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Plant–microbe interactions and nitrogen dynamics during wetland establishment in a desert stream

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Abstract In late-successional steady state ecosystems, plants and microbes compete for nutrients and nutrient retention efficiency is expected to decline when inputs exceed biotic demand. In carbon (C)-poor environments typical of early primary succession, nitrogen (N) uptake by C-limited microbes may be limited by inputs of detritus and exudates derived from contemporaneous plant production. If plants are N-limited in these environments, then this differential limitation may lead to positive relationships between N inputs and N retention efficiency. Further, the mechanisms of N removal may vary as a function of inputs if plant-derived C promotes denitrification. These hypotheses were tested using field surveys and greenhouse microcosms simulating the colonization of desert stream channel sediments by herbaceous vegetation. In field surveys of wetland (ciénega) and gravelbed habitat, plant biomass was positively correlated with nitrate (NO_3^-) concentration. Manipulation of NO_3^- in flow-through microcosms produced positive relationships among NO_3^- supply, plant production, and tissue N content, and a negative relationship with root:shoot ratio. These results are consistent with N limitation of herbaceous vegetation in Sycamore Creek and suggest that N availability may influence transitions between and resilience of wetland and gravelbed stable states in desert streams. Increased biomass in high N treatments resulted in elevated rates of denitrification and shifts from co-limitation by C and NO_3^- to limitation by NO_3^- alone. Overall NO_3^- retention efficiency and the relative importance of denitrification increased with increasing N inputs. Thus the coupling of plant growth and microbial processes in low C environments alters the relationship between N inputs and exports due to increased N removal under high input regimes that exceed assimilative demand.

Keywords Denitrification · Uptake · Nutrient retention · Ciénega · *Paspalum distichum* · Regime shift

Introduction

Ecosystem responses to variation in nutrient supply are influenced by the coupled interactions of primary

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producers and heterotrophic microbes (Knops et al. 2002; Danger et al. 2007; Jackson et al. 2008). The nature of these interactions can depend on the sensitivity of primary producers to variation in nutrient availability, and on the reliance of heterotrophic metabolism on contemporaneous organic matter (OM) supply from primary producers. When abundant OM fuels high microbial demand, plants and microbes compete for inorganic and, in some cases, organic nutrients (Kaye and Hart 1997; Schimel and Bennett 2004). When ambient supplies of labile OM are low, primary producers are likely to dominate nutrient dynamics (Bowden 1991; Berendse 1998), and assimilatory and dissimilatory nutrient demand of heterotrophic microbes will covary positively with OM inputs from primary producers (e.g., Griffiths et al. 1993; Hume et al. 2002; Baker and Vervier 2004). In this study, we use the colonization of organic-poor gravelbed desert streams by herbaceous vegetation as a model system to understand the consequences of these interactions for the relationship between nutrient inputs and the magnitude and mechanisms of nutrient assimilation, transformation, and export.

Nutrient export from ecosystems can vary depending on the temporal dynamics of retention and removal processes (e.g., vegetation succession and soil development). In early- to mid-successional systems where biomass accumulation is the predominant retention mechanism, retention of limiting nutrients increases with biomass increments then decline until biomass reaches steady state (Vitousek and Reiners 1975; Grimm 1987; but see Kaye et al. 2003). Inorganic nutrient export can remain below levels of inputs even at steady state biomass if removal occurs via other vectors such as export of dissolved or particulate organic materials (Hedin et al. 2003), long-term retention in recalcitrant soil OM pools (Kaye et al. 2003), or denitrification (Schade and Lewis 2006). Regardless of the present capacity of an ecosystem to retain or remove nutrients, high nutrient input regimes will generally saturate demand, leading to increased nutrient export in both absolute and relative terms (Aber et al. 1998; Earl et al. 2006). The resulting positive relationship between nutrient inputs and outputs is widely observed across systems and scales (e.g., Dise and Wright 1995; Mulholland et al. 2008).

The frequency of this observation notwithstanding, negative relationships between resource inputs and the relative magnitude of nutrient export (i.e., exports as a proportion of inputs) can occur if increased inputs produce large increases in retention or removal capacity. Such responses can occur during early succession, when high nutrient availability can promote rapid biomass accrual and thus dramatic increases in assimilative demand (Bowden 1991; Jones et al. 2004; Schade and Lewis 2006). A second mechanism by which nutrient additions can stimulate higher nutrient demand is the coupling of heterotrophic microbes to primary production under low OM conditions. If increased plant growth alleviates C limitation, then addition of nutrients would indirectly fuel additional microbial demand. The effect of this additional microbial activity on N export may be particularly large in systems that are favorable to denitrification, as the associated N removal capacity will not necessarily saturate over time. Because microbes acquiring N for dissimilatory pathways may be relatively poor competitors against plants (Bodelier et al. 1998; Silvan et al. 2005), coupled denitrification and primary production may be most important at input levels that exceed autotrophic demand.

Ecosystems with strong positive feedbacks, among species or between biota and the physical environment, often possess alternative stable states, meaning that more than one ecosystem configuration is stable across some range of environmental conditions (Scheffer et al. 2001). In desert streams, colonization of the active channel by herbaceous vegetation initiates the transition from a gravel-bed system dominated by algae toward densely vegetated wetlands (ciénegas: Heffernan et al. 2008). The strong effect of vegetation on sediment cohesion and channel stability permits dense ciénega vegetation to withstand flood regimes that completely scour channels with little or no vegetation, generating alternative (ciénega and gravelbed) states (Heffernan 2008). While the gravelbed state is currently far more common, ciénegas were historically widespread in drainages of the arid Southwestern US (Hendrickson and Minckley 1984). Efforts to restore ciénegas are hampered by a lack of information about nutrient dynamics and responses in these systems (Stromberg 1993).

The general importance of N for primary producers in desert streams is well-established. Within the wetted channel, the growth, composition, and spatial distribution of algal communities are highly sensitive to N availability, and thus to hydrologic connections between the surface stream and N-rich interstitial waters of hyporheic channel sediments (Grimm and Fisher 1986; Valett et al. 1994). In adjacent riparian habitats, the foliar chemistry and root:shoot allocation of trees and shrubs respond to multiple N sources including surface waters, floodwater inputs, and internal soil pools (Schade et al. 2002; Schade and Lewis 2006). Limitation of herbaceous plants by N availability is thus likely but unverified.

While primary producers in desert streams are strongly N-limited, microbes inhabiting low-organic-matter channel sediments are generally limited by the availability of labile C (Jones 1995). As a result, sediment microbial respiration and denitrification respond strongly to the inflow of surface waters and associated algal-derived OM (Jones et al. 1995; Holmes et al. 1996), and to the establishment of woody shrubs on sandbars (Schade et al. 2001). Anoxia and abundant methane in ciénega hyporheic zones suggest that herbaceous plants similarly fuel increased heterotrophic activity (Heffernan et al. 2008). However, the consequences of this OM subsidy for N transformations in ciénega sediments have not been addressed.

The objective of this study is to understand the effects of N supply on plant growth during the early stages of wetland establishment in desert streams, and to evaluate the consequences of interactions between production and denitrification for the fate of N inputs. We use a combination of field surveys and greenhouse microcosm experiments to evaluate three related hypotheses: first, that growth rates of herbaceous vegetation colonizing channel sediments are limited by N; second, that high plant growth alleviates C limitation of denitrification; and third, that the interplay between these processes influences the relationship between N supply and the magnitude and mechanisms of N transformation and loss. Based on these data and other studies, we discuss the implications of plant–N–microbe interactions for the resilience and ecosystem-scale N dynamics of desert stream wetlands and the relationships between nutrient inputs and outputs in ecosystems more generally.

Methods

Field surveys of N and plant abundance

To test the hypothesis that ambient variation in NO_3^- concentration influences herbaceous plant growth, we conducted longitudinal surveys in two sections of Sycamore Creek, AZ, a spatially-intermittent Upper Sonoran Desert stream. Reaches were selected because their upstream ends were known to have elevated NO_3^- concentrations, providing a natural gradient in N availability, and because they differed in the density and duration of herbaceous plant establishment. Because these surveys were originally part of two distinct sampling efforts, the particulars of vegetation sampling differ between the two sites. The first survey was conducted in April 2004 in a well-developed ciénega site that receives significant influx of N-rich groundwater. This site was dominated by knotgrass (*Paspalum distichum*), bulrush (*Schoenoplectus americanus*), horsetail (*Equisetum laevigatum*), and watercress (*Rorippa nasturtium-aquaticum*). We established nine transects, oriented perpendicular to streamflow, at 25 m intervals (200 m total length). At each transect, duplicate surface water samples were collected in pre-rinsed, acid washed 60-ml polyethylene bottles. We measured vegetation cover using the line-intercept method (resolution = 10 cm) along each transect, and harvested biomass from two randomly-placed 0.0625-m^2 quadrats. Biomass at each transect was calculated from vegetation cover and mean biomass of the two quadrats.

The second survey was conducted in June of 2005 along a 100-m side channel that was denuded of vegetation by large floods in the winter of 2005 but fed entirely by subsurface flow at the time of sampling. The plant community consisted of a relatively even mix of water speedwell (*Veronica anagallis-aquatica*), watercress (*R. nasturtium-aquaticum*), seep monkeyflower (*Mimulus guttatus*), rabbitsfoot grass (*Polypogon monspeliensis*), and Bermuda grass (*Cynodon dactylon*). We collected duplicate water samples and established biomass transects ($n = 21$) at 5-m intervals along the length of the channel. We used plant taxa and height, measured at 0.5-m intervals along transects, to estimate average biomass for each transect, based on taxon-specific height–biomass relationships (Heffernan 2008). We tested the hypothesis that variation in DIN influences biomass production in ciénega and gravelbed environments via regression analysis of relationships between surface water NO_3^- and aboveground biomass from field surveys.

Experimental manipulation of N availability

We used experimental mesocosms to determine whether field observations relationships between NO_3^- and vegetation biomass reflected causal relationships, and to evaluate the mechanistic interactions among N availability, plant growth, and hyporheic N cycling. In these experiments, we applied five N treatments (10, 25, 100, 500, 2000 μg NO_3^-–N l^{-1}; $n = 3$ per treatment) to *P. distichum* growing in flow-through microcosms over a period of 4 months (27 May–1 October 2006). In addition, three replicate controls were not planted with *P. distichum* runners and received the intermediate N concentration (100 μg NO_3^-–N l^{-1}). *P. distichum* was chosen for this experiment because it is a dominant species in ciénegas of Sycamore Creek and re-grows easily from cuttings.

Microcosms consisted of plastic shoeboxes (l × w × h: 32 × 18 × 10 cm) filled with coarse sediments (55–65% gravel, 35–45% sand, 0.2% AFDM) collected from within the wetted perimeter of Sycamore Creek, AZ, on the day prior to the start of the experiment and planted with freshly-collected *P. distichum* runners (5 g wet mass). Continuous flow of simulated stream water (i.e., with major ion chemistry similar to that of Sycamore Creek; Online Resource 1) was maintained using a multi-channel peristaltic pump. Water was pumped from reservoirs (120 l; one reservoir per treatment) via Tygon tubing (3.2 mm ID) into an inlet (2.5 cm PVC pipe) inserted into sediments at one end of each microcosm. Outflow occurred via gravity drainage through a plastic tube inserted at a height of approximately 9 cm into a screened well (5 cm PVC) inserted into sediments at the far end of the microcosm. Flow rates were measured and adjusted as necessary each time reservoirs were re-filled (every 3–4 days), and reservoirs were aerated using aquarium pumps. We replaced pump tubing monthly to minimize fouling.

Treatments were chosen to bracket a realistic range of N fluxes through hyporheic sediments of Sycamore Creek, where hyporheic concentrations can range from 5 to 200 μg NO_3^-–N l^{-1} (Valett et al. 1990; Holmes et al. 1994). If flow rates range from 0.3 to 3 m h^{-1} (Dent et al. 2007), water flux through a 180 cm^2 cross-section with porosity of 0.2 would range from 18 to 180 ml min^{-1}, and hyporheic N flux from 0.09 to 14.4 μg NO_3^-–N min^{-1}. The unlikely combination of rapid flow and extremely long residence times (and thus NO_3^- concentrations) could produce fluxes as high as 30 μg NO_3^-–N min^{-1}. At a target flow rate of 7.5 ml min^{-1}, NO_3^- fluxes through microcosms ranged from 0.07 to 15 μg N min^{-1}. Concentrations of ammonium (NH_4^+) and monophosphate (PO_4^{2-}) were also elevated above ambient concentrations to ensure that mesocosm fluxes were comparable to those that occur in the field (Online Resource 1).

Chemical characteristics of input and output waters were measured on days 4, 16, 39, 58, 96, and 115. Dissolved oxygen (DO), specific conductance, and temperature were measured in the screened PVC wells (i.e., at microcosm outflows) using a YSI 556 multiparameter probe (Yellow Springs Instruments, Inc., Yellow Springs, OH). Water samples were collected with a pre-rinsed syringe from input tubing and screened outflow wells, respectively, in pre-rinsed, acid washed 60 ml polyethylene bottles for analysis of NO_3^-, NH_4^+, total dissolved nitrogen (TDN), and dissolved organic carbon (DOC). All water samples were refrigerated until analysis. Water samples from microcosm inputs and outputs and field surveys were filtered immediately after collection or return to the lab using pre-ashed Whatman GF/F glass-fiber filters. Sub-samples for DOC analysis were acidified with HCl. NO_3^- and NH_4^+ were analyzed on a Latchat QC8000 Flow Injection Analyzer using the cadmium–copper reduction method (Wood et al. 1967) and the phenol–hypochlorite method (Solorzano 1969), respectively. DOC and TDN were measured from acidified samples using a Shimadzu TOC-VC total carbon analyzer followed by chemiluminescent analysis of TDN by a Shimadzu TNM-1 total nitrogen analyzer. Instrument failure precluded analysis of DOC and TDN from samples collected on day 95 and 115. Dissolved organic nitrogen (DON) was calculated as the difference between TDN and dissolved inorganic nitrogen ([DIN] = [NO_3^-–N] + [NH_4^+–N]). External source quality controls for all analyses were always within 10% of expected values. NO_3^- and NH_4^+ analyses were conducted within 24 h of sample collection, and TDN and DOC within 7 days.

We evaluated the effects of N supply and plant growth on hyporheic denitrification using an in situ denitrification limitation assay (DLA) with the acetylene block technique (Yoshinari and Knowles 1976). For each microcosm, denitrification was measured under ambient experimental conditions and in response to amendments of C (50 mg C l^{-1} as dextrose; Day 117), NO_3^- (20 mg N l^{-1}; Day 120), and NO_3^- and C (Day 123). We added acetylene by saturating 4 l of simulated streamwater, which was then mixed into 36 l of simulated streamwater (amended with C and/or N depending on treatment) in reservoirs using a peristaltic pump to achieve 10% acetylene saturation. Aerators were turned off during denitrification assays to minimize degassing of acetylene. Amendments were carried out for 24 h. This duration was chosen based on conservative tracer additions which indicated 80–90% hydrologic turnover within 6 h and complete hydrologic turnover after 18 h. Over the course of incubations, an unknown quantity of acetylene was almost certainly lost from reservoirs via degassing, potentially leading to declines in the efficiency of this assay. We have assumed that bias is minimal, and that any effects would be similar across long-term incubation treatments.

At the conclusion of each amendment, we collected dissolved gas samples by equilibrating 40 ml of water collected from the PVC outlet with 20 ml headspace in a sealed syringe, then transferring headspace to 3-ml glass vials with gas-tight septa after syringe headspace equilibration. To prevent carryover effects, the initiation of each amendment was delayed for 2 days after the end of the prior treatment. Headspace N_2O was analyzed using a Shimadzu GC-14 gas chromatograph with electron capture detector (Poropak Q, 80/100 column). Dissolved pN_2O was calculated from headspace values using temperature-corrected Bunsen coefficients.

At the conclusion of the experiment (Day 125), we collected plant tissue for mass and elemental analysis. Above- and below-ground plant tissue was weighed separately after drying at 60°C for 24 h, homogenized, then ground for elemental analysis. We measured tissue C and N content using a Perkin Elmer 2400 Series II CHN Analyzer.

Data analyses

We calculated DIN inputs over the course of the experiment from mean inflow rate and NH_4^+ and NO_3^- concentrations. For each solute (NO_3^-, NH_4^+, DON, DOC, and DO) and sampling date, we determined changes in concentration in each treatment on an absolute basis, and, for DIN, as a proportion of input concentration. We determined net DIN production and O_2 consumption (i.e., aerobic respiration) on a sediment mass basis from changes in concentration and hydrologic input. N mass balance budgets were constructed for each microcosm using hydrologic inputs and outputs of DIN and DON, ambient denitrification rate, and accumulation in plant tissue. Accumulation of N in sediments was assumed to be negligible owing to low abundance of fine sediments and OM. We determined DIN and DON outputs from linearly interpolated concentrations and mean water inflow rate, and plant N assimilation for above- and below-ground tissue from dry mass and %N. Estimates of total denitrification losses assumed that, in treatments where hyporheic sediments became anoxic, no denitrification occurred until the onset of hypoxia (<2 mg O_2 l^{-1}). To determine the effects of denitrification on hydrologic DIN losses, we calculated expected DIN losses in the absence of denitrification as the sum of estimated denitrification and observed DIN losses.

We evaluated the responses of total biomass accrual and root:shoot ratio to experimental DIN inputs using linear regression. Responses of C:N ratio of above- and below-ground tissue to DIN inputs were determined using a general linear model that included an interaction term between the two main effects (tissue type and DIN inputs). Our experimental design necessitates separate one-way analyses of the effects of N supply and vegetation on biogeochemical processes. To evaluate these effects, we compared among N treatments in vegetated microcosms and between bare and vegetated microcosms in 100 μg l^{-1} N treatments. We used repeated measures ANOVA to determine whether retention efficiency of DIN, aerobic respiration, and net changes in DO, NO_3^-, NH_4^+, DON, and DOC varied among treatments and over time. We used three-way ANOVA to evaluate the effects of nutrient amendments on the relationship between long-term N supply and denitrification, using total biomass production as a covariate of long-term N treatment. We used Tukey's HSD to evaluate significant differences among amendments within each long term treatment. We transformed data as necessary to meet assumptions of linearity and constant variance. Significance values reported for rmANOVA are Greenhouse-Geiser corrected. All statistical analyses were conducted using SPSS v.11.0 statistical software.

Results

Aboveground biomass tracked downstream decreases of surface water NO_3^- concentrations in both the ciénega and gravelbed field surveys (Fig. 1). Within the wetland site, NO_3^- decreased downstream from 180 to 60 μg N l^{-1}, and biomass ranged from 1050 to 410 g AFDM m^{-2}. Within the gravelbed seep, NO_3^- ranged from 75 to 5 μg N l^{-1}, and biomass ranged from 45 to 0 g AFDM m^{-2}. In both surveys, NO_3^- and aboveground biomass were significantly and positively related.

In microcosm experiments, production, allocation, and stoichiometry of biomass varied with DIN inputs (Fig. 2). Total biomass increased from an average of 7.7 g AFDM in the lowest N treatment to 124 g AFDM in the highest N treatments, and was significantly related to DIN inputs ($r^2 = 0.91$, $p < 0.001$; Fig. 2a). Root:shoot ratio varied significantly with N concentration ($r^2 = 0.82$, $p < 0.001$), declining from an average of 1:1 in the lowest N treatment to 1:6 in the highest. C:N ratios (Fig. 2b) decreased with increasing N ($F_{1,26} = 81.5$, $p < 0.001$), and were higher in belowground tissue ($F_{1,26} = 20.2$, $p < 0.001$), but C:N ratio of belowground tissue responded more strongly to increased N (tissue × N interaction: $F_{1,26} = 13.1$, $p < 0.001$).

Temporal patterns of aerobic respiration (Fig. 3a) varied with N supply (rmANOVA; treatment:$F_{4,10} = 17.95$, $p < 0.001$, time: $F_{5,50} = 6.17$, $p < 0.005$; interaction: $F_{20,50} = 5.20$, $p < 0.001$). In high N treatments, hyporheic DO dropped rapidly within a few weeks, becoming consistently hypoxic (<2 mg O_2 l^{-1}) on day 16 in the 2 mg l^{-1} N treatment and on day 58 in the 500 μg NO_3^-–N l^{-1} treatment. Other treatments remained moderately oxic (2–4 mg O_2 l^{-1}) over the course of the experiment. In the lowest N treatment and in unvegetated controls, net oxygen consumption declined over the course of the experiment. Respiration rates differed between vegetated and unvegetated microcosms (both receiving the 100 μg NO_3–N treatment), but those differences did not vary significantly over time (rmANOVA; treatment: $F_{1,4} = 24.1$, $p < 0.01$, time: $F_{5,20} = 9.59$, $p < 0.02$; interaction: $F_{5,20} = 2.01$, $p = 0.12$).

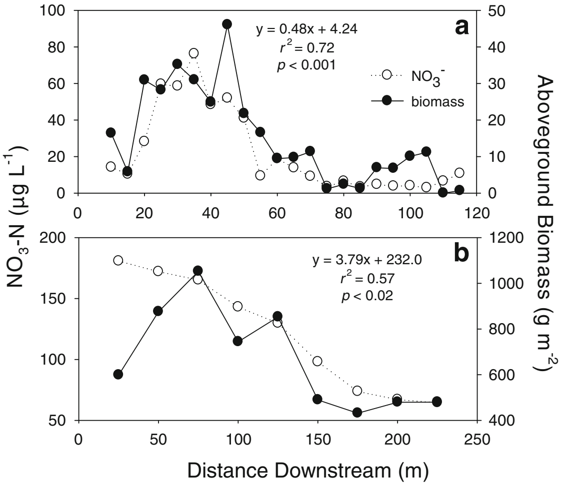

Fig. 1 Longitudinal changes in surface water NO_3^- and aboveground biomass in **a** a gravelbed side-channel (June 2005) and **b** ciénega reach (April 2004). Equations are best-fit least mean squares regressions of NO_3–N on aboveground biomass

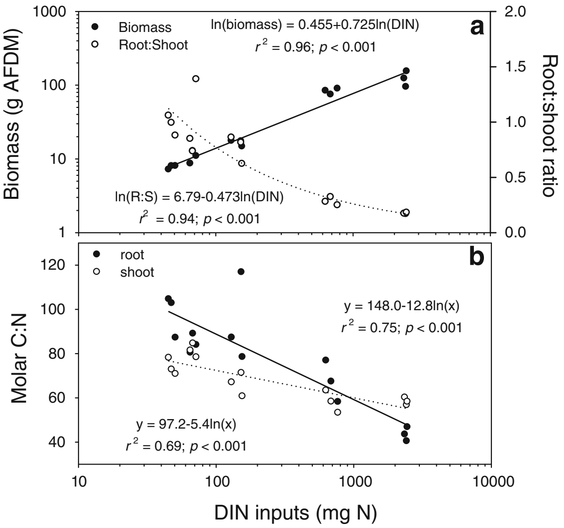

Fig. 2 Effects of NO_3–N concentration on **a** total net biomass accumulation and root:shoot ratio and **b** C:N of above- and below-ground tissue of *P. distichum*. *Lines* are best-fit least-mean-square regressions of log-transformed NO_3^- concentration on log-transformed biomass and root:shoot ratio and untransformed tissue C:N ratio

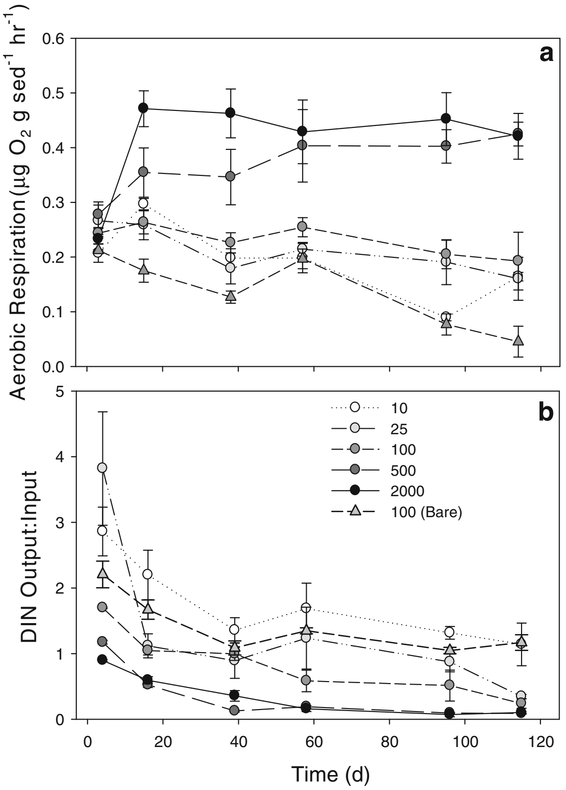

Fig. 3 Temporal patterns of **a** aerobic respiration and **b** DIN output:input ratios in experimental microcosms. Data are means (±SE) for each date and treatments ($n = 3$). Values greater than 1 in **b** indicate that DIN exports from mesocosms exceed DIN inputs

Net DIN export varied among treatments and declined in all treatments over the course of the experiment (Fig. 3b), but temporal dynamics of outputs:inputs did not vary significantly among N treatments (rmANOVA; treatment: $F_{4,10} = 108.7$, $p < 0.001$, time: $F_{5,50} = 27.4, p < 0.001$; interaction: $F_{20,50} = 2.41$, $p = 0.067$). Similarly, DIN output:input differed between vegetated and unvegetated treatments (at 100 µg NO_3–N), but temporal trajectories of DIN output:input were not significantly different (rmANOVA; treatment:$F_{1,4} = 79.0, p < 0.001$, time: $F_{5,20} = 25.3$, $p < 0.001$; interaction: $F_{5,20} = 2.39$, $p = 0.11$). All but the highest N treatment were initially sources of NO_3^-, but unvegetated controls were sources of NO_3^- throughout the experiment. In vegetated treatments, the timing and magnitude of transitions between sources and sinks of NO_3^- varied among treatments. High N treatments rapidly became net retainers of nitrate, and hydrologic outputs were less than 10% of inputs on the last two sampling dates. Lower N treatments became sinks of smaller magnitude later in the experiment. Inputs and outputs of NH_4^+ were similar throughout the experiment (Online Resource 2), and net changes in NH_4^+ did not vary significantly among treatments or over time. On average, microcosms were slight net sinks of DOC and DON, but DOC and DON removal did not vary among treatments or over time (Online Resource 3).

Responses of denitrification to short-term C and N amendments varied among long-term N treatments and thus as a function of plant biomass (Fig. 4; Table 1). Denitrification under ambient conditions was low in all but the two high N treatments. Addition of N consistently stimulated denitrification across all treatments. Addition of carbon resulted in elevated rates in the 25 and 100 µg NO_3^-–N l^{-1} treatments (both vegetated and bare controls), but not in the lowest or two highest N treatments. Addition of C and N resulted in high rates of denitrification in all treatments, but the magnitude of this effect decreased with increasing plant biomass.

The absolute and relative importance of denitrification and plant uptake as mechanisms of nutrient retention varied in response to nutrient inputs. In all treatments, plant uptake was the primary mechanism of N retention. The proportion of DIN inputs accounted for by plant uptake increased from 85 to 96% between the 10 and 500 µg NO_3^-–N l^{-1} treatments, but was less than 50% in the highest N treatment (Fig. 5a). Denitrification increased from less than 1% of DIN inputs but increased to 9 and 35% of DIN inputs, in the 500 and 2000 µg NO_3^-–N l^{-1}, respectively. In the low N treatments and the unvegetatated controls, the sum of uptake, denitrification, and hydrologic output was 50–60 mg N greater than DIN inputs; decreases in DON were comparable in magnitude in those treatments, but did not account for N budget imbalances (180–220 mg N) in the high N treatments (Online Resource 4). Over the course of the entire experiment, the 10 and 25 µg N l^{-1} treatments and the unvegetated controls were sources of DIN, while hydrologic outputs from the 500 and 2000 µg N l^{-1} treatments were 22 and 28% of inputs, respectively. The similarity of these retention efficiencies reflects the dramatic increase in denitrification in the 2,000 µg N l^{-1} treatment; absent denitrification, we estimate DIN outputs would be 31 and 63% of inputs, respectively, in these high N treatments (Fig. 5b).

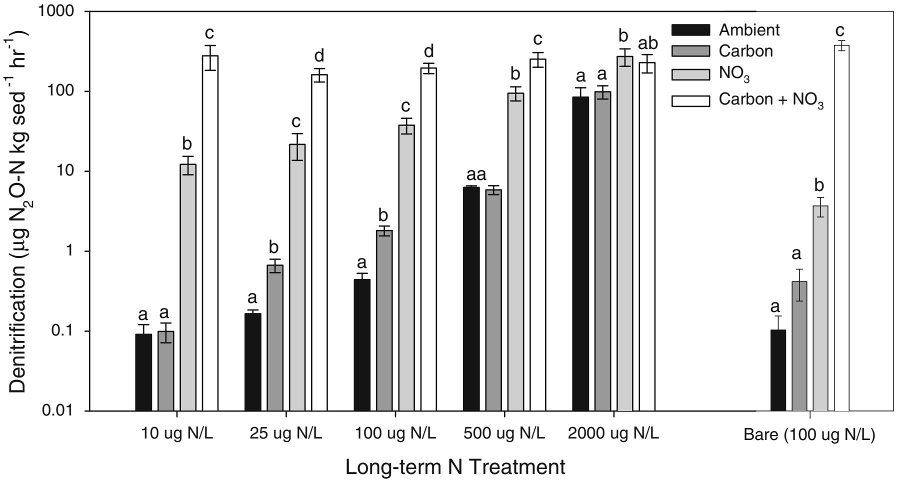

Fig. 4 Denitrification rates in experimental mesocosms under ambient conditions and after amendment with labile C, NO_3^-, and both C and NO_3^-. Data are shown as means for each amendment and long term NO_3^- concentration treatment. *Error bars* are ±SE. *Letters* indicate significant differences among short-term amendments within each long-term N fertilization treatment, based on Tukey's HSD

Table 1 Results of three-way ANOVA relating long-term NO_3^- inputs (N-AMB, upper panel) or presence of vegetation (lower panel) and carbon (C-ADD) and nitrogen (N-ADD) amendments to N_2O production during in situ acetylene block. Denitrification rates were log-transformed for this analysis

Source	Sum-of-squares	df	MSE	*F*-ratio	*p*
Nitrate inputs					
N-AMB	21.2	4	5.31	193.9	<0.001
C-ADD	2.91	1	2.91	106.3	<0.001
N-ADD	41.1	1	41.1	1501.7	<0.001
C-ADD × N-AMB	1.20	4	0.30	11.0	<0.001
N-ADD × N-AMB	7.35	4	1.84	67.1	<0.001
C-ADD × N-ADD	0.76	1	0.76	27.9	<0.001
N-AMB × C-ADD × N-ADD	0.90	4	0.22	8.236	<0.001
Error	1.095	40	0.027		
Vegetation					
Vegetation	1.20	1	1.20	36.4	<0.001
C-ADD	5.15	1	5.15	155.8	<0.001
N-ADD	24.0	1	24.0	727.0	<0.001
C-ADD × vegetation	0.49	1	0.49	14.8	<0.001
N-ADD × vegetation	0.04	1	0.04	1.25	0.281
C-ADD × N-ADD	1.24	1	1.24	37.5	<0.001
Vegetation × C-ADD × N-ADD	0.80	1	0.80	24.1	<0.001
Error	0.53	16	0.033		

Discussion

The hypothesis that herbaceous plants in desert streams are limited by N availability is supported by both the strong relationships between biomass and NO_3^- concentrations in the field (Fig. 1), and by the growth, tissue chemistry, and root:shoot allocation responses of *P. distichum* to experimental variation in N supply (Fig. 2). With the exception of the highest N treatment, observed biomass and hyporheic N fluxes correspond were within the range of field observations. The second highest N treatment

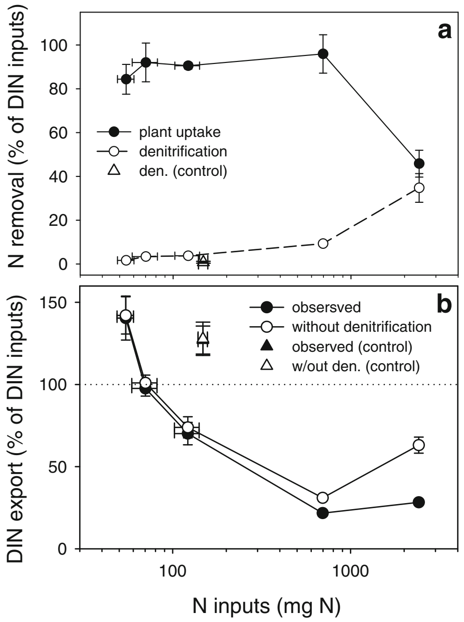

Fig. 5 Fates of N as a function of the magnitude of inputs in experimental microcosms. **a** Plant uptake and denitrification expressed as a percent of DIN inputs. **b** Hydrologic DIN export (as a percent of DIN inputs) as observed and estimated in the absence of denitrification. Data are shown as means (±SE) for each treatment

(500 µg NO_3^-–N l^{-1}) experienced fluxes corresponding to hyporheic [NO_3^-] of approximately 55 µg NO_3^-–N l^{-1}, a concentration frequently met or exceeded in desert stream hyporheic zones (Valett et al. 1990; Holmes et al. 1994), and had biomass (ca. 1100 g AFDM m^{-2}) comparable to observations from productive cienegas (Fig. 1, see also Heffernan 2008; Heffernan et al. 2008). Overall, these results indicate that herbaceous plants, like other primary producers in streams of the US Southwest, are sensitive to realistic spatial and temporal variation in N availability.

Results of this study also support the hypothesis that elevated plant production under high N inputs alleviates C limitation of heterotrophic microbes in the organic-poor sediments of desert streams. The persistence of oxic conditions (Fig. 3a) and declines in net nitrate production (Fig. 3b) in unvegetated controls and low N treatments are consistent with depletion of a limited pool of labile OM in channel sediments (Jones 1995). In the higher N treatments, the onset and persistence of hypoxia and net nitrate removal presumably reflect some combination of elevated respiration and N uptake by roots and the effects of plant production on carbon availability to heterotrophic microbes. With the exception of the two highest N treatments, net losses of DON were similar in magnitude to the difference between DIN inputs and the sum of denitrification, plant uptake, and hydrologic DIN export (Online Resource 4). This suggests that DON mineralization did in fact serve as an additional DIN source, and that plant uptake and/or denitrification was over-estimated or DIN export underestimated in the higher N treatments.

Increases in denitrification rate with increasing long-term N supply and shifts in responses to short-term C and N amendments further demonstrate the coupling of plant production and heterotrophic microbes (Fig. 4). Specifically, while addition of both C and N individually stimulated denitrification in the low N treatments and unvegetated controls, C additions alone did not stimulate additional denitrification in the two highest-N treatments. In addition, denitrification rates in the +NO_3^- and +C + NO_3^- treatments converged with increasing long-term N inputs (and thus with plant biomass). These results agree with other studies showing alleviation of C limitation and stimulation of denitrification by vegetation in sandy, organic poor aquatic environments (Weisner et al. 1994; Zhu and Sikora 1995; Schade et al. 2001; Hume et al. 2002). However, none of these studies examined these interactions along N concentration gradients.

Alternative states and interactions between biogeochemical and biogeomorphic processes

The persistence of wetlands in desert streams depends on the density-dependent stabilization of channel sediments by herbaceous vegetation (Heffernan 2008). The strong responses observed in this study therefore suggest that nutrient availability may influence the establishment and persistence of this alternative stable state. Ciénegas within Sycamore Creek are preferentially found in constrained canyons characterized by permanent surface water (Heffernan et al. 2008); this perennial flow is typically sustained by upwelling of deep alluvial flowpaths that are also enriched in NO_3^- (Dent and Grimm 1999). This positive covariation between the two resources most likely to limit plant establishment not only has implications for the distribution of ciénegas within drainage networks, but also confounds inference about the relative importance of nitrogen availability as a determinant of that distribution. While surface water is clearly a necessary and probably the primary determinant of ciénega persistence, the results of this study suggest that ciénega restoration efforts may be improved by fertilization of establishing vegetation in concert with hydrologic restoration and reductions in grazing pressure.

Many alternative stable states and other self-organized behavior (e.g., regular patterning) arise from the interaction of biogeochemical feedbacks that amplify the availability of nutrients and hydrogeomorphic feedbacks that influence their accumulation and redistribution. In shallow lakes, elevated nutrient loading leads to increased phytoplankton production and ultimately to hypolimnetic anoxia that mobilizes phosphorus (P). This biogeochemical feedback is magnified by a biogeomorphic feedback in which loss of macrophytes due to reduced water clarity permits more frequent re-suspension of benthic sediments (Horppila and Nurminen 2003). Coupling of biogeochemical and biogeomorphic feedbacks also influences stable state transitions in soft-bottom estuaries and oceans (Eriksson et al. 2010), riverine and wetland tree islands (Francis et al. 2009), and arid terrestrial landscapes (Ludwig et al. 2000). In ciénegas, the coupling of denitrification to plant growth, as observed in this study, would tend to produce antagonistic rather than synergistic interactions between biogeomorphic feedbacks. In other words, increased denitrification would tend to reduce plant growth, locally or downstream, and thus reduce the resilience of ciénegas to floods; however, other feedbacks on N supply may act in ways that are more favorable to ciénega establishment and persistence.

The responses of vegetation and denitrification to N inputs in this study correspond closely to the model developed by Schade and Lewis (2006) to describe the effects of nutrient supply on the growth, stoichiometry, and root:shoot allocation of *Bacharis salicifolia* on desert stream gravel bars and resulting responses of denitrification and N export. In that model, greater N inputs via subsurface flow led to increased initial productivity, thereby elevating denitrification and total N removal during the period of biomass accumulation, as was observed in our experimental system. If such responses were persistent, then denitrification might act as a negative feedback on ciénega establishment via reductions in available N within channel sediments. However, over the long term, physiological responses of vegetation may counteract these effects. In the same model (Schade and Lewis 2006), abundant N led to reduced belowground allocation and therefore denitrification, and increased foliar nutrient content led to higher quality litter and greater availability of soil N (Schade and Lewis 2006). Feedbacks on denitrification are thought to be responsible for alternative stable states in dune slack wetlands (Adema et al. 2005), and litter quality feedbacks produce alternative stable states in theoretical models of terrestrial systems (Manzoni and Porporato 2007), although empirical studies suggest that such feedbacks are generally weak (Knops et al. 2002; Ehrenfeld et al. 2005).

Biogeomorphic feedbacks that facilitate ciénega formation also have implications for the long-term dynamics of OM and N accumulation in these ecosystems. Fine sediments and associated OM accumulate quickly and preferentially in vegetated patches of desert streams (Heffernan et al. 2008). Trapping of particulate nutrients is thought to be one feedback promoting self-organized patterning of arid-land vegetation (Ludwig et al. 2000), and similar processes may occur in ciénegas. Rapid decomposition of ciénega vegetation (Norlin 2006) and strong correlations between OM content and fine sediment abundance in ciénega and gravelbed sediments (Heffernan et al. 2008) suggest that retention of sediments during floods, rather than accumulation of locally produced detritus, is the predominant mechanism of OM accrual in these systems. Deposition of OM may provide an additional source of N, potentially promoting further plant growth and biogeomorphic stability.

Vegetation establishment in ciénegas influences the N cycle in desert streams via both direct and indirect mechanisms whose net effect on N availability remains unclear. For example, the results of this study indicate a strong effect of vegetation on OM supply and thus denitrification potential; however, fine sediments that accumulate in ciénegas also tend to reduce exchange between the surface stream and hyporheic sediments (Heffernan et al. 2008; Predick and Stanley 2010). Given the strong effects of N on vegetation growth, the cumulative influence of these and other effects of vegetation on N dynamics has important implications for the resilience of ciénegas, and should be the focus of future research.

Plant–microbe interactions and the fate of N inputs

The negative relationship between nutrient inputs and nutrient export observed in our experiment reflects the large and rapid response of vegetation to variation in N supply and the coupled effect of increased production on N removal via denitrification. The extremity of the response reflects several features of our experimental system: first, because of minimal internal storage, nutrient inputs represented an overwhelming proportion of available N. Second, the combination of low initial biomass, abundant light and P, and warm temperatures permitted large, rapid, and compounding increases in autotrophic N demand under high N conditions. Third, hydrologic transport through our experimental system was slow and flowpaths were entirely accessible to plants. Finally, heterotrophic denitrifiers were tightly coupled to plant production, and thus N supply, due to minimal internal OM pools and large variation in production. On the basis of these characteristics, our experimental system may be viewed as an end-member in terms of potential responsiveness of nutrient demand to supply.

More muted versions of negative relationships between N input and output are observed in at least some ecosystem-scale studies. For example, N export from northern temperate forests increases minimally in response to small increases in N deposition above background, but increases rapidly beyond some threshold of inputs (9 kg N ha^{-1} year^{-1} in European temperate forests; Dise and Wright 1995). In other words, nutrient removal or retention efficiency increases initially as demand keeps pace with supply. Reflecting the relative responsiveness of biotic demand as a function of forest age, young secondary forests export less N when inputs increase than do older forests (Bredemeier et al. 1998). If the results of this study are more broadly applicable, then the threshold input level at which forests exhibit increased export may be influenced by the extent to which denitrification is coupled to primary production; to our knowledge no studies have addressed this coupled role of assimilatory and dissimilatory processes in shaping export responses to increased nutrient inputs.

The coupling of primary production and denitrification in response to N enrichment is most likely to occur in nutrient- and organic-poor environments, where N demand by both autotrophs and heterotrophs will should respond strongly and positively to variation in inputs (at the appropriate time scales). As a result, responses of nutrient export to variation in inputs should be muted relative to systems where nutrient limitation is less severe and heterotrophs are less dependent on recently fixed OM. Moreover, N limitation (primary or secondary) of autotrophs is a widespread feature of both terrestrial and aquatic systems (Elser et al. 2007), and limitation of heterotrophs by labile C availability occurs not only in OM-poor oceans (e.g., Cherrier and Bauer 2004), and terrestrial drylands (Sponseller 2007), but also in relatively OM rich temperate forest soils (e.g., Landi et al. 2006; Högberg et al. 2001). In response to increased N inputs, these linked dependencies may lead to outcomes other than increased denitrification depending on environmental conditions and plant and microbial community structure. Nonetheless, the interrelationships that couple plant and microbial responses to variation in N inputs are a common feature of ecosystems, with potentially important implications for the mechanisms and magnitude of N removal and export from ecosystems and their relation to inputs.

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