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THE INFLUENCE OF A RIPARIAN SHRUB ON NITROGEN CYCLING IN A SONORAN DESERT STREAM

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Abstract. Riparian zones often act as nutrient filters, removing NO₃ from water flowing through riparian soils. The role of vegetation in NO₃ retention remains unclear and may be direct (uptake) or indirect (stimulation of microbial activity). We studied the riparian shrub *Baccharis salicifolia* (seepwillow) in Sycamore Creek (Arizona, USA), to determine (1) if sites colonized by seepwillow were sinks for NO₃, and (2) the mechanism by which seepwillow causes NO₃ retention. Subsurface water was sampled along flowpaths from an uncolonized gravel bar through seepwillow sites at several depths and on several gravel bars. NO₃ concentration was significantly lower in seepwillow sites than in uncolonized sites, at least to 20 cm below the water table. Predictions of three hypotheses were tested to explain NO₃ losses: (H1) by plant uptake, (H2) by stimulation of denitrification by seepwillow, and (H3) a prior condition unrelated to seepwillow. Six experiments were used to test these hypotheses. Transplant experiments, plant size relationships, and root distribution experiments all demonstrated the importance of seepwillow (rejection of H3). Other tests involving removal of aboveground biomass, denitrification measures, and mass balance calculations showed a predominance of denitrification over uptake (rejection of H1). We conclude that the main effect of seepwillow is to produce organic matter creating conditions favorable to denitrification and a loss of NO₃ from subsurface water. Since denitrification is a permanent loss of N to the atmosphere, and uptake only temporarily retains N, the interaction between plants and microbes has important implications for the maintenance of water quality in streams and downstream reservoirs.

Key words: *Baccharis salicifolia*; denitrification; desert stream; N cycling; NO₃ retention; rhizosphere; riparian zone; seepwillow; Sonoran Desert, Arizona (USA).

INTRODUCTION

Riparian zones are known to act as nutrient filters, removing nutrients, particularly nitrogen, from water flowing through riparian soils. In agricultural watersheds, riparian buffer strips maintained along stream channels have proven useful in reducing nitrogen concentrations in agricultural runoff and thereby improving water quality (Peterjohn and Correll 1984, Hill 1996, Lowrance 1998). The mechanism by which riparian zones remove nitrogen has been difficult to establish because these ecosystems are not easily manipulated experimentally (Nelson et al. 1995, Hill 1996, Jacinthe et al. 1998). In particular, the role of riparian vegetation remains unclear.

Riparian vegetation may cause the loss of N both directly, through uptake and incorporation in plant biomass, and indirectly, through the stimulation of microbial processes in the rhizosphere. As plants allocate resources to the production of root biomass, a certain proportion of this organic matter is lost to the soil in a process known as rhizodeposition (Whipps and Lynch 1985). This input of organic matter fuels microbial production, generating high rates of microbial activity in

the vicinity of roots (Barber and Lynch 1977, Whipps and Lynch 1985, 1986, Van Veen et al. 1989, Whipps 1990). It is well known that stimulation of microbes in the rhizosphere can lead to increased rates of denitrification (Woldendorp 1962, Brar 1972, Bailey 1976, Volz et al. 1976, Smith and Tiedje 1979, Bakken 1988). In densely vegetated riparian zones, high rates of denitrification can cause significant loss of nitrogen from water flowing through the rooting zone (Groffman et al. 1992, Pinay et al. 1993).

Whether the predominant effect of riparian plants is direct or indirect has important implications for N retention. Uptake temporarily retains N in biomass, which is eventually returned to other ecosystem components when the plant dies. Denitrification leads to loss of N to the atmosphere, permanently removing it from the ecosystem. The relative importance of these two mechanisms, and the conditions under which each may be important, has been difficult to establish in any particular riparian ecosystem with much certainty, in part because of the difficulty of performing experimental manipulations to separate their effects (Hill 1996). A notable exception is a study by Verchot et al. (1997) in which plant uptake was suppressed in subplots of an experimental watershed in North Carolina. Their results showed denitrification to be significantly more important than uptake in attenuation of NO₃ from groundwater. This knowledge is of critical importance

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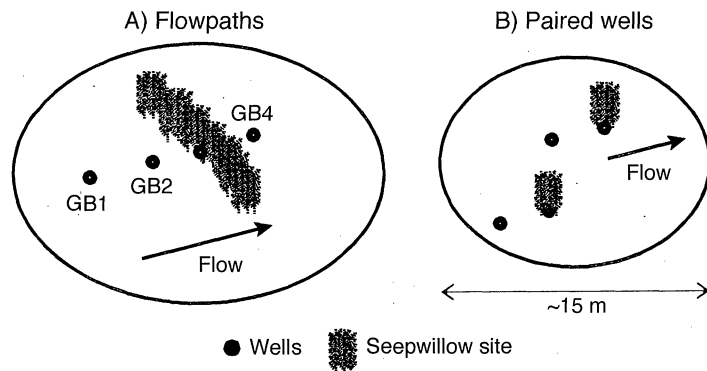


FIG. 1. Diagrams showing typical arrangement of seepwillow sites on a gravel bar: (A) an example of the arrangement of wells along a flowpath, and (B) an example of the arrangement of paired wells. The gravel bars in this study were highly variable in terms of the number of individual plants they supported. Seepwillow plants distributed in lines (A) were highly variable in area and consisted of several plants growing very close together. Individual seepwillow sites (B) are generally $<1 \text{ m}^2$ and are composed of one individual. This figure generally underestimates seepwillow cover on gravel bars in this study.

for effective management to maintain water quality in streams. One goal of this research is to increase our understanding of mechanisms of N retention by riparian vegetation.

Much of the work establishing the importance of riparian nutrient filters has been done in mesic regions of North America and Europe, particularly in agricultural watersheds. Little is known about nutrient cycling in arid land riparian zones, or their potential as nutrient filters in desert streams. Recent work on Sonoran Desert streams in Arizona suggests a strong hydrologic interaction between stream and riparian zone (Stromberg and Patten 1991, Stromberg et al. 1996), and the potential for a substantial influence of riparian vegetation on N concentrations in stream water. For instance, Marti et al. (2000) showed a prominent hydrologic connection from stream to riparian zone during floods in Sycamore Creek, a stream in Central Arizona. Furthermore, L. Dent et al. (*unpublished manuscript*) used a conservative tracer injection, also in Sycamore Creek, to show a net movement of water from stream to riparian zone during baseflow conditions. These studies show that stream water enters the riparian zone under a variety of conditions, leading to the possibility that riparian zones adjacent to Sycamore Creek may play a role in the retention of nitrogen from stream water. A second goal of this work is to investigate the influence of riparian vegetation on N cycling in desert streams.

Sycamore Creek displays several key features that lend themselves to the study of the influence of riparian plants on N cycling. Flash floods occur several times a year, removing vegetation from the active stream channel and leaving behind large gravel bars as flow subsides. When flood frequencies are low enough, seepwillow (*Baccharis salicifolia*), a woody riparian shrub, establishes on these gravel bars in discrete patches consisting of one or more individuals. Often a single gravel bar will contain several seepwillow sites growing independently of one another (Fig. 1). These seepwillow sites are generally surrounded by uncolonized gravel bar, are easy to manipulate, and present a good opportunity to study the effect of riparian plants

on N retention. In this system, we addressed the following questions: (1) Are seepwillow sites sinks for nitrogen, (2) If so, by what mechanism do they retain nitrogen?

APPROACH, HYPOTHESES, AND PREDICTIONS

Nutrient dynamics in uncolonized gravel bars

Holmes et al. (1994, 1998) worked in Sycamore Creek and other Sonoran Desert streams studying gravel bars that were free of vegetation. They measured nitrogen concentrations in water to a depth of 25 cm below the water table along flowpaths through gravel bar sediments, and demonstrated that dissolved inorganic N concentrations (mainly NO_3^-) increased along flowpaths due to mineralization of algal-derived organic N followed by nitrification. These increases generally occurred in the first few meters of flowpaths, with little further change thereafter. Eventually this water and its high NO_3^- load emerges from gravel bars and re-enters the stream. Due to these processes, NO_3^- is the dominant form of inorganic N in Sycamore Creek, and will be the focus of the rest of the paper.

Hypotheses and predictions: Are seepwillow sites sinks for NO_3^- ?

Based on previous work in other systems (summarized in Hill 1996), we hypothesized that seepwillow sites would be NO_3^- sinks, removing NO_3^- from subsurface water traveling through colonized gravel bar sites. This led us to predict lower NO_3^- concentrations in subsurface water collected from seepwillow sites relative to upflow bare sediment sites. Alternatively, seepwillow sites may not be NO_3^- sinks. If this is the case, NO_3^- will not change or will increase (Holmes et al. 1994, 1998) as water flows beneath seepwillows. If the pattern described by Holmes et al. occurs, then NO_3^- would increase and we would predict higher NO_3^- in water beneath seepwillows than in water beneath sediments without seepwillows.

Hypotheses and predictions: By what mechanism is NO_3^- retained?

We tested three hypothetical mechanisms of NO_3^- retention (here defined as hydrologic input minus hydro-

TABLE 1. Summary of hypotheses and predictions about the cause of nitrate declines in colonized sites of gravel bars in a Sonoran Desert stream.

Hypotheses	Predictions
H1: Plant causes NO ₃ decline directly through uptake and incorporation in biomass.	<p>P1a: Transplant of seepwillow will increase NO₃ in removal sites and decrease it in transplant sites.</p> <p>P1b: Larger plants will have a larger effect.</p> <p>P1c: Fine root biomass high at depths of NO₃ loss.</p> <p>P1d: Death of plant will increase NO₃.</p> <p>P1e: Retention in incremental growth of plant will explain NO₃ loss.</p>
H2: Plant causes NO ₃ declines through organic matter production leading to stimulation of microbial processes.	<p>P2a: Transplant of seepwillow will increase NO₃ in removal sites and decrease it in transplant sites.</p> <p>P2b: Larger plants will have a larger effect.</p> <p>P2c: Fine root biomass high at depths of NO₃ loss.</p> <p>P2d: Death of plant with roots intact will not effect NO₃ loss.</p> <p>P2e: Microbial process rates and bacterial numbers will be higher in colonized sediments.</p> <p>P2f: Retention in incremental growth of plant will not explain NO₃ loss.</p>
H3: Prior condition led to NO ₃ declines and to plant colonization.	<p>P3a: Removal and transplant will not cause any changes in NO₃.</p> <p>P3b: Size of plant will not explain severity of effect.</p> <p>P3c: Death of plant with root mass intact will have no effect on NO₃ loss.</p> <p>P3d: Microbial process rates and bacterial numbers will be higher in colonized sediments.</p> <p>P3e: Retention in incremental growth of plant will not explain NO₃ loss.</p>

Note: For a detailed description, see *Approach, hypotheses, and predictions*.

logic output) in seepwillow sites, with particular emphasis on the role of the plant in determining changes in NO₃ concentrations (Table 1). First, the plant may retain NO₃ directly through uptake and incorporation in biomass (H1). Alternatively, seepwillow may cause removal of NO₃ indirectly through stimulation of microbial processes, particularly denitrification (H2). This could occur through organic matter production by the plant, leading to increased microbial activity. Third, the plant may not influence NO₃ retention at all, but some prior condition led to both NO₃ declines and plant colonization of the site (H3). For instance, germination of seepwillow seedlings takes place in saturated sediments, often located along the edge of the stream as the stream dries and contracts laterally. These sediments are high in water and organic matter (due to the presence of stranded algae on the surface). These conditions may independently lead to both germination of seepwillow and high microbial activity, which then leads to NO₃ retention. In other words, a correlation between seepwillows and NO₃ decline may be spurious, not causative.

We generated several predictions based on each of these hypotheses (Table 1). Our approach was to use six independent tests, with each test generally pitting

two hypotheses against the third (Table 2). The first three tests were designed to establish whether or not the plant was responsible for NO₃ decline. Tests 4–6 were designed to determine if the mechanism of retention was microbial, or the direct result of plant uptake.

SITE DESCRIPTION

The study site was a 300-m reach in the middle section of Sycamore Creek, and consisted of several gravel bars that accounted for >75% of the active channel. During the study period, these gravel bars each supported several seepwillow sites surrounded by bare sediments. Alluvial sediments in the reach averaged >1.5 m deep, and active channel width was ~25 m. Water table elevation was variable, but was generally within 25 cm of the surface of gravel bars during the study period. Flow rates of water moving through gravel bar sediments average ~1 m/h (Holmes et al. 1994). Vegetation on gravel bars consisted almost entirely of seepwillow. Other minor species included Bermuda grass (*Cynodon dactylon*), and occasional seedlings of common riparian species, particularly mesquite (*Prosopis glandulosa*) and Gooding's willow (*Salix goodingii*). Due to severe flooding, gravel bars were relatively free of vegetation in the early 1990s. In 1995, flood mag-

TABLE 2. Summary of tests of predictions in Table 1.

Experiment	Variables measured	Hypotheses tested	Predictions tested	Result	Date
1) Transplant	[NO ₃] in shallow sub-surface water	H1 and H2 vs. H3	P1a, P2a, P3a	H1 and H2 supported, H3 not supported	Jul 1998–May 1999
2) Height vs. severity of effect	height of plant and change in [NO ₃] in wells in and out of colonized site	H1 and H2 vs. H3	P1b, P2b, P3b	H1 and H2 supported, H3 not supported	Jun 1998
3) Fine root biomass analyses	quantitative and qualitative analysis of fine root biomass	H1 and H2	P1c and P2c	H1 and H2 supported	Jun 1999
4) Removal of above ground biomass	[NO ₃] in shallow sub-surface water	H1 vs. H2 and H3	P1d, P2d, P3c	H1 not supported, H2 and H3 supported	Sep 1997
5) Sediment analyses	microbial respiration, potential denitrification and bacterial density	H2 and H3	P2e and P3d	H2 and H3 supported	Jan 1998–Jul 1998
6) Mass balance	estimated uptake, potential denitrification losses, and total NO ₃ loss	H1 vs. H2 and H3	P1e, P2f, P3e	H1 not supported, H2 and H3 supported	May–Aug 1998†

Notes: In general, tests pitted two hypotheses against the third. The first three tests were designed to test whether either plant hypothesis was supported, the second three to determine the relative importance of each plant hypothesis.

† Dates when data were collected.

nitude and frequency declined due to drought conditions, allowing vegetation heavily dominated by seepwillow to regrow. Establishment of seepwillow began in saturated sediments, with successful establishment dependent on the depth of the water table (Stromberg et al. 1996). Plants generally grew in a line of several individuals roughly parallel to the stream (Fig. 1B), maintaining this form as individual plants grew larger (eventually reaching heights >2 m).

METHODS

Changes in NO₃ concentration

We tested whether seepwillow sites were NO₃ sinks in three ways. First, paired groups of wells were sampled in seepwillow sites and upflow bare sediment sites within a single gravel bar, each member of a pair consisting of a group of wells (constructed from 2.5 cm diameter polyvinyl chloride pipe) reaching from 20 cm to 1 m below the water table. In a second gravel bar, several wells were placed and sampled along replicate flowpaths from bare sediment through seepwillow sites (Fig. 1A). Finally, an extensive survey was conducted in which 26 different seepwillow sites spread over four gravel bars were sampled along with corresponding upstream bare sediment sites (as in Fig. 1B). The survey extended over ~1 km of the stream channel.

In November 1996, six groups of wells were installed in one gravel bar, three groups in seepwillow sites paired with three groups 1 m upflow of the plants, in bare sediments. In November 1996 and February 1997, each group consisted of two wells, one installed to 20 cm and one to 1 m below the water table. In May 1997, a 60 cm deep well was added to each group. From each

well on each of the three dates, we measured dissolved oxygen (DO), NO₃, NH₄, soluble reactive phosphorus (SRP), and dissolved organic carbon (DOC). Samples for DO were collected by pumping water from each well into a plastic 250-mL bottle using a peristaltic pump. DO concentration was measured immediately in the field with an Orion 830 oxygen probe (Thermo Orion, Beverly, Massachusetts). For the rest of the analyses, triplicate water samples were collected from each well on each date and returned to the laboratory on ice for analysis. NO₃ and SRP concentrations were measured on a Bran and Leubbe TRAACS 800 autoanalyzer (Bran and Leubbe, Buffalo Grove, Illinois), NH₄ by the phenol-hypochlorite method (Solorzano 1969), and DOC on a Shimadzu TOC 5000 analyzer (Shimadzu, Columbia, Maryland) (high-temperature oxidation).

In January, May, and July 1998, we sampled three replicate flowpaths through shallow sediments, identified using dye injections as described by Holmes et al. (1994). Along each flowpath, 20 cm deep wells were installed at four locations 1 m apart (Fig. 1A). On each flowpath, sites GB1 and GB2 were bare sediment, site SW3 was a seepwillow site, and site GB4 was also bare sediment but 1 m downflow of the plant (Fig. 1A). We sampled DO, NO₃, and SRP from each well on each date, with the exception of July, when no SRP samples were collected. On each date, flowpaths through a different set of seepwillow patches were sampled, for a total of nine separate seepwillow sites over the course of the study.

In June 1998, we completed a survey of 26 seepwillow sites, in all of which plants were >150 cm tall,

distributed over several gravel bars. At each seepwillow site, we installed 20 cm deep wells immediately adjacent to each plant. Each of these wells was paired with a second well installed 1 m upflow from the plant in bare sediments (Fig. 1B). Triplicate water samples were collected from each well and analyzed for NO_3^- .

Role of the plant in NO_3^- retention

Transplant experiment (test 1).—This experiment involved transplanting individual seepwillow plants from NO_3^- sink locations to uncolonized locations within a gravel bar. The uptake and denitrification hypotheses both predicted an increase in NO_3^- at the original location and a decrease in NO_3^- at the new location. The prior condition hypothesis (H3) predicted no change at either location (Table 1). Wells were installed to 20 cm below the water table at 10 seepwillow sites and 10 bare sediment sites, within one gravel bar located in the reach described above, in June 1998. Triplicate water samples were collected before manipulation and analyzed for NO_3^- concentration on a Bran and Luebbe TRAACS 800 autoanalyzer. Five of the plants in seepwillow sites were removed with roots relatively intact and transplanted to bare sediments. The remaining 10 sites (5 colonized and 5 uncolonized) were left intact. Wells at all 20 sites were sampled for NO_3^- periodically between July 1998 and May 1999.

Plant height vs. severity of effect (test 2).—In July and August of 1998, 16 colonized sites were sampled to determine the relationship between the extent of NO_3^- reduction and the size of the plant. The uptake and denitrification hypotheses both predicted that larger plants would have a larger effect, while the prior condition hypothesis predicted no effect of the size of the plant on NO_3^- retention (Table 1). At each site, we measured the height of the plant and the amount of NO_3^- lost from water traveling through the patch. Wells were installed both in the seepwillow site and in bare sediments roughly 1 m upflow of the patch on the same flowpath (Fig. 1B). The difference in NO_3^- concentration was used to estimate the extent of NO_3^- reduction in the seepwillow site. The height of seepwillow plants is significantly related to both total plant biomass and root biomass using height–biomass regressions (J. D. Schade, unpublished data; $r^2 = 0.853$).

Fine root biomass analyses (test 3).—In the summer of 1999, the roots of seven plants, ranging from 56 to 254 cm in height, were excavated to determine rooting depths of plants of various sizes. This was done both quantitatively and qualitatively. Both uptake and denitrification predict, and, in fact, require, significant root mass at the depth of observed NO_3^- retention (Table 1). Total root mass in unsaturated sediments (20–25 cm deep from gravel bar surface to top of water table) and saturated sediments down to 20 cm below the water table was quantified by collecting all coarse (>1 mm) roots in a sector 0.4 m² in area on the upflow side of the plant. One sector was sampled near each plant.

Subsamples of sediments were collected and returned to the laboratory for quantification of fine (<1 mm) root biomass. Qualitative analysis was done by careful excavation of intact roots in the field for observation of rooting depth and architecture.

Removal of aboveground biomass (test 4).—In this experiment, aboveground biomass of several seepwillow plants was removed to kill the plant and eliminate plant uptake. Both the denitrification and prior condition hypotheses predicted no change in NO_3^- concentration, while the uptake hypothesis predicted an increase in NO_3^- concentration in cut seepwillow sites (Table 1). In the fall of 1997, we selected 10 seepwillow sites and 5 bare sediment sites of a large gravel bar in Sycamore Creek. At each site, we installed wells as described in the previous sections, and collected water samples to analyze for NO_3^- concentration. At 5 of the seepwillow sites, we removed all aboveground biomass but left the root mass intact. We sampled all sites ($n = 15$) for NO_3^- concentrations 2 d after the manipulation, then weekly for 30 d.

Sediment analyses (test 5).—Both the denitrification and prior condition hypotheses predicted higher microbial process rates and bacterial densities in sediments from seepwillow sites, relative to bare sediments (Table 1). We sampled saturated sediments from all flowpath locations described earlier (Fig. 1A) in January, May, and July 1998. Saturated sediments were collected 10–20 cm below the water table, and transported on ice to the laboratory. All analyses were completed within 72 h of collection. Subsamples were collected, dried, weighed, and combusted at 550°C for determination of ash-free dry mass (AFDM). Subsamples were also taken for measurement of bacterial densities using DAPI staining (Schallenberg et al. 1989), and for experiments to measure respiration and potential denitrification rates.

Respiration was measured in the laboratory at room temperature (25°C) as uptake of dissolved oxygen using respiration chambers filled with sediments and stream water. Chambers were constructed of clear cylindrical plastic 32 cm long, with a 4.4 cm inside diameter. Two replicate chambers were filled halfway with a known wet mass of sediments from each location, and all chambers were filled the rest of the way with stream water collected from a single location at Sycamore Creek. Each core was sealed on both ends with rubber stoppers and well mixed. After mixing, we measured initial dissolved oxygen and resealed chambers to eliminate air bubbles. All chambers were incubated for 4 h in the dark, during which time they were mixed by inverting the cores several times. After 4 h, cores were again well mixed and final DO concentrations measured. Ash-free dry mass methods described above were used to convert wet mass to AFDM.

Potential denitrification was measured using the acetylene block technique (Tiedje et al. 1989, Holmes et al. 1996), using sediments from all flowpath loca-

TABLE 3. Means (with 1 SE in parentheses) for all chemical constituents of water collected from nested sets of wells on all three dates at depths of 20 cm, 60 cm, and 1 m.

Chemical constituent	Date	20 cm		60 cm		1 m	
		Colonized	Uncolonized	Colonized	Uncolonized	Colonized	Uncolonized
NO ₃ (μg/L)	Nov	20 (4)	100* (10)	70 (12)	60 (4)
	Feb	520 (30)	660* (10)	580 (40)	590 (10)
	May	10 (0.9)	30* (1)	20 (3)	30 (3)	40 (3)	30 (4)
DO (mg/L)	Nov	4.87 (0.87)	9.22* (0.36)	8.94 (0.19)	8.98 (0.27)
	Feb	7.34 (0.53)	9.54* (0.38)	10.09 (0.27)	9.72 (0.39)
	May	2.06 (0.67)	6.58* (0.32)	3.36 (0.22)	6.27* (0.07)	4.92 (0.08)	5.19 (0.25)
SRP (μg/L)	Nov	40 (10)	60 (3)	50 (1)	60 (2)
	Feb	20 (2)	20 (2)	20 (4)	20 (3)
	May	10 (1)	20* (0.9)	10 (1)	10 (0.7)	20 (0.2)	20 (0.6)
NH ₄ (μg/L)	Nov	4 (2)	4 (1)	2 (1)	5 (1)
	Feb	9 (5)	4 (2)	5 (2)	4 (2)
	May	0.8 (2)	3 (3)	4 (1)	0.8 (0.3)	7 ± 3	2 ± 1
DOC (mg/L)	Nov	4.67 (0.10)	4.68 (0.26)	4.61 (0.21)	4.60 (0.19)
	Feb	3.62 (0.08)	3.90 (0.10)	3.56 (0.11)	3.90 (0.10)
	May	6.71 (0.76)	5.28 (1.02)	5.98 (0.35)	7.14 (1.14)	5.92 (0.51)	5.16 (0.37)

Notes: Asterisks denote significant differences between colonized and uncolonized sites ($P < 0.05$). Numbers are averages of three wells at each depth on each date.

tions on each date. Three replicate 150-mL serum bottles were filled with 150–200 g wet mass of sediments and amended with carbon and nitrogen by adding 40 mL of a solution containing 200 mg NO₃-N/L (as KNO₃) and 3.3 g C/L (as dextrose). Bottles were made anoxic by flushing with N₂ gas. These conditions allow for measurement of the maximum denitrification rate under close to ideal conditions. Acetylene (10 mL) was added to each bottle, and bottles were shaken to equilibrate acetylene between aqueous and vapor phases. After 15 min, pressure inside bottles was equilibrated with the atmosphere by briefly piercing bottle septa with a hollow needle. Bottles were incubated in the dark for 4 h. Initial and final headspace gas samples were collected in 3-mL evacuated containers. Denitrification rate was calculated from the difference between final and initial headspace nitrous oxide content (corrected for nitrous oxide dissolved in the aqueous phase, Bunsen coefficient = 0.54). Wet masses were converted into AFDM as above.

Mass balance (test 6).—In our mass balance approach, we estimated total uptake of nitrogen by plants, maximum loss of NO₃ due to denitrification, and total mass of NO₃ lost in seepwillow sites. The denitrification and prior condition hypotheses predicted that plant uptake would not explain NO₃ loss, while the uptake hypothesis predicted that it would (Table 1). Total uptake was estimated by measuring change in height of five individual seepwillow plants for 6 wk in the summer of 1998 and using a height–biomass regression to estimate net biomass increment, which was multiplied by measurements of %N of plant tissues to estimate total uptake. Potential denitrification rates were used to generate an estimate of maximum loss from denitrification by multiplying denitrification rate (N loss/grams dry mass of sediment) by sediment bulk density (mass of sediment (grams)/volume of sediment) and volume of the zone of influence of the plant. Total loss

of N in colonized sites was estimated by multiplying change in concentration of NO₃ (milligrams per liter) by the volume of water passing through the colonized site, which also relies on an estimate of the size of the zone of influence of the plant. Calculated uptake and denitrification losses were compared to estimates of total loss of NO₃.

We estimated the size of the zone of influence of the plant by sampling two small-scale transects of mini-piezometers through a seepwillow site, one parallel to the flow of water, the other perpendicular. The two transects crossed in the middle of the seepwillow site. The parallel transect consisted of piezometers placed 100, 75, 50, 30, 20, 10, and 0 cm upflow of the center of the plant, and 50, 100, and 150 cm downflow of the center of plant. The perpendicular transect consisted of piezometers 0, 20, 40, and 60 cm from the center of the plant both right and left of the parallel transect. All piezometers were sampled on the same day for NO₃, and changes in NO₃ concentration were used to determine the boundaries of the zone of influence of the plant.

RESULTS

Changes in NO₃ concentration

In groups of wells at multiple depths, NO₃ concentrations were significantly lower in seepwillow sites at 20 cm depths on all dates (Table 3). Differences in 1-m wells were not significant on any date, nor did concentrations in 60-cm wells differ between colonized and uncolonized pairs in May. DO concentrations in 20-cm wells were also significantly lower in seepwillow sites on all three dates, and in 60-cm wells in May (Table 3). DO concentrations in wells at 1 m depth were never significantly different. No other chemical constituents showed any significant differences, except SRP at 20 cm depth in May (Table 3).

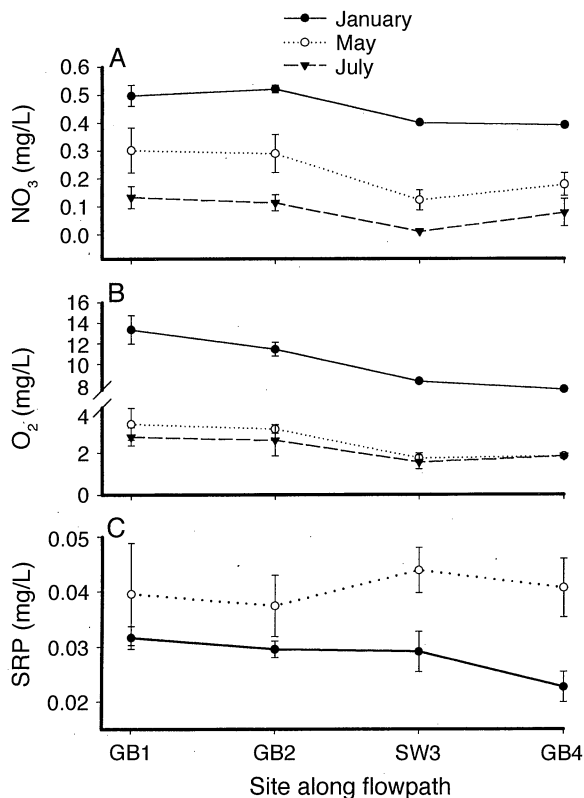


FIG. 2. (A) NO_3 , (B) dissolved oxygen, and (C) soluble reactive phosphorus (SRP) concentrations from flowpaths in January, May, and July. GB1, GB2, and GB4 are bare sediment, SW3 is the seepwillow site, corresponding to Fig. 1A. On all dates, the SW3 site is significantly lower in NO_3 and dissolved oxygen than GB1 and GB2. GB4 is never different from SW3. Differences in SRP are never significant. Symbols represent means of three flowpaths; error bars represent ± 1 SE.

In the analysis of flowpaths, we found significantly lower NO_3 concentrations in samples from SW3 of flowpaths (seepwillow sites) compared to sites GB1 and GB2 (Fig. 2A; ANOVA followed by Tukey's test, $P < 0.05$) on all dates. The proportion of NO_3 lost between GB2 and SW3 was 23%, 59%, and 91% in January, May, and July, respectively. GB4, bare sediments downstream of the plant, was never significantly different from SW3 (Fig. 2A). Declines in DO concentration between GB2 and SW3 were significant in January and May, but not in July (Fig. 2B; ANOVA followed by Tukey's test). Differences in DO concentration between GB1 and GB2 or between SW3 and GB4 were not significant (Fig. 2B). SRP did not vary significantly along flowpaths (Fig. 2C).

Because we did not observe any consistent differences between seepwillow sites and bare sediments for nutrients besides NO_3 and DO, and N often limits production in Sycamore Creek (Grimm and Fisher 1986), we sampled only NO_3 in the June 1998 survey. In all 26 pairs of wells, NO_3 concentrations were lower in

samples from wells in seepwillow sites than those in bare sediments. When all 26 sites were pooled, seepwillow sites were significantly lower in NO_3 concentration than bare sediment sites (0.06 vs. 0.17 mg NO_3/L , paired t test, $P < 0.05$), and on average 65% of NO_3 was lost between seepwillow sites and bare sediments.

Role of the plant in NO_3 retention

Transplant experiment (test 1).— NO_3 concentrations in removal locations were not significantly different from those in colonized locations before the manipulation, day 0 (Fig. 3B). As predicted by both plant-associated hypotheses (H1 and H2), NO_3 increased rapidly after removal of the plant and, by day 21, NO_3 concentrations at removal locations were indistinguishable from those at uncolonized sites and remained so for the rest of the experiment (Fig. 3A). On days 21, 39, and 256, NO_3 concentrations at removal sites were significantly higher than colonized sites.

Concentrations of NO_3 in transplant locations were not significantly different from concentrations in uncolonized locations before manipulation (Fig. 3B). It took 256 d for NO_3 concentrations at transplant locations to drop to levels significantly lower than those at

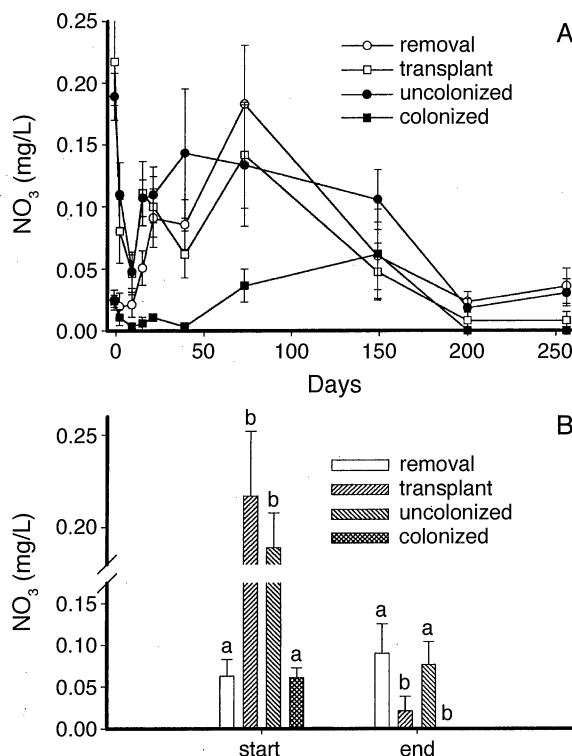


FIG. 3. Changes in NO_3 concentrations for (A) the entire course of the transplant experiment (means ± 1 SE) and (B) day 0 before the manipulation and day 256 at the end of the experiment (means ± 1 SE). Explanation of treatments: removal = previously seepwillow sites; transplant = sites that received plants from removal sites; uncolonized = unmanipulated, bare sediment sites; colonized = unmanipulated, seepwillow sites. In all cases, $n = 5$ replicates for each treatment.

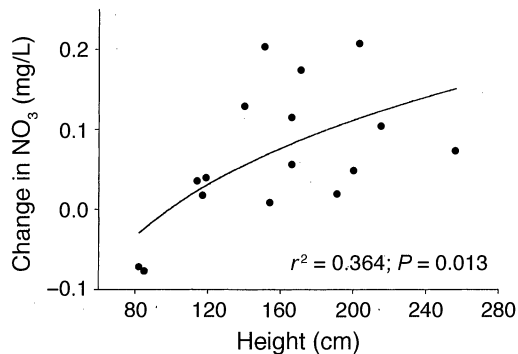


FIG. 4. The relationship between the height of seepwillow plants and the severity of NO_3 reduction, measured as the change in concentration in samples collected from wells upstream of and immediately adjacent to each plant (see Fig. 1). The regression line is from linear regression using data up to a height of 170 cm. The relationship breaks down for taller plants.

uncolonized and removal locations. By the end of the experiment, NO_3 concentrations at removal locations were not significantly different from uncolonized sites, and were significantly higher than at colonized sites (Fig. 3B). At transplant locations, NO_3 concentrations were not significantly different from colonized sites, and were significantly lower than at uncolonized sites (Fig. 3B).

Plant height vs. severity of effect (test 2).—In the survey of 16 colonized sites, the difference in NO_3 concentration between wells in seepwillow sites vs. wells 1 m upstream in bare sediments (i.e., the magnitude of NO_3 decline) was positively related to the height of seepwillow plants (Fig. 4). Regression analysis yielded a significant positive relationship between height and NO_3 reduction ($r^2 = 0.614$, $P = 0.004$) up to a height of 170 cm. Above 170 cm, we found no relationship between height and severity of effect (Fig. 4).

Fine root biomass analyses (test 3).—Plant excavations showed considerable fine root biomass in both unsaturated sediments and saturated sediments down to 20 cm below the water table, particularly for larger plants (Table 4). Fine root mass was significantly related to the height of the plant in both saturated ($r^2 = 0.70$, $P = 0.02$) and unsaturated sediments ($r^2 = 0.76$, $P = 0.01$), with large plants supporting higher fine root mass at both depths. Coarse root mass was only significantly related to height in unsaturated sediments ($r^2 = 0.62$, $P = 0.04$). Qualitative observations showed most coarse root mass in unsaturated sediments or near the surface of the saturated zone (roughly the upper 10 cm), with roots tending to spread horizontally rather than downward.

Removal of aboveground biomass (test 4).—Removing aboveground biomass was designed to eliminate uptake by killing the plant while leaving the root mass

TABLE 4. Root mass of *Baccharis salicifolia* plants from unsaturated sediments above the water table (shallow) and the top 20 cm of the saturated zone (deep).

Height (cm)	Shallow (g/m^3)		Deep (g/m^2)	
	Fine (<1 mm)	Coarse (>1 mm)	Fine (<1 mm)	Coarse (>1 mm)
56	90	0	0	0
84	60	2	40	0
137	70	0	60	0
167	300	34	490	10
227	360	170	270	20
233	550	530	690	440
254	840	830	550	1030

intact. Plants did not resprout at any time during the experiment, indicating that the plants were dead and no nutrient uptake was occurring. Cut and uncut treatments were never significantly different, and both were always lower in NO_3 than bare sediment sites (Fig. 5). Removing aboveground biomass had no effect on NO_3 reductions over the course of this experiment.

Sediment analyses (test 5).—Respiration rates were significantly higher in sediments from seepwillow sites (SW3) than bare sediments for all flowpaths on all dates (Fig. 6A; ANOVA followed by Tukey's test, $P < 0.05$). Respiration rates of sediments from sites GB1, GB2, and GB4 were never significantly different from each other; thus the plant's effect on respiration was highly localized. Results from potential denitrification experiments were similar to respiration experiments (Fig. 6B). Again, seepwillow sites showed significantly higher rates of potential denitrification than all bare sediment sites in May and July, and all but GB4 in January (Fig. 6B). Furthermore, potential denitrification rates were significantly higher in colonized sites sampled in May and July than those sampled in January.

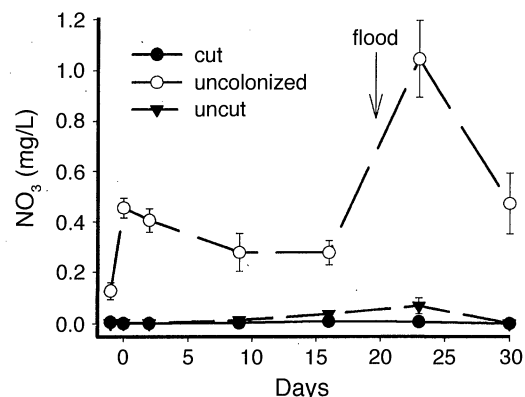


FIG. 5. NO_3 concentrations during the aboveground biomass removal experiment. "Cut" indicates plants from which aboveground biomass was removed. Symbols represent means of five replicates for each treatment; error bars indicate ± 1 SE.

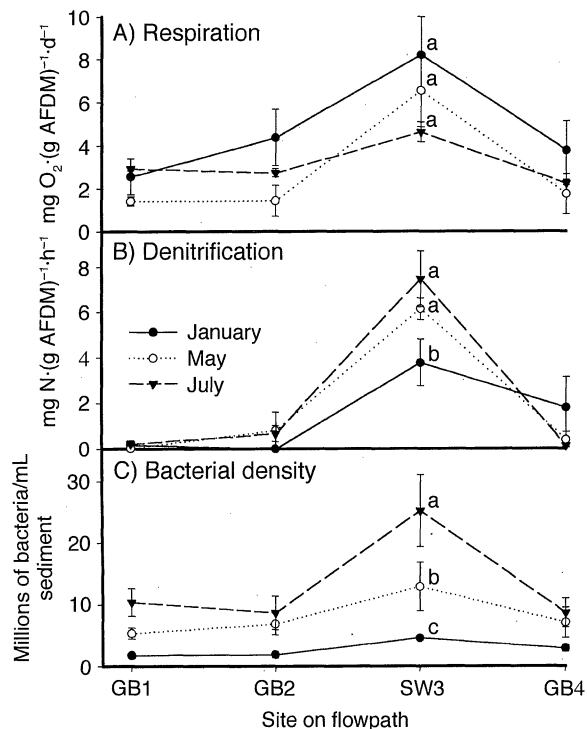


FIG. 6. Results from analyses of sediments collected from three replicate flowpaths on each of the three dates (means \pm 1 SE): (A) respiration rates, (B) potential denitrification rates, and (C) bacterial densities. SW3 is the seepwillow site, as in Fig. 1A. For all three measures, values for SW3 are significantly greater than those for GB1, GB2, and GB4, except in the cases of GB4 for denitrification in January and bacterial densities in May. Letters indicate significant differences for SW3 across dates.

Bacterial densities were consistently higher in seepwillow sites (SW3) of flowpaths (Fig. 6C) than GB 1, 2, and 4. Differences were significant in January and July (ANOVA, Tukey's test, $P < 0.05$), but not in May ($P = 0.08$). This effect was also localized. Bacterial densities were highest in seepwillow sites sampled in July, and higher in May than January, consistent with results for potential denitrification.

Mass balance (test 6).—Denitrification could potentially account for all of the NO₃ loss, whereas uptake accounted for only a very small proportion of NO₃ loss. Total N uptake by plants was estimated at 0.20 mg·m⁻²·h⁻¹, maximum loss due to denitrification at 1200 mg·m⁻²·h⁻¹, and total loss of NO₃ in seepwillow sites averaged 11 mg·m⁻²·h⁻¹. Plant uptake accounted for 2% of total NO₃ removed, while maximum denitrification (rates after amendment with NO₃ and dextrose) losses could account for 110× the total loss. Denitrification could account for all of observed NO₃ loss if rates were only 1% of the maximum rate. Most of the NO₃ loss in the small-scale transect parallel to the flow of water occurred between 10 and 20 cm from the center of the plant, and the zone of low NO₃ extended roughly 50 cm past the center of the plant (Fig.

7A). The affected zone from the perpendicular transect extended roughly 40 cm to either side of the center of the plant, and was more symmetrical (Fig. 7B). We used this information to calculate the size of the zone of influence, assuming 20 cm depth, to be ~0.1 m³, which was used to calculate total losses from denitrification and total loss of NO₃ in colonized locations.

DISCUSSION

Holmes et al. (1994) showed that, in the absence of vegetation, nutrient concentrations increased in water moving along subsurface flowpaths. We hypothesized that after colonization, seepwillow sites would be NO₃ sinks, leading us to predict that NO₃ concentrations should be lower in water from seepwillow sites. This hypothesis was supported for shallow wells; NO₃ and DO concentrations at shallow depths were lower in all seepwillow sites than in upstream bare sediment sites. However, deeper wells showed no significant differences between sites. Furthermore, NH₄, SRP, and DOC were not consistently different between sites, and thus we conclude that they are unaffected by the plants. The change in NO₃ concentration between February and May in both colonized and uncolonized sites (Table 3) is due to large changes in surface stream NO₃ concentration. In general, streamwater nutrient concentrations are higher in the spring than the summer (Grimm 1987). The hydrologic linkage between gravel bars and the stream is very strong in Sycamore Creek. Essentially

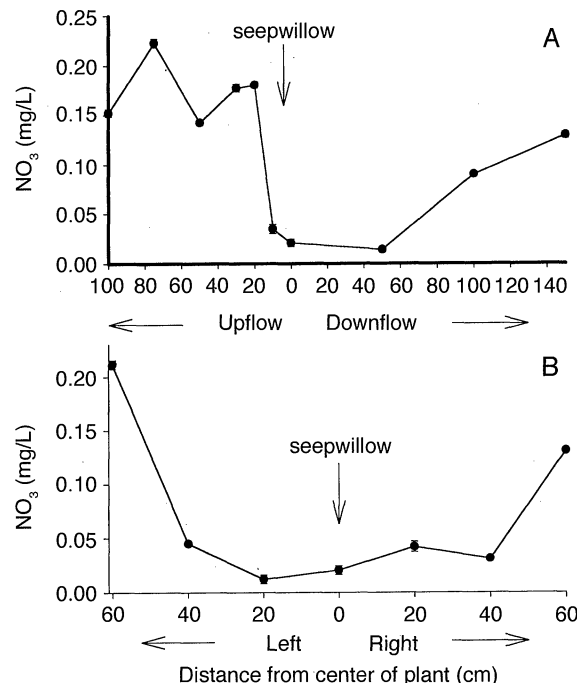


FIG. 7. Small-scale changes in NO₃ concentrations in (A) parallel and (B) perpendicular transects through a seepwillow site. Transects cross in the center of the seepwillow plant. Symbols are means of triplicate samples.

all interstitial water originates in the stream during baseflow (C. L. Dent, J. W. Edmonds, J. C. Henry, N. B. Grimm, E. Marti, and J. R. Welter, *unpublished manuscript*), and seasonal changes in nutrient concentrations in gravel bars are generally associated with changes in surface water.

Detailed analysis of water chemistry variables described above, as well as sediment analyses, were restricted to one or two gravel bars. We recognize that this limits our ability to generalize our results. However, since in this work and in preliminary work (J. D. Schade, *unpublished data*), NO_3 was the only chemical constituent of the water that varied significantly between colonized and uncolonized sites, we restricted our extensive sampling to analysis of changes in NO_3 concentration as an indicator of a plant effect. Including seepwillow sites sampled in the survey and all other experiments, we sampled >50 seepwillow sites spread over six gravel bars covering ~1 km of the stream. We found lower NO_3 in seepwillow at >90% of the sites sampled, suggesting that the effects on NO_3 found in the intensive work described above are likely to apply to most seepwillow sites on most gravel bars (Fig. 4).

Our observations on rooting depths suggest that the great majority of roots are shallow, restricted to either unsaturated sediments or the top 20 cm of the saturated zone (Table 4). Shallower water is flowing through the rooting zone, where NO_3 is removed primarily by denitrification and DO is consumed by microbial activity, fueled by root production by the plants. Deeper water flows below the rooting zone, and is unaffected by plant activities. Other recent studies of riparian zones also emphasize the importance of the depth of the water table in determining the degree of nutrient filtration (Groffman et al. 1992, Simmons et al. 1992, Hill 1996, Lowrance 1998). In these studies, riparian zones that acted as effective nutrient filters featured shallow bedrock or some other geologic feature underlying riparian soils, which forced water to flow laterally through the rooting zone in its passage through riparian soils. Furthermore, in our study, NO_3 concentrations often remained low after water exited seepwillow sites (GB4 along flowpaths, Fig. 2A), indicating that seepwillow may reduce the input of available N from gravel bar to stream, possibly leading to reduced algal production in the surface stream.

The first three tests in Table 2 and described above were designed to determine whether the plant was responsible for NO_3 decline, either directly (plant uptake, H1) or indirectly (denitrification, H2). In tests 1 and 2, predictions of these hypotheses were opposed to predictions of the prior condition hypothesis (H3, Table 1). In the transplant experiment (test 1), NO_3 concentrations increased shortly after plants were removed, suggesting that removal of the root mass was effective and that any root material left behind quickly decomposed. Transplant sites did not become NO_3 sinks until the next spring, suggesting significant time was re-

quired to reestablish an active rhizosphere. The fact that transplant sites did eventually become NO_3 sinks, combined with the loss of this effect at removal sites, is strong support for the two plant-related hypotheses (uptake (H1) and denitrification (H2)), and strong evidence against the prior condition hypothesis (H3). One caution in interpretation of this experiment comes from the lack of a sufficient control for the comparison between removal and colonized sites. These sites differed in both the presence or absence of a plant, and also in the level of disturbance of the site. However, the lack of a significant difference between removal sites and uncolonized sites at the end of the experiment leads to the conclusion that disturbance of removal sites had no lasting effect. This leads us to attribute the difference between removal and colonized sites at the end of the experiment (Fig. 1A) to the loss of an effect of the plant, not to any effect of disturbance.

Further support comes from the significant relationship between the height of the plant and the extent of effect on NO_3 concentrations (test 2 and Fig. 4), indicating that the effect strengthens as the plant grows and produces a larger root mass, up to a threshold plant size of ~170 cm in height. The presence of a large biomass of fine roots below the water table (test 3 and Table 4) is a necessary requirement for the plausibility of either plant-associated hypothesis and supports them both. The results from tests 1–3 support both plant hypotheses, while tests 1 and 2 also lead to the rejection of a prior condition (H3 and Table 2). The evidence of these three tests leads to the conclusion that the activity of the plants is responsible for reductions in NO_3 concentrations in seepwillow sites.

But which plant-related mechanism is most important? Tests 4–6 were designed to determine whether NO_3 concentrations were reduced through uptake by the plant (H1) or by microbial activities (i.e., denitrification (H2) and a prior condition (H3)). In the biomass removal experiment (test 4 and Fig. 5), NO_3 concentrations were unaffected by the removal of all aboveground biomass from seepwillow plants. Since the plants never sprouted, we assumed that uptake was no longer occurring. The elimination of uptake had no effect on NO_3 concentrations, at least over the short term, leading to the conclusion that uptake was not causing NO_3 declines (rejection of uptake (H1)). These results are similar to results from a study of an experimental watershed in North Carolina, in which the suppression of plant uptake had no effect on NO_3 removal from groundwater flowing through a forested riparian zone (Verchot et al. 1997). Our mass balance calculations (test 6) estimate that uptake explains 2% of observed NO_3 loss, while denitrification could easily have removed all of the missing NO_3 . This also leads to rejection of uptake (H1), and the conclusion that uptake is not responsible for NO_3 retention. Sediment experiments (test 5 and Fig. 6) show higher rates of respiration and potential denitrification, and higher bacterial densities in sediments from seepwillow sites,

supporting denitrification (H2) and prior condition (H3) hypotheses, that microbial processes are responsible for loss of NO_3 . Considered by themselves, tests 4–6 support hypotheses related to microbial processes, while tests 4 and 6 also lead to the rejection of plant uptake (H1).

We conclude from the above analysis that the most likely mechanism of NO_3 retention in seepwillow-colonized gravel bars is denitrification fueled by organic matter production by the plant (H2), either through the death of fine roots or the release of dissolved organic carbon (DOC) from actively growing roots. We base this conclusion on a consideration of all six tests. Although all individual tests supported two of the three hypotheses, we can use the entire battery of six tests to eliminate plant uptake (H1) and a prior condition (H3). The first three tests lead to the rejection of a prior condition (H3), while the second three tests lead us to reject plant uptake (H1). We are left with denitrification (H2), the only hypothesis of the three supported by all six tests. In particular, eliminating plant uptake through the removal of aboveground biomass, with subsequent death of the plant, had no effect on NO_3 losses, as long as the root mass was left intact. Comparing these results with the transplant experiment, in which the root mass was also removed, we conclude that an intact root mass is both necessary and sufficient for NO_3 retention to occur, and a living plant actively taking up nitrogen is not necessary, at least on a short time scale. Since the transplanting process destroys much of the fine root mass, the long time lag (256 d) before transplant locations became NO_3 sinks probably reflects the time required for accumulation of a sufficient fine root mass. The main influence of seepwillow, then, is to continue to produce root material or exude organic matter from roots, which maintains an active microbial community in the rhizosphere of the plant, leading to removal of NO_3 from subsurface water.

The importance of denitrification is somewhat surprising in the generally well-oxygenated sediments that exist in these gravel bars, even under the plants. However, previous work in Sycamore Creek has demonstrated that denitrification rates in uncolonized sediments are highest at the interface between stream and gravel bar, where oxygen rates are also highest (8–10 mg/L; Holmes et al. 1996). The most likely explanation of this result is the occurrence of anoxic microsites generated by the input of algal-derived organic matter from the stream. Samples for DO measurements in both that study and in this one were collected at too large a scale to detect these microsites. Furthermore, studies of microbial processes in the rhizosphere of plants show that microbial activity is tightly linked to the presence of plant roots, and can change over a very small spatial scale (Barber and Lynch 1977, Whipps 1990). Our results are consistent with a tight linkage between microbes and seepwillow roots leading to anoxic zones around roots at a very small spatial scale

(millimeters to centimeters), resulting in higher rates of denitrification than expected based on bulk DO measurements.

It also seems surprising at first glance that we found no difference in DOC concentrations between treatments, even though exudation of DOC is one possible source of material stimulating microbial activity. However, the tight linkage that exists between microbes and plant roots leads us to hypothesize that any DOC lost from the plant is rapidly used by microbes in the rhizosphere and does not lead to any changes in concentration in bulk water samples.

Our mass balance calculations showed quite clearly that plant uptake cannot account for our observed loss of NO_3 , while denitrification could. Two factors must be considered, however, in evaluating this conclusion. First, using measurement of height in combination with height–biomass regression introduces potentially significant error to the calculation of production. The regression used here is not discussed in detail, but the relationship between height and biomass in seepwillow is relatively strong ($r^2 = 0.853$, $P < 0.001$, $n = 81$), giving us confidence in our measurement of net production. A second and more significant problem, however, is that plant uptake was measured during a limited part of the growing season, and we do not know how it varies throughout the year. Seepwillow sheds its leaves in response to water stress, not seasonal changes, and usually supports green leaves year-round (J. D. Schade and N. B. Grimm, *personal observations*), suggesting production by these plants may be less variable than seasonally deciduous plants. This may dampen variation in uptake over the course of the year, reducing the magnitude of error in our measurements. During this project, seepwillow showed little sign of water stress, and plants maintained a healthy crop of leaves throughout the study period. Furthermore, if our estimate of plant uptake is low by an order of magnitude, uptake would explain 20% of NO_3 retention, still a relatively small proportion compared to denitrification. Nevertheless, uptake by seepwillow is certain to be more important during other times of the year, particularly when flowers and seeds are produced. Our observations suggest that flowers and seeds are generally produced in the late spring and fall. During this study, plants were not flowering in July and August; however, previous observations during a wetter period in the early 1980s suggest that flowering can continue into August under the right conditions (N. B. Grimm, *personal observations*). Annual NO_3 retention, then, is undoubtedly due to some combination of uptake and denitrification, and their relative importance may vary from year to year. We can, however, say with confidence that during the summer in which we studied these plants, denitrification played a larger role in NO_3 retention than plant uptake.

Our estimates of maximum loss of NO_3 due to denitrification far exceeded observed NO_3 losses (1200

$\text{mg}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$ vs. $11 \text{ mg}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$); however, potential denitrification may far exceed actual rates. In this case, actual rates of denitrification of $11 \text{ mg}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$, or $\sim 1\%$ of potential rates, would be sufficient to account for observed NO_3 losses. Previous studies in gravel bars of Sycamore Creek suggest that actual denitrification rates may be as much as 30% of potential rates (Holmes et al. 1996). In addition, Holmes et al. suggest that their methods underestimated actual denitrification by an unknown factor, suggesting actual denitrification rates could be high enough to account for our observed NO_3 losses. Furthermore, denitrification rate is probably not maximized throughout a seepwillow site, and spatial variability of denitrification is likely to be high, with highest rates at the interface between colonized and uncolonized sites of gravel bars. At these interfaces, water rich in NO_3 enters the rooting zone of seepwillow, which should lead to conditions favorable to high rates of denitrification. We used measurements of potential denitrification and an estimate of the velocity at which water moves through gravel bar sediments (1 m/h , Holmes et al. 1994), to calculate how far into a seepwillow site water would have to travel before all of the observed NO_3 loss would occur, assuming maximum denitrification rates ($1200 \text{ mg}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$) in the seepwillow site. All NO_3 loss would occur 1.1 cm into the zone of influence of the plant, if denitrification rates were at their maximum. We should observe, then, a rapid loss of NO_3 at the upflow edge of the zone of influence as NO_3 -rich water moves into an area of high potential denitrification. This calculation matches very well with our observation of changes in NO_3 concentration as water moves along a flowpath into an area influenced by the plant (parallel transect, Fig. 7A). We do indeed see a rapid loss of NO_3 at the upflow edge of the colonized site, with 83% of NO_3 loss occurring between 10 and 20 cm from the base of the stem (Fig. 7A). This zone of rapid loss corresponds in space to the zone of high fine-root biomass. A recent study by Jacinthe et al. (1998) also showed small-scale variability in denitrification rates in soils collected from a riparian forest in Rhode Island, USA, with high denitrification rates associated with patches of organic matter, particularly decomposing roots. These observations reinforce our conclusion that the production of root material by seepwillow leads to high rates of NO_3 removal.

The management implications of these results are significant. Seepwillow sites are sinks for NO_3 , which is often found in high concentrations in ecosystems experiencing high nitrogen loading either through agricultural practices or atmospheric deposition. Understanding the mechanism of removal will help to determine the time scale at which retention by seepwillow sites will help mitigate this form of pollution. Denitrification causes permanent loss of N from the ecosystem, while uptake only temporarily stores N in plant biomass (Lowrance 1998). Nitrogen stored in plant bio-

mass eventually becomes available again when the plant, or some part of the plant, dies. Using plants to reduce N loading to downstream ecosystems will be effective on a long time scale if plant activities indirectly lead to more permanent mechanisms of removal, such as denitrification, as we have shown here for seepwillow sites. Furthermore, as Nadelhoffer et al. (1999) have shown, as N deposition increases, the proportion of total N removal by forest ecosystems that is retained in plant biomass increases, at least in the short term. Since N in plant biomass is eventually made available again, the long-term ability of riparian forests to mitigate the effects of fertilizer additions may be reduced under increased N loading.

Through its effects on NO_3 concentrations, seepwillow may have a considerable influence on ecosystem processes in other subsystems, particularly algal production in the surface stream of Sycamore Creek. Since gravel bars make up a high proportion of the active channel during baseflow, N dynamics in gravel bars can have a strong effect on ecosystem processes in the stream. A significant effect of seepwillow on gravel bar N dynamics may also have a strong influence on the surface stream. The magnitude of the effect of seepwillow depends on several factors, including the abundance and size of plants. The plant effect is limited to shallower subsurface water, which places a bound on how large an effect colonization by seepwillow can have on algal production in the surface stream. If we limit the effect to the top 20 cm of subsurface flow, and assume that gravel bars average 1.5 m deep, then the maximum proportion of NO_3 removed by colonized patches is $\sim 12\%$. This may underestimate the effect on stream productivity, since shallower water is probably what is supplied to the stream at areas of hydrologic exchange between gravel bar and stream. Deeper water may move under several gravel bars before emerging to join surface flow (Fisher et al. 1998). Furthermore, the size of the plant has a large influence on the root mass and the depth of the rooting zone, both of which increase as the plants grow.

The abundance of seepwillow varies tremendously in time and space, and is tied to large-scale climatic cycles, such as El Niño. During the early 1990s, frequent, severe floods reduced seepwillow abundance. The onset of drought in the summer of 1995 allowed the establishment of seepwillow sites. As the severity of the drought increased, new seepwillow seedlings attempting to establish during the spring of 1996 eventually died as the water table dropped rapidly. We suggest that seepwillow and climate interact to determine the importance of seepwillow sites as nitrogen sinks. In wet years (during El Niño in southwestern United States), floods prevent the establishment of seepwillow in the channel, and nitrogen sinks do not develop. In severely dry years (La Niña), the water table may be too deep for seedlings to establish, water is not in contact with the rooting zone of established plants (often

leading to their deaths), and no nitrogen sinks develop. In intermediate years, the water table remains shallow enough for seepwillow to establish and water remains in contact with the rooting zone of plants already present. It is in these years that seepwillow sites would be nitrogen sinks. Furthermore, when little flooding occurs for several years, N availability in the stream tends to be low (Grimm and Fisher 1992). If seepwillow remains in contact with the water table during these years, it may exacerbate N limitation in the surface stream at a time when delivery of N to the stream from the watershed is low.

We have established in this work that colonization of gravel bars by seepwillow leads to the development of NO₃ sinks, mainly due to stimulation of microbial activity in the rhizosphere of plants, with a smaller influence through uptake and incorporation of N in plant biomass. The most important mechanism of NO₃ loss in this case, denitrification, leads to permanent removal of N from the system by returning it to the atmosphere. In an N-limited ecosystem (such as Sycamore Creek) this may have important consequences for ecosystem productivity. In systems experiencing heavy N loading, this may lead to effective, long-term mitigation of the effects of N enrichment on freshwater ecosystems.

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