Ecology, 83(2), 2002, pp. 412–422

THE INFLUENCE OF SUBSTRATE HETEROGENEITY ON BIOFILM METABOLISM IN A STREAM ECOSYSTEM

BRADLEY J. CARDINALE,[1,3] MARGARET A. PALMER,[1] CHRISTOPHER M. SWAN,[1] SHANE BROOKS,[1] AND N. LEROY POFF[2]

[1]*University of Maryland, Department of Biology, College Park, Maryland 20782 USA*
[2]*Colorado State University, Department of Biology, Fort Collins, Colorado 80523 USA*

Abstract. Simplification of natural habitats is a growing global concern demanding that ecologists better understand how habitat heterogeneity influences the structure and functioning of ecosystems. While there is extensive evidence that physical habitat heterogeneity affects the structure of biotic communities (i.e., organismal abundance, distribution, diversity, etc.), ecologists know little about how variability in physical conditions within habitats regulates ecological processes that are important for the functioning of an ecosystem. We performed a field experiment to assess the effects of geomorphic heterogeneity (i.e., variation in substrate size) on rates of benthic productivity and respiration at the scale of whole riffle habitats in a stream ecosystem. While holding median sizes constant, we manipulated variation in the size of stream bed sediments in replicate riffles to create two treatments representing increased and decreased levels of physical habitat heterogeneity relative to natural conditions in the stream. Physical habitat heterogeneity had an immediate and significant impact on the primary productivity of stream algae and on the respiration of the benthic biofilm. The rates of both ecological processes were elevated in the high-heterogeneity riffles, probably as a result of quantified alterations to near-bed flow velocity and turbulence intensity. Results presented here provide support for the widely held, but largely untested, assumption that physical habitat heterogeneity exhibits control over ecosystem-level processes, and it suggests that human-induced simplification of habitats may indeed be altering the functioning of ecosystems.

Key words: abiotic variation; biofilm metabolism; ecological processes; ecosystem functioning; physical habitat simplification; stream algae; stream riffles.

INTRODUCTION

One of ecology's foremost challenges is to understand how and when physical habitat heterogeneity regulates the structure and function of biotic communities. Ecologists are well aware that habitat heterogeneity exerts a strong influence on the distribution and abundance of species, on species interactions, and on the trophic structure of biological communities (MacArthur and MacArthur 1961, Hilborn 1975, Levin 1976, Hassell 1980, Hanksi 1981, Pacala and Roughgarden 1982, Abrams 1988, Kareiva 1990, Holt and Hassell 1993, and many others). There is general consensus that loss of habitat heterogeneity is one of the most serious problems threatening the persistence of natural communities (Bell et al. 1991, Pickett et al. 1996, Dobson et al. 1997). This problem is being exacerbated by many human activities that are simplifying the structure of ecosystems—physical habitat heterogeneity is declining, natural disturbance regimes are being removed or simplified, and species pools are being homogenized worldwide (Stanford et al. 1996, Daily 1997, Rahel 2000).

Manuscript received 28 September 2000; revised 14 March 2001; accepted 15 March 2001; final version received 16 April 2001.

[3] E-mail: bc84@umail.umd.edu

While there has been a great deal of research on the coupling between habitat simplification and the dynamics of populations and communities, ecologists have only recently begun to explore how changes in physical habitat heterogeneity influence important ecosystem-level processes such as primary production, decomposition, or the cycling of nutrients (Gustafson 1998). A limited, but growing number of studies suggest that the physical complexity of a habitat (i.e., the number of distinct habitat or patch types composing an ecosystem) is an aspect of heterogeneity likely to exert strong control over ecological processes that maintain the functioning of ecosystems (e.g., Pierce and Running 1995, van Zyll De Jong et al. 1997, Gao et al. 2000). However, the potential importance of physical habitat variability (i.e., the spatial variation of physical properties within or between habitats) for ecosystem functioning remains largely unexplored. Rarely have researchers held mean or median habitat conditions constant in space or time and asked how variability of a physical parameter influences ecosystem processes (Palmer et al. 1997*a*, *c*). Thus, there are few examples where changes in ecosystem functioning can be unequivocally attributed to habitat variability per se, without confounding by changes in the most common physical environment. If physical habitat hetero-

geneity is critical to the functioning of ecosystems as many ecologists have assumed, and habitat simplification continues at its current pace globally, then much additional work is required to understand how rates of productivity, decomposition, and other ecosystem processes respond to variation in the physical environment.

Streams and rivers have arguably experienced some of the most dramatic forms of habitat simplification of any type of ecosystem (Brooks and Gregory 1988, Allan and Flecker 1993, Stanford et al. 1996, Sala et al. 2000). Within watersheds, the damming and straightening of stream channels to control discharge have reduced spatial and temporal variability in the flow of water through lotic ecosystems (Ligon et al. 1995, Poff et al. 1997, Graf 1999). Within stream reaches, the removal of physical structures such as woody debris or beaver dams has eliminated important types of stream habitat (Naiman et al. 1986, Frissell and Nawa 1992, Shields and Smith 1992). At smaller spatial scales, the benthic habitats crucial to many stream organisms have been homogenized by increased rates of erosion and sedimentation (Phillips 1993, Palmer et al. 2000). To the extent physical habitat heterogeneity is required to maintain the diversity of ecological processes that underlie the ecological integrity of ecosystems, all of these forms of habitat simplification might be expected to alter stream ecosystem functioning (Power 1995, Meyer 1996). At present, however, there is little empirical or theoretical basis from which to draw predictions about how habitat simplification might alter ecological processes in streams, or for that matter, any other type of ecosystem.

In this paper, we present the results of a field experiment that examined the effects of physical habitat heterogeneity on key biotic processes in a stream. Our research was unique in that we (1) carefully characterized physical habitat heterogeneity in a quantifiable manner, (2) manipulated habitat heterogeneity without altering median values of the abiotic parameter of interest, and (3) performed our experiment at the relatively large spatial scale of whole riffle habitats. Thus, we were able to unambiguously relate changes in ecological processes to variation in the abiotic environment at a spatial scale that is relevant to stream functioning. We focused our attention on geomorphic heterogeneity (variation in the size of stream bed sediments) within riffle habitats of streams because (1) riffles are ''hot spots'' for many ecological processes considered to be crucial for stream ecosystem functioning (Allan 1995) and (2) geomorphic heterogeneity within riffles can govern the dynamics of near-bed flow (Nowell and Jumars 1984, Davis and Barmuta 1989, Carling 1992) in ways that may influence ecosystem processes (reviewed in Hart and Finelli 1999). Thus, we hypothesized that geomorphic heterogeneity in stream riffle habitats is an important determinant of ecosystem processes. To test this general hypothesis, we established reference sites as well as two treatments of substrate heterogeneity by experimentally altering variation in the size of benthic substrates in entire riffles habitats. We then tracked the development of two crucial stream processes, benthic productivity and respiration, over a 28-d period and tested for differences in these processes between the two treatments.

Methods

The study site

The experiment was performed in Milltown Creek, a third-order tributary of the Catoctin River that drains into the Potomac River in the Piedmont region of northern Virginia, USA. Milltown Creek is a low-gradient (1:125) mesotrophic stream fed by groundwater. Human impacts on the stream are relatively minimal as the watershed is dominated by deciduous forest interspersed with low-density housing. The particular reach of the stream chosen for the experiment was a 1-km section having a distinct pattern of alternating riffles and pools flowing through a wooded area with an intact riparian zone characterized by sycamore (*Platanus occidentalis*), box elder (*Acer negundo*), hackberry (*Celtis occidentalis*), river birch (*Betula nigra*), tulip poplar (*Lioriodendron tulipfera*), and the introduced multiflowered rose (*Rosa multiflora*). The channel in the experimental section ranged from 2 to 5 m wide and was dominated by coarse gravel (median particle size 55 mm). Water depth in the stream riffles averaged 5–10 cm and near-bed velocity averaged 12 cm/s (Brooks et al., *in press*). Our experiment was performed during a 28-d interval in the late summer of 1998 (16 July–12 August) when the stream had relatively constant summer baseflow. The experiment was terminated after changes in discharge on day 30 prevented us from maintaining the experimental treatments.

Experimental design and manipulation of heterogeneity

The experimental units were 10 riffle habitats, each separated by at least one deep pool and, in most cases, an unmanipulated riffle that was not part of the study. The three most upstream study riffles were assigned to be unmanipulated references, used to make a qualitative assessment as to whether or not measured variables were within the range of what occurs naturally in the stream (as in ''background reference sites,'' Power et al. 1998). All reference riffles were upstream of the treatment riffles and were, therefore, unaffected by manipulations that took place in the treatment riffles. Of the seven remaining study riffles, three were randomly assigned to a low heterogeneity (LH) treatment and four were randomly assigned to a high heterogeneity (HH) treatment.

Prior to our manipulations of substrate heterogeneity (<2 h), we imposed a disturbance of equal magnitude on each of the treatment riffles to ensure that experi-

mental units were comparable at the onset of the experiment. The goal of this disturbance was to minimize potential differences in initial conditions among riffles so that our results could be decisively attributed to treatment effects (i.e., not driven by differences in initial conditions). Working in an upstream to downstream direction, we removed all substrates >5 cm (second-axis diameter) from the stream bed of each riffle on 16 July 1998. Rocks were individually scrubbed on the stream bank with a bottle brush, and rinsed of flora and fauna, which were discarded (i.e., not returned to the stream bed). Entire riffles were then raked three times to a depth of 5–8 cm. Following raking, a team of workers formed a line across the riffle and, while walking upstream to downstream, disturbed all substrates to a depth of ~10 cm with their feet.

Immediately after disturbing the treatment riffles, we manipulated habitat heterogeneity by altering spatial variation in the size of benthic substrates in the riffle habitats. In riffles assigned to the LH treatment, we withheld the largest and smallest rocks from the stream bed to narrow the particle size distribution (i.e., decrease variance) around the median particle size. In riffles assigned to the HH treatment, we added a range of rocks smaller and larger than the median to broaden the particle size distribution (i.e., increase variance). We periodically measured the size (second-axis diameter) of 100 randomly selected rocks in each riffle and iteratively adjusted particle size until we reached predetermined levels of substrate heterogeneity. Substrate heterogeneity was measured in two ways. First, we used the particle size ratio d_{84}/d_{50} (where d_i represents the particle size larger than the ith percent of particles in the riffle), which is a measure of heterogeneity widely used by stream geomorphologists (Hey and Thorne 1983, Wiberg and Smith 1991). As a second, complementary, measure of substrate heterogeneity, we used the standard deviation from the median particle size. Target levels of heterogeneity for the treatments (d_{84}/d_{50} of 2.5 for the HH treatment, and 1.5 for the LH) were greater than and less than background levels in the stream ($d_{84}/d_{50} \approx 2.0$) and spanned the maximum contrast that could be achieved using naturally occurring bed material. We verified the integrity of the treatments on day 20 of the experiment (August 5) by measuring the size of 100 randomly selected particles along established transects in each riffle. Median particle size (d_{50}) was compared between treatments using a two-tailed t test, while the two measures of substrate heterogeneity (the d_{84}/d_{50} ratio, and the SD of median particle size) were compared between treatments using one-tailed t tests (because of the a priori expectation that HH > LH).

Current velocity and turbulence

Flow velocity and turbulence intensity were measured at 20 randomly selected locations in each riffle midway through the experiment (days 16–18). Readings were collected using an acoustic Doppler velocimeter (model 10-MHz ADV, Sontek, San Diego, California, USA) positioned to measure flow at 6–7 mm above the stream bed. Interference by acoustic reflection from the stream bed (Finelli et al. 1999) prevented data collection at ~30% of the selected locations. When there was acoustic interference, the ADV was moved within a 30-cm radius until a reliable reading could be taken. We could not visually identify features of the stream bed that consistently led to acoustic interference; thus, we have no reason to believe this sampling protocol was biased towards any particular substrate type.

At all of the 20 randomly selected locations, three-dimensional velocity was recorded at 25 Hz for two minutes, yielding a time series of $n = 3000$ data points. Prior work indicated this length of time was required to characterize turbulence within 5% of the true mean. From each time series we computed velocity and turbulent kinetic energy (TKE) at a sampling location as:

$$\text{velocity} = \frac{\sum_{n=1}^{3000} \sqrt{\mathbf{u}_i^2 + \mathbf{v}_i^2 + \mathbf{w}_i^2}}{n} \quad (1)$$

$$\text{TKE} = \frac{1}{2}\rho(u'^2 + v'^2 + w'^2) \quad (2)$$

where **u, v,** and **w** are the orthogonal velocity vectors measured by the ADV, u', v', and w' are the mean deviations from the respective vector means, and ρ is the density of water (Bradshaw 1971). TKE was used for this study (as opposed to other measures of turbulence) because we felt that, as a measure of the temporal variance in flow that is not standardized for mean velocity, it best represents the true fluctuating forces experienced by benthic organisms. The median of the 20 measurements of velocity and turbulence were used to characterize the most common flow environment near the stream bed of every riffle. Median velocity and turbulence were compared between LH and HH riffles using two-tailed t tests.

Benthic productivity and respiration

Productivity and respiration of the benthic biofilm were measured on standardized substrates (unglazed ceramic tiles) that were deployed in treatment riffles at the start of the experiment (Fig. 1A). Clay or ceramic tiles are commonly used media for measuring aquatic biofilm metabolism, and are particularly useful for making relative comparisons among treatments (Vollenweider 1974, Steinman and Lamberti 1996). Immediately after manipulation of substrate heterogeneity, 10 clean (i.e., bare) tile ''units'' were staked flush with the surface of the stream bed at five equidistant positions along two upstream–downstream transects in each treatment riffle. Tile units (6.9 × 13.8 cm) consisted of 18 individual tiles (5.29 cm^2) connected by

FIG. 1. (A) One of ten tile units that were placed on the benthic habitat of a low heterogeneity riffle. (B) The central incubation site showing tiles sealed inside 0.5-L metabolism chambers being held at a constant temperature in water baths. Also shown are examples of (C) low heterogeneity (LH) and (D) high heterogeneity (HH) riffles after manipulation of substrate variability.

nylon lines in a 3×6 rectangular array. We also placed tile units in the three reference riffles 90 d prior to our manipulations of substrate heterogeneity. We assumed that tiles in the reference riffles were fully colonized by the time our study was begun, and that measurements taken from these tiles represented ambient rates of biofilm metabolism in the stream.

On several sampling dates after the manipulation (days 4, 8, 15, 25 for treatment riffles; days 1, 4, 8, 15, 25 for reference riffles) we removed one tile from eight randomly selected tile units in each riffle. These eight individual tiles, meant to serve as subsamples of a riffle, were placed together in a small tray containing stream water and brought to a central incubation site within 10 min. The central incubation site was a 1.0-m^2 open canopy area of the stream (the same location for all sampling dates) equipped with a portable generator and two water baths. All eight tiles (i.e., subsamples) from a given riffle were sealed together inside a 0.5-L clear acrylic chamber (1 chamber per riffle) that was filled with filtered (45 μm) stream water, and the chamber was placed in a randomly selected position in one of the two water baths (see Fig. 1B). The airtight chambers had submersible pumps (run using the portable

generator) that internally circulated water at a velocity of 12.5 ± 2.7 cm/s (mean ± 1 SD). This velocity was chosen because it was comparable to the median velocity of the stream (Brooks et al., *in press*), and was an available setting on the pumps. Using the water baths, we were able to hold water temperature in the chambers at ambient stream temperature (the mean difference between chamber and stream temperature for all sampling dates = 0 ± 1°C [mean ± 1 SD]), and we were able to keep temperature equal between treatments ($P > 0.24$ for all ANOVAs comparing incubation temperature between LH and HH treatments for the four sampling dates). Further, by performing the incubations at a central location we ensured that all tiles experienced identical lighting conditions within a given sampling date.

The rate of respiration of the benthic biofilm, which included both autotrophic (R_A) and heterotrophic (R_H) components, was determined by measuring oxygen uptake in the chambers (Model 830 oxygen probe, Orion, Beverly, Massachusetts, USA) during an incubation in the dark (chambers were covered with sleeves of dark fabric that prevented sunlight penetration). Following the dark incubations, tiles inside the chambers were exposed to ambient sunlight and the net metabolism of the biofilm (gross primary production $-[R_A - R_H]$) was determined as the change in oxygen concentration over a second incubation period. Gross primary production of the biofilm, GPP, was calculated as the sum of net metabolism and respiration (Bott 1996). Our technique allowed incubations to be relatively short (45–60 min), which is important for minimizing nutrient depletion and supersaturation of oxygen that can be problematic when using enclosed chambers to measure metabolism (Bott et al. 1997).

Following measurements of productivity and respiration, tiles were removed from the chambers, placed in a cooler, and transported to the laboratory where the biofilm was removed with a toothbrush. The biofilm was suspended in a constant volume of water and homogenized by vigorous stirring. Subsamples ranging from 10% to 100% of the total volume (depending on biofilm density) were filtered onto 0.70-μm Whatman GF/F filters (Whatman, Clifton, New Jersey), which were stored in 90% ethanol for a minimum of 48 h before being analyzed for algal biomass. Algal biomass was determined spectrophotometrically as chlorophyll *a* using methods of Steinman and Lamberti (1996) and substituting the specific absorption coefficient for samples extracted in ethanol derived by Nusch (1980). Biofilm respiration, GPP, algal biomass, and biomass-specific productivity (i.e., GPP/algal biomass) were compared between treatments using mixed model repeated measures ANOVAs (SAS 1996). We used gaussian covariance structures for these models, which is recommended for repeated measures taken over unequal time intervals allowing correlations between measurements to decline as a function of time (Littell et al. 1996). The probability of a Type I error was set at $\alpha = 0.05$ for all analyses.

Results

There was no difference in the median size of particles (d_{50}) between the LH and HH treatments ($t = 0.01$, df = 5, $P = 0.99$, Fig. 2A). Median substrate size for both treatments was within the range of what occurs naturally in Milltown Creek (Fig. 2A), indicating that our manipulations did not lead to aberrant particle sizes. There was a significant difference in the heterogeneity of particle sizes between the treatments (Fig. 2B, C). The geomorphic ratio d_{84}/d_{50} averaged 1.5 times greater in HH than in LH riffles ($t = 3.18$, df = 5, $P = 0.01$, Fig. 2B), and the SD of the median particle size averaged two times higher in HH than in LH riffles ($t = 7.97$, df = 5, $P < 0.01$, Fig. 2C). These complementary measures of heterogeneity showed that variance in substrate size in the HH riffles was elevated beyond what occurs naturally in Milltown Creek (compare the HH treatment to maximum values of the reference riffles, Fig. 2B, C), while variance in the LH riffles was reduced below what occurs naturally in the stream (compare the LH treatment to minimum values of the reference riffles, Fig. 2B, C).

Fig. 2D displays how the difference in substrate heterogeneity between the two treatments was achieved. Increased heterogeneity in the HH riffles resulted from a reduction in the proportion of particles ranging in size from 55 to 140 mm and a concurrent increase in the proportion of particles >140 mm. Reduced heterogeneity in the LH riffles was the result of an increased proportion of particles ranging in size from 40 to 75 mm and a concurrent reduction in the proportion of all particles >75 mm. Although our intention was to alter both sides of the particle size distribution equally, changes in riffle substrate size variability were mostly achieved by altering the relative proportions of medium (40–90 mm) and large (>90 mm) substrate sizes.

Manipulation of substrate heterogeneity led to contrasting flow environments in the treatment riffles. Median near-bed velocity was significantly faster in HH riffles than in LH riffles (16.0 vs. 9.7 cm/s, $t = 3.17$, df = 5, $P = 0.03$, Fig. 3A). Presumably, this occurred because increasing the proportion of large particles in HH riffles (Fig. 2D) restricted the area available for water flow and caused velocity to increase via conservation of momentum. The distribution of velocity measurements in HH riffles was considerably wider (i.e., more variable) than in LH riffles due to both subtle increases in the proportion of locations having extremely low near-bed velocity and a substantial increase in the proportion of locations having velocities >20 cm/s (Fig. 3B). Flow of water near the stream bed was also more turbulent in HH than in LH riffles ($t = 3.25$, df = 5, $P = 0.02$) with TKE at any given point on the stream bed averaging 2.35 times higher in the HH treatment (Fig. 3C). Greater turbulence in the HH riffles resulted pri-

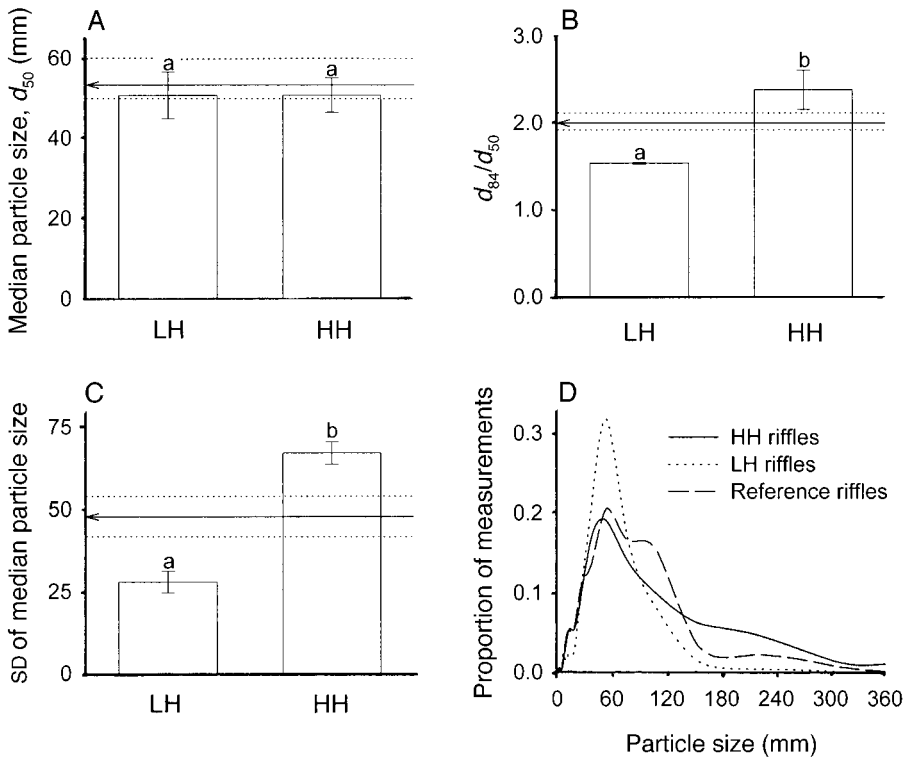

FIG. 2. (A) The median particle size in a riffle (d_{50}) and two measures of particle size heterogeneity: (B) the geomorphic ratio d_{84}/d_{50}, and (C) the standard deviation from the median particle size. Histograms show the means ± 1 SE for $N = 3$ low and $N = 4$ high heterogeneity riffles measured on day 20 of the experiment. Columns marked with different letters are significantly different from each other (t tests, $P < 0.05$). For comparison to natural characteristics of substrata in the stream, dotted lines show the maximum and minimum values, and the solid arrows show the mean value of $N = 3$ reference riffles that were not manipulated during the experiment. Also shown is (D) the frequency distribution of all particle measurements in the treatment and reference riffles. Smoothed trend lines are presented for clarity.

marily from a greater proportion of localities having highly variable flow (Fig. 3D)—a pattern consistent with how increased ''roughness'' of a stream bed can lead to more complex flow patterns (Davis and Barmuta 1989). In general, near-bed velocity and turbulence in LH riffles were comparable to that documented in the reference riffles, but velocity and turbulence in HH riffles were substantially elevated above natural levels (compare treatments to values for the reference riffles, Fig. 3).

Substrate heterogeneity had significant effects on the rate of metabolism of the stream biofilm. The development of benthic respiration in the two treatments followed similar trajectories over the course of the experiment (i.e., no treatment × day interaction, $F_{4,20} = 1.10$, $P = 0.38$, Fig. 4A), yet the biofilm from the HH riffles consistently consumed more oxygen than the biofilm from the LH riffles ($F_{1,5} = 17.06$, $P < 0.01$, Fig. 4A). Indeed, benthic respiration averaged 65% faster in HH than in LH riffles over the course of our measurements. The development of benthic respiration in the treatment riffles occurred rapidly, with measurements taken on day 4 ranging from 43% to 56% of the maximum values observed in riffles during the study. There was a decline in benthic respiration in the treatments on day 8 that tracked an unexplainable decrease in respiration in the stream as a whole (i.e., note the simultaneous decline in treatment and reference riffles, Fig. 4A). Following this, respiration increased to asymptotic values by day 15 of the experiment. For all sampling dates, mean benthic respiration of LH treatment riffles was below the minimum value that occurred naturally in the stream (compare the LH treatment to the range given for the reference riffles, Fig. 4A). In contrast, respiration in the HH treatment slightly exceeded natural levels of respiration by day 25 of the experiment (compare the HH treatment to the range given for the reference riffles, Fig. 4A).

Substrate heterogeneity also had a significant affect on the rate of benthic primary production. There was no treatment × day interaction for GPP ($F_{4,20} = 0.87$, $P = 0.50$, Fig. 4B), indicating that the development of benthic productivity followed similar trajectories for the two treatments over the course of the experiment. However, the biofilm from the HH riffles was more productive per unit area than the biofilm from the LH riffles ($F_{1,5} = 6.98$, $P < 0.05$, Fig. 4B). On average, HH riffles exhibited 39% more gross productivity than did LH riffles. Like biofilm respiration, the develop-

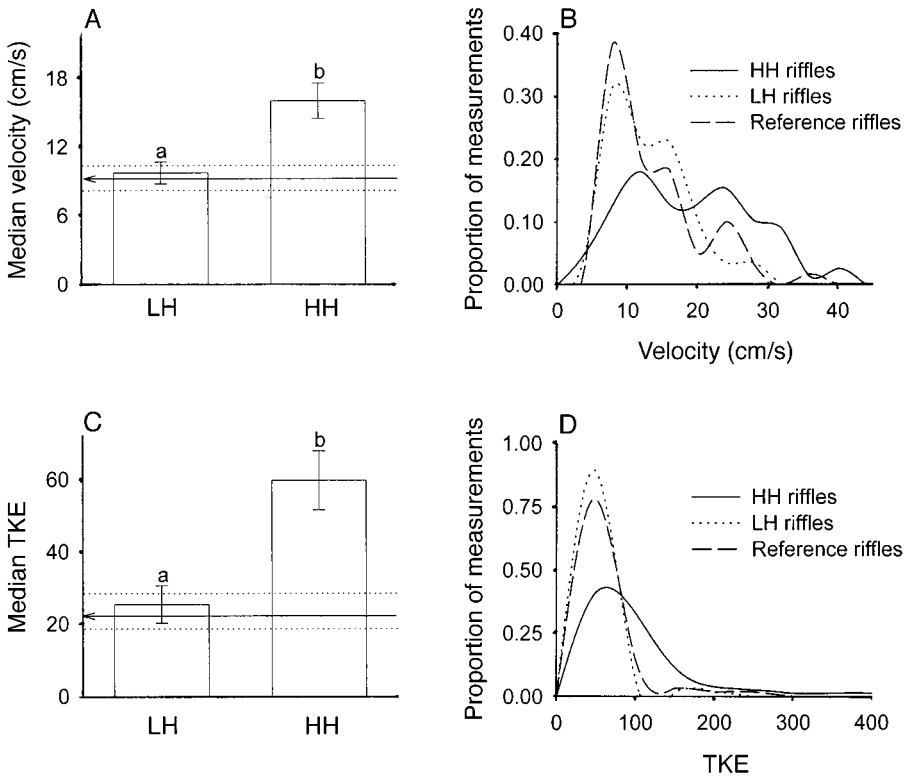

FIG. 3. (A) Median velocity, (B) the frequency distribution of velocity measurements, (C) median turbulent kinetic energy (TKE), and (D) the frequency distribution of TKE measurements in riffles on day 20 of the experiment. Data in plots (A) and (C) are mean values ± 1 SE of $N = 3$ low and $N = 4$ high heterogeneity riffles. Columns with different letters are significantly different from each other (t tests, $P < 0.05$). For comparison to natural characteristics of the stream, dotted lines show the maximum and minimum values, and the solid arrows show the mean value of $N = 3$ reference riffles that were not manipulated during the experiment. Data in plots (B) and (D) represent all measurements collected in the treatments and reference riffles with smoothed trend lines presented for clarity.

ment of GPP in the treatment riffles followed an asymptotic response curve. GPP increased to within the range that occurs naturally by day 8 of the experiment (compare treatment to reference values in Fig. 4B). Maximum values of GPP were reached by day 15 for both treatments, after which, changes in GPP paralleled ambient productivity of the stream.

Increased productivity in the HH riffles was not the result of differing amounts of algal biomass in the two treatments. Chlorophyll *a*, a commonly used estimate of algal biomass (Nusch 1980, Steinman and Lamberti 1996), accumulated on tiles at the same rate in both treatments (i.e., no treatment × day interaction, $F_{4,20} = 0.23$, $P = 0.92$). The amount of chlorophyll *a* on tiles in the treatment riffles was within naturally occurring levels by day 8 of the experiment, after which biomass accrual paralleled changes that were occurring naturally in the stream (i.e., compare trends for treatment riffles to trends for reference riffles in Fig. 4C). There was no main effect of the treatments on chlorophyll *a* densities ($F_{1,5} = 0.01$, $P = 0.94$, Fig. 4C), suggesting that, per unit algal biomass, the stream biofilm collected from the HH riffles was more productive than the biofilm from the LH riffles. Indeed, we found a main effect of the treatments on biomass-specific productivity ($F_{1,5} = 9.14$, $P = 0.03$), which was consistently higher in HH riffles over the duration of the experiment (Fig. 4D).

DISCUSSION

There is currently much concern that human-induced simplification of natural habitats may be altering the functioning of ecosystems (Cairns 1995, Meyer 1996, Daily 1997, Dobson et al. 1997, Palmer et al. 1997*a*, *c*, Poff et al. 1997, Graf 1999). Yet, experimental evidence that ecosystem-level processes respond to changes in habitat heterogeneity, particularly changes in the variability of physical parameters within a habitat, is scarce. This study shows that physical habitat heterogeneity does indeed influence the rates of key ecological processes in a stream ecosystem. We were able to manipulate variation in the size of benthic substrata in entire riffle habitats without altering the most common (i.e., median) particle size. We tracked the development of two ecological processes, benthic productivity and respiration, and found that both responded immediately and significantly to changes in riffle habitat heterogeneity. The rate of respiration by the

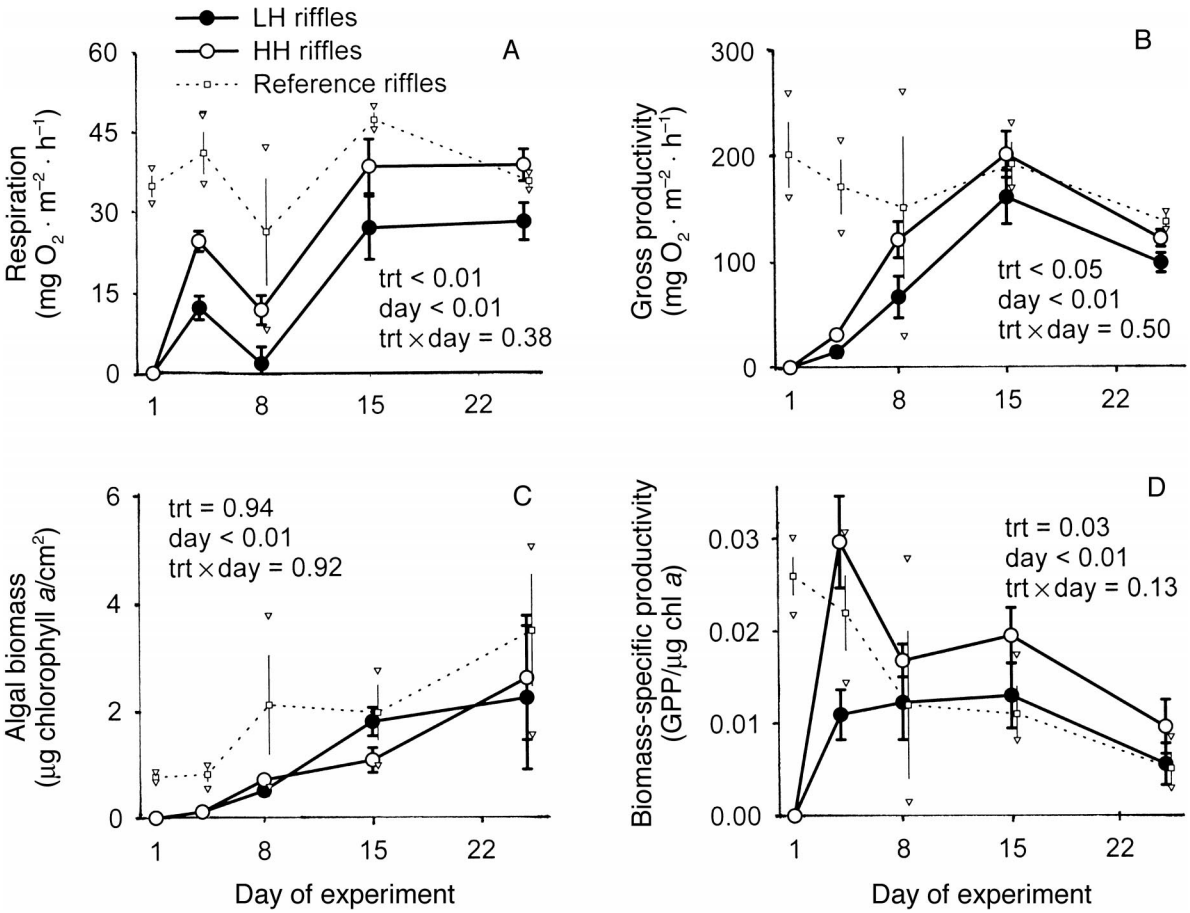

FIG. 4. (A) Respiration, (B) GPP, (C) algal biomass, and (D) biomass-specific productivity of the benthic biofilm on standardized ceramic tiles colonized in the riffle habitats. Data points are the mean ± 1 SE of $N = 3$ low heterogeneity, $N = 4$ high heterogeneity, and $N = 3$ reference riffles. Open triangles show the maximum and minimum values for the reference riffles on each date. *P* values from repeated-measures ANOVAs comparing the LH and HH treatments are displayed for each variable. Mean temperatures during incubation of the tiles were held at ambient stream temperature (day 1 = 23.5°C, day 4 = 23.3°C, day 8 = 23.4°C, day 15 = 23.6°C, and day 25 = 24.7°C), and lighting conditions were identical for all tiles within a date (see *Methods*).

stream biofilm averaged 65% greater in HH vs. LH riffles while the rate of productivity in HH riffles was 39% higher on average. Elevated rates of productivity did not result from greater accrual of algal biomass, but rather, higher levels of biomass-specific productivity in HH riffles suggest that increasing substrate variability accelerated benthic metabolism. Because our experimental design allowed us to attribute changes in metabolic processes to substrate variability per se, this study indicates that altering the physical heterogeneity of stream riffle habitats can indeed alter stream ecosystem functioning.

We originally intended to run this experiment beyond 25 d, but changes in stream discharge prevented us from maintaining the experimental treatments. There is, however, reason to believe the study was sufficiently long to assess the full development of biofilm functioning. Benthic respiration and productivity in this stream reached maximum values within 15 d, and by the end of the experiment (day 25) both processes were comparable to and were tracking changes in metabolism in the references riffles. These trends are similar to the development of biofilm metabolism documented in other stream studies where respiration and productivity on bare or recently disturbed substrata frequently attain ≥50% of maximal values in 3–8 d, and maximal values within 12–20 d (Fisher et al. 1982, Osborne 1983, Grimm 1987, Stock and Ward 1988, Jones and Lock 1989, Claret 1998, Uehlinger and Naegeli 1998, Romani and Sabater 1999). Thus, the duration of our experiment should have been sufficient to identify more than just transient responses of biofilm functioning to changes in riffle heterogeneity.

Our data indicate a rapid response of biofilm activity to the treatments of substrate heterogeneity. The lack of any treatment × day interaction for biofilm respiration or productivity (Fig. 4A, B) indicates that divergence in metabolism between treatments was estab-

lished by the first sampling date. Thus, the proximate cause of differences in metabolism between the treatments must have been established immediately after manipulation of the riffles, and was influential during early colonization of the tiles. We can think of at least three mechanisms that potentially explain how geomorphic heterogeneity in riffles affected the rates of benthic respiration and productivity in this manner. First, increased velocity and turbulence associated with greater physical heterogeneity may have directly stimulated metabolism of the stream biofilm. Substrate heterogeneity can influence near-bed hydraulics in streams (Nowell and Jumars 1984, Davis and Barmuta 1989, Carling 1992), and hydraulics constrain processes that are mediated by biota (reviewed by Hart and Finelli 1999). At subscouring levels of discharge, algal productivity generally increases as velocity and turbulence increase (Stevenson 1996, Biggs et al. 1998, Hart and Finelli 1999), presumably because velocity and turbulence are associated with the flux of nutrients, gasses, and organic matter that can limit the metabolism of benthic biota. Thus, changes in the flow environment of the high heterogeneity riffles may have stimulated benthic productivity and respiration by increasing the delivery rate of nutrients and gasses and/or by reducing the size of the benthic boundary layer that limits diffusion.

A second possibility is that productivity and respiration differed between the treatments because of changes in the species composition of the biofilm. The structure and composition of the biofilm is sensitive to near-bed flow, and increasing velocity and turbulence can induce shifts in dominance to algal taxa that have a more prostrate physiognomy adapted to higher flow conditions (Stevenson 1990, 1996, Biggs et al. 1998). Because taxa with different physiognomy can differ in their rates of metabolism (Steinman et al. 1992), changes in productivity and respiration could have resulted from shifts in community composition as greater substrate heterogeneity increased flow velocity and turbulence. While we did not note any qualitative differences in the physiognomy of the algae that colonized the tiles (i.e., all tiles appeared to be dominated by diatoms with no evidence of filamentous algae), a full characterization of the structure of the biofilm was beyond the scope of this study. Therefore, we do not have any evidence that helps us to distinguish between these first two potential explanations. However, whether substrate heterogeneity influenced ecological processes via stimulation of metabolism by individual taxa in the biofilm, or via changes in the species composition of the biofilm, alterations to near-bed flow were the most likely intermediary.

A third potential explanation for the differences in productivity and respiration between treatments is that there were differences in the magnitude of top-down control by grazers. Herbivores can increase or decrease the metabolism of a biofilm depending on how they influence competitive interactions among algae and bacteria (see Feminella and Hawkins 1995). If substrate heterogeneity affected the abundance or composition of grazers, either directly by means of substrate preferences or indirectly via changes in flow, then this could have resulted in differential grazing intensity between the two treatments and contributed to contrasting rates of ecological processes. However, data collected concurrently with ours suggest this was not the case. A companion study, which monitored the abundance and species composition of the dominant consumers of organic matter (macroinvertebrates) during the same period our study was performed, found no effect of habitat heterogeneity on the total abundance of macroinvertebrates in the riffles, or on the abundance of invertebrate herbivores (Brooks et al., *in press*). That study also could not identify any significant difference in the species composition of herbivores inhabiting LH and HH riffles. The fact that LH and HH riffles contained approximately the same abundance of a similar complement of herbivores suggests that the effects of habitat heterogeneity on ecosystem functioning were not mediated by a consumer response to heterogeneity.

Streams are known to play a vital role in the maintenance of the biosphere by influencing the flux of minerals, nutrients, and energy between terrestrial and marine environments, by influencing the decomposition of organic and inorganic wastes, and by contributing to the overall productivity of landscapes (Freckman et al. 1997, Palmer et al. 1997*b*, Covich et al. 1999). But it is widely recognized that humans are simplifying the physical structure of streams and rivers in ways that may compromise their ability to perform these vital ecological functions (Allan and Flecker 1993, Cairns 1995, Poff et al. 1997, Richter et al. 1997, Ricciardi and Rasmussen 1999). While human-induced habitat simplification is becoming increasingly common at all scales within watersheds, it is particularly prevalent at the scale our experiment was performed. Indeed, localized changes in the rates of erosion and sedimentation exert great impacts at the riffle and reach scales eliminating critical habitat for stream biota (Phillips 1993, Palmer et al. 2000). Common management and restoration practices attempt to conserve physical habitat heterogeneity or increase it via morphological improvements of the stream bed and/or the addition of in-stream structures (Gore and Shields 1995, Jungwirth et al. 1995, Muhar et al. 1995, Muhar 1996, Stanford et al. 1996, Palmer et al. 1997*a*). Therefore, our finding that variation in particle sizes at the riffle scale influences ecosystem functioning (primary production and benthic respiration) is pertinent to both real world perturbations in streams and to the common management responses to those perturbations. Given that human-induced simplification of habitats may be altering numerous aspects of physical heterogeneity potentially important for ecosystem-level processes, much additional research is needed to understand the links be-

tween heterogeneity and the functioning and sustainability of ecosystems.

ACKNOWLEDGMENTS

This work was supported by grants from the National Science Foundation to M. A. Palmer (DEB-9741101, DEB-9981376) and to M. A. Palmer and N. L. Poff (DEB-9622288). We thank the following persons for their help with labor intensive field manipulations and sampling: Rachel Alvestad, Justin Benoit, Chris Bertz, Jeremy Goetz, Amanda Graham, Sadie Jernigan, Karen Nelson, Suzanna Ribblett, Christopher Smith, and Dave Wooster. We also are indebted to Scott Collins, Stan Dodson, Karen Nelson, Dave Wooster, and two anonymous reviewers for constructive comments that improved the final version of this manuscript. We also thank the Douglas' and Vazques' for allowing us access to their land during this study.

LITERATURE CITED

Abrams, P. 1988. Resource productivity–consumer species diversity: simple models of competition in spatially heterogeneous environments. Ecology **69**:1418–1433.

Allan, D. J. 1995. Stream ecology: structure and function of running waters. Chapman and Hall, London, UK.

Allan, D. J., and A. S. Flecker. 1993. Biodiversity and conservation in running waters. BioScience **43**:32–43.

Bell, S. S., E. D. McCoy, and H. R. Mushinsky. 1991. Habitat structure: the physical arrangement of objects in space. Chapman and Hall, London, UK.

Biggs, B. J. F., D. G. Goring, and V. I. Nikora. 1998. Subsidy and stress responses of stream periphyton to gradients in water velocity as a function of community growth form. Journal of Phycology **34**:598–607.

Bott, T. L. 1996. Primary production and community respiration. Pages 533–556 *in* F. R. Hauer and G. A. Lamberti, editors. Methods in stream ecology. Academic Press, San Diego, California, USA.

Bott, T. L., J. T. Brock, A. Baattrup-Penderson, P. A. Chambers, W. K. Dodds, K. T. Himbeault, J. R. Lawrence, D. Planas, E. Snyder, and G. M. Wolfaardt. 1997. An evaluation of techniques for measuring periphyton metabolism in chambers. Canadian Journal of Fisheries and Aquatic Sciences **54**:715–725.

Bradshaw, P. 1971. Introduction to turbulence and its measurement. Permangon Press, New York, New York, USA.

Brooks, A., and K. Gregory. 1988. Channelization, river engineering, and geomorphology. Pages 145–167 *in* J. M. Hooke, editor. Geomorphology in environmental planning. Wiley and Sons, Chichester, UK.

Brooks, S., M. A. Palmer, B. J. Cardinale, C. Swan, and S. Riblett. Assessing the success of streambed restoration: limitations of community structure data. Restoration Ecology, *in press.*

Cairns, J., Jr. 1995. Rehabilitating damaged ecosystems. Second edition. Lewis Publishers, Ann Arbor, Michigan, USA.

Carling, P. 1992. The nature of the fluid boundary layer and the selection of parameters for benthic ecology. Freshwater Biology **28**:273–284.

Claret, C. 1998. A method based on artificial substrates to monitor hyporheic biofilm development. International Review of Hydrobiologie **83**:135–143.

Covich, A. P., M. A. Palmer, and T. A. Crowl. 1999. The role of benthic invertebrate species in freshwater ecosystems. BioScience **49**:119–127.

Daily, G. C., editor. 1997. Natures' services; societal dependence on natural ecosystems. Island Press, Washington, D.C., USA.

Davis, J. A., and L. A. Barmuta. 1989. An ecologically useful classification of mean and near-bed flows in streams and rivers. Freshwater Biology **21**:271–282.

Dobson, A. P., A. D. Bradshaw, and A. J. M. Baker. 1997. Hopes for the future: restoration ecology and conservation biology. Science **277**:515–522.

Feminella, J. W., and C. P. Hawkins. 1995. Interactions between stream herbivores and periphyton: a quantitative analysis of past experiments. Journal of the North American Benthological Society **14**:465–509.

Finelli, C. M., D. D. Hart, and D. M. Fonseca. 1999. Evaluation the spatial resolution of an acoustic Doppler velocimeter and the consequences for measuring near-bed flows. Limnology and Oceanography **44**:1793–1801.

Fisher, S. G., L. J. Gray, N. B. Grimm, and D. E. Busch. 1982. Temporal succession in a desert stream ecosystem following flash flooding. Ecological Monographs **52**:93–110.

Freckman, D. W., T. H. Blackburn, L. Brussaard, P. Hutchings, M. A. Palmer, and P. V. R. Snelgrove. 1997. Linking biodiversity and ecosystem functioning of soils and sediments. Ambio **26**:556–562.

Frissell, C. A., and R. K. Nawa. 1992. Incidence and causes of physical failure of artificial habitat structures in streams in western Oregon and Washington. North American Journal of Fisheries Management **12**:182–197.

Gao, Q., M. Yu, and X. Yang. 2000. A simulation analysis of the relationship between regional primary production and vegetation structure under climatic change scenarios. Ecological Modelling **131**:33–45.

Gore, J. A., and F. D. Shields, Jr. 1995. Can large rivers be restored? BioScience **45**:142–152.

Graf, W. L. 1999. Dam nation: a geographic census of American dams and their large scale hydrologic impacts. Water Resources Research **35**:1305–1311.

Grimm, N. B. 1987. Nitrogen dynamics during succession in a desert stream. Ecology **68**:1157–1170.

Gustafson, E. J. 1998. Quantifying landscape spatial pattern: what is the state of the art? Ecosystems **1**:143–156.

Hanksi, I. 1981. Coexistence of competitors in patchy environments with and without predation. Oikos **37**:306–312.

Hart, D. D., and C. M. Finelli. 1999. Physical–biological coupling in streams: the pervasive effects of flow on benthic organisms. Annual Review of Ecology and Systematics **30**:363–395.

Hassell, M. P. 1980. Some consequences of habitat heterogeneity for population dynamics. Oikos **35**:150–160.

Hey, R. D., and C. R. Thorne. 1983. Accuracy of surface samples from gravel bed material. Journal of Hydraulic Engineering **109**:842–851.

Hilborn, R. 1975. The effect of spatial heterogeneity on the persistence of predator–prey interactions. Theoretical Population Biology **8**:346–355.

Holt, R. D., and M. P. Hassell. 1993. Environmental heterogeneity and the stability of host–parasitoid interactions. Journal of Animal Ecology **62**:89–100.

Jones, S. E., and M. A. Lock. 1989. Hydrolytic extracellular enzyme activity in heterotrophic biofilms from two contrasting streams. Freshwater Biology **22**:289–296.

Jungwirth, M., S. Muhar, and S. Schmutz. 1995. The effects of recreated instream and ecotone structures on the fish fauna of an epipotamal river. Hydrobiologia **303**:195–206.

Kareiva, P. 1990. Population dynamics in spatially complex environments: theory and data. Philosophical Transactions of the Royal Society of London B **330**:175–190.

Levin, S. A. 1976. Population dynamic models in heterogeneous environments. Annual Review of Ecology and Systematics **7**:287–310.

Ligon, F. K., W. E. Dietrich, and W. J. Trush. 1995. Downstream ecological effects of dams. BioScience **45**:183–192.

Littell, R. C., G. A. Miliken, W. W. Stroup, and R. D. Wolfinger. 1996. SAS system for mixed models. SAS Institute, Inc. Cary, North Carolina, USA.

MacArthur, R. H., and J. W. MacArthur. 1961. On bird species and diversity. Ecology **42**:594–598.

Meyer, J. L. 1996. Conserving ecosystem function. Pages 136–145 *in* S. T. A. Pickett, R. S. Ostfeld, M. Shachak, and G. E. Likens, editors. The ecological basis of conservation: heterogeneity, ecosystems, and biodiversity. Chapman and Hall, New York, New York, USA.

Muhar, S. 1996. Habitat improvement of Austrian rivers with regard to different scales. Regulated Rivers: Research and Management **12**:471–482.

Muhar, S., S. Schmutz, and M. Jungwirth. 1995. River restoration concepts—goals and perspectives. Hydrobiologia **303**:181–194.

Naiman, R. J., J. M. Melillo, and J. E. Hobbie. 1986. Ecosystem alteration of boreal forest streams by beaver (*Castor canadensis*). Ecology **67**:1254–1269.

Nowel, A. R. M., and P. A. Jumars. 1984. Flow environments of aquatic benthos. Annual Review of Ecology and Systematics **15**:303–328.

Nusch, E. A. 1980. Comparison of different methods for chlorophyll and phaeopigment determination. Archiv fur Hydrobiologie Beih Ergebnisse der Limnologie **14**:14–36.

Osborne, L. L. 1983. Colonization and recovery of lotic epilithic communities: a metabolic approach. Hydrobiologia **99**:29–36.

Pacala, S. W., and J. Roughgarden. 1982. Spatial heterogeneity and interspecific competition. Theoretical Population Biology **121**:92–113.

Palmer, M. A., R. F. Ambrose, and N. L. Poff. 1997*a*. Ecological theory and community restoration ecology. Restoration Ecology **5**:291–300.

Palmer, M. A., A. P. Covich, B. J. Findlay, J. Gilbert, K. D. Hyde, R. K. Johnson, T. Kairesalo, S. Lake, C. R. Lovell, R. J. Naiman, C. Ricci, F. Sabater, et al. 1997*b*. Biodiversity and ecosystem processes in freshwater sediments. Ambio **26**:571–577.

Palmer, M. A., A. P. Covich, S. Lake, P. Biro, J. J. Brooks, J. Cole, C. Dahm, J. Gibert, W. Goedkoop, K. Martens, J. Verhoeven, and W. J. van de Bund. 2000. Linkages between aquatic sediment biota and life above sediments as potential drivers of biodiversity and ecological processes? BioScience **50**:1062–1075.

Palmer, M. A., C. C. Hakenkamp, and K. Nelson-Baker. 1997*c*. Ecological heterogeneity in streams: why variance matters. Journal of the North American Benthological Society **16**:189–202.

Phillips, J. D. 1993. Pre- and post colonial sediment sources and storage in the lower Neuse basin, North Carolina. Physical Geography **14**:272–284.

Pickett, S. T. A., R. S. Ostfeld, M. Shachak, and G. E. Likens, editors. 1996. The ecological basis of conservation: heterogeneity, ecosystems, and biodiversity. Chapman and Hall, New York, New York, USA.

Pierce, L. L., and S. W. Running. 1995. The effects of aggregating subgrid land surface variation on large-scale estimates of net primary production. Landscape Ecology **10**: 239–253.

Poff, N. L., J. D. Allan, M. B. Bain, J. R. Karr, K. L. Prestegaard, B. D. Richter, R. E. Sparks, and J. C. Stromberg. 1997. The natural flow regime: a paradigm for river conservation and restoration. BioScience **47**:769–784.

Power, M. E. 1995. Floods, food chains, and ecosystem processes in rivers. Pages 52–60 *in* C. G. Jones and J. H. Lawton, editors. Linking species and ecosystems. Chapman and Hall, New York, New York, USA.

Power, M. E., W. E. Dietrich, and K. O. Sullivan. 1998. Experimentation, observation, and inference in river and watershed investigations. Pages 113–132 *in* W. J. Resetarits and J. Bernardo, editors. Experimental ecology: issues and perspectives. Oxford University Press, New York, New York, USA.

Rahel, F. J. 2000. Homogenization of fish faunas across the United States. Science **288**:854–856.

Ricciardi, A., and J. B. Rasmussen. 1999. Extinction rates of North American freshwater fauna. Conservation Biology **13**:1220–1222.

Richter, B. D., D. P. Braun, M. A. Mendelson, and L. L. Master. 1997. Threats to imperiled freshwater fauna. Conservation Biology **11**:1081–1093.

Romani, A. M., and S. Sabater. 1999. Effect of primary producers on the heterotrophic metabolism of a stream biofilm. Freshwater Biology **41**:729–736.

Sala, O. E., F. S. Chapin, J. A. Armesto, E. Berlow, J. Bloomvield, R. Dirzo, E. Huber-Sanwald, L. F. Huenneke, R. B. Jackson, A. Kinzig, R. Leemans, D. M. Lodge, et al. 2000. Global biodiversity scenarios for the year 2010. Science **287**:1770–1774.

SAS Institute Inc. 1996. SAS/STAT user's guide. Release 6.03 Edition. SAS Institute, Inc., Cary, North Carolina, USA.

Shields, F. D., Jr., and R. H. Smith. 1992. Effects of large woody debris removal on physical characteristics of a sandbed river. Aquatic Conservation: Marine and Freshwater Ecosystems **2**:145–163.

Stanford, J. A., J. V. Ward, W. J. Liss, C. A. Frissell, R. N. Williams, J. A. Lichatowich, and C. C. Coutant. 1996. A general protocol for restoration of regulated rivers. Regulated Rivers: Research and Management **12**:391–413.

Steinman, A. D., and G. A. Lamberti. 1996. Biomass and pigments of benthic algae. Pages 295–311 *in* F. R. Hauer and G. A. Lamberti, editors. Methods in stream ecology. Academic Press, New York, New York, USA.

Steinman, A. D., P. J. Mulholland, and W. R. Hill. 1992. Functional responses associated with growth form in stream algae. Journal of the North American Benthological Society **11**:229–243.

Stevenson, R. J. 1990. Benthic algal community dynamics in a stream during and after a spate. Journal of the North American Benthological Society **9**:277–288.

Stevenson, R. J. 1996. The stimulation and drag of current. Pages 321–341 *in* R. J. Stevenson, M. L. Bothwell, and R. L. Lowe, editors. Algal ecology: freshwater benthic ecosystems. Academic Press, New York, New York, USA.

Stock, M. S., and A. K. Ward. 1989. Establishment of a bedrock epilithic community in a small stream: microbial (algal and bacterial) metabolism and physical structure. Canadian Journal of Fisheries and Aquatic Sciences **46**:1874–1883.

Uehlinger, E., and M. W. Naegeli. 1998. Ecosystem metabolism, disturbance, and stability in a prealpine gravel bed river. Journal of the North American Benthological Society **17**:165–178.

van Zyll De Jong, M. C., I. G. Cox, and D. A. Scruton. 1997. An evaluation of instream habitat restoration techniques on salmonid populations in a Newfoundland stream. Regulated Rivers Research and Management **13**:603–614.

Vollenweider, R. A. 1974. A manual on methods for measuring primary production in aquatic environments. I.B.P. Handbook 12. Second edition. Blackwell Scientific, Oxford, UK.

Wiberg, P. L., and J. D. Smith. 1991. Velocity distribution and bed roughness in high-gradient streams. Water Resources Research **27**:825–838.