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# Relationship between sediment organic matter, bacteria composition, and the ecosystem metabolism of alpine streams

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*Abstract*

We tested whether sediment bacteria abundance (4′,6-diamidino-2-phenylindole–stained cell counts) were related to sediment organic content (ash-free dry mass [AFDM]) in 11 nonforested streams of three different Alpine catchments during summer 2003. We used terminal restriction fragment–length polymorphism (T-RLFP, a molecular genetic technique) to test for seasonal and spatial differences in bacterial composition in these same streams. We then related the above parameters, in conjunction with periphyton biomass and hyporheic respiration, to whole stream estimates of gross primary production (GPP) and ecosystem respiration (ER) in a glacial and nonglacial stream, representing environmental extremes, in one of the catchments. The percentage of organic matter of sediments was 4–14% (0.01–0.04 g AFDM ml sediment$^{-1}$), and counts of bacteria cells per millimeter of sediment averaged $2 \times 10^6$–$4 \times 10^6$. Bacteria counts correlated with sediment AFDM only for streams in the catchment with highest sediment AFDM levels. Bacteria composition (based on the presence and absence of terminal restriction fragments from T-RFLP analysis) changed seasonally in the different streams and differed between glacial- and groundwater-fed streams. In the one catchment, hyporheic respiration averaged 0.0004 and 0.0003 g $O_2$ h$^{-1}$ kg sediment$^{-1}$ and was positively correlated with AFDM ($r^2 = 0.23$). Ecosystem metabolism displayed a strong seasonality, with GPP averaging 4.5 and 8.4 and ER averaging 5.4 and 9.9 g $O_2$ m$^{-2}$ d$^{-1}$ for the two sites, respectively, thus indicating a predominance of heterotrophy (P : R $< 1$) in these high-elevation, open-canopied systems. Bacteria play a strong role in the trophic dynamics of alpine streams.

Most running-water ecosystems are considered to be net-heterotrophic (Benke et al. 1988), although autotrophic production supplies the metabolic fuel for many streams and rivers with open canopies, such as desert streams (Minshall 1978; Jones et al. 1995). Surprisingly, the trophic character of running waters above the tree line (i.e., alpine streams), although globally abundant and periodically accruing high levels of periphyton biomass, has yet to be determined (Zah and Uehlinger 2001). Autotrophic production in streams can supply substantial quantities of organic matter (OM) that is eventually decomposed by heterotrophic microbial assemblages associated with surface biofilms and hyporheic sediments (Bott and Kaplan 1985; Cole et al. 1988). As a consequence, the metabolic activities of the microbial assemblage, through high rates of respiration, can cause these ecosystems to be net heterotrophic (Grimm and Fisher 1984). Indeed, microbes such as bacteria play a crucial role in the trophic dynamics of temperate running waters (Edwards et al. 1990; Meyer and Edwards 1990), being major drivers of metabolic processes in hyporheic sediments (Naegeli and Uehlinger 1997) and a trophic link with secondary consumers (Hall and Meyer 1998).

Recent molecular developments (e.g., terminal restriction fragment–length polymorphism [T-RFLP] analysis of 16S rRNA) now provide ecologists with powerful tools for characterizing the composition and dynamics (spatial and temporal) of bacteria assemblages in relation to physical habitat properties (Liu et al. 1997; Tiedje et al. 1999; Osborn et al. 2000), with major implications toward better elucidation of ecosystem function (Lindström 2000; Kuske et al. 2002; Lindström and Bergström 2004). Relating the microbial composition to ecosystem function should enhance the mechanistic understanding and give new insight into the role of heterotrophs in ecosystem metabolism, in this study alpine streams. The primary objectives of our study were threefold. First, we tested whether the abundance of bacteria in sediment was related to the OM content of sediments in different alpine streams. Other studies of temperate forested streams have documented a strong correlation between bacteria abundance and OM (Bott and Kaplan 1985; Hall and Meyer 1998). Second, we tested whether the bacteria composition, using T-RFLP analysis, in the study streams differed spa-

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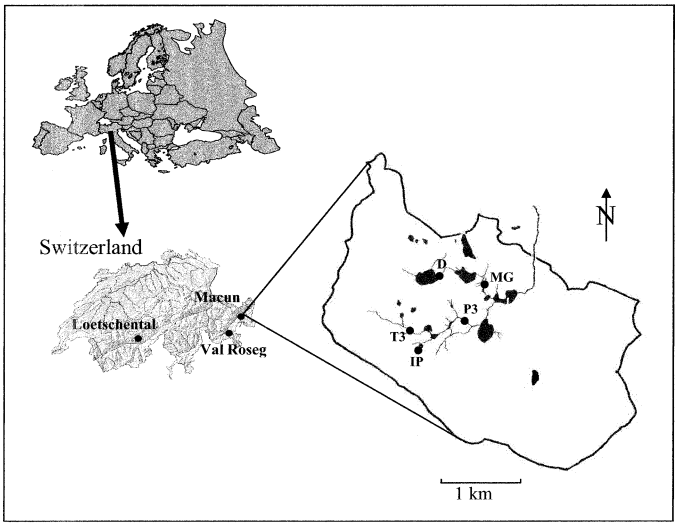


Fig. 1. Location of the Val Roseg, Loetschental, and Macun catchments within Switzerland. The enlargement shows the five streams sampled within the Macun Lakes area in the Swiss National Park. See Table 1 for site coordinates and characteristics of the study sites.

tially and seasonally. This objective examined the feasibility of using this relatively new molecular technique in lotic ecosystem assessment. Finally, we tested whether seasonal changes in ecosystem metabolism (gross primary production [GPP], ecosystem respiration [ER], and P : R) were related to changes in primary production, hyporheic respiration, bacteria assemblage structure, and sediment organic content in a glacial versus nonglacial alpine stream. We used a glacial and nonglacial system to represent the environmental extremes in the types of streams found in alpine regions (Ward 1994).

## Materials and methods

*Study sites and field sampling*—Eleven streams in three alpine catchments, Val Roseg, Loetschental, and Macun (Fig. 1), were included in the study for cross-catchment analyses of bacteria and sediment OM. The Val Roseg catchment is situated in the Bernina Massif in the upper Engadin, Graubünden, Switzerland. The catchment is 1,768–4,049 m a.s.l., with ~30% of its drainage water being of glacial origin (Tockner et al. 2002). Three groundwater-fed springs (G1, G2, and G3) were selected for study within the main floodplain area, which is 2,000–2,100 m a.s.l. (Table 1). The Loetschental catchment lies embedded between the Walliser and Berner Alps in the Canton Wallis, Switzerland. Here, three groundwater-fed streams (LT1, LT2, and LT3) were selected for study at altitudes between 1,900 and 2,000 m a.s.l. The main river of the Loetschental is glacial-fed, and the overall catchment is 50% glaciated (Robinson et al. 2001). Five study streams were selected in the Macun catchment, a high alpine cirque in the Canton Graubünden, Switzerland, on the basis of their water regime (S. Matthaei unpubl. data). Two sites (D and MG) were groundwater-fed streams, whereas sites T3 and IP were fed primarily by glacial meltwater from nearby rock glaciers. The remaining stream (P3) was intermediate, being influenced by glacial meltwater and, to a much smaller degree, by groundwater. All streams in the Macun catchment were at ~2,600 m a.s.l. (Table 1).

The ecosystem metabolism study was carried out in the Macun catchment at sites MG and P3 (Fig. 1). Added to the Swiss National Park in 2000, the 3.6 $km^2$ area is surrounded by mountains at altitudes of 2,800–3,100 m a.s.l. in the east, south, and west. Facing north, the mountains reach an elevation just above 2,600 m a.s.l. The area is composed of 26 lakes, in which the five larger ones are interconnected by streams. The stream network system of Macun is subject to high spatial and temporal variability. Decreasing discharges

Table 1. Average ($n$ = 3, SD in parentheses) physical and chemical characteristics of the study sites. The Val Roseg and Loetschental catchments were sampled in June and August 2003, and the Macun catchment was sampled in July, August, and Semptember 2003. Site IP was not sampled in September because the site went dry.

| Catchment and sites | Location East | Location North | Water source | Elevation (m a.s.l) | Temperature (°C) | Conductivity ($\mu$S $cm^{-1}$) | $NO_2$-N + $NO_3$-N (mg $L^{-1}$) | $PO_4$-P ($\mu$g $L^{-1}$) | DOC (mg $L^{-1}$) | POC (mg $L^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|
| Val Roseq | | | | | | | | | | |
| G1 | 9°51′50″ | 46°25′33″ | Groundwater | 2,031 | 4.2(0.8) | 78.5(23.1) | 0.36(0.07) | <5 | 0.91(0.11) | 0.14(0.07) |
| G2 | 9°51′44″ | 46°25′15″ | Groundwater | 2,040 | 6.4(0.9) | 56.6(21.6) | 0.15(0.01) | <5 | 0.89(0.02) | 0.11(0.05) |
| G3 | 9°51′26″ | 46°25′26″ | Groundwater | 2,022 | 9.8(0.7) | 28.4(0.9) | <0.1 | <5 | 0.90(0.20) | 0.23(0.10) |
| Loetschental | | | | | | | | | | |
| LT1 | 7°53′34″ | 46°26′35″ | Groundwater | 1,948 | 10.3(6.0) | 88.2(1.4) | 0.18(0.02) | <5 | 0.48(0.39) | 0.27(0.20) |
| LT2 | 7°53′43″ | 46°26′37″ | Groundwater | 1,970 | 15.7(5.8) | 85.6(1.5) | <0.1 | <5 | 0.89(0.41) | 0.21(0.71) |
| LT3 | 7°53′55″ | 46°26′43″ | Groundwater | 1,985 | 12.2(0.9) | 86.3(1.7) | 0.18(0.02) | <5 | 0.56(0.54) | 0.18(0.05) |
| Macun | | | | | | | | | | |
| D | 10°07′36″ | 46°43′45″ | Groundwater | 2,637 | 13.4(2.7) | 6.3(0.9) | <0.1 | <5 | 1.53(0.50) | 1.17(0.91) |
| MG | 10°07′51″ | 46°43′44″ | Groundwater | 2,623 | 14.2(4.0) | 5.9(0.3) | <0.1 | <5 | 1.72(0.60) | 1.52(0.73) |
| P3 | 10°07′45″ | 46°43′34″ | Glacial | 2,642 | 9.8(1.8) | 13.3(9.7) | 0.30(0.10) | <5 | 1.06(0.30) | 0.28(0.32) |
| T3 | 10°07′24″ | 46°43′32″ | Glacial | 2,675 | 8.7(0.9) | 11.9(6.5) | 0.40(0.09) | <5 | 1.00(0.50) | 0.17(0.11) |
| IP | 10°07′27″ | 46°43′27″ | Glacial | 2,675 | 2.8(3.1) | 24.6(20.7) | 0.48(0.12) | <5 | 0.99(0.30) | 0.34(0.32) |

(ceasing snowmelt runoff and reduced glacial meltwater) in early autumn results in ~60% contraction of the channel network (S. Matthaei unpubl. data). The outlet stream, Zeznina, drains north, flowing into the Inn River in the Engadin valley (1,412 m a.s.l.). The bedrock geology is granite/gneiss (crystalline) and concentrations of micronutrients ($Mg^{2+}$, $SO_4$, $Cl^-$, $K^+$, $Na^+$, $Ca^{2+}$, Cd, Pb, Fe, Mn, and Cu) in the streams were below analytical detection limits (S. Matthaei unpubl. data), and these levels are typical for local/regional streams flowing on crystalline bedrock.

Field work began in mid-June 2003 and was completed by the end of September 2003. The three catchments are relatively remote and are accessible only on foot. The Val Roseg and Loetschental were sampled on two dates in mid-June and mid-August. The Macun catchment was sampled for the various parameters on 22–25 July, 19–22 August, and 16–19 September. Site IP in Macun was not sampled in September because it was dry. On each visit to each study site, the water temperature and conductivity ($T_{ref}$ = 20°C) were measured in the field with a portable LF323 conductivity meter (WTW). In addition, a 1-liter water sample was collected on each date and site, filtered through preashed, preweighed glass-fiber filters (MILLIPORE) and returned to the laboratory for analyses of nitrate-N, phosphate-P ($PO_4$-P), and dissolved organic carbon (DOC), as described in Tockner et al. (2002). The filters were used for the determination of particulate organic carbon (POC).

*Bacteria abundance and sediment OM*– Sediment samples ($n$ = 3 per date and site) for bacteria abundance and OM content were collected using a 60-ml plastic syringe (Sanitex tube with the tube cut even at the 0-ml tick mark) and each immediately placed in a 50-ml Greiner tube (TPP) for transport to and storage in the laboratory. Samples were stored at 4°C until they were analyzed in the laboratory, usually within days. The bacterial abundance in each sample was determined using fluorochrome 4′,6-diamidino-2-phenylindole (DAPI) staining. Here, 50 ml of homogenized sediment sample was diluted to 1 : 10 with 10× phosphate-buffered saline (PBS) buffer (0.58 mol $L^{-1}$ $Na_2HPO_4$, 0.17 mol $L^{-1}$ $NaH_2PO_4$, and 0.68 mol $L^{-1}$ NaCl). After vortexing for 4 min at intensity 8 (Vortex Genie 2), 10 ml of aliquot was further diluted to 1 : 10 with 10× PBS buffer. The sample was vortexed again for 2 min at intensity 8, and the aliquot was further diluted to 1 : 20 with sterile-filtered $H_2O$ (MILLEX™GP; Millipore); the aliquot was therefore diluted to 1 : 2,000. Subsequently, 10 ml of sample was filtered through an ethanol-cleaned suction filter (PC MB 25 mm 0.2 $\mu$m B; Sterico AG), and the suction filter was carefully rinsed with 2 ml of sterile-filtered water. A microscope slide was cleaned with ethanol and mounted with the filter, and 50 $\mu$l of DAPI solution was added to the filter before being covered with a cover slide. Then, the microscope slide was incubated in the dark for 30 min before counting DAPI-stained cells using a microscope at ×100 magnification (Olympus BX50F; Olympus). The number of bacteria was based on counts of 10 microscopic fields and then standardized to number per milliliter of sediment.

The organic content of each sediment sample was determined by taking a homogenized subsample from each Greiner tube. The subsample was dried at 60°C, weighed, combusted at 540°C, and reweighed. The difference between weights was considered the organic fraction of the sediment as ash-free dry mass (AFDM). This value was used to calculate the percentage organic content of each sediment sample from the various sites on each date.

*Bacteria composition*—Bacteria composition was determined from the same sediment sample in which bacterial abundance and organic content were assessed. Here, 5–7 ml of sediment sample was transferred to a 50-ml Greiner tube, to which 25 ml of lysis buffer (50 mmol $L^{-1}$ Tris-HCl, 20 mmol $L^{-1}$ $Na_2$ diaminetetraacetic acid [EDTA], 100 mmol $L^{-1}$ NaCl, and 1% wt/vol polyvinylpolypyrrolidone) was added and vortexed vigorously for 2 min. Before purification, bacteria DNA samples were shaken for 45 s in a bead beater (365 $g$) (FastPrep FP120; Savant Instruments), placed on ice for 1 min, and shaken again for 45 s. Samples were centrifuged (Biofuge Fresco; Heraeus Kendro) for 5 min at 673 $g$ at 4°C. The DNA was purified with an equal volume of phenol : chloroform : isoamyl alcohol (25 : 24 : 1) and washed twice with an equal volume of chloroform : isoamyl alcohol (24 : 1). A 500-$\mu$l extracted and purified DNA sample was precipitated with 1 : 10 vol of 3 mol $L^{-1}$ sodium acetate (pH 5.5) and 2 vol of 100% ethanol. After freezing at −80°C for 2 h, samples were centrifuged for 20 min at 2004 $g$ at 4°C. Precipitated DNA was washed twice with 750 $\mu$l ice-cold 70% ethanol, dried, and dissolved in 50 $\mu$l TE (10 mmol $L^{-1}$ Tris-HCl, and 1 mmol $L^{-1}$ EDTA [pH 7.6]). The DNA was visualized on a 1% agarose gel and further purified according to the GENECLEAN III protocol (Q-Bio Gene).

Universal primers 16S 8f (AGAGTTTGATCCTGGCTCAG) and 16S 926r (CCGTCAATTCCTTTRAGTTT), labeled with a fluorescent dye (IRDye™700 and IRDye™800, respectively) at the 5′ end were used for the amplification of DNA. These primers produce a 900-bp fragment of 16S rDNA. The polymerase chain reaction (PCR) mixture was composed of 9 $\mu$l PCR buffer (Sigma Chemical), 0.4 $\mu$l dNTPs (25 mmol $L^{-1}$ each), 0.25 $\mu$l *Taq* DNA polymerase (Sigma Chemical), and 30.35 $\mu$l sterile $H_2O$. The DNA suspension, which was solubilized in 40 $\mu$l of PCR mixture, contained the two primers at 100 pmol each, 1 $\mu$l target DNA, and 7 $\mu$l sterile $H_2O$. The cycling conditions used included an initial denaturation step at 93.5°C for 3 min, followed by 35 cycles of denaturation at 93.5°C for 30 s, annealing at 49°C for 30 s, and extension at 72°C for 2 min. The PCR run (Genius FGENO5TD; Techne) was completed by a final extension at 72°C for 4 min. Five microliters of the PCR product were removed to determine the success and yield of amplification reaction by agarose gel electrophoresis.

The PCR-amplified products were subsequently digested with restriction endonuclease *Hae*III. The DNA solution (45 $\mu$l) was mixed with 6.5 $\mu$l 10× mol $L^{-1}$ restriction buffer, 1.5 $\mu$l restriction enzyme *Hae*III, and 12 $\mu$l of sterile water and then incubated in a water bath (JULABO Water Bath; Julabo Labortechnik) for 2 h at 37°C. The resulting terminal restriction fragments (T-RFs) were precipitated for 2 h at −80°C, washed twice with 750 $\mu$l ice-cold 70% ethanol,

dried, and then dissolved in 6 $\mu$l TE. Digested amplification products were resolved by electrophoresis on a polyacrylamide gel using a LI-COR DNA sequencer. Electrophoresis was run at 800 V for 6 h on 25-cm plates. The number of T-RFs was determined using BASE IMAGIR and GENE IMAGIR 4.03 analytical software (Scanalytics Inc.). Accurate counts of T-RFs were possible for 100–615 bp of each 900-bp rDNA fragment for both fluorescent dyes. The presence and absence of T-RFs provided a genetic measure of the bacterial composition in each sample.

*Hyporheic respiration and periphyton biomass*—Respiration rates of hyporheic sediments and periphyton biomass on stones were determined for sites MG (groundwater-fed) and P3 (glacial-fed) in the Macun catchment on 24 June, 20 August, and 16 September. Five replicate samples were measured on each date at each site. Sediment respiration was determined as the decrease in dissolved oxygen over time, according to the method of Jones et al. (1995). After first removing the uppermost 10 cm of benthic sediments, circular plexiglas chambers (5.35 cm diameter and 30.1 cm length) were filled half with hyporheic sediments collected at a depth of 10–30 cm and the remaining half with stream water. Sediments were sieved to exclude particles >8 mm. Chambers were then gently inverted three times (to allow any air trapped within the sediments to escape), dissolved oxygen was measured, and they were sealed with a rubber stopper. Sealed chambers were buried in the stream channel, covered with stones to exclude light, and incubated in situ for 3–7 h. After incubation, chambers were again inverted three times before measuring final dissolved oxygen concentrations (Jones et al. 1995). Dissolved oxygen concentrations were quantified with an Oxi330i oxygen meter calibrated in the field (WTW). The contents (water and sediment) of each chamber were placed in sealed plastic bags, frozen (at −25°C), and returned to the laboratory for analysis.

In the laboratory, chamber contents were analyzed for particulate organic matter (POM) in four fractions: <0.125 mm, 0.125–1 mm, >1 mm, and coarse sediment. Sediment fractions were filtered through preashed, preweighed glass-fiber filters (Millipore), dried (at 60°C for 48 h) and weighed. POM content was determined by loss of ignition at 540°C for 4 h. Hyporheic respiration rates were normalized by sediment weight (g $O_2$ $h^{-1}$ $kg^{-1}$ sediment).

Periphyton biomass was quantified for both sites (MG and P3) from five stones collected randomly within the study reach on each date. Stones were individually brushed and the resulting slurry filtered through preashed (450°C) glass-fiber filters (Whatman GF/F; 0.45-$\mu$ pore size). Filters were then frozen (at −25°C), returned to the laboratory, and processed for organic content as AFDM. Here, filters were weighed, combusted at 550°C, and reweighed. The difference in weight before and after combustion was used to determine AFDM, with values expressed per unit stone area after Uehlinger (1991).

*Ecosystem metabolism*—Open stream metabolism was calculated for sites MG and P3 in the Macun catchment. ER and GPP were determined using the dual-station diel $O_2$ method, as refined by Marzolf et al. (1994). Oxygen concentrations were measured continuously over a 4-d period each in July, August, and September 2003. Stream temperature and dissolved oxygen concentrations at the upstream and downstream ends of the two sites were recorded with field-calibrated Oxi330i oxygen meters (WTW) at 30-min intervals.

The net oxygen production rate, $\beta(t)$, in g $O_2$ $m^{-2}$ $d^{-1}$, was calculated using

$$\beta(t) = [K_s(O_2 - O_{2sat}) + \Delta O_2/\Delta t]z \quad (1)$$

where $K_s(T, Q)$ is the reaeration rate coefficient (1/$h$) as a function of temperature $T$ (°C) and discharge $Q$ ($m^3$ $s^{-1}$), $z$ represents the mean depth (m), $\Delta O_2/\Delta t$ is the change in oxygen concentration between the upstream and downstream station, and $O_{2sat}$ is the saturation concentration of $O_2$ (mg $O_2$ $L^{-1}$).

To estimate $K_s$, the gas exchange of a volatile tracer (sulfur hexafluoride, $SF_6$) was measured. $SF_6$ (a gas mixture of $SF_6$ and $N_2$, 10% vol/vol $SF_6$) was continuously injected sufficiently above the upstream station of each site, to ensure complete lateral mixing when entering the study reach (Naegeli and Uehlinger 1997). After reaching plateau $SF_6$ concentrations (usually ~2 h after injection), seven water samples per station were collected using 50-ml glass syringes. The syringes were transferred to the laboratory in a cooler, where they were analyzed for $SF_6$ on a gas chromatograph within 24 h. The $K_s$ of $SF_6$ was calculated as

$$K_s(T, Q) = \ln(c_u/c_d)(1/\tau) \quad (2)$$

where $\tau$ is the travel time of water (h) between the upstream and downstream section and $c_u$ and $c_d$ are corresponding upstream (u) and downstream (d) steady-state concentrations of $SF_6$. The travel time of water through each study reach was quantified by monitoring a NaCl-tracer solution (1 kg NaCl 10 L $water^{-1}$) with a portable LF323 conductivity meter (WTW). The reaeration rate coefficient of oxygen was calculated by multiplying the $K_s$ of $SF_6$ by 1.4 (Cirpka et al. 1993). Thyssen et al. (1987) described temperature dependence with an exponential temperature function,

$$K_s(T) = K_s(20°C) \times 1.024^{(T-20°C)} \quad (3)$$

where $K_s$(20°C) represents the reaeration rate coefficient at the reference temperature of 20°C.

ER, GPP, and P:R were determined on the basis of estimates of $\beta(t)$. To calculate ER, the average net oxygen production rate during the dark period was extrapolated to 24 h, and GPP was calculated as the sum of $\beta(t)$ and ER. Calculation of these metabolic parameters rested on the assumption that day respiration equals night respiration (Bott and Kaplan 1985).

*Statistical analysis*—Catchment differences in bacteria abundance (DAPI counts) and sediment organic content (AFDM) were tested using one-way analysis of variance (ANOVA; Zar 1984). A factorial ANOVA (general linear model) was used to test for site and date effects within each catchment. When differences were found using ANOVA, the post hoc Tukey's test was used to indicate parameter differences between catchments or dates, respectively (Zar 1984). To detect and visualize site and date differences in bacteria

assemblages, a principal component analysis (PCA) using presence and absence data of T-RFs was performed (Varimax rotation constrained to four factors). The 30 most frequent T-RFs, excluding those found at all sites, based on T-RFLP data for the primer 16S 926r (IRDye™800) was used in the PCA. The same PCA performed on the primer 16S 8F (IRDye™700) showed similar results and is not presented here. Furthermore, we used a factorial ANOVA to test for site and date effects in hyporheic respiration and OM (AFDM) and completed a simple correlation analysis between hyporheic respiration and AFDM for the two sites (MG and P3) in the Macun catchment (Zar 1984). The Statistica software package was used for all statistical analyses (Statsoft, version 6.0).

## Results

*Physical and chemical characteristics of the study sites*—Groundwater-fed streams in Val Roseg had average temperatures <10°C during the study period, whereas groundwater-fed streams in the other catchments ranged 10–16°C (Table 1). In contrast, the glacial-influenced streams in Macun had average temperatures <10°C, with site IP at <3°C. Conductivities ranged from >50 to ~88 $\mu$S cm$^{-1}$ in the Val Roseg (except site G3 at ~30 $\mu$S cm$^{-1}$) and Loetschental but averaged <30 $\mu$S cm$^{-1}$ in the crystalline region of Macun. Nitrate-nitrogen levels were generally high, ranging ~100–480 $\mu$g L$^{-1}$, with the highest values in the Macun glacial sites. Phosphate concentrations ($PO_4$-P), on the contrary, were below detection limits, and this was commonly the limiting nutrient in most Swiss Alpine streams (Robinson et al. 2003). Dissolved and particulate OM levels were low, ranging ~0.5–1.7 mg L$^{-1}$ (DOC) and ~0.1–1.5 mg L$^{-1}$ (POC). Levels of DOC and POC tended to be highest in the Macun streams (Table 1).

*Sediment OM and bacteria abundance*—The percentage of OM and AFDM of sediment was higher in the Macun streams than in the Val Roseg and Loetschental streams (Tukey's, $p < 0.05$), whereas similar levels were found in the Val Roseg and Loetschental streams (Tukey's, $p > 0.05$) (Fig. 2). The percentage of OM in sediment was <5% in the Val Roseg and Loetschental streams (~0.01 g AFDM ml$^{-1}$ sediment) but was ~15% in Macun streams (~0.04 g AFDM ml$^{-1}$ sediment). A significant catchment effect in bacteria abundance was found ($p < 0.0001$), with DAPI fluorescent counts of bacteria being highest in the Val Roseg (Fig. 2). Average counts of DAPI-stained cells per milliliter of sediment ranged $1.96 \times 10^6$ in the Macun streams to $2.77 \times 10^6$ in the Loetschental streams and $4.09 \times 10^6$ in the Val Roseg streams. DAPI bacteria counts and sediment AFDM were negatively correlated ($r^2 = -0.24$) using data from all sites and dates.

Within-catchment analysis revealed the Val Roseg to have no significant site, date, or site by date effect for OM but a significant site ($p = 0.014$) and date ($p = 0.018$) effect for AFDM (Fig. 2, site values not shown). Bacteria counts showed a highly significant site, date, and site by date effect (site by date, $p = 0.0017$) in the Val Roseg. The Loetschental showed no site, date, or site by date effect for OM or AFDM ($p < 0.05$), although bacteria counts had a significant site effect ($p = 0.014$). The Macun catchment, on the other hand, had a significant site effect for OM ($p < 0.0001$) and AFDM ($p = 0.003$). Furthermore, the Macun showed a significant date effect ($p = 0.004$) and highly significant site and site by date effects ($p < 0.001$) in bacteria abundance (Fig. 2, site values not shown). Within-catchment correlations of bacteria counts and AFDM indicated a positive relationship ($r^2 = 0.30$) in Macun and negative relationships in the Val Roseg ($r^2 = -0.40$) and Loetschental ($r^2 = -0.09$).

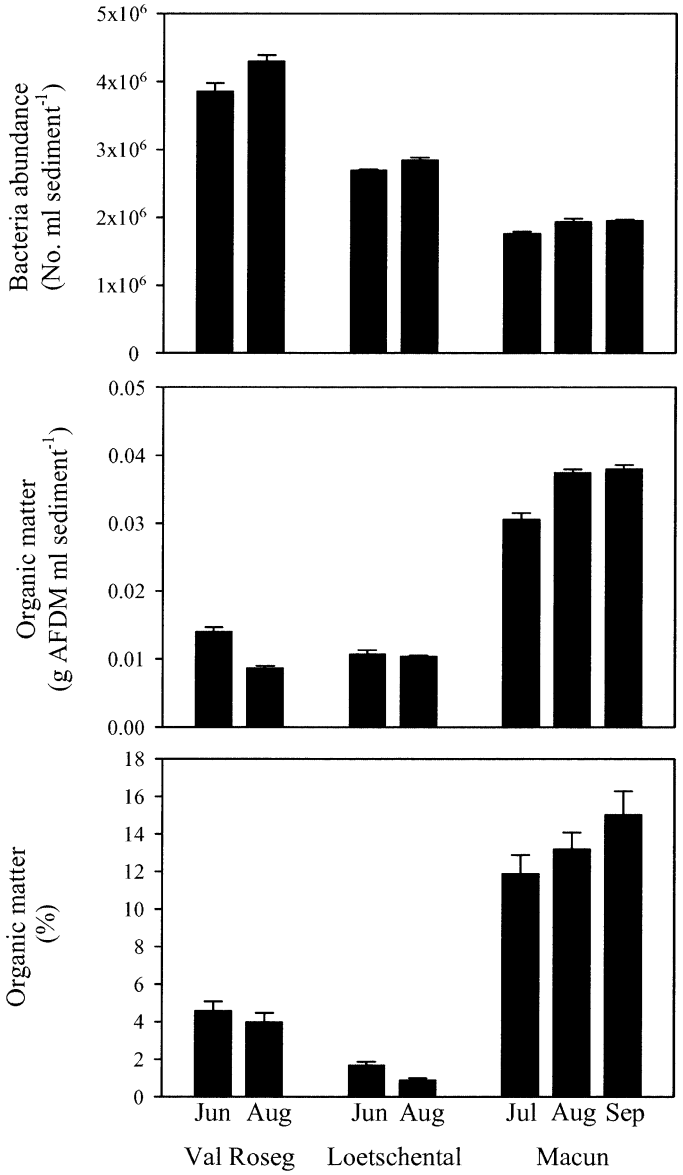


Fig. 2. Average (±SE, $n = 9$ except $n = 8$ in September for Macun because one stream was dry and not sampled) percentage of OM, AFDM, and bacteria abundance (DAPI-stained cell counts) in sediment samples collected from study streams in the Val Roseg, Loetschental, and Macun catchments. Streams in the Val Roseg and Loetschental were sampled in June and August 2003, whereas the Macun Lakes area was sampled in July, August, and September 2003.

*Bacteria composition*—The principal components analysis using data from primer 16S 926r are plotted for each catchment separately in Fig. 3. The first two components explained 35% of the variation among samples collected at all sites and dates. A seasonal shift in bacteria composition within streams and catchments was evident along axis 1 with late summer (August in Loetschental and Val Roseg, except G2) or autumn (September in Macun) samples generally located toward the right side of each plot. Sites LT1 and LT2 showed no clear seasonal separation, but we believe that this was the result of a major sediment debris input between sample dates at these streams. For instance, the July samples clustered together for all three streams in the Loetschental and the August sample for site LT3 (unaffected by the debris flow) showed the same temporal pattern as streams in the other catchments. The PCA for the Macun data further showed that bacteria assemblages in groundwater-fed streams (D and MG) differed distinctly from streams with a glacial influence (sites P3, T3, and IP) along axis 2 (Fig. 3).

*Ecosystem comparison of glacial and nonglacial streams*—Hyporheic respiration, periphyton biomass, and bacteria abundance: Hyporheic respiration rates ranged $-0.0003$ to $-0.0005$ g $O_2$ $h^{-1}$ $kg^{-1}$ sediment for MG (a nonglacial stream) and $-0.0002$ to $-0.0004$ g $O_2$ $h^{-1}$ $kg^{-1}$ sediment for P3 (a glacial stream) in the Macun catchment (Fig. 4). Respiration significantly increased from July to September in MG but peaked in August in P3. Hyporheic respiration was significantly different between streams only in September, being two times higher in MG than in P3 (Tukey's, $p < 0.05$). Site MG had higher $r^2$ values between respiration and $AFDM_{Total}$ on each respective date than P3 (Table 2). In both streams, respiration had the highest correlations with the smallest AFDM category ($AFDM_{<0.125mm}$) in July, the intermediate size category $AFDM_{0.125–1mm}$ in August, and the largest size categories ($AFDM_{>1mm.\ and\ C.S.}$) in September.

Site MG had higher AFDM and bacteria abundance in sediments across all dates (except September for bacteria) than site P3 ($p < 0.001$) (Fig. 4). Sediment AFDM values were lower in July than August and September in P3 (Tukey's, $p < 0.05$). Periphyton biomass (as AFDM) displayed similar increases in biomass over the study period in both streams, even though the periphyton biomass was $\sim 2\times$ higher in P3 than MG in August (Tukey's, $p < 0.05$).

Ecosystem metabolism: From July to September 2003, GPP decreased from 10.00 to 2.35 to 1.18 g $O_2$ $m^{-2}$ $d^{-1}$ in MG and from 10.98 to 10.78 to 3.58 g $O_2$ $m^{-2}$ $d^{-1}$ in P3 (Fig. 4). ER essentially mirrored GPP in both streams, increasing over the study period in MG, while decreasing from July to August and then increasing in September in P3. Net primary production (NPP) followed the same pattern as ER, although less pronounced, in both streams. Consequently, P:R indicated net heterotrophy in July and August ($P:R < 1$) and net autotrophy ($P:R \geq 1$) in September in MG, whereas P3 was net autotrophic in July but net heterotrophic in August and September (Fig. 4).

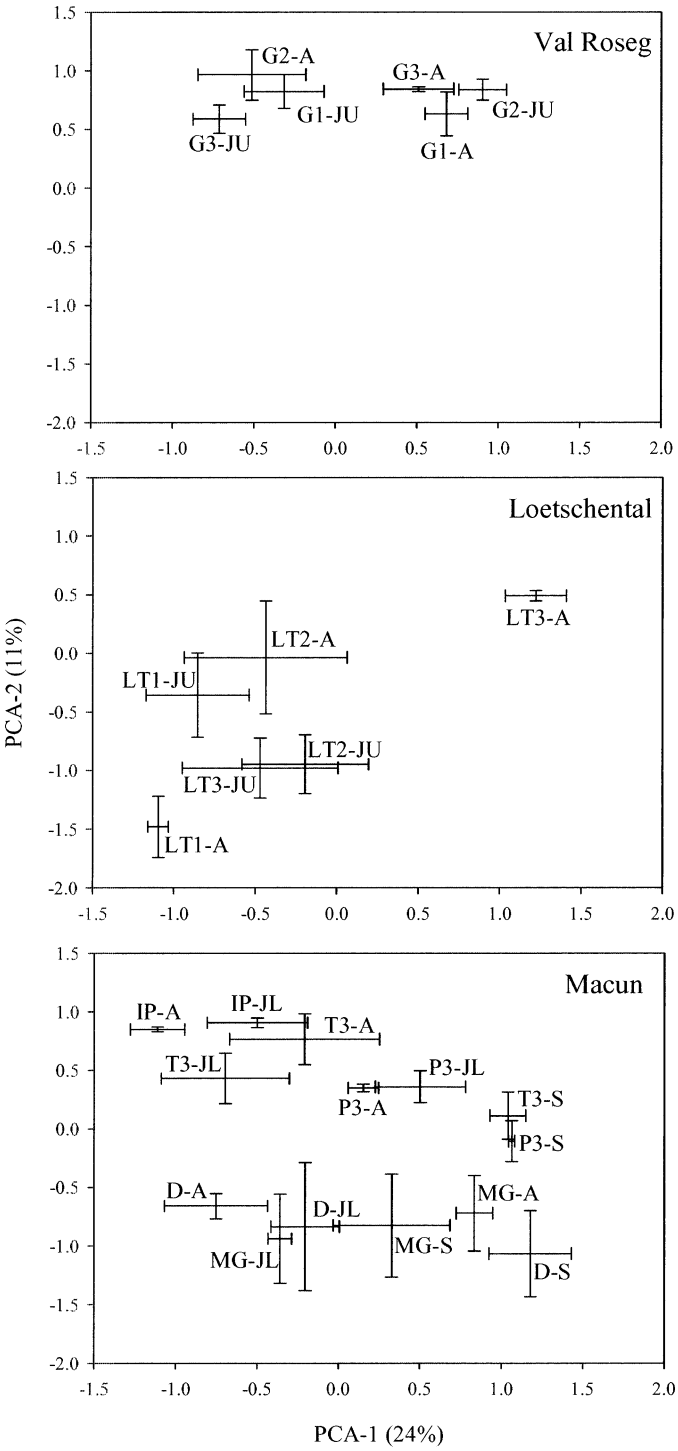


Fig. 3. Scatter plots of a PCA of the 16S 926r (IRDye™800) TRFP data set using the 30 most frequent T-RFs, excluding those found at all streams. The data set was derived from digestion with restriction enzyme *Hae*III (see methods). PCA-1 explained 24% and PCA-2 11% of the variation among samples. Each catchment was plotted separately for better visualization of data. Labels include each study stream followed by the sample month where JU = June, JL = July, A = August, and S = September. Bars are SEs based on three samples analyzed for each stream (78 sediment samples in total were analyzed for bacteria genetics).

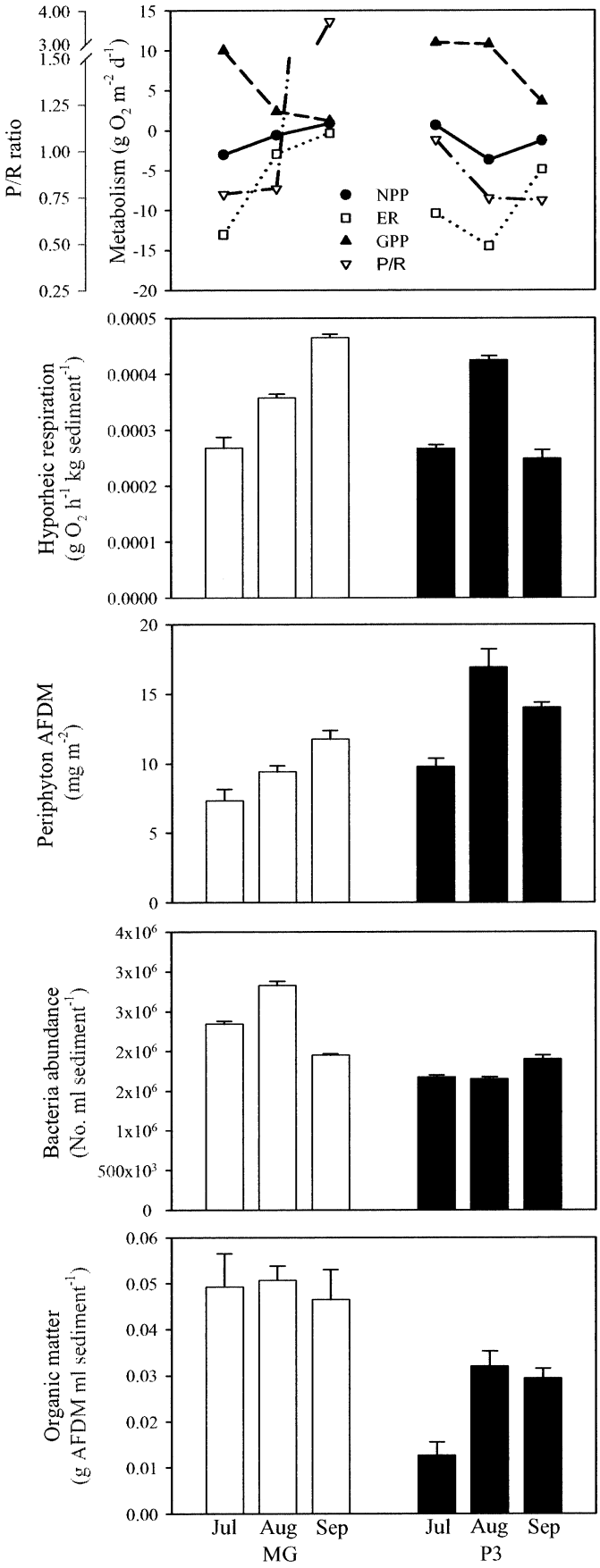


Fig. 4. Average (±SE) sediment AFDM and bacteria abundance (DAPI-stained cell counts), AFDM of periphyton ($n = 5$), hyporheic respiration ($n = 5$), and ecosystem metabolism (GPP, ER, NPP, and P : R, see ''Materials and methods'' section) for study sites MG and P3 in the Macun Lakes area in July, August, and September 2003.

Relationships between ecosystem compartments and metabolism: In July, both streams had relatively low periphyton biomass and low hyporheic respiration, but MG had greater bacteria abundances than P3 (Fig. 4). The higher bacteria levels, equating to higher respiration, in MG may have resulted in that system being net heterotrophic (P : R $<$ 1), whereas autotrophic production in P3 may have offset the respiration produced by the lower bacterial numbers in P3, thus allowing that system to be net autotrophic (P : R $>$ 1). In August, the higher hyporheic respiration rates clearly caused both streams to be net heterotrophic, regardless of the increase in periphyton biomass. In September, lower bacteria numbers and higher periphyton levels shifted MG to being net autotrophic, even though hyporheic respiration was still relatively high. MG displayed a continual increase in NPP over the study period. In contrast, decreasing hyporheic respiration rates along with somewhat lower periphyton levels maintained P3 in a state of heterotrophy in September. Here, NPP decreased in August from July and increased again in September but not to levels observed in MG.

## Discussion

*Bacteria abundance and sediment OM*—Bacteria counts were on the order of 2–4 $\times$ $10^6$ cells $ml^{-1}$ sediment, being similar to levels observed in small mountain streams (Geesey et al. 1978). Other studies have documented cell counts in the range of $10^8$–$10^{12}$ from blackwater streams and low gradient systems with high organic loads (Bott et al. 1984; Edwards 1987). A common finding is that bacteria abundance is correlated with the amount of benthic OM and dissolved OM (Bott and Kaplan 1985; Hall and Meyer 1998). We found this relationship at only one of the study areas (Macun), and streams in this catchment had the highest amounts and percentages of OM in sediments. In fact, most high alpine streams are above tree line and thus have minimal allochthonous inputs of OM (Zah and Uehlinger 2001). In contrast, the Val Roseg and Loetschental streams had almost two-fold greater bacteria abundances than the Macun streams, which suggests other factors influenced bacteria assemblages in these alpine streams than simply the amount of OM. It is of interest that the specific conductance was substantially greater in the Val Roseg and Loetschental streams than in the Macun streams, supporting a strong geologic control on ecosystem productivity and perhaps bacteria abundances. The productive capacity of streams has been linked to differences in ionic potential (Koetsier et al. 1996).

Low OM concentrations in sediments were found in the two streams in the Loetschental catchment that had a debris torrent in July caused by melting permafrost (C.T. Robinson pers. observation). The introduced material appeared to be a

Table 2. Correlation results ($r^2$) of the five sediment ash-free dry mass (AFDM) categories with hyporheic respiration rate (values in bold are significant).

| | MG | | | P3 | | |
|---|---|---|---|---|---|---|
| Category | July | August | September | July | August | September |
| $AFDM_{<0.125mm}$ | **0.87** | 0.00 | 0.11 | 0.76 | 0.04 | 0.07 |
| $AFDM_{0.125\text{-}1mm}$ | 0.44 | **0.79** | 0.19 | 0.23 | 0.62 | 0.16 |
| $AFDM_{>1mm}$ | 0.48 | 0.09 | 0.63 | 0.59 | 0.34 | 0.65 |
| $AFDM_{CS}$ | 0.00 | 0.03 | 0.76 | 0.00 | 0.40 | 0.01 |
| $AFDM_{Total}$ | **0.93** | 0.31 | 0.34 | 0.74 | 0.04 | 0.03 |

mixture of coarse sand and gravel that probably contained a low percentage of OM, as indicated by the lower percentage OM in the August samples from these streams. However, bacteria abundances were higher in August than in June, suggesting bacterial inputs from the adjacent landscape. Leff et al. (1992) found high numbers of bacteria being transported with inorganic and organic particles. The increased sediment levels, although being lower in the percentage of OM, may have enhanced habitat conditions that promoted microbial metabolism, as shown by Cardinale et al. (2002). Indeed, Cardinale et al. (2002) attributed this effect to changes in near-bed flow velocity and turbulence intensity. We suspect that the added sediment increased benthic porosity, thus increasing flow exchange between surface water and hyporheic sediments that stimulated bacteria activity (Hargrave 1972; Findlay et al. 1993). However, bacteria cell counts provide only a measure of abundance (numbers) and not one of productivity (Proctor and Souza 2001); thus, other measures are needed to fully elucidate the proposed mechanisms resulting in differences in bacteria abundances. Regardless, the bacteria abundances found in this study demonstrate that bacteria can be an important heterotrophic component in alpine streams above the tree line.

*Bacteria composition*—The T-RFLP results showed two major findings: (1) a clear separation of bacteria assemblages from glacial streams compared with nonglacial streams and (2) a clear seasonal shift in bacteria assemblages in most of the study streams. Both findings have important implications for the assessment of ecosystem metabolism in running waters. Studies on soils, using the same technique, also differentiated bacteria assemblages in relation to soil type and respective soil functioning (Bruce 1997; Liu et al. 1997). Because bacteria have been shown to be key agents in stream respiration (Hall and Meyer 1998) and OM dynamics (Kaplan and Bott 1983) and stream respiration shows high seasonality (Fuss and Smock 1996; Uehlinger and Naegeli 1998) and spatial differences (Mulholland et al. 2001), knowledge about the bacteria assemblage structure in concert with functional measures can provide insight on spatial and temporal processes in stream ecosystem function. For instance, the genetic differentiation between glacial and nonglacial bacteria assemblages suggests a mechanistic linkage between the environmental and functional differences found for these common alpine stream types (see below). Furthermore, the seasonal shift in bacteria assemblages was related to the observed seasonal changes in ecosystem metabolism in our study streams. For example, a major shift in assemblage structure occurred in September in P3, and this was the period when system metabolism also shifted. Similarly, bacteria composition shifted in August in MG, and this was the period when system metabolism also shifted substantially.

*Functional patterns between glacial and nonglacial streams*—Hyporheic respiration: Our results on hyporheic respiration in both Macun streams are in general consensus with findings of other streams and rivers, being associated with sediment OM content. The processing of OM, a fundamental ecosystem function of running waters (Minshall et al. 1983; Benke et al. 1988), within the hyporheic zone can contribute a substantial fraction (>80% in some streams) to stream ecosystem respiration (Grimm and Fisher 1984). The majority of OM is stored within the hyporheic zone, indicating its importance as a site of metabolism and nutrient cycling in stream ecosystems (Mulholland et al. 1997). In this study, bacteria abundances were related to the differences in the OM content of sediments in the two streams and were highest in the groundwater-fed stream. As mentioned above, the seasonal changes in bacteria composition also reflected changes in the metabolic properties and, thus, ecosystem functioning observed in these two contrasting stream types.

Correlation of hyporheic respiration with different OM size fractions revealed a temporal change in organic stores used by sediment bacteria. For example, hyporheic respiration was associated with small loosely attached organic particles ($AFDM_{<0.125mm}$) in July, with intermediate-sized particles ($AFDM_{0.125–1mm}$) in August, and with large particles ($AFDM_{>1mm}$) in September. Pusch and Schwoerbel (1994) also found a strong correlation between loosely associated particulate OM and hyporheic respiration (Naegeli and Uehlinger 1997), and the type of organic particles such as size and organic content can influence sediment respiration dynamics (Hargrave 1972; Amon and Benner 1996). The temporal sequence in respiration with organic size fractions suggests that the smaller more loosely attached particles were being consumed by microbes and may became limiting as the season progresses. These smaller particles may be renewed each spring with the breakdown of senescent primary producers during winter and organic matter inputs from the catchment via snowmelt runoff.

Ecosystem metabolism: The ecosystem metabolism results showed these alpine streams to be generally net heterotrophic ($P:R < 1$), although net autotrophy was observed on

some dates. Because allochthonous inputs are known to be low in alpine streams (Zah and Uehlinger 2001), we suspect that ecosystem metabolism is fueled by primary production as seen in other stream ecosystems (e.g., desert streams) receiving full sunlight (Jones 1995; Young and Huryn 1999). Primary producers can reach high biomass in alpine streams (Robinson et al. 2003). We found a relationship between sediment OM and periphyton biomass, supporting the view that autotrophic production provided a substantial fraction of the OM to hyporheic sediments in these alpine streams. Some OM may have been derived from lake sources upstream of our study streams, although seston OM was not quantified in the present study. Our data suggest that ecosystem metabolism in these open-canopied high-elevation streams may share similar functional attributes as open-canopied streams at lower elevations that rely on autotrophic production as an energy base.

Both streams had relatively high rates of GPP in July with decreasing rates later in summer, perhaps reflecting the more intense solar radiation in alpine environments in summer. Although photosynthetically active radiation strongly correlates with system GPP (Mulholland et al. 2001), intense ultraviolet radiation can inhibit primary production in high-elevation systems (Kelly et al. 2003), and both streams were shallow (<20 cm deep on average) and clear. Other physical factors also may have contributed to the temporal differences in metabolism between the two sites, such as water temperature, transient storage, velocity, discharge, nutrients, and disturbance (Benke et al. 1988; Uehlinger and Naegeli 1998; Young and Huryn 1999). Of these factors, water temperature was higher and water flow-through (an indicator of transient storage) was threefold slower in MG than in P3. Furthermore, MG had higher levels of OM and bacteria abundance than P3 and greater hyporheic respiration in September. The interplay among water temperature, sediment OM, transient storage, and bacteria abundance, along with compositional shifts in the bacteria assemblage, suggests a strong microbial role in the ecosystem metabolism of these alpine systems. Additional research comparing glacial and nonglacial alpine streams is needed to more fully understand the relationships among the various controlling factors on ecosystem metabolism and for a better comparison among open-canopied stream ecosystems in general.

The combination of measures used in the present study showed that ecosystem metabolism can vary considerably among alpine streams. Although P3 was glacially influenced, water turbidity was low and similar to MG. However, its bacterial assemblage was characteristic of other glacial streams in the Macun catchment and differed from groundwater-fed streams like MG. As was noted by Hargrave (1972), metabolic features cannot be related simply to bacterial abundances but must incorporate some measure of microbial activity. For instance, the increase in ecosystem respiration for P3 in August could be a result of increased metabolic activity in the hyporheos associated with changes in bacteria assemblage structure and OM content. Our results show that bacteria are important players in the functioning of alpine stream ecosystems, and new insights on stream function can be gained through better knowledge of bacteria composition.

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