearman rank correlation coefficients (whole data set n = 44) were calculated to relate changes in bacterial community composition and activity to environmental parameters. For that purpose, we used the relative abundance of different bacterial groups expressed as percentage and the physico-chemical variables measured. Attempts to normalize some of these variables proved unsuccessful. Statistical analyses were done using SigmaStat (Systat Software).

RESULTS

Environmental conditions and bulk bacterial production

Data on the changes in water temperature, chl *a* concentration, DOC, TDN and bulk bacterial production (Fig. 1) were used to define periods when important physical and biological processes had taken place in the lake. Accordingly, we selected the following 5 dates to look in detail at the composition of the bacterial community and at leucine incorporation by the dominant bacterial groups:

(1) 14 June 2005, representing the situation before ice-out, characterized by low water temperature and relatively high concentrations of DOC and TDN (Fig. 1B,D); (2) 29 June 2005, after the spring overturn, which led to an increase in concentrations of TDN and bacterial production; (3) 28 July 2005, corresponding to the maximum of bacterial production, high water temperature, and high concentrations of DOC at the surface, as well as high values of chl *a* at the bottom; (4) 9 August 2005, deep chlorophyll maximum; (5) 12 October 2005, after the autumn overturn, when water

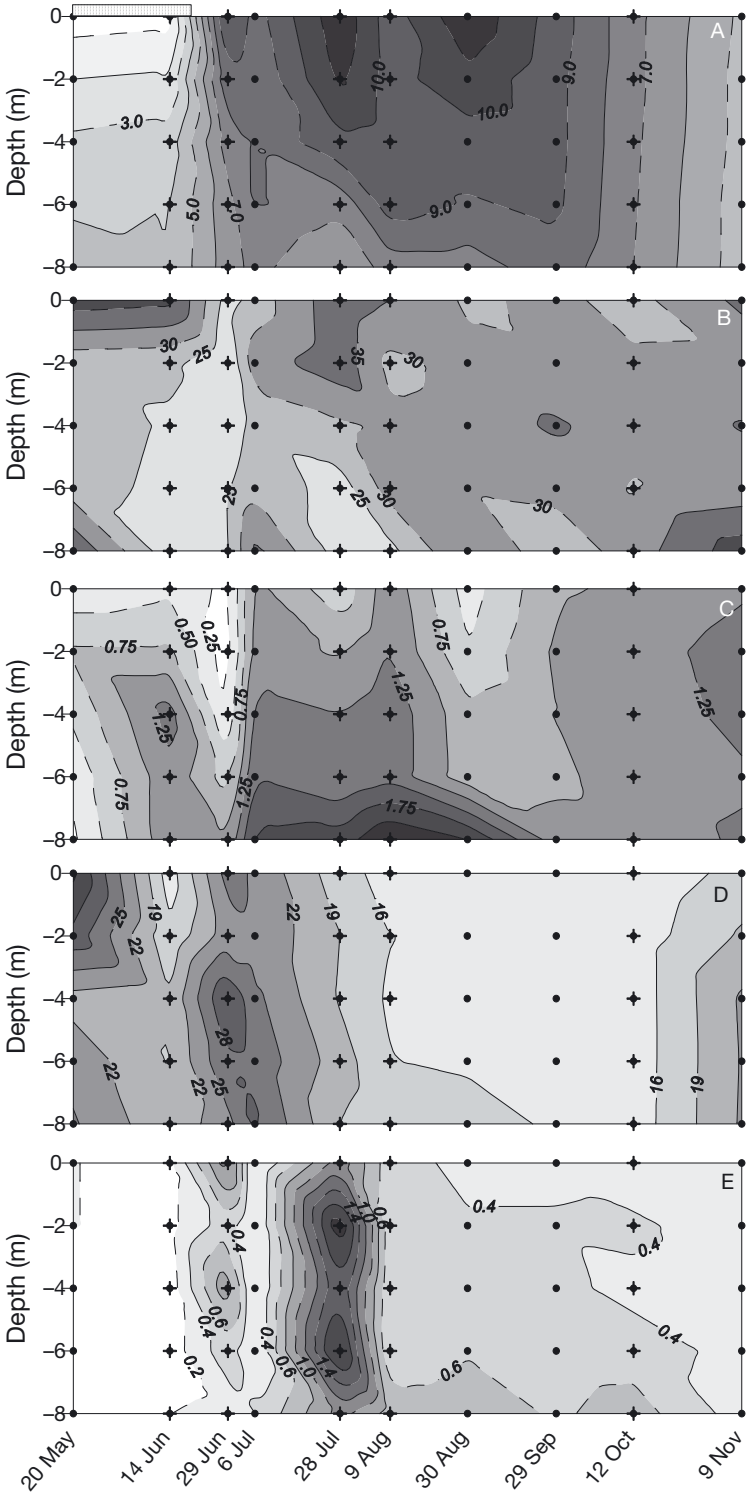

Fig. 1. Temporal and vertical fluctuations in (A) temperature (°C), (B) dissolved organic carbon (µM), (C) chlorophyll *a* concentration (µg l^{-1}), (D) total dissolved nitrogen (µM), and (E) bacterial production (nmol Leu l^{-1} d^{-1}) in the Gossenköllesee (GKS) during the study period in 2005. The grey box above Panel A represents ice cover. Crosses indicate the dates chosen for a more detailed analysis of the bacterial community structure and leucine incorporation at the single-cell level. Dots show time and depth of the measured data set

temperature, and concentrations of TDN, chl *a* and DOC were homogeneous in the water column (Fig. 1).

Bacterial abundance and community structure

Hybridization efficiency varied during the seasonal cycle. The lowest detection rate of probe EUBI-III (~62% of DAPI counts) corresponded to samples collected under ice, and the highest detection rate corresponded to those samples taken immediately after the spring overturn (86 to 90% of DAPI counts). The group-specific probes that we used covered >60% of the domain *Bacteria* in the ice-covered period and ~91% during the ice-free season (range 74 to 100%). Probe ARC915 detected generally <1% of DAPI-stained cells, except for the samples from October and November, when *Archaea* accounted for 2 to 6% of DAPI-stained cells (data not shown).

The ice-covered period (Fig. 2A) was characterized by low bacterial abundances, ranging from 1.2 to 1.4 × 10^5 cells ml^{-1}, and the dominance of *Actinobacteria* (~20 to 30% of DAPI counts). After the spring overturn, bacterial abundance doubled in the whole water column (Fig. 2B), and the relative abundance of *Actinobacteria* increased to 50% of DAPI counts at 6 m depth. Further, the relative abundance of *Cytophaga*-like bacteria also increased, representing ~22% of DAPI counts throughout the water column. The relative abundance of *Betaproteobacteria* remained low (~10% of DAPI counts).

On 28 July, bacterial abundance increased only below 6 m depth (Fig. 2C). *Betaproteobacteria* dominated the bacterial community at the surface, whereas *Actinobacteria* did so at 4 m and in deeper layers of water. The contribution of the R-BT subgroup to *Betaproteobacteria* was ~72% in the upper water layers and ranged from 87 to 96% below 4 m depth. The relative abundance of *Cytophaga*-like bacteria increased slightly with depth from 7% of DAPI counts at 0 m to 12% of DAPI counts at 8 m depth.

On 9 August (Fig. 2D), bacterial abundance peaked. *Betaproteobacteria* was the most abundant bacterial group (~30% of DAPI counts), with high absolute abundances in the

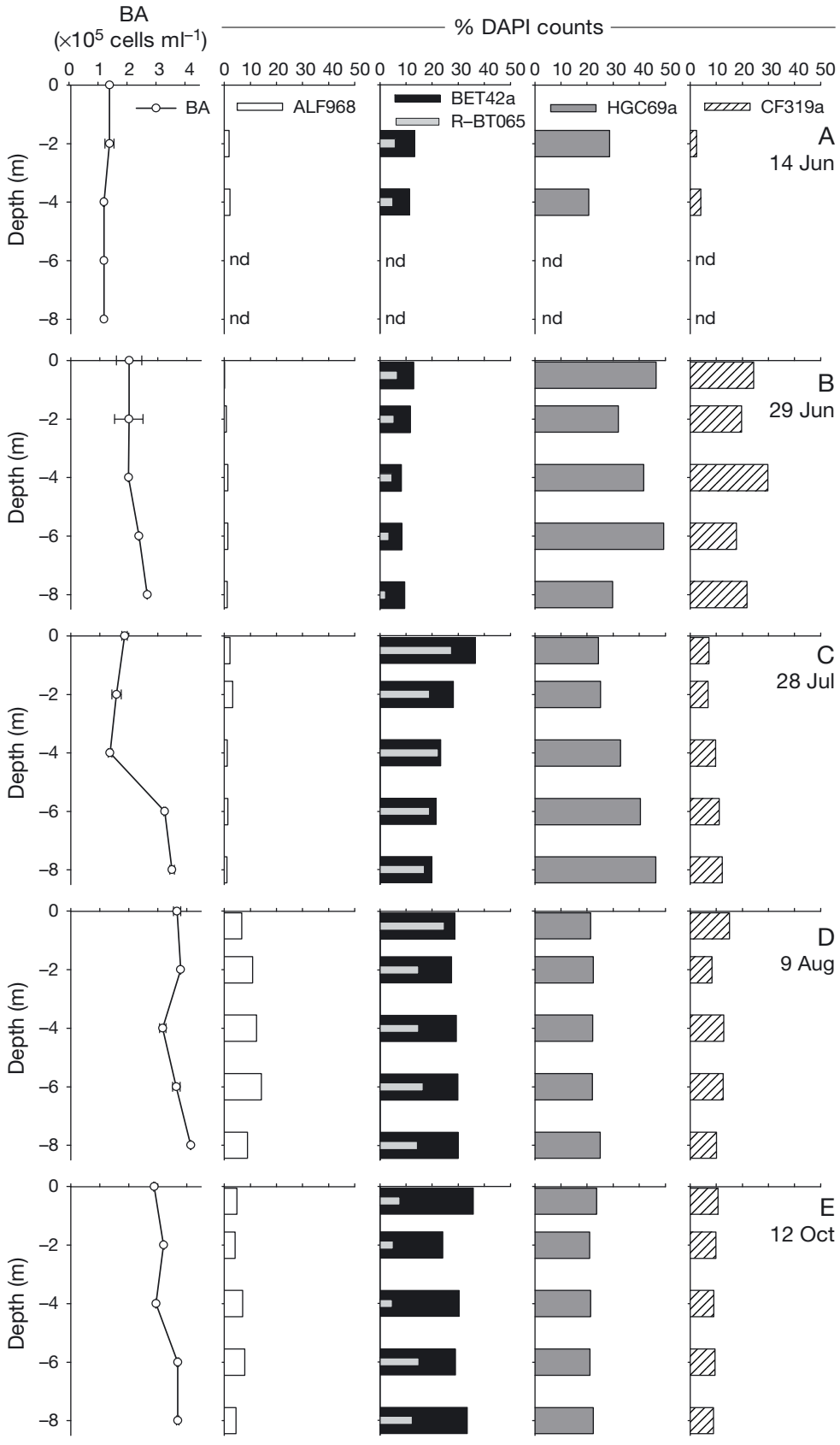

Fig. 2. Vertical distribution of bacterial abundance (BA) and bacteria targeted by probes ALF968 (*Alphaproteobacteria*), BET42a (*Betaproteobacteria*), R-BT065 (R-BT subgroup of *Betaproteobacteria*), HGC69a (*Actinobacteria*), and CF319a (*Cytophaga*-like bacteria) in the Gossenköllesee (GKS) during the most relevant events of the seasonal cycle. On 14 June 2005, the bacterial community structure was assessed only at 2 and 4 m depths. nd: no data. DAPI: 4′,6-diamidino-2-phenylindole

water column ranging from 0.9 to 1.1 × 10^5 cells ml^{-1}. The contribution of the R-BT subgroup decreased with depth and represented 86% of *Betaproteobacteria* at the surface, but only 48% at 8 m depth. The relative abundance of *Alphaproteobacteria* reached its maximum at 6 m depth (14% of DAPI counts).

After the autumn overturn (12 October, Fig. 2E), bacterial abundance remained high (~3.6 × 10^5 cells ml^{-1}). *Betaproteobacteria* still dominated the bacterial assemblage, in terms of both relative and absolute abundance, but the R-BT subgroup comprised <30% of *Betaproteobacteria*. The relative abundance of *Actinobacteria* and *Cytophaga*-like bacteria was rather constant throughout the water column.

Seasonal dynamics in single-cell activity

In general, *Betaproteobacteria* and *Actinobacteria* accounted for >50% of all cells with visible leucine incorporation detected by MAR, and sometimes they even comprised all of the leucine-positive cells, particularly in the epilimnion (the upper 4 m). *Betaproteobacteria* represented between 25 and 72% of leucine-positive bacteria, whereas the contribution of *Actinobacteria* showed a 7-fold variation, ranging from 7 to 48% of leucine-positive cells (Fig. 3). The contribution of *Betaproteobacteria* to leucine incorporation was always higher than expected from their relative abundance, whereas that of the *Actinobacteria* was either lower or equivalent to their numerical contribution to the community structure (Fig. 3).

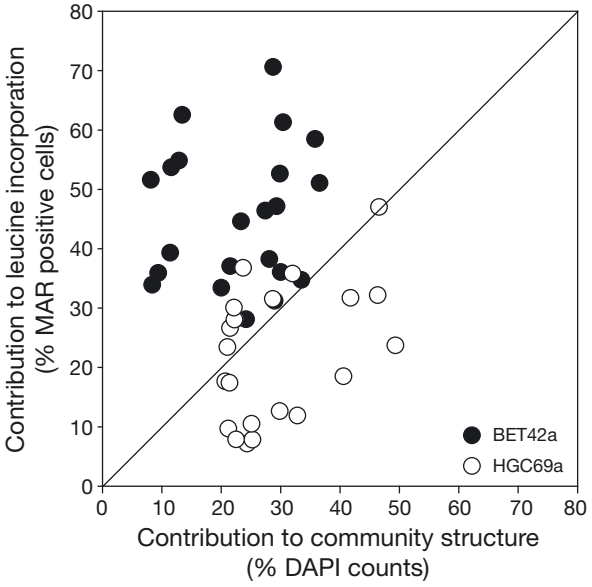

Fig. 3. Relative contribution of the dominant bacterial groups to leucine incorporation versus community structure. BET42a: *Betaproteobacteria*; HGC69a: *Actinobacteria*. The diagonal line represents a 1:1 relationship. MAR: microautoradiography

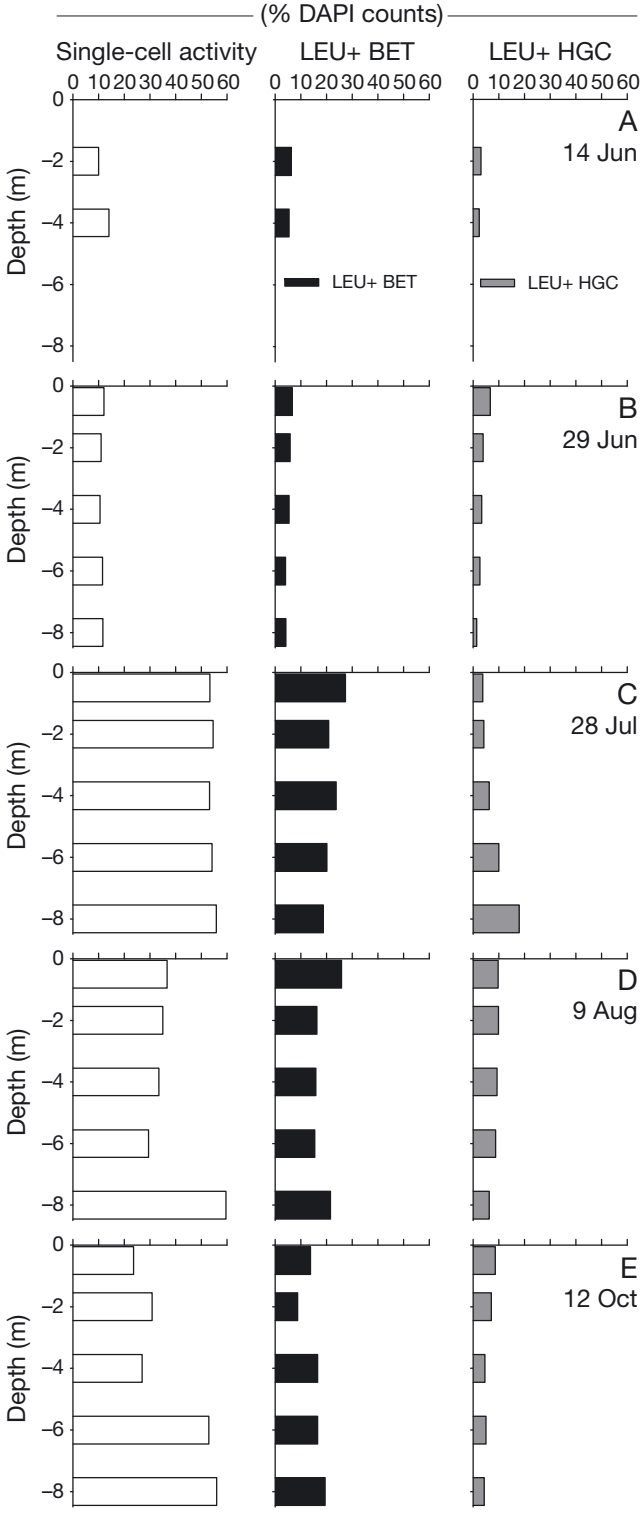

Fig. 4. Vertical distribution of the single-cell activity (total leucine-positive bacteria), and leucine-positive *Betaproteobacteria* (LEU+ BET) and leucine-positive *Actinobacteria* (LEU+ HGC) in the Gossenköllesee (GKS) during the most relevant events of the seasonal cycle. On 14 June 2005, samples were taken only at 2 and 4 m depths. DAPI: 4′, 6-diamidino-2-phenylindole

The relative abundance of leucine-incorporating cells under ice was low (on average ~11% of DAPI counts), and most of them were *Betaproteobacteria* (Fig. 4A). Unlike bulk bacterial production rates, the relative abundance of leucine-positive cells did not increase after the spring overturn (Fig. 4B). In this vertical profile, *Actinobacteria* represented ~50% of the cells incorporating leucine at 0 m, but at any other depth the *Betaproteobacteria* dominated the assemblage of MAR+ cells (Fig. 4B). In July, leucine-incorporating cells represented ~50% of DAPI counts throughout the water column (Fig. 4C). The relative abundance of leucine-positive cells increased for both groups, but showed different trends. The contribution of *Actinobacteria* to leucine incorporation increased with depth, whereas that of the *Betaproteobacteria* slightly decreased. In August, the abundance of leucine-positive cells decreased at every depth, except at 8 m, where ~58% of DAPI-stained cells showed leucine incorporation (Fig. 4D). Leucine-positive *Betaproteobacteria* contributed on average to 17% of DAPI counts, except for depths of 0 and 8 m, where they represented >22% DAPI counts. Active *Actinobacteria* were less numerous, and their contribution to leucine incorporation decreased with depth. After the autumn overturn, activity decreased in the upper 4 m of the water column, but it was >40% of DAPI counts below this depth (Fig. 4E).

Relationships between the dominant bacterial groups and environmental variables

Betaproteobacteria, and particularly its R-BT subgroup (Table 1), showed significant positive relationships with bacterial activity parameters (bulk production and leucine-incorporating cells). Additionally, *Betaproteobacteria* were positively related to the TDP concentration, and the R-BT cluster to temperature. *Actinobacteria* were positively related only to the TDN concentration.

Table 1. Spearman rank correlation coefficients between the relative abundance (% DAPI counts) of *Alphaproteobacteria* (ALF), *Betaproteobacteria* (BET) and its R-BT subgroup (R-BT), *Actinobacteria* (HGC), and *Cytophaga*-like bacteria (CF), and different bacterial and environmental parameters. BP: bulk bacterial production; Activity: relative abundance of leucine-incorporating cells; Temp: water temperature; Chl *a*: chlorophyll *a*; DOC: dissolved organic carbon; TDN: total dissolved nitrogen; TDP: total dissolved phosphorus; ns: not significant. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$

	ALF	BET	R-BT	HGC	CF
BP	ns	0.37*	0.52**	ns	0.69***
Activity	ns	0.51**	0.65***	ns	ns
Temp	ns	ns	0.56***	ns	0.41*
Chl *a*	0.38*	ns	ns	ns	ns
DOC	0.48**	0.38*	ns	ns	ns
TDN	−0.66***	−0.86***	−0.56***	0.65***	ns
TDP	ns	0.42*	ns	ns	ns
Nitrate	−0.64***	−0.72***	−0.73***	ns	ns

DISCUSSION

Seasonal dynamics in bacterial community composition and single-cell activity

Our results show that the bacterial community of the alpine lake studied, Gossenköllesee (GKS), responds to the main physical events (melting of the ice cover, mixis, stratification) and biological events (maxima in bacterial production, development of the deep chlorophyll maximum) in the lake, with clear changes in their activity and structure.

The heterotrophic bacterial assemblage of the GKS and other alpine lakes in the area is often co-dominated by *Betaproteobacteria* and *Actinobacteria* (Glöckner et al. 2000, Warnecke et al. 2005, Pérez & Sommaruga 2006), which was also the case for our study. Although the predominance of *Betaproteobacteria* in freshwater systems has been described (Glöckner et al. 1999), recent evidence indicates that *Actinobacteria* is a prevailing group in numerous alpine lakes (Warnecke et al. 2005). Our study shows that both bacterial groups are subjected to marked seasonal changes and that their dominance alternates during the ice-free season.

In the present study, *Actinobacteria* was the most abundant group under the ice cover, in agreement with the report by Glöckner et al. (2000), but we also showed for the first time that this group profited most from the input of organic matter during ice-out. The significant positive relationship of *Actinobacteria* with TDN (but not with inorganic nitrogen forms), which reached its highest concentration under the ice and after the disappearance of the ice cover (Fig. 1D), suggests that these organisms were favored by the organic nitrogen compounds accumulated and produced in the ice cover. Recently, Hörtnagl et al. (2010) found that *Actinobacteria* in the neuston of the GKS were positively related to the TDN concentration in this layer, which acts as a collector for atmospheric deposition. The fact that the material present in the snow cover is mainly of microbial and atmospheric origin (Felip et al. 1999) suggests that *Actinobacteria* respond positively not only to soil-derived DOM (Pérez & Sommaruga 2006) or algal-derived DOM (Stepanauskas et al. 2003, Nelson 2009), but to atmospheric sources of DOM as well.

The relative abundance of *Betaproteobacteria* increased concomitantly with the concentrations of chl *a* and DOC and a peak in bacterial production. The high abundances of *Betaproteobacteria*, in both absolute and relative terms, from August until the autumn overturn, seem to be a recurrent seasonal pattern in the GKS which was also observed in the study of Pernthaler et al. (1998) and its accompanying work (Glöckner et al. 2000). However, our study shows, additionally, that the increase in the abundance of *Betaproteobacteria* was caused by a single cluster of the *Betaproteobacteria*, the R-BT subgroup. The abundance of this subgroup was highest at the same time as the bulk bacterial production reached its maximum. This finding supports our previous results that showed that the R-BT subgroup is the most active bacterial group under different experimental manipulations (Pérez & Sommaruga 2006, Pérez et al. 2010). In other freshwater systems, such as the meso-eutrophic Řimov Reservoir (Czech Republic), the R-BT subgroup is correlated to the fraction of cells with high nucleic acid content, which in turn, is tightly coupled to bacterial production (Šimek et al. 2005).

Water temperature was positively related to changes in the abundance of the R-BT cluster in the GKS. Members of this cluster are known to be fast-growing cells (Šimek et al. 2006), and because growth rate is usually positively affected by temperature, one hypothesis is that, during warmer periods, R-BT bacteria overgrow other members of the *Betaproteobacteria*. In a cold and oligotrophic lake such as the GKS, with a relatively short 'warm' season, this strategy might be advantageous. Although a positive relationship between phytoplankton dynamics (particularly cryptophytes) and the presence of the R-BT subgroup has been found in the Řimov Reservoir (Šimek et al. 2008), we could not find any significant relationship between this subgroup and the chl *a* concentration. However, our previous work showed that the R-BT subgroup was greatly stimulated by the addition of organic matter derived from algae (Pérez & Sommaruga 2006).

Our study shows a clear temporal and vertical separation of the dominant bacterial groups in the GKS, as suggested by Glöckner et al. (2000), that translates into a highly significant negative relationship ($r = -0.48$, $p < 0.01$). As described before, *Actinobacteria* dominated the community structure under the ice cover and immediately after ice-out, indicating a preference for low temperatures and presumably less labile DOM, whereas *Betaproteobacteria* were more successful during the summer stratification using the autochthonously produced DOM. Further, we observed not only a temporal, but also a vertical, separation of these 2 groups in summer. In July, for example, *Betaproteobacteria* were more abundant at the surface of the lake, whereas *Actinobacteria* dominated in the deeper zone. Most likely, temperature influenced the vertical segregation of these 2 groups during the summer stratification. Another non-exclusive explanation for this vertical pattern might be the vertical distribution of heterotrophic flagellates in the GKS. Wille et al. (1999) found higher abundances of heterotrophic flagellates at the bottom of the lake (8 m) during the summer than at the surface. This is in agreement with reports indicating that *Actinobacteria* are favored in the presence of grazers due to their small cell size (Pernthaler et al. 2001, Jezbera et al. 2005). In contrast, members of *Betaproteobacteria* are known to be very sensitive to predation (Pernthaler et al. 2001). In particular, members of the R-BT subgroup, although fast-growing, are also very vulnerable to bacterivory (Šimek et al. 2005).

Cytophaga-like bacteria were the third most abundant group during our study, and they had high relative abundances after the ice-break and the subsequent thermal mixing — in agreement with Battin et al. (2001), who related their abundance to inputs of allochthonous organic material in a glacial stream. In addition, their relative abundance was positively related to bacterial production, as found by others (Eiler & Bertilsson 2007), but not to the single-cell leucine incorporation (Table 1). This suggests that *Cytophaga*-like bacteria were favored by inputs of organic matter that drove both bacterial production bursts, but that they are not specialized in the uptake of monomers such as leucine (Cottrell & Kirchman 2000). These authors associated *Cytophaga*-like bacteria with the degradation and uptake of high-molecular-weight DOM.

Alphaproteobacteria, although not very abundant, were consistently present during the whole study period and were positively related to concentrations of chl *a* and DOC (Table 1). The increase in their relative abundance in our study, when chl *a* reached its highest value, suggests that limnetic *Alphaproteobacteria* might profit from actively growing phytoplankton as observed for their oceanic counterparts (Teira et al. 2008).

Contribution of the dominant bacterial groups to community structure and leucine incorporation

Although bacterial abundance was initially dominated by *Actinobacteria*, leucine incorporation at the single-cell level was driven mainly by *Betaproteobacteria* during the whole ice-free season. Independently from their relative abundance, *Betaproteobacteria* generally comprised the highest number of leucine-positive cells (Fig. 3), in agreement with the findings of Hornák et al. (2010). This was reflected by a highly

significant positive relationship between *Betaproteobacteria* and the relative abundance of leucine-incorporating cells, but not between the latter and *Actinobacteria*. We also observed a remarkable correlation ($r = 0.81$, $p < 0.001$) between the relative abundance of active *Betaproteobacteria* and that of the R-BT subgroup. Only few recent studies (Hornák et al. 2006, 2010, Salcher et al. 2010) have assessed the contribution of particular freshwater bacterial groups to bacterial production, but in agreement with a study in the Římov Reservoir (Hornák et al. 2006), *Betaproteobacteria* — and particularly their R-BT subgroup — were found to drive the activity of the bacterial community. Although freshwater *Actinobacteria* are known to be an active component of the bacterial community (Warnecke et al. 2005, Pérez & Sommaruga 2006), able to incorporate leucine in a wide range of freshwater systems (Buck et al. 2009, Hornák et al. 2010, Pérez et al. 2010), a positive relationship between this group and bacterial production has, to our knowledge, not been reported. The recent work of Salcher et al. (2010) showed that *Actinobacteria* in an oligomesotrophic lake have a preference for amino acids other than leucine, which could explain their proportionally low contribution to single-cell activity assessed with this amino acid. However, our preliminary data indicate that, at least in the late ice-free season (August and September), similar proportions of *Actinobacteria* incorporated leucine and an amino acid mixture in the GKS (M. T. Pérez unpubl. data).

Concluding remarks

Even at the large taxonomic resolution level used in this study, we detected changes in the dominance of the main bacterial groups that were associated with crucial biological and physical events in the lake. *Actinobacteria* dominated during the colder period under ice and immediately after the ice-out, profiting — together with the *Cytophaga*-like bacteria — from the input of allochthonous material accompanying the ice-out, whereas *Betaproteobacteria*, and particularly its R-BT subgroup, increased in importance during the summer stratification. Interestingly, the dominance of a certain group in terms of abundance did not imply that it was also responsible for driving the production of the bacterial assemblage, as shown for *Actinobacteria*. On the contrary, the contribution of *Betaproteobacteria* to total leucine-incorporating cells was always higher than expected by its relative abundance. This underlines the importance of assessing concomitantly both the structure and the single-cell activity of the bacterial community to understand the functioning of a particular ecosystem.

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