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Effects of nutrient availability on periphyton growth and diversity in the Middle Rio Grande: top-down and bottom-up factors

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Prepared by:
Rebecca Bixby and Ayesha Burdett
University of New Mexico

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Prepared by:
Drs. Rebecca Bixby and Ayesha Burdett
Department of Biology and Museum of Southwestern Biology
University of New Mexico
MSC 03-2020
Albuquerque, New Mexico 87131
Email: bbixby@unm.edu

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Bureau of Reclamation
and
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555 Broadway NE
Albuquerque, NM 87102

Agency representative:
Jeanne Dye
ph: 505-462-3564
email: JDye@usbr.gov

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Executive Summary

In lotic and lentic ecosystems, primary producers serve as a food resource for higher trophic levels. In the Middle Rio Grande, invertebrate and fish grazers, including the federally endangered Rio Grande silvery minnow (*Hybognathus amarus*), are dependent on attached algae (periphyton) as a food resource. However, the relationships between environmental factors and algal biomass/community structure in the Middle Rio Grande are poorly understood. In many aquatic systems, primary production is often limited by nutrient availability; it is not known how nutrient levels affect algal food resources for grazers in the Rio Grande or whether other factors limit productivity (e.g. high turbidity and decreased light penetration). Seasonal changes in precipitation also influence environmental parameters, including nutrients; periphyton may be limited by increased turbidity and nutrient concentrations and turbidity levels.

The research presented here is two-fold:

1. **We examined the longitudinal relationship between periphyton and environmental parameters, including nitrogen (N) and phosphorus (P).** Seasonally at five locations, from Angostura to Bosque del Apache, we collected physical, chemical, and biological (i.e., algal) data from November 2007-November 2008. Multiple physical and chemical parameters varied significantly both seasonally and among locations. Generally, turbidity was much lower at upstream locations than at downstream locations and tended to be lower in winter/spring than in later summer. Also, nutrient concentrations (NO_3-N and PO_4-P) differed significantly among locations. Concentrations were consistently low at Angostura and Bosque del Apache, but varied seasonally at the other locations that were more heavily influenced by urban and agricultural inputs. By contrast, algal biomass (measured as chlorophyll *a*) was consistently low with some significant variation among sites and seasons. However, there were marked differences among locations in diatom community structure – upstream sites tended to have more epilithic and alkaliphilic diatom taxa that prefer lower turbidity, whereas downstream sites contained taxa tolerant to poor water conditions and high turbidity and live on sediment substrates.

2**. Nutrient-diffusing substrates (NDS) were used to investigate effects of nitrogen and phosphorus availability on algal biomass and species composition in the Middle Rio Grande.** Algal biomass was extremely low on all nutrient treatments (control, N, P, N+P), largely due to light limitation caused by consistently high turbidity levels and a series of flooding and drying events which inevitably decimated organismal growth on the substrates. Similarly, it was difficult to detect differences in the abundance of invertebrate grazers among nutrient treatments. While these results are somewhat inconclusive, there are indications that nutrients, and grazers, do influence algal community dynamics. The experiment is proposed to be repeated in 2009 with a modified experimental design.

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List of Important Acronyms/ Special Terms Used in Report

AFDW – Ash-free dry weight (a measure of organic biomass)
N – nitrogen
NDS – nutrient-diffusing substrate
P – phosphorus
TN – total nitrogen
TP – total phosphorus

Introduction

In the Middle Rio Grande, primary producers play a critical role in the aquatic food web. Many invertebrate and fish grazers, including the federally endangered Rio Grande silvery minnow (*Hybognathus amarus*), are dependent on attached algae (periphyton) as a food resource (Cowley 2006, Pease et al. 2006). Previous research has verified that all life stages of the Rio Grande silvery minnow ingest diatoms and other algal groups during at least part of the year (Shirey 2004, Pease et al. 2006). Diatoms are generally a more nutritious food source due to their high lipid content, while cyanobacteria are less palatable and often contain toxins (Steinman 1996). Therefore, shifts in algal community composition can affect food quality for grazers.

The relationships between environmental factors and algal growth/community structure in the Middle Rio Grande are currently poorly understood. In aquatic systems, primary production is often limited by nutrient availability (usually nitrogen and/or phosphorus). Currently, it is not known whether nutrient levels affect algal food resources for fish grazers in all reaches of the Rio Grande. Other factors such as light limitation (resulting from increased turbidity) and temperature may also play roles in determining patterns of primary productivity. Seasonal changes in precipitation also influence environmental parameters, including nutrients and turbidity, which can subsequently influence periphyton growth and community structure. River flow dynamics differ significantly among seasons and among years. High flows can result in increased scouring and decreased substrate availability for algal growth. From a management perspective, research has not determined if habitat restoration efforts for the Rio Grande silvery minnow are creating appropriate habitat for periphyton communities.

Project Justification

This research identifies nutrient availability for algal periphyton biomass and species composition in the Middle Rio Grande. More broadly, this baseline research addresses the potential of anthropogenic impacts and habitat restoration projects to provide sufficient nutrients to support food supply (i.e., periphyton) for grazers such as the Rio Grande silvery minnow. **This study also examines the longitudinal relationship between periphyton and environmental parameters, including nitrogen (N) and phosphorus (P). In conjunction with seasonal survey work, a detailed experiment was conducted to investigate the role of N and P availability and how these nutrients affect biomass and species composition of algae in the Middle Rio Grande.**

The bottom-up influence of nutrient enrichment generally shows increased algal biomass and shifts towards greater diversity and a community composed of tolerant and nutrient-loving taxa (Lowe et al. 1986, Tank and Dodds 2003). Combining nutrient enrichment with top-down grazing influence from fish, snails or macroinvertebrates often show varying periphyton responses from strong top-down effects due to grazing to stronger stimuli from nutrient enrichment (Stewart 1987, Rosemond et al. 1993). In our nutrient enrichment experiment, **we expect that stronger bottom-up effects will result in an increase in periphyton biomass, reflecting a response related to limitation or co-limitation by one or more nutrients. If grazing is a stronger factor than nutrient enrichment, periphyton biomass will be limited in nutrient treatments where grazers are present.**

In general, it has been shown that rivers in the southwestern US are nitrogen-poor with few nutrient inputs (Grimm et al., 1981; Passell et al., 2005). It is difficult to infer historical nutrient levels, some information can be gleaned from fish gut content analysis of Rio Grande silvery minnow collected in 1874. This analysis indicated that diets were dominated by diatoms from nutrient-loving epipelic diatoms (i.e., those that grow on sediment), which provides evidence that the floodplain may have been wider than it is currently and provided areas for grazing by fish (Cowley et al. 2006, Shirey et al., 2008). This historic

floodplain also provided a connection to the river via nutrient cycling in an arid landscape that otherwise provides little nutrient input. While the diatom information infers higher levels of nutrients and higher sediment deposition in the floodplain compared to the modern river, these data were not appropriate to hypothesize on nutrient levels in the main channel.

Today, nutrient concentrations are heavily influenced by anthropogenic inputs. Wastewater treatment effluents contribute the majority of phosphorus and nitrogen. Additionally, grazers are restricted to diets dominated by smaller, epipsammic diatoms (i.e., those that grow on sand), based on gut content (Cowley et al. 2006, Shirey et al., 2008). This indicates that the fish are no longer grazing in the floodplain areas and have been forced to forage in the main channel (where there is less fine sediment accumulation due to channel incision).

The study presented here examined the temporal and spatial distributions of not only diatoms but other algal taxa that may play a role as food resources for Rio Grande silvery minnow. Furthermore, the arid southwest U.S. is not well understood from a phycological perspective and this work will provide baseline information about the natural history of algal taxa and increase our understanding of the primary producer role in aridland river systems.

Project hypotheses and objectives

In a system with naturally low nutrient levels like the Middle Rio Grande (Passell et al. 2005), we expect that low nutrient availability (N and/or P) in the upper reaches of the river system will limit the primary production and biomass of benthic algae. Work on streams in the Gila watershed and Zuni Mountains has shown that NO_3-N, rather than PO_4-P, is consistently a limiting factor for primary production (Grimm et al. 1981, Coleman and Dahm 1990). The Rio Grande watershed has predominantly volcanic soils, which tend to be phosphorus-rich (Triska et al. 2006) and may provide sufficient nutrients for algal production. Therefore, it is likely that in the Middle Rio Grande, **nitrogen may be the limiting factor** for primary producers in areas upstream of the Albuquerque area waste treatment plants.

Nutrient and turbidity levels tend to be relatively higher downstream from Albuquerque compared to upstream, due to inputs from wastewater treatment facilities and from landscape runoff. Wastewater treatment effluent appears to be the major source of nutrient loading to the main river channel in the Rio Grande (Van Horn et al. 2006, Zeglin et al. 2006), so nutrients may not be limiting to algae directly downstream from the wastewater facilities. In contrast, it has been demonstrated the agricultural fields along the Rio Grande serve as sinks for total dissolved nitrogen; water in agricultural return channels has lower nitrogen levels than the water diverted from the Rio Grande to fields for irrigation (Oelsner et al., 2007). Therefore, sources of nutrients are may be attributed to wastewater treatment effluent. Additionally, it is more likely that **light becomes limiting with increases in turbidity** downstream from Albuquerque. Further downstream, nutrients may be absorbed by sediment and biota, causing a decline in nutrient levels.

Nutrient availability and light limitation are not the only factors influencing periphyton communities in the Middle Rio Grande. Substrate availability, along with slower water velocities, is also important in determining the health of periphyton communities in a river with incised channels. Also, flow and temperature changes through the year are expected to be associated with seasonal shifts in benthic algal communities, from a diatom-dominated community during the winter months through early summer to a community with a larger proportion of filamentous green algae and cyanobacteria during late-summer to autumn during low-flow.

The research presented here is composed of a quarterly longitudinal survey and a nutrient enrichment experiment in the Middle Rio Grande. The longitudinal survey provides informational about temporal and spatial shifts and relationships between nutrient levels and the biomass and diversity of algae. The nutrient enrichment experiment examined the effects of nitrogen and/or phosphorus availability on algal biomass and community composition. The experiment was conducted in combination with the exclusion of algal grazers. Invertebrate grazers potentially limit some biomass accumulation but nutrients generally play a stronger role in shaping periphyton communities.

This two-tiered study examined the following questions:

1. Is there a relationship between periphyton biomass and species composition and environmental parameters in any given reach in the Middle Rio Grande?
2. Along a longitudinal reach, is there a relationship between periphyton biomass and species composition and environmental parameters in the Middle Rio Grande?
3. Do seasonal patterns in environmental variables affect periphyton biomass and species composition?
4. Does increased nutrient availability affect algal biomass and change species composition?
5. Is grazing a strong factor in determining periphyton communities? How do factors of top-down and bottom-up control affect primary producers?

Background

Periphyton as a food resource

Diet of the Rio Grande silvery minnow: Research indicates that the Rio Grande silvery minnow has a primarily herbivorous diet composed of benthic algae. For example, larval and juvenile fish in the Middle Rio Grande consume benthic algae as part of their diet during high flow periods in spring (Pease et al. 2006). This isotopic analysis did not, however, separate diatoms from other common algal divisions such as filamentous chlorophytes and cyanobacteria.

Further research on minnow diets verifies the consumption of periphyton as a major portion of nutrition. Gut content analysis of adult *Hybognathus amarus* shows a dominance of diatom frustules in the guts (Shirey 2004, Cowley et al. 2006). Green algae and several taxa of cyanobacteria, including *Merismopedia* and *Anabaena*, were also identified from guts in *H. amarus* (Shirey 2004). Gut content analysis is likely to show disproportionately higher percentages of diatoms compared to other algal taxa because diatoms have cell walls composed of biogenic silica, allowing the frustules to pass through guts intact. Other algal taxa have cell walls composed of less durable substances, such as cellulose, which are more easily digested and often unidentifiable in the gut (Gelwick and Matthews 2006).

Magaña (2007) also found that Rio Grande silvery minnow prefer diatoms in a series of food preference studies. However, results from food preference studies are often not verified with gut analysis, or skewed by failure to select representative food resources (i.e., cyanobacteria, chlorophytes) (Steinman 1996). The genus *Hybognathus* has pharyngeal teeth and pharyngeal taste buds, which may allow the fish to selectively filter diatoms (Hlohowskyj et al. 1989). It is likely that herbivorous fish do not selectively graze only diatoms, but may also get nutrition from other periphytic taxa (Shirey 2004). Diatoms are generally considered to be a superior food source with high lipid content, while cyanobacteria are less palatable (Steinman 1996). Additionally, shifts in diatom species composition can result in changes of overall lipid content (Sicko-Goad and Andresen 1991). Therefore, shifts in algal community composition can affect food quality and quantity for grazers.

Periphyton dynamics

Periphyton as environmental indicators: Diatoms and other algae often live within narrow environmental conditions, making them important environmental indicators in aquatic and terrestrial ecosystems (Lowe 1974). Conductivity, pH, dissolved oxygen, turbidity/light availability, salinity, flow, and microhabitat are all known to affect periphyton growth, production, and species composition (Van Dam et al. 1994, Potapova and Charles 2002, Pan et al. 2004, Potapova and Charles 2005). Rapid response time to environmental change (often in days) and ease of collection make diatoms robust environmental indicators of aquatic ecosystems, including rivers and streams (Stevenson and Pan 1999). For example, light limitation can shape species assemblages, with species-specific adaptation to low light levels (Greenwood and Rosemond 2005). Periphyton communities respond to environmental factors by changes in biomass, shifts in taxa at different taxonomic levels (division, genera, or species shifts), or changes in photosynthetic stress. Understanding the diversity and role of diatoms and other algae in aridland rivers is crucial to our understanding of how management in the Middle Rio Grande watershed affects this riverine ecosystem.

Endemism in aridland algae: Algal taxonomic studies of the arid lands in the southwestern U.S. are limited (Czarnecki and Blinn 1978, Czarnecki et al. 1981, Spaulding et al. 2002), but such studies document a flora containing both tolerant, cosmopolitan taxa and species that are regionally endemic to the southwestern U.S. Adaptation to new or variable habitats has been documented in many ecosystems (e.g., Kociolek and Spaulding 2000, Bixby et al. 2005a). Evolving to fit into a new environment would be especially important for periphyton in the Middle Rio Grande, which is a flood-pulsed system with high levels of salinity and water temperature.

Factors influencing periphyton biomass and community composition

Nutrients in the Rio Grande: Nutrients are one of the main determinants affecting algal growth and species composition. Termed a "bottom-up" factor, nutrient levels often control periphyton biomass and species composition. Nutrients can be obtained by periphyton communities from the water column or can be available from sediment through absorption (Vadeboncoeur et al. 2006). Patchiness in algal communities is also closely linked with heterogeneous nutrient distributions in the water and sediment interfaces (Coleman and Dahm 1990). Often nutrients can be co-limiting. In temperate lakes that are usually P-limited, research has shown that the system may be co-limited by both N and P (Elsner et al. 1988). It is possible that this situation may also be true for the oligotrophic northern reaches of the Rio Grande.

The geomorphology of the Middle Rio Grande changes from a more channelized river north of Albuquerque to a sandy bottom riverbed with a wide floodplain in the southern reaches of the study site. This change is reflected in the dominant sediment type in the river bottom. This morphology may affect how nutrients can be retained and cycled (D. Van Horn, pers. comm.). Overall, it is unclear if rates and patterns of nutrient processing in the Rio Grande are similar to other large river systems in which low nutrient retention is found (Alexander et al. 2000).

Both irrigation return flows and wastewater treatment plant effluent are likely to affect nutrient loading in the Middle Rio Grande. Wastewater treatment effluent in the Middle Rio Grande consistently contributed the largest source of nitrogen loading (Oelsner et al. 2007). In comparison, agricultural return flows has been shown to have much lower nitrogen concentrations than diversion water during both wet and dry years (Oelsner et al. 2007), indicating agricultural lands may act as a sink for nitrogen. Current monthly water quality sampling by C. Dahm and D. Van Horn (UNM) includes sampling of the irrigation return ditches.

Turbidity and light availability: In some reaches of the Middle Rio Grande, primary production is most likely limited by light availability in the water column. Chlorophyll *a* measurements taken from the river thalweg show extremely low levels of algal biomass (chl *a* < 0.005 mg/L) (D. Van Horn and C. Dahm (UNM), unpublished data). Furthermore, there is no diurnal O_2 signal, supporting the hypothesis that photosynthesis is minimal in the water column (D. Van Horn and C. Dahm (UNM), unpublished data). Similar conditions exist in aridland Australian rivers with high turbidity levels (Secchi depth 6-15 cm) (Bunn et al. 2003). In that study, the primary production was restricted to an a "bathtub ring" along the shallow, littoral margins of the river where some light is available (Bunn et al. 2003). Turbidity data collected along the littoral zone of the Middle Rio Grande show a similar type of ecosystem with high turbidity readings (~25-240 N.T.U.) (Eichhorst et al. 2006).

Interdependence of light and nutrients can change the predicted response of organisms. In short-term nutrient enrichment additions, heavy shading can negate the effects of elevated nutrient levels (Bernhardt and Likens 2004, Greenwood and Rosemond 2005). However, research has also demonstrated distinct periphyton community shifts between high and low nutrient streams in stream systems with low light (in this case, because of dense canopy cover) and naturally high levels of nutrients (Mosisch et al. 2001, Bixby et al. 2005b).

River morphology and seasonal changes: Seasonal differences in flow and changing hydroperiod cycles may influence growth and species composition of primary producers by altering substrate availability in the floodplain (see below) and increasing the effects of scouring (Biggs and Hickey 1994). In aridland streams and rivers, the temporal and spatial fluctuation in flow and stream channel can vary substantially (Stanley et al. 1987). Nutrient concentrations are diluted during high flow, which could result in decreased periphyton growth. In contrast, Pease et al. (2006) show that ephemerally flooded backwaters and channels with low flow are utilized by very high densities of larval and juvenile fish for nurseries and feeding. Isotope data confirm that epibenthic algae are temporally important as a food resource for grazers during high flow (Pease et al. 2006).

Substrate availability for periphyton: Microhabitats, such as sediment, sand and woody debris (e.g., tumbleweeds) provide different conditions for periphyton growth and species diversity; this is reflected in differences in chlorophyll *a* (Vadeboncoeur et al. 2006) and community structure differences (Stevenson and Hashim 1989, Potapova and Charles 2005). In one study, chlorophyll levels were 10× higher in sediments compared to on hard substrates, possibly because of nutrient sorption in the sediment (Vadeboncoeur et al. 2006). Lotic systems are especially patchy in terms of habitat quantity and quality (Pringle et al. 1988). For example, differences in light availability (related to riparian cover and water column turbidity (because of local sediment mixing) may play important roles in shaping algal communities. Finally, epipsammic diatoms tend to be smaller in size because shifting sand may crush their frustules. Generally, larger growth forms of diatoms and other algae characterize epipelic communities. This may imply that better grazer food resources may be available in epipelic habitats.

Top-down effects from grazers: Top-down factors such as grazing by fish and invertebrates can also alter periphyton productivity, growth and community composition (Steinman et al. 1987, Feminella and Hawkins 1995, Ranvestel et al. 2004). Generally, a reduction in grazers results in periphyton biomass increases and shifts in growth forms (to more upright taxa) (i.e., Connelly et al., 2008; Steinman et al., 1991). Additionally, several studies indicate that top-down predation pressure from fish can alter abundances of invertebrate populations and algal standing crops in a three-tiered food web. For example, the presence of fathead minnows was associated with lower abundances of many invertebrate fauna in wetlands (Hanson et al. 2005). Rio Grande silvery minnows are likely to consume periphyton, as well as some aquatic invertebrates and detritus, so their presence can have significant effects on the local food web.

Methods

Longitudinal survey

Survey locations

Field research was conducted in the Middle Rio Grande from the Angostura Diversion Dam downstream to Bosque del Apache, north of Elephant Butte Reservoir (Figure 1). Five locations along the Rio Grande were monitored seasonally (four times per year). The locations were chosen based on access, location relative to wastewater treatment effluent discharge and irrigation return drains, and urban development.

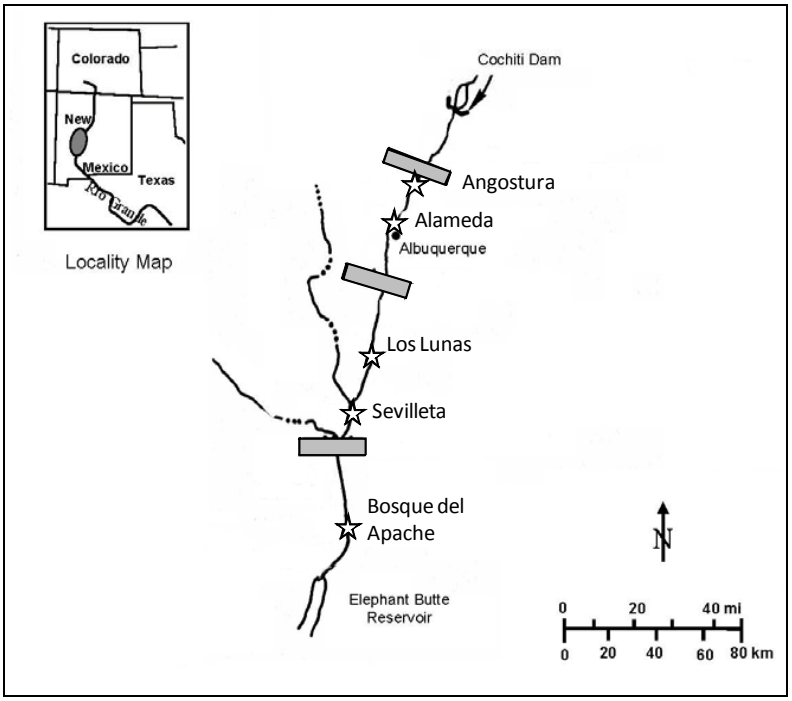

Figure 1. Locations for longitudinal sampling on the Middle Rio Grande (indicated with stars). From upstream to downstream: Angostura, Rio Rancho, Los Lunas, Sevilleta, and Bosque del Apache. Grey boxes indicate diversion structures.

1. Angostura directly below the diversion dam, River Mile 209.7 (Middle Rio Grande Conservancy District). This location is upstream from Albuquerque area wastewater treatment effluent. Data show that nitrogen and phosphorus are low above the greater Albuquerque metro area (Van Horn et al., 2006).

2. Alameda bridge crossing, New Mexico State Highway 528, River Mile 192.2 (City of Albuquerque) (downstream from the Rio Rancho wastewater treatment plant effluent)

3. Los Lunas, two miles above the Peralta wasteway input, River Mile 161.4 (Middle Rio Grande Conservancy District). This location is downstream from the Los Lunas WWTP effluent, and is

noted to have some of the highest nutrient loadings in the Middle Rio Grande (Van Horn et al., 2006).

4. Sevilleta National Wildlife Refuge, Bernardo, River Mile 120.0 (U.S. Fish and Wildlife Service), south of the confluence with the San Francisco drain (from west, contains water from the Rio Puerco) and the La Joya drain (from east, continuous flows). Our site is at the confluence with the Rio Salado and downstream from several irrigation ditch returns.

5. Bosque del Apache National Wildlife Refuge, River Mile 79.1 (U.S. Fish and Wildlife Service). Downstream from San Acacia Dam (influences of inputs from the low flow conveyance channel)

Longitudinal Survey Methods

Physical and Chemical Parameters

Each location along the Rio Grande was sampled for physical, chemical and biological (i.e., algal) parameters on a quarterly basis. We were unable to sample all parameters at sites during some sampling periods (i.e., May 2008) because of high water; in these situations, water and physical measurements were collected but n biological data was collected which requires entering the river. At each location, three subsites (usually backwaters or pools) were selected in the river in low flow areas where algae could colonize on substrates. This multiple site sampling design accounted for variability in the river because of differences in shading, habitat, and mixing within the water column. These subsites may change from season to season depending on the river flow and geomorphology. Both sides of the river were sampled (as required in the original RFP) in some cases but it often was logistically impossible to cross the river at certain locations and seasons.

Physical and chemical measurements were taken in backwaters and pools. Water depth and a brief habitat description were recorded at each site. Water temperature (°C), specific conductance (µS/cm), pH, dissolved oxygen (mg/L), and salinity (ppt) were measured using a multiparameter water quality meter (YSI Model 85D). Turbidity (NTU) (as a surrogate for light attenuation) was measured using a portable turbidity meter (La Motte 2020e). Velocity (m/s) was measured using a Marsh–McBirney Flo-Mate water velocity meter. Water samples were collected in replication (n = 3) from the water column from each of the three sites at each location, filtered in the field or in the lab at the University of New Mexico using a 47mm diameter Millipore membrane filter (0.45 µm pore size) and a Swinnex filter apparatus and syringe. Unfiltered water samples were also collected for analysis of total nitrogen (TN) and total phosphorus (TP). Filtered and unfiltered water samples were frozen at the lab until analysis.

In addition to analyzing nutrient levels in the water column, sediment samples were collected at each subsite and analyzed for TN and TP, as an indication of nutrient availability for algal communities through sorption from sediment. These samples were collected as bulk samples in clean wide-mouth bottles and transported to the University of New Mexico and frozen until analysis.

Anions (nutrients) were analyzed from replicate filtered water samples at the University of New Mexico Biology Annex Analytical Laboratory. PO_4-P (µg/L), NO_3-N (µg/L), Cl^- (mg/L), Br^- (µg/L), and SO_4 (mg/L) were analyzed using a Dionex DX-100 Ion Chromatograph, using Chromeleon 6.60 software (AWWA et al. 1998, USEPA 1997). NH_4-N (µg/L) was analyzed using a colorimetric spectrophotometric method (AWWA et al. 1998, Technicon Industrial Systems, 1973).

Total P and TN were extracted from unfiltered water by oxidation with persulfate and boric acid (Stelzer and Lamberti 2001) and then analyzed using a Technicon AutoAnalyzer.

Total N from sediments was analyzed by combustion with a Thermoquest CE Instruments NC2100 Elemental Analyzer. Total P was extracted from sediments using combustion, followed by HCl addition. Samples were analyzed using a Technicon AutoAnalyzer.

Unmarked distilled water blanks and lab standards were included in all analyses for machine and sample calibration.

Periphyton Parameters

Benthic periphyton were quantitatively sampled from epipelic/epipsammic (sediment/sand) and epilithic (i.e., rock) habitats, depending on availability. Three replicate epipelic/epipsammic samples were collected from each subsite using a 0.5 cm core made from a modified 60 ml syringe. Epilithic samples were scrubbed from 2-3 rocks from each subsite (when available), and then surface area was calculated for each rock. Sampling of benthic periphyton from the surface of submerged tumbleweed was planned, but tumbleweeds occurred rarely and did not represent an important substrate for periphyton.

Ash free dry weight (AFDW) was measured from a subsample from each replicate. Each AFDW replicate was oven-dried (60^{o}C, overnight), weighed, ashed (540^{o}C, 2hr) in a muffle furnace and then reweighed. AFDW was calculated as the difference between dried and ashed weights.

Chlorophyll *a* was analyzed as a second measure of algal biomass. Chlorophyll *a* was extracted from a subsample from each replicate core sample (~2g sediment), by immersing the sediment in ethanol (95%, 10mL) which was then heated (70^{o}C, 5min) (modified from Sartory and Grobbelaar 1984). The supernatant was analyzed using a HP 8452A diode spectrophotometer. Chlorophyll *a* content was calculated from optical densities measured at 660nm and 750nm pre- and post-acidification (Sartory and Grobbelaar 1984).

An additional epipelic/epipsammic or epilithic sample was collected from each site to determine periphyton community composition and preserved in 10% formalin. Periphyton community composition was determined from replicates from each substrate type at each site (Stevenson and Bahls 1999). Densities of filamentous cyanobacteria and unicellular green algae (both termed "soft" algae) were determined using a Palmer-Maloney counting chamber at 400× magnification (brightfield optics) on a Zeiss Universal research microscope. Taxa were identified to genus and enumerated along a transect(s) until 500 live cells/units are recorded. Some cyanobacterial filaments that lack cell differentiation were counted in 10 micrometer lengths (one length = one unit) (Lowe and Laliberte 2006). In samples with extremely low cell densities, a maximum of 10 transects were examined.

To determine diatom richness (number of taxa) and species abundance (cells/mm^2), 2 mL aliquots from each sample were processed using a method developed for sediment samples (30% hydrogen peroxide and concentrated nitric acid) (Stoermer et al. 1995). These samples were then rinsed six times with distilled water to remove oxidation by-products. Processed samples were evaporated onto coverslips and mounted to microscope slides with Naphrax mounting medium, making permanent slides. Specimens along transects were examined under oil immersion at 1250× magnification using phase and brightfield optics. 500 valves were enumerated from each sample. In samples with extremely low diatom densities, counting ceased after 10 transects. Diatoms were enumerated and identified to the species level. Identification of taxa was based on taxonomic literature including work from the southwestern U.S. (NAQWA data, NM region, D.F. Charles, personal comm., Czarnecki and Blinn 1978, Czarnecki et al. 1981). Digital images of each taxon were recorded and compiled in a taxonomic database. Duplicate

slides and periphyton subsamples will be accessioned in the newly formed algal collection at the Museum of Southwestern Biology (MSB).

Because only a subset of algae was processed and analyzed, duplicate periphyton samples have been processed from 5% of the samples collected (both from the longitudinal survey and NDS experiment) and processed and analyzed using standard methods. Quality control was monitored by calculating a percent community similarity index for proportional data from two duplicate diatom slides; the similarity index should be greater than 75% to be considered good replication. These duplicate slides have been assessed for variability related to microhabitats in the reach, sample preparation, and analytical variability (Stevenson and Bahls 1999).

Nutrient-diffusing substrate experiment

Nutrient diffusion substrates (NDS) were used in combination with electrical grazer exclusion to investigate bottom-up and top-down effects on periphyton biomass and species composition.

NDS experiment location

Initially, we proposed to conduct the nutrient-diffusing substrate (NDS) experiment at Sevilleta National Wildlife Refuge (U.S. Fish and Wildlife Service). However, due to continuous high flows in the main channel, we were unable to safely secure the experimental apparatus at this location. Consequently, the experiment was conducted at Bosque del Apache National Wildlife Refuge, River Mile 79.1 (U.S. Fish and Wildlife Service) in July 2008. The floodplain was inundated throughout the summer because of high flows in the main channel and sediment deposition upstream from the selected location, so the experiment was conducted on the floodplain rather than the main channel.

NDS construction

The nutrient-diffusing substrates were made from inverted terracotta saucers (4″ dia.). Terracotta provides a suitable substrate with a textured surface for algal colonization. Four treatments were assigned to the saucers – control, N, P, and N+P. The saucers were filled with agar and a combination of KNO_3 (= NO_3-N), KH_2PO_4 (= PO_4-P), both NO_3-N and PO_4-P or neither (as a control) (modified from Tank and Dodds 2003, Pringle and Triska 2006). Filled saucers were attached to a piece of Plexiglas which had holes drilled into each corner. Each saucer was randomly placed in a frame built from PVC pipe, although control treatments furthest upstream to avoid any nutrient contamination (Figure 2) although contamination from cross-over treatment is unlikely (Tank et al. 2006). The frame was then placed into the river and held in place with T-posts (Figure 3). Saucers were quickly covered by a thin layer of sediment and sand, mimicking an epipelic/epipsammic habitat. There were four replicate frames for the non-exclusion treatment. Four replicate samples of each nutrient treatment (control, N, P, N+P) were collected weekly (with the exception of week 4, when the saucers were out of the water) and processed for algal parameters and invertebrates.

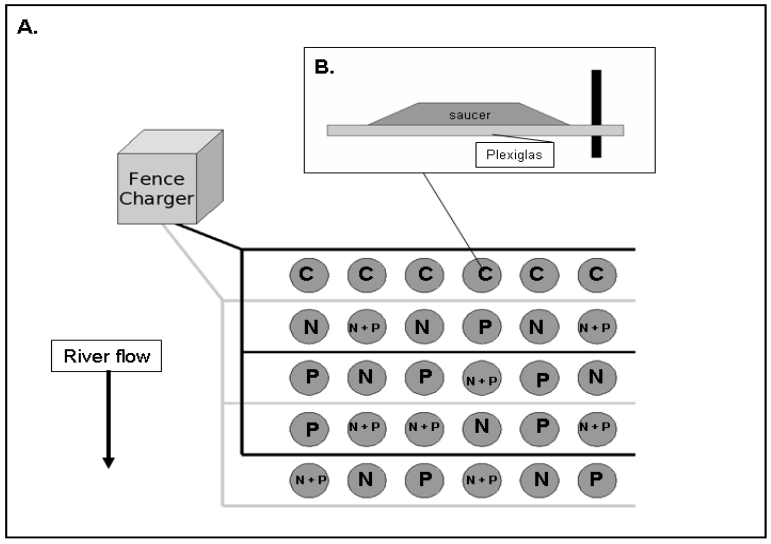

Figure 2. Schematic diagram of nutrient-diffusing substrate experiment (grazer exclusion component). A. Proposed experimental setup with electric grazer exclusions powered by fence charger; B. Enlarged view of nutrient-diffusing saucer.

The design of the frame was modified for the grazer exclusion treatment. Invertebrates and fish were excluded from the saucers using an electrical field. Exposed electrical wires were distributed between the saucers on the PVC frame and then attached to a 12V solar-powered fence charger (Pringle and Blake 1994, Moulton et al. 2004). However, because of the high conductivity of the river, the solar fence chargers shorted out when the entire frame (16 saucers) was electrified. To compensate for the effect of high conductivity, the area electrified by each fence charger was reduced to two saucers. These two saucers were one control saucer (no nutrients) and one NDS (N+P), rather than including a control and three nutrient treatments (N, P, N+P). Because there were only two saucers, saucers from the exclusion were only collected on the final week of the experiment rather than every week during the experiment. Four replicate samples of each treatment (control, N+P) were collected from the non-grazer treatment.

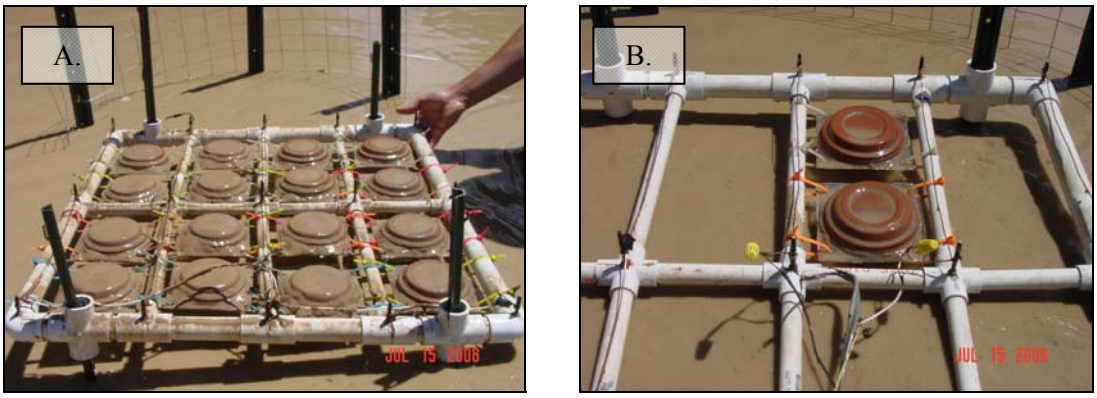

Figure 3. Nutrient diffusing substrates in the river. A. Sixteen saucers in an array without grazer exclusion. Note sediment deposits on the surface of the saucers after one week of immersion in the river; B. Two saucers in an array with electrical exclusion of grazing invertebrates and fish.

NDS Sampling

For an outline of sampling techniques used each week, refer to Table 1.

Physical and chemical parameters were measured at the experimental location each week (pH, temperature, specific conductivity, dissolved oxygen, water depth, turbidity, and water velocity). Replicate water samples (n = 3) were also collected for ambient water chemistry in the river (PO_4-P, NO_3-N, NH_4-N, Cl^-, Br^-, and SO_4). Collection and analysis methods are described in the longitudinal monitoring section above.

Each week, four replicates of each treatment (control, N, P, N + P) of the non-grazed experiment were randomly selected and removed from the experiment. In the final week, saucers were also collected from the grazer experiment. In the field, before the saucers were scrubbed, invertebrates were hand-picked from the NDS and preserved in formalin. In the laboratory, invertebrate samples were enumerated and identified to the lowest possible taxonomic level (generally, microcrustacea were identified to order and insects were identified to family). Voucher specimens of invertebrates will be accessioned in the Arthropod Division at the Museum of Southwestern Biology.

Each collected saucer was then scraped with a toothbrush and rinsed into a graduated cylinder using distilled water. A subsample was taken for AFDW and chlorophyll *a*. Samples for algal species identification were collected every week and preserved with 10% formalin. However, only samples from week 3 were analyzed during the week of maximum colonization. Collection and analysis methods are described in the longitudinal monitoring section above.

We had planned to assess overall periphyton community structure *in situ* using an Ocean Optics spectral analyzer (HR2000CG-UV-NIR), fiberoptic reflectance probe and fiberoptic oxygen sensor. Pigment reflectance allows an assessment of gross community structure (cyanobacteria, green algae, diatoms). However, this technique was unable to be used because of low biomass of algae in the field, in conjunction with technical difficulties in the laboratory. The technique continues to be refined in the laboratory and it is hoped that it will become a useful tool for *in situ* analysis of periphyton community structure in the future.

Table 1. Sampling schedule for nutrient-diffusing substrate experiment

sample week	parameters
Week 0	physical/chemistry
Week 1	physical/chemistry, nutrients
Week 2	physical/chemistry, nutrients, chl *a*, algal species[1], invertebrates
Week 3	physical/chemistry, nutrients, chl *a*, algal species[1,2], invertebrates
Week 4	physical/chemistry, NO BIOLOGICAL SAMPLE COLLECTION [saucers out of water]
Week 5	physical/chemistry, nutrients, chl *a*, algal species[1], invertebrates

[1] For accession at the Museum of Southwestern Biology

[2] For community analyses

Statistical Analyses

SPSS (SPSS for Windows Release 16.0. SPSS Inc 2007) was used for all univariate statistical analyses. Data was transformed as necessary to meet assumptions of normality when using parametric statistical tests. Data from the longitudinal survey were tested for significant differences among season and location, while data from the NDS experiment were tested for significant differences among sample week and nutrient treatment, or nutrient treatment and grazer exclusion. One-way analysis of variance (ANOVA) was used to test for differences followed by Tukey's HSD *post-hoc* tests to examine differences among treatments when appropriate.

Indicator species analysis (ISA) (Dufrêne and Legendre 1997) was used to determine which diatom taxa were characteristic of either low or high nitrate levels (based on significant ANOVA results by site). This analysis generates an indicator value [0 (non-indicator) – 100 (perfect indicator)] for each taxon based on the product of relative abundance and relative frequency of each taxon in each treatment (i.e., low/high nutrient). Monte Carlo tests (1500 randomizations) were run to determine if the indicator value was greater than expected by chance. Indicator species have both an indicator value greater than 25 and $p < 0.05$. This classification was calculated using PC-ORD software (Version 4.37, McCune and Mefford 1999).

Results

Longitudinal survey

General water quality

Quarterly surveys have been conducted five times at five sampling locations since this project was initiated. Partial sampling was completed in August 2007 but extremely high water and method development resulted in an incomplete data set for that period. In the end, we have treated that sampling period as a trial run and have not included these data in this annual report. Most water quality parameters differed significantly among either season, location, or both (Table 2).

Turbidity was highly variable throughout the survey period, differing from less than 10 NTU (minimum = 4.97 NTU at Angostura in November 2008) to over 4000 NTU (Figure 4). Turbidity was significantly different among sites and seasons and was generally low at Angostura throughout the year and higher at Alameda, the Sevilleta and Bosque del Apache. Turbidity was generally highest in August following monsoon rains which increased tributary inputs throughout the Middle Rio Grande.

Water temperature differed significantly ($P < 0.001$) among sample seasons and among locations (Figure 4). Generally, water temperatures were lower at Angostura where relatively deep, cobbled subsites were sampled and the site is located downstream from the cold hypolimnetic water releases from Cochiti reservoir, while temperatures were slightly higher at the Sevilleta and Los Lunas where the river becomes more shallow with sand substrate.

Flow measured at individual sites differed significantly among seasons ($P = 0.001$), even though low-flow habitats with potentially higher algal biomass were specifically selected for surveys. In general, flow was relatively high in May 2008 (particularly at Alameda) and relatively low in November 2008 (at all locations) (Figure 4). The flows at individual sites remain relatively consistent at a given time of year throughout the river.

Dissolved oxygen (DO) levels were generally fairly consistent among seasons and locations. Generally, DO levels ranged from 5-14 mg/L (median = 5.2 mg/L), and were slightly higher in November 2008 compared to other seasons with the marked exception of May 2008 ($P < 0.001$, Figure 4). In May 2008, DO levels were extremely high at some sites within locations (maximum = 20 mg/L at Los Lunas and Bosque del Apache), although many sites had DO levels less than 10 mg/L at this sampling season.

pH also differed significantly among seasons ($P < 0.001$). Interestingly, there was no longitudinal trend in pH levels – locations which were relatively high in one season could be relatively low in another season. For example, pH levels were relatively low and there was less variation among locations in May 2008.

Salinity and specific conductivity differed significantly among location and among seasons. In general, locations further upstream (Angostura, Alameda) have relatively low levels of salinity and specific conductivity compared to locations further downstream (Sevilleta, Bosque del Apache).

Table 2. Summary of results from ANOVA analysis of all water quality parameters, testing for differences among seasons and locations. Significant results ($P<0.05$) are shown in bold.

	season		**Location**		**season × location**	
	$F_{4, 47}$	**P**	**$F_{4, 47}$**	**P**	**$F_{16, 47}$**	**P**
turbidity (NTU)	20.885	**<0.001**	28.395	**<0.001**	3.718	**<0.001**
temperature (°C)	155.240	**<0.001**	16.126	**<0.001**	7.163	**<0.001**
flow (m/s)	7.457	**<0.001**	1.551	0.203	1.432	0.168
DO (mg/L)	20.162	**<0.001**	1.442	0.235	1.920	**0.042**
pH	21.434	**<0.001**	0.279	0.890	2.286	**0.014**
salinity (ppt)	8.882	**<0.001**	20.715	**<0.001**	3.807	**<0.001**
specific conductivity (µS/cm)	5.398	**0.001**	12.089	**<0.001**	2.260	**0.016**
	$F_{4, 191}$	**P**	**$F_{4, 191}$**	**P**	**$F_{16, 191}$**	**P**
NO_3-N (µg/L)	25.654	**<0.001**	102.834	**<0.001**	8.025	**<0.001**
PO_4-P (µg/L)	14.999	**<0.001**	84.963	**<0.001**	9.233	**<0.001**
bromide (µg/L)	34.338	**<0.001**	29.956	**<0.001**	2.360	**0.003**
chloride (mg/L)	138.289	**<0.001**	470.478	**<0.001**	10.094	**<0.001**
sulfate (mg/L)	33.235	**<0.001**	142.577	**<0.001**	12.160	**<0.001**
	$F_{4, 402}$	**P**	**$F_{4, 402}$**	**P**	**$F_{16, 402}$**	**P**
ammonium (µg/L)	11.884	**<0.001**	29.912	**<0.001**	5.365	**<0.001**
	$F_{3, 125}$	**P**	**$F_{4, 125}$**	**P**	**$F_{8, 125}$**	**P**
water TN (mg/L)	65.742	**<0.001**	25.479	**<0.001**	25.734	**<0.001**

water (mg/L)	TP	19.133	**<0.001**	11.941	**<0.001**	26.173	**<0.001**
		$F_{3, 37}$	**P**	**$F_{4, 37}$**	**P**	**$F_{12, 37}$**	**P**
sediment (%)	TN	2.844	0.051	7.117	**001**	2.127	**0.039**
sediment (%)	TP	0.516	0.674	2.341	0.073	2.598	**0.013**
		$F_{4, 189}$	**P**	**$F_{4, 189}$**	**P**	**$F_{15, 189}$**	**P**
chlorophyll *a* (mg/m^2)		23.249	**<0.001**	8.883	**<0.001**	6.521	**<0.001**
		$F_{4, 187}$	**P**	**$F_{4, 187}$**	**P**	**$F_{15, 187}$**	**P**
AFDW (g/m^2)		18.022	**<0.001**	31.575	**<0.001**	6.919	**<0.001**

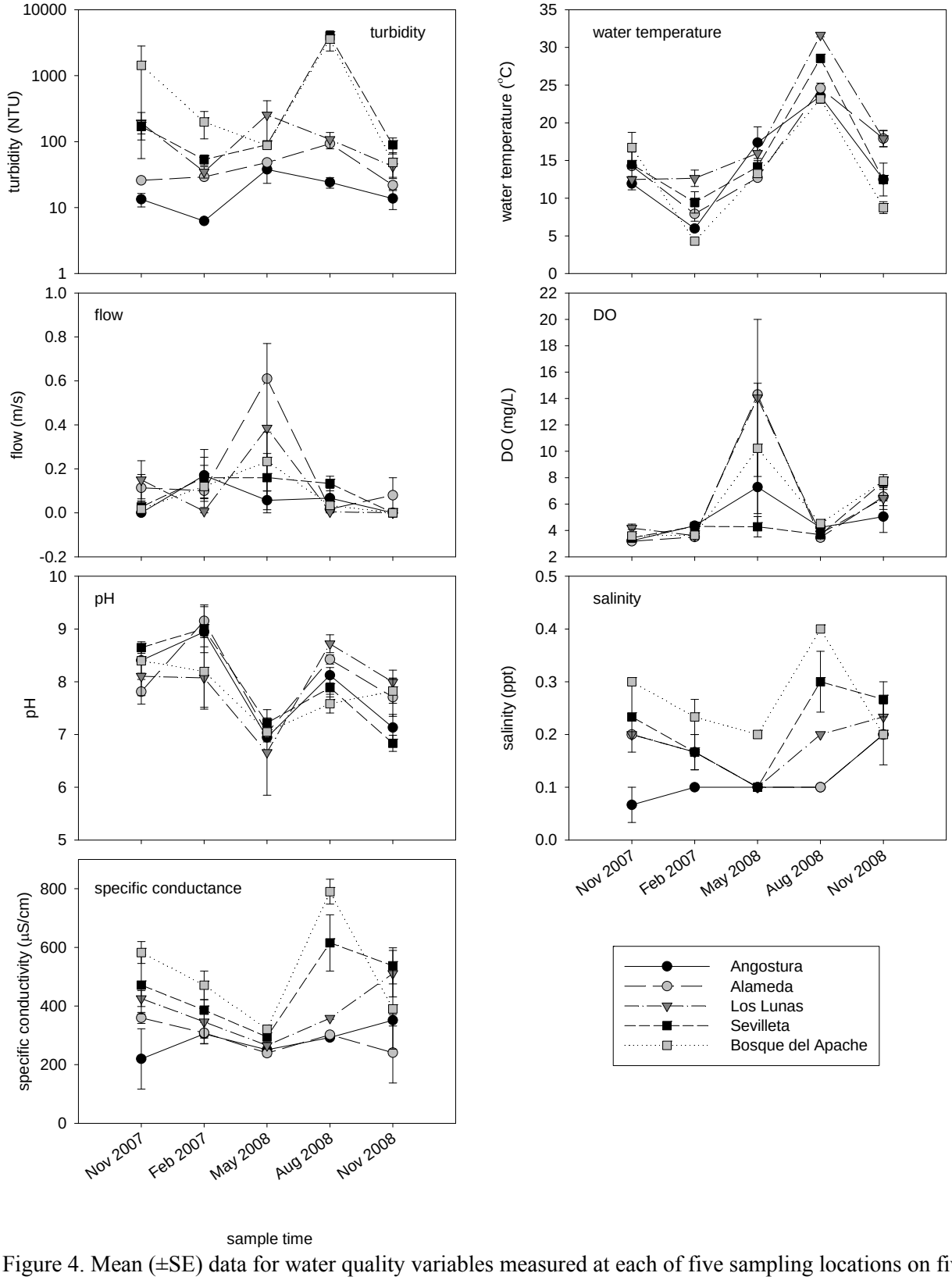

Figure 4. Mean (±SE) data for water quality variables measured at each of five sampling locations on five sampling periods. Note log scale for turbidity.

All of the nutrients differed significantly among seasons and locations, with significant interactions (Table 2; Figure 5). There is a trend for NO_3-N and PO_4-P levels to increase in the cooler seasons (November, February) and then decline in the warmer seasons (May, August) at all locations with the exception of the far north locations (Angostura, Alameda) (Figure 5). The trend is not as clear for the other anions (bromide, chloride, sulfate), although there are significant differences among seasons. Concentration of these anions generally increase longitudinally – levels are relatively low at the upstream locations (Angostura, Alameda) compared to the downstream locations (Sevilleta, Bosque del Apache). Ammonium concentration was relatively high at Los Lunas in the cooler months (November, February) compared to the two northern locations and to Bosque del Apache, where ammonium concentrations were comparably low throughout the year.

TN and TP measured from water samples differed significantly among seasons and locations (Table 2; Figure 6). Generally, TN concentrations decreased throughout the year whereas TP concentrations tended to increase. TN was usually relatively lower at the downstream locations (Sevilleta, Bosque del Apache) compared to the upstream locations, although TN concentration at Angostura was very variable among seasons. TP differed among locations but it was difficult to determine a longitudinal trend for TP concentrations.

TN and TP measured from sediment samples were also variable among seasons and locations (Table 2; Figure 7). Upstream locations (Angostura, Alameda) had relatively low levels of TN compared to downstream locations. By comparison, Angostura generally had high levels of sediment TP whereas Alameda had relatively low levels. The downstream locations (Sevilleta, Bosque del Apache) had intermediate levels of sediment TP compared to the upstream locations.

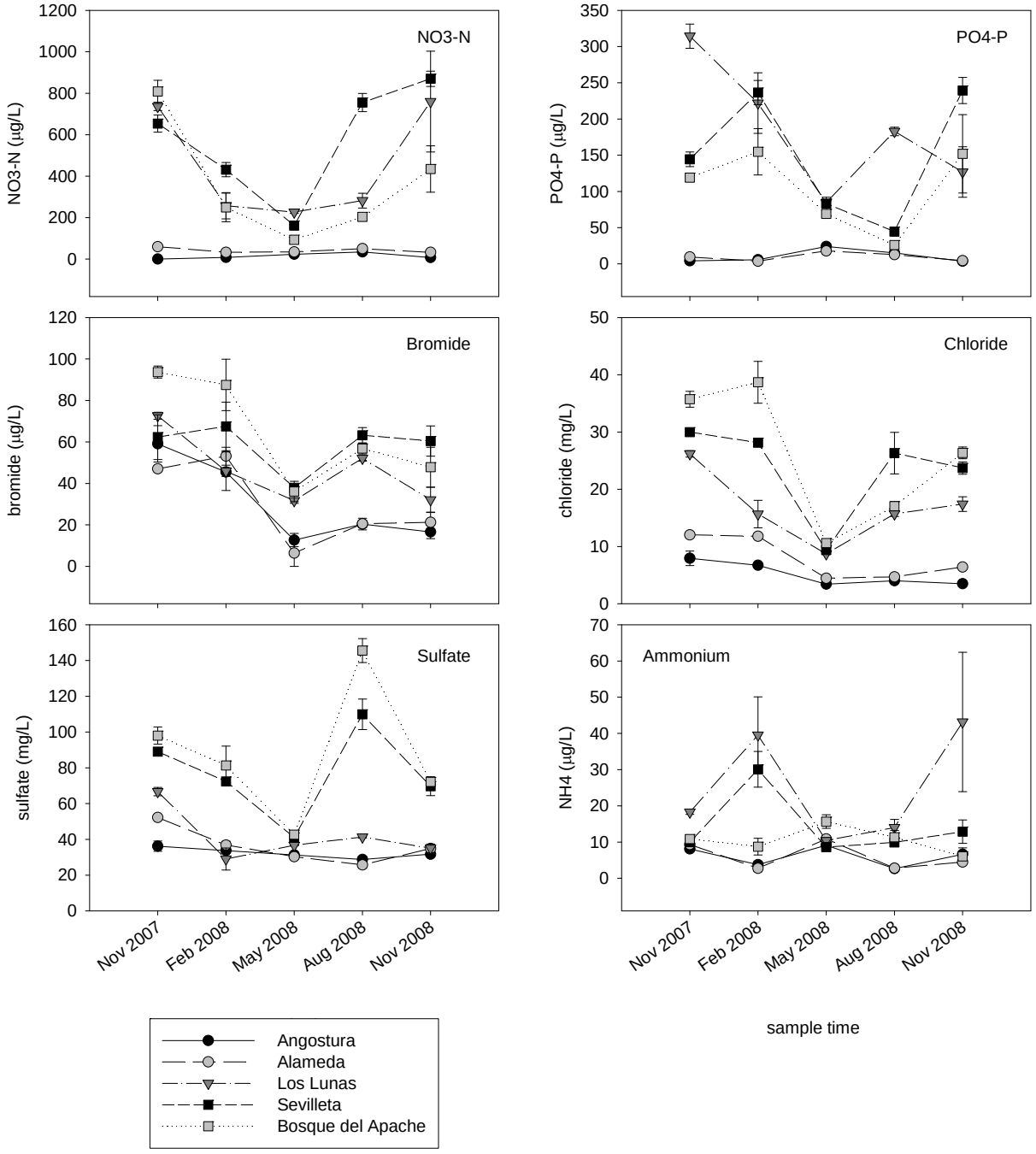

Figure 5. Mean (±SE) data for nutrient data analyzed from water samples collected at each of five locations on five sampling periods.

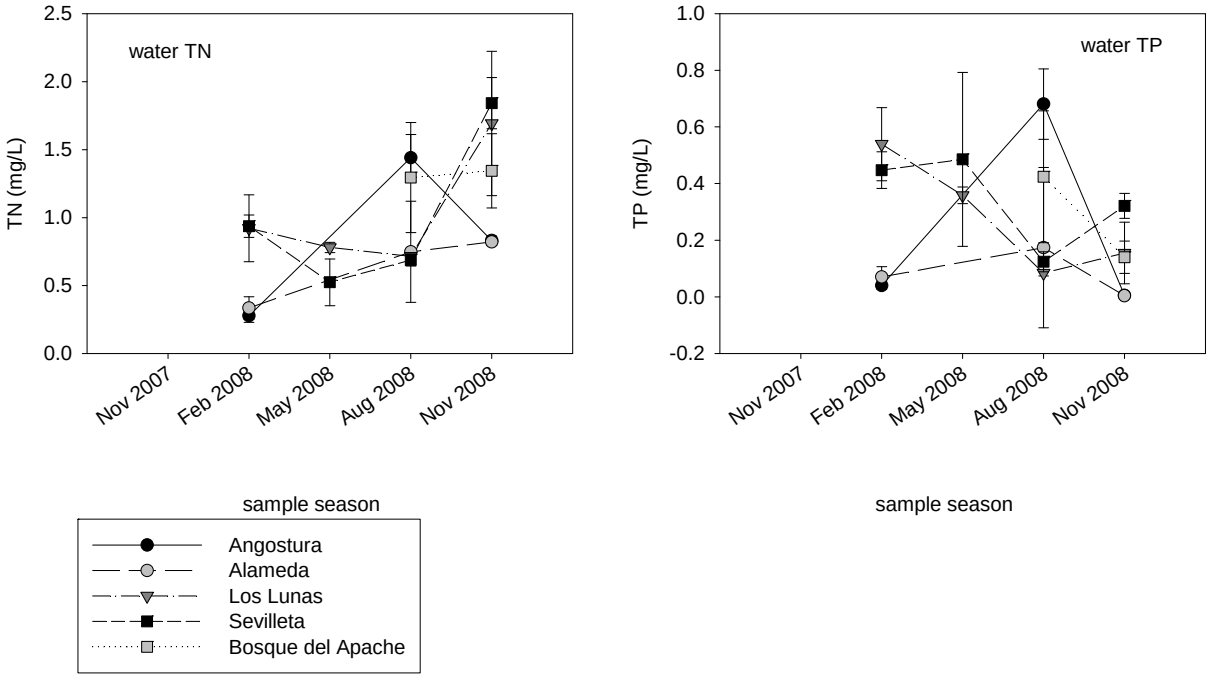

Figure 6. Mean (±SE) data for TN and TP measured from water samples collected at each of five sampling locations on four sampling periods.

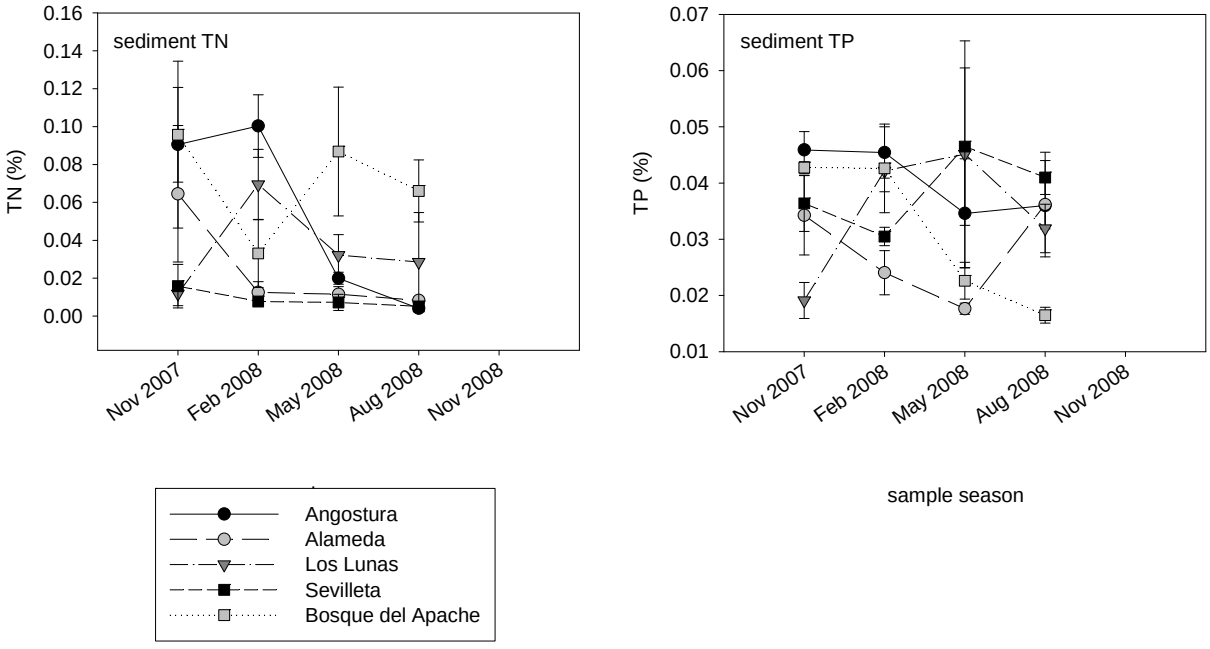

Figure 7. Mean (±SE) data for TN and TP data analyzed from sediment samples collected at each of five locations on four sampling periods. Note that TN and TP sediment samples are still being prepared from November 2008 surveys.

Biomass collected on natural substrates

Biomass of chlorophyll *a* differed significantly among seasons and locations (Table 2; Figure 8). Generally, chlorophyll *a* biomass was lower in August than in any other season. Biomass was highest upstream at Angostura (particularly in February 2008) and lowest further downstream at the Sevilleta and Bosque del Apache. Ash-free dry weight (AFDW) also differed significantly among seasons and locations. Trends were difficult to detect – AFDW is heavily influenced by recent floods and flow conditions, which also differs among seasons and between years.

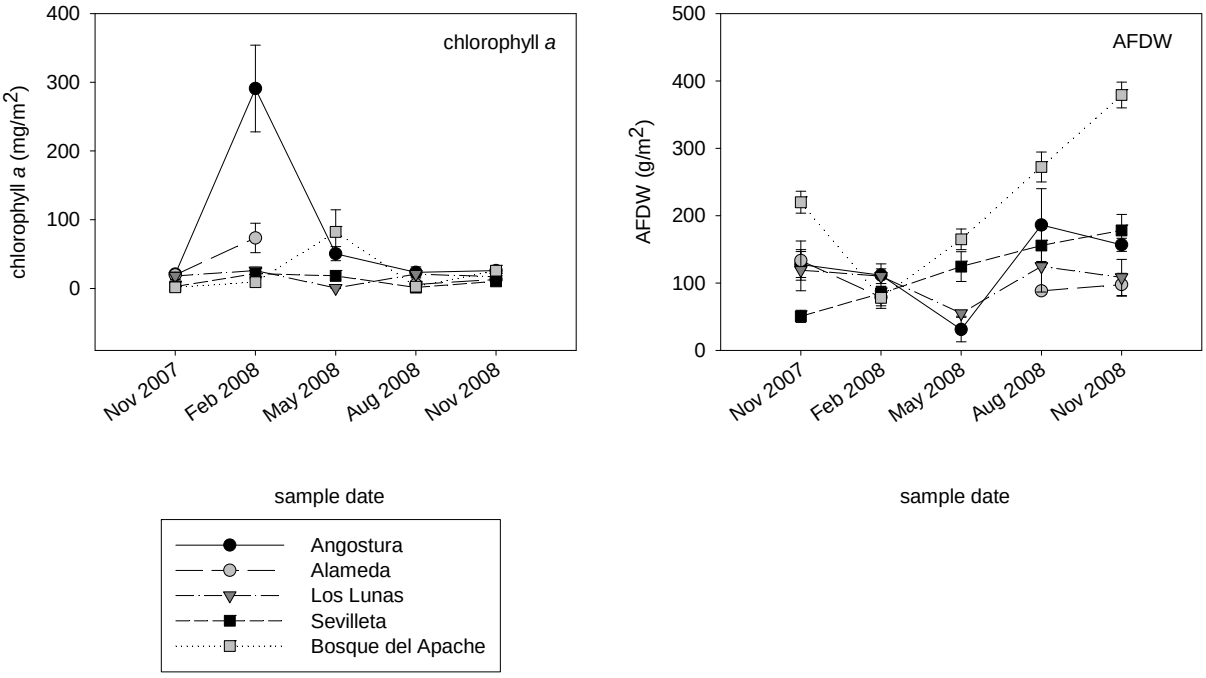

Figure 8. Mean (±SE) data for chlorophyll *a* and AFDW analyzed from samples collected at each of five locations on five sampling periods.

Diatom community structure

Diatom communities have been analyzed from the five sites from November 2007, February 2008, May 2008, and August 2008. To date, 245 taxa have been recorded, including the Q/A slides (Appendix A1-7). A larger number of diatom taxa recorded from the Rio Grande are unidentified and noted by a number or "cf" in the taxa list (Table A1-7). The southwestern US has a paucity of taxonomic literature and studies; it is likely that a number of the taxa reported in the research are new to science. This work represents some of the first work to record algal communities in larger rivers in the southwestern US. Initial results indicate that there were differences in diatom community structure among the five locations. Overall species richness was greatest at Angostura (152 taxa recorded) and least at Sevilleta (68 taxa recorded). Seasonal species richness ranges from 36-80 taxa recorded at any given site, with an average of 60 diatom taxa at a site/season). There were no significant differences in species richness among sites ($P = 0.64$) or season ($P = 0.228$). Angostura, Alameda and the Sevilleta shared many taxa, whereas Los Lunas and Bosque del Apache had communities very different to each other, and to all other locations.

Indicator species analysis showed a number of diatom taxa that are characteristic of either the upstream sites (Angostura and Alameda) or downstream sites (Los Lunas, Sevilleta, and Bosque del Apache) (Table 3). This analysis showed that alkaliphilous *Epithemia sorex and Amphora pediculus* and cosmopolitan taxa *Cocconeis placentula* var. *lineata*, *Planothidium lanceolatum*, and *Cocconeis pediculus* were most indicative of environmental conditions of the upper reaches of the Middle Rio Grande, as well as the most common taxa.

The quality control measures taken in the analysis show that the percent similarity community index to be 80.5% among duplicate slides counted. Soft algae are still being enumerated; preliminary results show that algal taxa other than diatoms play an extremely minor role in total algal community. Diatom taxa dominate both the epipelic/epipsammic and epilithic habitats.

Table 3: Indicator species values for strongest diatom indicators of low (i.e., upper reaches) and high (i.e., lower reaches) concentrations of nutrients in the Middle Rio Grande.

Species	Indicator value	P value
Low nutrients		
Epithemia sorex	90	0.009
Cocconeis placentula var. lineata	83	0.038
Planothidium lanceolatum	79	0.046
Amphora pediculus	78	0.024
Cocconeis pediculus	74	0.018
Gomphonema gracile	71	0.035
Navicula seminulum	68	0.050
High nutrients		
Surirella angusta	92	0.013
Nitzschia gracilis	67	0.057

Nutrient-diffusing substrate experiment

The NDS experiment was set up in the river on 9 July 2008 (week 0). The first collection was made two weeks after the experiment was initiated (22 July). Further collections were made at week 3 (29 July) and week 5 (12 August). Unfortunately, no collections were made at week 4 (5 August) as water levels had dropped significantly and the saucers were left exposed for at least two days.

General water quality

Water quality parameters were recorded each week that the NDS experimental location was visited (Table 4). Most of these water quality parameters were relatively consistent throughout the experimental period, with the exception of turbidity. Turbidity was much lower at week 0 (when the experiment was first established) compared to every other sample week (>4000 NTU).

Concentrations of all anions differed significantly among sample weeks (Figure 9;

Table 5). In general, the concentration of NO_3-N increased over time with the exception of week 4 where NO_3-N was extremely low. By contrast, the concentration of PO_4-P generally decreased over time. Concentrations of chloride and bromide also tended to increase over time, whereas concentration of sulfate tended to decrease. The concentration of ammonium did not differ significantly among sample weeks ($F_{4, 22} = 0.74$, $P = 0.574$).

Table 4. Water quality parameters recorded on site each week that the NDS arrays were in place.

week	flow (m/s)	water temperature (°C)	DO (mg/L)	specific conductivity (µS/cm)	salinity (ppt)	turbidity (NTU)	pH
week 0	0.11	23.4	4.40	389.4	0.2	251	8.13
week 1	0.30	23.1	4.19	787.0	0.4	>4000	8.04
week 2	0.27	24.6	3.89	461.5	0.2	>4000	8.07
week 3	0.11	24.2	4.39	615.0	0.3	>4000	8.16
week 4	0.17	22.4	4.12	591.0	0.3	>4000	8.26
week 5	0.10	24.2	3.67	542.0	0.3	>4000	8.11

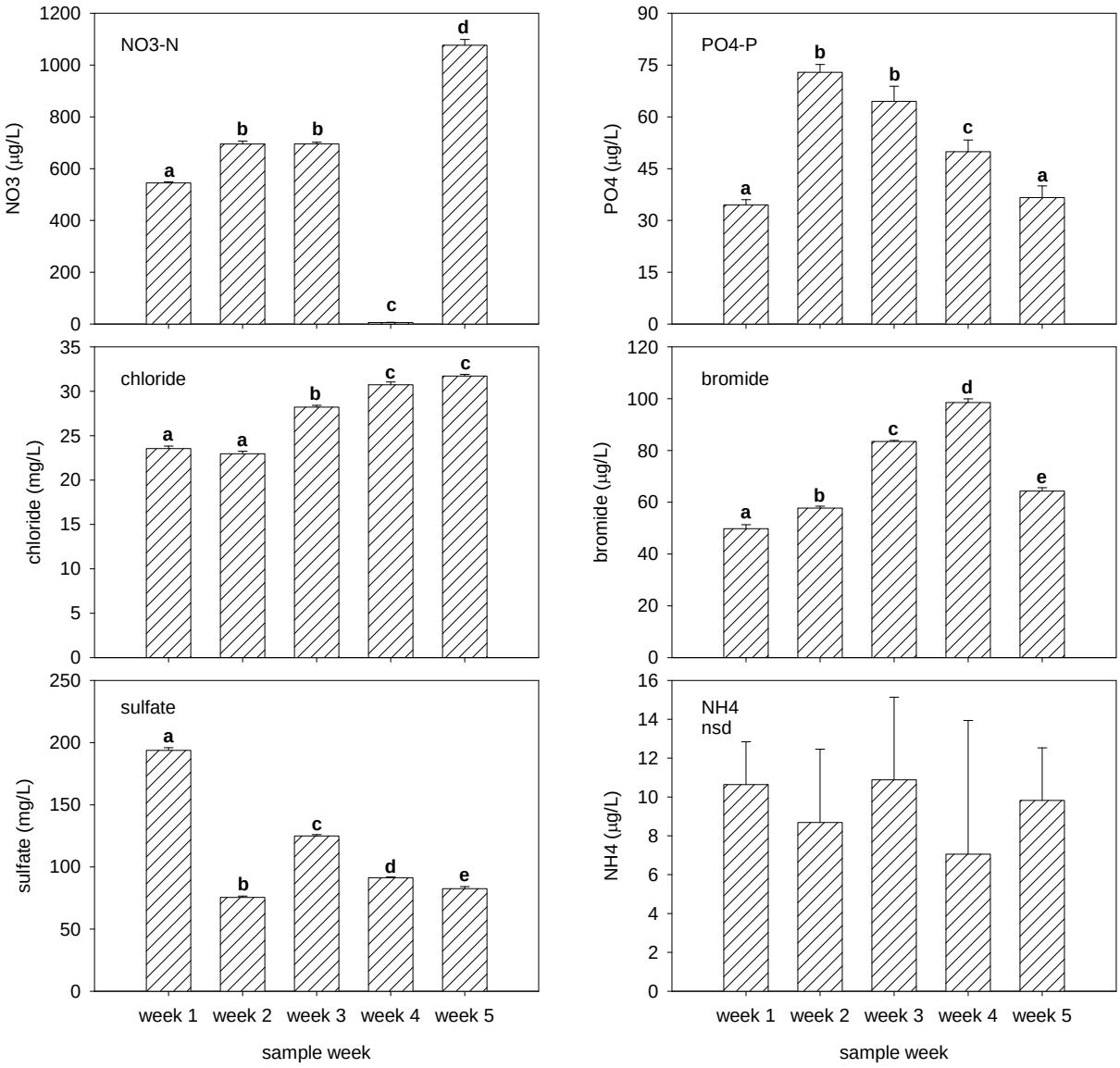

Figure 9. Mean (±SE) data for anions and ammonium analyzed from water column samples collected each week at the experimental location. Letters above bars indicate significant differences (nsd = no significant difference).

Table 5. Summary of results from ANOVA analyses of anions, ammonium, chlorophyll *a* and ash-free dry weight of organic matter (AFDW), testing for differences among seasons and locations. Note that water samples were collected five times (each sampling week) for analysis of anions and ammonium whereas biofilm samples were only collected three times (weeks 2, 3 and 5) for analysis of organic biomass.

nutrient	$F_{4, 25}$	P
Chloride (mg/L)	235.60	**< 0.001**
Bromide (µg/L)	279.17	**< 0.001**
NO_3-N (µg/L)	1108.24	**<0.001**
PO_4-P (µg/L)	28.40	**<0.001**
Sulfate (mg/L)	885.13	**<0.001**

Biomass collected on saucers

Biomass of chlorophyll *a* and AFDW was highly variable among replicate saucers, both within nutrient treatments and among sample weeks (Figure 10). No significant differences could be detected among treatments for chlorophyll *a*, but there were significant differences among treatments for AFDW (Table 6). Notably, many of the saucers had no detectable chlorophyll *a* biomass (25 out of the total 48 samples). Generally, AFDW was lower on control saucers than on saucers with nutrients and lower at week 5 than any other week.

Biomass of chlorophyll *a* and AFDW were also very variable among replicate saucers used in the electrical exclusion experiment (Figure 11). Again, eleven of the sixteen samples had negligible levels of chlorophyll *a*. No significant differences were detected between nutrient treatments (Table 7) or grazing treatments (Table 7). Initial surveys of the algal species slides showed extremely low numbers of diatoms (1 specimen/transect) and further analysis was ceased. We suspect that the multiple low measures of primary productivity are related to high turbidity/low light availability and the design of the experiment which doesn't allow the arrays float and expose the saucers to enough light for algal biomass accumulation.

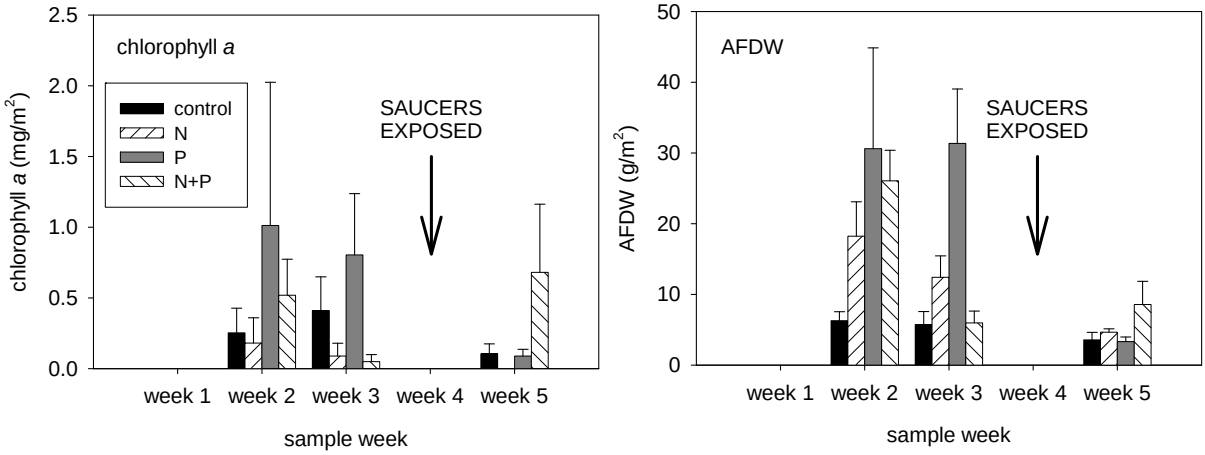

Figure 10. Mean (±SE) data for chlorophyll *a* and AFDW from NDS samples. Note that no saucers were collected at week 1 or at week 4.

Table 6. Summary of results from ANOVA analyses for chlorophyll *a* and AFDW, testing for differences among sample weeks and nutrient treatments. Significant differences (P<0.05) are highlighted in bold.

	week		**nutrients**		**week × nutrients**	
	$F_{2, 36}$	**P**	**$F_{3, 36}$**	**P**	**$F_{6, 36}$**	**P**
chlorophyll *a* (mg/m^2)	0.31	0.736	1.63	0.200	1.03	0.422
AFDW (g/m^2)	16.54	**<0.001**	5.82	**0.002**	3.46	**0.008**

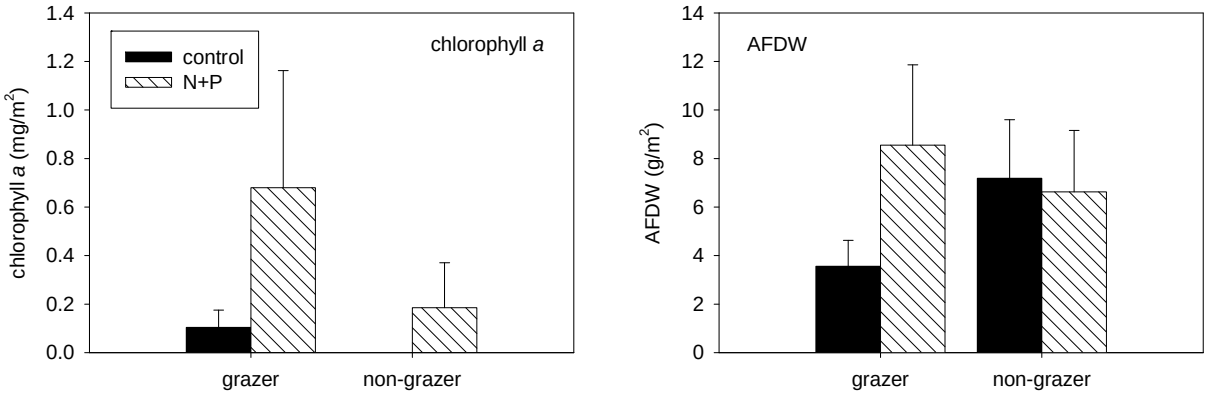

Figure 11. Mean (±SE) data for biomass of chlorophyll *a* and ash-free dry weight collected from NDS control and N+P nutrient treatments on grazer arrays (non-electrified) and non-grazer arrays (electrified). These samples were only collected at week 5.

Table 7. Summary of results from ANOVA analyses for chlorophyll *a* and AFDW, testing for differences between exclusion treatments. No significant differences were detected.

	exclusion		**nutrients**		**exclusion × nutrients**	
	$F_{1, 12}$	**P**	**$F_{1, 12}$**	**P**	**$F_{1, 12}$**	**P**
chlorophyll *a* (mg/m^2)	2.09	0.174	0.97	0.343	0.01	0.932
AFDW (g/m^2)	0.05	0.829	1.83	0.201	0.16	0.694

Invertebrate colonization

Invertebrates were collected from NDS saucers on weeks 2, 3 and 5 (Figure 12). There was an extremely low diversity of organisms. Simuliids and chironomids clearly dominated the fauna, and other taxa only occurred rarely.

There were no significant differences among nutrient treatments, but there were significant differences among weeks for total abundance, chironomid abundance and simuliid abundance (Table 8). Total abundance was significantly lower at week 2 than at the later collection dates. There was also a taxonomic shift between week 3 and week 5: simuliids were most abundant at week 3 whereas chironomids were most abundant at week 5.

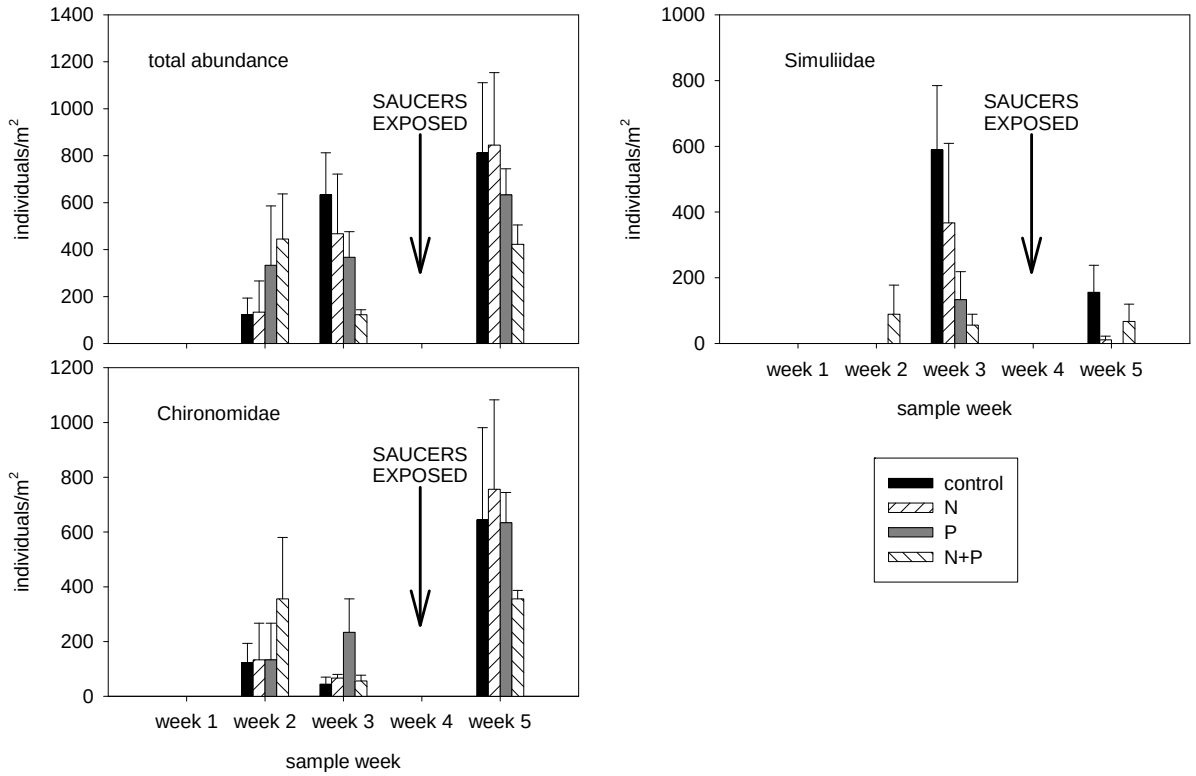

Figure 12. Mean (± SE) abundance of invertebrates collected from NDS arrays on each of the sampling weeks (weeks 2, 3, 5) from four different nutrient treatments (control, N, P, N+P).

Table 8. Summary of results from ANOVA analyses of total abundance of invertebrates, Chironomidae and Simuliidae, testing for differences between sample week (week 2, week 3, week 5) and nutrient treatment (control, N, P, N+P). Significant differences (P<0.05) are highlighted in bold.

	Week		**nutrients**		**week × nutrients**	
	$F_{2, 44}$	**P**	**$F_{3, 44}$**	**P**	**$F_{6, 44}$**	**P**
total abundance	3.970	**0.026**	0.320	0.811	1.246	0.302
Chironomidae	9.298	**<0.001**	0.488	0.692	0.748	0.614
Simuliidae	11.987	**<0.001**	3.127	0.035	1.588	0.173

Invertebrates were collected from NDS saucers with non-grazer treatment only on week 5, and only from control and N+P saucers (Figure 13). The abundance of organisms was variable and no significant differences were detected between ambient and exclusion treatments for total abundance, simuliids or chironomids (Table 9). Simuliids were significantly more abundant on control saucers than on N+P saucers (P = 0.044), while the abundance of other organisms did not differ significantly between nutrient treatments.

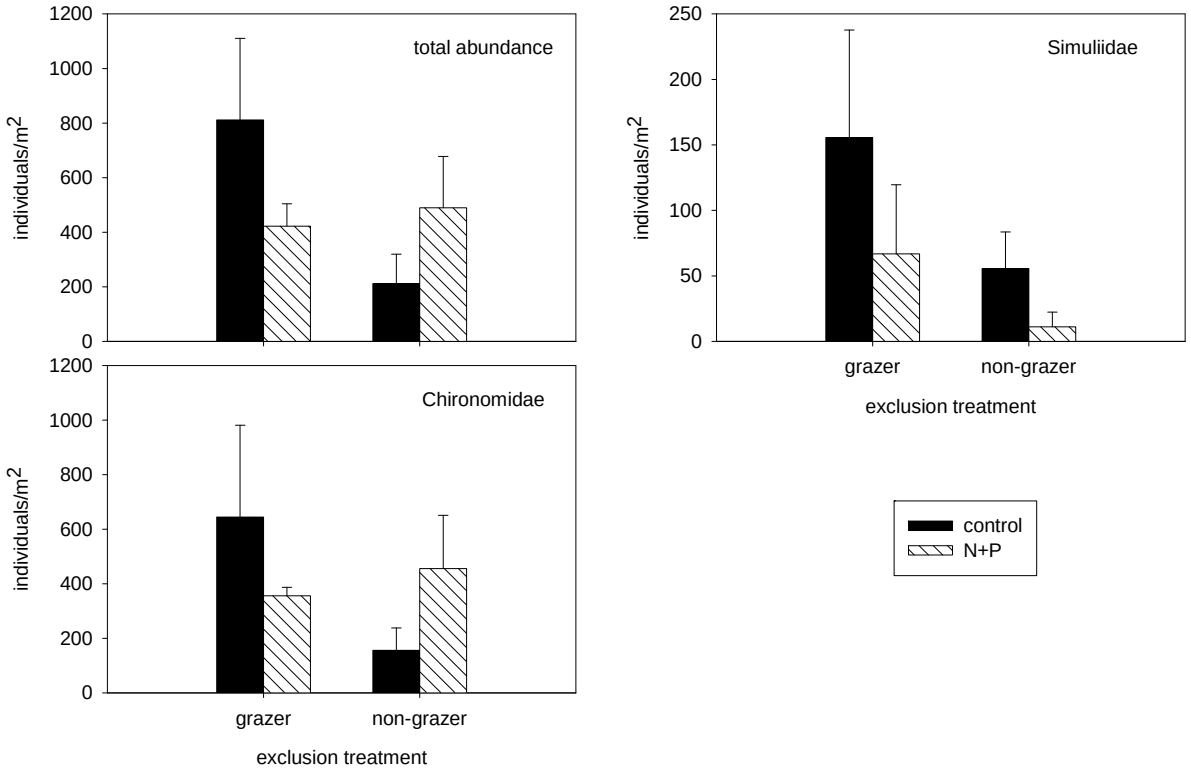

Figure 13. Mean (± SE) abundance of invertebrates from NDS samples collected at sample week 5 from NDS arrays with two nutrient treatments (control, N+P) and two grazer exclusion treatments (grazer, non-grazer): total abundance, simuliid (black fly) abundance and chironomid (midges) abundance. Note differences in scale on y-axis.

Table 9. Summary of results from ANOVA analyses of total abundance of invertebrates, Chironomidae and Simuliidae, testing for differences between nutrient treatments (control, N+P) and grazer exclusion (grazer, non-grazer). Significant differences (P<0.05) are highlighted in bold.

	nutrients		**exclusion**		**nutrients × exclusion**	
	$F_{1, 12}$	**P**	**$F_{1, 12}$**	**P**	**$F_{1, 12}$**	**P**
total abundance	0.090	0.774	1.980	0.185	3.090	0.104
Chironomidae	0.000	0.978	0.950	0.349	2.180	0.166
Simuliidae	5.040	**0.044**	2.050	0.178	0.010	0.917

Discussion

Longitudinal survey

The longitudinal survey indicates that there were significant differences in the physical and chemical character in the river, both among locations and among seasons.

Differences among seasons can be attributed to seasonal regional climate and influence of agricultural activities. For example, turbidity at the downstream locations was extremely high following rainfall events (e.g. monsoonal rains in August), which increase flow from tributaries such as the Rio Jemez in the north and the Rio Puerco and Rio Salado in the south. Both of these tributaries are intermittent streams and tend to have extremely high sediment loads when they are flowing. Seasonally, shifts in turbidity played a major role in shaping algal communities. In the summer months, high turbidity associated with tributary inputs created a light-limited environment where primary production was limited to a littoral zone "bathtub ring." This restricted habitat is demonstrated by the concentrations of chlorophyll a concentrations which at their lowest during the summer months.

Downstream sites are also influenced by overall land use and seasonal shifts in agricultural activity in the watershed. There is a decline in NO_3-N and PO_4-P concentrations in the water column during the summer irrigation season when alfalfa crops are growing. It has been shown that agricultural fields work as a nutrient sink, rather than source the Rio Grande (Oelsner et al. 2007) so reduced nutrient levels in the river are expected. Additionally, there was a gradient of nutrient inputs as the river flowed through urban landscapes. Concentrations of PO_4-P and NO_3-N were consistently low at sites furthest from urban influence, but varied seasonally at locations that were more heavily affected by anthropogenic inputs.
For example, it appears that nutrient concentrations were lowest at Angostura, upstream from Albuquerque, because Angostura receives fewer anthropogenic inputs (from agriculture or wastewater treatment). Similar nutrient patterns are noted in Bosque del Apache, the southernmost site in this study.

Data indicate periphyton communities influenced by low nitrogen and turbidity levels in sites upstream of wastewater inputs. Diatom communities were dominated by *Cocconeis placentula* var. *lineata, Cocconeis pediculus,* and *Epithemia sorex* in the summer months. During winter months when turbidity was relatively low, *Diatoma vulgare*, *Rhopalodia gibberula,* and *Cocconeis placentula* var. *lineata* were most common. *Epithemia sorex*, *Rhopalodia gibberula*, *R. gibba*, and *Reimeria sinuata* were common during all sampling periods, associated with low nitrogen levels and influence of alkaline soils. Substrate, flow and elevated nutrients shaped algal communities at the downstream sites. In reaches downstream from wastewater effluents with a wide, sand-silt riverbed, epipelic diatoms (e.g., *Surirella minuta*, *S. angusta*, *Nitzschia dissipata*, *Navicula* cf *radiosa*, and *Navicula symmetrica*) were abundant during summer months with high turbidity levels. Months with lower turbidity were dominated by *Nitzschia palea* and several *Surirella* taxa.

In addition to the turbidity and nutrient factors shaping communities, the type of substrate present at the sites have been important. For example, *Cocconeis placentula* var. *lineata* was common at Angostura, Alameda and the Sevilleta, but less common at Los Lunas and Bosque del Apache. Optimally, this diatom grows on solid rock substrates, which were not available at Los Lunas and Bosque del Apache. It is likely that the Alameda site functions as a transition site with a wider, sandy riverbed but lower nutrients. This is evident by the overlap in "low nutrient" and "epipelic" taxa from both the northern and southern sampling sites being recorded from the Alameda site. It should be noted that indicator species analysis has focused on taxa that were located exclusively (or almost exclusively) upstream or downstream of Albuquerque. We have highlighted those taxa, but have also included a number of taxa with explanatory autecology located in low and higher nutrient localities have been mentioned. These taxa occur in higher numbers in one site or the other but also are recorded from other sites, therefore not functioning as good

indicators of a specific parameter. Additional data for multiple years will alleviate some of the seasonal and annual variability and noisiness and add to more definitive patterns of algal diversity and biomass in the Middle Rio Grande. Overall, insight into diversity and drivers of periphyton dynamics is crucial to the understanding of stochasticity and seasonality in aridland riverine ecosystems.

NDS experiment

River conditions made it challenging to conduct the NDS experiment with great success. Water levels were variable and turbidity levels were extremely high. It was difficult to submerge the arrays at the correct distance below the water surface so that there was enough light penetrating to encourage algal growth, without risking exposing the saucers. In fact, the saucers were exposed between week 3 and week 4 when water levels decreased more than 15cm. Because of these physical limitations, algal production was very low. Biomass of chlorophyll *a* on the saucers was negligible. Initial surveys of the diatom communities indicate extremely low densities.

While dense periphyton communities did not develop on the NDS saucers, invertebrate populations did colonize. Invertebrate abundances differed significantly among sample weeks – blackfly larvae (Simuliidae) were most abundant at week 3 whereas chironomid larvae (Chironomidae) were most abundant at week 5. Also, blackfly larvae were significantly more abundant on control saucers than on N+P saucers in the grazer/non-grazer experiment. Whether or not these differences in invertebrate population densities are related to the availability and diversity of periphyton for grazing is yet to be explored.

Despite the poor growth and colonization of periphyton on NDS saucers, there are indications that this experiment can be conducted again with greater success. We are currently developing a new method for immersing the arrays in the water so that they can float and continually adjust to fluctuating water levels. This would avoid the saucers being either submersed too much so that light penetration is negligible or being elevated out of the river so that saucers dry out and aquatic organisms perish.

We propose to use the first year's NDS data as a pilot project. The second year will include a broad-scale experiment to examine the effects of turbidity on periphyton communities. We proposed to extend the experiment using NDS to test the effects of reduced irradiance due to suspended particulates in the water column. Comparative experiments will be conducted in a downstream reach (Sevilleta) and a less turbid reach north of Albuquerque (Angostura). If river flows or weather conditions impede our ability to conduct the experiments in the river, we have access to a number of mesocosms used for another related ecological experiment at the Sevilleta. The sampling design and methods will be the same as the NDS experiment presented here. We expect that light will play a major role in reducing algal biomass and decreasing algal diversity and this may have subsequent effects on grazing invertebrates and fish.

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Appendices

Appendix 1 – Data from the longitudinal survey

Table A1-1. Mean (±SE) data for water quality variables at each of five sampling locations on five sampling periods. *samples not collected because of high river flows, na = not applicable

season	location	N	turbidity (NTU)			temperature (−C)			flow (m/s)			DO (mg/L)		
Nov 07	Angostura	3	13.31	±	3.09	11.93	±	0.83	0	±	0	3.21	±	0.05
	Alameda	3	25.8	±	1.97	14.27	±	0.15	0.11	±	0.06	3.17	±	0.12
	Los Lunas	3	191.63	±	85.71	12.47	±	0.38	0.15	±	0.09	4.17	±	0.31
	Sevilleta	3	169.67	±	39.19	14.47	±	1.65	0.03	±	0.03	3.44	±	0.32
	Bosque del Apache	3	1433.93	±	1378.61	16.7	±	2.02	0.02	±	0.02	3.58	±	0.49
Feb 08	Angostura	3	6.25	±	0.34	5.97	±	0.35	0.17	±	0.12	4.35	±	0.27
	Alameda	3	29.17	±	0.81	7.9	±	0.95	0.1	±	0.04	3.49	±	0.23
	Los Lunas	3	34.73	±	6.79	12.63	±	1.08	0.01	±	0.01	3.6	±	0.24
	Sevilleta	3	53.2	±	9.72	9.43	±	1.42	0.16	±	0.09	4.3	±	0.15
	Bosque del Apache	3	198.03	±	87.97	4.3	±	0.12	0.12	±	0.1	3.63	±	0.12
May 08	Angostura	3	37.8	±	14.56	17.37	±	2.1	0.06	±	0.04	7.27	±	3.22
	Alameda*	1	48.2	±	na	12.7	±	na	0.61	±	na	14.3	±	na
	Los Lunas*	2	253.75	±	162.25	15.95	±	1.25	0.39	±	0.39	14.05	±	5.96
	Sevilleta	3	89.4	±	4.09	14.17	±	0.82	0.16	±	0.11	4.27	±	0.77
	Bosque del Apache	3	87.5	±	7.35	13.27	±	0.29	0.23	±	0.13	10.22	±	4.94
Aug 08	Angostura	3	24.1	±	4.4	23.53	±	0.63	0.07	±	0.04	4.22	±	0.32
	Alameda	3	92.83	±	7.3	24.57	±	0.7	0.02	±	0.02	3.45	±	0.24
	Los Lunas	3	107.5	±	29.85	31.63	±	0.22	0	±	0	3.74	±	0.26
	Sevilleta	3	4000	±	0	28.53	±	0.52	0.13	±	0.03	3.66	±	0.11
	Bosque del Apache	3	3585	±	1216.24	23.17	±	0.58	0.03	±	0.02	4.51	±	0.11
Nov 08	Angostura	3	13.72	±	4.41	12.47	±	0.09	0	±	0	5.04	±	1.2
	Alameda	3	21.77	±	3.07	17.9	±	1.1	0.08	±	0.08	6.55	±	0.68
	Los Lunas	3	41.87	±	14.3	17.9	±	1.05	0	±	0	6.43	±	0.84
	Sevilleta	3	89.2	±	24.21	12.47	±	2.17	0	±	0	7.67	±	0.56
	Bosque del Apache	3	48.33	±	19.56	8.73	±	0.79	0	±	0	7.73	±	0.24

Table A1-1 continued.

Season	location	N	pH			salinity (ppt)			specific conductivity (µS/cm)		
Nov 2007	Angostura	3	8.40	±	0.24	0.07	±	0.03	219.37	±	103.13
	Alameda	3	7.81	±	0.24	0.20	±	0.00	359.43	±	18.60
	Los Lunas	3	8.10	±	0.37	0.20	±	0.00	425.33	±	27.38
	Sevilleta	3	8.65	±	0.11	0.23	±	0.07	471.13	±	97.95
	Bosque del Apache	3	8.40	±	0.06	0.30	±	0.00	582.33	±	37.24
Feb 2008	Angostura	3	8.94	±	0.11	0.10	±	0.00	304.87	±	1.13
	Alameda	3	9.15	±	0.27	0.17	±	0.03	307.93	±	35.74
	Los Lunas	3	8.07	±	0.59	0.17	±	0.03	345.97	±	75.23
	Sevilleta	3	9.00	±	0.45	0.17	±	0.03	385.87	±	94.85
	Bosque del Apache	3	8.19	±	0.68	0.23	±	0.03	470.67	±	48.50
May 2008	Angostura	3	6.94	±	0.08	0.10	±	0.00	250.67	±	22.58
	Alameda*	1	7.03	±	na	0.10	±	na	239.00	±	na
	Los Lunas*	2	6.66	±	0.81	0.10	±	0.00	265.10	±	12.00
	Sevilleta	3	7.22	±	0.10	0.10	±	0.00	294.30	±	7.08
	Bosque del Apache	3	7.05	±	0.07	0.20	±	0.00	320.57	±	2.37
Aug 2008	Angostura	3	8.12	±	0.15	0.10	±	0.00	292.37	±	2.34
	Alameda	3	8.42	±	0.09	0.10	±	0.00	301.70	±	3.12
	Los Lunas	3	8.72	±	0.17	0.20	±	0.00	358.37	±	3.60
	Sevilleta	3	7.89	±	0.17	0.30	±	0.06	615.33	±	95.99
	Bosque del Apache	3	7.58	±	0.18	0.40	±	0.00	790.00	±	42.25
Nov 2008	Angostura	3	7.13	±	0.25	0.20	±	0.00	351.30	±	19.42
	Alameda	3	7.71	±	0.36	0.20	±	0.00	240.59	±	103.31
	Los Lunas	3	7.99	±	0.23	0.23	±	0.03	510.40	±	79.15
	Sevilleta	3	6.83	±	0.15	0.27	±	0.03	537.10	±	61.69
	Bosque del Apache	3	7.82	±	0.23	0.20	±	0.06	390.23	±	137.47

Table A1-2. Mean (±SE) data for nutrient data analyzed from water samples collected at each of five locations on five sampling periods. *samples not collected because of high river flows

Season	location	N	NO_3-N (µg/L)			PO_4-P (µg/L)		
Nov 2007	Angostura	9	0.37	±	0.20	4.16	±	1.06
	Alameda	9	59.70	±	7.36	9.35	±	2.87
	Los Lunas	9	736.32	±	19.94	314.27	±	16.69
	Sevilleta	9	653.56	±	41.12	144.42	±	10.15
	Bosque del Apache	9	808.63	±	53.56	119.14	±	3.05
Feb 2008	Angostura	9	8.28	±	1.12	5.74	±	3.05
	Alameda	9	32.88	±	4.35	3.70	±	1.34
	Los Lunas	9	257.71	±	63.39	221.86	±	41.76
	Sevilleta	9	431.70	±	34.41	236.48	±	16.68
	Bosque del Apache	9	249.50	±	69.16	154.72	±	32.11
May 2008	Angostura	9	23.64	±	3.70	24.03	±	3.52
	Alameda*	3	34.68	±	6.17	17.88	±	4.04
	Los Lunas*	6	226.30	±	5.93	84.55	±	7.33
	Sevilleta	9	161.37	±	6.26	83.07	±	2.85
	Bosque del Apache	9	93.10	±	7.96	68.86	±	4.81
Aug 2008	Angostura	9	34.62	±	7.59	15.08	±	1.64
	Alameda	9	50.09	±	4.06	12.67	±	1.36
	Los Lunas	9	281.99	±	35.52	182.83	±	5.93
	Sevilleta	9	755.19	±	43.42	44.47	±	3.36
	Bosque del Apache	9	204.04	±	6.93	26.01	±	1.97
Nov 2008	Angostura	9	7.97	±	1.14	3.69	±	1.42
	Alameda	9	32.58	±	5.79	4.34	±	1.17
	Los Lunas	9	759.72	±	243.09	126.69	±	34.95
	Sevilleta	9	869.71	±	36.65	239.35	±	18.02
	Bosque del Apache	9	434.37	±	111.62	151.85	±	54.05

Table A1-2 continued.

Season	location	N	bromide (ug/L)			chloride (mg/L)			sulfate (mg/L)		
Nov 2007	Angostura	9	59.11	±	8.76	7.92	±	1.26	36.19	±	2.82
	Alameda	9	46.99	±	1.00	12.01	±	0.20	52.18	±	0.22
	Los Lunas	9	72.62	±	1.66	26.18	±	0.55	66.69	±	2.34
	Sevilleta	9	62.31	±	10.79	29.97	±	0.44	89.04	±	1.39
	Bosque del Apache	9	93.73	±	2.92	35.73	±	1.39	98.00	±	4.82
Feb 2008	Angostura	9	45.54	±	2.10	6.70	±	0.04	33.70	±	0.24
	Alameda	9	53.15	±	4.33	11.76	±	0.34	36.78	±	2.10
	Los Lunas	9	45.95	±	9.35	15.66	±	2.41	29.00	±	6.13
	Sevilleta	9	67.48	±	11.69	28.13	±	0.40	72.33	±	1.41
	Bosque del Apache	9	87.51	±	12.45	38.68	±	3.66	81.31	±	10.87
May 2008	Angostura	9	12.66	±	3.22	3.41	±	0.06	31.26	±	0.41
	Alameda*	3	6.38	±	6.38	4.45	±	0.02	30.27	±	0.13
	Los Lunas*	6	31.69	±	1.34	8.70	±	0.09	36.70	±	0.39
	Sevilleta	9	37.85	±	1.76	9.36	±	0.06	40.96	±	0.18
	Bosque del Apache	9	36.09	±	4.86	10.59	±	0.11	42.64	±	0.30
Aug 2008	Angostura	9	20.35	±	2.78	4.02	±	0.36	28.77	±	1.62
	Alameda	9	20.48	±	1.71	4.70	±	0.33	25.64	±	1.77
	Los Lunas	9	52.08	±	1.40	15.68	±	0.42	41.30	±	0.53
	Sevilleta	9	63.19	±	3.71	26.30	±	3.62	109.92	±	8.52
	Bosque del Apache	9	56.83	±	2.55	17.05	±	0.79	145.53	±	6.68
Nov 2008	Angostura	9	16.65	±	3.18	3.47	±	0.26	31.69	±	1.63
	Alameda	9	21.19	±	5.05	6.40	±	0.30	34.88	±	0.99
	Los Lunas	9	32.13	±	6.13	17.40	±	1.28	35.00	±	2.38
	Sevilleta	9	60.38	±	7.32	23.70	±	1.07	69.69	±	5.26
	Bosque del Apache	9	47.83	±	9.64	26.34	±	1.02	72.16	±	2.57

Table A1- 3. Mean (±SE) data for ammonium (μg/L) analyzed from water samples collected at each of five locations on five sampling periods. *samples not collected because of high river flows,**lab equipment error

season	location	N	NH_4 (μg/L)		
Nov-07	Angostura	18	8.13	±	0.69
	Alameda	18	9.30	±	0.81
	Los Lunas	18	18.26	±	0.59
	Sevilleta	18	9.96	±	0.61
	Bosque del Apache	18	10.86	±	0.43
Feb-08	Angostura	18	3.77	±	0.96
	Alameda**	16	2.74	±	0.91
	Los Lunas	18	39.56	±	10.53
	Sevilleta	18	30.09	±	4.93
	Bosque del Apache	18	8.75	±	2.32
May-08	Angostura	18	9.10	±	0.76
	Alameda *	6	10.84	±	0.94
	Los Lunas *	12	10.41	±	0.79
	Sevilleta	18	8.61	±	0.55
	Bosque del Apache	18	15.66	±	1.85
Aug-08	Angostura	18	2.66	±	0.70
	Alameda	18	2.77	±	0.63
	Los Lunas**	17	13.99	±	2.26
	Sevilleta**	17	9.90	±	0.91
	Bosque del Apache**	17	11.34	±	1.89
Nov-08	Angostura	18	6.62	±	1.79
	Alameda	18	4.45	±	0.39
	Los Lunas	18	43.15	±	19.23
	Sevilleta	18	12.87	±	3.21
	Bosque del Apache	18	6.04	±	0.27

Table A1-4. Mean (±SE) TN and TP **(mg/L)** measured from water samples collected at each of five sampling locations on five sampling periods. *samples not collected because of high river flows, **lab processing errors, na = not applicable

season	location	N	TN (mg/L)			TP (mg/L)		
Nov 2007			no data collected					
Feb 2008	Angostura	9	0.2773	±	0.0479	0.0396	±	0.0130
	Alameda	9	0.3348	±	0.0820	0.0703	±	0.0366
	Los Lunas	9	0.9213	±	0.2459	0.5392	±	0.1293
	Sevilleta	9	0.9366	±	0.0826	0.4472	±	0.0647
	Bosque del Apache**	0	na	±	na	na	±	na
May 2008	Angostura**	0	na	±	na	na	±	na
	Alameda*	0	na	±	na	na	±	na
	Los Lunas*	6	0.780	±	0.038	0.358	±	0.029
	Sevilleta	9	0.5239	±	0.1716	0.4856	±	0.3069
	Bosque del Apache**	0	na	±	na	na	±	na
Aug 2008	Angostura	9	1.4397	±	0.1711	0.6805	±	0.1243
	Alameda	9	0.7476	±	0.3717	0.1740	±	0.2829
	Los Lunas	9	0.7153	±	0.0272	0.0838	±	0.0097
	Sevilleta	9	0.6883	±	0.0446	0.1237	±	0.0353
	Bosque del Apache	9	1.2940	±	0.4051	0.4240	±	0.2326
Nov 2008	Angostura	9	0.8307	±	0.0349	0.0053	±	0.0087
	Alameda	9	0.8213	±	0.0276	0.0040	±	0.0060
	Los Lunas	9	1.6920	±	0.5300	0.1547	±	0.1089
	Sevilleta	9	1.8413	±	0.1881	0.3213	±	0.0440
	Bosque del Apache	9	1.3440	±	0.2726	0.1400	±	0.0569

Table A1-5. Mean (±SE) TN (%) and TP (%) measured from sediment samples collected at each of five sampling locations on four sampling periods. Note that TN and TP sediment samples are still being prepared from November 2008 surveys. *samples not collected because of high river flows, **lab processing errors

season	location	N	TN (%)			TP (%)		
Nov 2007	Angostura	3	0.091	±	0.044	0.046	±	0.003
	Alameda	3	0.064	±	0.036	0.034	±	0.007
	Los Lunas	3	0.012	±	0.006	0.019	±	0.003
	Sevilleta	3	0.016	±	0.011	0.036	±	0.005
	Bosque del Apache	3	0.096	±	0.025	0.043	±	0.001
Feb 2008	Angostura **	2	0.100	±	0.017	0.045	±	0.005
	Alameda	3	0.012	±	0.006	0.024	±	0.004
	Los Lunas	3	0.069	±	0.019	0.042	±	0.004
	Sevilleta	3	0.008	±	0.002	0.030	±	0.002
	Bosque del Apache	3	0.033	±	0.018	0.043	±	0.008
May 2008	Angostura	3	0.020	±	0.003	0.035	±	0.010
	Alameda*	2	0.011	±	0.004	0.018	±	0.001
	Los Lunas*	2	0.032	±	0.011	0.045	±	0.020
	Sevilleta	3	0.007	±	0.004	0.046	±	0.014
	Bosque del Apache	3	0.087	±	0.034	0.023	±	0.003
Aug 2008	Angostura	3	0.004	±	0.001	0.036	±	0.004
	Alameda	3	0.008	±	0.001	0.036	±	0.009
	Los Lunas	3	0.029	±	0.026	0.032	±	0.004
	Sevilleta	3	0.005	±	0.002	0.041	±	0.003
	Bosque del Apache	3	0.066	±	0.016	0.016	±	0.001

Table A1-6. Chlorophyll *a* and AFDW data analyzed from samples collected at each of five locations on five sampling periods. *samples not collected because of high river flows, **lab processing

		chlorophyll *a* (mg/m^2)				AFDW (g/m^2)			
season	**location**	**N**	**mean**	**±**	**se**	**N**	**mean**	**±**	**se**
Nov 2007	Angostura	9	20.73	±	2.37	10	127.35	±	19.20
	Alameda	9	19.74	±	6.46	9	133.30	±	29.11
	Los Lunas	9	18.04	±	5.47	9	119.00	±	30.49
	Sevilleta	9	2.80	±	0.83	9	50.53	±	8.99
	Bosque del Apache	9	1.75	±	0.45	9	219.87	±	16.33
Feb 2008	Angostura**	6	290.93	±	63.15	6	111.56	±	16.90
	Alameda	9	73.46	±	21.34	9	78.69	±	16.49
	Los Lunas	9	25.87	±	4.85	9	110.02	±	10.86
	Sevilleta	9	21.54	±	3.60	9	84.48	±	8.82
	Bosque del Apache	9	9.17	±	2.71	9	77.79	±	11.74
May 2008	Angostura	9	50.62	±	10.22	9	30.89	±	18.17
	Alameda*	0	na	±	na	0	na	±	na
	Los Lunas*	2	0.4861	±	0.3681	3	55.78	±	4.06
	Sevilleta	9	18.35	±	5.76	9	124.30	±	22.03
	Bosque del Apache	9	82.31	±	31.98	9	164.90	±	15.40
Aug 2008	Angostura**	3	23.36	±	8.11	3	185.96	±	53.99
	Alameda	9	5.77	±	0.79	9	88.23	±	5.04
	Los Lunas	9	20.79	±	4.01	9	124.62	±	38.11
	Sevilleta	9	1.22	±	0.23	9	155.45	±	25.04
	Bosque del Apache	9	2.59	±	0.70	9	272.29	±	22.30
Nov 2008	Angostura	9	25.89	±	2.55	9	156.63	±	9.71
	Alameda	9	13.07	±	1.96	9	97.40	±	16.94
	Los Lunas	9	17.01	±	3.55	9	108.19	±	26.82
	Sevilleta	9	10.39	±	2.80	9	178.03	±	23.80
	Bosque del Apache	9	26.03	±	3.89	9	379.18	±	19.03

Table A1-7. Summary of diatom taxa identified from the longitudinal survey (excluding Q/A slides) and relative abundance (%) at each of five sampling locations.

Diatom taxon	Angostura	Alameda	Los Lunas	Sevilleta	Bosque del Apache
Achnanthes cf *levanderi*	0.00	0.00	0.90	0.00	0.00
Achnanthes sp. 1	0.00	0.00	0.26	0.00	0.00
Achnanthes sp. 2	0.00	0.10	0.00	0.00	0.00
Achnanthes sp. 3	0.00	0.10	0.00	0.00	0.00
Achnanthes subatomoides	0.05	0.00	0.00	0.00	0.00
Achnanthes subsalsa	0.00	0.00	0.26	0.00	0.00
Achnanthidium cf *linearis (or biasolittiana)*	0.34	0.00	0.13	0.00	0.16
Achnanthidium linearis	0.05	0.00	0.00	0.00	0.00
Achnanthidium minutissimum	0.15	0.00	0.13	0.98	0.16
Amphipleura pellucida	0.11	0.05	0.00	0.10	0.00
Amphora acutiuscula	0.00	0.00	0.00	0.00	0.08
Amphora ovalis	0.00	0.00	0.00	0.00	0.22
Amphora pediculus	0.68	0.39	0.65	0.00	0.00
Amphora perpusilla	0.92	0.48	0.00	0.78	0.48
Amphora sp. 1	0.00	0.00	0.00	0.20	0.00
Anomoeoneis sphaerophora	0.00	0.00	0.23	0.10	0.06
Asterionella formosa	0.38	0.48	0.19	0.44	0.00
Aulacoseira granulata	0.85	0.00	0.32	1.13	0.48
Aulacoseira italica	0.00	0.00	3.20	0.00	0.56
Bacillaria paxillifer	0.00	0.00	0.00	0.05	0.00
Caloneis amphisbaena	0.00	0.00	0.23	0.73	0.76
Caloneis bacillum	0.15	0.00	0.00	0.00	0.16
Caloneis schumanniana	0.00	0.00	0.13	0.00	0.00
Cocconeis pediculus	4.19	6.64	2.49	1.03	2.43
Cocconeis placentula var. *lineata*	29.33	12.94	7.24	5.77	5.12
Craticula ambigua	0.00	0.00	0.00	0.00	0.22
Craticula sp.	0.00	0.00	0.03	0.00	0.00
Cyclotella antiqua?	0.04	0.00	0.00	0.00	0.00
Cyclotella cf *meneghiniana*	0.00	0.00	0.13	0.00	0.00
Cyclotella meneghiniana	0.49	0.19	0.23	0.39	0.24
Cyclotella sp.	0.05	0.00	0.00	0.00	0.00
Cymatopleura solea var. *apiculata*	0.10	0.05	0.10	0.10	0.24
Cymbella affinis	0.52	0.48	0.00	0.00	0.24
Cymbella caepitosa	0.04	0.00	0.00	0.00	0.00
Cymbella cf *affinis*	0.10	0.17	0.00	0.00	0.08
Cymbella sp. 1 *(Amphora?)*	0.05	0.00	0.00	0.00	0.00
Cymbella tumida	0.01	0.14	0.13	0.00	0.02
Diadesmis confervacea	0.00	0.00	0.78	0.00	0.58
Diatoma (round)	0.00	0.19	0.00	0.20	0.00
Diatoma vulgare	10.64	9.10	1.94	15.51	4.27
Diatoma-capitate	3.87	15.31	2.20	15.07	0.08
Diatoma-oval	2.43	3.16	0.26	2.89	0.16
Encyonema minutum	0.18	0.27	0.13	0.20	0.32
Encyonema silesicum	0.59	0.10	0.00	0.00	0.08
Eolimna minima	0.05	0.07	0.00	0.00	0.08
Epithemia cf *sorex*	0.00	0.07	0.00	0.00	0.00
Epithemia sorex	1.36	1.86	0.29	0.00	0.24

Eunotia incisa	0.10	0.00	0.00	0.00	0.00
Eunotia intermedia	0.05	0.00	0.00	0.00	0.00
Fallacia insocialibilis	0.15	0.00	0.00	0.00	0.00
Fragilaria capucina var. *mesolepta*	2.26	0.19	0.00	0.39	2.19
Fragilaria sp. (GV)	0.00	0.00	0.39	0.00	0.00
Fragilaria sp. 1	0.00	0.00	0.13	0.00	0.00
Fragilaria vaucheriae	0.19	0.00	0.00	0.00	0.88
Geissleria decussis	0.05	0.19	0.36	0.00	0.00
Gomphoneis cf *herculeana*	0.39	0.24	0.00	0.00	0.00
Gomphonema acuminatum	0.00	0.00	0.00	0.00	0.06
Gomphonema angustatum	0.00	0.10	0.00	0.00	0.00
Gomphonema cf *lagenula*	0.00	0.10	0.00	0.00	0.00
Gomphonema cf *pumilum*	0.00	0.00	0.13	0.20	0.00
Gomphonema clavatum	0.00	0.00	0.00	0.00	0.08
Gomphonema gracile	0.25	0.07	0.13	0.00	0.00
Gomphonema insigne	0.05	0.00	0.00	0.00	0.00
Gomphonema lagenula	0.00	0.00	0.00	0.00	0.08
Gomphonema minutum	0.34	0.00	0.13	0.00	0.00
Gomphonema olivaceum	0.76	0.87	0.52	0.68	1.47
Gomphonema parvulum	0.35	0.07	0.00	0.20	0.24
Gomphonema parvulum?	0.00	0.10	0.00	0.00	0.00
Gomphonema pumilum	0.68	0.19	0.00	0.20	0.24
Gomphonema rhombicum	0.35	0.00	0.00	0.00	0.00
Gomphonema sp. 1	0.02	0.00	0.00	0.00	0.00
Gomphonema sp. 2	0.00	0.10	0.00	0.00	0.00
Gomphonema sp. 3	0.00	0.00	0.00	0.00	0.06
Gomphonema subclavatum	0.05	0.00	0.00	0.00	0.00
Gomphonema truncatum	0.00	0.00	0.26	0.00	0.00
Gomphonema/Gomphoneis sp.	0.10	0.00	0.00	0.00	0.00
Gyrosigma acuminatum	0.07	0.00	0.00	0.00	0.00
Gyrosigma cf *sciotoense*	0.00	0.00	0.00	0.00	0.08
Gyrosigma scalproides	0.00	0.10	0.84	0.00	0.18
Gyrosigma sciotoense	0.00	0.02	0.13	0.00	0.02
Gyrosigma sp.	0.00	0.00	0.13	0.00	0.00
Hanzschia amphioxys	0.00	0.10	0.19	0.00	0.24
Hippodonta capitata	0.19	0.00	0.26	0.00	0.00
Hippodonta cf. *capitata*	0.05	0.19	0.26	0.20	0.00
Hippodonta sp. 1	0.05	0.00	0.00	0.00	0.00
Karayevia clevei	0.10	0.00	0.00	0.00	0.08
Luticola goeppertiana	0.00	0.00	0.00	0.00	0.08
Luticola mutica	0.10	0.00	0.13	0.00	1.14
Luticola mutica var. ventricosa	0.00	0.00	0.00	0.00	0.08
Luticola muticoides	0.10	0.00	0.65	0.00	0.04
Luticola sp.	0.00	0.00	0.00	0.00	0.08
Mastogloia sp.	0.00	0.00	0.00	0.00	0.08
Melosira varians	1.27	0.77	0.06	0.00	11.40
Navicula angusta	0.02	0.00	0.00	0.00	0.00
Navicula capitatoradiata	1.37	1.93	1.39	0.68	1.12
Navicula cf *accomoda*	0.19	0.10	0.52	1.76	0.00
Navicula cf *buderi*	0.23	0.00	0.00	0.00	0.00
Navicula cf *cinta*	0.00	0.00	0.13	0.00	0.00
Navicula cf *cocconeiformis*	0.05	0.00	0.39	0.00	0.00
Navicula cf *constans*	0.00	0.00	0.13	0.00	0.44

Navicula cf *constans* var. *symmetrica*	0.10	0.00	0.00	0.00	0.00
Navicula cf *cryptocephala*	0.05	0.00	0.00	0.00	0.00
Navicula cf *cryptotenella*	0.00	0.00	0.26	0.00	0.00
Navicula cf *elginensis*	0.05	0.00	0.00	0.00	0.00
Navicula cf halophiloides	0.29	0.14	0.19	0.00	0.36
Navicula cf *radiosa*	0.00	0.00	0.00	5.77	0.24
Navicula cf *rhynchocephala*	0.02	0.00	0.00	0.00	0.00
Navicula cf *symmetrica*	0.05	0.63	0.00	0.00	0.00
Navicula cf *tripartita*	0.00	0.00	0.00	0.20	0.00
Navicula cf *upsaliensis*	0.00	0.29	0.00	0.00	0.00
Navicula cf *viridula*	0.00	0.00	0.00	0.00	0.26
Navicula clementis	0.04	0.00	0.00	0.00	0.00
Navicula cryptocephala	0.29	0.77	2.23	0.20	0.24
Navicula cryptotenella	1.43	2.00	1.94	0.78	0.16
Navicula germainii	0.05	0.00	0.00	0.39	0.08
Navicula gregaria	0.00	0.00	0.00	0.39	0.00
Navicula lanceolata	0.00	0.00	1.45	0.00	0.64
Navicula libonensis	0.05	0.00	0.13	0.00	0.00
Navicula pseudoanglica	0.15	0.10	0.00	0.00	0.00
Navicula radiosa	0.19	0.17	0.00	0.20	0.38
Navicula recens	0.00	0.17	0.00	0.00	0.00
Navicula rostellata	0.41	0.65	2.04	0.59	1.08
Navicula seminulum	0.49	0.34	0.26	0.00	0.08
Navicula sp. 1	0.06	0.00	0.00	0.00	0.00
Navicula sp. 2	0.05	0.00	0.00	0.00	0.00
Navicula sp. 3	0.10	0.00	0.00	0.00	0.00
Navicula sp. 4	0.12	0.00	0.00	0.00	0.00
Navicula sp. 5	0.00	0.00	0.13	0.00	0.00
Navicula sp. 6	0.00	0.10	0.00	0.00	0.00
Navicula sp. 7	0.00	0.00	0.00	0.20	0.00
Navicula sp. 8	0.00	0.00	0.00	0.59	0.00
Navicula sp. 9	0.00	0.00	0.00	0.20	0.00
Navicula sp. 10	0.05	0.00	0.00	0.00	0.00
Navicula sp. 11	0.19	0.00	0.00	0.00	0.00
Navicula sp. 12	0.00	0.00	0.13	0.00	0.00
Navicula sp. 13	0.00	0.00	0.00	0.00	0.24
Navicula sp. 14	0.00	0.00	0.00	0.00	0.08
Navicula sp. 15	0.00	0.10	0.00	0.00	0.00
Navicula sp. 16	0.00	0.00	0.65	0.00	0.08
Navicula subminuscula	0.00	0.29	0.13	0.00	0.00
Navicula symmetrica	0.00	11.01	5.85	0.59	2.63
Navicula tripartita	0.56	0.19	0.00	0.20	0.16
Navicula trivialis	0.00	0.10	0.13	0.00	0.00
Navicula veneta	0.52	0.82	3.56	0.20	1.04
Navicula viridula	0.00	0.00	0.90	2.20	0.24
Navicula viridula var. linearis (or rostellata)	0.00	0.00	0.00	0.00	0.16
Navicula/Fallacia sp.	0.00	0.00	0.90	0.20	0.00
Nitzschia acicularis	0.47	0.00	0.00	0.00	0.00
Nitzschia amphibia	0.62	0.02	1.20	0.00	0.32
Nitzschia angustata	0.00	0.00	0.00	0.00	0.08
Nitzschia capitellata	0.72	0.19	1.10	0.00	0.70
Nitzschia cf filiformis	0.00	0.00	0.00	0.20	0.00
Nitzschia cf frustulum	0.00	0.00	0.26	0.00	0.00

Nitzschia cf perminuta	0.05	0.00	0.00	0.00	0.00
Nitzschia clausii	0.00	0.19	0.00	0.00	0.08
Nitzschia communis	0.76	0.00	0.00	0.00	0.00
Nitzschia dissipata	3.08	5.79	3.43	3.67	2.43
Nitzschia filiformis	0.64	0.17	0.13	0.00	0.16
Nitzschia frustulum	0.15	1.11	1.45	0.59	0.34
Nitzschia gracilis	0.00	0.00	0.29	0.00	1.67
Nitzschia heufleriana	0.19	0.82	0.36	0.20	2.37
Nitzschia inconspicua	0.58	0.24	0.97	0.20	0.08
Nitzschia linearis	0.69	0.10	0.71	0.54	0.22
Nitzschia microcephala	0.05	0.00	0.00	0.00	0.00
Nitzschia palaeaformis	0.00	0.14	0.00	0.00	0.08
Nitzschia palea	3.00	3.26	16.52	1.57	19.95
Nitzschia perminuta	1.07	0.19	0.26	0.00	2.17
Nitzschia recta	0.07	0.48	1.00	0.00	0.90
Nitzschia sinuata var. *delognei*	0.00	0.00	0.00	0.20	0.00
Nitzschia sp. 1	0.05	0.00	0.00	0.00	0.00
Nitzschia sp. 2	0.00	0.65	0.00	0.00	0.00
Nitzschia sp. 3	0.00	0.10	0.00	0.78	0.08
Nitzschia sp. 4	0.00	0.07	0.00	0.00	0.00
Nitzschia sp. 5	0.00	0.10	0.00	0.00	0.00
Nitzschia sp. 6	0.00	0.00	0.00	0.00	0.08
Nitzschia sp. 7	0.01	0.00	0.00	0.00	0.00
Nitzschia sp. 8	0.00	0.00	0.36	0.00	0.00
Nitzschia sp. 9	0.00	0.00	0.00	0.00	0.06
Nitzschia supralitorea	0.00	0.00	0.39	0.00	0.00
Nitzschia terrestris	0.00	0.00	0.13	0.00	0.12
Nitzschia wuellerstorfii	0.00	0.00	0.06	0.00	0.00
Pinnularia borealis	0.00	0.00	0.13	0.00	0.00
Pinnularia sp. 1	0.00	0.00	0.19	0.00	0.00
Pinnularia sp. 2	0.00	0.10	0.00	0.00	0.00
Planothidium delicatulum	0.30	0.29	0.52	0.00	0.46
Planothidium lanceolatum	0.81	0.85	0.23	0.29	0.28
Planothidium lanceolatum v. rostrata	0.25	0.48	0.23	0.20	0.08
Planothidium lanceolatum var. *dubium*	0.62	0.12	0.23	0.00	0.00
Planothidium lanceolatum var. *frequentissimum*	0.19	0.36	0.61	0.00	0.00
Planothidium sp. 1	0.02	0.00	0.00	0.00	0.00
Planothidium sp. 2	0.05	0.00	0.00	0.00	0.00
Pleuroseira laevis	0.02	0.00	0.00	0.00	0.00
Pseudostaurosira brevistriata	2.80	0.29	0.32	0.20	1.57
Pseudostaurosira brevistriata var. *inflata*	0.00	0.00	0.00	0.00	0.08
Reimeria sinuata	1.10	0.00	0.00	0.00	0.08
Reimeria cf *uniseriata*	0.35	0.19	0.23	0.00	0.08
Rhoicosphenia abbreviata	1.18	0.80	0.65	0.34	0.58
Rhopalodia brebissoni	0.00	0.00	0.39	0.00	0.00
Rhopalodia gibba	0.15	0.60	0.06	0.29	0.12
Rhopalodia gibberula	0.16	0.94	0.74	0.00	0.24
Sellaphora cf bacillum	0.00	0.10	0.00	0.00	0.00
Sellaphora cf pupula	0.00	0.00	0.00	0.20	0.00
Sellaphora pupula	0.18	0.27	0.61	0.20	0.54
Stauroneis anceps	0.05	0.00	0.00	0.00	0.00
Staurosira cf. construens	0.00	0.10	0.00	0.00	0.00
Staurosira construens	0.64	0.87	0.90	0.00	1.04

Staurosira construens var. *binodis*	0.72	0.58	0.13	0.00	0.56
Staurosira construens var. *subsalina*	0.00	0.00	0.65	0.00	0.00
Staurosira construens var. *venter*	2.14	1.83	4.27	1.76	2.47
Staurosira elliptica	0.00	0.10	0.26	0.00	0.00
Staurosira sp.	0.00	0.10	0.00	0.00	0.00
Staurosirella leptostauron	0.13	0.29	0.00	0.10	0.08
Staurosirella pinnata	0.00	0.29	0.00	0.20	0.00
Staurosirella pinnata var. intercedens	0.00	0.10	0.00	0.00	0.00
Staurosirella sp.	0.00	0.10	0.00	0.00	0.00
Stephanodiscus hantzschiana	0.15	0.00	0.00	0.00	0.24
Stephanodiscus niagarae	0.00	0.10	0.00	0.15	0.08
Stephanodiscus sp.	0.00	0.02	0.00	0.00	0.00
Surirella angusta	0.40	0.00	4.14	0.44	4.58
Surirella linearis var. *constricta*	0.00	0.00	0.00	0.00	0.08
Surirella minuta	1.00	0.19	3.68	23.97	1.65
Surirella minuta (raised sternum)	0.00	0.00	0.00	0.20	0.00
Surirella minuta forma 1	0.15	0.00	0.13	0.20	0.00
Surirella ovalis	0.05	0.00	0.03	0.00	1.81
Surirella sp. 1	0.05	0.00	0.00	0.00	0.00
Surirella sp. 2	0.00	0.00	0.13	0.00	0.00
Surirella sp. 3	0.00	0.00	0.00	0.00	0.02
Synedra cf *acus*	0.00	0.00	0.00	0.00	0.02
Synedra cf *ulna*	0.00	0.24	0.00	0.34	0.02
Synedra delicatissima	0.05	0.00	0.00	0.00	0.00
Synedra goulardii	0.00	0.00	0.06	0.00	0.04
Synedra parasitica	0.00	0.00	0.26	0.00	0.00
Synedra rumpens var. *familaris*	0.44	0.00	0.00	0.00	0.00
Synedra ulna	0.58	0.22	0.29	0.00	1.46
Tryblionella angustata	0.05	0.00	0.13	0.00	0.16
Tryblionella constricta	0.05	0.10	0.13	0.00	0.24
Tryblionella hungarica (not undulate)	0.00	0.00	0.00	0.00	1.47
Tryblionella hungarica (undulate)	0.04	0.12	0.39	0.20	1.91
Tryblionella lacunarum	0.00	0.00	0.00	0.00	0.08

Appendix 2 – Data from the NDS experiment

Table A2-1. Mean (±SE) anions **(µg/L)** and ammonium **(µg/L)** analyzed from water column samples collected each week during the NDS field experiment.

week	NO_3-N (µg/L)	PO_4-P (µg/L)	chloride (mg/L)	bromide (µg/L)	sulfate (mg/L)	NH_4 (µg/L)
week 1	544.50 ± 4.26	34.48 ± 1.48	23.54 ± 0.29	49.76 ± 1.56	193.81 ± 2.04	10.64 ± 2.21
week 2	695.00 ± 11.01	72.86 ± 2.31	22.94 ± 0.29	57.67 ± 0.82	75.38 ± 1.14	8.69 ± 3.77
week 3	695.39 ± 6.83	64.47 ± 4.41	28.21 ± 0.21	83.43 ± 0.46	124.86 ± 1.02	10.89 ± 4.25
week 4	5.51 ± 0.75	49.90 ± 3.39	30.74 ± 0.30	98.49 ± 1.44	91.32 ± 0.56	7.06 ± 6.88
week 5	1076.80 ± 22.19	36.59 ± 3.41	31.70 ± 0.21	64.28 ± 1.30	82.46 ± 1.66	9.83 ± 2.70

Table A2-2. Mean (± SE) chlorophyll *a* (mg/m^2) and ash-free dry weight (AFDW) (g/m^2) analyzed from NDS arrays from weeks 2, 3, and 5 with four different nutrient treatments (control, N, P, N+P).

week	nutrients	chlorophyll *a* (mg/m^2)			AFDW (g/m^2)		
week 2	control	0.252	±	0.174	6.290	±	1.251
	N	0.180	±	0.179	18.241	±	4.833
	P	1.012	±	1.012	30.603	±	14.264
	N+P	0.518	±	0.254	26.048	±	4.336
week 3	control	0.410	±	0.238	5.733	±	1.828
	N	0.090	±	0.090	12.410	±	3.043
	P	0.804	±	0.433	31.344	±	7.702
	N+P	0.050	±	0.050	5.964	±	1.658
week 5	control	0.104	±	0.071	3.556	±	1.070
	N	0.000	±	0.000	4.649	±	0.463
	P	0.089	±	0.047	3.316	±	0.646
	N+P	0.680	±	0.482	8.551	±	3.305

Table A2-3. Mean (± SE) chlorophyll *a* (mg/m^2) and AFDW (g/m^2) analyzed from NDS arrays from week 5 with two nutrient treatments (control, N+P) and two grazer exclusion treatments (grazer, non-grazer).

exclusion	nutrients	chlorophyll *a* (mg/m^2)			AFDW (g/m^2)		
grazer	control	0.104	±	0.071	3.556	±	1.070
	N+P	0.680	±	0.482	8.551	±	3.305
non-grazer	control	0.000	±	0.000	7.182	±	2.414
	N+P	0.185	±	0.185	6.622	±	2.530

Table A2-4. Mean (± SE) invertebrate abundance (individuals/m^2) collected from NDS arrays on each of the successful sampling weeks (weeks 2, 3, and 5) from four different nutrient treatments (control, N, P, N+P).

week	nutrient treatment	N	total abundance (indiv/m^2)	Simuliidae (indiv/m^2)	Chironomidae (indiv/m^2)
week 2	control	4	122.2 ± 71.1	0.0 ± 0.0	122.2 ± 71.1
	N	4	133.3 ± 133.3	0.0 ± 0.0	133.3 ± 133.3
	P	4	333.3 ± 252.4	0.0 ± 0.0	133.3 ± 133.3
	N+P	4	444.4 ± 192.9	88.9 ± 88.9	355.6 ± 224.4
week 3	control	4	633.3 ± 179	588.9 ± 195.7	44.4 ± 25.7
	N	4	466.7 ± 255	366.7 ± 242.3	66.7 ± 12.8
	P	4	366.7 ± 109.4	133.3 ± 85.1	233.3 ± 122.2
	N+P	4	122.2 ± 21.3	55.6 ± 33.3	55.6 ± 21.3
week 5	control	4	811.1 ± 299.5	155.6 ± 82.2	644.4 ± 336.8
	N	4	844.4 ± 309.5	11.1 ± 11.1	755.6 ± 326.6
	P	4	633.3 ± 110.9	0.0 ± 0.0	633.3 ± 110.9
	N+P	4	422.2 ± 82.2	66.7 ± 52.9	355.6 ± 31.4

Table A2-5. Mean (±SE) invertebrate abundance (individuals/m^2) analyzed from NDS samples collected at sample week 5 from NDS arrays with two nutrient treatments (control, N+P) and two grazer exclusion treatments (grazer, non-grazer).

nutrients	grazer exclusion	N	total abundance (indiv/m^2)	Simuliidae (indiv/m^2)	Chironomidae (indiv/m^2)
control	grazer	4	811.1 ± 299.5	155.6 ± 82.2	644.4 ± 336.8
	non-grazer	4	211.1 ± 107.9	55.6 ± 28.0	155.6 ± 82.2
N+P	grazer	4	422.2 ± 82.2	66.7 ± 52.9	355.6 ± 31.4
	non-grazer	4	488.9 ± 188.6	11.1 ± 11.1	455.6 ± 194.9

Appendix 3 - Diffusion experiment

Introduction

In order to test the effectiveness of nutrient diffusing substrates (NDS), the diffusion rate of N and P was tested over time in the laboratory. Diffusion rates were measured from control substrates as well as for N, P and N+P treatments.

Methods

Three replicate NDS saucers were made for each of four treatments (control, N, P, N + P), following the same protocol described earlier for the *in situ* NDS experiment (p. 11). Each NDS saucer was placed in a plastic container filled with 2.5 L of distilled water. The containers were arranged in arrays of four, with one replicate of each treatment placed randomly in each array. Three aquarium aerators were used to ensure that the water in the containers was homogenous and to prevent a boundary layer of high nutrient concentration around the substrates. A single aerator was used for each array, with Tygon® tubing directing air flow into each individual container. The containers were loosely covered throughout the experiment to prevent dust and other particulates from contaminating the containers.

Approximately 150 ml of water was collected for nutrient analysis from each container on a weekly basis. Samples were collected each week for four weeks. Water in the containers was then replaced completely with 2.5 L of fresh distilled water. Three samples of water from the distilled water source were taken each week as blanks for the following week. All samples were stored frozen for processing.

Samples were thawed, filtered using a 47mm diameter Millipore membrane filter (0.45 µm pore size) and a Swinnex filter apparatus and syringe, and then analyzed at the University of New Mexico Biology Annex Analytical Laboratory. PO_4-P (µg/L) and NO_3-N (µg/L) were analyzed using a Dionex DX-100 Ion Chromatograph using Chromeleon 6.60 software (AWWA et al. 1998, USEPA 1997).

Statistical analyses

Concentrations of nutrients were corrected for weekly variations in the source water by subtracting the mean concentration of blank samples from each of the nutrient treatment samples (control, N, P, N+P):

(corrected concentration) = (analyzed concentration) – (blank concentration)

SPSS (SPSS for Windows Release 16.0. SPSS Inc 2007) was used for statistical analyses. The calibrated concentrations of NO_3-N and PO_4-P were checked for normality using Probability-Probability plots; data for PO_4-P were log-transformed. Repeated measures one-way analysis of variance (ANOVA) was used to test for differences among sample weeks and nutrient treatments. Tukey's HSD *post-hoc* tests were used to compare the means of each nutrient treatment.

Results and discussion

Nutrient concentrations were negligible in control treatments and were very high in some treatments (Figure A3-1). There were significant differences among sample weeks ($P < 0.001$) and nutrient treatments ($P < 0.001$) for both NO_3-N and PO_4-P (Table A3-1). Concentration of NO_3-N was highest in the N and N+P treatments, whereas concentration of PO_4-P was highest in the P and N+P treatments, indicating that nutrients were successfully diffusing from the substrates.

Concentrations of both nutrients peaked at week 2 and then declined. Notably, concentrations of NO_3-N were similar in all treatments at week 4, whereas concentration of PO_4-P was always higher in P-treated

substrates. Theoretically, concentrations should decline progressively over time. We hypothesize that the concentrations peaked at week as a result of initial P-sorption to the terracotta of the saucers, releasing later in the experiment (Brown et al., 2001). The distilled water blank samples also had high concentrations of both nutrients during the second week, so it is also possible that there may have been an issue with the source water.

This study indicates that NDS diffuse nutrients for an extended period of time (weeks). This indicates that NDS could act as a point source of nutrients for an extended period of time, and thereby influence the algal community colonizing on each saucer. We plan to rerun the experiment sampling more frequently to better resolve the diffusion pattern.

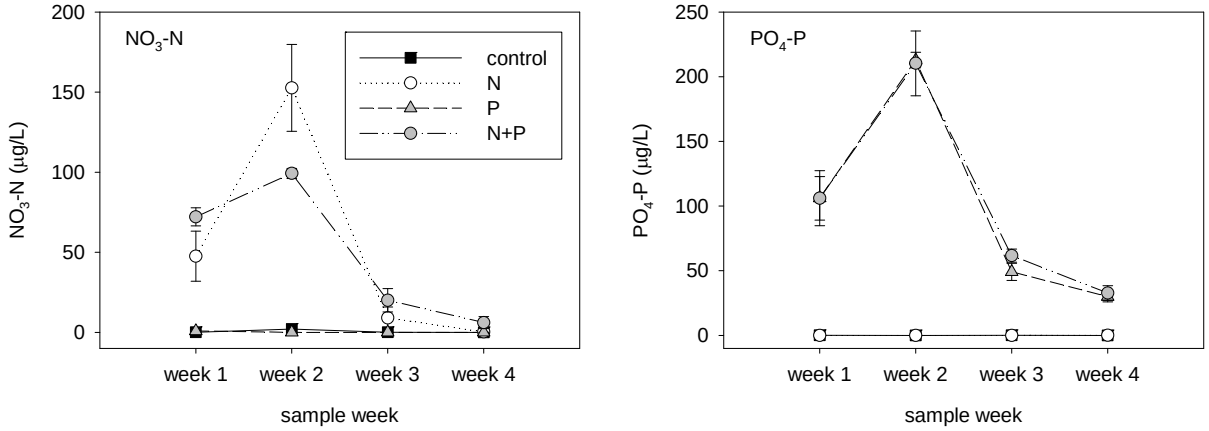

Figure A3-1. Concentrations of NO_3-N and PO_4-P collected from each of the four nutrient treatments (control, N, P, N+P) over the four week diffusion study. The y axis for the PO_4-P graph has been log-transformed.

Table A3-1. Summary of results from ANOVA analysis of NO_3-N and PO_4-P, testing for differences between sample week (weeks 1-4) and nutrient treatment (control, N, P, N+P). Significant results ($P<0.05$) are highlighted in bold.

	week		**nutrient**		**week × nutrient**	
	$F_{3, 32}$	P	$F_{3, 32}$	P	$F_{9, 32}$	P
NO_3-N	26.752	**0.000**	78.534	**0.000**	7.191	**0.000**
PO_4-P	55.800	**0.000**	2309.922	**0.000**	22.433	**0.000**

Appendix 3 - Tumbleweed as an algal substrate

During the initial review process for this contract, reviewers hypothesized on the role of submerged tumbleweed as an algal substrate in the Middle Rio Grande. Given that submerged tumbleweed often provide good fish habitat, tumbleweed may be an important component for evaluating algal food resources in the Middle Rio Grande. An experiment to determine colonization rates and diversity of algae on tumbleweed was performed in the Middle Rio Grande in October-November 2007. Tumbleweed segments were placed in the river at the Angostura, Alameda, and Sevilleta locations. These sites were chosen based on nutrient inputs above and below the Albuquerque reach. Segments of tumbleweeds were attached to posts with nylon fishing line and placed in the river to be colonized by algae in the water column. Segments for tumbleweed were removed and sampled weekly for 4 weeks. Diatoms and soft algae are in the process of being enumerated from tumbleweeds and compared within and among sites. Preliminary results show that tumbleweeds at the Angostura site was dominated by diatoms *Epithemia sorex*, *Cocconeis placentula*, and *Gomphonema parvulum* while the Alameda site were dominated by diatoms *Nitzschia dissipata*, *E. sorex*, and *Navicula capitatoradiata*, and the Sevilleta site by *N. dissipata*, *Nitzschia palea*, and *Navicula subminuscula*. All dominant taxa are nutrient tolerant and all species, excluding *N. palea,* are alkaliphilous.

We have also developed a calibration data set to test the relationship between tumbleweed biomass and surface area to assist in surface area calculations for quantitative algal sampling from tumbleweed for future work. Dried segments of tumbleweed were weighed from 20 dry tumbleweeds and segment surface area was calculated using geometric equations. There was a very good correlation (R^2 = 0.88) between mass and surface area (Figure A4-1). The surface area calibration provides a tool for subsequent research of diatom communities on tumbleweed.

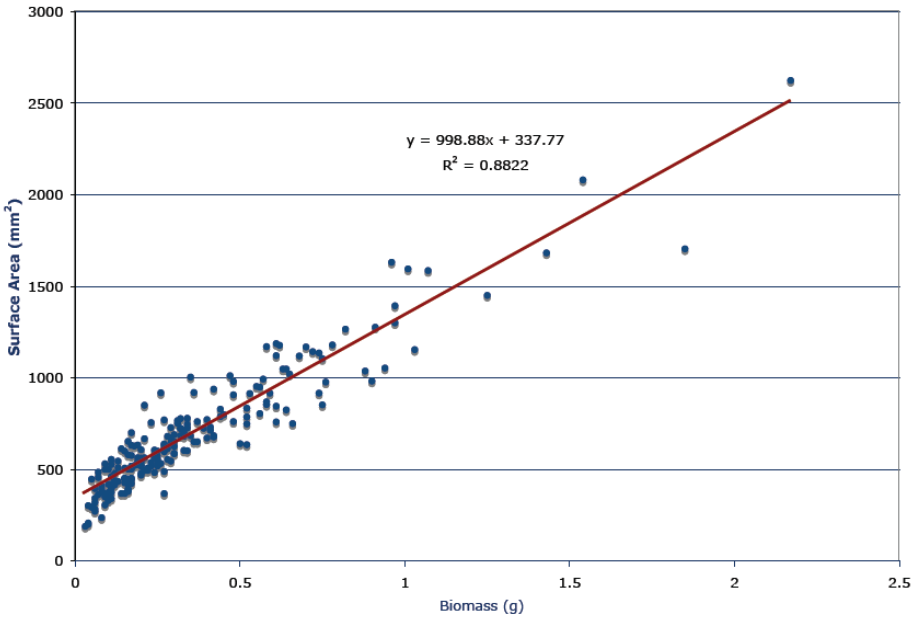

Figure A4-1: Surface area calibration for tumbleweed segments showing biomass (g) and surface area (mm^2)