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STREAM ECOSYSTEM RESPONSE TO LIMESTONE TREATMENT IN ACID IMPACTED WATERSHEDS OF THE ALLEGHENY PLATEAU

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Abstract. Restoration programs are expanding worldwide, but assessments of restoration effectiveness are rare. The objectives of our study were to assess current acid-precipitation remediation programs in streams of the Allegheny Plateau ecoregion of West Virginia (USA), identify specific attributes that could and could not be fully restored, and quantify temporal trends in ecosystem recovery. We sampled water chemistry, physical habitat, periphyton biomass, and benthic macroinvertebrate and fish community structure in three stream types: acidic (four streams), naturally circumneutral (eight streams), and acidic streams treated with limestone sand (eight streams). We observed no temporal trends in ecosystem recovery in treated streams despite sampling streams that ranged from 2 to 20 years since initial treatment. Our results indicated that the application of limestone sand to acidic streams was effective in fully recovering some characteristics, such as pH, alkalinity, Ca^{2+}, Ca:H ratios, trout biomass and density, and trout reproductive success. However, recovery of many other characteristics was strongly dependent upon spatial proximity to treatment, and still others were never fully recovered. For example, limestone treatment did not restore dissolved aluminum concentrations, macroinvertebrate taxon richness, and total fish biomass to circumneutral reference conditions. Full recovery may not be occurring because treated streams continue to drain acidic watersheds and remain isolated in a network of acidic streams. We propose a revised stream restoration plan for the Allegheny Plateau that includes restoring stream ecosystems as connected networks rather than isolated reaches and recognizes that full recovery of acidified watersheds may not be possible.

Key words: acid precipitation; Allegheny Plateau (West Virginia, USA); benthic macroinvertebrates; fishes; limestone-sand treatment; nutrients; restoration effectiveness; stream ecosystem attributes; stream remediation.

Introduction

The use of limestone to mitigate the biological effects of acid deposition and surface-water acidification has become widespread (Weatherley 1988). Large-scale liming programs have been developed in the northeastern United States and Canada (Olem 1991), Wales (Ormerod et al. 1990), Sweden (Appelberg and Svenson 2001), Finland (Kauppi et al. 1990), and Norway (Sandoy and Romundstad 1995). However, despite numerous attempts to assess the effectiveness of these programs, several questions remain as to whether acidified watersheds of the eastern United States are being successfully restored, and, if not, whether there are opportunities for improved restoration approaches.

Biological degradation of aquatic ecosystems resulting from acid precipitation is well documented (Schindler 1988, Herlihy et al. 1993). This is particularly true in the central Appalachian Mountains of the eastern United States, which experiences one of the highest acid-loading rates of any area in the nation (Herlihy et al. 1993). Many streams in the central Appalachians with poor buffering capacity are being altered structurally and functionally at all trophic levels due to acidification. The primary causes of these changes are elevated inorganic monomeric aluminum concentrations and reduced pH associated with episodic acidification (Baker et al. 1996, Wigington et al. 1996).

Acidification has been shown to alter periphyton and macroinvertebrate communities (Kobuszewski and Perry 1993, Meegan and Perry 1996). Studies have demonstrated both reduced and elevated periphyton biomass and a shift in periphyton community composition in response to acidification (Meegan and Perry 1996). Acidification also has been shown to reduce overall macroinvertebrate species richness, density, and biomass (Krueger and Waters 1983). However, some acidified streams may not show a reduction in macroinvertebrate density or evenness, which may be attributed to the proliferation of acid-tolerant species (Kobuszewski and Perry 1993).

Fish communities may be indirectly (reduced and altered food sources) and directly (decreased survival rates) impacted by acidification (Krueger and Waters 1983, Baker et al. 1996, Van Sickle et al. 1996). Impacts

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to brook trout (*Salvelinus fontinalis*) populations are especially severe because they typically inhabit small, high-elevation catchments, which are highly vulnerable to acidification (Herlihy et al. 1993). In fact, the West Virginia Division of Natural Resources approximates that 25% of streams within West Virginia (WV) that historically supported brook trout populations have been degraded by acid deposition (Menendez et al. 1996). Episodic acidification has been found to reduce brook trout abundance and biomass (Baker et al. 1996), and reduce survival rates for all early life-history stages (Marschall and Crowder 1996, Petty et al. 2005).

Numerous techniques have been developed to combat these impacts. Remediation techniques include the application of limestone sand directly into acid-impacted stream channels (i.e., Clayton et al. 1998, Keener and Sharpe 2005), whole-catchment liming using powdered $CaCO_3$ (i.e., Bradley and Ormerod 2002), and automated limestone dosers (i.e., Sandoy and Romundstad 1995, Clayton and Menendez 1996, Appelberg and Svenson 2001). State agencies in the central Appalachians typically use annual in-stream applications of limestone sand along with hydropowered limestone dosers (Downey et al. 1994, Zurbuch et al. 1996, Hudy et al. 2000). WV state agencies began treating acidic streams in 1970 with automated dosers, but now focus on in-stream sand application once it was found to be a more effective and cost-efficient form of treatment (Clayton et al. 1998). By 2002, a total of 51 streams were being treated in West Virginia with >5000 kg of limestone sand high (>97%) in calcium carbonate. The approach taken in West Virginia is to add twice the amount of limestone needed to treat the annual acid load in the targeted stream in the first year of treatment. This amount is then reduced by half in subsequent years. This approach is taken in order to prevent extreme reductions in pH and/or in Ca:H ratios during acidic episodes (Clatyon et al. 1998).

Limestone treatment of acid-impaired streams has proven to be beneficial to in-stream water chemistry with post-treatment trends including elevated pH and dissolved calcium, and reduced total and monomeric aluminum concentrations (Weatherley et al. 1991, Downey et al 1994, Menendez et al. 1996). Limestone treatment also has been shown to increase macroinvertebrate and fish species richness compared to pre-treatment conditions (Downey et al. 1994, Clayton and Menendez 1996). In West Virginia, Menendez et al. (1996) and Clayton et al. (1998) observed the colonization of reproducing fish populations into streams lacking resident fish populations prior to limestone treatment. However, other studies have shown little or no recovery in macroinvertebrate or fish communities following limestone treatment (Eggleton et al. 1996, Simmons and Doyle 1996, Bradley and Ormerod 2002, LeFevre and Sharpe 2002).

Despite extensive study, numerous questions remain about whether acid-impacted streams are being fully restored by limestone mitigation programs. As is the case with most stream restoration projects throughout the United States and worldwide, the ability of limestone mitigation to successfully restore the full range of stream ecosystem attributes has not been adequately evaluated (Ormerod 2004, Bernhardt et al. 2005, Palmer et al. 2005). Most previous studies have examined responses in only a small number of streams or have failed to fully quantify responses across a range of chemical and biological attributes. Furthermore, proper temporal scale is well recognized as being a critical component in judging the ecological success of a restoration project (Lake 2001, Lepori et al. 2005). To our knowledge, there have been no studies to examine temporal trends in the recovery process of acid-impacted streams following limestone treatment in the central Appalachians. Given these uncertainties, the overriding objective of our study was to determine the extent of ecological recovery (chemical and biological) and temporal trends in recovery of acid-impaired streams treated with limestone sand.

Materials and Methods

Study area

Our study was conducted within the Cheat River and Gauley River watersheds in the Central Appalachian Mountains of east-central West Virginia, USA (Appendix A; see Plate 1). Both watersheds are part of the Allegheny Plateau ecoregion and originate in Pocahontas County, West Virginia. The Cheat River flows north draining ~3400 km^2 from an elevation of 1425 m in the headwaters to 248 m at its confluence with the Monongahela River. The Gauley River drains a 3683-km^2 watershed and flows west–southwest ranging in elevation from 1402 m in its headwaters to 183 m at its confluence with the New River. Land cover in both watersheds is predominately forested. The geology of both watersheds consists predominantly of sandstones and shales. Outcrops of limestone are rare. This area receives one of the highest acid loading rates in the eastern U.S., and both the Gauley and Cheat River watersheds have been targeted by the West Virginia Division of Natural Resources (WVDNR) as high priorities for restoration.

Site selection

We studied a total of 20 streams within these watersheds: 4 acid-impaired (acidic), 8 naturally circumneutral (circumneutral) and 8 historically acidic streams treated annually with limestone sand (treated) (Appendix A). We chose naturally circumneutral streams within the study area to serve as reference conditions against which to judge the success of acid-remediation programs. Circumneutral streams with minimal anthropogenic impairment are representative of the "best available conditions" in this region and provide the only reasonable reference condition against which to evaluate limestone remediation (Campbell 2000). Treated streams used in this study have been treated regularly

TABLE 1. Descriptive characteristics of 20 study streams in the Cheat River and Gauley River watersheds, West Virginia, USA.

Site	Status†	River basin	Basin area (km^2)	Mean pH (SE)	Pre-treatment mean pH‡	Distance from treatment location (km)	Distance to mainstem (m)
Upper Second Fork	A	Cheat	3.6	4.74 (0.24)			4026
South Fork Red Creek	A	Cheat	22.0	4.74 (0.22)			153
North Fork Cranberry River	A	Gauley	24.1	5.01 (0.26)			1416
Red Creek	A	Cheat	77.0	4.93 (0.20)			0
Little Odey	C	Cheat	0.9	6.42 (0.36)			3241
Grants Branch	C	Cheat	3.5	6.96 (0.22)			78
Gandy Run	C	Cheat	5.5	6.66 (0.26)			106
Jakeman	C	Gauley	7.5	7.14 (0.30)			158
Rattlesnake Run	C	Cheat	9.5	7.30 (0.14)			832
Big Run	C	Cheat	10.2	7.02 (0.26)			67
Little Black Fork	C	Cheat	12.3	7.22 (0.16)			196
South Fork Cranberry River	C	Gauley	36.1	6.69 (0.17)			0
McGee Run	T-8	Cheat	5.8	6.69 (0.33)	4.30	1.3	30
Crouch Run	T-13	Cheat	7.2	6.58 (0.41)	4.60	1.8	62
Red Run	T-7	Cheat	12.7	6.23 (0.14)	4.50	1.2	7728
Big Rocky Run	T-2	Gauley	22.2	7.11 (0.36)	5.60	4.7	708
First Fork	T-13	Cheat	22.9	6.52 (0.28)	5.70	1.5	45
Dogway Fork	T-16	Gauley	24.8	7.46 (0.18)	4.70	10.3	555
North Fork Cherry River	T-7	Gauley	57.3	7.09 (0.31)	5.20	10.0	0
Otter Creek	T-20	Cheat	75.1	6.65 (0.30)	5.50	18.3	196

Note: There are no data available for pre-treatment pH and distance to treatment location for acidic and circumneutral streams because they have not been treated.

† Sites are sorted by status (A, acidic; C, circumneutral; and T, treated, with number of years since initial treatment) and by basin area.

‡ Data sources: Clayton et al. (1998) for McGee Run, Crouch Run, and First Fork; West Virginia Division of Natural Resources (*unpublished data*) for Red Run, Big Rocky Run, and North Fork Cherry River; Menendez et al. (1996) for Dogway Fork; and Menendez (1972) for Otter Creek.

following WVDNR protocols and were known to possess highly acidic conditions prior to treatment, including: low pH (Table 1), acidophilic macroinvertebrate communities, and lack of reproducing fish populations (Menendez et al. 1996, Clayton et al. 1998, Menendez 1972; WVDNR, *unpublished data*). Acidic streams were selected to match the pretreatment conditions of treated streams. All streams chosen for this study were coldwater systems (in-stream temperatures never exceeding 20°C) located within predominantly mixed deciduous–coniferous forested catchments within the Monongahela National Forest (Appendices A and B).

Physical characteristics and treatment

To control for the effects of physical habitat we selected streams possessing relatively good habitat quality as determined using U.S. Environmental Protection Agency (EPA) rapid visual habitat assessment (RVHA) protocols (Barbour et al. 1999). All streams possessed RVHA scores that exceeded 150 (maximum possible RVHA = 200) (Appendix B). Furthermore, we selected study sites to cover a broad range of canopy cover (34–96%) (Appendix B). We measured canopy cover within each stream reach with a concave spherical densiometer. Four directional readings were taken every 10 m within each study reach (Bopp 2002). All readings were averaged to yield a total average canopy-cover percentage (±2.4%) per stream reach surveyed (Appendix B). We monitored daily mean flow from a central USGS gaging station in each basin to quantify the regional hydrologic regime during the study period.

ArcGIS (version 8.3, ESRI 2003) was used to calculate stream basin area, which ranged from 0.9 to 77.0 km^2, and to measure the distance from each sampled stream reach to the nearest downstream mainstem (≥4th-order stream) (Table 1). In treated streams, we also used ArcGIS to determine the distance from each study reach to the furthest upstream limestone application point designed to treat the entire acid load of the study reach (Table 1). The number of years since initial treatment in treated streams varied from 2 to 20 years (Table 1). Otter Creek is treated near its headwaters with a single, hydropowered limestone doser (Menedez 1972). Dogway Fork is treated near its headwaters through direct application of limestone sand along with a limestone doser near the midpoint of the watershed (Zurbuch et al. 1996). The remaining six treated streams are treated through the in-stream application of limestone sand following treatment guidelines outlined by Clayton et al. (1998).

Water chemistry

A total of four water-chemistry samples were collected at each site in April, September, and October 2003 and April 2004. Previous studies in this region have focused on temporal variability in water chemistry and the effectiveness of limestone remediation in preventing acidic episodes in treated streams (Menendez et al. 1996, Zurbuch et al. 1996, Clayton et al. 1998). To avoid redundancy, our water sampling was designed simply to obtain an estimate of average chemistry at each site. Consequently, water samples were taken across a range of low to moderate flow periods in what was a typical, highly variable flow year (Appendix C). Each season, one 250-mL water sample was filtered using a Nalgene polysulfone filter holder and receiver with mixed cellulose ester membrane disc filters with a 0.45-μm pore size. Filtered samples were immediately treated with 2 mol/L 2% nitric acid to maintain pH <2. Filtered samples were used to analyze dissolved constituents. An unfiltered 250-mL water sample was also collected and treated with 2 mol/L 2% nitric acid. This unfiltered acidified sample was used for the analysis of total cations and anions. The third sample was an unfiltered 472-mL water sample. This sample was used to analyze alkalinity and inorganic monomeric aluminum. All samples were kept on ice after collection, and stored in the laboratory at 4°C until analysis could be completed at the National Research Center for Coal and Energy (NRCCE) at West Virginia University (Morgantown, West Virginia, USA).

The following chemical variables were analyzed: calcium (Ca^{2+}), magnesium (Mg^{2+}), potassium (K^{+}), sodium (Na^{2+}), chloride (Cl^{-}), nitrate (NO_3^{-}), sulfate (SO_4^{2-}), total phosphorus (P_t) and dissolved phosphorus (P_d), phosphate (PO_4^{-}), alkalinity (alk), total aluminum (Al_t), dissolved aluminum (Al_d), and inorganic monomeric aluminum (Al_{im}) according to EPA standard methods (EPA 1991). Calcium-to-hydrogen ionic ratios (Ca:H) were calculated using in-stream pH measurements and Ca^{2+} results from the laboratory. Ca:H ratios are an effective index for determining suitable water chemistry for fish survival (Brown and Skeffington 1992, Clayton et al. 1998). Alkalinity in the form of $CaCO_3$ was analyzed with an auto-titrator method (EPA 1991; method 310.1). Cations were measured with inductively coupled plasma optical emission spectroscopy (ICP-OES) (EPA 1991; method 200.7). Anions such as Cl^{-}, NO_3^{-}, SO_4^{2-}, and PO_4^{-} were quantified with a flow-injection analyzer according to EPA methods 325.3, 353.2, 375.2, and 365.1, respectively (EPA 1991). Al_{im} was analyzed with cation exchange through a strongly acidic exchange resin followed by ICP-OES (EPA 1991; method 200.7).

Temperature (in degrees Celsius, °C), specific conductance (SpC) (in microseconds per centimeter, μs/cm), and pH were measured in the field with a YSI 600 XL multi-parameter water-quality monitor (YSI, Yellow Springs, Ohio, USA) during each water-chemistry sample ($n = 4$ samples). We used area-velocity techniques to calculate discharge at each site during each sampling season with a Marsh-McBirney Flo-Mate (Marsh-McBirney, Frederick, Maryland, USA).

Biofilm

We collected biofilm samples from each study site in June and August 2003 to provide a relative estimate of the biomass of primary producers in acidic, circumneutral, and treated streams. Reach length for all biological sampling was set at 40 times average wetted width (width measured every 10 m) with a 150-m minimum and 300-m maximum length (Lyons 1992). We followed EPA's Environmental Monitoring and Assessment Program (EMAP) standard protocols for periphyton sampling in erosional habitat, which involved randomly selecting 11 cobbles from riffle habitats scattered throughout the study reach (Hill 1998). We then followed methods outlined by Lowe and Laliberte (1996) for rock scraping and biofilm processing to obtain estimates of ash-free dry mass (AFDM) in milligrams of total biofilm from each site. AFDM was divided by the total area sampled per stream to yield amount of biofilm present per unit area of stream bed (in milligrams per square centimeter, mg/cm^2).

Benthic macroinvertebrates

We sampled benthic-macroinvertebrate communities at each site in May 2003. We decided to sample macroinvertebrates in one season based upon previous studies showing small seasonal variation in macroinvertebrate communities relative to spatial variation (Bopp 2002). Consequently, we attempted to maximize the number of streams sampled, while minimizing sampling intensity over time. We chose spring as the benthic-macroinvertebrate sample period, because it is the season when stream water chemistry in this region tends to be most acidic due to high flows from precipitation and/or snow melt (DeWalle and Swistock 1994, Petty and Barker 2004). In addition, taxon richness of stream invertebrates tends to be highest in spring (Cuffney et al. 1993). A modified Hess sampler (0.10 m^2, 250-μm mesh net) was used to collect benthic macroinvertebrates at five randomly selected locations within targeted riffle habitat at each study reach (Rosemond et al. 1992). The five samples were combined, elutriated through a 250-μm mesh sieve and preserved with a mixture 95% ethanol and Rose Bengal solution to facilitate sample processing (Williams and Williams 1974).

In the laboratory, benthic samples were filtered through 1-mm and 250-μm sieves. All macroinvertebrates retained in the 1-mm sieve were identified using Merritt and Cummins (1996) and Wiggins (1998). Oligocheates and Nematomorphs were identified to order, Ephemeroptera, Plecoptera, and Trichoptera (EPT) were identified to genera, and the remaining taxa collected were identified to the family level. The 250-μm samples were subsampled using a Folsom phytoplankton splitter (Wildco, Buffalo, New York, USA) and 12.5% of this sample was identified as described above. Subsamples were multiplied by 8 and added to the 1-mm samples for an overall estimate of macroinvertebrate community structure. A subset of individual identifications was verified by Janet Clayton (West Virginia Division of Natural Resources) and through reference collections at West Virginia University. An ocular micrometer was used to measure all head-capsule widths to the nearest 0.1 mm. We then used regression equations to convert head-capsule widths to dry mass (DM) (Benke et al. 1999) in order to estimate relative macroinvertebrate biomass per stream (in milligrams of dry mass per square meter, mg DM/m^2). Oligocheatea and Nematomorpha were removed and analyzed separately due to incomplete specimens with indistinguishable anterior ends. Decapoda ($n = 48$ specimens) were removed from all subsequent benthic-macroinvertebrate analyses, because they have been shown to significantly increase the variance in stream macroinvertebrate biomass estimates (Miller 1985). Macroinvertebrates of terrestrial origin were also removed from in-stream density and biomass estimates. Finally, Collembola ($n = 1$ specimen), Copepoda ($n = 2$ specimens), Hydracarina ($n = 8$ specimens), and Hemiptera Gerridae ($n = 2$ specimens) were removed from biomass estimates due to lack of biomass/head-capsule width regressions for these taxa.

We calculated benthic-macroinvertebrate density and biomass as the number of individuals and biomass collected within the stream reach divided by the area of stream sampled for all taxa at the lowest taxonomic level identified. All taxa collected were grouped according to their level of acid tolerance (acid tolerant, acid sensitive, or tolerance unknown) following Rosemond et al. (1992), Clayton and Menendez (1996), and Simmons and Doyle (1996) (Appendix D). Percentage of acid-tolerant and acid-sensitive taxa biomass and number of acid-tolerant and acid-sensitive species per stream were then calculated. All aquatic taxa also were assigned to one of the following functional feeding groups (FFGs): collector/filterer, collector/gatherer, scraper, shredder, predator, or unknown following Merritt and Cummins (1996) at the lowest taxonomic level identified. Percentage of biomass for each FFG was then calculated for each stream reach sampled.

Fishes

We sampled fish communities at each site in July 2003. Fish sampling was conducted at one time to minimize disturbance to the study sites and maximize the number of streams that could be sampled. Sampling was conducted in summer, because (1) flows are low enough to allow efficient sampling, (2) fish distributions tend to remain relatively stable in summer (Barbour et al. 1999), and (3) it is the best time to obtain a reliable estimate of brook trout recruitment success (Petty et al. 2005).

A one-pass electrofishing procedure modified from EPA-EMAP protocols (McCormick et al. 2001) was used to sample fishes in each stream reach. Electrofishing was conducted with a backpack electrofisher (DC, 60 Hz, 400–600 V; Smith-Root, Vancouver, Washington, USA) and a combination of dip nets and a portable seine (Petty et al. 2005). All fish collected were anesthetized in clove oil (20% ethanol) and measured for standard length (± 1 mm) and mass (± 0.1 g). Brook trout, brown trout, rainbow trout, rock bass, and smallmouth bass were measured individually. All other fishes were sorted into species and weighed as a group. Minimum and maximum standard length (in millimeters, mm) for each species batch also was measured. All fish were allowed to recover from anesthetic and returned to their approximate location of capture.

Density (in number per square meter, no./m^2) and biomass (in grams per square meter, g/m^2) were calculated for each species at each site. We also quantified the density and biomass of juvenile and adult trout separately. Juvenile trout were identified on the basis of length (≤ 75 mm) from published and unpublished data on length frequency distributions in this region (Petty et al. 2005).

Statistical analysis

The central purpose of our statistical analysis was to quantify differences in chemical and biological attributes among acidic, circumneutral, and treated streams and determine which, if any, attributes were fully or partially recovered through limestone treatment. A secondary objective was to quantify relationships between recovery in treated streams and the amount of time since treatment began. All statistical tests were conducted at an a priori alpha level of 0.05. To approximate normality, it was necessary to transform nearly all measured variables, including basin area (in square kilometers, km^2) [$\ln(x + 1)$], water-chemistry concentrations [$\ln(x + 1)$], biofilm biomass (in milligrams per square centimeter, mg/cm^2) [$\ln(x + 1)$], benthic-macroinvertebrate density (in number per square meter, no./m^2) [$\log(x)$] and biomass (in milligrams of dry mass per square meter, mg DM/m^2) [$\log(x)$], all benthic-macroinvertebrate percentage data (arcsine square-root of proportion), fish density (in number per square meter, no./m^2) [$\log(x + 1)$], and fish biomass. Minimum detection-limit values for water-chemistry parameters were used for analysis if results were below detectable range.

We used repeated-measures ANOVA to assess the effects of stream status (i.e., acidic, circumneutral, or treated) and season on water chemistry. For treated streams, simple linear regression was used to assess the effect of time since initial treatment and distance from treatment location on water chemistry. We used two-way ANOVA to assess the effects of stream status and season on biofilm biomass. Simple linear regressions were used to assess the effect of canopy cover, time since initial treatment, and distance from treatment location on biofilm.

We used analysis of covariance (ANCOVA) to determine the effects of stream status, basin area (in square kilometers, km^2), and canopy cover on benthic-macroinvertebrate richness, density, total site biomass, percentage acid-sensitive and acid-tolerant biomass, number of acid-sensitive and acid-tolerant taxa, and

TABLE 2. Water-chemistry parameters by stream status across seasonal sampling periods, together with F values from repeated-measures ANOVA designed to detect an effect of stream status and season on each water-chemistry variable.

Chemical parameter†	Detection limit	Stream status: Acidic, Mean	Acidic, SE	Circumneutral, Mean	Circumneutral, SE	Treated, Mean	Treated, SE	Status, $F_{2,17}$	Season, $F_{3,49}$	Status × Season, $F_{6,49}$
pH	±0.2	4.89^{a}	0.14	6.91^{b}	0.09	6.79^{b}	0.12	70.68 ***	23.62***	2.61*
Alk ($CaCO_3$) (mg/L)	±0.2	1.55^{a}	0.35	9.81^{b}	1.43	6.59^{b}	0.96	5.2*	0.86^{NS}	1.84^{NS}
Ca^{2+} (mg/L)	0.03	1.32^{a}	0.10	4.09^{b}	0.52	3.21^{b}	0.32	5.19*	49.0***	1.07^{NS}
Ca^{2+}:H^{+}	NA‡	10^{a}	3	3300^{b}	789	3364^{b}	1062	43.30***	15.55***	0.93^{NS}
Mg^{2+} (mg/L)	0.02	0.50^{a}	0.08	0.80^{b}	0.05	0.44^{a}	0.03	12.63***	14.26***	2.32*
Na^{+} (mg/L)	0.02	0.25^{a}	0.08	0.41^{b}	0.04	0.21^{a}	0.04	5.11*	16.48***	3.66**
K^{+} (mg/L)	0.01	0.30^{a}	0.08	0.46^{b}	0.05	0.21^{a}	0.02	7.5**	40.71***	6.61***
SO_4^{2-} (mg/L)	0.1	4.14^{a}	0.33	4.22^{a}	0.24	4.26^{a}	0.33	0.07^{NS}	22.74***	2.29^{NS}
NO_3^{-} (mg/L)	0.02	0.19^{a}	0.04	0.36^{b}	0.03	0.21^{a}	0.03	9.65**	50.15***	4.25***
Cl^{-} (mg/L)	1.0	1.02^{a}	0.01	1.15^{b}	0.07	1.08^{a}	0.03	0.95^{NS}	0.45^{NS}	1.36^{NS}
P_t (mg/L)	0.03	0.05^{a}	0.01	0.09^{a}	0.03	0.05^{a}	0.006	0.74^{NS}	5.88**	1.57^{NS}
P_D (mg/L)	0.03	0.03^{a}	0.002	0.04^{a}	0.002	0.04^{a}	0.003	3.5^{NS}	2.55^{NS}	1.55^{NS}
PO_4^{-} (mg/L)	0.001	0.002^{a}	0.0004	0.003^{a}	0.0003	0.002^{a}	0.0003	3.1^{NS}	1032.4***	1.38^{NS}
Al_t (mg/L)	0.01	0.29^{a}	0.08	0.04^{b}	0.008	0.14^{c}	0.01	29.68***	11.92***	49.0***
Al_d (mg/L)	0.01	0.14^{a}	0.02	0.02^{b}	0.004	0.09^{a}	0.01	21.29***	8.10***	1.74^{NS}
Al_{im} (mg/L)	0.01	0.08^{a}	0.03	0.02^{b}	0.001	0.04^{ab}	0.009	4.91*	1.51^{NS}	3.08*
Temperature (°C)	±0.15	10.27^{a}	0.92	12.05^{b}	0.53	10.05^{a}	0.64	4.86*	35.96***	2.11^{NS}
SpC (μs/cm)	±0.5%	21.14^{a}	1.47	42.38^{b}	6.73	27.59^{ab}	1.90	3.12*	7.79***	0.83^{NS}

Note: Parameter means with the same lowercase superscript letter did not differ significantly among stream status ($P > 0.05$). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant.

† Alk, alkalinity; P_t, total phosphorus; P_d, dissolved P; Al_t, total aluminum; Al_d, dissolved Al; Al_{im}, inorganix monomeric Al; SpC, specific conductivity.

‡ NA = not applicable.

percentage FFGs. In treated steams, we used linear regression to assess the effect of time since initial treatment and distance to treatment location on all benthic-macroinvertebrate parameters.

To further investigate the response of benthic communities to limestone treatment, we used Jaccard similarity followed by nonmetric multidimensional scaling (NMDS) and mean-similarity (MeanSIM) analysis to assess entire community similarities in circumneutral, acidic and limestone-treated streams. Based on preliminary results, we divided treated streams into those sampled <2 km from treatment (T1) and those sampled >3 km from treatment (T2). Jaccard similarity was calculated based on presence and absence of maroinvertebrate taxa at the lowest taxonomic level identified. NMDS was then used to visually compare community similarities among sites. To create the NMDS ordination, the similarity matrix was first converted to a dissimilarity matrix (Van Sickle 1997). The more similar two sites are in terms of taxa present the nearer they are to each other on the NMDS map (Clarke and Green 1988). To facilitate interpretation of the NMDS axes, we used t tests to compare NMDS scores between sites where specific taxa were present or absent. A significant difference along a given axis was interpreted to mean that the particular taxon was an important contributor to the NMDS axis. This was done for NMDS axes 1 and 2. We assessed the strength of the stream-classification scheme (Acidic, Circumneutral, Treatment Group 1, and Treatment Group 2) by comparing both within-class and between-class community similarities with MeanSIM analysis (MEANSIM6 software; Smith et al. 1990, Van Sickle 1997). Permutation tests (10 000) were used to test the null hypothesis of "no class structure" (Van Sickle 1997) given the stream-classification scheme above.

Finally, we used ANOVA to assess the effect of stream status on fish species richness, total site density, and biomass, trout density and biomass, and trout young-of-year density and biomass. Simpler linear regression was used to assess the effects of basin area, time since initial treatment, distance from treatment location, and distance to nearest mainstem on fish-community variables in treated streams.

RESULTS

Water chemistry

We found a significant effect of stream status on water chemistry in 10 of the 16 parameters measured. Specifically, we found that pH, alk (alkalinity), Ca^{2+}, Ca:H (calcium-to-hydrogen ionic ratio), and SpC (specific conductance) were significantly higher in both circumneutral and treated streams compared to acidic streams (Table 2, Fig. 1). Average pH range was 4.7–5.0 in acidic streams, 6.4–7.3 in circumneutral streams, and 6.2–7.5 in treated streams (Table 1). Furthermore, average Mg^{2+}, Na^{+}, K^{+}, and NO_3^{-} concentrations were significantly higher in circumneutral streams compared to both acidic and treated streams (Table 2). However, this trend was highly variable within sampling periods (Fig. 1). In addition, Al_t (total aluminum) and Al_d (dissolved aluminum) levels were significantly lower in circumneutral streams compared to both acidic and

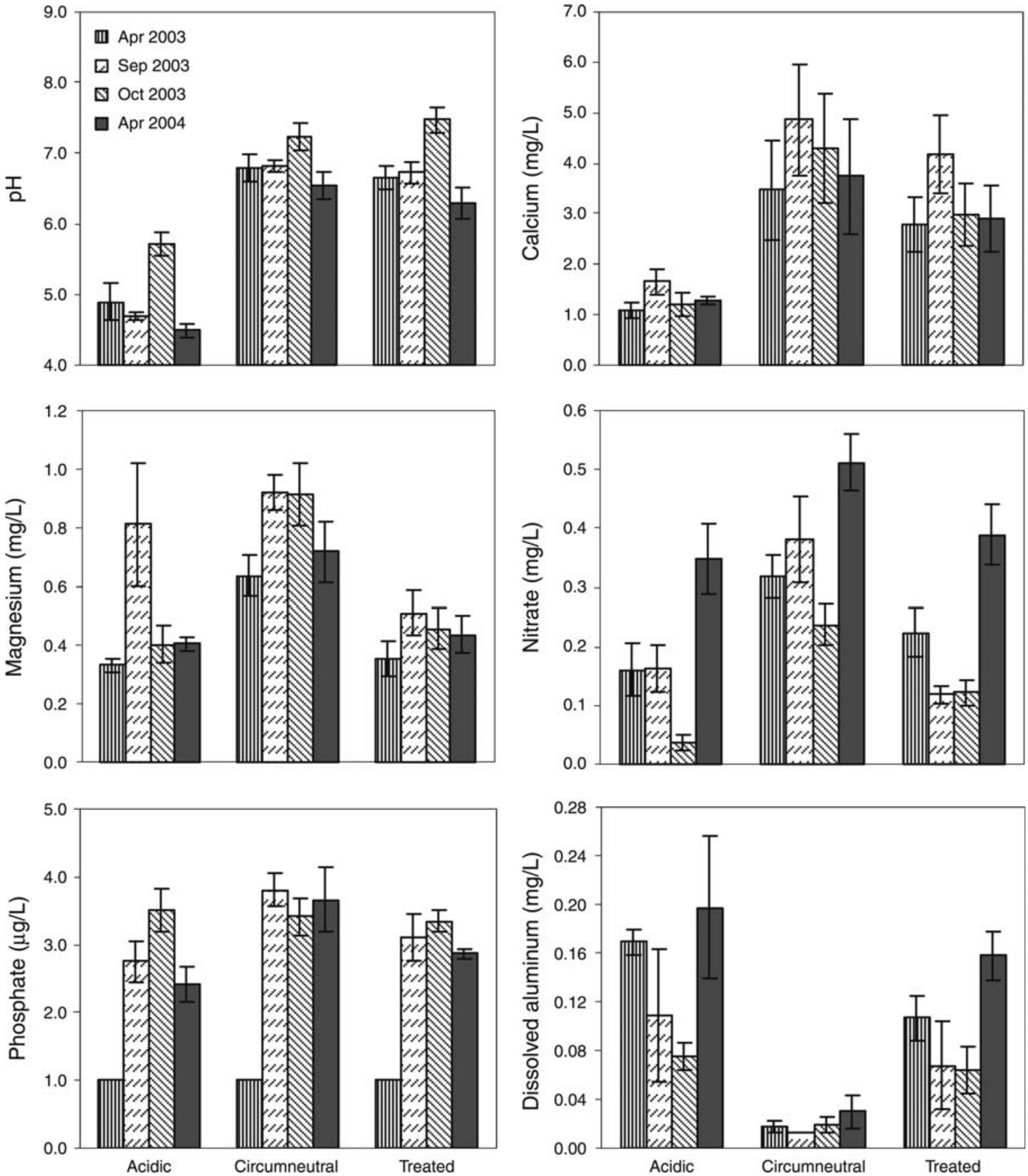

FIG. 1. Selected water-chemistry parameters, by stream status, for each seasonal water-chemistry sample. Data are means ± SE.

treated streams when averaged across sampling periods (Table 2) and within all sampling periods (Fig. 1). We also found that Al_t in treated streams was lower than those concentrations found in acidic streams (Table 2, Fig. 1). We observed no significant status effect on SO_4^{2-}, Cl^-, P_t (total phosphorus), P_d (dissolved P), and PO_4^- (Table 2).

We observed high seasonal variability in water chemistry independent of stream status. Only 4 of the 18 water-chemistry parameters analyzed did not differ significantly among seasons (Table 2). We found a significant effect of season on pH, alk, Ca^{2+}, Ca:H, Mg^{2+}, Na^+, K^+, SO_4^{2-}, NO_3^-, P_t, PO_4^-, Al_t and Al_d, and SpC (Table 2). For example, we found that pH values were typically lowest and Al concentrations highest during spring samples (Fig. 1).

The time since initial treatment had no detectible effect on water chemistry in treated streams (R^2 ranged

TABLE 3. Biofilm, benthic-macroinvertebrate, and fish community variables averaged by stream status along with corresponding statistical summaries.

	Stream status							
	Acidic		Circumneutral		Treated			
Variables	Mean	SE	Mean	SE	Mean	SE	F	df
Biofilm								
June biomass (g/cm^2)	3.1[a]	0.3	2.9[a]	0.8	2.5[a]	0.4	0.37	2, 17
August biomass (g/cm^2)	0.8[a]	0.1	1.3[a]	0.4	1.1[a]	0.2	0.81	2, 17
Benthic macroinvertebrates								
Taxon richness†	17[a]	1.3	34[b]	1.4	19[a]	2.4	17.02	4, 15
Density (no./m^2)	2040[ab]	504	3051[a]	579	1259[b]	365	9.27	4, 15
Biomass (mg DM/m^2) ‡	544.9[a]	49.1	1679.2[b]	229.4	473.1[a]	194.6	17.52	4, 15
Tolerant taxa biomass (%)	96[a]	1.6	41.2[b]	5.1	67[c]	7.8	28.17	4, 15
Sensitive taxa biomass (%)	0[a]	0.0	52[b]	6.3	21[c]	7.3	35.67	4, 15
Tolerant taxa (no.)	15[a]	0.9	15[a]	0.5	12[b]	0.6	6.83	4, 15
Sensitive taxa (no.)	0[a]	0.0	11[b]	0.6	4[a]	1.4	22.66	4, 15
Filterer biomass (%)	4[a]	1.7	6[a]	1.9	5[a]	1.4	0.51	4, 15
Gatherer biomass (%)	25[a]	8.8	23[a]	2.5	28[a]	6.2	0.94	4, 15
Predator biomass (%)	40[a]	9.1	35[a]	5.7	29[a]	7.6	0.49	4, 15
Scraper biomass (%)	24[a]	9.0	17[a]	2.5	19[a]	4.5	0.29	4, 15
Shredder biomass (%)	7[a]	1.1	19[a]	6.5	18[a]	5.2	0.49	4, 15
Fishes								
Species richness (no.)§	2[a]	0.7	5[b]	1.6	6[b]	1.3	3.26	2, 17
Biomass (g/m^2)	0.1[a]	0.02	2.4[b]	0.28	1.3[c]	0.25	13.17	2, 17
Trout density (no./m^2)	0.001[a]	0.0006	0.08[b]	0.03	0.03[b]	0.009	2.52	2, 17
Trout biomass (g/m^2)	0.09[a]	0.04	1.4[b]	0.25	0.9[b]	0.27	4.64	2, 17
Trout young-of-year, yoy								
Density (no./m^2)	0.0001[a]	0.0001	0.05[a]	0.02	0.01[a]	0.005	2.37	2, 17
Biomass (g/m^2)	0.0003[a]	0.0001	0.13[a]	0.05	0.03[a]	0.01	3.13	2, 17

Note: Variable means with the same lowercase superscript letter did not differ significantly among stream status ($P > 0.05$).
† Taxon richness is the number of taxa found at each study location, averaged by stream status.
‡ DM, dry mass.
§ Species richness is the number of fish species found at each study location, averaged by stream status.

from <0.0001 to 0.3, P values ranged from 0.99 to 0.16). There was a tendency for pH to increase with increasing distance from treatment ($y = 0.5x + 4.8$; $R^2 = 0.431$); however, this relationship was not significant ($P = 0.07$). Statistical trends for all other water-chemistry variables based on distance to treatment location were not significant.

Biofilm and macroinvertebrates

Stream status had no significant effect on biofilm biomass in either the June or August sample (Table 3). We collected a total of 12 419 benthic macroinvertebrates from 70 taxa across a range of acid sensitivity (Appendix D). We observed a significant effect of stream status on all benthic-macroinvertebrate variables measured, except those associated with functional feeding groups (FFGs) (Table 3). However, unlike water chemistry, macroinvertebrate communities in treated streams as a group were not equal to circumneutral streams. Instead, community variables in treated streams were either statistically indistinguishable from those in acidic streams (e.g., taxon richness, density, biomass, and the number of sensitive taxa) or intermediate to acidic and circumneutral streams (e.g., percentage sensitive- and tolerant-taxa biomass) (Table 3). Macroinvertebrate communities in treated streams were highly variable from stream to stream (Table 4). Several treated streams possessed communities that were as low as or lower in taxon richness, density, biomass and the number of acid-sensitive taxa than those found in acidic streams. In contrast, other treated streams possessed communities that were substantially more similar to, if not identical to circumneutral reference sites (e.g., Dogway Fork; Table 4).

Variation in benthic-macroinvertebrate attributes among treated streams was not significantly correlated with time since initial treatment (P values ranged from 0.38 to 0.19). However, much of the variation in macroinvertebrate communities among treated streams could be explained by the distance of treated sites to the treatment location (Fig. 2). In every case, (with the exception of the number of acid-tolerant taxa, $P = 0.06$) we observed a significant linear relationship between distance from treatment and benthic macroinvertebrate community variables (Fig. 2). There also was some evidence that a threshold distance of 2–3 km resulted in a shift from communities representative of acidic conditions to communities more representative of circumneutral conditions (Fig. 2). Nevertheless, despite the generally improved conditions in treated streams far from treatment, only one stream (Dogway Fork) consistently possessed community attributes that were

TABLE 4. Aquatic benthic-macroinvertebrate calculations by site at the lowest taxonomic level identified.

Site	Stream status †	Taxon richness	Macroinvertebrate Density (m^2)	Macroinvertebrate Biomass (g DM/m^2)	Biomass (%) Tolerant	Biomass (%) Sensitive	No. taxa Tolerant	No. taxa Sensitive
Upper Second Fork	A	20	1412	599	93	0	17	0
South Fork Red Creek	A	14	1222	398	96	0	13	0
North Fork Cranberry	A	16	2076	575	100	0	16	0
Red Creek	A	17	3450	604	95	0	15	0
Little Odey	C	34	4288	948	75	11	17	10
Grants Branch	C	33	2332	2039	37	56	15	8
Gandy Run	C	29	2408	1284	36	62	14	11
Jakeman Run	C	25	1208	676	40	55	12	9
Rattlesnake Run	C	35	2062	1970	29	66	15	12
Big Run	C	37	2120	2134	47	43	16	11
Little Black Fork	C	34	3668	1801	30	62	15	11
South Fork Cranberry	C	34	6322	2581	36	59	16	13
McGee Run	T1	15	372	71	87	2	11	1
Crouch Run	T1	11	90	63	93	0	12	0
Red Run	T1	14	836	161	91	2	13	1
First Fork	T1	16	580	167	77	6	10	2
Big Rocky	T2	22	1342	312	48	33	13	5
Dogway Fork	T2	32	1896	1670	53	38	15	11
North Fork Cherry	T2	23	1680	493	39	37	11	9
Otter Creek	T2	19	3276	848	49	51	13	4

† Key: A, acidic; C, circumneutral. T1 refers to treated streams sites located <2 km from treatment. T2 refers to treated sites located >3 km from treatment.

within the range observed in circumneutral streams (Fig. 2).

Unfortunately, a simple interpretation of these results is not possible, because distance to treatment was correlated with basin area ($R^2 = 0.84$, $P = 0.01$). Nevertheless, we believe that distance was the more important determinant of benthic macroinvertebrate communities for two reasons. First, community attributes were more strongly correlated to distance than to basin area. Second, streams that were near to treatment consistently possessed communities indicative of acidic conditions, regardless of basin area. For example, in treated streams, we observed that the percentage of acid-sensitive taxa biomass was more strongly correlated to distance to treatment location ($R^2 = 0.96$, $P < 0.0001$) than to basin area ($R^2 = 0.73$, $P = 0.007$).

Mean similarity (MeanSIM) analysis supported our finding that benthic-macroinvertebrate communities in treated streams <2 km from treatment (i.e., T1 streams) were comparable to communities in acidic streams, whereas communities in streams >3 km from treatment (i.e., T2 streams) were more similar to but not identical to circumneutral streams (Table 5). Specifically, we observed extremely low mean similarity between T1 and circumneutral streams and high similarity between T2 and circumneutral streams (Table 5). Furthermore, permutation tests resulted in strong evidence (P values <0.0001) that the stream classifications we used (A, C, T1, and T2) effectively separated macroinvertebrate communities into four distinct groups. NMDS (nonmetric multidimensional scaling) clarified the specific taxonomic differences among the groups (Fig. 3). Specifically, several acid-sensitive taxa tended to be present only in circumneutral streams and T2 streams (Fig. 3). Interestingly, there also was a tendency for Isoperla and Corydalidae, two acid-tolerant predatory insects, to be present in circumneutral streams, but absent in T2 streams (Fig. 3).

Fishes

Fish were collected in all stream reaches except for the acidic North Fork of Cranberry. We observed a significant status effect on five of the seven fish-community variables measured; however, this effect was highly variable (Table 3). We found that fish species richness, trout density, and trout biomass were significantly higher in treated and circumneutral streams than in acidic streams (Table 3, Fig. 4). Conversely, we found that total fish density and biomass in treated streams were intermediate to circumneutral and acidic streams (Table 3, Fig. 4). We did not observe a status effect on trout young-of-year (yoy) density or biomass (Table 3, Fig. 4).

In circumneutral streams, three of the seven fish-community variables analyzed were significantly correlated with basin area (Fig. 4). We observed a negative correlation between basin area and both trout density and trout yoy density and a positive correlation between basin area and fish species richness in circumneutral streams (Fig. 4). Fish species richness in small treated streams was similar to that of equally sized circumneutral streams. However, larger treated streams consistently had lower species richness than expected (Fig. 4). We did not find a significant basin-area effect on acidic or treated stream fish-community variables (Fig. 4).

Finally, we did not observe a significant effect of distance to mainstem on any fish community variable

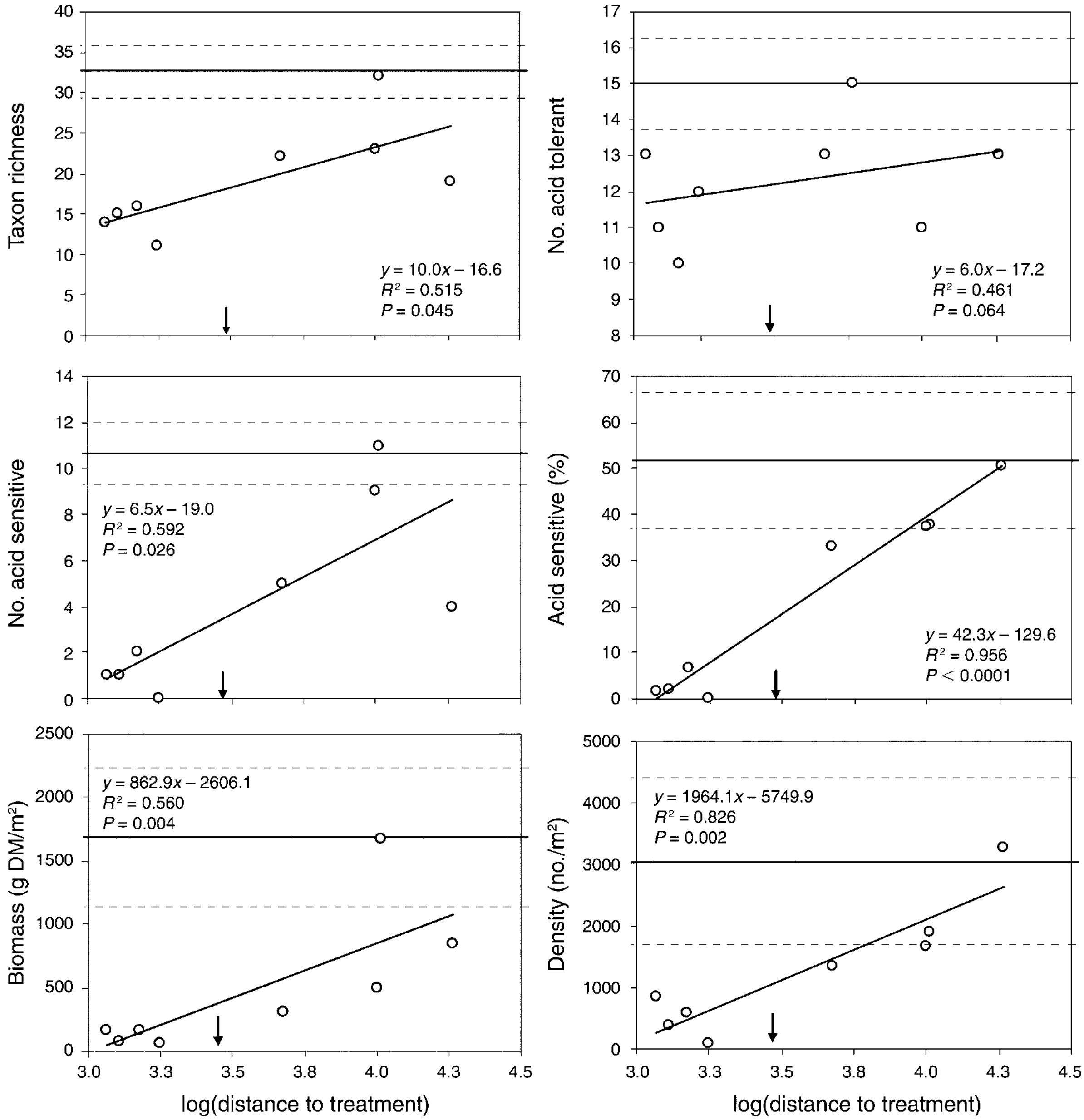

FIG. 2. Relationship between benthic-macroinvertebrate attributes and log-transformed distance to treatment location (measured in meters) in limestone-treated streams. Means (solid horizontal lines) and 95% confidence interval values (dashed horizontal lines) from circumneutral streams are shown for comparison.

analyzed. Furthermore, in treated streams, we did not observe a distance-to-treatment or time-since-initial-treatment effect on fish-community variables. As with benthic macroinvertebrate community variables, only one treated stream, Dogway Fork, consistently possessed fish-community attributes that were statistically equivalent to circumneutral streams.

Discussion

The need for aquatic ecosystem restoration has grown dramatically in recent years (National Research Council 1992). Consequently, stream and river restoration projects are becoming increasingly common worldwide (Ormerod 2004, Bernhardt et al. 2005, Palmer et al. 2005). Unfortunately, however, there are several important criticisms of ecological restoration generally, and stream restoration specifically. For example, Hildebrand et al. (2005) argued that most restoration projects are focused on inappropriate time scales and uncertain or inappropriate restoration targets (e.g., structural habitat features rather than ecosystem processes). In addition, a recent survey of river restoration projects in the United States has identified an alarming lack of pre- and post-restoration monitoring (Bernhardt et al. 2005), as well as

TABLE 5. Matrix showing mean Jaccard similarities for macroinvertebrate communities (presence and absence of lowest taxa indentified) between and within stream classes.

Stream class	Stream class			
	C	A	T1	T2
C	0.586			
A	0.338	0.538		
T1	0.296	0.436	0.403	
T2	0.534	0.386	0.358	0.515

Notes: Key to stream classes: C, circumneutral; A, acidic; T1, <2 km from treatment; T2, >3 km from treatment. Values along the diagonal are the mean within-group similarities; off-diagonal values are the mean between-group similarities.

a lack of agreement on what constitutes successful restoration and how success should be measured (Palmer et al. 2005). Given these criticisms, our objective was to assess the benefits and shortcomings of the limestone-remediation program of the Allegheny Plateau ecoregion in West Virginia, USA. In doing so, we hoped to gain a realistic view of the successes and failures of restoration and obtain the information needed to adapt the restoration program.

Restoration successes

The current limestone-remediation program has successfully restored self-sustaining trout fisheries in acidic streams of the Allegheny Plateau ecoregion in West Virginia. Limestone additions increase pH, alkalinity, and Ca^{2+} concentrations to levels that are similar to circumneutral reference streams during base-flow conditions. These chemical improvements apparently were enough to offset dissolved aluminum concentrations that remain elevated in treated streams and allow for successful trout reproduction and survival. High levels of calcium have been shown to reduce the toxic effects of elevated aluminum levels in fish bioassays (Ingersoll et al. 1990). Our finding is consistent with numerous studies conducted in this region that have shown improved pH and recovered trout populations following limestone additions (Weatherley et al. 1991, Downey et al. 1994, Menendez et al. 1996, Clayton et al. 1998, Hudy et al. 2000).

Young-of-the-year trout were found in all circumneutral and treated streams with highest densities observed in the smallest streams. Petty et al. (2005) found that streams with a basin area <3 km^2 supported $>80\%$ of spawning activity in a Central Appalachian watershed. Petty et al. (2005) also found that successful brook trout reproduction was limited to streams with alkalinity levels >5–10 mg $CaCO_3$/L. All acidic streams in this study were below this threshold and the lack of juvenile trout within these streams reflects this finding. However, three circumneutral streams (Big Run, Gandy Run, and Grants Branch) and four limestone-treated

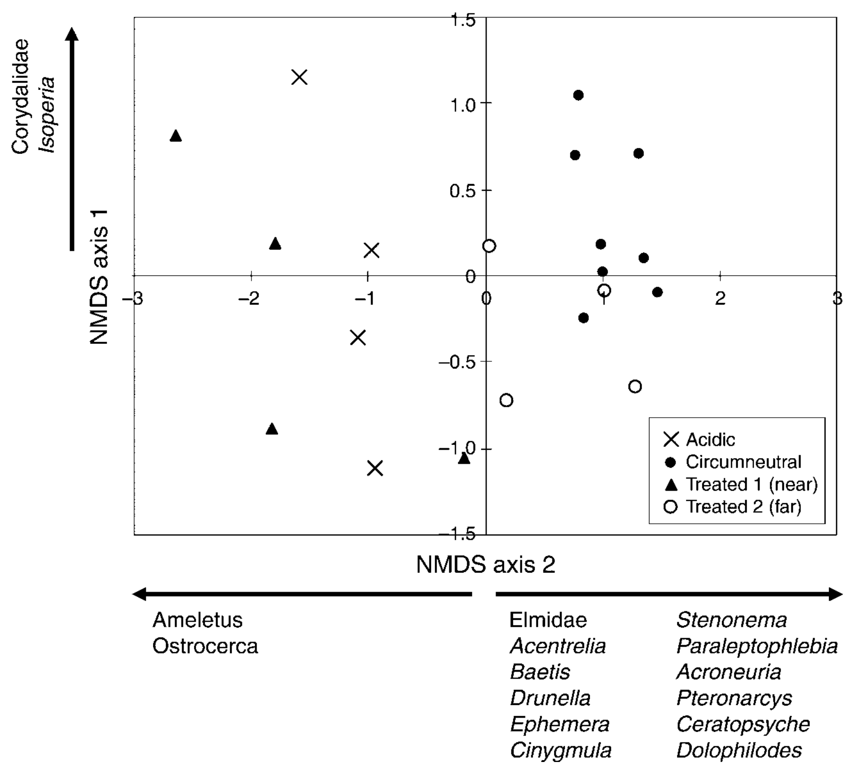

FIG. 3. Benthic-macroinvertebrate-based nonmetric multidimensional scaling (NMDS) axes 1 and 2 scores for acidic, circumneutral, and limestone-treated streams. Macroinvertebrate taxa for which we observed a strong significant relationship ($P < 0.005$) between presence/absence and NMDS scores are shown for each axis.

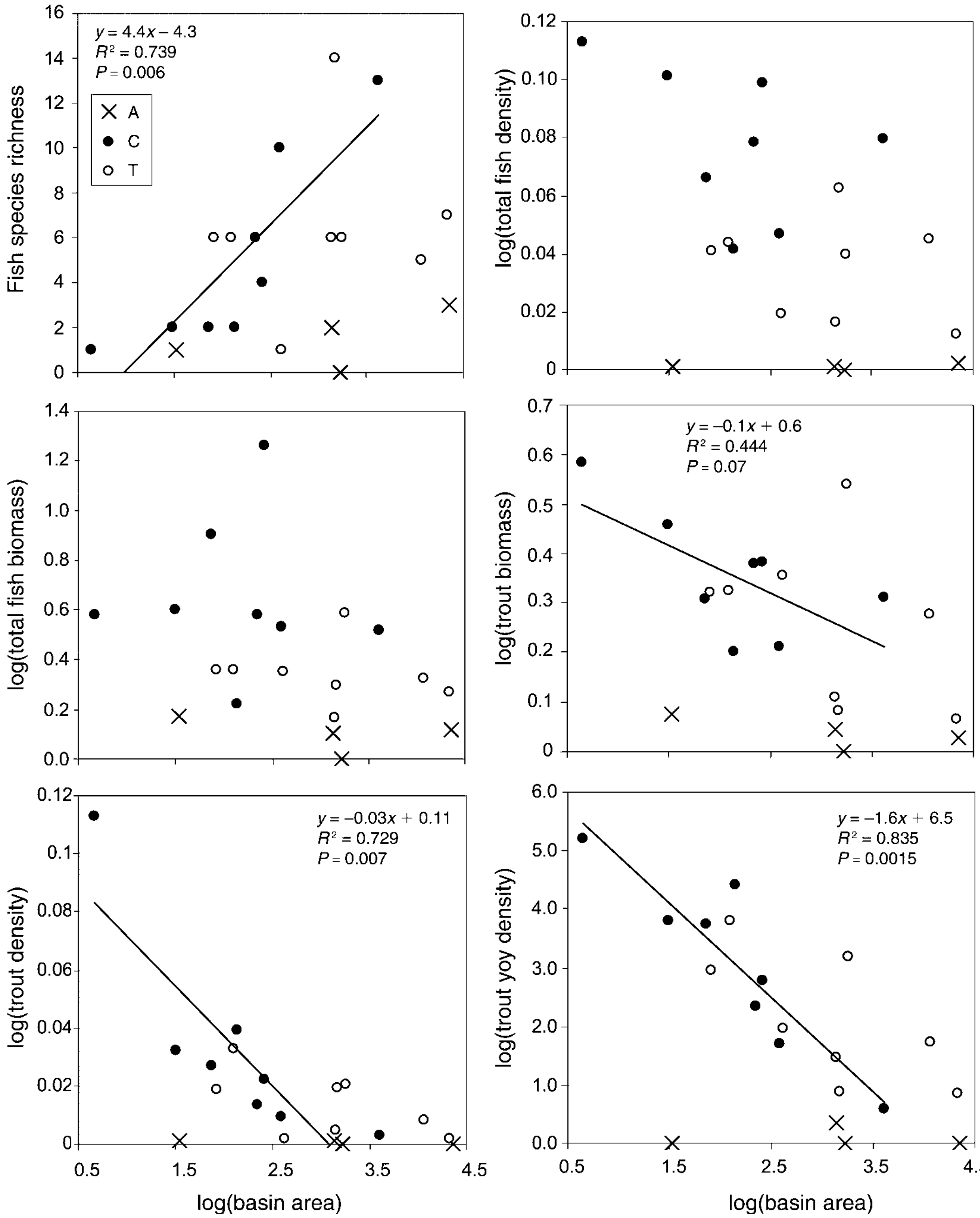

FIG. 4. Fish species richness and log-transformed values for total fish density and biomass, trout biomass and density, and trout young-of-year (yoy) density (no./km^2) as a function of basin area. Biomass was originally measured as g/m^2, and density was measured as no./m^2. Stream status: A, acidic; C, circumneutral; T, limestone treated.

streams (Otter Creek, Crouch Run, First Fork, and Red Run) had average alkalinity concentrations <5 mg $CaCO_3$/L, but had reproducing trout populations. We conclude that average Ca:H ratio $\geq$10 is a better indicator of water quality necessary for reproducing brook trout populations. All circumneutral and treated streams had Ca:H ratios above this threshold. This finding supports previous suggestions made by Clayton et al. (1998).

Restoration shortcomings

Incomplete ecosystem recovery is common in stream-restoration studies that are fully assessed. For example, Lepori et al. (2005) found limited recovery of stream macroinvertebrate and fish communities following habitat restoration. Similarly, full restoration of most chemical and biological attributes in limestone-treated streams of the Allegheny Plateau is not occurring,

PLATE 1. Limestone sand treatment of First Fork to Shavers Fork in the Cheat River watershed, West Virginia, USA. Photo credit: Pete Lamothe.

despite recovery of pH, Ca^{2+}, and trout populations. Our research identified three important factors that potentially limit recovery in limestone-treated streams: (1) poor water quality in mixing zones immediately downstream of treatment, (2) treated stream reaches that continue to drain acidic watersheds, and (3) treated stream reaches that remain isolated within acidic stream networks.

Mixing-zone complications

We found incomplete recovery of several important measures of ecological condition near to treatment in limestone-treated streams. Specifically, we found that benthic-macroinvertebrate density, and the percentage of acid-sensitive taxa biomass were strongly dependent on spatial proximity to limestone treatment. Our findings are consistent with those of several studies that have observed incomplete recovery of benthic-macroinvertebrate communities in limestone-treated streams (Menendez et al. 1996, Bradley and Ormerod 2002, Keener and Sharpe 2005).

The incomplete recovery of macroinvertebrate communities in limestone-treated streams may be the result of highly variable water chemistry downstream of the treatment location. We found that treated streams sampled near to treatment (<2 km) had slightly lower pH and Ca:H ratios, and significantly lower numbers of acid-sensitive macroinvertebrate taxa compared to those sampled >3 km from treatment. Bradley and Ormerod (2002) also observed a low occurrence of acid-sensitive species in treated streams and attributed this trend to highly variable water chemistry (e.g., periods of low pH). Water quality is most variable immediately downstream of treatment, because it takes time for the limestone to dissolve and influence pH and alkalinity. It also takes time for metals, such as aluminum, to precipitate out of solution once pH increases above a critical threshold or to change to a less toxic form. These chemical processes result in chemical mixing zones immediately downstream of treatment that are characterized by highly variable pH and alkalinity, increased metal precipitation, and increased suspended solids (Menendez et al. 1996, Bradley and Ormerod 2002, Petty and Barker 2004).

Increased sediments may also have impacted macroinvertebrate communities in treated streams sampled <2 km from treatment. Increased sedimentation has been shown to lead to reductions in macroinvertebrate abundances and altered communities (Keener and Sharpe 2005). Several limestone-remediation studies have noted a negative impact of limestone addition on macroinvertebrate communities sampled <500 m downstream of treatment location (Menendez et al. 1996, Keener and Sharpe 2005). However, all of the treated streams in our study were sampled >1 km from treatment location in an effort to avoid potential negative impacts from sedimentation. Consequently, we think it was unlikely that incomplete recovery in treated streams was a direct result of sedimentation from limestone sand.

Acidic watershed

Numerous studies have shown that the loss of base cations (i.e., Ca^{2+}, Mg^{2+}, Na^{2+}, K^{+}) from soils of acidified watersheds is a significant factor influencing surface water chemistry (DeWalle and Swistock 1994, Likens et al. 1996, Lawrence et al. 1999). Consequently, it is not surprising that streams within acidic watersheds treated with limestone at the reach scale did not recover base-cation concentrations other than Ca^{2+}. This may be important biologically, because Mg^{2+}, Na^{2+}, and K^{+} are essential elements for cellular processes such as ion transport, enzyme activation, and control of protein conformation in all organisms (Hynes 1970, Phipps 1976). Nevertheless, these nutrients are not considered limiting in most stream ecosystems (Dodds 2002), and it is unclear whether the incomplete recovery of biological communities was a result of low base-cation concentrations. Additional studies in central Appalachian watersheds are needed to fully determine the long-term effects of base-cation losses on stream communities.

Nitrogen and phosphorus, however, potentially are limiting nutrients in lotic ecosystems (Rosemond et al. 1993, Tank and Webster 1998). We did not detect differences in total P concentrations among acidic, circumneutral, and treated streams. However, phosphate concentrations in all streams sampled were extremely low (<5 μg/L), which is typical of forested streams in the eastern United States (Rosemond et al. 1993). Nitrate, in contrast, was significantly lower in limestone-treated streams than in either circumneutral or acidic streams. This finding is consistent with those of Hindar et al. (1996) but is contrary to observations of Cirmo and Driscoll (1996). It is possible that reduced nitrate concentrations in treated streams, due to historical acidification, coupled with naturally low phosphate concentrations could depress productivity levels compared to circumneutral conditions.

Another consequence of treated streams draining acidic watersheds is that dissolved aluminum continues to be delivered from the surrounding watershed at a high rate. Indeed, we found that limestone treatment was not effective at reducing aluminum concentrations to circumneutral reference conditions. In our study, aluminum concentrations in treated steams were either intermediate to circumneutral and acidic-stream concentrations or more similar to acidic conditions. These findings are very similar to those of Keener and Sharpe (2005). In contrast, most historical limestone-remediation studies have shown that limestone treatment significantly reduces aluminum concentrations compared to acidic control reaches (Downey et al. 1994, Menendez et al. 1996, Simmons and Doyle 1996, Hudy et al. 2000, LeFevre and Sharpe 2002) and even to concentrations similar to those found in circumneutral streams (Rundle et al. 1995). We observed dissolved-aluminum concentrations that exceeded chronic impairment levels of aquatic biota according to West Virginia water-quality standards (>0.087 mg/L) in a number of acidic and treated stream reaches. Also, inorganic monomeric aluminum concentrations exceeded 0.06 mg/L, a level thought to be unsuitable for the survival and reproduction of many fish species (Baker et al. 1996), in several acidic streams and a few treated streams. In contrast, dissolved and inorganic monomeric aluminum concentrations never surpassed toxic levels in any circumneutral stream.

Isolation of treated streams

Limestone-treated streams in this region are often isolated within acidic stream networks, and this isolation may limit the recovery of both macroinvertebrate and fish communities, especially species richness. For example, both downstream dispersal and overland flight of macroinvertebrates have been found to influence colonization rates (Fenoglio et al. 2002, Sanderson et al. 2005). Therefore, the recolonization of acid-sensitive benthic-macroinvertebrate species into treated stream reaches may be limited, because treated reaches remain surrounded by acidophilic communities. Indeed, we found that benthic-macroinvertebrate communities in treated streams were never fully recovered to reference conditions, with the exception of Dogway Fork. This finding supports those of numerous studies that observed limited recovery of benthic-macroinvertebrate communities (Downey et al. 1994, Rundle et al. 1995, Eggleton et al. 1996, Simmons and Doyle 1996, Bradley and Ormerod 2002, Keener and Sharpe 2005). However, recent research indicates that acid-sensitive macroinvertebrates are capable of recolonizing geographically isolated streams although dispersal rates necessary for full recovery of these populations is still unknown (Masters et al., *in press*). We found that treatment allowed for the recolonization of a few acid-sensitive macroinvertebrate taxa; however; the abundance of intolerant taxa remained low in treated streams. These findings suggest that isolation of treated streams may be just as important as local water quality to the recovery of benthic macroinvertebrate communities.

Geographic isolation from high-quality streams may also limit recovery of fish communities in treated streams. We found that limestone treatment was effective at restoring fish species richness in smaller streams; however, larger treated streams consistently had fewer fish species than was expected. In addition, total fish density and biomass were never fully recovered in limestone-treated streams compared to circumneutral reference conditions, with the exception of Dogway Fork. Several studies have shown a dependence of fish communities on the interconnectivity of drainage networks (Fausch et al. 2002, Petty et al. 2005). Consequently, extensive losses of fish species in acid-impacted watersheds may limit the ability of limestone treatment to fully recover fish communities in isolated stream reaches.

Temporal trends in recovery

We found that the number of years an acid-impaired steam is treated with limestone sand did not influence the overall level of recovery of the system. Unfortunately, there are only a few studies that have been able to examine the effect of time on stream recovery (Appelberg et al. 1995, Simmons and Doyle 1996, Appelberg and Svenson 2001, Bradley and Omerond 2002). Simmons and Doyle (1996) and Bradley and Omerond (2002) concluded that extended time periods (>10 years) were needed to assess the duration of effective treatment effects and because some biological recovery processes require time. However, Appelberg and Svenson (2001) concluded that the long-term changes resulting from limestone treatment were small relative to changes observed immediately following treatment. Our study included streams that have been treated anywhere from 2 to 20 years, and we found no evidence of temporal trends in the recovery process. For example, treatment on First Fork, Crouch Run, and Dogway Fork began more than 10 years ago; however, these streams had extremely different macroinvertebrate communities. Both First Fork and Crouch Run were dominated by acid-tolerant taxa and had extremely depressed macroinvertebrate densities and biomass compared to Dogway Fork. In addition, Big Rocky Run, which has been treated with limestone sand for only 2 years, possessed greater macroinvertebrate density and biomass and a larger percentage biomass and number of acid-sensitive species than McGee Run, Crouch Run, Red Run, and First Fork, which have all been treated for $\geq$7 years. Finally, temporal trends in the recovery process of fish populations also were not detected, indicating an immediate but ultimately limited recovery of fishes following limestone treatment.

Implications for acid-remediation programs

In order to maximize ecological recovery through limestone remediation in the Allegheny Plateau ecoregion, our research suggests that limestone applications should be placed, (1) as high in the headwaters as possible, (2) at multiple locations, and (3) in a manner that will recover interconnected stream networks rather than isolated reaches. These practices will reduce mixing-zone complications, increase watershed connectivity, and allow for the movement and full recolonization potential of both fish populations and benthic macroinvertebrate communities. In this study, Dogway Fork provided an excellent example of how the full ecological-recovery potential of acidic streams may be achieved using the practices outlined above. Unlike any other treated stream in this study, Dogway Fork had full recovery of nearly all macroinvertebrate and fish community variables. We attribute this recovery to Dogway Fork's unique treatment regime, which includes multiple mainstem treatment locations starting high in the watershed and its direct connection with a healthy river system downstream.

Regardless of treatment practices, we recognize that some elements of acidified stream ecosystems may never be restored. Of particular concern is the issue of depressed productivity levels. Future research in acidic-stream restoration might include the addition of micro- and macro-nutrients, such as magnesium, potassium, nitrogen and phosphorus. The in-stream addition of N and P in tandem has been shown to increase microbial activity, biofilm biomass, and grazer biomass in Appalachian streams (Rosemond et al. 1993). Increases in productivity at lower trophic levels in treated streams could propagate to higher trophic levels leading to increased fish growth rate and overall fish biomass (Peterson et al. 1993). However, because eutrophication of rivers and estuaries is a major, worldwide problem (Carpenter et al. 1998), the potential local benefits of adding nutrients to limestone-treated streams must be weighed against the potential contribution to a much larger problem. Also, we believe that the spatially explicit limestone-treatment practices discussed above should be implemented before nutrient additions are conducted.

Future studies of limestone-treatment programs in the eastern United States should examine the effects of acidic episodes on the recovery potential of treated streams, particularly small treated streams. Recent studies have proven that acidic episodes caused by spring runoff may play a large role in preventing biological recovery in streams recovering from acidification (Laudon et al. 2004, Lepori and Ormerod 2005, Kowalik and Ormerod 2006). Previous studies in West Virginia have indicated that the treatment techniques currently used are effective in preventing extreme reductions in pH and Ca:H ratios during high-flow events. However, concentrations of other harmful water-chemistry parameters (e.g., aluminum) during acidic episodes remain unknown. Therefore, we recommend future, detailed water-chemistry studies that focus on spring high-flow events in treated streams.

Finally, we recommend large-scale monitoring of benthic-macroinvertebrate indicator taxa associated with acid-stream restoration programs throughout the central Appalachians. Based upon our results, the following taxa appear to be good indicators of low acid impairment in circumneutral and fully restored streams in this region: the mayflies *Baetis, Acentrella, Drunella, Ephemerella, Ephemera,* and *Paraleptophlebia*; the caddisflies *Ceratopsyche, Cheumatopsyche, Diplectrona, Chimmara* and *Dolophilodes*; and the stonefly families Perlidae and Pteronarcyidae. Such programs are needed to determine if our findings are consistent over time and applicable to other watersheds. Long-term monitoring programs also are needed to assess the effectiveness of adaptive limestone-treatment programs in this region.

Integrated efforts to assess the effectiveness of stream and river restoration programs are lacking worldwide (Ormerod 2004, Bernhardt et al. 2005), and this is especially true of acid-remediation efforts in the central Appalachians. The results of our study indicate that it is possible to determine which ecological attributes are being recovered through limestone-remediation programs and which attributes are not. Such information is necessary to establish realistic expectations of recovery and to design restoration programs that maximize ecological recovery at the watershed scale.

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Literature Cited

Appelberg, M., P. E. Lingdell, and C. Andren. 1995. Integrated studies of the effects of liming acidified waters (ISELAW-programme). Water, Air, and Soil Pollution 85:883–888.

Appelberg, M., and T. Svenson. 2001. Long-term ecological effects of liming—the Iselaw Programme. Water, Air, and Soil Pollution 130:1745–1750.

Baker, J. P., J. Van Sickle, C. J. Gagen, D. R. DeWalle, W. E. Sharpe, R. F. Carline, B. P. Baldigo, P. S. Murdoch, D. W. Bath, W. A. Kretser, H. S. Simonin, and P. J. Wigington. 1996. Episodic acidification of small streams in the Northeastern United States: effects on fish populations. Ecological Applications 6:422–437.

Barbour, M. T., J. Gerritsen, B. D. Snyder, and J. B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: periphyton, benthic macroinvertebrates and fish. Second edition. EPA 841-B-99-002. U.S. Environmental Protection Agency, Office of Water, Washington, D.C., USA.

Benke, A. C., A. D. Huryn, L. A. Smock, and J. B. Wallace. 1999. Length–mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. Journal of the North American Benthological Society 18:308–343.

Bernhardt, E. S., et al. 2005. Synthesizing U.S. river restoration efforts. Science 308:636–637.

Bopp, J. A. 2002. Combined effects of water chemistry, canopy cover, and stream size on benthic macroinvertebrates along a central Appalachian stream continuum. Thesis. West Virginia University, Morgantown, West Virginia, USA.

Bradley, D. C., and S. J. Ormerod. 2002. Long-term effects of catchment liming on invertebrates in upland streams. Freshwater Biology 47:161–171.

Brown, D. J. A., and R. A. Skeffington. 1992. Timescales of recovery from acidification: Implications of current knowledge for aquatic organisms. Environmental Pollution 77:227–234.

Campbell, D. E. 2000. Using energy systems theory to define, measure, and interpret ecological integrity and ecosystem health. Ecosystem Health 6:181–204.

Carpenter, S., N. F. Caraco, D. L. Correll, R. W. Howarth, A. N. Sharpley, and V. H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. Issues in Ecology 3:1–12.

Cirmo, C. P., and C. T. Driscoll. 1996. The impacts of a watershed $CaCO_3$ treatment on stream and wetland biogeochemistry in the Adirondack Mountains. Biogeochemistry 32:265–297.

Clarke, K. R., and R. H. Green. 1988. Statistical design and analysis for a 'biological effects' study. Marine Ecology Progress Series 46:213–226.

Clayton, J. L., E. S. Dannaway, R. Menendez, H. W. Rauch, J. J. Renton, S. M. Sherlock, and P. E. Zurbuch. 1998. Application of limestone to restore fish communities in acidified streams. North American Journal of Fisheries Management 18:347–360.

Clayton, J. L., and R. Menendez. 1996. Macroinvertebrate responses to mitigative liming of Dogway Fork, West Virginia. Restoration Ecology 4:234–246.

Cuffney, T. F., M. E. Gurtz, and M. R. Meador. 1993. Methods for collecting benthic invertebrate samples as part of the National Water-Quality Assessment Program. USGS Open-file Report 93-406. United States Geological Survey, Denver, Colorado, USA.

DeWalle, D. R., and B. R. Swistock. 1994. Causes of episodic acidification in five Pennsylvania streams on the northern Appalachian Plateau. Water Resources Research 30:1955–1963.

Dodds, W. K. 2002. Freshwater ecology concepts and environmental applications. Academic Press, New York, New York, USA.

Downey, D. M., C. R. French, and M. Odom. 1994. Low cost limestone treatment of acid sensitive trout streams in the Appalachian mountains of Virginia. Water, Air, and Soil Pollution 77:49–77.

Eggleton, M. A., E. L. Morgan, and W. L. Pennington. 1996. Effects of liming on an acid-sensitive southern Appalachian stream. Restoration Ecology 4:247–263.

EPA [U.S. Environmental Protection Agency]. 1991. Methods for the determination of metals in environmental samples. EPA 600-91-010. United States Environmental Protection Agency Office of Research and Development, Washington, D.C., USA.

ESRI [Environmental Systems Research Institute]. 2003. ArcView version 8.3. Environmental Systems Research Institute, Redlands, California, USA.

Fausch, K. D., C. E. Torgersen, C. V. Baxter, and H. W. Li. 2002. Landscapes to riverscapes: bridging the gap between research and conservation of stream fishes. BioScience 52:483–498.

Fenoglio, S., P. Agosta, T. Bo, and M. Cucco. 2002. Field experiments on colonization and movements of stream invertebrates in an Apennine river (Visone, NW Italy). Hydrobiologia 474:125–130.

Herlihy, A. T., P. R. Kaufmann, M. R. Church, P. J. Wigington, Jr., J. R. Webb, and M. J. Sale. 1993. The effects of acidic deposition on streams in the Appalachian mountain and piedmont region of the Mid-Atlantic United States. Water Resources Research 29:2687–2703.

Hildebrand, R. H., A. C. Watts, and A. M. Randles. 2005. The myths of restoration ecology. Ecology and Society 10:19–29.

Hill, B. H. 1998. Periphyton. Pages 199–132 *in* J. M. Lazorchak, D. J. Klemm, and D. V. Peck, editors. Environmental monitoring and assessment program—surface waters. Field operations and methods for measuring the ecological condition of wadeable streams. EPA/620/R-94/004F. U.S. Environmental Protection Agency Washington, D.C., USA.

Hindar, A., F. Kroglund, E. Lydersen, A. Skiple, and R. Hogberget. 1996. Liming of wetlands in the acidified Lake Roynelandsvatn catchment in southern Norway: effects on stream water chemistry. Canadian Journal of Fisheries and Aquatic Sciences 53:985–993.

Hudy, M., D. M. Downey, and D. W. Bowman. 2000. Successful restoration of an acidified stream through mitigation with limestone sand. North American Journal of Fisheries Management 20:453–466.

Hynes, H. B. N. 1970. The ecology of running waters. University of Toronto Press, Toronto, Ontario, Canada.

Ingersoll, C. G., D. R. Mount, D. D. Gulley, T. W. LaPoint, and H. L. Bergman. 1990. Effects of pH, aluminum, and calcium on survival and growth of eggs and fry of brook trout (*Salvelinus fontinalis*). Canadian Journal of Fisheries and Aquatic Sciences 47:1580–1592.

Kauppi, P., P. Anttila, and K. Kenttamies. 1990. Acidification in Finland. Finnish acidification research programme HAP-RO 1985–1990. Springer-Verlag, New York, New York, USA.

Keener, A. L., and W. E. Sharpe. 2005. The effects of doubling limestone sand applications in two acidic southwestern Pennsylvania streams. Restoration Ecology 13:108–119.

Kobuszewski, D. M., and S. A. Perry. 1993. Aquatic insect community structure in an acidic and circumneutral stream in the Appalachian Mountains of West Virginia. Journal of Freshwater Ecology 8:37–45.

Kowalik, R. A., and S. J. Ormerod. Intensive sampling and transplantation experiments reveal continued effects of episodic acidification on sensitive stream invertebrates. Freshwater Biology 51:180–191.

Krueger, C. C., and T. F. Waters. 1983. Annual production of macroinvertebrates in three streams of different water quality. Ecology 66:840–850.

Lake, P. S. 2001. On the maturing of restoration: linking ecological research and restoration. Ecological Management and Restoration 2:110–115.

Laudon, H., O. Westling, A. Bergquist, and K. Bishop. 2004. Episodic acidification in northern Sweden: a regional assessment of the anthropogenic component. Journal of Hydrology 297:162–173.

Lawrence, G. B., M. B. David, G. M Lovett, P. S. Murdoch, D. A. Burns, J. L. Stoddard, B. P. Baldigo, J. H. Porter, and A. W. Thompson. 1999. Soil calcium states and the response of stream chemistry to changing acidic deposition rates. Ecological Applications 9:1059–1072.

LeFevre, S. R., and W. E. Sharpe. 2002. Acid stream water remediation using limestone sand on Bear Run in Southwestern Pennsylvania. Restoration Ecology 10:223–236.

Lepori, F., D. Palm, E. Brannas, and B. Malmqvist. 2005. Does restoration of structural heterogeneity in streams enhance fish and macroinvertebrate diversity? Ecological Applications 15:2060–2071.

Lepori, F., and S. J. Ormerod. 2005. Effects of spring acid episodes on macroinvertebrates revealed by population data and *in situ* toxicity tests. Freshwater Biology 50:1568–1577.

Likens, G. E., C. T. Driscoll, and D. C. Buso. 1996. Long term effects of acid rain: response and recovery of a forest ecosystem. Science 272:244–246.

Lowe, R. L., and G. D. Laliberte. 1996. Biomass and pigments of benthic algae. Pages 295–314 *in* F. R. Hauer and G. A. Lamberti, editors. Methods in stream ecology. Academic Press, San Diego, California, USA.

Lyons, J. 1992. The length of stream to sample with a towed electrofishing unit when fish species richness is estimated. North American Journal of Fisheries Management 12:198–203.

Marschall, E. A., and L. B. Crowder. 1996. Assessing population responses to multiple anthropogenic effects: a case study with brook trout. Ecological Applications 6:152–167.

Masters, Z., I. Peteresen, A. G. Hildrew, and S. J. Ormerod. *In press*. Insect dispersal does not limit the biological recovery of streams from acidification. Aquatic Conservation: Marine and Freshwater Ecosystems.

McCormick, F. H., R. M. Hughes, P. R. Kaufmann, D. V. Peck, J. L. Stoddard, and A. T. Herlihy. 2001. Development of an index of biotic integrity for the Mid-Atlantic Highland Region. Transactions of the American Fisheries Society 130: 857–877.

Meegan, S. K., and S. A. Perry. 1996. Periphyton communities in headwater streams of different water chemistry in the central Appalachian mountains. Journal of Freshwater Ecology 11:247–256.

Menedez, R. 1972. Limestone treatment of an acid stream: effects on chemistry, benthos, and trout. Unpublished report; available upon request from Department of Natural Resources, Wildlife Resources Division, Charleston, West Virginia, USA.

Menendez, R., J. L. Clayton, and P. E. Zurbuch. 1996. Chemical and fishery responses to mitigative liming of an acidic stream, Dogway Fork, West Virginia. Restoration Ecology 4:220–233.

Merritt, R. W., and K. W. Cummins. 1996. An introduction to the aquatic insects of North America. Third edition. Kendall and Hunt, Dubuque, Iowa, USA.

Miller, C. 1985. Correlates of habitat favorability for benthic macroinvertebrates at five stream sites in an Appalachian Mountain drainage basin, U.S.A. Freshwater Biology 15: 709–733.

National Research Council. 1992. Restoration of aquatic ecosystems. National Academy Press, Washington, D.C., USA.

Olem, H. 1991. Liming acidifies surface waters. Lewis Publishers Chelsea, Michigan,USA.

Ormerod, S. J. 2004. A golden age of river restoration science? Aquatic Conservation: Marine and Freshwater Ecosystems 14:543–549.

Ormerod, S. J., N. S. Weatherley, W. J. Merrett, A. S. Gee, and P. G. Whitehead. 1990. Restoring acidified streams in upland Wales: a modeling comparison on the chemical and biological effects of liming and reduced sulphate deposition. Environmental Pollution 64:67–85.

Palmer, M. A., et al. 2005. Standards for ecologically successful river restoration. Journal of Applied Ecology 42:208–217.

Peterson, B. J., et al. 1993. Biological responses of a tundra river to fertilization. Ecology 74:653–672.

Petty, J. T., and J. Barker. 2004. Water quality variability, trace metals, and implications for restoring a mined Appalachian watershed. Proceedings of the American Society of Mining and Reclamation 21:1484–1504.

Petty, J. T., P. J. Lamothe, and P. M. Mazik. 2005. Spatial and seasonal dynamics of brook trout populations in a central Appalachian watershed. Transactions of the American Fisheries Society 134:572–587.

Phipps, D. A. 1976. Metals and metabolism. Clarendon Press, Oxford, UK.

Rosemond, A. D., P. J. Mulholland, and J. W. Elwood. 1993. Top-down and bottom-up control of stream periphyton: effects of nutrients and herbivores. Ecology 74:1264–1280.

Rosemond, A. D., S. R. Reice, J. W. Elwood, and P. J. Mulholland. 1992. The effects of stream acidity on benthic invertebrate communities in the southeastern United States. Freshwater Biology 27:193–209.

Rundle, S. D., N. S. Weatherley, and S. J. Ormerod. 1995. The effects of catchment liming on the chemistry and biology of upland Welsh streams: testing model predictions. Freshwater Biology 34:165–175.

Sanderson, R. A., M. D. Eyre, and S. P. Rushton. 2005. The influence of stream invertebrate composition at neighbouring sites on local assemblage composition. Freshwater Biology 50:221–231.

Sandoy, S., and A. J. Romundstad. 1995. Liming of acidified lakes and rivers in Norway an attempt to preserve and restore biological diversity in the acidified regions. Water, Air, and Soil Pollution 85:997–1002.

Schindler, D. W. 1988. Effects of acid rain on freshwater ecosystems. Science 239:149–157.

Simmons, K. R., and K. Doyle. 1996. Limestone treatment of Whetstone Brook, Massachusetts III. Changes in the invertebrate fauna. Restoration Ecology 4:284–292.

Smith, E. P., K. W. Pontasch, and J. Cairns. 1990. Community similarity and the analysis of multi-species environmental data: a unified statistical approach. Water Research 24:507–514.

Tank, J. L., and J. R. Webster. 1998. Interaction of substrate and nutrient availability on wood biofilm processes in streams. Ecology 79:2168–2179.

Van Sickle, J. 1997. Using mean similarity dendrograms to evaluate classifications. Journal of Agricultural, Biological and Environmental Statistics 2(4):370–388.

Van Sickle, J., J. P. Baker, H. A. Simonin, B. P. Baldigo, W. A. Kretser, and W. E. Sharpe. 1996. Episodic acidification of small streams in the northeastern United States: fish mortality in field bioassays. Ecological Applications 6:408–421.

Weatherley, N. S. 1988. Liming to mitigate acidification in freshwater ecosystems: a review of the biological consequences. Water, Air, and Soil Pollution 39:421–437.

Weatherley, N. S., G. P. Rutt, S. P. Thomas, and S. J. Ormerod. 1991. Liming acid streams: aluminum toxicity to fish in mixing zones. Water, Air, and Soil Pollution 55:345–353.

Wiggins, G. B. 1998. Larvae of the North American caddisfly genera. Second edition. University of Toronto Press Toronto, Ontario, Canada.

Wigington, P. J., J. P. Baker, D. R. DeWalle, W. A. Kretser, P. S. Murdoch, H. A. Simonin, J. Van Sickle, M. K. McDowell, D. V. Peck, and W. R. Barchet. 1996. Episodic acidification of small streams in the northeastern United States: episodic response project. Ecological Applications 6: 374–388.

Williams, D. D., and N. E. Williams. 1974. A counter staining technique for use in sorting benthic samples. Limnology and Oceanography 19:152–154.

Zurbuch, P. E., R. Menendez, and J. L. Clayton. 1996. Limestone neutralization of Dogway Fork, West Virginia, by means of a rotary-drum system. Restoration Ecology 4: 206–219.

APPENDIX A

A figure showing study locations and corresponding stream status within the Cheat River and Gauley River watersheds, West Virginia, USA (*Ecological Archives* A017-038-A1).

APPENDIX B

A table containing descriptive characteristics and distribution coordinates of the 20 study sites in the Cheat River and Gauley River watersheds, West Virginia, USA (*Ecological Archives* A017-038-A2).

APPENDIX C

A figure showing mean daily flow and annualized mean daily flows (2003–2004) for the Cheat River at Parsons, West Virginia, USA (*Ecological Archives* A017-038-A3).

APPENDIX D

A table listing acid tolerance and functional family feeding-group (FFG) classifications and number of sites captured for all benthic-macroinvertebrate taxa collected (*Ecological Archives* A017-038-A4).