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The Microbial Link in Ecosystem Processing in the East Fork of the Jemez River:

Extracellular Enzyme Response to Habitat, Seasonal Fluctuations, and Wildfire Disturbance

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Abstract

Mounting evidence suggests that a warming climate in the southwestern United States threatens the quality and quantity of mountain source waters for New Mexico's largest surface water source, the Rio Grande. These waters, fed primarily by snowmelt, are critical to the ecological, agricultural, municipal, and cultural requirements of New Mexico. In an effort to construct a baseline of stream metabolism in the East Fork of the Jemez River (EFJR), a major tributary of the Rio Grande, our research team sampled macrophytes, algae, macroinvertebrates, and benthic microbial communities to assess changes pertaining to habitat and seasonal fluctuations and in response to a devastating wildfire in the headwaters during the summer of 2011.

This project focused specifically on the role of benthic heterotrophs in organic matter and nutrient cycling and export. Within the benthic microenvironments, retention, mineralization, assimilation, and recycling of particulate and dissolved organic matter occur. Particularly in autochthonous-dominated systems like the EFJR where algal-bacterial interaction controls organic matter processing, benthic microbes contribute significantly to ecosystem processes and are a key to understanding fluvial carbon and nutrient cycling. We conducted assays quantifying biofilm extracellular enzyme activity, the first step in microbial degradation of organic compounds, targeting prominent extracellular enzymes that change with the availability of carbon and nutrients in the sediments. Benthic sediments from three pools and three riffles on the EFJR were collected over a year's period to coincide with major seasonal events: the end of spring snowmelt, early summer, mid-summer, early fall after the monsoon season, and late fall. Water quality data on temperature, pH, dissolved oxygen, turbidity, specific conductivity, nitrate and soluble reactive phosphorus concentrations were collected using continuous monitoring equipment installed in the stream.

Data on extracellular enzyme activities to specific substrates of organic carbon, nitrogen, and phosphorus were correlated with stream water quality parameters and nutrient data, macrophyte and algal biomass, and whole-stream metabolism to ascertain microbial response to habitat and seasonal fluctuations pertaining to metabolic processes and changes attributable to the summer wildfire.

Results of the extracellular enzyme assays suggest that the EFJR is highly productive, generating high levels of activity of carbon (C)- and nitrogen (N)-acquisition enzymes correlating to high rates of gross primary productivity and community respiration. C- and N-acquisition ecoenzyme activities peaked in the month of greatest algal and macrophyte production (July) and were least active after spring snowmelt. Activities were generally higher in pools than in riffles, correlated with the greater biomass of macrophytes and algae in the pools. Stoichiometric ratios of the ecoenzyme activities were calculated and C:N, C:P, and N:P ratios were consistent throughout the year with little seasonal fluctuation, suggesting autotroph-heterotroph interactions are in dynamic equilibrium.

Post-fire effects were pronounced, with drops in C- and N-acquisition enzyme activities and spikes in phosphorus (P)-acquisition ecoenzyme activities, up nearly 9 X pre-fire levels. Instream nutrients, specific conductivity, and turbidity increases, dissolved oxygen sags, and a 70% drop in macrophyte biomass at our site was attributed to major physical disturbances from unusually high overland flow upstream in the burned headwater forests. Increased nutrients mobilized from meadow soils and leached from the phosphate-rich ash load may have triggered the dramatic phosphatase response.

Extracellular enzyme activity may prove to be an important metric for understanding the complex processes of organic matter cycling in aquatic ecosystems. Coupled with traditional parameters of water

quality as monitoring tools, ecoenzyme assays may provide evidence of ecosystem changes due to climactic and anthropogenic alterations in watersheds critical to the water supply of New Mexico.

Introduction

Climate models predict accumulating greenhouse gases will drive a poleward expansion of the subtropical dry zone, resulting in a drier southwestern United States (Seager and Vecchi, 2010). Furthermore, climate change is expected to lead to severely reduced regional winter precipitation and mountain snow pack, a shift to earlier spring runoff, a change to more rain and less snow, and severe reductions in runoff rates (Milly et al., 2008). How this drying trend will affect New Mexico's water quality and quantity is of primary interest to research efforts in the UNM Departments of Biology and Earth and Planetary Sciences funded by New Mexico's Experimental Program to Stimulate Competitive Research (NM EPSCoR). In an effort to support and augment efforts to determine the effects of climate change on New Mexico's largest source of fresh water, the Rio Grande, we studied the function of aquatic heterotrophic microbes in connection to organic matter and nutrient cycling in the East Fork of the Jemez River (EFJR), a third-order stream that meanders through a large valley situated in the Valles Caldera National Preserve in the mountainous region near the headwaters of this Rio Grande tributary.

Our specific objectives in studying biofilm extracellular enzyme activity were to determine if heterotrophic activity varied by habitat, in pools vs. riffles, or with changing season, and whether these changes correlated with whole-stream metabolism, stream biogeochemical parameters, or nutrient availability. Secondarily, we wanted to study the effects of a major forest fire in the headwaters of the East Fork on specific extracellular enzyme activities in downstream sediments.

Literature Review

Importance of heterotrophs to organic matter cycling

Microorganisms in benthic biofilm communities are critical to organic matter cycling in aquatic ecosystems (Meyer et al. 1988; Findlay et al. 2010; Van Horn et al. 2011). Organic matter necessary for biotic growth in streams is derived from either allochthonous (external plant and animal detritus) or autochthonous (derived from internal aquatic plant, algal or microbial) sources. The activity of the microbial community in cycling detrital organic matter and releasing energy in the process is a major key in driving stream nutrient cycling. Heterotrophic organisms break down complex organic matter and convert this material to smaller carbon-containing compounds for assimilation by both autotrophs and heterotrophs (Cummins et al. 1979; Meyer et al. 1988). The aptly named "microbial loop" describes the central link of microbes in the bottom-up food webs of lotic systems (Meyer 1994). Microbes are integral to the processes of organic matter decomposition and production of assimilable carbon- and nitrogen-

containing inorganic compounds for energy and nutrients as well as serving as a principal source of carbon for many taxa of macroinvertebrates (Edwards and Meyer 1987; Hall and Meyer 1998).

The majority of microbes in stream ecosystems exist in biofilms, attached colonies of organisms embedded within a complex of self-produced extracellular polymeric substances (Konhauser 2007). Biofilms are highly organized and resilient ecosystems where organic and inorganic compounds are cycled between community members. These natural ecosystems are composed of autotrophic (algae and cyanobacteria) and heterotrophic (bacteria and fungi) organisms that vary by season and biome. Battin et al. (2003) illustrated the importance of biofilms in stream metabolism by demonstrating a correlation between an increase in biofilm mass with increased hydrodynamic storage and particle retention in artificially-constructed stream mesocosms. It is within these microenvironments in which retention, mineralization, assimilation and recycling of essential elements occur. Benthic biofilms contribute significantly to aquatic nutrient cycling and, as such, are a valuable ecosystem component to monitor.

Factors affecting carbon and nutrient cycling

Carbon, nitrogen, and phosphorus cycling in aquatic systems are influenced by a host of biogeochemical factors. Stream nutrient concentrations (Hillebrand et al. 2002; Stelzer et al. 2003), temperature (Cummins and Klug 1979), light (Fellows et al. 2006; Roberts et al. 2007), pH (Strauss et al. 2002; Fierer et al. 2007), flow (Dahm et al. 2003; Sabater et al. 2006; Liu et al. 2008), quality and quantity of organic matter (Dahm 1981; Findlay et al. 2003; Kominoski and Rosemond 2011), and the biological community structure (Hoellein et al. 2010) all influence how carbon and nutrients are utilized. These biogeochemical factors are engendered in large part by the hydrology and climate of the particular stream in question.

Sabater et al. (2006) demonstrated that in times of low flow in Mediterranean streams detritus accumulates, particularly in pools as opposed to riffles, and contributes to increases in ecoenzyme activity. In addition to the effects of scouring in periods of high flows that can dramatically alter community structure, seasonal changes in algal, macrophyte, and macroinvertebrate populations would suggest that ecoenzyme activity could vary both spatially and seasonally. That nutrient concentrations may change seasonally was shown in a study conducted in the vicinity of our study site in the Valles Caldera (Liu et al. 2008). The authors showed that nutrient concentrations increased after snowmelt and monsoonal events and suggested that the nutrients were leached from organic-rich soils with the runoff. Thus, seasonal variations in flow may affect community structure and detrital biomass and subsequently ecoenzyme activity.

Thanks to instruments funded by NM EPSCoR, continuous monitoring of stream nutrient concentrations and stream parameters such as pH, dissolved oxygen (DO), temperature, turbidity, and specific conductivity was available at our study site. These data were useful in drawing conclusions about seasonal changes in instream biogeochemical factors and their influence on metabolic activity.

Biofilm role in water quality: Microbial enzyme function

In an early paper on stream ecosystems, Cummins (1974) stressed that stream water quality relies on the continued functioning of critical micro- and macrobiota. To the end of preserving relationships in

the benthic community, he advocated including functional assays in water quality monitoring efforts. This study employed an assay to quantify extracellular enzyme activity specifically targeting the function of microbes in organic matter processing. Because our study stream is autochthonous-dominated, the role of fungi, critical in decomposition of allochthonous debris such as twigs and leaves, was considered to be of less importance than bacteria, which have been shown to be more active on stream sediments and fine particles (Hieber and Gessner 2002).

The first step in microbial breakdown of organic matter is the hydrolytic activity of extracellular enzymes that accumulate in biofilms. These enzymes catalyze the degradation of complex organic compounds into low molecular weight compounds that are then available for assimilation by heterotrophic organisms (Sinsabaugh et al. 1991). The activity of these extracellular enzymes exuded in the biofilm has been correlated with mass loss of detrital matter in streams (Sinsabaugh et al. 1994). Romani et al. (2004) found a positive relationship between the types of dissolved organic carbon (DOC) and extracellular enzyme activity (EEA), concluding that the microbial activity in the biofilms mediated DOC dynamics.

Enzyme activity in biofilms is stimulated by both microbial growth (Sinsabaugh et al. 2009) and by increases in and types of available organic substrates (Findlay et al. 2003). Increases in polysaccharides will be followed by amplified activity of polysaccharide-hydrolyzing enzymes such as β–glucosidase and N-acetyl-glucosiminidase. Alkaline phosphatase activity will be stimulated by increases in organic phosphate in the biofilms. Artigas et al. (2008) established a relationship between EEA and the type of organic matter available and the C:N:P ratio of the stream organic matter.

The most widely studied extracellular enzymes in aquatic systems are those that catalyze the hydrolysis of terminal ends of primary sources of C-, N-, and P-containing organic compounds (Sinsabaugh et al. 2010). The five enzymes we used and the reactions they catalyze are β-glucosidase (βG), hydrolysis of structural polysaccharides such as lignocellulose, a principal component of plant cell walls; α-glucosidase (αG), hydrolysis of smaller, more labile polysaccharides; N-acetyl-glucosaminidase (NAG), hydrolysis of aminosaccharides such as chitin, a C- and N-containing component of macroinvertebrates; alkaline phosphatase (AP), hydrolysis of phospholipids and phosphosaccharides; and leucine amino peptidase (LAP), hydrolysis of proteins and polypeptides. The activities of these enzymes were individually quantified and reported and also used in ratios to simulate the stoichiometric ratios, C:N, C:P, and N:P.

Stoichiometry of enzyme activity

In his seminal paper on carbon, nitrogen and phosphorus stoichiometry, Redfield (1958) established the correlation existing between sea water levels of nitrate and phosphate and demonstrated that living marine phytoplankton have similar molar ratios of N:P at 16:1. This natural balance of nutrients can be found in all aquatic systems (although at different ratios) and reflects the fact that trophic interactions occur in equilibrium and the biochemical composition of algae is remarkably consistent for these elements.

Heterotrophic streams may be less responsive than autotrophic streams to changes in the N:P ratio. Schade et al. (2011) found this was true in several California headwater streams that are traditionally N-limited. The authors conjectured that algae have a greater capability to store nutrients than bacteria and therefore have greater stoichiometric plasticity. Bacteria have no storage vacuoles so their internal stoichiometry varies less. Presumably, then, bacteria must ramp up ecoenzyme activity to hydrolyze the available organic sources of nitrogen and phosphorus to generate assimilable nutrients when nitrate and inorganic phosphate concentrations are low.

Several authors have utilized extracellular enzyme activity data to produce stoichiometric ratios that can be used to reflect the changes in community metabolism in response to nutrient limitations or seasonal variations. Stating that extracellular enzyme activity is reflective of both microbial growth and nutrient and carbon availability, Sinsabaugh et al. (2009) proposed that the C:N:P ratios of organic matter in soils and streams could be modeled using ratios of the EEA specific for dominant sources of C, N, and P. Artigas et al. (2008) demonstrated a connection between EEA and biofilm stoichiometry by showing that biofilm LAP activity increased as biofilm biomass C:N ratio decreased. Van Horn et al. (2011) reported an increase in EEA with increased nutrient supply in their study using artificial stream mesocosms. These studies suggest that C:N:P ratios of ecoenzyme activities can be utilized as a sensitive measure of stream nutrient availability and biotic demand.

Sinsabaugh et al. (2010) surveyed ecoenzyme data from nine published studies and produced stoichiometric ratios from the data. The authors suggest that the results were consistent with other established conceptual models to assess biofilm and planktonic community function. Coupled with the advantage that ecoenzyme activities are more easily assayed than many other methods of assessing microbial function, the ecoenzyme activity ratios are a practical method for assessing community metabolism in response to nutrient limitations or seasonal changes in community structure.

Forest fire effects on stream water chemistry and biota

In June of 2011, a wildfire in the Jemez Mountains quickly consumed more than 635 km^2 of the Santa Fe National Forest, Valles Caldera National Preserve, Bandelier National Monument, and Santa Clara Pueblo. By the time the Las Conchas fire was finally declared to be out in early August, its size and intensity made it one of the most destructive wildfires in New Mexico's history. Although our study site escaped the flames, the forests of the headwaters of the EFJR were extensively burned.   Reported effects of the burn in the forest and the EFJR were increased overland flows and flooding after summer monsoon events, increased turbidity in the stream (Figure 1), major fish kills, and decreased DO that persisted intermittently for weeks and was reported more than 110 km downstream in the Rio Grande (C. Dahm, unpublished). Long-term effects of the Las Conchas fire on the quality of the EFJR have yet to be determined.

Other research has reported wildfire effects on stream ecosystems. Earl and Blinn (2003) conducted a five-year study on the effect of wildfires on water chemistry and biota in the Gila River of New Mexico. They reported increases in nutrients (ammonium, nitrate and phosphate), turbidity, alkalinity, and pH and decreases in DO that were short-lived. However, high phosphate levels continued to persist for more than a month post-fire. Effects on the macroinvertebrates were severe, but periphyton showed few effects. There was no mention of microbial alterations due to wildfires, but drops in DO and increases in turbidity would likely affect microbial biomass and high phosphate levels could influence ecoenzyme activity.   A study in Australian pine and eucalyptus forests post-wildfires (Smith et al. 2012) demonstrated that total suspended solids (TSS), turbidity, nitrate and phosphate levels in nearby streams returned to normal three years after the fires, but sediment exports from these forests into nearby catchments continued to persist after storm events, particularly in the pine forests. These studies suggest that the effects of the Las Conchas fire on the Jemez River watershed will persist for some years.

Figure 1: Photos of our study site on the EFJR in June, 2011 (left), and post-fire in August of the same year (right). The high turbidity in August was due to the post-fire overland flows and ash fall in the headwaters of the EFJR.

Biofilm composition and effects on extracellular enzyme activity

The composition of the communities in biofilms has been shown to affect extracellular enzyme activity (Romani and Sabater 1999; Romani and Sabater 2000; Rier et al. 2007). Romani and Sabater (1999) found a positive correlation between chlorophyll-*a* concentration (algal activity) and increased bacterial enzyme activity, especially β-glucosidase. Algal photosynthetic activity was reported to alter ecoenzyme activity in Romani and Sabater (2000). The authors demonstrated that biofilms grown in light had higher hydrolytic enzyme activities than those grown in the dark. Rier et al. (2007) tested EEA from biofilms grown in light and artificially-shaded stream channels and found that increased levels of light increased alkaline phosphatase, β-glucosidase, and leucine aminopeptidase activities. They also showed a significant correlation between algal biomass and the activities of alkaline phosphatase and leucine aminopeptidase, enzymes involved in the mineralization of organic phosphates and organic nitrogen. If algal photosynthesis plays a role in stimulating bacterial enzymatic activities, then algae may ultimately influence decomposition rates. Algal and macrophyte biomass and parameters assessing stream metabolism, that is the P:R ratio, were correlated with biofilm ecoenzyme activity in an effort to ascertain if there was a seasonal interplay between algal growth and the enzymatic function of microbes.

Materials and Methods

Study site

      The study site that supports the efforts of a wide variety of NM EPSCoR projects is located in the Valles Caldera National Preserve approximately 120 km north and west of Albuquerque, New Mexico, in the Jemez Mountains (Figure 2). Our biotic sampling sites and continuous monitoring instruments are located on the EFJR, within an elk exclosure (Figure 3) that encloses approximately 300 m of meandering stream. The site, a high mountain third-order stream situated in a large meadow with few riparian trees or shrubs, is situated less than 12 km from the headwaters of the study stream. Overall, the basal flow in the EFJR near our site ranges from 0.08-0.12 $m^3 s^{-1}$, but spring snowmelt and periodic monsoonal events in the months of July and August have increased discharge many times the base flows. The study stream consists of pools with mainly sandy bottoms and riffles primarily composed of cobbles and gravel.
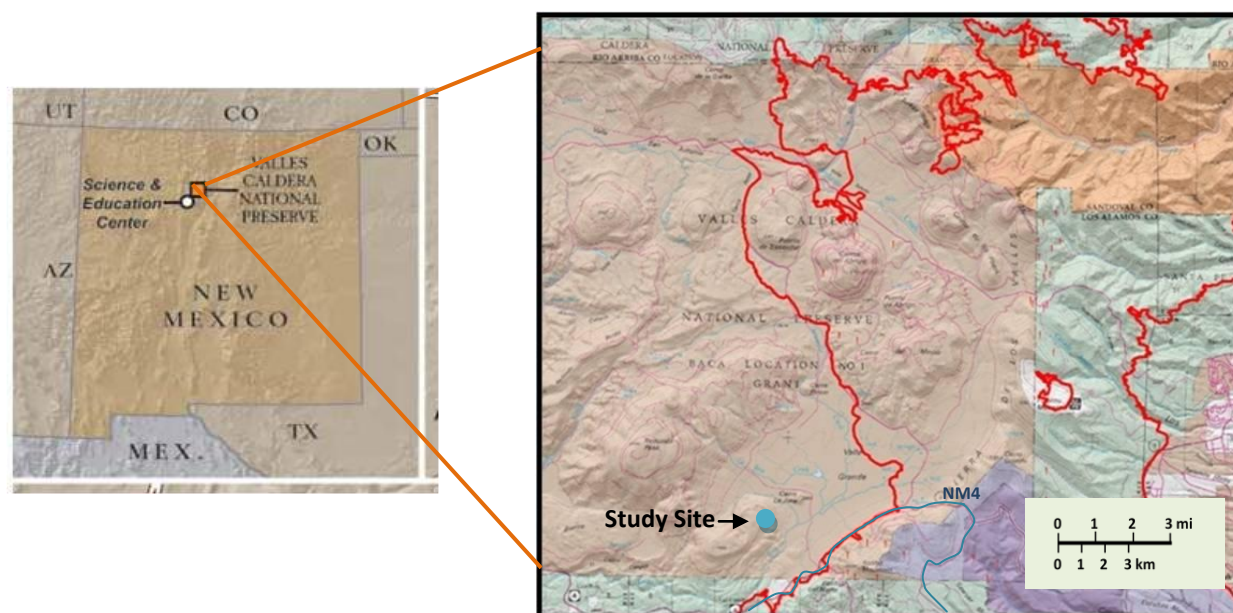

Figure 2: The Valles Caldera National Preserve (VCNP) is located in north central New Mexico. The sampling site within the Valles Caldera, indicated by a blue circle, is situated on the East Fork of the Jemez River, about 3 km from the main southern entrance of the VCNP. The red outline on the inset map shows the boundary of the Las Conchas wildfire in late June to early August, 2011.

Figure 3: The sampling site on the East Fork of the Jemez River, outlined in grey, is located within an elk exclosure in the Valles Caldera National Preserve that encloses roughly 300 meters of meandering stream.

Sampling procedure

Benthic samples were collected from the EFJR on six occasions over the course of a year. These dates were chosen to coincide with major seasonal events: early summer, mid- summer (during the monsoon season), early fall (post-monsoon season), late fall, post-spring runoff, and early summer. At each sampling date, six sites were sampled, three pools and three riffles. Within each pool and riffle site, nine samples were obtained including three cross-channel samples at an upstream transect, three cross-channel samples at a midstream transect, and three cross-channel samples at a downstream transect. Surface benthic sediments to approximately 2 cm in depth were sampled because the surface layer of the benthos is purported to contain the greatest microbial biomass in most streams (Findlay et al. 2002). Sampling was conducted with a coring apparatus (5 cm in diameter by 2 cm in depth) to demarcate the site and sediments were then aspirated into sterile polycarbonate tubes (Figure 4). Samples were kept on ice in the dark during transport and frozen within 5 hours of collection at -80°C until assayed.

Figure 4: The sampling procedure in the EFJR involved sinking a coring apparatus 2 cm into the sediments and aspirating the contents into sterile tubes .

Biofilm function

Biofilm sediments were assayed for the activity of five hydrolytic extracellular enzymes: alpha-glucosidase (αG), beta-glucosidase (βG), alkaline phosphatase (AP), N-acetylglucosaminidase (NAG), and leucine aminopeptidase (LAP). Table 1 lists the enzymes chosen, their enzyme commission number, and the function of each enzyme. These enzymes were selected to target the processing of organic carbon (the glucosidases), organic nitrogen (the glucosaminidase and aminopeptidase), and organic phosphorus compounds (phosphatase) and are the most widely studied enzymes in ecoenzyme assays. The assay was run according to an adaptation of the protocol of Sinsabaugh et al. (1997). Substrate and sediment solutions were prepared in 5 mM bicarbonate buffer at pH 8. Sediments were pre-weighed in 0.06-0.07 g aliquots in microtubes. To start the assay, the sediments were suspended in 400 µL of buffer and 100 µL of substrate to affect the appropriate concentration. During incubations, the microtubes were tumbled continuously at room temperature in the dark 0.8 to 2.5 hours, depending on the optimal enzyme activity dynamics. Incubation timing was established in preliminary linearity trials. At the end of incubation, the samples were centrifuged and 250 µL aliquots of supernatant were added to wells in black, 96-well polystyrene flat-bottom microtiter plates (Sigma-Aldrich, Missouri, USA). Four replicate wells were run per sample. Each plate contained reference standards, substrate, sample, and quench controls. The quench control was included to quantify nonspecific reduction of fluorescence by the sediment alone. Fluorescence was measured using a Dynatech MicroFLUOR plate reader set to an excitation wavelength of 365 nm and an emission wavelength of 450 nm. Extracellular enzyme activities were reported as nmol substrate converted per hour for each gram of sediment dry mass (nmol h^{-1} g^{-1}).

Stoichiometric ratios

Stoichiometric ratios of extracellular enzyme activities were produced using $\log_e$-transformed activity data. The stoichiometric ratios of the EEA were calculated as follows: for C:N ratio, the BG: (NAG + LAP) ratio was used, for the C:P ratio, the BG:AP ratio was used, and for the N:P ratio, the (NAG + LAP):AP was used.

Biomass assessment

Dry mass (DM), ash-free dry mass (AFDM), and organic matter percentages were determined on all samples. Sediment samples were dried overnight at 80°C on pre-weighed aluminum pans and reweighed to determine dry mass. The samples and pans were then incinerated for 3 hours at 500°C and allowed to cool overnight. Following incineration, the samples and pans were reweighed and AFDM was determined. The organic matter percentage was calculated from the mass lost in the incineration process.

Macrophyte and algal biomass

Data on filamentous algal and macrophyte biomass assessments from samples collected on the same in pools and riffles were generously provided by fellow researchers associated with the NM EPSCoR water quality project (V. Thompson, personal communication).

Water quality, chemistry, and nutrient data

Measurements of depth, width, and flow velocity were conducted at each pool and riffle transect. Discharge was calculated from these data. Water quality and nutrient data were collected using continuous monitoring instruments that were deployed instream from ice-out (early March) through November (Figure 5). Parameters such as DO, pH, turbidity, specific conductivity, and temperature were collected with a SONDE (YSI, Inc., Ohio, USA) while nutrient data were collected with a SUNA UV nitrate sensor (SAtlantic LP, Halifax, Canada) and a Cycle-P phosphate sensor (Wet Labs, Oregon, USA). SUNA and SONDE data were generously supplied by L. Sherson.

Figure 5: The continuous monitoring equipment set up in the EFJR (left) includes a SONDE (YSI, Inc., Ohio, USA), a SUNA UV nitrate sensor (SAtlantic LP, Halifax, Canada) and a Cycle-P phosphate sensor (Wet Labs, Oregon, USA).   At the right is a photo of a SONDE that collects temperature, pH, dissolved oxygen, specific conductivity, and turbidity data at 15-minute intervals.

Whole stream metabolism

Whole stream metabolism data were calculated using a model that derives gross primary productivity (GPP) and community respiration (CR) from PAR, % saturation of dissolved oxygen, barometric pressure, and water temperature data using a protocol developed by Grace and Imberger (2006). These data were calculated and generously provided by a fellow researcher associated with the NM EPSCoR water quality project (B. Shafer, personal communication).

Data Analysis

Data analysis consisted of calculating the enzyme activity per gram of sediment organic matter. Input values were fluorescence and aliquot masses from 4 replicates, fluorescence averaged from 4 substrate controls and 4 sample controls, the dry mass to wet mass ratios for each pool and riffle sample, the volumes of supernatant used (0.5 mL) and volume in the plate wells (250 µL), the quench and emission coefficients, and the number of hours of incubation for the specific enzyme.

The quench coefficient is a measure of the decrease in fluorescence caused by interactions of the substrate with the sample itself. The quench coefficient is estimated by taking the ratio of the fluorescence of the sample + standard divided by the fluorescence of the standard alone and adjusting for an emission

coefficient calculated from standards. According to Sinsabaugh et al. (1997), the quench coefficient measures between <10-40%.

Statistical Procedures

Analysis of variance (ANOVA) was used to detect differences in ecoenzyme activity between the sampling periods and to determine differences in ecoenzyme activities and stoichiometric ratios between pools and riffles. Determinations of correlations among ecoenzyme activities, gross primary productivity, community respiration, stream nutrient concentrations, and algal and macrophyte biomass were conducted using Pearson correlation analysis and linear regression. Because of the high variances of the raw EEA data, $\log_e$-transformed data were used for all calculations and comparisons of stoichiometric ratios. Standardized major axis (Type II) (SMA) regressions were run on the stoichiometric ratios using SMATR, version 2 (Warton et al. 2006).

Results

Spatial comparisons of ecoenzyme activities

Sampling averages of minimum and maximum temperatures, minimum dissolved oxygen, pH, specific conductivity, and stream levels of nitrate and phosphate ions are given in Table 2. Mean data for the biomass of stream biofilm sediments, macrophytes (combined species) and filamentous green algae (*Cladophora*) as well as estimates for GPP, CR, and the P:R ratio are summarized in Table 3.   Ecoenzyme activities for all five enzymes over the six sampling dates are summarized (Table 4). Data were given for pools and riffles separately as these overall means for pools differed significantly from riffles for all but AG. Analysis of variance (ANOVA) comparison of means of ecoenzyme activities of BG ($p < 0.01$), AP ($p < 0.05$), NAG ($p < 0.01$), and LAP ($p < 0.05$) showed significant differences between pools and riffles. The biomass of macrophytes and filamentous green algae was also significantly greater in pools than in riffles (averages for pools, 1270 g OM m^{-2}, and for riffles, 55.3 g OM m^{-2}; $p < 0.001$).

Temporal comparisons of ecoenzyme activities

Seasonal variations were documented in all five enzyme activities (Table 4). The carbon-acquiring enzymes (BG and AG) showed the greatest activity in July, 2011, and the lowest activity in April. Gross primary productivity (GPP) and community respiration (CR) were at the highest levels in July (74.0 and 68.7 mg O$_2$· L^{-1} ·s^{-1}, respectively; P:R ratio = 1.1) and lowest in April (14.4 and 9.3 mg O$_2$· L^{-1} ·s^{-1}, respectively; P:R ratio = 1.6) (Table 3). The activities of BG and AG were positively correlated with GPP (BG: r^2 = 0.250, $p < 0.01$; AG: r^2= 0.392, $p < 0.01$) and with the biomass of macrophytes and filamentous green algae (BG: r^2 = 0.120, $p < 0.05$; AG: r^2 = 0.127, $p < 0.05$).

The nitrogen-acquiring enzymes (LAP, NAG) paralleled the BG activity especially in the pools, peaking in July and showing the lowest activity in the April sampling.   Unlike the BG, AG and NAG, LAP activity was higher in riffles at 4 of 6 time points. Pool activities were greater than riffles in the two months

of maximum LAP activity (July and October). Like BG and AG, significant correlations were demonstrated between NAG (r^2 = 0.131, $p < 0.01$) and LAP (r^2 = 0.127, $p < 0.05$) activities and biomass of macrophytes and algae. Correlations with GPP were significant for NAG (r^2 = 0.255, $p < 0.01$) and LAP (r^2 = 0.461, $p < 0.01$) as well.

Negative correlations were seen with BG, AG, NAG and LAP activities and stream nitrate levels, and for BG and NAG with stream phosphate levels as well. For NO_3^-, p values for BG, AG, and NAG were $p <$ 0.001 while LAP was $p < 0.01$. For PO_4^{-3}, regression p values for BG were $p < 0.05$; for NAG, $p < 0.001$.

The peak activity in the pools for AP, the phosphorus-acquiring enzyme, was also in July and in June 2012, and lowest in April, showing a relationship with the peak activities of C- and N-acquiring enzymes. AP peak activity was not correlated with the biomass of macrophytes and algae. AP activity was correlated with GPP (r^2 = 0.300, $p < 0.05$) only in the pools. AP activity in the riffles was unlike the other enzymes as AP exhibited minimal seasonal shifts in activity except for a dramatic spike in September where the mean (24,030 nmol $hr^{-1} g^{-1}$ OM) was nine times greater than the mean AP activity for the rest of the sampling dates (2639 nmol $hr^{-1} g^{-1}$ OM).   And, unlike the other ecoenzymes, AP activity in the riffles was positively correlated with specific conductivity ($p < 0.001$), and stream nutrients, NO_3^- ($p < 0.05$) and PO_4^{-3} ($p < 0.001$).

Stoichiometric ratios

Stoichiometric ratios of log_e-transformed ecoenzyme activities varied spatially, in the pools vs. riffles, and seasonally (Table 5). ANOVA comparison of all sampling dates showed statistically significant differences between pools and riffles for the ratios: C:P ($p < 0.001$), C:N ($p < 0.001$), and N:P ($p < 0.005$).

Standard major axis (Type II) regression slopes for BG:AP (C:P) ratios were 0.822 for pools (r^2 = 0.140, $p < 0.01$) and 0.990 (r^2 =0.087, *N.S.*) for riffles. BG:(NAG + LAP) (C:N) ratio regression slopes were 0.987 for pools (r^2 = 0.513, $p < 0.001$) and 0.787 (r^2 = 0.633, $p < 0.001$) for riffles. (NAG + LAP):AP ratio (N:P) averages were 1.032 for pools (r^2 = 0.428, $p < 0.001$) and 0.902 for riffles (r^2 = 0.066, *N.S.*).

Fire effects on ecoenzyme activity and on stream physical and chemical parameters

Effects of the Las Conchas wildfire in late June to early August, 2011 were evident in stream primary productivity and in extracellular enzyme activities in the sediments.   With one dramatic exception, all ecoenzyme activities were significantly lower in September than in July and rebounded again in October (Table 4). In fact, ANOVAs of the activities of BG, AG, and NAG showed that the September ecoenzyme activities were different compared to all other dates ($p < 0.05$) except the early spring sampling in April when activities were very low. The notable exception to reduced post-fire ecoenzyme activity was the extremely high alkaline phosphatase activity in the riffles during the September sampling. On this date, the mean AP activity was 24,030 nmol $hr^{-1} g^{-1}$ OM compared with the July and October AP activities of 2580 and 2600 nmol $hr^{-1} g^{-1}$ OM, respectively. This AP activity was greater by 4 to 11 times the maximum activity of the other four enzymes over the study's duration. Stream nitrate levels also increased from 6 µM in July to 13.4 µM post-fire, while phosphate levels went up from 0.22 µM to 2.20 µM post-fire (Table 2).

Macrophyte biomass in riffles (Table 3) dropped nearly 70% from July levels to September levels (from 650 to 218 g OM m^{-2}) after post-fire scouring events. Filamentous green algae were eradicated from the riffles and pools. The P:R ratio, consistently between 1.2 and 1.5 the rest of the year, fell to 0.9, and both GPP and CR dropped to half the July rates, signaling a severe ecosystem disruption.

Stream physical and chemical parameters also showed evidence of fire effects. Discharge data for the two weeks prior to this sampling date showed peak flows of 0.63 $m^3 s^{-1}$ and flows of 0.32 $m^3 s^{-1}$ in the riffles on the post-fire sampling date (Table 3) compared with 0.05 $m^3 s^{-1}$ on the July sampling date. Depths in the pools and riffles on this sampling date were the greatest of the seven sampling dates (Table 3) and were four times the depth of the pools and riffles in September a year after the fire (personal communication, data not shown). Increases in specific conductivity, lowered dissolved oxygen (DO) levels, and increases in stream nitrate and phosphate ion concentrations indicated a chemical change in water quality. AP activity in the riffles was positively correlated with specific conductivity ($r^2 = 0.486$, $p < 0.001$), stream nitrate ion concentration ($r^2 = 0.121$, $p < 0.05$), and stream phosphate ion concentrations ($r^2 = 0.459$, $p < 0.001$).

Because of this major change in AP activity post-fire, we analyzed the stoichiometric ratios for September 2011 and compared them with data compiled from all other sampling dates. Figure 6 illustrates the change in slopes for September stoichiometric ratios in riffles vs. pools and as compared with combined data from the other sampling dates. Table 6 summarizes the pool and riffle statistical data for this comparison.

Discussion

Comparison of EEA data with other studies

Our data on extracellular enzyme activities compare favorably with comparable studies in other rivers. Romani and Sabater (1999) found a correlation between algal activity and increased BG activity. Sinsabaugh and Foreman (2001) showed seasonal variation in EEA and related the increases in activity to times of increased productivity. Our results support these data. Stoichiometric ratios relating the $\log_e$-transformed EEA of BG to the other enzymes were comparable to the ratios from Sinsabaugh et al. (2010) for BG:AG and BG:NAG. We had higher BG:AP ratios, if the unusual data from the post-fire sampling is excluded, and lower BG:LAP ratios, reflecting the nutrient status of our stream which is nitrogen-deficient for most of the year. Our data were also compiled from a year of sampling and exhibit seasonal variations that may be a source of differences with other researchers' data.

Comparing our data to those of Hill et al. (2009), our July 2011 data suggest that the East Fork Jemez River is a very productive stream, with extracellular enzyme maximum activity levels at or higher than Hill et al.'s data for all five enzymes. These authors reported data from 447 sites in 3 rivers of the Mississippi basin, showing mean BG activities ranging from 47-125 nmol $h^{-1} g^{-1}$ dry mass (DM). Using our data calculated in the same units (nmol $h^{-1} g^{-1}$ DM), our average BG activities for the 6 sampling dates ranged from 35 in April to 219 nmol $h^{-1} g^{-1}$ DM in our July sampling.   Maximum means for Hill et al. (2009) for AG, NAG, LAP, and AP were 12, 60, 197, and 123 nmol $h^{-1} g^{-1}$ DM compared to our 99, 162, 256, and 173 nmol $h^{-1} g^{-1}$ DM.   Our maximum activities for all five enzymes were documented in July 2011, excluding the extremely high phosphatase activity in September associated with the post-fire disruption, and this may have been an unusually productive month overall. Indeed, the P:R ratio was 1.1 in July, with the greatest GPP and CR levels of the year, suggesting both primary productivity and microbial respiration were operating at high levels. P:R ratios in the East Fork Jemez River generally reflect an autotrophic

environment. Our P:R ratios for June, October, 2011, and April, June, 2012, were 1.3, 1.4, 1.5, and 1.3, respectively.

Habitat effects on extracellular enzyme activities

Carbon-acquisition activity (BG and AG) was greater in pools than in riffles overall. NAG, which hydrolyzes aminopolysaccharides, yielding both amino acids and glucosamine, was also greater in pools than riffles. The nitrogen-acquiring (LAP) and phosphorus-acquiring (AP) enzymes were more active in the riffles. These data support the findings of Romani and Sabater (2001) who found that the ecoenzyme activity involved in the degradation of polysaccharides was greater on sandy substrates, like those in pools, than on rocky substrates while phosphatase activity was greater on rocky substrates such as those found in riffles. We found that the pools were also the site of roughly 70% of macrophyte growth, the predominant source of primary production in the EFJR. The macrophyte biomass was greatest in October, and more balanced between pools (67%) and riffles (33%). During this October sampling, we found the greatest carbon-acquiring enzyme activity in riffles for all the study dates. In September, the pools contained 88% of the macrophyte biomass, due to the scouring post-fire that affected growth in the riffles. This sampling date also showed the lowest BG and AG activity outside of April when macrophytes and filamentous green algae were not yet growing. The activities of BG and AG are correlated with biomass of macrophytes and algae, supporting the explanation that the greater carbon-acquisition activity occurs in the pools than in the riffles. The peptidase and phosphatase activities were higher in the riffles than in the pools except for the peak month for primary productivity when the riffles were replete with actively growing macrophytes and green algae. AP did not show a correlation with macrophyte and filamentous green algal biomass. It appears that in the East Fork of the Jemez River, the pools support greater rates of decomposition for organic carbon-containing compounds than do the riffles.

Ecoenzyme activities varied seasonally

Temporal differences in enzyme activities were evident. The carbon-acquiring enzymes (BG and AG) showed the greatest activity in July when both green algal biomass and macrophyte biomass were reaching peak productivity. The carbon-acquisition enzymes showed the lowest activity in April when filamentous green algae and macrophytes were not present.   The nitrogen-acquiring enzymes (LAP, NAG) mimicked the BG activity especially in the pools, peaking in July and showing the lowest activity in the April sampling. These results were similar to those reported by Romani and Sabater (2000) in various streams in Spain where they found that when the growth of algae was at its maximum, the ecoenzyme activity was also at the maximum. Sinsabaugh and Foreman (2001) also found elevated EEA at times of increased productivity. Hill et al. (2009) reported that BG, NAG, and LAP activity will increase in response to the increase in organic matter in the sediment. These data and ours support the contention that in autochthonous streams such as the EFJR, exudates from algae and macrophytes are a primary carbon source for biofilm communities and increases in algal and macrophyte biomass will trigger increases in the C- and N-acquisition enzyme activities.

For each of the enzymes responsible for C- and N-acquisition, a negative correlation with stream nitrate levels was documented. BG and NAG also showed a negative correlation with stream phosphate

levels. This would suggest that the activities of carbon and nitrogen acquisition may be influenced by drops in available stream nutrients. Researchers have suggested that increases in stream nutrients may actually decouple the bacterial-algal interactions (Scott et al. 2008), and this may be particularly important in autochthonous streams such as the EFJR.

The peak activity in the pools for AP, the phosphorus-acquiring enzyme, was in July, 2011, and in June, 2012, and lowest in April, showing a relationship with the peak activities of C- and N-acquiring enzymes. However, even though AP activity peaked with the BG, NAG, and LAP activities in the pools, this increase was not correlated with the biomass of macrophytes and filamentous green algae. Our results corroborate those of Romani and Sabater (2000), who showed no correlation between phosphatase activity and peaks in primary productivity. We showed that increased AP activity is correlated with the season of highest activities of C-acquisition (BG: r^2 = 0.595; p < 0.05), and N-acquisition (NAG+ LAP: r^2 = 0.896; p < 0.001). In contrast to a study by Hill et al. (2009), we found that AP activity, unlike the other enzymes, was positively correlated with stream nitrate and phosphate levels. Hill et al. (2009) compared ecoenzyme activities from 3 rivers in the Mississippi River basin and found no differences in phosphatase activities even though the rivers had significantly different water and sediment phosphate concentrations.

Stoichiometric ratios

Extracellular enzyme activities themselves clarify only a part of the picture of microbial response to changing organic carbon and nutrient content in streams. A more important metric to consider might be the stoichiometric ratios associated with carbon, nitrogen, and phosphorus acquisition. According to Sterner and Elser (2002) in their publication on ecological stoichiometry, maintaining a balance of C:N:P ratios is critical in regulating the biofilm organisms' response to changes in their environment.

We compared $\log_e$-transformed EEA ratios for C:P (BG:AP), C:N [BG: (NAG + LAP)] and N:P [(NAG + LAP): AP] using SMA (type II) regression analysis to those reported by Sinsabaugh et al. (2009). These authors computed ratios on $\log_e$ -transformed data from 445 lotic sediments in the Mississippi River basin and reported mean ratios of 1.063 for C:P, 1.008 for C:N, and 1.050 for N:P. Our mean ratios for C:P, C:N, and N:P for combined pool and riffle sites were 0.932, 0.869, and 1.062, respectively. For this comparison, we excluded the post-fire sampling data because those data were considerably different than the EEA the rest of the year due to the physical disturbance of the stream.

The nutrient acquisition ratio N:P was much the same as for data reported by Sinsabaugh et al. (2009), suggesting slightly greater N-acquisition activity, and the lower average C:P and C:N ratios found in our stream may be reflective of its highly productive nature. Carbon availability is not a limiting factor for the majority of the year.

Stoichiometric ratios were fairly consistent throughout the year (Table 5) with C:N ratios in the pools at or just below 1 (0.98-1.03) and C:P ratios around 1.1 (1.0-1.16). Riffles for both C:N and C:P were consistently just below 1 (0.91-1.06), excluding the post-fire sampling.

Fire effects on ecoenzyme activities

The Las Conchas wildfire, one of the largest in New Mexico's history, burned 634 km^2 of forests in northern NM, including the headwater forests of the EFJR, between the end of June and the early part of

August, 2011. The scorching of the Ponderosa pine forest in the EFJR headwaters caused hydrophobicity of forest soils and subsequent monsoonal rains created severe erosion that led to channel scouring downstream and produced high sediment and ash loads in the stream.

On-site observational evidence of high turbidity due to the heavy sediment load (Figure 1) and the severe scouring of the riffles was supported by the reduction in macrophyte biomass (Table 3), dropping 70% from July levels in the September sample (from 232 to 73 g OM m^{-2}). Filamentous green algae were scoured in high stream flows one to two weeks prior to the sampling date. Increases in nitrates (2 X) and phosphates (10 X) post-fire were similar to the results of Smith et al. (2011) who reported that post-fire nitrate levels in the water column increased as much as 250-fold and total P levels increased up to 141-fold over unburned levels.

Unlike the four other enzymes, AP activity in the riffles showed minimal seasonal shifts in activity except for the dramatic spike post fire (September sampling) (Table 4) where the mean AP activity was nine times greater than the mean activity for the rest of the sampling dates. This was likely an effect of post-fire disruption and was positively correlated with the instream nitrate and phosphate levels that also increased 2 to 10-fold at this time.   These increases in nutrient levels were likely a result of mobilization from the soils in the valley and leaching from the ash load. Spencer and Hauer (1991) documented 5- to 60-fold increases in phosphate, ammonium, and nitrate concentrations after a severe wildfire in Glacier National Park in 1988.

This dramatic increase in AP in the riffles and drops in BG, NAG and LAP in both pools and riffles affected the C:P stoichiometric ratios, consistently at or just below 1.0 the rest of the year (range 0.95-1.06) (Figure 6). The C:P ratio dropped significantly in the riffles to 0.77 ($p < 0.001$) and the N:P ratio to 0.78 ($p < 0.001$). The consistent ratios during the rest of the year support the contention that biofilm communities will reach stoichiometric balance despite the heterogeneity of structural and nutrient input regimes found in lotic systems (Sinsabaugh et al. 2010), thereby making these significant changes in ratios in the post-fire sampling all the more unusual.

Because of the severe disruption of primarily the riffle habitats just prior to the September sampling due to flood events and severe erosion upstream, and heavy ash fall and chemical changes in the aftermath of the fire, the September sampling date was analyzed separately from all other dates for stoichiometric ratios (Table 6). Figure 6 illustrates the marked differences in the slopes for the September C:N, C:P, and N:P riffle ratios as compared with all other sampling dates. Higher phosphorus-acquiring enzyme activity and correspondingly lower carbon- and nitrogen-acquiring activities accounted for the altered ratios.

The month following the devastating scouring events showed a significant rebound for the EEA, but the low rate of GPP compared to the July peak (Table 4) suggests that this was a microbial response to the senescence of the primary producers that would supply ample organic carbon to degrade. Whether this was a normal end-of-season level of response from the microbial community or an unusually high rate due to the devastation post-fire remains to be seen.

The effects of the wildfire on ecosystem processing in the EFJR appear to be persisting. The DO concentrations in the stream before ice-out were less than 1.0 mgL^{-1} (personal observation) and April 2012 activities for all the enzymes reflected a sluggish start to the spring season. And, although the GPP and CR modeling data suggest that the primary productivity and respiration rates were rebounding by June 2012, the microbial, macrophyte and filamentous green algal responses suggest otherwise.   The June 2012 carbon- and nitrogen-acquisition EEA were lower than in June 2011, and C:P and N:P stoichiometric ratios

were lower. AP activity was increased from the previous year. In June, 2012, the combined macrophyte and algal biomass was only 42% of the levels of June 2011 (Table 3). How long these effects will persist and when conditions in the East Fork of the Jemez River will return to pre-fire levels will be answered with future studies.

Conclusion

For a low-order stream that relies primarily on autochthonous carbon and nutrient sources, the East Fork of the Jemez River is a productive ecosystem. Implications of this study are that the primary producers and the microbes in the biofilms are intrinsically coupled through biofilm recycling of carbon, nitrogen and phosphorus to contribute to a highly efficient and responsive ecosystem. The data documenting seasonal differences support the responsiveness of the biofilm community to changes in carbon and nutrient input and contributes to a baseline for future comparison. Spatial comparisons elucidate that the structure of the substrate contributes to function. Pools show more consistency in activity over time due to their sandy substrates and to the fact that their greater depths serve to buffer the sediments from fluctuations in stream discharge.

The post-fire disturbance of the East Fork of the Jemez River produced some surprising results in ecoenzyme activities. While the decrease in carbon- and nitrogen-acquisition activities could be explained by the decline in autotrophic activity due to the severe scouring, particularly of the riffles, the extreme increases in phosphatase activity in response to wildfire effects have not been documented before. The implication is that microbial phosphatase activity was responding to increased instream phosphorus-containing compounds such as ash residue. Ongoing efforts in monitoring ecoenzyme activities in this stream over the next few years and in other streams subjected to severe disturbance will be helpful in determining if this response is sustained and widespread.

In nitrogen-poor streams such as ours, primary productivity may be more dependent on microbial activity to generate nutrients than on the nutrients in the water column. So, although stream chemistry parameters are useful to monitor, the dynamics of ecosystem processing require additional monitoring of the biological interaction of algae and bacteria to understand the complexities of stream metabolism. Data on extracellular enzyme activities are important indicators of microbial regulation of carbon and nutrient dynamics in lotic systems and used as a monitoring tool may provide evidence of ecosystem changes due to climactic and anthropogenic alterations in watersheds.

Tables and Figures

Table 1. The ecoenzymes targeted in this study and the reactions they catalyze.

Ecoenzyme	Abbreviation	Enzyme Commission Number	Function of Enzyme
β-1,4-glucosidase	BG	3.2.1.21	Carbon acquisition. Catalyzes the hydrolysis of terminal β-1,4-bonds of cellobiose to yield glucose.
α-1,4-glucosidase	AG	3.2.1.20	Carbon acquisition. Catalyzes the hydrolysis of α-1,4-terminal bonds of starch and disaccharides to yield glucose.
alkaline phosphatase	AP	3.1.3.1	Phosphate acquisition. Catalyzes the hydrolysis of terminal bonds of phospholipids and phosphosaccharides to yield phosphate.
β-1,4-N-acetylglucosaminidase	NAG	3.2.1.14	Nitrogen and carbon acquisition. Catalyzes the hydrolysis of chitins and peptidoglycans to yield glucosamine.
Leucine aminopeptidase	LAP	3.4.11.1	Nitrogen acquisition. Catalyzes the hydrolysis of proteins and polypeptides to yield leucine and other amino acids.

Table 2. Stream chemistry parameters measured with instream continuous monitoring instruments installed at the study site. Data are averages of instrument readings from the 72 hours preceding each sampling date.

Sampling Date	Temperature Min (°C)	Temperature Max (°C)	Dissolved O$_2$ Max (mg·L^{-1})	Dissolved O$_2$ Min (mg·L^{-1})	Specific Conductivity (μS·cm^{-1}]	pH	Nitrate (μM)	Phosphate (μM)
June 2011	5.0	20.4	9.85	5.59	87	8.44	3.9	0.43
July 2011	12.1	25.5	9.87	2.08	97	8. 56	6.1	0.22
September 2011	12.7	20.7	8.08	4.04	171	8.06	13.4	2.20
October 2011	3.5	13.6	10.44	7.31	102	8.15	7.7	0.30
April 2012	3.7	15.3	10.15	6.53	84	7.64	NA	NA
June 2012	9.9	23.8	10.26	4.51	94	9.25	NA	NA

Table 3. Data for habitat depth, discharge, biofilm organic matter (OM), macrophyte and algal biomass in the EFJR for each collection date. Data for gross primary productivity (GPP), community respiration (CR), and net ecosystem production (NEP) were modeled using stream and meteorological data from local data loggers.

Sampling Date	Habitat	Depth (cm)	Discharge Q (m³·s⁻¹)	Biofilm OM (% OM)	Macrophyte biomass (g OM ·m⁻²)	Filamentous green algal biomass (g OM ·m⁻²)	Gross primary productivity GPP (mg O₂· L⁻¹ ·d⁻¹)	Community respiration CR (mg O₂· L⁻¹ ·d⁻¹)	Net ecosystem production NEP (mg O₂· L⁻¹ ·d⁻¹)	P:R
June 2011	Pools	NA	NA	5.5	1394	144	39.9	31.2	8.7	1.3
	Riffles	NA	NA	4.6	578	242				
July 2011	Pools	32.4	0.06	4.8	1494	337	74.0	68.7	5.3	1.1
	Riffles	17.2	0.05	6.3	650	101				
September 2011	Pools	43.3	0.24	6.5	1600	0	30.1	32.0	-2.0	0.9
	Riffles	28.4	0.32	5.2	218	0				
October 2011	Pools	30.1	0.06	3.7	2670	0	21.4	15.9	5.5	1.4
	Riffles	13.0	0.06	2.6	1284	0				
April 2012	Pools	35.8	0.20	2.7	0	0	14.4	9.3	5.1	1.6
	Riffles	25.9	0.23	1.3	0	0				
June 2012	Pools	28.2	0.03	2.8	529	123	39.7	31.5	8.1	1.3
	Riffles	11.2	0.03	2.2	239	270				

Table 4. Temporal changes in ecoenzyme activity in the EFJ R. Data are in units of nmol·hr^{-1}·g^{-1}OM. Standard deviations follow in parentheses. ANOVA results documenting significant differences in ecoenzyme activity between pools and riffles for the sample date are indicated by * ($p < 0.05$) and ** ($p < 0.01$).

Sampling Date	Habitat	Beta-glucosidase BG nmol·hr^{-1}·g^{-1}OM	Alpha-glucosidase AG nmol·hr^{-1}·g^{-1}OM	Alkaline phosphatase AP nmol·hr^{-1}·g^{-1}OM	n-Acetyl glucosaminidase NAG nmol·hr^{-1}·g^{-1}OM	Leucine amino peptidase LAP nmol·hr^{-1}·g^{-1}OM
June 2011	Pool	4032.3 (1175.1)	742.2 (368.6)	1276.0 (382.5)	2527.4 (762.7)	1099.2 (957.9)
	Riffle	2111.3 ** (933.2)	702.6 (426.8)	2551.0 ** (315.5)	1825.1 * (573.4)	2651.3 ** (1206.3)
July 2011	Pool	5545.6 (3104.5)	2133.2 (1650.0)	3823.2 (2553.9)	3327.9 (1918.9)	5672.9 (3730.1)
	Riffle	2761.8 ** (1897.1)	742.5 * (459.8)	2081.0 (1782.1)	2080.4 (972.6)	3892.7 (2645.8)
September 2011	Pool	2307.7 (1091.5)	421.5 (237.9)	2147.1 (1546.7)	1043.8 (371.2)	773.1 (573.9)
	Riffle	1610.4 (819.2)	433.3 (203.3)	24031.7 ** (21912.2)	567.6 * (372.1)	1512.3 (1428.5)
October 2011	Pool	4119.1 (1189.4)	750.6 (757.8)	1293.6 (341.9)	1782.6 (506.1)	1489.7 (1441.3)
	Riffle	4312.1 (3620.3)	980.4 (784.7)	2599.6 ** (803.4)	1252.4 (655.8)	3701.7 ** (1112.0)
April 2012	Pool	2123.6 (876.1)	260.6 (209.4)	1183.1 (633.8)	1418.2 (530.3)	254.6 (286.1)
	Riffle	828.3 ** (595.1)	214.4 (120.8)	1159.6 (525.2)	493.9 ** (479.9)	554.3 (333.3)
June 2012	Pool	3174.8 (1130.5)	642.0 (204.9)	3786.3 (3224.3)	2088.4 (325.4)	1746.9 (788.0)
	Riffle	2171.8 * (475.4)	676.0 (587.5)	3064.6 (1155.5)	1578.8 * (526.4)	3412.0 * (1976.5)
Averages	Pool	3512.9 (1891.2)	800.3 (922.0)	2221.9 (2063.4)	2006.9 (1118.5)	1767.1 (2344.6)
	Riffle	2299.3 ** (2005.7)	624.7 (476.3)	5997.9 * (11,830)	1300.0 ** (838.2)	2527.3 * (1788.5)

Table 5. Stoichiometric ratios of ecoenzyme activities over time. C:N ratio is calculated by dividing β-glucosidase (BG) by n-acetyl glucosaminidase + leucine amino peptidase (NAG + LAP) activities. C:P ratio is calculated by dividing β-glucosidase (BG) by alkaline phosphatase (AP)activities and N:P ratio is calculated by dividing n-acetyl glucosaminidase + leucine amino peptidase (NAG + LAP) activities by alkaline phosphatase (AP) activities. ANOVA results documenting significant differences in ratios between pools and riffles are indicated by ** ($p < 0.01$).

Sampling Date	Habitat	C:N BG:(NAG + LAP)	C:P BG: AP	N:P (NAG + LAP):AP
June 2011	Pool	1.016	1.164	1.146
	Riffle	0.909	0.967	1.066
July 2011	Pool	0.941	1.039	1.101
	Riffle	0.932	1.055	1.129
September 2011	Pool	1.033	1.016	0.985
	Riffle	0.986	0.765	0.783
October 2011	Pool	1.033	1.161	1.126
	Riffle	0.952	1.011	1.059
April 2012	Pool	1.033	1.098	1.063
	Riffle	0.913	0.961	0.990
June 2012	Pool	0.975	0.998	1.023
	Riffle	0.913	0.952	1.042
Averages	Pool	1.007 ** (0.057)	1.082** (0.090)	1.075** (0.076)
	Riffle	0.947 (0.069)	0.949 (0.119)	1.000 (0.135)

Table 6. Standardized major axis (Type II) regression results for $\log_e$-transformed stoichiometric ratios comparing the post-fire ratios with the other five sampling dates. Slopes, R^2 values, and *p* values are given for pools and riffles.

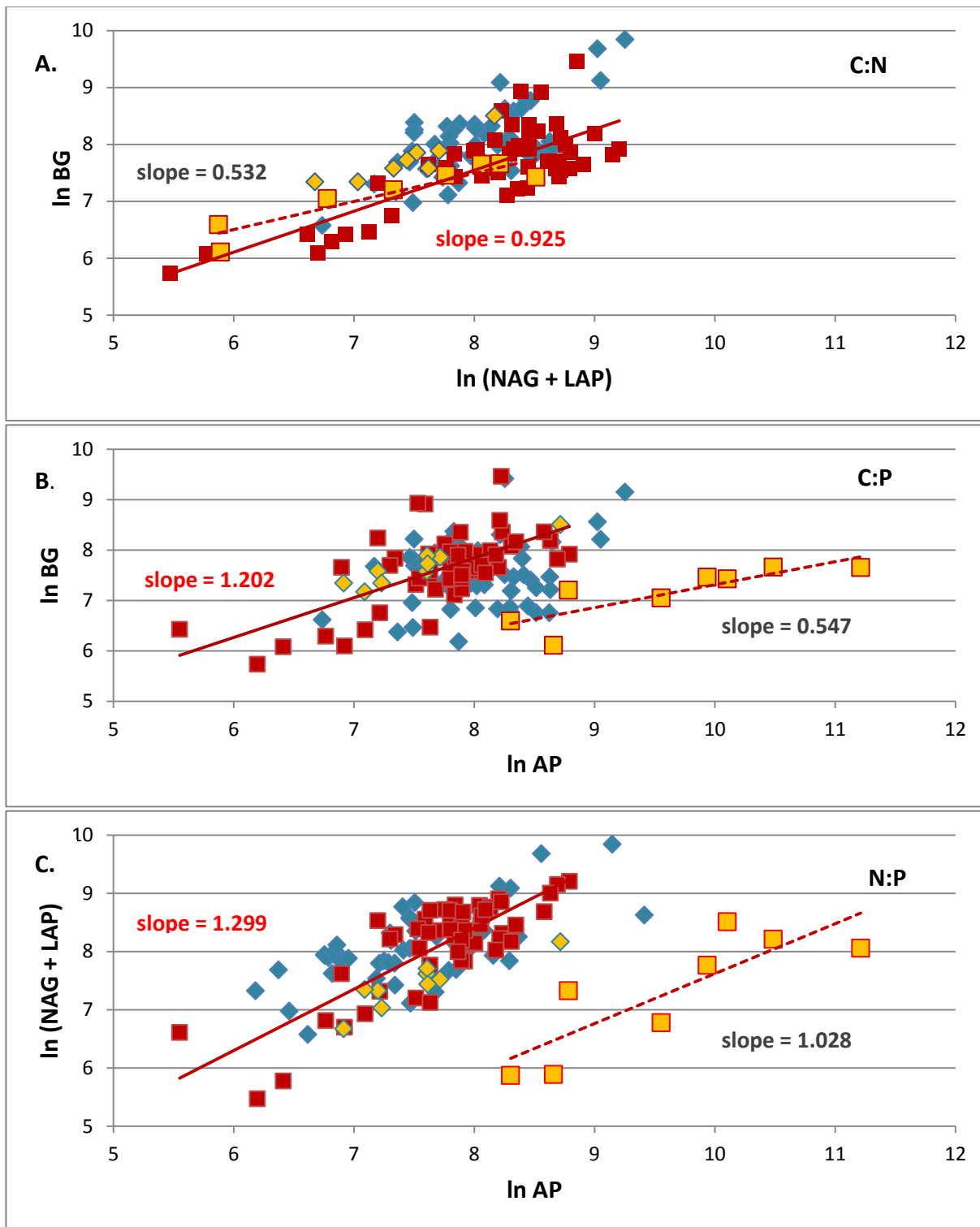|	All Sampling Dates except September 2011		September 2011 Sample	
	Pools	**Riffles**	**Pools**	**Riffles**
C:N				
Slope	0.814	0.925	0.945	0.532
R^2 value	0.438	0.606	0.686	0.859
***p* value**	< 0.001	< 0.001	< 0.01	< 0.01
C:P				
Slope	0.744	1.202	0.751	0.547
R^2 value	0.076	0.431	0. 896	0.689
***p* value**	< 0.05	< 0.01	< 0.001	< 0.05
N:P				
Slope	0.914	1.299	0.795	1.028
R^2 value	0.410	0.657	0.795	0.692
***p* value**	< 0.001	< 0.001	< 0.01	< 0.01

Figure 6. Stoichiometric ratios of log$_e$-transformed organic carbon to nitrogen ecoenzyme activities (A), organic carbon to phosphorus activities (B), and organic nitrogen to phosphorus activities (C). Fire effects are illustrated by comparing the post-fire sample (yellow markers) to all other sample dates. Pools (blue and yellow diamonds) and riffles (red and yellow squares) are compared. SMA (Type II) regression lines compare the slopes of the year's riffles samples (solid line) with those of the post-fire sampling in September (dotted line). Values for slopes for solid lines (**in red**) and dotted lines (**in grey**) are given. SMA (Type II) regression results are given in Table 6.

Acknowledgements

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