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Highly Active Microbial Communities in the Ice and Snow Cover of High Mountain Lakes

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An exploratory study carried out in Pyrenean and Alpine lakes shows that a rich, active microbial community lives in the slush layers of the winter cover of such lakes in spite of the low temperature and the seasonal occurrence of the habitat. Bacteria were very diverse in morphology, with filaments reaching up to 100 μm long; flagellates, both autotrophic (chrysophytes, cryptophytes, dinoflagellates, and volvocales) and heterotrophic, and ciliates were abundant, reaching biovolume values up to 2.7×10^6 μm^3 ml^{-1}. Species composition was very variable, with dominance depending on date and depth. Although many species were typical of lake plankton communities, some were restricted to the slush, for instance the predatory ciliates *Dileptus* sp. and *Lacrymaria* sp., and others were restricted to the surface pools, such as the snow algae *Chlamydomonas nivalis*. Microbial biomasses and usually bacterial and algal activities were greater in the slush layers than in the lake water. Photosynthesis rate in the upper cover layers reached values up to 0.5 μg of C $liter^{-1}$ h^{-1}, and high bacterial activities up to 226 pmol of leucine incorporated $liter^{-1}$ h^{-1} and 25 pmol of thymidine incorporated $liter^{-1}$ h^{-1} were measured. For most species, lake water flooding the ice and snow cover could provide an inoculum. Differential growth depending on the environmental conditions (nutrients, organic matter, light) of a particular slush layer could provide dominance of different groups or species. However, there was no obvious colonizing mechanism for those species not appearing either in plankton or in communities on top of the snowpack.

Temperature is one of the main factors determining the distribution and abundance of species because of its effects on enzymatic activities (2). Nevertheless, microorganisms have colonized snow, ice, and waters with temperatures at or below 0°C. Some algal species bloom at the snow surface during spring (14, 19), and complex microbial communities have been found in cryoconite holes on glaciers (38), but marine ice habitats are probably the most widely studied examples. Distinct microbial communities composed of psychrophilic microalgae, bacteria, and protozoa colonize and grow in melt pools on the ice surface, in brine channels within the ice, attached to the bottom of the ice, and in the sub-ice platelet layer (1, 10, 21, 25). Recent studies in the Antarctic pack ice indicate that microbial production and community development are likely even during winter at extremely low temperatures (11). Microbial communities attached onto or within the ice of freshwater lakes comparable to marine ice communities have not been described (22). Nevertheless, covers of freshwater lakes are a complex physical structure, especially in snowy regions, worthy of further exploration.

High mountain lakes are covered by ice and snow during several months of the year. From the cover formation to the complete melt, changes in the physical structure of the cover occur, driven by snow deposition, melting, refreezing, and the flooding of water that rises from the lake through ice cracks until the cover reaches a hydrostatic equilibrium (6). At the same time, there is a parallel evolution of the chemical composition of the different layers, which can either concentrate or dilute the original composition by partial melting or mixing materials of atmospheric, catchment, and lake origin. As a result, slush layers enriched in nutrients (soluble reactive phosphorus, ammonium and nitrate) are common (6, 16). Some of them are located at depths where enough light arrives for photosynthesis, especially at the end of the winter, when the specific absorbance of snow decreases with thawing. In addition, flood water and atmospheric deposition, predominantly in spring when meadows and forests at lower altitudes flourish, deliver organic matter and nutrients to the cover. All these facts suggest that the ice and snow cover of lakes can sustain a particular microbial community, although the low temperatures may prevent them from reaching measurable activities. Some indirect evidence already exists. Jones and Ouellet (17) measured ATP concentration in slush layers and suggested that some bacteria or algae transported from the lake to the slush cover could survive for a short time. Catalan (6) found a C:N ratio of the particulate matter and an accumulation of nitrite indicative of bacterial activity in slush layers.

In this paper, we present an exploratory study, carried out in some high mountain lakes of the Pyrenees and Alps, which describes the presence of abundant, active, and very diverse microbial communities in the slush layers of the winter cover of those lakes.

MATERIALS AND METHODS

Study sites and sampling. The study was conducted in three high altitude lakes: Lake Redó in the Pyrenees and Schwarzsee ob Sölden and Gossenköllesee in the Tyrolean Alps. All are highly oligotrophic, but they present different morphology and chemical composition. Lake Redó and Gossenköllesee are weakly buffered, while Schwarzsee ob Sölden is acidic (Tables 1 and 2). They are covered by ice and snow for at least half the year; the thickness of the ice cover reaches several meters during its maximum in April.

Sampling was done in the central area of each lake during the period of

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TABLE 1. Morphological and chemical parameters of Lake Redó (LR) (Pyrenees) and Schwarzsee ob Sölden (SOS) and Gossenköllesee (GKS) (Tyrolean Alps)[a]

Morphology or chemistry	Result for lake		
	LR	SOS	GKS
Morphological parameters			
Altitude (m)	2,240	2,799	2,415
Geographical position	42°N 38°E	46°N 10°E	47°N 11°E
Geology	Granodiorite	Crystalline	Crystalline
Area (ha)	24	3.5	1.7
Maximum depth (m)	73	18.6	9.9
Length (m)	655	400	203
Mean depth (m)	32	10	4.6
Width (m)	565	164	120
Lake water chemistry			
pH	6.6	5.5	6.8
Conductivity (μS/cm)	10	10	18
Alkalinity (μeq/liter)	34	2	92
NO_3^- (μeq/liter)	14	12	20
SO_4^{2-} (μeq/liter)	32	40	61
Cl^- (μeq/liter)	8	12	7
NH_4^+ (μeq/liter)	3	2	1
Ca^{2+} (μeq/liter)	63	46	130
Mg^{2+} (μeq/liter)	6	14	19
Na^+ (μeq/liter)	12	4	11
K^+ (μeq/liter)	3	2	8
Total phosphorus (μg/liter)	2	3	3

[a] Chemistry data from Catalan and Camarero (8) (LR) and Wögrath and Psenner (42) (GKS, SOS).

maximum cover thickness: 1992 and 1994 in Lake Redó and 1994 in the two Tyrolean lakes. In Lake Redó, the water phase of the slush layers, the surface lake water (0.5 m beneath the ice), and the small pools formed on the cover during spring were sampled. In the Tyrolean lakes, the interstitial water of the cover and occasionally the lake water column were sampled. Water from the slush layer was pumped from small holes drilled in the cover, as shown in Fig. 1. Progressively deeper slush layers were consecutively sampled.

Microbial abundance and biomass. Bacterial numbers were determined by epifluorescence microscopy using DAPI staining on black Nuclepore filters (pore size, 0.2 μm) by the method of Porter and Feig (28). Bacterial biomass, size, and shape were determined by image analysis in a Zeiss Axioplan microscope with a UV-light lamp, with an interference filter (from 450 to 490 nm) connected to a high-sensitivity video camera (Hamamatsu C2400-08). The software LUCIA (Laboratory Imaging, Prague, Czech Republic) was used in combination with a real-time image processor (MATROX MVP-AT, Dorval, Quebec, Canada). Digitized images taken at ×2,000 magnification were resolved with pixels (512 by 512) with 8 bits of memory for every pixel. Background subtraction was used to minimize variability associated with nonuniformity in illumination and electronic noise. The digital image was filtered with a nonlinear filter (General Filtration). Measurement parameters included area, perimeter, elongation, circularity, length, and width. Conversion to cell volume was calculated by applying the relationship $V = (w^2 \times \pi/4) \times (l - w) + (\pi \times w^3/6)$, where V is volume (in cubic micrometers) and w and l are cell width and length (in micrometers), respectively. To transform biovolume into carbon biomass, the following allometric equation was used (24): $C = 0.12 \times V^{0.72}$, where C is carbon content (picograms of C per cell).

TABLE 2. Precipitation chemistry[a]

Chemical	Precipitation (μeq/liter) at site	
	Pyrenees	Alps
NO_3^-	20	22
SO_4^{2-}	50	38
Cl^-	19	9
NH_4^+	22	27
Ca^{2+}	89	30
Mg^{2+}	7	6
Na^+	23	11
K^+	9	4

[a] Data are from Camarero et al. (5).

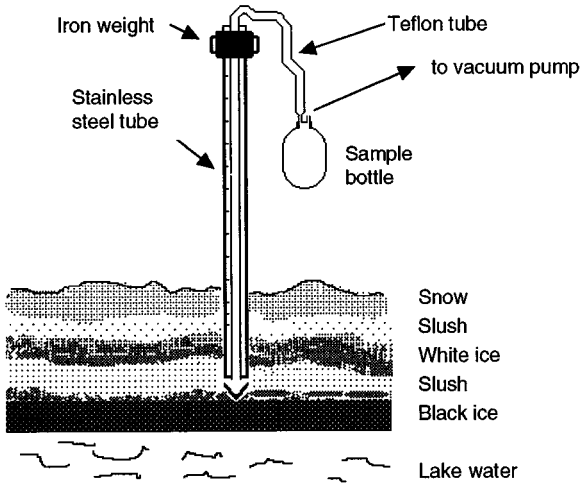

FIG. 1. Equipment for sucking slush water from winter cover. White ice originates from slush freezing, while black ice comes from lake water.

Abundances of algae, heterotrophic flagellates, and ciliates were estimated with the Utermöhl method after fixation with Lugol's solution (34). Biovolume was estimated by shape assimilation to known geometric forms and direct measurement of the main cell dimensions. Absorbance spectra of photosynthetic pigment extracts in 90% acetone were used for chlorophyll (Chl) determinations (15). The A_{430}/A_{410} ratio was used as a phaeopigment indicator (23). The relation between the molar ratio of Chl*a* and total Chl*a*-related pigments (Chl + phaeophorbide + phaeophytin) and the A_{430}/A_{410} index was calibrated for Lake Redó by Catalan (7) [Chl*a*/tChl*a* = $(A_{430}/A_{410} - 0.62)/0.483$]. The A_{480}/A_{665} ratio was used as an indicator of relative abundance of carotenoids (35).

Microbial activity. Photosynthetic activity was determined by incubations in situ with radioactively labeled carbon (31). Immediately after sampling, 500 ml of sample was inoculated with 5 ml of 10 μCi of $NaH^{14}CO_3$ ml^{-1} and vigorously stirred. Then, 100 μl of sample was stored in radioassay vials containing 0.5 ml of β-phenyl-ethyl-amine (Scharlau), in order to determine the amount of radioactivity added. The rest of the sample was dispensed into 130-ml Pyrex bottles under dim light. At each sampling level, two clear bottles and one dark bottle were incubated for 2 to 3 h. After incubation, cells were collected onto a Whatman GF/F filter, which was dried overnight and then exposed for 3 min to HCl fumes and stored in a radioassay vial. ^{14}C incorporation was measured in a Packard Tri-Carb 1500 liquid scintillation counter after the addition of 5 ml of Biogreen 1 (Scharlau) scintillation cocktail.

Bacterial activity was estimated in situ by using radiolabeled thymidine and/or leucine incorporation rates. In Lake Redó, incorporation of [^3H]thymidine into material insoluble in cold trichloroacetic acid (TCA) (4, 9) was measured in the spring of 1992. One control and three replicate samples (10 ml) were incubated with 18 nM of [^3H]thymidine (47 Ci $mmol^{-1}$) for 120 to 150 min. Incorporation was stopped by adding formaldehyde (2% final concentration). Then, 10 ml of ice-cold 10% TCA was added. After 20 min, the mixture was filtered through 0.2-μm-pore-size Whatman cellulose nitrate filters, previously wetted and washed in ice-cold 5% TCA. Then the filters were rinsed twice in ice-cold 10% TCA. Subsequently, the filters were placed in a scintillation vial and dissolved with 1 ml of ethylacetate (30 min); 10 ml of Biogreen 1 scintillation cocktail was added, and after 6 h the samples were radioassayed on a liquid scintillation counter (Packard Tri-Carb 1500). [^3H]leucine incorporation into hot TCA precipitates was measured in 1994 samples, according to the method described by Kirchman (18), slightly modified. One control and three replicate samples (15 ml) were incubated with 25.5 nM of [^3H]leucine (60 Ci $mmol^{-1}$) for 120 to 150 min. For extraction, 2 ml of 50% TCA was added, and the mixture was then heated in a water bath at 85°C for 30 min and left at room temperature for 30 min. Samples were filtered through 0.2-μm-pore-size Whatman cellulose nitrate membrane filters presoaked in cold leucine; sample tubes were then rinsed in 5% ice-cold TCA, and the rinse was poured through the filter. Filters were subsequently rinsed twice in 5% TCA, once in cold 80% ethanol, and once in distilled water. Radioassay was as for thymidine. In Schwarzsee and Gossenköllesee bacterial activity was estimated by dual labeling with [^3H]thymidine and [^{14}C]leucine incorporation in cold TCA-insoluble material (a modification of the method described in reference 32). [^3H]thymidine (70 to 90 Ci $mmol^{-1}$) at a final concentration of 10 nM and [^{14}C]leucine (310 mCi $mmol^{-1}$) at a final concentration of 40 nM were added to three replicates and one control sample (20 ml). Samples were incubated at lake temperature for 24 h, and subsequently bacteria

were killed with formaldehyde. Cellulose nitrate filters (0.2-μm pore size; Sartorius) were rinsed first with 5 ml of 5% ice-cold TCA and then with ice-cold distilled water to reduce the background. Samples were tipped onto the filter with 5 ml of 5% ice-cold TCA for the extraction, and 5 min later samples were filtered. Filters were rinsed twice with 5 ml of 5% ice-cold TCA; they were left to dry and placed in scintillation vials. Scintillation cocktail (15 ml) (Packard High Flash Emulsifier) was added just before counting. Radioassay was done by Beckman LS 6000 IC.

The active fraction of bacterioplankton was determined by INT (2-*p*-iodophenyl-3-*p*-nitrophenyl-5-phenyltetrazoliumchloride) staining (43).

RESULTS

Microbial community composition. Bacterial communities in the slush layers of the winter cover were morphologically diverse in all lakes. Lake water bacteria occurred as small free-living cells, mostly short rods and cocci (ca. 0.05 μm^3). However, in the slush layers, together with these small forms, long, thin filaments, sometimes longer than 100 μm, dominated bacterial biomass (Fig. 2a). Branched long filaments and stalked bacteria were not uncommon, and in a few cases, *Ancalomicrobium*-like morphologies were found (Fig. 2b).

Eucaryote communities were also highly diverse in most samples and showed a large variability between layers, periods, and lakes. This is illustrated in Fig. 3, where an ordination of the samples from Lake Redó is plotted according to their scores in the first two axes of variability of the species composition. The ordination was carried out by a detrended correspondence analysis (13) of the species biovolumes. The main axis of variability differentiates samples from 1992 and 1994, since some species appeared or were highly abundant in only one year. For instance, *Cryptomonas marsonii*, an *Ochromonas* sp., *Dictyosphaerium subsolitarium*, *Chrysolykos skujae*, and several ciliates were characteristic of 1992 samples, while *Pseudoquadrigula*, *Oocystis parva*, *O. borgei*, a *Monoraphidium* sp., a *Gymnodinium* sp., a *Chromulina* sp., *Sphaerocystis schroederi*, and some heterotrophic flagellates appeared exclusively in 1994. These interannual differences in the composition of organisms in the slush layers reflect the planktonic composition of the lake water flooding the snow cover.

More interesting is the second axis of variability, because it clearly separates lake water samples from cover samples (Fig. 3). Main differences between lake water and slush layers are due to ciliate species. Ciliates typical for lake water were *Strombidium* spp., a *Holophrya* sp., and *Askenasia acrostomia*, whereas slush layers were characterized by nondetermined species of heterotric ciliates and some clearly nonplanktonic species of genera such as *Urosoma*, *Lacrymaria*, and *Dileptus.*

The only autotrophic species exclusive to the cover were those found in surface pools (*Chlamydomonas nivalis*, a *Chlamydomonas* sp., a *Chromulina* sp.). Nonflagellate species seldom appeared in the slush layers where different *Chlamydomonas species*, and some autotrophic species which also show phagotrophy such as *Dinobryon cylindricum*, *Cryptomonas* spp., *Gymnodinium* spp., and *Chromulina* spp., grew in higher concentrations than in lake water. At the end of the winter season, the upper slush layers, and especially surface pools, were rich in allochthonous material, such as pollen, conidia, leaves and stem debris, and amorphous organic material.

Biomass. No significant differences in bacterial biomass were found between the diverse slush layers and lake water samples in Lake Redó, except that in the deeper slush layer from 7 June 1994, a significantly higher value was measured (Table 3). However, bacterial biomass is the product of two terms, cell size and cell abundance, and both changed significantly and in opposite ways among samples. At the beginning of May, in both 1992 and 1994 surveys, size distribution showed a stratigraphical tendency: the shallower the slush layers, the larger the bacterial cells (Fig. 4A and B). In later samples, less heterogeneous cell size and cell abundance vertical distribution were observed. Large filaments provided a very particular size distribution in Gossenköllesee (Fig. 4C), and in that case the mean cell value has little sense.

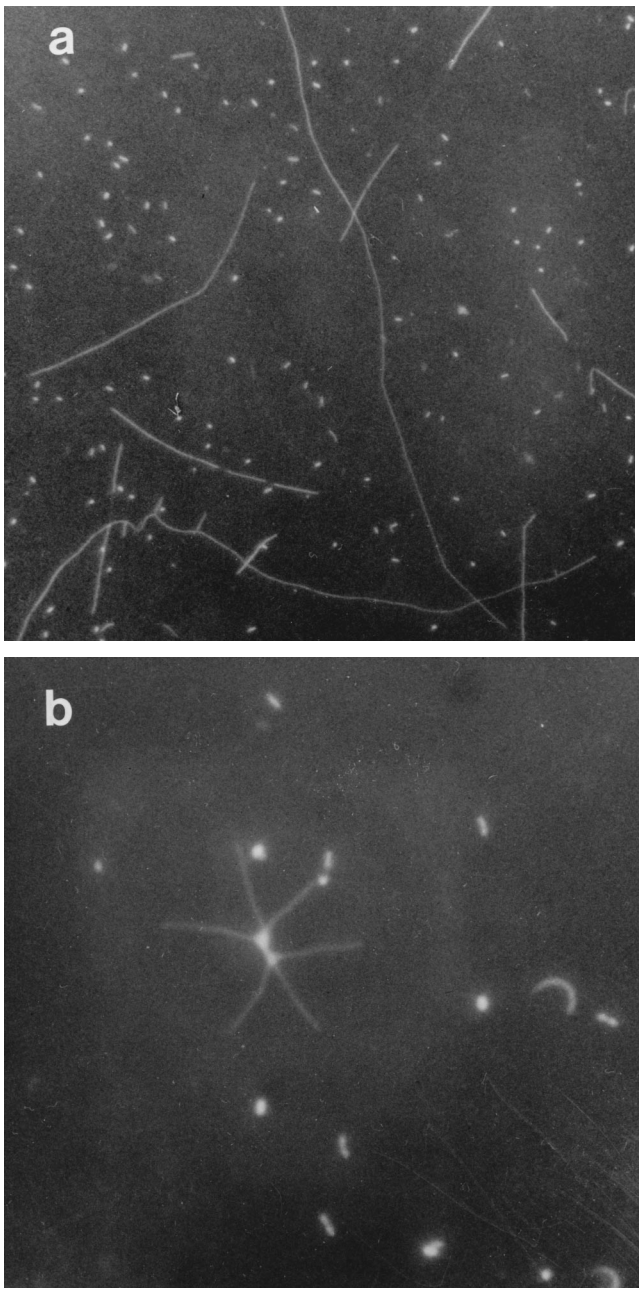

FIG. 2. Bacteria in slush layers of Gossenköllesee (26 April 1994). Epifluorescence microscopy with DAPI staining. (a) Single filaments (magnification, ×500); (b) star-shaped filaments (magnification, ×1,600).

Algal and protozoan biomasses in slush layers were always higher than in lake water (Fig. 5). However, although the distribution of abundance of the different groups in the cover did not show a constant stratigraphical pattern, it always clearly differed from that of lake water. In the Lake Redó 1992 survey, there was an increment in the relative importance of colorless nanoflagellate and ciliate biomasses in the slush communities

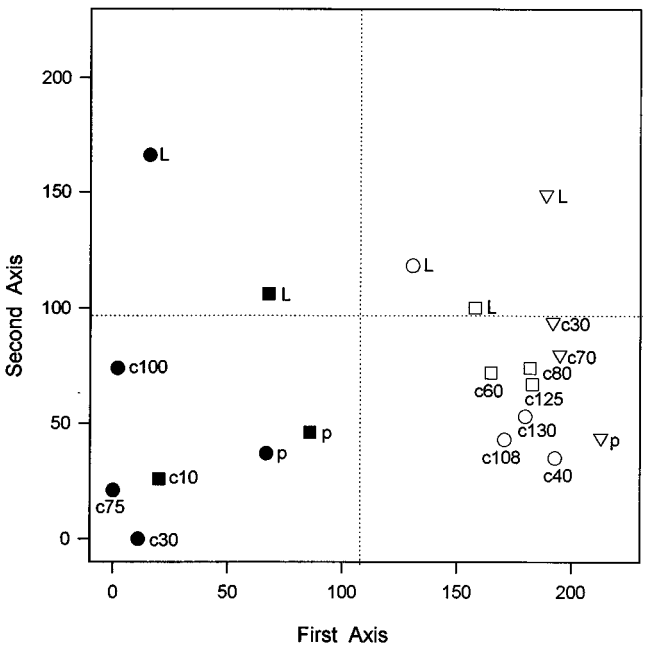

FIG. 3. Plot of the standardized scores of Lake Redó samples in the two main axes generated by detrented correspondence analysis of the biovolumes of flagellate, algal, and ciliate species (37). First axis explains 23% of species variability and is characterized by positive loads of several chlorococcal chlorophytes (*Oocystis*, *Pseudoquadrigula*, *Monoraphydium*, and *Sphaerocystis* spp., etc.) and negative loads of diverse ciliate species (*Askenasia* sp., *Holophrya* sp., and others), *Cryptomonas marsonii*, and *Ochromonas* sp. Second axis explains 9% of species variability and is characterized by positive loads of *Mallomonas* sp., *Ankistrodesmus* sp., *Peridinium* sp., and some heterotrophic flagellates and negative loads of some heterotric ciliates, cyst forms of *Askenasia* sp., carnivorous ciliates such as *Lacrymaria* and *Dileptus* sp., and several species of *Chlamydomonas*. Many species had an intermediate load on both axes. First axis mainly reflects interannual variability in species composition, while second axis shows species differentiating cover communities from lake water ones. Dark symbols correspond to 1992 samples (●, 12 May; ■, 26 May); light symbols represent 1994 samples (○, 14 May; □, 25 May; ▽, 7 June). c, cover sample at the corresponding slush layer depth (in centimeters); L, lake water; p, pool.

TABLE 3. Lake Redó bacterial biovolume, biomass, average cell volume, and average cell carbon content determined by image analysis

Date and slush layer depth (cm)	Biovolume (mm^3/liter)	Biomass (μg of C/liter)	Abundance (10^6 cells/liter)	Cell vol (μm^3)	C content (fg/cell)
12 May 1992					
30	0.033	4.54	73	0.456	62
75	0.031	4.65	110	0.294	44
100	0.032	5.39	200	0.155	27
Lake water	0.043	7.92	380	0.115	21
26 May 1992					
10	0.083	10.99	250	0.338	45
Lake water	0.075	10.27	270	0.281	39
14 May 1994					
40	0.015	1.91	30	0.498	64
130	0.013	1.99	41	0.313	48
Lake water	0.011	2.55	170	0.068	15
25 May 1994					
80	0.027	5.08	350	0.077	14
125	0.028	5.75	380	0.073	15
Lake water	0.019	4.56	350	0.055	13
7 June 1994					
30	0.046	7.97	360	0.127	22
70	0.146	24.33	640	0.229	38
Lake water	0.019	4.44	310	0.061	14

compared to those of lake water, which was also reflected by lower photosynthetic pigment levels (Table 4). A higher percentage of degraded Chl*a* in the slush layers than in lake water indicates either a certain decay of algal populations or, less likely, a higher grazing pressure. In the Lake Redó 1994 survey, enrichments by cryptophytes, dinoflagellates, or chrysophytes were found, with different dominance of one of these groups in each sample (Fig. 5). The only clear stratigraphical tendency in both surveys was an increment in abundance and dominance of volvocales (flagellated chlorophytes) in shallower slush layers, which reached a maximum on surface pools. On some occasions these pools had a blood-red color because of the high accumulation of carotenoids by some *Chlamydomonas* species (Table 4).

Activities. Regarding bacterial activity, we present [^{3}H]thymidine and [^{3}H]leucine incorporation rates instead of cell production in order to avoid confusion arising from the use of different conversion factors (3). Most samples from slush layers showed very high activities, whereas significantly lower activities were found in lake water (Table 5). The percentage of active bacteria was also usually much higher in the cover than in the lake water (Table 5). In Lake Redó neither bacterial size nor bacterial biomass was correlated with higher activities (Tables 3 and 5). At the end of the cover period, when melting processes become important, no significant differences between cover and lake water bacterial activities were found. In Schwarzsee and Gossenköllesee, vertical production profiles changed over time and usually showed a clear bacterial activity increase in deeper slush layers correlated with bacterial number and frequency of active bacteria. The latter reaches high values, up to 40%.

Measurements of autotrophic carbon fixation during the Lake Redó 1992 survey showed significant activities throughout the cover (Table 4). Photosynthesis rate decreased from shallower to deeper slush layers as could be expected, but even in the deepest layer (1 m below surface) the activity was much higher than in the lake. Only the surface pool showed lower cell carbon fixation than other slush layers.

DISCUSSION

In winter, slush layers with a high water content are common in the ice and snow cover of high mountain lakes (6). This exploratory study carried out in Pyrenean and Alpine lakes shows that a very diverse microbial community, composed of bacteria, different taxa of autotrophic and heterotrophic flagellates, and ciliates, can be found in these cold microhabitats. Microbial biomass and heterotrophic and, occasionally, photosynthetic activities are higher than in lake water. Although many species of the slush layers also occur in lake plankton, some species are characteristic of the former habitat, especially ciliates. A number of algae known to grow on snow beds, especially in late spring, appear in slush layers down to a depth of 30 cm and in surface pools.

Bacteria in the slush layers are morphologically variable, including filaments and branched forms, and their cell sizes (0.07 to 0.45 μm^3 on average in Lake Redó, higher in Tyrolean lakes) are larger than in the lake water and generally much larger than freshwater bacterioplankton (0.083 and 0.185 μm^3 [39]) but equivalent to cell volumes reported from Arctic sea ice (0.47 μm^3 [33]). Sullivan and Palmisano (36) described similar, morphologically diverse bacterial communities in sea ice. Bacterial activity is also generally higher than in sea ice (12, 20) and marine and freshwater bacterioplankton from oligo-

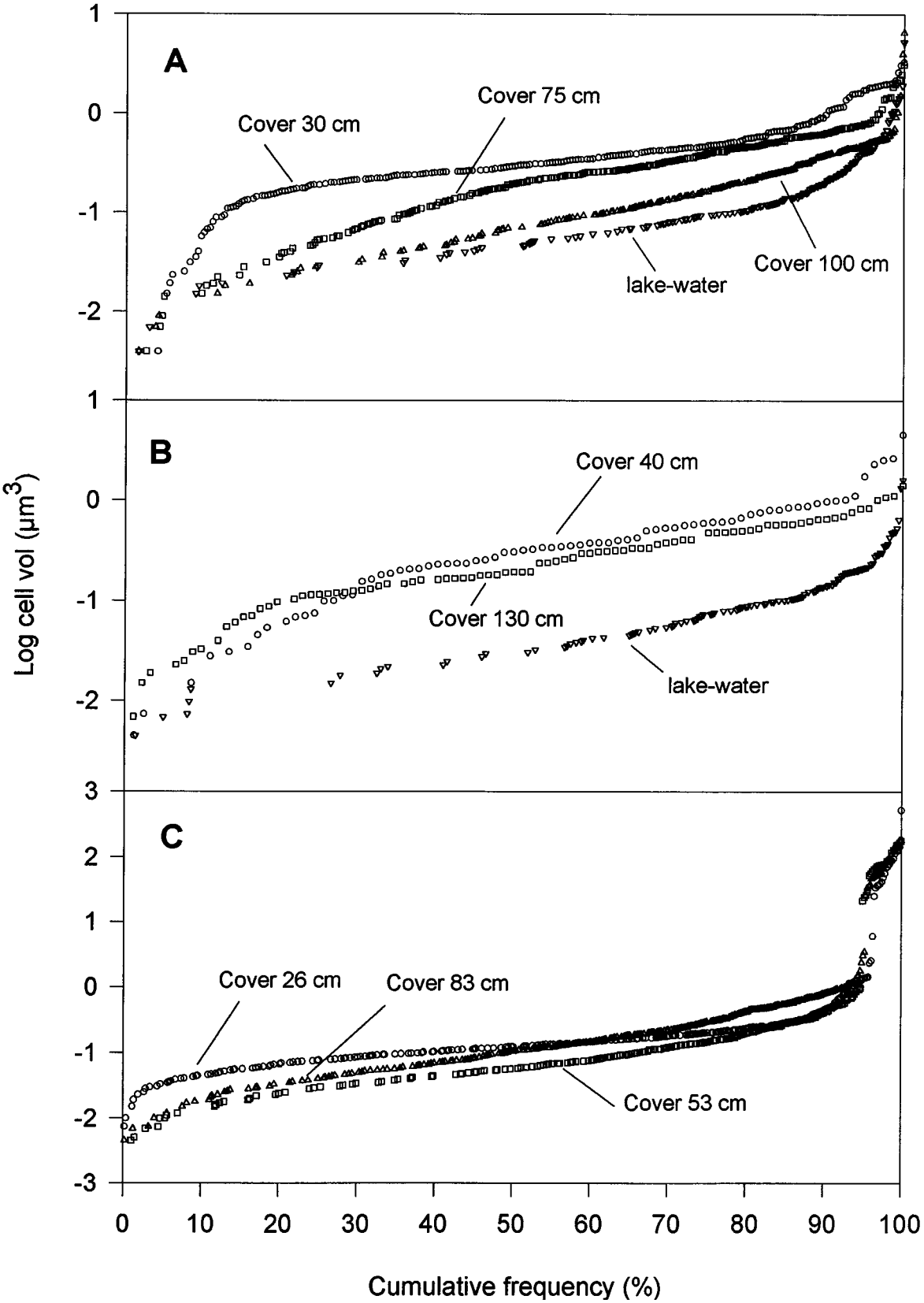

FIG. 4. Bacterial cell size distributions (cumulative frequency) from Lake Redó (LR) and Gossenköllesee (GKS). (A) LR, 12 May 1992; (B) LR, 14 May 1994; (C) GKS, 26 April 1994 (note the large bacteria found in this survey).

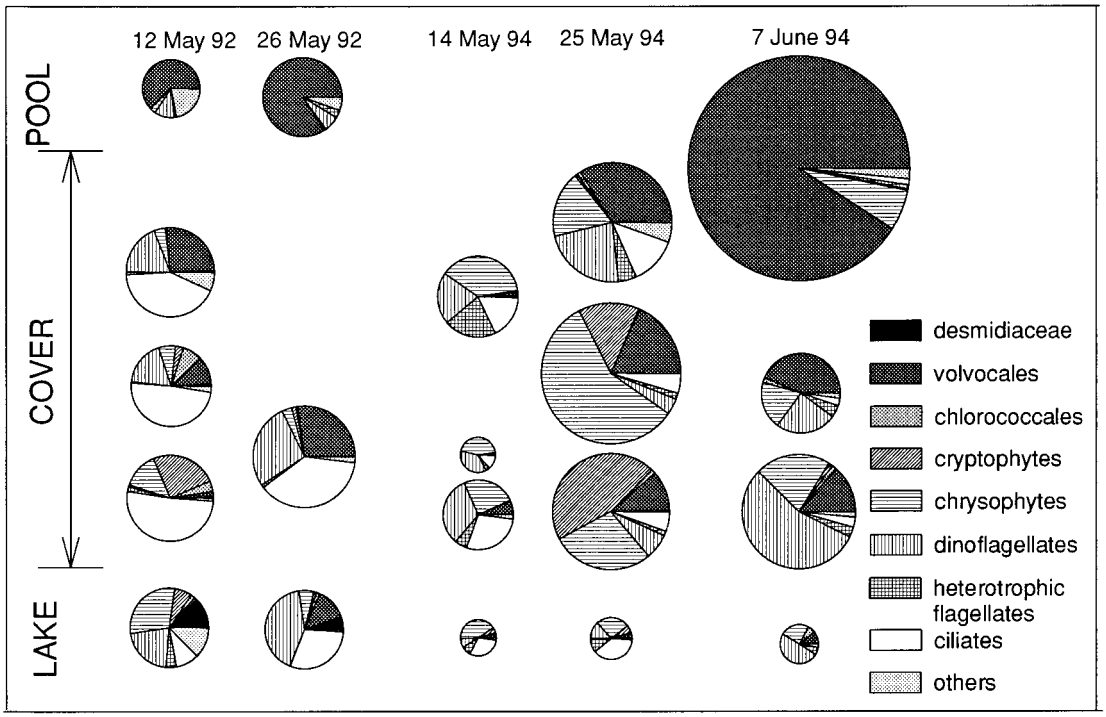

FIG. 5. Biovolume distribution of microbial groups in the different samples of pool, slush, and lake water from Lake Redó. Diameters of the circles are proportional to the square root of the total community biovolume; the pool sample from 7 June 1994 is reduced to 50% of the corresponding size.

trophic systems (26, 30). In some cases, percentages of active bacteria are extremely high, up to 40%. It has been shown by Pomeroy et al. (27) and Wiebe et al. (40, 41) that the lower the temperature, the larger the amount of substrate required to reach a given activity. Therefore the high activities found in this study, although possibly episodic, raise the questions of substrate availability and origin.

In slush layers organic matter can originate from allochthonous and autochthonous sources. Two different external sources can contribute to nutrient enrichment. (i) Water from the lake flooding the snow cover brings dissolved organic matter and organisms. Some of them will decay in the slush, as shown by the increase in the proportion of degraded chlorophyll, and in the fate of some groups such as nonflagellate chlorophytes (chlorococcales) and desmidiaceae. (ii) There is a significant deposition of particulate matter on the snow surface, especially during spring, in the form of pollen, vegetation debris, and insects. The chemical composition of snow and rain reflects the higher contribution of alkaline dust in the Pyrenees (Table 2), but it represents only a small fraction of total atmospheric input. On the other hand, our measurements of photosynthetic fixation of carbon show that in situ production of organic matter can, in some cases, be a sufficient source of carbon, especially in slush layers closer to the snow surface. Therefore, the whole spectrum from completely heterotrophic assemblages to communities subsisting by their own primary production can probably be found. The nature of slush layers, a matrix of ice grains with nutrient-rich interstitial water, provides a better environment for bacterial activity than lake water; interaction between cells and substrate and development of filamentous forms are facilitated.

TABLE 4. Lake Redó, 1992 survey: pigment content and photosynthesis rates in surface pool, slush layers, and lake water

Site or slush layer depth (cm)	Chl (μg/liter)		Nonaltered Chl*a* (%)	A_{480}/A_{665}	Photosynthesis rate (μg of C/liter/h)
	a	Total			
12 May					
30	0.40	0.81	58	1.62	0.405
75	0.34	0.83	47	2.55	0.353
100	0.39	1.06	45	1.32	0.277
Lake water	0.58	1.31	72	1.44	0.002
26 May					
Pool	0.27	1.31	52	6.28	0.06
10	0.40	1.00	64	2.99	0.513
Lake water	0.22	0.62	81	2.01	0.115

Flagellates and ciliates differ notably from lake to lake, from year to year, and among slush layers, even between those a few centimeters apart. Layers dominated by cryptophytes, chrysophytes, dinoflagellates, ciliates, or bacteria have been found. Most algal species identified in the slush layers correspond to organisms also found in the lake, if *Chlamydomonas* species peculiar to snow habitats are excluded. Probably, lake water flooding the cover provides an inoculum of species and then differential growth starts, depending on the particular conditions of the layer in which the species are trapped. It may be symptomatic that algal species which are found dominating the layers (*Cryptomonas marsonii*, a *Gymnodinium* sp., *Dinobryon cylindricum*, a *Chromulina* sp.) show facultative bacterial feeding (29); nevertheless, our in situ measurements of photosynthesis show that, at least episodically, autotrophic growth can be significant.

Ciliate communities in slush layers show many species that are not present in the lake plankton community, in contrast to algae. Some of them are predaceous gymnostomes (*Dileptus* sp., *Lacrymaria* sp.), which point to the complex trophic rela-

TABLE 5. Summary of all study sites[a]

Site, date, and slush layer depth (cm)	Thy (pmol/liter/h)	Leu (pmol/liter/h)	Bacteria (10^6 cells/liter)	FAB (%)
Lake Redó				
12 May 1992				
30	4.65		73	
75	25.41		110	
100	22.94		200	
Lake water	5.63		380	
26 May 1992				
Pool	8.15		260	
10	17.03		250	
Lake water	14.89		270	
25 May 1994				
60		3.3	270	
80		1.3	350	
125		3.1	380	
Lake water		2.5	350	
07 June 1994				
Pool		28.2	480	
30		3.5	360	
70		6.0	640	
Lake water		3.4	310	
Schwarzee ob Sölden				
20 April 1994				
21	0.07	3.2	520	7
45	0.14	3.4	780	14
87	0.21	4.9	820	20
135	0.53	30.0	1,030	35
11 May 1994				
30	0.01	1.3	460	9
55	0.63	51.4	530	38
95	0.57	41.7	700	33
125	0.80	46.8	950	40
Lake water	0.30	4.9	200	8
Water column	0.30	2.8	220	5
31 May 1994				
42	0.41	55.5	600	16
53	0.14	26.7	670	11
62	0.87	129.4	1,400	30
86	2.05	211.5	1,600	42
Lake water	1.79	226.2	160	4
Water column	0.52	36.0	140	6
07 July 1994				
Pool	0.06	16.5	210	6
12	0.11	11.45	260	14
25	0.16	13.1	320	20
32	0.22	25.7	500	24
Gossenköllesee				
26 April 1994				
65	0.07	4.1	270	9
105	0.24	16.4	410	11
128	0.53	36.7	480	27
150	1.16	54.3	590	35
Lake water	0.09	6.6	300	22
Water column	0.08	5.1	420	19
07 May 1994				
36	0.07	9.0	310	12
52	0.09	6.4	360	18
78	0.17	14.5	590	29
135	0.32	2.5	630	38
15 June 1994				
44	0.09	3.6	610	15
89	0.06	3.5	580	8
200	0.01	4.8	210	6

[a] Shown are bacterial production by [^{3}H]thymidine (Thy) and [^{3}H]leucine or [^{14}C]leucine (Leu) incorporation, bacterial abundance, and frequency of active bacteria (FAB). Lake water samples are from a 0.5-m depth; values for the water column are calculated as weighted averages of samples at different depths.

tionships that may be present in these microbial communities. The mechanism by which the slush layers are colonized by those species absent in the lake plankton is a question of interest, because this habitat is ephemeral and apparently not easily colonizable except from plankton or the atmosphere.

Since the proportion of slush is higher in shallow lakes, processes in the winter cover can greatly influence the cycling of elements in the whole system.

In conclusion, this study provides evidence of a new, highly diverse, active microbial community at low temperature, which, given the seasonal restriction and highly dynamic environment, merits further investigation in terms of colonization mechanisms, growth resources, factors conditioning species assemblages, and succession trends.

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