AN ABSTRACT OF THE DISSERTATION OF

Daniel J. Sobota for the degree of Doctor of Philosophy in Fisheries Science presented on September 24, 2007.
Title: Linkages among Land Use, Riparian Zones, and Uptake and Transformation of Nitrate in Stream Ecosystems

Abstract approved:

Stanley V. Gregory

Land use alters the physical and biological structure of stream ecosystems and potentially alters their capacity to process nitrogen (N), an essential nutrient that has nearly doubled in abundance on the biosphere during the past century from human activities. In this dissertation, I quantified uptake and transformation of nitrate (NO_3^-) in small (≤ third-order) streams and related these dynamics to aquatic ecosystem processes, including primary production and organic matter decomposition, and attributes of riparian zone structure and vegetation composition. I also analyze patterns of stream NO_3^- processing among three classes of adjacent land use practices (forest, agriculture, and urban).

In Chapter 2, ambient rates of NO_3^- uptake and transformation were measured with 24-hr releases of ^{15}N-labeled NO_3^- in nine stream reaches in the Willamette River Basin of western Oregon during summer low flow (July – August). Three reaches each were surrounded by forested, agricultural or

urban land use. After standardizing reaches to a 500-m length, I estimated that ≥ 20% of tracer $^{15}NO_3^-$ was taken up by detrital and autotrophic biomass in eight of the reaches. In the remaining stream, which had the largest discharge (120 L s^{-1}) in this study, only 8% of the tracer was taken up in 500 m. Tracer labeling of detritus and autotrophic biomass and a positive correlation (r_s=0.81) of uptake with gross primary production suggested that assimilation was the dominant uptake pathway in all streams. Denitrification, dissimilatory reduction of NO_3^- to N_2 and N_2O gases, composed 3 – 15% of ^{15}N budgets over 500 m in two agricultural reaches and in one urban reach dominated by large slowly-turning over pools. However, denitrification was below detection limit at five of the remaining six reaches. This study showed that pathways of stream NO_3^- uptake and transformation differed among streams adjacent to three diverse land use practices.

In Chapter 3, I quantified effects of substrate nutritional quality and inorganic N loading (as NO_3^-) on wood breakdown in western Oregon streams. Short-term (< 2 month) breakdown rates of wood substrates of high nutritional quality (*Alnus rubra*; red alder) and low quality (*Pseudotsuga menziesii*; Douglas-fir) increased with dissolved inorganic N (11 to 111 µg N L^{-1}) across six streams ($p = 0.04$), but this relationship was confounded with concurrent increases in stream temperature. Across the six streams, breakdown rates of red alder were consistently double that of Douglas-fir. A longer-term study (313 d) in a coniferous forest Oregon Cascades stream suggested effects of increased NO_3^- availability on wood breakdown became evident after cellulose and lignin components of woody tissues began to decompose (> 4 months of incubation). Average breakdown rates substrates enriched with NO_3^- were higher than those incubated in low NO_3^- conditions, but this difference was not statistically significant. However, microbial biofilm respiration rates and activity of two enzymes involved in the breakdown of woody tissues (β-glucosidase and phenol oxidase) on red alder had significantly greater responses to NO_3^- additions than on Douglas-fir after four months of incubation in the stream. Results suggest that increases in N loading to streams bordered by riparian forests with fast-growing deciduous species could increase wood breakdown rates. On the other hand, increases to N loading may have a smaller effect on wood breakdown in streams surrounded by long-lived coniferous species.

In Chapter 4, I quantified patterns of stream channel and riparian zone attributes for 72 streams equally distributed among forests or grasslands, agriculture, and urban land use practices on from eight major North American regions. I also related these patterns to stream NO_3^- uptake determined from $^{15}NO_3^-$ tracer releases. Agricultural and urban streams had a simplified channel structure (low width-to-depth ratio, low variation in stream depth, and high stream banks) relative to forest or grassland streams. Agricultural and urban streams also had a significantly smaller median sediment diameter (D_{50}) and fraction of benthic sediments composed by silt than in forest and grassland streams. Overstory canopy cover over the channel and in the riparian zone was lowest for agricultural streams but did not significantly differ between forest or grassland streams and urban streams. A multiple regression model showed that stream NO_3^- uptake decreased with increasing canopy cover, but also increased with abundance of silt in benthic sediments. This suggested NO_3^- uptake was strongly influenced by in-stream primary production and extent of anoxic environments (conducive for denitrification). A multiple regression model for fractional NO_3^- uptake by denitrification further supported the concept that extent of anoxic environments influenced overall NO_3^- uptake in streams.

Through these studies, I demonstrated that attributes of riparian zone structure and vegetation composition can strongly influence NO_3^- uptake and transformation in stream ecosystems by controlling organic matter dynamics. I also have shown that riparian zone attributes vary significantly among three different land use types (forest or grassland, agriculture, and urban). Similarly, pathways of NO_3^- uptake and effects of NO_3^- on wood breakdown did or were expected to differ among different land use types / riparian zone characteristics. However, other factors besides riparian attributes, particularly level of nutrient loading, alteration of stream channel physical structure, and basin position of the stream, must be considered in concert when evaluating effects of land use on riparian zone and stream ecosystem structure and function.

Linkages among Land Use, Riparian Zones, and Uptake and Transformation of Nitrate in Stream Ecosystems

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APPROVED:

Major Professor, representing Fisheries Science

Head of the Department of Fisheries and Wildlife

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Daniel J. Sobota, Author

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Stan Gregory, Sherri Johnson, and Linda Ashkenas contributed to the interpretation of data and content of Chapter 2. Stan Gregory and Peter Bottomley contributed to data interpretation of Chapter 3. The Lotic Intersite Nitrogen eXperiment research group, consisting of over 20 universities, federal agencies, and private companies across North America, contributed data to Chapter 4.

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Chapter 1

INTRODUCTION

Streams are distinct ecosystems mediating the quantity and quality of nutrients transported from terrestrial environments to receiving water bodies (Triska et al. 1984). Movement of nutrients in stream ecosystems consists of cycling between biotic and abiotic ecosystem components extended spatially on the longitudinal axis of the channel (Elwood et al. 1983). Therefore, nutrients do not cycle at fixed locations in streams but instead spiral through multiple locations in a downstream direction. The nutrient spiraling concept (Webster and Patten 1979; Elwood et al. 1983) quantifies this process as an interaction between stream hydrology, benthic biological processes, and abiotic adsorption-desorption processes (Valett et al. 1996).

This dissertation specifically focuses on spiraling of nitrogen (N) in flowing water (lotic) ecosystems. This element often is a limiting nutrient in unmanaged streams and rivers, especially in western North America (Triska et al. 1984; Vitousek et al. 1997; Thomas et al. 2001). A conceptual diagram of major components of the N cycle, which also depicts major components of N spiraling in streams and rivers, is presented in Fig. 1. While N constitutes 78% of the troposphere, it is largely inaccessible to biological use due to the molecular structure of triple bonded N_2 gas (valence = 0) (Schlesinger 1997). However, biological processes are directly responsible for the conversion of N_2 into an accessible form in the biosphere. The process of N fixation is an anaerobic process carried out by an assortment of free-living heterotrophic bacteria, bacteria in symbiotic relationships with higher plants, cyanobacteria, and green-sulfur and purple-sulfur bacteria (Wetzel 2000). In this process, N_2

is reduced to ammonia (NH_3; valence = -3) in an oxygen-free environment and then incorporated into an amino acid group ($-NH_2$). This fixed N is then available for cycling into other biological components of the ecosystem, either through direct consumption of organic nitrogen or assimilation of excreted N waste products, most notably uptake of ammonium (NH_4^+) by microbes, algae, and higher plants (Schlesinger 1997).

Besides assimilatory uptake, NH_4^+ also can be oxidized to a variety of NO_x compounds. Nitrate (NO_3^-; valence = +5) is the most oxidized form of N found naturally and is highly mobile on the landscape due to its high water solubility (Wetzel 2000). Nitrification is the microbial process responsible for the production of most NO_3^- in terrestrial and aquatic ecosystems (Atlas and Bartha 1998). Chemoautotrophic nitrification is a two-step biologically-mediated process carried out by specialized bacteria in which NH_4^+ is first oxidized to nitrite (NO_2^-) and then converted to NO_3^-. Energy derived from NH_4^+ and NO_2^- (electron donors) is used to fix CO_2 into organic carbon (Schlesinger 1997). In addition to autotrophic nitrification, two other sources of NO_3^- naturally exist. NO_3^- also is produced through heterotrophic nitrification, which is carried out by a diverse array of bacteria (most notably the Archeabacteria) and fungi (Atlas and Bartha 1997; Hart et al. 1997) and in some systems (i.e., coastal forest soils) may be an important production pathway for NO_3^- (Hart et al. 1997; Perakis et al. 2006; Boyle 2007). NO_3^- also is produced through the coupled abiotic fixation of dinitrogen gas (N_2) to NO_x compounds associated with atmospheric lightning; but this process is minor compared to other sources of fixed nitrogen, constituting < 10% of total non-anthropogenic N inputs to the biosphere (Schlesinger 1997).

Fixed N is returned to the atmosphere as N_2 gas predominantly through the process of denitrification (Schlesinger 1997). Denitrification is a microbially-mediated process conducted by obligate- and facultative-anaerobic bacteria in which NO_3^- is used as an electron acceptor in this respiration process. NO_3^- yields the second-highest energy yield per mole of reduced organic matter (free energy yield = -476 kJ at pH = 7) compared to oxygen (free energy yield = -501 kJ at pH = 7) and reduced through the pathway of $NO_3^- \rightarrow NO_2^- \rightarrow NO^- \rightarrow N_2O \rightarrow N_2$ by a consortium of eubacteria (Hedin et al. 1998; Atlas and Bartha 1998). This process represents a complete removal of fixed N from active ecosystem cycling and in pre-industrial times, largely balanced with rate of biological N fixation (Schlesinger 1997). Denitrification also produces nitrous oxide (N_2O) (also a byproduct of nitrification) which 300 times more potent than CO_2 as a greenhouse gas and a catalyst for the destruction of stratospheric ozone (Atlas and Bartha 1998).

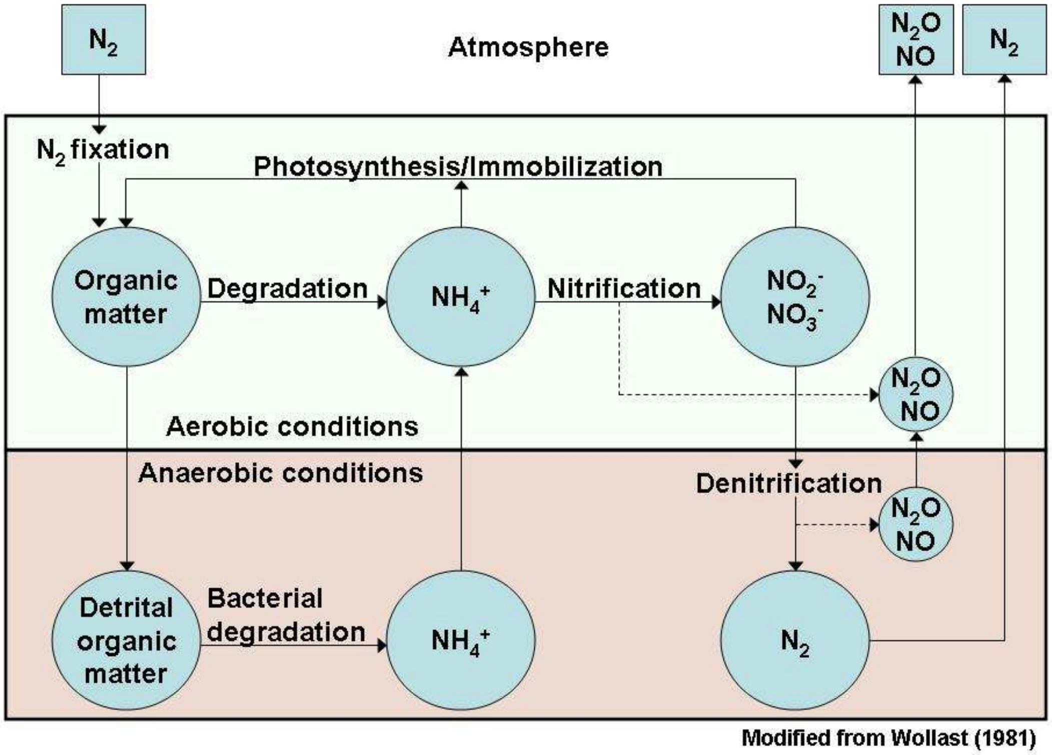


Fig. 1. A conceptual model of major components of nitrogen (N) cycling in terrestrial and aquatic ecosystems.

Advances in agricultural and industrial technologies over the past century have more than doubled the annual input rate of fixed N to the biosphere (Vitousek et al. 1997; Holland et al. 2005). Globally, the loading of N to streams and rivers has more than doubled, with localized systems receiving loading more than 50 times pre-industrial averages (Meybeck 1982). Increases in concentrations of NO_3^- and the shift of dominance of total dissolved nitrogen (TDN) from dissolved organic nitrogen (DON) to NO_3^- in streams and rivers are typical symptoms of human alteration of the nitrogen cycle on the landscape (Perakis and Hedin 2002; Bernot et al. 2006). Two mechanisms are responsible for this phenomenon. First, direct and indirect loading of NO_3^- to streams and rivers in human populated areas increases as a consequence of fertilizer runoff (direct), sewage inputs (mediated by nitrification of NH_4^+), and atmospheric deposition of NO_x produced in fossil fuel combustion (Vitousek et al. 1997; Atlas and Bartha 1998). Second, increased input of N can saturate the capacity for assimilatory uptake of N by plants and microbes, increasing the availability of NH_4^+ through increased N mineralization rates and consequently increasing nitrification rates (Aber et al. 1998; Bernot et al. 2006). Anthropogenic increases in stream NO_3^- concentrations have several important negative impacts on ecological conditions and human health (Townsend et al. 2003). Increased loading of NO_3^- to small streams has been implicated as a cause of eutrophication in river systems and coastal marine estuaries (Howarth et al. 1996; Alexander et al. 2000), contributing to the formation of “dead zones” in major water bodies including the Gulf of Mexico and the Chesapeake Bay. High levels of N inputs to ground and surface waters also pose several significant threats to municipal water quality, including increased risk of methemoglobinemia and several types of cancer (Townsend et al. 2003).

The nutrient spiraling concept provides quantifiable metrics of NO_3^- dynamics in stream ecosystems (Mulholland et al. 2000; Peterson et al. 2001; Webster et al. 2003). Using this framework, general predictions of uptake and downstream transport of NO_3^- are possible using basic information on stream flow, NO_3^- concentration, and characteristics of ecosystem metabolism (Peterson et al. 2001; Hall and Tank 2003; Ensign and Doyle 2006; Wollheim et al. 2005; O'Brien et al. 2007). However, quantitative information on specific NO_3^- uptake mechanisms in a wide range of landscape settings and response of uptake to alteration of bordering riparian zones is still limited. Land use activities alter the physical and biological structure of stream – riparian interactions (Harding et al. 1995; Paul and Meyer 2001), potentially altering the capacity of streams to process N. Thus, information on responses of stream ecosystem N processing to riparian zone alteration may be critical for mitigating effects of increased N loading to aquatic ecosystems.

In this dissertation, I quantified uptake and transformation of nitrate (NO_3^-) in small (< third-order) streams and related these patterns to aquatic ecosystem processes—including primary production and decomposition—and attributes of stream – riparian interactions with particular focus on effects of land use activities. I present results from whole-stream releases of tracer levels of the stable isotope ^{15}N in the form of NO_3^- (Chapter 2) for nine streams in forest, agricultural, and urban land uses, manipulative field experiments on effects of N enrichment on decomposition of an important riparian-derived organic matter component (wood) in streams (Chapter 3), and a large-scale statistical analysis that quantifies the influence of channel morphological and riparian zone attributes on uptake rates of NO_3^- in streams (Chapter 4).

Chapter 2

A ^{15}N TRACER STUDY OF NITRATE UPTAKE AND TRANSFORMATION IN STREAMS INFLUENCED BY DIFFERENT LAND USES IN WESTERN OREGON, USA

Daniel J. Sobota, Sherri L. Johnson, Stan V. Gregory, and Linda R. Ashkenas

ABSTRACT

Human land use changes physical and biological characteristics of streams and potentially alters the capacity of these ecosystems to process nitrogen (N). We report results from 24-hr releases of ^{15}N-labeled nitrate (NO_3^-) in nine stream reaches in the Willamette River Basin of western Oregon, USA, conducted during low flow in late summer (July – August). Three reaches each were surrounded by forested, agricultural or urban land use. Twenty percent or more of the tracer $^{15}NO_3^-$ was taken up by detritus or autotrophic biomass within a 500-m stream length in eight reaches. Only 8% of the tracer was taken up in 500 m for the stream reach with the largest discharge (120 L s^{-1}). The dominance of uptake into detritus and autotrophic biomass and a strong correlation of whole-stream uptake with gross primary production (r_s=0.81) showed that assimilation was the most important uptake pathway in all reaches. Denitrification composed a larger proportion (20-38%) of total uptake in two reaches where inorganic N:P molar ratios exceeded the Redfield Ratio (15:1) and in one urbanized reach dominated by large slowly-turning over pools. Estimated nitrification rates fell within 95% confidence limits of gross NO_3^- uptake in all reaches, suggesting concurrent uptake and regeneration of NO_3^- even in streams where N was limiting. Overall, our study shows that N uptake and transformation in streams differed among forest, agricultural, and urban land uses and these differences are linked to biophysical characteristics of riparian zones and stream channels.

INTRODUCTION

While input of biologically-available nitrogen (N) to terrestrial landscapes has more than doubled during the past century (Meybeck 1982; Vitousek et al. 1997), only 20 – 40% of this loading can be accounted for in stream and river export to downstream water bodies (Howarth et al. 1996; Alexander et al. 2000). Biological processing in small streams has been hypothesized to play an important role in accounting for this missing N (Alexander et al. 2000; Peterson et al. 2001; Bernhardt et al. 2005). Using results from the Lotic Intersite Nitrogen eXperiment (LINX), which released tracer levels of ^{15}N in the form of NH_4^+ for six weeks during low flow in eleven streams in North America and Puerto Rico, Peterson et al. (2001) suggested that 15% to 87% of dissolved inorganic N (ammonium (NH_4^+) and nitrate (NO_3^-)) would be removed from the water column by biological mechanisms within 1 km in a typical headwater stream not impacted by human activities. Human land use changes physical and biological characteristics of streams and potentially alters capacity of these ecosystems to process N, yet specific impacts of human land use practices on the capacity of streams to process N remain largely unknown.

Quantitative information on uptake and transformation of NO_3^- in streams is of particular importance because high concentrations (i.e., > 10 mg L^{-1}) of this mobile N species pose threats to water quality and human health (Townsend et al. 2003). One way in which NO_3^- is removed from stream transport is through denitrification, an anaerobic microbial respiration process in which NO_3^- is reduced to N_2O and N_2 gases (Atlas and Bartha 1998). This process constitutes a complete removal of N from the ecosystem and has been suggested as a possible means to remove excess anthropogenic N from aquatic ecosystems. Routing anthropogenic NO_3^- through small streams (where surface area of stream bed to water volume is greatest) to maximize denitrification has been specifically been recommended to attenuate N runoff from agricultural fields in the upper Mississippi River basin (Alexander et al. 2000). Previous studies indicate that denitrification removes < 2% to > 50% of NO_3^- in small streams and is controlled by availability of organic carbon, low-oxygen environments, and NO_3^- (Swank and Caskey 1982; Martin et al. 2001; Mulholland et al. 2004; Bohlke et al. 2004).

In addition to denitrification, assimilation by stream biota constitutes an important uptake pathway for NO_3^- in stream ecosystems (Mulholland et al. 2000; Simon et al. 2004). Assimilatory uptake transforms oxidized N into reduced organic forms and, unlike denitrification, does not completely remove N from the ecosystem. Previous studies estimate assimilation of NO_3^- indirectly through coupled NH_4^+ uptake-nitrification of tracer $^{15}NH_4^+$ and show that NO_3^- uptake accounts for > 50% of total assimilation by autotrophs and heterotrophs (Mulholland et al. 2000; Webster et al. 2003; Simon et al. 2004). The relative contribution of stream autotrophic and heterotrophic processes to assimilatory NO_3^- uptake should depend on light levels, degree of N limitation, and abundance of detrital substrates (Webster et al. 2003).

One additional factor in considering effects of land use activities on stream ecosystem N processing is background biogeochemistry. In North America, studies that have investigated the relationship of NO_3^- dynamics in streams to human land use have largely been conducted in eastern North America (e.g., Alexander et al. 2000; Wollheim et al. 2005; Bernot et al. 2006). While stream NO_3^- dynamics have been studied in western North America (e.g., Triska et al. 1989; Grimm 1987; Munn and Meyer 1990; Davis and Minshall 1999), specific effects of land use generally have not been considered. One of the primary biogeochemical differences between eastern and western North America is distribution of N on the landscape. In eastern North America, non-point source atmospheric deposition and large-scale fertilizer applications lead to high levels of N in aquatic systems (Vitousek 1977; Aber et al. 1998; Perakis and Hedin 2002), though stream NO_3^- concentrations have been consistently declining possibly a result from increasing N retention in terrestrial (Dail et al. 2002; Goodale et al. 2005) or aquatic ecosystems (Bernhardt et al. 2005). Coupled with the older sedimentary geologies, phosphorus is the limiting nutrient to biological production in many eastern North American streams (Meyer et al. 1980; Munn and Meyer 1990; Valett et al. 2002). In western North America, natural levels of N are variable and related to N-fixation rates in aquatic (Triska et al. 1984) and terrestrial (Compton et al. 2003) systems. Variable geology in western North America, including ancient marine sediments and young volcanic basalts rich in P, suggests that N-limitation in stream ecosystems depends strongly on local context of terrestrial vegetation composition and underlying geology. Thus, N uptake and transformation in western streams may show different responses than eastern streams to increases in load rates of N depending on background vegetation and biogeochemical settings.

Our objective in this study was to quantify whole-system and individual pathways of NO_3^- uptake and transformation in forested, agriculture, and urban streams in the Willamette River basin, Oregon, using a stable isotope tracer (^{15}N). Many streams in this region appear to b N-limited based on molar ratios dissolved N to dissolved P < 30:1 (Anderson et al. 1978; Gregory 1980; Murphy et al. 1998), but human land use also has increased loading of NO_3^- to many streams (Poor and McDonnell 2007). One hypothesis is that uptake of NO_3^- increases asymptotically with increasing NO_3^- concentration, reflecting a saturating response of N assimilation by stream biota (O'Brien et al. 2007). However, alteration of biological and physical characteristics of riparian zones and channels by forestry, agriculture, and urban land use practices may also alter stream NO_3^- uptake by reducing assimilatory uptake associated with the decomposition of allochthonous organic matter while at the same time increasing assimilatory uptake by aquatic primary producers that have been released from light-limitation through reduction of riparian canopy shade. We also thought whole-stream denitrification rates would be related to NO_3^- concentration, detrital organic matter standing stocks, and degree of transient water storage in the stream reach.

SITE DESCRIPTIONS

This study was conducted in the Willamette River basin (45ºN, 123ºW) of western Oregon, USA (Fig. 1). The climate is characterized by cool, wet winters (mean temperature = 4ºC) and warm, dry summers (mean temperature = 19ºC) (Taylor and Hannan 1999). Annual precipitation (mostly rain) ranges from 1000 to 2000 mm from late autumn (November) through spring (May) (Taylor and Hannan 1999). This study was conducted during summer low flow (June – September). Nine stream reaches (second- or third-order) were selected in urban, agriculture, or forested land uses (three reaches per land use) (Appendix A). Surrounding land cover (2001 National Land Cover Database, Multi-Resolution Land Characteristics Consortium, US EPA, Washington, D.C.) generally matched our field classifications (Table 1). Length of reaches ranged from 125 to 590 m. Concentrations of dissolved N often are naturally low in regional streams ($NO_3^- < 50$ μg N L^{-1}; > 50% of dissolved N as dissolved organic nitrogen (DON)) from low atmospheric N deposition (< 2 kg ha^{-1} yr^{-1}) and low aquatic N fixation rates (Buckley 1977; Triska et al. 1984). High natural levels of phosphorus (P) in basalts common in the region often lead to N-limitation in these streams (Gregory 1987). However, streams in sedimentary geologies and those surrounded by red alder forests (*Alnus rubra*; an N-fixer) or in developed areas often have higher N levels (Compton et al. 2003; Perakis et al. 2006; Poor and McDonnell 2007).

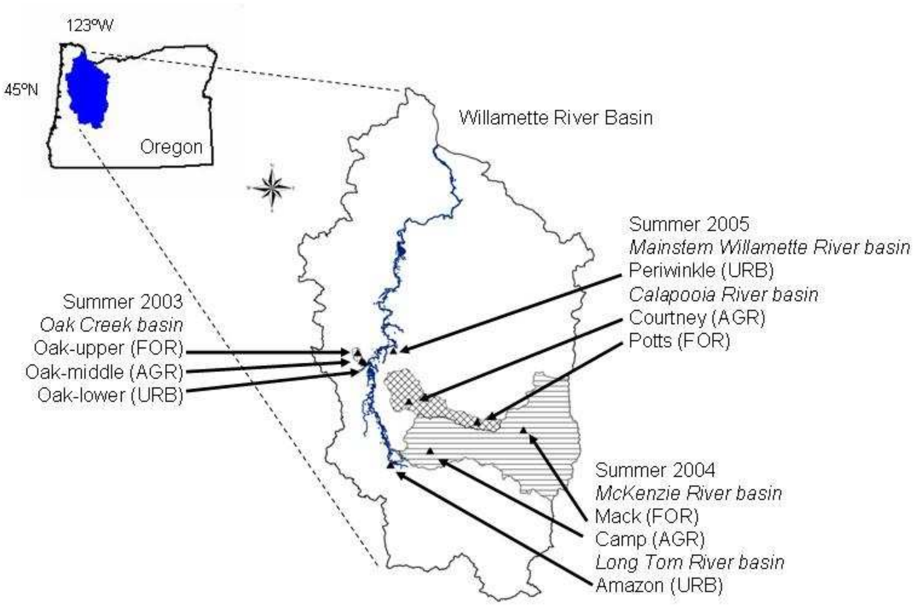


Fig. 2. Location of study reaches in the Willamette River basin, Oregon, USA. FOR = forest; AGR= agriculture; URB = urban.

Table 1**.** Physiographic setting and land use cover of the nine study reaches in the Willamette River Basin, Oregon.

	Forest			Agriculture			Urban		
Variable	Oak -upper	Mack	Potts	Oak -middle	Camp	Courtney	Oak -lower	Amazon	Periwinkle
Year of study	2003	2004	2005	2003	2004	2005	2003	2004	2005
Geographic location	44º37’N 123º20’W	44º13’N 122º10’W	44º16’N 122º29’W	44º34’N 123º18’W	44º07’N 122º49’W	44º22’N 123º58’W	44º34’N 123º17’W	44º03’N 123º06’W	44º37’N 123º05’W
Elevation (m)	180	760	550	80	200	100	70	130	70
Catchment area (ha)	617	531	349	3051	2681	4169	3221	2179	1026
Reach length (m)	423	404	590	350	462	352	223	545	119
Stream order†	2	3	2	3	4	2	3	3	3
Specific land use⫯	SG	OG	SG	GS/LS	PS/LS	GS/LS	U	R/C	R
Catchment land cover (%)↑									
Native vegetation	97	100	100	74	98	75	71	31	0
Agriculture	1	0	0	18	2	25	18	0	70
Urban	2	0	0	8	0	0	11	69	30

†Based on 1:24,000 USGS topographic maps.

⫯Refers to land use immediately surrounding the study reach. SG = second-growth forest; OG = old-growth forest; GS = grass seed agriculture; LS = livestock; PS = pasture; U = university campus; R = residential neighborhood; C = commercial property.

↑Catchment land cover data from 30-m pixel GIS layers from the National Land Cover Database (NLCD 2001).

METHODS

Channel, riparian, and hydrologic characteristics

Channel and riparian characteristics were assessed using US EPA Environmental Monitoring and Assessment Program (EMAP) protocols (http://www.epa.gov/emap2/index.html). Fifteen channel transects were designated on each reach. At each transect, we measured wetted width and 10 evenly-spaced measurements of depth and benthic sediment diameter. Cover over the stream channel was assessed with a concave spherical densitometer at the midpoint of the wetted channel at each transect. Discharge was measured during the tracer ^{15}N release by dilution of a conservative tracer (Cl^- or Br^-) downstream of the release site (Webster and Valett 2006). Transient water storage also was estimated from a conservative tracer (Cl^-) release over 4 – 24 hrs in the week preceding or during the tracer release (Stream Solute Workshop 1990). Specific conductance (conductivity at 25ºC) of Cl^- was measured every 5 min with a Hydrolab 4a Minisonde (Hach Environmental, Loveland, Colorado, USA) at a downstream location > one-third the reach length. One-dimensional Transport with Inflow and Storage (OTIS) (Runkel 1998) was used to estimate transient storage parameters, three of which we emphasize (α, A_s/A, and $F_{med}200$). α quantifies exchange between advection and transient storage, A_s/A characterizes transient storage zone area versus stream cross-sectional area, and $F_{med}200$ quantifies transient storage over 200 m (Runkel 2002).

Stream chemistry and biology

Immediately prior to the isotope release, filtered (Whatman GF/F, pore size = 0.45 μm; Florham Park, New Jersey, USA) water samples were collected from six points in the study reach for the analysis of NO_3^- (cadmium reduction method), NH_4^+ (phenate method), total dissolved nitrogen (TDN; high temperature persulfate digestion (Valderrama 1981) followed by the cadmium reduction method), soluble reactive phosphorus (SRP; abscorbic acid method), total dissolved phosphorus (TDP; microwave digestion followed by abscorbic acid method), and dissolved organic carbon (DOC; Combustion Infrared Method) (APHA 2005). Additional samples for NO_3^- were collected from the same six points during each tracer release.

Standing stocks (g m^{-2}) of detrital organic matter and autotrophic biomass were measured in each reach. Leaves, conifer needles, fine benthic organic matter (FBOM), epilithon, bryophytes, macrophytes, and filamentous algae, were quantitatively sampled from an area of 0.25 to 1 m^2 at ten points distributed among pool and riffle habitats in each reach (Mulholland et al. 2000; Ashkenas et al. 2004). Samples were dried (60ºC) to a constant weight and combusted at 500ºC for 1 hr to estimate ash free dry mass (AFDM). Standing stocks were calculated by weighted-averaging by area of habitat type in the reach. Additionally, N content of samples were measured on a Heraeus CHN elemental analyzer (Hanau, Germany; performed at the Ecosystems Center, Woods Hole, Massachusetts, USA). Carbon (C) content was estimated as 45% of AFDM (Simon et al. 2004) for biomass sampled on Oak Creek reaches (summer 2003) or was measured directly. Small (< 10 cm diameter) and large (≥ 10 cm diameter) wood were estimated with 10 to 15 linear transects (Wallace and Benke 1984) perpendicular to flow in the reach and assuming a wood density of 0.4 g cm^{-3} (Harmon et al. 1986).

Whole-system gross primary production (GPP) and ecosystem respiration (ER) was measured according to one- (three reaches) or two-station methods (Young and Huryn 1999). Dissolved oxygen concentrations were measured with Hydrolab minisondes at 5-min intervals for a 24 – 48 hr period beginning at noon on the day of the tracer release. Where the two-station method was used, minisondes were placed 100 – 200 m apart in the reach. Atmospheric exchange rates of dissolved oxygen were determined from downstream decline of a conservative gas tracer (SF6) corrected for dilution by a conservative tracer (Hall and Tank 2005).

Tracer $^{15}NO_3^-$ releases

A solution of 98% pure ^{15}N in the form of KNO_3^- and a conservative tracer in distilled water was injected at a constant rate for 24 hr on each reach starting at 1300 hr. The conservative tracer estimated downstream dilution of injected $^{15}NO_3^-$. All ^{15}N values were quantified as:

(1) $\delta^{15}N$ (‰) = $(R_{sam}/R_{std} - 1) * 1000$

where R_{sam} is the sample atomic ratio of $^{15}N/^{14}N$ and R_{std} is an air standard (0.003663). We targeted an enrichment of $\delta^{15}N$ = 20,000‰ while increasing NO_3^--N concentrations < 7% to trace NO_3^- uptake without fertilization (Mulholland et al. 2004). Immediately before the start of the release, background samples for $^{15}NO_3^-$ were collected at six points in the reach. $^{15}NO_3^-$ samples were collected at the six points plus one upstream background site at 12 (midnight) and 24 (noon) hrs after the start of the release. One day after the release, ^{15}N samples for dissolved N (NO_3^-, NH_4^+, and DON) and suspended particulate organic N (SPON) were collected (O'Brien et al. 2007).

Dissolved N samples were filtered and analyzed within one week (kept at 4ºC until analysis) or frozen until analyses were performed. $^{15}NO_3^-$ and $^{15}NH_4^+$ samples were processed according to Mulholland et al. (2004) and O'Brien et al. (2007); $TD^{15}N$ samples were digested with persulfate reagent (Valderrama 1981) and processed as $^{15}NO_3^-$ samples. Known aliquots of unlabeled NO_3^- or NH_4^+ were added to samples to reduce the $\delta^{15}N$ to the range required by the isotope laboratory (~4,000‰). Three to five replicates of distilled water with identical aliquots of unlabeled NO_3^- or NH_4^+ were made during each release. All ^{15}N samples were analyzed at the Ecosystems Center (Woods Hole, Massachusetts, USA) on a duel inlet Finnigan MAT Delta S mass spectrometer (Finnigan, Sacramento, CA, USA).

We also sampled $^{15}N_2$ and $^{15}N_2O$ at ten points in each reach at the same time of water collections (O'Brien et al. 2007). $^{15}N_2O$ was analyzed at the UC-Davis Stable Isotope Laboratory (Davis, California, USA) on a Finnigan MAT 251 Isotope Ratio Mass Spectrometer (Finnigan, Sacramento, California, USA). $^{15}N_2$ from Oak Creek reaches also was analyzed at UC-Davis; but $^{15}N_2$ for other reaches was analyzed on a multi-collector GV Instruments Isoprime Mass Spectrometer (GV Instruments, Manchester, UK) at the MSU Stable Isotope Laboratory (Lansing, MI, USA). ^{15}N in benthic biomass was sampled at six points in reaches plus one upstream site one day after the release (Hamilton et al. 2001). $SPO^{15}N$ was collected on a pre-combusted GF/F (Wollheim et al. 2001). $SPO^{15}N$ and biomass were dried, ground to a fine powder and analyzed at the Ecosytems Center.

Uptake lengths (S_w) of $^{15}NO_3^-$ during midnight and noon were estimated as the inverse of the slope from the log – linear regression model of dilution-corrected $^{15}NO_3^-$ mass flux versus distance downstream (Mulholland et al. 2004). Uptake velocity (V_f) and areal uptake rate (U) of NO_3^--N were calculated from discharge (Q), average stream width (w), and stream NO_3^- concentration (C) (Stream Solute Workshop 1990):

$$V_f = \frac{Q}{wS_w} \tag{2}$$

$$U = V_fC \tag{3}$$

Nitrification was estimated from a mass balance of stream flux of NO_3^-, groundwater input of NO_3^-, and gross NO_3^- uptake (O'Brien et al. 2007). Lateral inflow of groundwater was estimated from downstream dilution of the conservative tracer co-injected with $^{15}NO_3^-$. Groundwater NO_3^- samples were collected from riparian wells (50 cm deep) except at Amazon, Periwinkle, and Camp, where groundwater NO_3^- was assumed identical to surface water. Denitrification rates were estimated in a linear combination model fitted to $^{15}NO_3^-$ and $^{15}N_2$ or $^{15}N_2O$ flux by minimizing squared residuals in Microsoft Solver (Microsoft Corporation, Redmond, WA, USA) (Mulholland et al. 2004). Denitrification rates only are reported where $\delta^{15}N$ in both N_2 and N_2O for three or more samples was greater than the upper 97.5% confidence limit of average background $\delta^{15}N$ in each gas. ^{15}N exported as NH_4^+ and SPON was estimated by fitting a linear combination model from Mulholland et al. (2000) in Microsoft Solver. $DO^{15}N$ export was not calculated because recovery efficiencies for $\delta^{15}N$ of TDN were highly variable.

^{15}N taken up by detritus and autotrophic biomass was estimated according to Hamilton et al. (2001). We considered epilithon as autotrophic biomass and FBOM as detritus though we recognize that both consist of algae, cyanobacteria, heterotrophic microbes, and amorphous organic matter. ^{15}N budgets for individual reaches were estimated by scaling standing stock of biomass ^{15}N to the entire reach, estimating fraction of overall $^{15}NO_3^-$ uptake attributable to denitrification (Mulholland et al. 2004), and calculating reach export of ^{15}N as NO_3^-, NH_4^+, and SPON.

For comparisons of ^{15}N budgets, we scaled ^{15}N budgets to 500-m reaches. We estimated export of ^{15}N in the form of NO_3^- by solving a negative exponential decay model with the average uptake length (S_w) of midnight and noon. We assumed the same proportion of $^{15}NO_3^-$ uptake by biomass and denitrification as in the original reach. Error (difference between released and recovered ^{15}N) was distributed among biomass, denitrification, and export as NH_4^+ and SPON based on relative proportion of ^{15}N fate in original budgets. Therefore, all 500-m budgets summed to 100%.

Statistics

Confidence intervals (95%) for uptake lengths were taken as the inverse of 95% confidence limits for the slope of $^{15}NO_3^-$ flux (natural log) versus distance downstream (Hamilton et al. 2001). Confidence intervals (95%) for V_f and U were calculated from the 95% confidence bounds on S_w; error in discharge, stream width, and NO_3^- was not considered. Log-log regression models quantified correlation between S_w and stream velocity * depth (Wollheim et al. 2001). Spearman correlation coefficients (r_s) were used to correlate detrital biomass and riparian canopy cover with GPP and ER. r_s also was used to correlate V_f with GPP and ER. Linear regression models were used to relate % of DIN in TDN and U to NO_3^- and TDN concentrations. Statistics were performed in R 2.4.0 (R Development Core Team 2006).

RESULTS

Channel, riparian, and hydrologic characteristics

All forest reaches were well-shaded (> 56% canopy cover) while agriculture and urban reaches exhibited a wide range of canopy cover (0.1 – 82%) (Table 2). Wetted widths ranged from an average of 2.2 to 6.5 m and depths ranged from average of 0.06 to 0.37 m (Table 2). Median diameter of sediment ranged from 0.008 to 0.126 m. Average discharge ranged from 2.7 to 118 L s^{-1}, with eight reaches < 40 L s^{-1} (Table 2). Low influence of transient water storage was observed among reaches, with estimates for two urban streams not possible because of fluctuations in background Cl^- and incomplete mixing of the conservative tracer (Table 2). One reach (Oak-lower) showed a relatively large influence of transient storage from the presence of large pools.

Stream chemistry and biology

Lowest concentrations of NO_3^- and highest concentrations of DON occurred in two urban reaches (Amazon and Periwinkle) (Table 3). NH_4^+ concentration was consistently lower than NO_3^- except in one urban reach where NH_4^+ and NO_3^- were < 5 μg N L^{-1} (Table 3). Highest TDN concentrations occurred in agriculture and urban reaches (Table 3). SRP ranged from 5 to 209 μg L^{-1} and TSP ranged from 24 to 258 μg L^{-1} (Table 3). Dissolved inorganic N (DIN; NH_4^+ + NO_3^-) to SRP molar ratios fell below

Table 2. Riparian, geomorphic and hydrologic characteristics of the nine reaches. Standard deviations are in parentheses. Transient storage parameters were estimated using OTIS (Runkel 1998). NA = not available.

	Forest			Agriculture			Urban		
Variable	Oak -upper	Mack	Potts	Oak -middle	Camp	Courtney	Oak -lower	Amazon	Periwinkle
Channel cover (%)	92	56	88	75	69	27	82	49	1
Wetted width (m)	2.2 (0.7)	5.8 (2.8)	2.9 (0.7)	2.7 (0.8)	5.9 (1.4)	3.1 (1.2)	4.1 (1.4)	6.5 (0.7)	3.4 (0.8)
Depth (m)	0.11 (0.10)	0.14 (0.14)	0.11 (0.10)	0.12 (0.10)	0.22 (0.18)	0.11 (0.10)	0.28 (0.22)	0.06 (0.02)	0.37 (0.14)
D_{50} (m)†	0.064	0.128	0.045	0.023	0.045	0.032	0.016	0.008	0.064
Pool/riffle (m^2 m^{-2})	0.79	0.55	0.15	7.15	0.89	5.40	4.37	no pools	no riffles
Discharge (L s^{-1})	8.9	30.7	19.0	6.7	118.8	38.7	7.9	22.8	2.7
α (s^{-1})	0.00003	0.00012	0.00020	0.00068	0.00008	0.00017	0.00370	NA	NA
A_s/A	0.18	1.00	0.16	0.31	0.07	0.16	2.28	NA	NA
$F_{med}200$	0.02	0.13	0.04	0.24	0.01	0.05	0.52	NA	NA
DaI‡	0.15	0.36	1.45	34.20	0.15	1.79	0.15	NA	NA

† D_{50} = median substratum diameter.

‡ DaI = Damkohler Index; the ratio of transport in and out the transient storage zone due to convection (Ge and Boufadel 2005).

Table 3. Water chemistry and temperature of the nine study reaches during isotope releases. Concentrations are averages of six points distributed in each reach collected immediately before and during the isotope release at midnight and noon sampling (NO_3^- only) or from immediately before the release (all other chemical species). Temperatures are minimum and maximum values from 5-min recording intervals taken during releases.

	Forest			Agriculture			Urban		
Variable†	Oak -upper	Mack	Potts	Oak -middle	Camp	Courtney	Oak -lower	Amazon	Periwinkle
NO_3^- (µg N L^{-1})	72	57	57	82	50	100	143	3	8
NH_4^+ (µg N L^{-1})	1	2	5	9	6	11	19	5	4
DON (µg N L^{-1})	80	39	169	89	51	100	115	321	347
TDN (µg N L^{-1})	153	98	231	180	107	211	177	329	359
GW NO_3^- (µg N L^{-1})	10	52	89	8	--	10	233	--	--
SRP (µg L^{-1})	51	13	25	48	5	5	45	18	209
TDP (µg L^{-1})	345	24	38	53	14	21	67	45	258
DIN:SRP	4.7	10.0	5.5	4.2	23.2	49.1	8.0	0.9	0.1
TDN:TSP	6.7	8.6	12.2	7.1	16.1	22.5	8.8	16.1	3.1
DOC (mg C L^{-1})	2.05	0.88	1.27	1.70	0.89	2.57	1.87	3.45	7.04
DOC:DON	29.9	26.3	8.8	22.3	20.4	30.0	19.0	12.5	23.7

† GW NO_3^- = groundwater concentration of NO_3^-; TDN = total dissolved nitrogen; SRP = soluble reactive phosphorus, TDP = total dissolved phosphorus, DOC = dissolved organic carbon, DIN:SRP = molar ratio of dissolved inorganic N (NO_3^--N + NH_4^+-N) to SRP. DOC:DON = molar ratio of dissolved organic carbon to dissolved organic nitrogen.

15:1 in seven reaches while TDN:TSP ratios all fell below 22.5:1 in all reaches (Table 3). DOC ranged from 0.88 to 7.04 mg L^{-1} and molar DOC:DON ratio ranged from 8.8 to 29.9 (Table 3).

Detritus was the largest component of benthic biomass in eight reaches, with autotrophic biomass dominant in one urban reach (Table 4). FBOM and wood composed >99% of detritus in all reaches. Large and small wood were most abundant in forest reaches and one urban reach (Table 4). Large wood was more abundant than small wood in six of the seven reaches with wood. Leaves and conifer needles made up < 2% of detritus (Table 4). Autotrophic biomass composed < 1 to 66% of benthic biomass in reaches (Table 4). Composition, but not standing stock, of autotrophic biomass exhibited a pattern among land uses (Table 4). Bryophytes were only found in two forest reaches and composed > 77% of autotrophic biomass. Filamentous green algae (*Cladophora* spp.) occurred in agriculture and urban reaches and composed 10 to 90% of autotrophic biomass. Algal fines made up 5% and macrophytes composed 33% of autotrophic biomass in two separate urban reaches (Table 4). C:N ratio of detritus exhibited a wide range within and among reaches (Table 4). Large and small wood had the highest C:N, ranging from 26:1 in one agriculture reach to 194:1 in one forest reach. FBOM had a C:N > 20:1 in six reaches with one agriculture and two urban reaches exhibiting C:N similar to autotrophic biomass (Table 4). Autotrophic biomass components exhibited lower C:N, though filamentous green algae had C:N ratios > 26:1 in one agricultural and two urban reaches (Table 4).

Table 4. Habitat-weighted standing stocks of stream benthic biomass in the nine reaches during the isotope releases. Molar ratios of carbon-to-nitrogen (C:N) are in parentheses.

	Forest			Agriculture			Urban		
Biomass type (g AFDM m^{-2})	Oak -upper†	Mack	Potts	Oak -middle†	Camp	Courtney	Oak -lower†	Amazon	Periwinkle
FBOM	181.6	64.2	94.2	844.5	120.3	166.2	3257.3	57.7	99.5
	(22:1)	(29:1)	(21:1)	(22:1)	(26:1)	(12:1)	(21:1)	(13:1)	(8:1)
Leaves/needles	6.3	0.2	32.2	21.0	14.0	4.8	8.6	0.2	--
	(25:1)	(70:1)	(19:1)	(26:1)	(29:1)	(26:1)	(30:1)	(53:1)	
Small wood	524.1	251.4	272.2	285.0	269.6	49.5	363.8	--	--
	(45:1)	(194:1)	(63:1)	(26:1)	(48:1)	(39:1)	(100:1)		
Large wood	1798.0	4660.3	1801.5	438.6	156.1	63.0	3519.1	--	--
	(45:1)	(194:1)	(63:1)	(26:1)	(48:1)	(39:1)	(100:1)		
Total detritus	2510.0	4972.1	2200.1	1589.1	558.0	283.5	7148.9	57.9	99.5
	(42:1)	(189:1)	(58:1)	(23:1)	(44:1)	(20:1)	(36:1)	(13:1)	(8:1)
Epilithon	3.0	4.3	2.3	1.4	7.5	3.9	21.1	3.3	--
	(8:1)	(20:1)	(9:1)	(4:1)	(8:1)	(9:1)	(20:1)	(14:1)	
Filamentous algae	--	--	--	19.6	--	0.2	10.2	12.8	194.5
				(10:1)		(26:1)	(28:1)	(26:1)	(10:1)
Algal fines	--	--	--	--	--	--	--	1.3	--
								(44:1)	
Macrophytes	--	--	--	--	--	--	--	--	52.0
									(11:1)
Aquatic bryophytes	--	37.8	7.7	--	--	--	--	--	--
		(18:1)	(14:1)						
Total autotrophic	3.0	42.1	10.0	21.0	7.5	4.1	31.3	17.4	246.5
	(8:1)	(18:1)	(13:1)	(9:1)	(8:1)	(10:1)	(22:1)	(29:1)	(10:1)

†Carbon assumed to be 45% of AFDM (Simon et al. 2004).

Table 5. Ecosystem metabolism estimates for the nine reaches.

	Forest			Agriculture			Urban		
Variable†	Oak-upper‡	Mack	Potts	Oak-middle‡	Camp	Courtney	Oak-lower	Amazon	Periwinkle‡
GPP	0.45	0.21	0.28	1.03	0.32	3.03	0.83	2.83	11.76
O_2 loss	-0.99	-4.78	-14.34	-0.98	-4.89	-4.04	-6.92	-4.87	-9.85
ER_{nitcor}	-0.91	-4.69	-14.29	-0.57	-4.77	-3.37	-6.87	-4.83	-9.74
GPP/ER_{nitcor}	0.49	0.04	0.02	1.82	0.07	0.90	0.12	0.59	1.21

†GPP = gross primary production, O_2 loss = uncorrected ER, ER_{nitcor} = ER corrected for O_2 consumed by nitrification (2 moles O_2 consumed per mole of N). Units are g O_2 m^{-2} d^{-1}.

‡One-station method; two-station method for all other reaches.

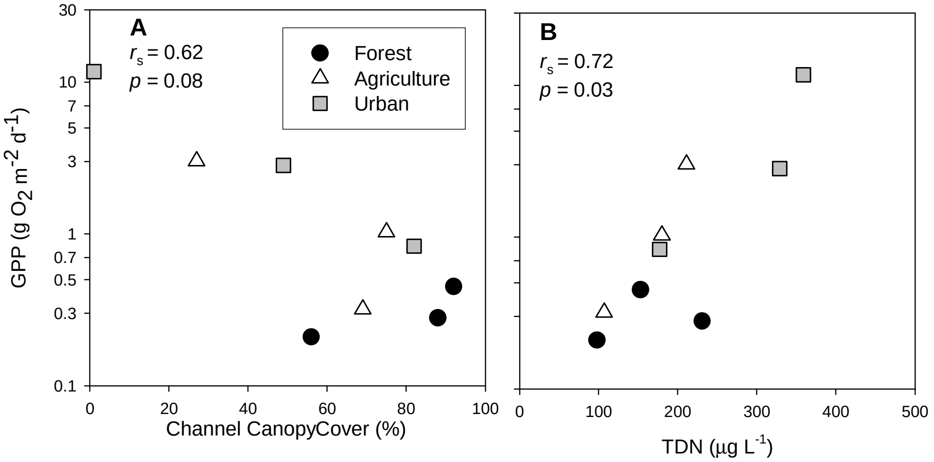


Fig. 3. Gross primary production versus (A) canopy cover and (B) TDN. r_s = Spearman rank correlation coefficient.

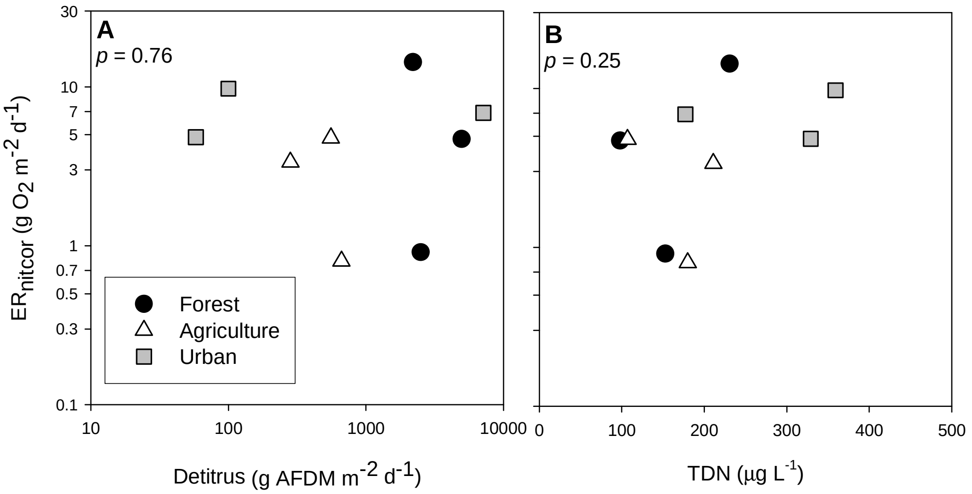


Fig. 4. Ecosystem respiration corrected for nitrification (ER_{nitcor}) versus (A) detritus and (B) TDN.

GPP ranged from 0.21 to 11.76 g O_2 m^{-2} d^{-1} among reaches and was lowest in forest (Table 5). GPP was correlated to riparian canopy cover(r_s = 0.62; *p* = 0.08; 7 d.f.) and TDN (r_s = 0.72; *p* = 0.03; 7 d.f.) (Fig. 3), but not DIN (*p* = 0.86). ER was corrected for O_2 consumed by nitrification by assuming 2 moles of O_2 consumed for every mole of N oxidized (Schlesinger 1997) because nitrification accounted for > 16% of oxygen consumption in several reaches (Tables 5 and 6). Nitrification-corrected ER (ER_{nitcor}) ranged from -0.91 to -14.29 g O_2 m^{-2} d^{-1} and did not correlate with detrital biomass (*p* = 0.76) or TDN (p = 0.25) (Fig. 4). Two agricultural and urban reaches exhibited P/R > 0.5, indicating that autochthonous production was the dominant organic matter source during the time of study (Table 6). The remaining reaches exhibited GPP/ER_{nitcor} < 0.49 with four < 0.12, indicating decomposition of allochthonous organic matter dominated ecosystem metabolism (Table 5).

$^{15}NO_3^-$ spiraling and mass balance

Uptake of $^{15}NO_3^-$ was observed in all reaches during at least one sampling period (midnight or noon) (Fig. 5). One complete uptake length (S_w) for tracer $^{15}NO_3^-$ within the reach length was only observed in two urban reaches (Fig. 5). While five reaches exhibited a shorter S_w during noon than midnight, only one agricultural reach exhibited non-overlapping 95% confidence intervals for S_w between sampling periods (Fig. 5). Three reaches had undefined upper 95% confidence limits for midnight while only one reach had a completely undefined S_w during noon. At one agricultural and one

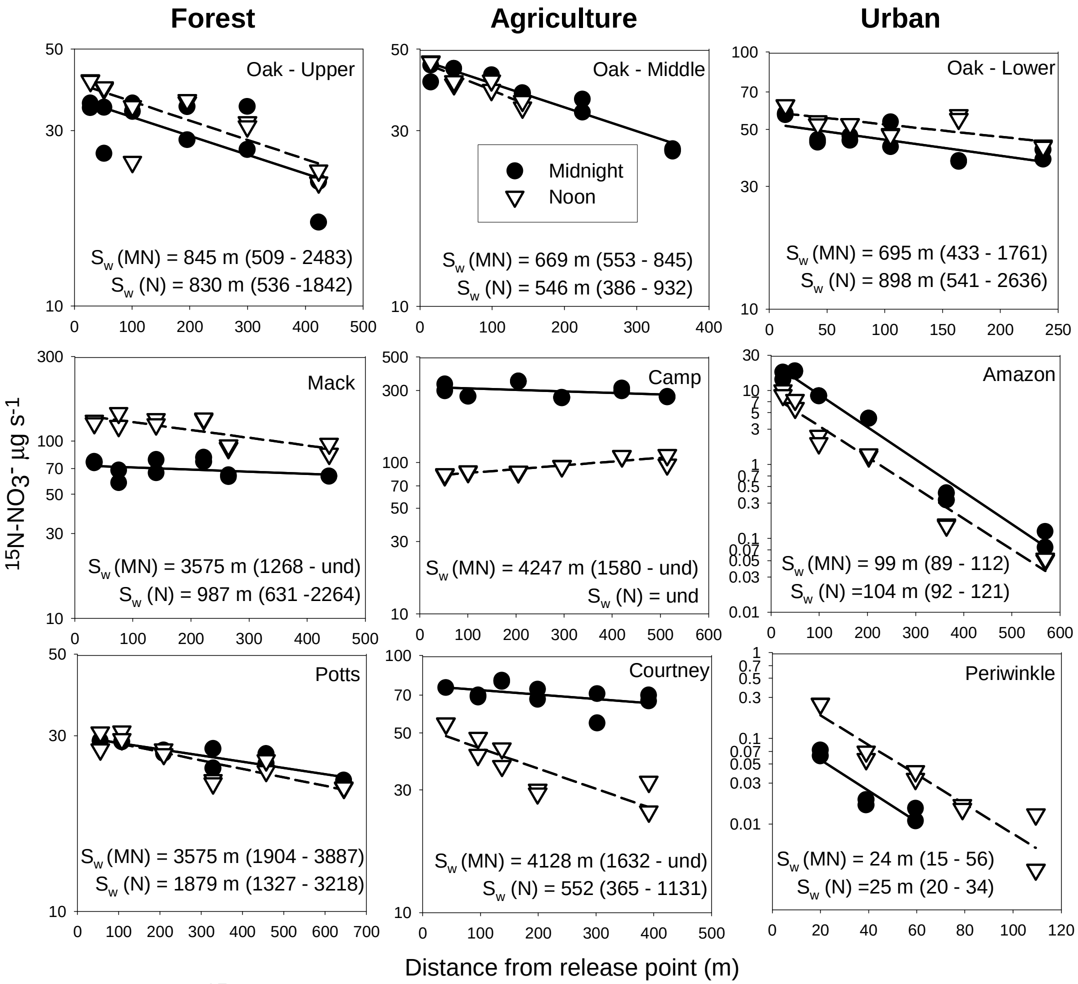


Fig. 5. Flux of $^{15}NO_3^-$ tracer downstream of release point for the nine reaches. Lines are the least-squares best fit natural log-linear regression models. Uptake lengths (S_w) of NO_3^- estimated from tracer releases for midnight (MN) and noon (N) are in each panel. Values in parentheses are 95% confidence intervals; und = positive slope estimate and S_w cannot be estimated.

urban reach, only the lower four and three sampling points, respectively, were used to estimate S_w due to irregular patterns in discharge at the lower two sampling points or minimal transport of $^{15}NO_3^-$ and the conservative tracer beyond the third sampling point. S_w (natural log) for midnight and noon increased proportionally with the natural log of velocity * depth, with an average log-log slope of 1.39 for midnight and 0.80 for noon (Fig. 6). The 95% confidence limits for both slopes included one (Fig. 6).

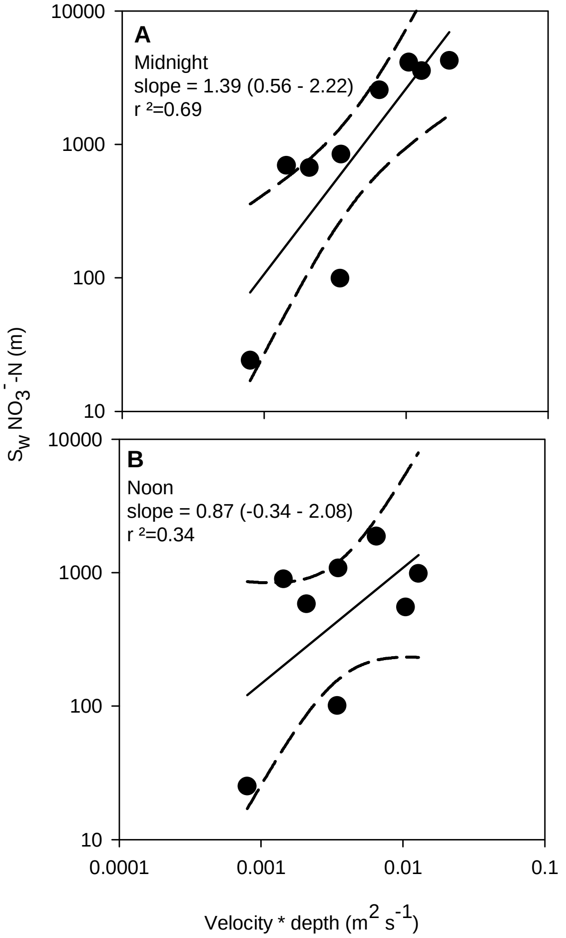


Fig. 6. Uptake length (S_w) of NO_3^- estimated from tracer releases for (A) midnight and (B) noon versus the product of stream velocity and stream depth for the nine reaches. Dashed lines are 95% confidence intervals.

NO_3^- uptake ranged from 4.5 to 35.6 μg N m^{-2} min^{-1} at midnight and 5.6 to 109.8 μg N m^{-2} min^{-1} at noon (Table 6). Uptake rates were slightly higher at noon for six reaches, though 95% confidence intervals for midnight and noon overlapped in all but one agriculture reach (Table 6). The lowest observed uptake rate occurred in an urban stream with the second-lowest NO_3^- concentration among reaches (Table 3). We could not obtain NO_3^- uptake for a noon estimate on one agriculture reach (Camp) since S_w was undefined.

Denitrification was observed in four reaches (Table 6). We did not detect tracer ^{15}N in either N_2 or N_2O at forest reaches and two urban reaches (Table 6). Denitrification was observed in all three agricultural reaches and one urban reach at midnight (Table 6). We only observed ^{15}N tracer in both N_2 and N_2O gases at noon in one agricultural (Courtney) and urban (Oak-lower) reach. Denitrification made up 4% of total NO_3^- uptake at midnight in one agricultural reach, but comprised 14 – 45% of total NO_3^- uptake in the other three reaches where denitrification was observed (Table 6). Where denitrification was measured, N_2O never accounted for more than 1.5% of total gas production (Table 6).

Nitrification rates were the same order of magnitude as NO_3^- uptake rates, ranging from 1.7 to 216.4 $\mu g\ N\ m^{-2}\ min^{-1}$ (Table 6). All three forest reaches had higher average nitrification rates at noon than midnight; this pattern was reversed at agriculture and urban reaches (Table 6). There was overlap of the 95% confidence intervals for gross NO_3^- uptake and nitrification rates (Table 6).

Table 6. Whole-system uptake of NO_3^-, denitrification, and nitrification rates estimated from tracer releases. Nitrification rates are calculated from a mass balance of NO_3^- (see text for description). Values in parentheses are 95% confidence half widths based on error estimates in the slope of the log-linear regression model of $^{15}NO_3^-$ flux versus reach distance. n.d. = not detected.

		Forest			Agriculture			Urban	
Variable†	Oak -upper	Mack	Potts	Oak -middle	Camp	Courtney	Oak -lower	Amazon	Periwinkle
NO_3^--N U (µg m^{-2} min^{-1})									
Midnight	19.1	4.5 (8.1)	10.8	18.0	14.8	20.4	35.6	5.7	13.9
	(12.6)		(3.7)	(3.8)	(24.9)	(31.1)	(21.5)	(0.6)	(7.9)
Noon	24.4	18.6	14.1	28.1		109.8	15.6	5.6	16.1
	(13.4)	(10.5)	(5.9)	(11.6)	n.d.	(56.2)	(10.3)	(0.7)	(4.3)
Denitrification (µg N m^{-2} min^{-1})									
Midnight N_2	n.d.	n.d.	n.d.	0.8	3.6	9.3	9.0	n.d.	n.d.
Midnight N_2O	n.d.	n.d.	n.d.	0.005	0.001	0.08	0.02	n.d.	n.d.
Noon N_2	n.d.	n.d.	n.d.	n.d.	n.d.	15.8	3.3	n.d.	n.d.
Noon N_2O	n.d.	n.d.	n.d.	n.d.	n.d.	0.09	0.05	n.d.	n.d.
Nitrification (µg NO_3^--N m^{-2} min^{-1})									
Midnight	8.7	10.0	5.4	62.5	19.1	216.4	18.9	5.3	34.1
Noon	20.7	19.5	9.5	1.7	n.d.	101.7	7.4	4.7	11.0

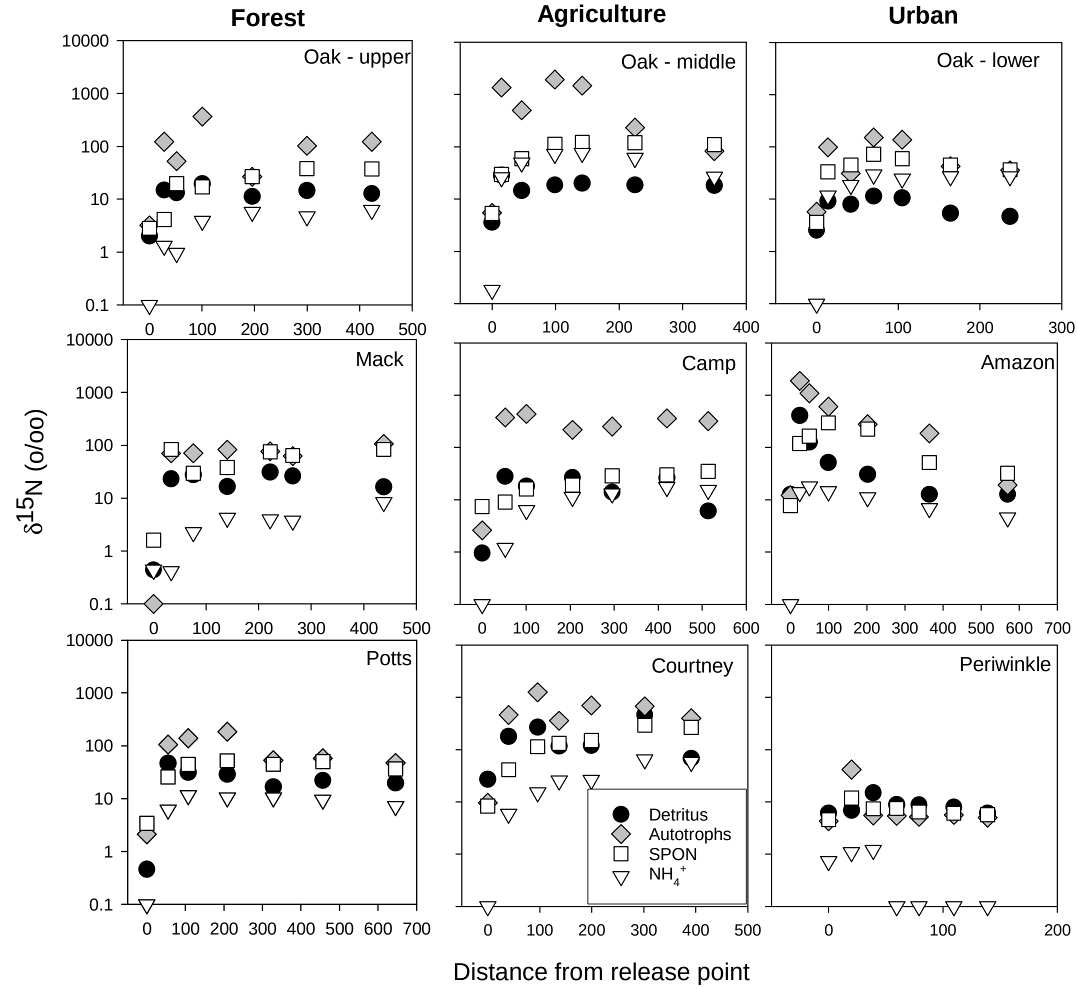


Fig. 7. Label of ^{15}N tracer in stream biomass, suspended particulate organic nitrogen (SPON), and NH_4^+ downstream for the release point 24 hr after the end of tracer releases. Values at 0 m are background δ^{15}N values. Negative δ^{15}N values were replaced with 0.1 to allow log transformation; the lowest δ^{15}N replaced among all compartments was -5‰ for NH_4^+ at the downstream four sampling points on Periwinkle Creek.

Autotrophic biomass was labeled with 100 - 1000‰ of tracer ^{15}N and generally was more labeled than detritus, SPON, or NH_4^+ (Fig. 7). The highest ^{15}N labeling of autotrophic biomass occurred at one agriculture reach (500 to 1934‰) where filamentous green algae were abundant (Table 4). Detritus was labeled with 10 to 100‰ in seven reaches (Fig. 7). Labeling of SPON was intermediate or more similar to autotrophic biomass components in all reaches. Labeling of NH_4^+ was lower than benthic biomass and SPON except for two reaches (agriculture and urban) where NH_4^+ was more highly labeled than detritus (Fig. 7). Uptake rates of benthic biomass are in Appendix B.

Percent of tracer $^{15}NO_3^-$ accounted for ranged from 68.7 to 147.1% among reaches (Appendix C). At six reaches, 85.9 to 109.1% of tracer was accounted for. We over-accounted tracer ^{15}N in two reaches (agriculture and urban) by 47.1 and 31.6%, respectively. We could not account 31.7% of tracer ^{15}N in one forest reach. Export of ^{15}N as NH_4^+ and SPON accounted for < 0.01% in all reaches and is not considered further. Likewise, we assumed DON export was negligible. Appendix D displays NH_4^+ and SPON estimates.

After standardizing reach lengths and proportionally distributing missing ^{15}N in original mass balances among biomass types, we found that 20.6 to 99.9% of tracer $^{15}NO_3^-$ was taken up in a 500-m length for eight reaches (Table 7). The largest stream in the study (Camp) was projected export 88.9% of the tracer ^{15}N as NO_3^- in 500 m (Table 7). Detritus composed the major fate of taken up ^{15}N tracer in two forest, two agricultural and one urban reach (Table 7). In seven reaches, FBOM was the largest pool of retained ^{15}N in detritus (Table 4). Small and large wood constituted 30 – 47% of ^{15}N uptake by detritus in all forest reaches and two agricultural reaches (Table 7). Uptake of ^{15}N by leaves constituted 22 - 45% of total ^{15}N uptake by detritus in two forest reaches with deciduous riparian trees (Oak-upper and Potts).

Table 7. Distribution of $^{15}NO_3^-$ tracer recovered in the nine reaches adjusted to a 500-m stream length. Error in original ^{15}N budgets is assumed to be distributed proportionally among uptake by benthic biomass and denitrification. Export of ^{15}N in the form of NH_4^+ and SPON are not considered (< 0.01% of original budgets; Appendix B).

	Forest			Agriculture			Urban		
Distribution of recovered ^{15}N (%)	Oak -upper	Mack	Potts	Oak -mid	Camp†	Courtney	Oak -lower	Amazon	Periwinkle
Retention									
FBOM	15.2	2.3	2.0	7.7	1.2	20.1	29.4	38.0	42.6
Leaves/needles	7.8	0.02	7.0	0.8	1.3	0.5	2.5	0.02	--
Small wood	6.6	1.0	5.3	3.8	2.7	0.4	0.5	--	--
Large wood	4.9	1.0	1.9	0.7	0.4	0.1	0.9	--	--
Detrital retention	34.5	4.3	16.1	12.9	5.6	21.1	33.3	38.1	42.6
Epilithon	10.4	10.6	3.2	3.7	2.4	3.6	3.0	5.3	--
Filamentous algae	--	--	--	39.1	--	0.3	1.3	46.6	34.3
Algal fines	--	--	--	--	--	--	--	9.3	--
Macrophytes	--	--	--	--	--	--	--	--	23.1
Aquatic bryophytes	--	12.8	1.3	--	--	--	--	--	--
Autotrophic retention	10.4	23.3	4.5	42.8	2.4	3.9	4.3	61.2	57.4
Total retention	44.9	27.6	20.6	55.7	8.0	25.0	37.6	99.3	99.99
Denitrification	--	--	--	0.7	3.1	15.2	9.6	--	--
Export as NO_3^-	55.1	72.4	79.4	43.6	88.9	59.8	52.8	0.7	0.01
Recovery in original budget (%)‡	68.3	95.2	89.4	147.3	99.2	85.9	109.1	131.6	99.3

†Only whole-system uptake and denitrification rates from midnight used.

‡Recovery in original budget refers to difference between observed estimates of ^{15}N released and recovered

Uptake of ^{15}N by autotrophic biomass was the dominant fate of total ^{15}N uptake in one forest, one agricultural, and two urban reaches (Table 7). Epilithon composed 69 – 100% of ^{15}N uptake by autotrophic biomass in five reaches (two forest, two agricultural, and one urban) (Table 7). Aquatic bryophytes composed 29 and 55% of total autotrophic uptake of ^{15}N two forest reaches (Table 7). Filamentous green algae made up 8 to 100% of total autotrophic uptake of ^{15}N in five reaches (Table 7). Algal fines in the matrix of filamentous green algae and vascular macrophytes only were sampled in one reach each, yet these components contribute 15 and 40%, respectively, to total autotrophic uptake of ^{15}N in these reaches. The error-corrected percent of tracer ^{15}N denitrified in the four reaches where it was measured ranged from 0.7 to 15.1% of total $^{15}NO_3^-$ released (Table 7). Denitrification composed 1 to 38.8% of total ^{15}N uptake observed in these reaches.

V_f measured at noon was strongly correlated with whole-system GPP ($r_s = 0.81$; $p = 0.01$; 6 d.f.) with agricultural (*sans* Camp) and urban reaches exhibiting higher V_f and GPP than forest reaches (Fig. 8A). V_f measured at midnight did not significantly correlate with ER_{nitcor} ($p = 0.67$) (Fig. 8B). Percent of DIN composing TDN concentration significantly increased with increasing NO_3^- concentration ($p = 0.001$; $r^2 = 0.77$) but decreased with increasing TDN concentration ($p = 0.01$; $r^2 = 0.57$) (Fig. 9A and 9B). However, average NO_3^- uptake rates between noon and midnight did not show significant trends across NO_3^- ($p = 0.11$) and TDN ($p = 0.77$) concentrations (Fig. 9C and 9D). There also was no evidence for an asymptotic response of

NO_3^- uptake to increasing NO_3^- (*p* = 0.13) or TDN (*p* = 0.77) concentration. Furthermore, standard errors for average NO_3^- uptake rates overlapped across all ranges of NO_3^- and TDN concentrations (Fig. 9C and 9D).

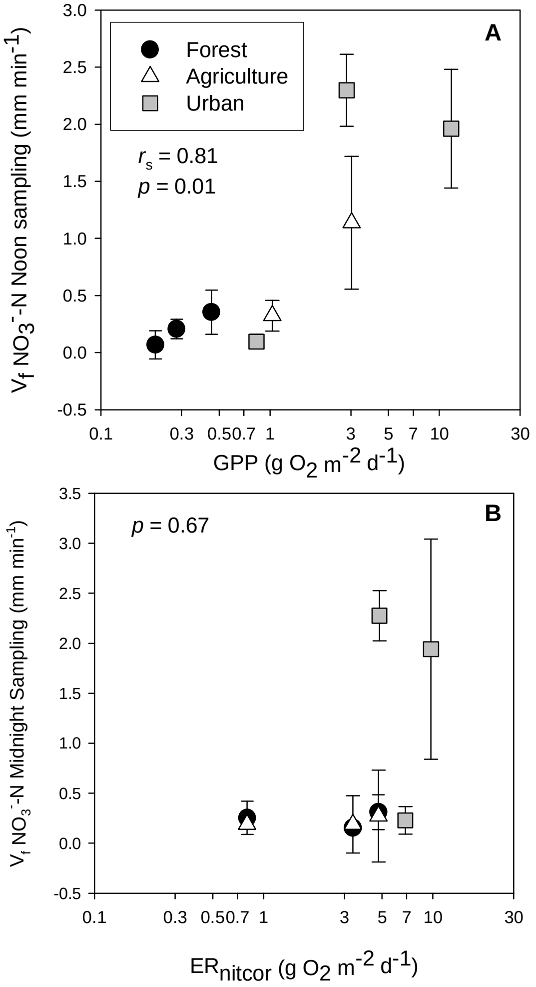


Fig. 8. (A) Comparison of observed NO_3^- uptake velocity (V_f) at noon to gross primary production (GPP) and (B) V_f at midnight versus nitrification-corrected ecosystem respiration (ER_{nitcor}). r_s = Spearman rank correlation coefficient. Bars are 95% confidence interval half-widths.

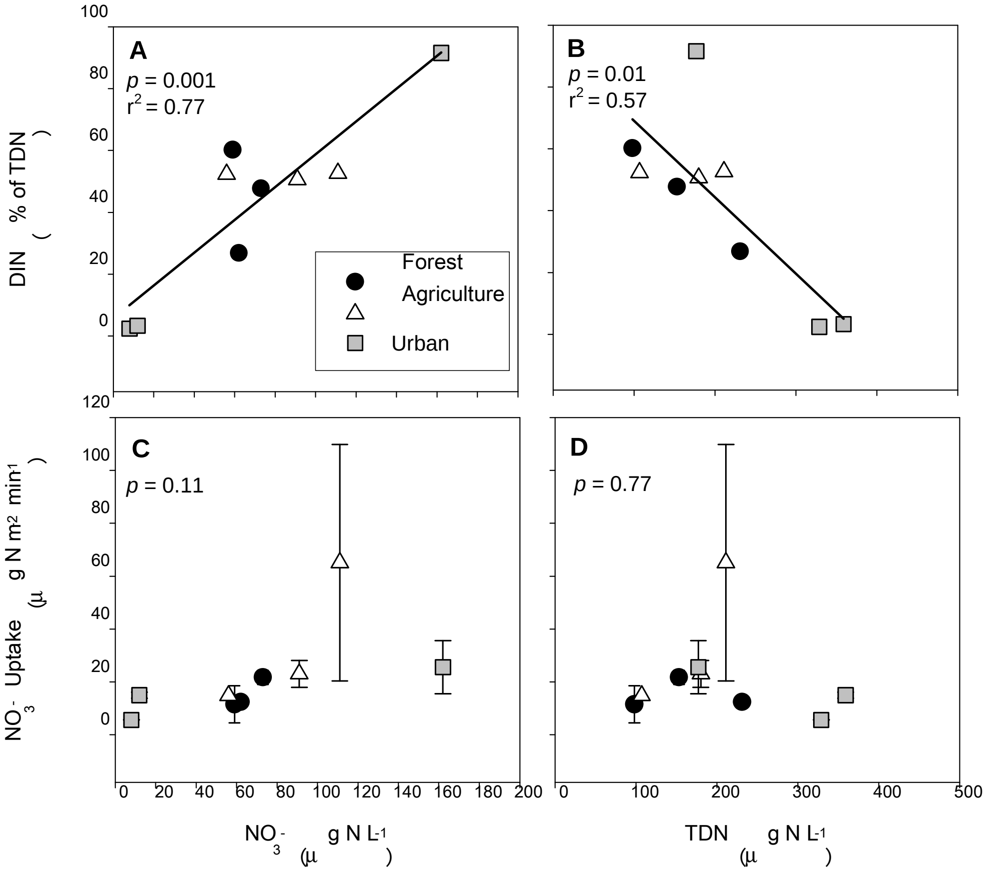


Fig. 9. Percent of dissolved inorganic nitrogen (DIN) in total dissolved nitrogen (TDN) versus (A) NO_3^- and (B) TDN and average whole-system NO_3^- uptake rate versus (C) NO_3^- and (D) TDN. Bars represent standard errors.

DISCUSSION

While the concept of small streams as efficient landscape processing sites for NO_3^- is not new (Munn and Meyer 1990; Valett et al. 1996; Peterson et al. 2001), results from this study quantify several previously undescribed patterns in the uptake and transformation of NO_3^- in stream ecosystems across a broad range of land use types. Regardless of stream water N concentration or adjacent land use practices, ambient uptake of NO_3^- in small streams during the period of low flow and relatively high biological activity (summer) was dominated by assimilation into autotrophic and heterotrophic biomass. Correspondingly, denitrification of tracer $^{15}NO_3^-$ was a small proportion of N fate in the majority of study reaches, with only three reaches exhibiting 3 -15% of tracer $^{15}NO_3^-$ denitrified. Moreover, estimates of whole-stream nitrification were not statistically differentiated that of gross NO_3^- uptake in all reaches, showing that NO_3^- was released back to stream transport nearly at the same rate it was taken up across a wide range of N concentrations and biophysical conditions. Comparisons of data from these whole-system $^{15}NO_3^-$ tracer studies with other ^{15}N tracer studies, including those employing both $^{15}NH_4^+$ and $^{15}NO_3^-$ (Tank et al. 2000; Mulholland et al. 2000; Hamilton et al. 2001; Ashkenas et al. 2004; Bohlke et al. 2004; Mulholland et al. 2004; Bernot et al. 2006; Earl et al. 2007; O'Brien et al. 2007), reveal striking similarities in uptake rates and fate of $^{15}N^-$ in small streams despite several strong differences in stream water chemistries, natural biophysical characteristics of channels and riparian zones, and patterns of surrounding land use.

Relationship between S_w and stream discharge

The relationship between uptake length (S_w) and stream discharge must first be considered when examining differences in nutrient dynamics among streams (Wollheim et al. 2001). Previous studies have shown a strong correlation between S_w and stream discharge (Peterson et al. 2001; Ensign and Doyle 2006). This correlation is not surprising since stream discharge factors into the empirical equation for determining S_w (Stream Solute Workshop 1990). The nature of the relationship between S_w and discharge is critical for making cross-system comparisons. If physical differences (i.e., width and depth) are primarily responsible for differences in S_w among different stream sizes, then a one-to-one relationship is expected between the log-log plot of S_w versus the product of stream velocity and depth (v*d) (Wollheim et al. 2001). If gradients of biological or chemical characteristics co-vary with physical differences in streams of different sizes, then the slope of log-log plot of S_w versus v*d should be significant different from one (Wollheim et al. 2001). Since the slopes of the relationship of S_w to v*d on log-log scale for both midnight and noon could not be differentiated from one base on overlap of 95% confidence intervals for (Fig. 6), physical differences largely accounted for the trend of increasing uptake length with increasing stream size among reaches. Thus, differences in uptake rates (U) and uptake velocities (V_f) of NO_3^- among our study reaches represent reach-specific effects on stream chemistry and biology (Wollheim et al. 2001).

Assimilatory fate of ^{15}N

Previous studies (Earl et al. 2006; O'Brien et al. 2007) have shown asymptotic increases of areal N uptake (U) with increasing N concentration associated with agricultural and urban land use practices, suggesting that N uptake becomes saturated with increased N loading associated with these land use practices. However, we did not find statistical support for this hypothesis (Fig. 8 and 9). In fact, U did not appear to strongly differ among all study reaches (Fig. 9) despite ranges of NO_3^- concentrations ranging two orders of magnitude (3 to 143 μg N L^{-1}) (Table 3). The main difference from the above studies and our study was range of channel and riparian zone biophysical characteristics encountered. Sites selected by Earl et al. (2006) and O'Brien et al. (2007) had relatively similar stream and riparian biophysical characteristics despite being surrounded by different land use conditions. In this study, however, selected reaches showed several profound differences in channel and riparian characteristics, including a wide range of riparian canopy cover and abundance of benthic organic matter (Table 2). Thus, rather than showing a clear asymptotic increase with NO_3^- concentration, pathways of NO_3^- uptake and transformation among our reaches either were N-limited or exhibited a response to land use effects on benthic detritus and in-stream primary production.

The similarity of U among study reaches may be partially explained by the magnitude of NO_3^- uptake by detritus in agricultural and urban streams (Table 7). Experimental tracer additions of $^{15}NH_4^+$ in eastern and western North America have demonstrated that detritus, including FBOM, leaves, and wood, dominates uptake of NH_4^+ and NO_3^- in forested stream ecosystems (Mulholland et al. 2000; Tank et al. 2000; Sanzone et al. 2001; Hamilton et al.

2001; Ashkenas et al. 2004). In this study, uptake on detritus also constituted a substantial (16 – 78% of error-corrected budgets) pathway for NO_3^- uptake in forested streams (Table 7). Yet we also found that uptake on benthic detritus constituted a substantial (23 – 61% of error-corrected budgets) pathway for tracer $^{15}NO_3^-$ uptake in streams bordered by agricultural and urban land uses, even where benthic detritus standing stocks were only 1 to 3% of that found in unmanaged forest stream reaches, primarily due to the absence of small and large wood. This runs contrary to our original expectation that contribution of benthic detritus to total U would be substantially lower in human-altered reaches than in forest reaches. Two explanations may account for this observation: (1) detrital pools sampled in highly-altered, relatively un-shaded streams likely included a substantial fraction of photoautotrophic organisms or (2) heterotrophic demand for inorganic N may have been higher in agricultural and urban stream reaches due to higher overall quality of carbon resources (indicated by C:N ratio in detritus and DOC). We cannot test these hypotheses with these data from our study, though the second hypothesis may also help explain why a clear trend of whole-system ER (nitrification-corrected) was not observed with standing stock of detritus, even if respiration by autotrophs is assumed to account for 50-70% of total ER (Webster et al. 2003).

In addition to contribution of detritus to U, our results show a substantial influence of in-stream primary production on biological demand for NO_3^- from the stream water column. V_f of NO_3^-, a proxy for biological N demand (Hall and Tank 2003), showed a strong positive correlation with whole-system GPP; this trend also has been seen in studies of forest streams using both unlabeled and tracer $^{15}NO_3^-$ (Fellows et al. 2006; Mulholland et al. 2006) (Fig. 7). Additionally, Hall and Tank (2003) showed that 75% of the variation in NO_3^- V_f was explained by GPP across 11 low N streams in Wyoming. Highest rates of GPP were seen in agricultural and urban streams likely due to combined influence of lower riparian canopy cover and higher concentrations of TDN (Fig. 3). However, U did not differ significantly across all reaches, suggesting that the uptake by autotrophic and detrital benthic biomass changed proportionally relative to stream-riparian biophysical conditions (Fig. 9).

Denitrification and nitrification

The inability to detect substantial incorporation of ^{15}N tracer into dissolved N_2 and N_2O gases in two-thirds of the study reaches suggests that denitrification was not as an important fate for NO_3^- compared to assimilatory uptake during the time of study in these streams. Other researchers using $^{15}NO_3^-$ also have observed < 16% of total NO_3^- U in streams to attributable to denitrification (Mulholland et al. 2004; O'Brien et al. 2007). But other ^{15}N tracer studies also have observed a relatively large proportion of U in small streams (50-91%) by denitrification (Bolhke et al. 2004; O'Brien et al. 2007). These contrasting findings probably are due to the interplay of three limiting factors to denitrification: carbon resources (Groffman et al. 2006), anoxic environments (Atlas and Bartha 1998), or availability of NO_3^- (Martin et al. 2001). We likely saw the influence at least two of these limiting factors: anoxic environments and availability of NO_3^-. We also originally expected abundance of detrital resources to influence denitrification rates, but we could not evaluate this hypothesis with only four reaches where denitrification was measurable, all which had similar DOC concentrations and detrital standing stocks. The only reaches where we could detect substantial denitrification included two agricultural streams that were potentially P-limited based on molar ratios of inorganic N:P ratios and one urban reach with the highest influence of transient water storage (Table 2). However, we did not measure denitrification associated with the flux of groundwater through riparian soils, which has been shown to be a “hotspot” area for this process (Hedin et al. 1998). Our tracer releases also did not encompass large enough temporal and spatial scales to provide estimates for denitrification tightly coupled with nitrification at sediment-water interfaces, which has been indicated as a major process for N removal in streams and rivers (Laursen and Seitzinger 2004) and account for a major portion of denitrification where NO_3^- is < 300 μg N L^{-1} (Seitzinger et al. 2006), which included all stream reaches in this study. Had we conducted the ^{15}N tracer releases during wet season in the Pacific Northwest (November – May), we likely would have seen much higher stream water concentrations of NO_3^-, regardless of adjacent land use (Compton et al. 2003; Evans 2007). However, it is likely that NO_3^- uptake during this time still does not represent a complete removal of N from the landscape: assimilatory uptake dominated total NO_3^- uptake when denitrification activity should have been at its peak (corresponding with peak annual temperatures; Triska et al. 2007).

Patterns of whole-system nitrification reveal two interesting aspects of this anabolic process in streams. First, nitrification rates could not be statistically differentiated from U among all reaches. This suggests that even in systems that exhibit a high demand (V_f) for N, the release of NO_3^- was similar to immobilization from the water column. This finding is nearly the same as results from another LINX 2 study region that used identical methods to estimate whole-system nitrification (Kansas; O'Brien et al. 2007). This suggests a high degree of N turnover within various spiraling compartments in the stream. These results are similar to results of tracer ^{15}N studies in forest soils that have found concurrently high gross nitrification and gross NO_3^- uptake (Hart et al. 1995; Hart et al. 1998), leading to small net rates of nitrification or NO_3^- uptake. Nitrification rates estimated from this study also were similar to rates reported by $^{15}NH_4^+$ tracer studies (Hamilton et al. 2001; Ashkenas et al. 2004), further confirming this approach to estimate whole-system nitrification. The second important aspect of whole-system nitrification observed in this study was the apparent contribution of nitrification to daily oxygen consumption, especially in two agricultural streams (Table 5). In these reaches, nitrification could account for 46 and 17% of total daily oxygen consumption. This observation may have implications for methods of estimating whole-stream metabolism (Young and Huryn 1999; Hall and Tank 2005) since the contribution of nitrification in oxygen consumption is not currently considered in estimates of ecosystem respiration.

Conclusions

Impacts of land use on the capacity of headwater stream ecosystems to influence water quality are important considerations for land managers charged with reducing N loading to downstream rivers, lakes, reservoirs, and estuaries (Alexander et al. 2007). Recent estimates suggest that first and second-order headwater streams contribute nearly 40 – 65% of N supplied to downstream water bodies (Alexander et al. 2007) and land use practices can significantly alter the capacity of these systems to retain or remove (denitrify) NO_3^- (Triska et al. 2007). The contribution of this research to understanding effects of headwater streams on downstream water quality was that the fate of tracer $^{15}NO_3^-$ in streams differed among land use settings. In streams surrounded by extensive riparian forests, small and large wood constituted a low but consistent pool (> 2%) for the ^{15}N recovered. In streams where riparian forests had been modified or removed, a substantial fraction (> 34%) of ^{15}N recovered in the stream was found in filamentous green algae blooms. While the fates differed, whole-stream NO_3^- uptake rates were similar across all reaches despite an order of magnitude differences in both NO_3^- and TDN concentrations and the wide variety of channel/riparian biophysical characteristics across forest, agricultural and urban land use settings.

CHAPTER 3

INFLUENCES OF SUBSTRATE QUALITY AND NITROGEN AVAILABILITY ON BREAKDOWN OF WOOD IN STREAMS OF WESTERN OREGON, USA

Daniel J. Sobota, Stan V. Gregory, and Peter J. Bottomley

ABSTRACT

In this study, we investigated effects of substrate nutritional quality and inorganic nitrogen (N) availability on wood breakdown in western Oregon streams. Short-term (< 2 month) breakdown rates were significantly faster for a high quality wood substrate (red alder) versus a low quality substrate (Douglas-fir). Breakdown rates for both substrates increased with dissolved inorganic N concentration (11 to 111 μg N L^{-1}) across six streams surrounded by different land uses ($p = 0.04$). A longer-term study (313 d) in a forested Oregon Cascades stream suggested effects of increased N availability on wood breakdown became apparent only after lignocellulose components of woody tissues began to decompose. There were only slightly faster breakdown rates exhibited for wood substrates enriched with N (red alder +N: $k = 0.036$; Douglas-fir +N: $k = 0.0008$) than un-enriched substrates (red alder: $k = 0.024$; Douglas-fir: $k = 0.0004$). However, microbial biofilm respiration rates and activity of two extracellular enzymes involved in the decomposition of lignocellulose showed that red alder had a greater response to N enrichment than Douglas-fir. These results support the concept that wood turnover in streams with fast-growing deciduous riparian forests is faster than streams with riparian forests composed of long-lived conifers and that increases in loading of N to fast-growing deciduous forest streams may accelerate wood breakdown rates while having lesser effect on wood breakdown in coniferous forest streams.

INTRODUCTION

Influences of wood on the structure and function of forested stream ecosystems have been well-documented for over 30 years (Swanson and Lienkaemper 1978; Beschta 1979; Harmon et al. 1986; Bilby and Ward 1989; Gregory et al. 2003). One important component of stream wood dynamics is residence time of wood in channels, which influences geomorphology and rate of carbon supply in these ecosystems (Melillo et al. 1983; Lienkaemper and Swanson 1987). Longevity of wood in streams is mediated by two factors: (1) physical processes that transport whole wood pieces, leach relatively soluble tissue constituents, and fragment pieces to smaller particles and (2) biological processes that degrade wood (Harmon et al. 1986). In large streams and rivers, physical processes largely control residence time of wood in streams. Linkages to upstream sources, spatial arrangement of large wood jams, and lateral transport on adjacent floodplains are all important factors in determining wood turnover rates in these systems (Nakamura and Swanson 1994; Piegay et al. 1999). However, in smaller headwater streams where stream transport capacity is much lower, physical leaching and fragmentation (Ward and Aumen 1986) and biological decomposition (Melillo et al. 1984) (collectively referred to in this paper as “breakdown”) have stronger influence on wood turnover rate.

A large body of evidence shows that substrate quality is a strong influence on wood breakdown rate in small streams (Anderson et al. 1978;

Melillo et al. 1983; Harmon et al. 1986; Spänoff and Meyer 2004). In a review of factors controlling plant litter decomposition in northern streams, Melillo et al. (1984) concluded that initial litter quality, specifically lignin content, was the dominant factor determining the rate of wood breakdown. As an example, they showed a strong inverse relationship between first-order decay rates of five wood species and initial lignin or initial lignin-to-initial nitrogen (N) content in two northern Quebec streams (Melillo et al. 1983; Melillo et al. 1984). This relationship and other similar relationships for breakdown of leaf litter in streams are hypothesized to result from decreases in relatively labile carbon (C) resources (proteins, simple sugars) available for use by heterotrophic bacteria and fungi (Melillo et al. 1984; Gessner and Chauvet 1994).

Availability of N in stream water also may be a key factor regulating the breakdown of wood in headwater streams (Aumen et al. 1985; Golladay and Webster 1988; Tank and Webster 1998; Gulis et al. 2004). In a comparison of wood breakdown of three different wood size classes in two southern Appalachian hardwood forest streams, Golladay and Webster (1988) hypothesized that stimulation of microbial biofilm activity by increased N supply was responsible for faster wood breakdown in all size classes in the stream with highest nitrate (NO_3^-) concentrations. In another southern Appalachian stream, addition of dissolved inorganic N (DIN) by two orders of magnitude greater than background levels (24 μg N L^{-1}) resulted in a two-to-tenfold increase in mass loss rates for manufactured and natural wood substrates and a threefold increase in the standing stock of wood biofilm fungal biomass (Gulis et al. 2004). Additions of 1-10 mg NO_3^--N L^{-1} stimulated mineralization of ^{14}C-labeled lignocellulose in wood of a conifer species (Douglas-fir, *Pseudotsuga menziesii*) for up to 30 days following amendments in a laboratory microcosm study (Aumen et al. 1985).

While these studies provide compelling evidence for a direct influence of inorganic N supply on wood breakdown in streams, several important aspects remain unexplored. First, while the response of decomposition of leaf litter with different nutritional qualities (indicated by lignin and N content) to N enrichment has been well-studied (e.g., Triska and Sedell 1976; Gulis and Suberkropp 2003), fewer studies have directly compared the response of different wood substrate qualities to N enrichment. In fact, we are aware of only one study that has investigated the interactive effects of substrate quality and stream nutrient concentration on wood breakdown rates in streams. In a laboratory microcosm, Melillo et al. (1984) stimulated breakdown of alder (13% lignin) wood shavings, but not spruce (25% lignin) shavings, by raising external phosphorus concentrations from 5 to 25 μg P L^{-1}. The response of wood breakdown in streams in response to N enrichment has not been well investigated. Melillo et al. (1984) proposed four distinct phases in C and N accrual and release from decomposing wood: uptake of soluble carbohydrates and phenolics by colonizing wood biofilms (phase I; immobilization), mineralization of cellulose and lignin constituents (phase II; mineralization), recondensation of lignin degradation products with inorganic N or nitrogenous compounds contained in microbial exoenzymes (phase III; immobilization), and degradation of these humification products by microbial processing (phase IV; mineralization). This model provides a useful framework for predicting response of different wood substrate qualities to N enrichment, though we are not aware of specific evaluations through manipulative experiments. Moreover, this model does not consider interaction with physical fragmentation or leaching processes which may account for a significant fraction of mass loss (Ward and Aumen 1986).

In this study, we evaluated the influence of inorganic N availability on decomposition of high- and low-quality wood substrates in streams in western Oregon. Our specific questions were (1) do increases in stream water concentration of inorganic N correspond with increases in rates of wood decomposition? (2) Does initial wood substrate quality alter responses of wood decomposition to inorganic N availability? (3) Does the rate of wood decomposition change through time in response to increased N? We evaluated these questions in two separate experiments. In the first experiment, wood substrates from Douglas-fir (*Pseudotsuga menziesii*; low nutritional quality) and red alder (*Alnus rubra*; high nutritional quality) were incubated for three-to-six weeks in six different streams in western Oregon with a gradient of inorganic N concentrations. In the second experiment, wood substrates from the two species were exposed to either high or low N concentrations over ten months in a single stream with low natural concentrations of inorganic N.

SITE DESCRIPTIONS

Six second- and third-order streams in the Willamette River Basin of western Oregon were selected for the first field experiment (Fig. 10; Table 8). The climate is Mediterranean with warm, dry summers (June – September) with mean air temperature of 19ºC and cool, wet winters with a mean temperature of 4 ºC (Taylor and Hannan 1999). Annual precipitation ranges from 1000 – 2200 mm with most falling as rain from October through May (Taylor and Hannan 1999). This study was conducted during summer (June-July), which corresponds to low flow on stream hydrographs in this region (Jones and Grant 2000). Two streams each were located adjacent to urban areas, agricultural fields, or forest (old-growth and second-growth). Riparian vegetation bordering urban streams consisted of mixed ornamental trees, shrubs, and lawns. Agricultural streams were bordered by a 12-m riparian buffer of native tree and shrub species. One forested stream (Mack Creek) was surrounded by a 500-year old Douglas-fir forest while the other was located in a 40 – 60 year-old second-growth riparian forest dominated by red alder, a prolific N-fixer (25 – 100 kg N ha^{-1} yr^{-1}) (Binkley et al. 1994).

Mack Creek, a third-order stream draining a 640 ha basin in the central Oregon Cascades, was the site for the second field experiment. Stream flow varies seasonally, with high flows occurring from late October through May and gradually-declining low flow occurring from June through late September/October (Fig. 11A). During the study period, mean daily discharge ranged from a low of 9 L s^{-1} in early October 2006 to a high of 2,016 L s^{-1} in the middle of December 2006 (Fig. 11B). Stream temperature also exhibits a strong seasonal pattern (Fig. 11A). Mean daily temperature ranged from a high of 15.1ºC in August 2006 to a low of 2.1ºC in January 2007 (Fig. 11A).

Previous studies in Mack Creek have found primary production to be N-limited (Gregory 1980). Based on proportional water samples collected every three-to-four weeks, concentrations of NO_3^- ranged from 33 to 116 μg N L^{-1}; NH_4^+ ranged from 1 to 25 μg N L^{-1}; and total dissolved N (TDN; NO_3^- + NH_4^+ + dissolved organic N (DON)) ranged from 70 to 200 μg N L^{-1} (Fig. 11B). Concentrations of other biologically-important solutes (dissolved organic carbon (DOC), soluble reactive phosphorus (SRP), and total soluble phosphorus (TSP)), were relatively stable. DOC concentrations ranged from 0.60 to 1.29 mg C L^{-1}; SRP ranged from 5 to 12 μg P L^{-1}, and TSP ranged from 12 to 30 μg P L^{-1} (H.J. Andrews Experimental Forest; http://www.fsl.orst.edu/lter/). Stream water pH was close to neutral, ranging from 7.3 to 7.5 over the entire study period. Molar ratios of dissolved inorganic N (NO_3^-+NH_4^+) to SRP ranged from 12:1 to 42:1 with an average of 24:1 while ratios of TDN to TSP ranged from 8:1 to 22:1 with an average of 15:1 (H.J. Andrews Experimental Forest; http://www.fsl.orst.edu/lter/). Benthic organic matter in Mack Creek is dominated by conifer wood > 5 cm in diameter, with > 99% of organic matter in the form of large boles, branches, and rootwads (Ashkenas et al. 2004).

Table 8**.** Physiographic, riparian, hydrologic, and in-channel wood attributes of the six study sites used in surveys of short-term wood breakdown in the Willamette River basin, Oregon.

Study site	Geographic coordinates	Dates of Study	Riparian forest type‡	Riparian Cover (%)↑	Discharge (L s^{-1})	Temperature (ºC)	Stream wood (m^3 ha^{-1})
Forest							
Mack Creek	44º13′00″N 122º10′57″W	19 July & 9 Aug – 1 Sep 2004†	C	67	30.7	13 (11 – 15)	489
Potts Creek	44º16′50″N 122º29′24″W	5 July – 30 July 2005	D	87	19	13 (11 – 15)	7
Agricultural							
Camp Creek	44º07′55″N 122º49′12″W	30 June – 30 July 2004	Mixed C/D buffer strip	29	118.8	16 (11 – 24)	11
Courtney Creek	44º22′49″N 122º58′03″W	28 June – 15 July 2005	D buffer strip	11	38.7	19 (16 – 28)	3
Urban							
Amazon Creek	44º03′38″N 123º06′46″W	16 June – 8 July 2004	None	16	22.8	21 (15 – 27)	0
Periwinkle Creek	44º37′10″N 123º05′46″W	30 July – 30 August 2005	None	1	2.7	23 (20 – 28)	0

† Two separate sets of wood substrates incubated on stream

‡ C = coniferous; D =deciduous

↑ Riparian cover is average of 11-15 transect measures each consisting of five measures with a concave spherical densitometer laterally spaced at: center of the channel (1 measurement), 12.5 later distance from channel (2), and 25 m lateral distance from the channel (2).

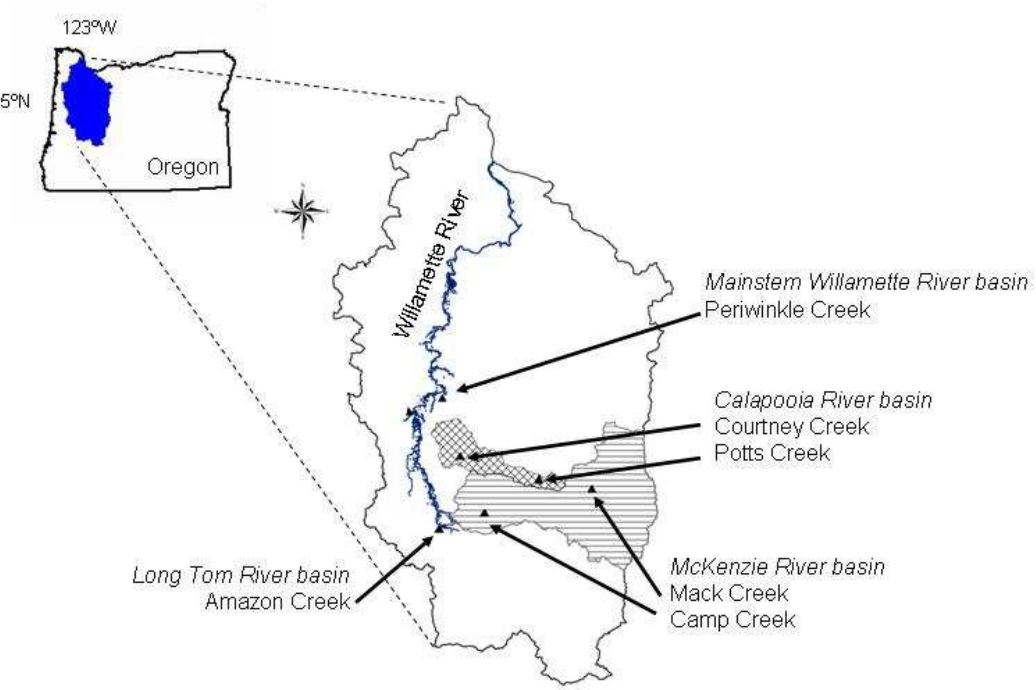


Fig. 10. Location of six study sites used in surveys of short-term wood breakdown in the Willamette River basin, Oregon, USA.

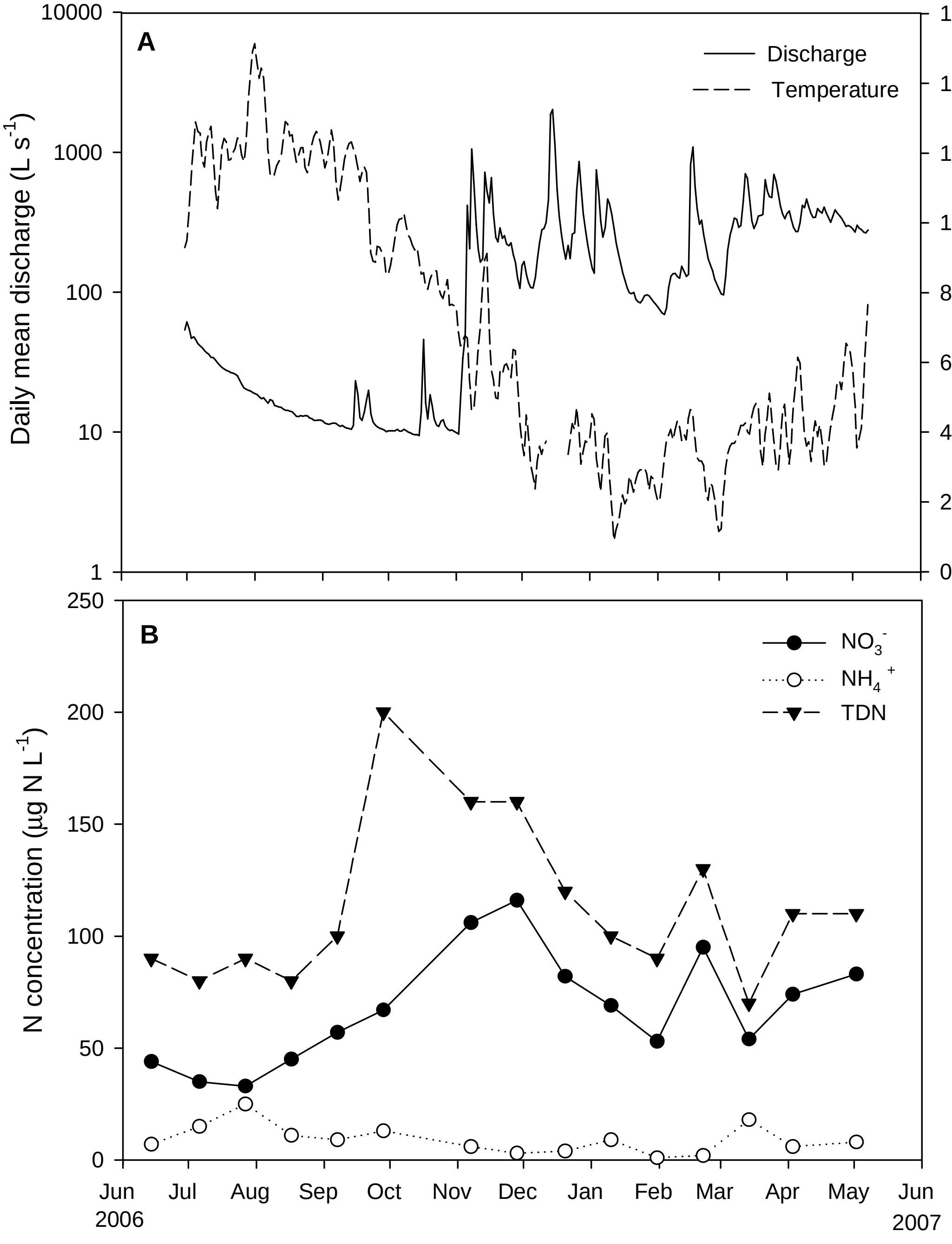


Fig. 11. (A) Mean daily discharge and temperature and (B) stream water N concentrations during the wood incubation experiment on Mack Creek from 30 June 2006 to 8 May 2007. TDN = total dissolved nitrogen, which includes NO_3^-, NH_4^+, and dissolved organic N (DON).

METHODS

Experiment 1

In the summers of 2004 and 2005, ten replicate substrates (10 cm long; 2.5 cm wide; 5 mm thick) of Douglas-fir (low quality) and red alder (high quality) were in on each of the six streams for 17 to 44 d (Table 8). Substrates were made from kiln-dried milled lumber. The molar C/N of Douglas-fir substrates was 542±1 (mean±standard error) while red alder was 210±1 determined on a Europa Scientific Roboprep C/N analyzer at the Stable Isotope Research Unit, Oregon State University, Corvallis, Oregon. Lignin content of Douglas-fir was approximately 26-28% and red alder was approximately 13% based on published values (Melillo et al. 1983; Harmon et al. 1986).

Following incubation on each stream, substrates were returned to the laboratory on ice and processed within 24 – 48 hr. Individual substrates were partitioned into subsamples for mass loss, microbial biofilm respiration, and chlorophyll *a* standing stocks. Mass loss rates were determined by solving for mass loss rate (k; units of d^{-1}) in a negative exponential decay model:

$$(4) \qquad k = \ln(N_t/N_0)/-t$$

Where t is time (d), N_t is % ash-free dry mass (AFDM) remaining relative to a control (determined from triplicate subsamples of non-incubated wood), and

N_0 is original AFDM for each wood type. *k* was solved for individual samples and then averaged according to substrate type and stream location.

Biofilm respiration was measured as the change in dissolved oxygen (O_2) over time at 15ºC. A known portion of each sample was placed in a 65-ml glass bottle. Bottles were filled with filtered stream water, acclimated to the atmosphere at 15ºC for 1 - 2 hr, and incubated sealed for 4 - 12 hrs. All incubations occurred in the dark to prevent photosynthesis. Initial and final O_2 concentrations (mg O_2 L^{-1}) were measured with a calibrated Orbisphere 2607 oxygen sensor (Orbisphere Laboratories, Geneva, Switzerland). Two to three filtered water blanks served as controls. Respiration was calculated as the control-corrected change in O_2 mass per time. We expressed respiration both as per area and as per mass of substrate.

Chlorophyll *a* of wood subsamples (frozen for < 30 d) was extracted with hot ethanol (Sartory and Grobbelar 1984). Absorbance of extract was measured at 665 and 750 nm (initial and acidified with 0.1 N HCl) on a HP 8452 diode array spectrophotometer (The Hewlett-Packard Company, Palo Alto, California, USA). Water samples for dissolved N were collected at the time of substrate collection and filtered through a Whatman GF/F. Samples were analyzed for NO_3^- (cadmium reduction method), NH_4^+ (phenate method), and TDN (persulfate digestion followed by cadmium reduction) (APHA 2005) (all analyses performed at the Central Cooperative Analytical Laboratory, Corvallis, Oregon). Soluble reactive phosphorus (SRP) (ascorbic acid method) and total soluble phosphorus (TSP) (persulfate digestion followed by ascorbic acid method) also were measured (APHA 2005). Hourly temperature on each reach during incubations was measured with a Stowaway water temperature logger (Onset Corporation, Bourne, Massachusetts, USA; resolution of ± 0.2ºC).

Experiment 2

Red alder and Douglas-fir substrates were incubated for 313 days beginning on 28 June 2006 and ending 8 May 2007 in Mack Creek. Six locations 40-m apart were selected within a randomly selected 200-m reach on Mack Creek (Appendix E). At each location, paired sets of red alder and Douglas-fir (eight replicates each species) were submerged. Substrates of each species fastened on top of 5% agar gel plates (Bacto^{TM} Agar Lot 6080254, Becton, Dickinson, and Company, Sparks, Maryland, USA). Half of the plates were made with 1 M KNO_3^- solution while half were made with distilled water. Laboratory incubations confirmed that 1 M KNO_3^- plates released 5.1% (95% confidence interval of 1.6 to 8.6%; 6 d.f.) of N remaining in plate per day for five months. Controls contained < 4 μg N L^{-1} (NO_3^- and NH_4^+). Plates were replaced with fresh gels at each collection. Each substrate by N treatment was collected on 1 August 2006 (32 d), 14 September 2006 (75 d), 16 November 2006 (135 d), and 8 May 2007 (313 d). For August and September, four stations were sampled; due to losses in high flow, only three stations were sampled in November and May.

Substrates were placed on ice and subsampled for assays of microbial biofilm respiration, activity of two enzymes involved in the degradation of woody tissues, chlorophyll *a*, and mass loss within 48 hr of collection. Biofilm respiration and chlorophyll *a* standing stocks were assayed according to the first experiment. The enzymes β-1,4-glucosidase (Enzyme Commission (EC) number 3.2.1.21) and phenol oxidase (EC 1.10.3.2 and 1.14.18.1), were assayed using methods described in Sinsabaugh et al. (1992). Substrate samples were divided into three analytical replicates plus one control. Each replicate was submerged in 2 mL of 5 mM enzyme substrate dissolved in 50 mM acetate buffer at pH 5.0 (β-1,4-glucosidase: p-nitrophenyl-β-D-glucopyranoside; phenol oxidase: L-dihydroxyphenylalanine (DOPA); 50 mM acetate buffer for controls). Replicates were sonicated and incubated on a shaker table at 15ºC for 1 hr. Incubated replicates were centrifuged and β-glucosidase samples were fixed with 0.2 mL of 1 N NaOH and diluted to 10 mL with distilled water. Absorbance at 410 nm quantified β-glucosidase and absorbance at 460 nm quantified phenol oxidase. Activities were expressed as μmol of substrate digested corrected for wood substrate and enzyme substrate controls. We also quantified enzyme activity on non-incubated substrates. Assimilatory efficiency was indexed by dividing activity of measured enzymes per mass by respiration per mass, reasoning that production of these enzymes expressed biosynthesis (Sinsabaugh et al. 1993) while respiration was loss of C to maintenance. We assumed all respiration was attributable to heterotrophs though previous studies indicate ~50% of respiration on wood in Mack Creek is attributable to photoautotrophs (Sabater et al. 1998). While this proportion likely changes over time and differs among substrates, for simplicity we assumed that the proportion of photoautrophic respiration was constant across substrates and N treatments. It also was possible that respiration could have been due to uptake of organic C from the water column, but we could only assume that this was a constant fraction across substrates and N treatments.

Statistics

In the first experiment, each response variable (*k*, respiration, and chlorophyll *a*) was regressed on mean temperature, DIN concentration, and atomic ratio of DIN:SRP with a species (Douglas-fir or red alder) interaction term. Variables were natural log transformed if values ranged over two orders of magnitude. Multiple regression models were evaluated with backwards extra-sum-of squares *F* tests (Ramsey and Schafer 1999). Variables were dropped if $p > 0.05$. In second experiment, breakdown rates were estimated from the slope of the regression model for AFDM remaining versus the time period of the incubation (Webster and Benfield 1986). Comparison of 95% confidence intervals of regression slopes for each species by treatment assessed differences in breakdown rates (Ramsey and Schafer 1999). We also compared relative effects of species, N treatment, and incubation time on respiration, chlorophyll a, AFDM, extracellular enzymes, and assimilatory efficiency index for substrates collected at individual sites in the experimental reach (Appendix F). We used this approach to remove site-specific effects (e.g., localized groundwater upwelling, exposure to different stream velocity profiles, etc.). We specifically compared natural logged ratios of ambient red alder to Douglas-fir (R/D) to N-treated substrates (R_N/D_N) in two-way ANOVA models (treatment by incubation time). We also compared D_N/D to R_N/R over time. Significant ($p < 0.05$) differences were assessed with Tukey's multiple comparison procedure (Ramsey and Schafer 1999). Statistics were calculated in R 2.4.0 (R Development Core Team 2006).

RESULTS

Experiment 1

First-order breakdown rates (*k*) ranged from a low of -0.0002 ± 0.0016 d^{-1} (mean ± standard error) for Douglas-fir in Courtney Creek (agricultural) to a high of 0.009 ± 0.0014 d^{-1} in Camp Creek (agricultural) (Table 9). Average breakdown rate was higher for red alder than Douglas-fir in all streams (Table 9). However, differences were only significant for agricultural streams and Set 1 on Mack Creek ($p<0.05$).

Among all sites, chlorophyll a standing stocks ranged from 0.41 ± 0.09 mean μg chlorophyll *a* cm^{-2} on red alder in Camp Creek (agricultural) to 3.10 ± 0.41 μg chlorophyll *a* cm^{-2} on red alder in Courtney Creek (agricultural). Douglas-fir had significantly higher standing stocks of chlorophyll *a* than red alder in Mack Creek (Set 2; forest), Potts Creek (forest), and Amazon Creek (urban). However, differences were not consistent within or among sites, with chlorophyll *a* standing stocks on each species not significantly different for the three other streams and Set 1 on Mack Creek.

Respiration rates expressed as per area and as per mass showed similar ranges for both species except in Potts Creek (forest), where respiration on red alder substrates was two times greater than Douglas-fir (Table 10). Expressed per area, respiration rates ranged from not detectable on red alder in Periwinkle Creek (urban) to 1.54 ± 0.15 μg O_2 cm^{-2} hr^{-1} for red alder in Potts Creek (Table 9). Expressed per mass, respiration rates ranged from not detectable for red alder on Periwinkle to 115.9 ± 8.5 μg O_2 g $AFDM^{-1}$ hr^{-1} for red alder in Potts (Table 9).

N and P concentrations varied by an order of magnitude among sites (Table 9). Sampling of DIN one week prior to collections suggested that water chemistry remained similar throughout the incubation period (data not shown). DIN ranged from 8 μg N L^{-1} in Amazon Creek (urban) to 111 μg N L^{-1} in Courtney Creek (agricultural) (Table 9). DON concentrations were lowest in Mack Creek (forest) and highest in urban streams, ranging from 321 to 347 μg N L^{-1} (Table 9). SRP ranged from 5 μg P L^{-1} in agricultural streams to 209 μg P L^{-1} in one urban stream (Periwinkle). TSP followed a similar pattern to SRP concentrations (Table 9). DIN/SRP molar ratios ranged from 0.1 to 49.1.

Table 9. Short-term decay rates (*k*), respiration rates, and chlorophyll *a* standing stocks for Douglas-fir and red alder wood substrates and stream water chemistry on the six study streams. Values in parentheses are standard errors.

	Substrate sample size (*n*)		Decay rates (d^{-1})		Chlorophyll *a* ($\mu g\ cm^{-2}$)	
Study site	Douglas-fir	red alder	Douglas-fir	red alder	Douglas-fir	red alder
Forest						
Mack Creek†						
Set 1	10	8	0.0005 (0.0008)	0.0084 (0.0015)	2.68 (0.62)	2.84 (0.95)
Set 2	10	10	0.0052 (0.0012)	0.0060 (0.0014)	1.22 (0.28)	0.50 (0.07)
Potts Creek	10	10	0.0030 (0.0012)	0.0081 (0.0015)	2.72 (0.65)	0.71 (0.15)
Agricultural						
Camp Creek	10	10	0.0037 (0.0010)	0.0090 (0.0014)	1.19 (0.47)	0.41 (0.09)
Courtney Creek	10	10	-0.0002 (0.0016)	0.0074 (0.0015)	2.55 (0.19)	3.10 (0.27)
Urban						
Amazon Creek	10	10	0.0004 (0.0011)	0.0039 (0.0009)	0.96 (0.10)	0.56 (0.08)
Periwinkle Creek	10	10	0.0012 (0.0008)	0.0022 (0.0007)	0.51 (0.17)	0.85 (0.21)

† Set 1 = 44 d; Set 2 = 23 d

‡ Temperatures only for two days prior to substrate collection.

Table 9. Continued.

	Respiration rates (μg O_2 cm^{-2} hr^{-1})		Respiration rates (μg O_2 g $AFDM^{-1}$ hr^{-1})		Water chemistry (μg L^{-1})				
Study site	Douglas-fir	red alder	Douglas-fir	red alder	NO_3^--N	NH_4^+-N	DON	SRP	TSP
Forest									
Mack Creek†									
Set 1	0.30 (0.04)	0.38 (0.03)	20.9 (2.7)	27.1 (2.6)	57	2	39	13	24
Set 2	0.44 (0.02)	0.42 (0.05)	32.5 (1.2)	23.6 (2.8)	57	2	39	13	24
Potts Creek	0.78 (0.06)	1.54 (0.15)	46.7 (3.4)	115.9 (8.5)	57	5	169	25	38
Agricultural									
Camp Creek	0.19 (0.02)	0.21 (0.02)	14.2 (1.5)	14.2 (2.0)	50	6	51	5	14
Courtney Creek	0.31 (0.04)	0.58 (0.05)	17.2 (2.7)	38.4 (3.2)	100	11	100	5	21
Urban									
Amazon Creek	0.35 (0.05)	0.29 (0.02)	19.7 (1.9)	18.7 (2.8)	3	5	321	18	45
Periwinkle Creek	0.12 (0.08)	-0.23 (0.07)	6.1 (4.5)	-16.3 (5.0)	8	4	347	209	258

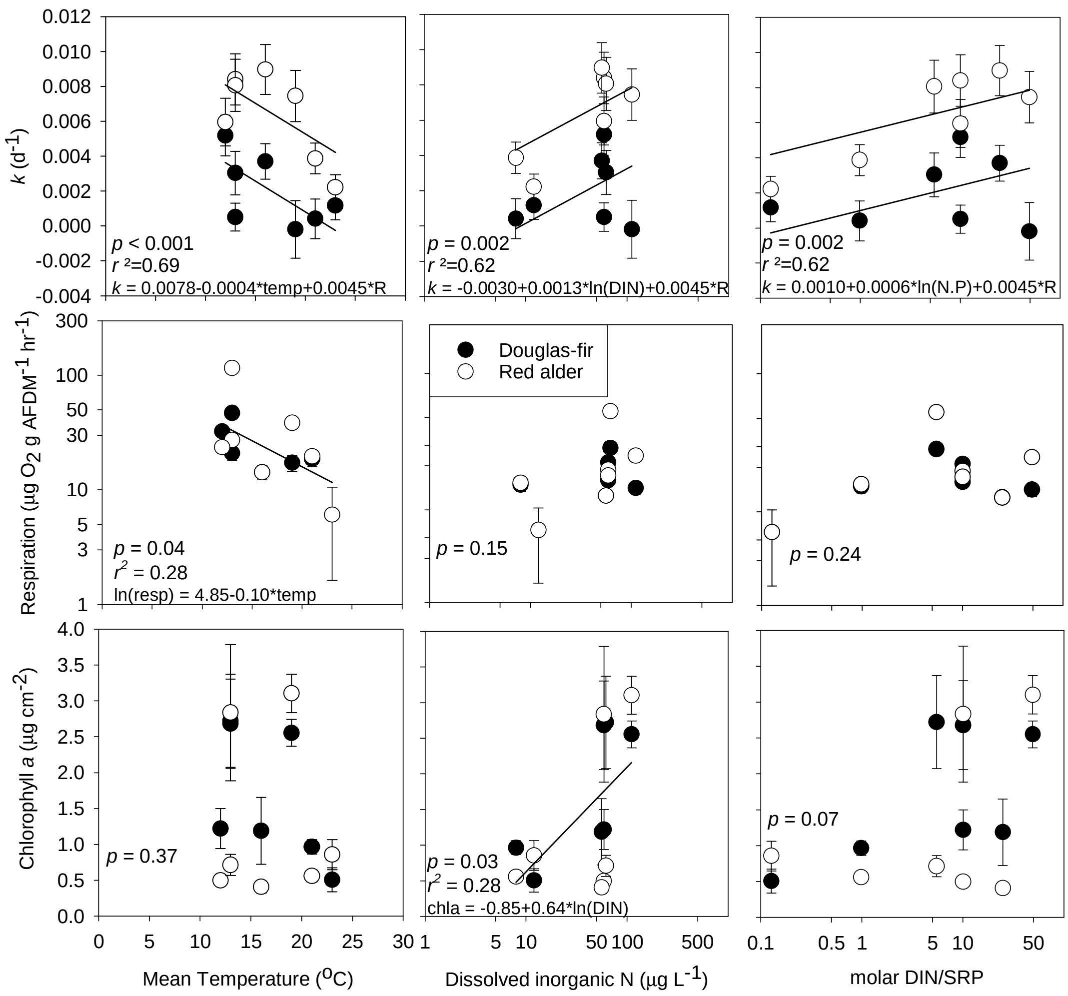


Fig. 12. Means of short-term wood breakdown rates (*k*), respiration rates (resp), and chlorophyll *a* (chla) versus mean temperature during incubation (temp), dissolved inorganic N concentration (DIN), and molar DIN/SRP (N.P). Error bars represent standard errors. Regression models were selected through a backwards elimination procedure; R = indicator variable (0 or 1) denoting species (Douglas-fir=0; red alder=1) (Appendix F).

Only short-term breakdown rates showed significant correlations to mean temperature, DIN, or DIN/SRP and differences according to species substrate type (Fig. 12; Appendix F). Across the range of each of these explanatory variables, red alder had a breakdown rate of 0.0045 ± 0.0010 d^{-1} greater than Douglas-fir (Fig. 12). Breakdown rates declined with increasing temperature and increased with increasing DIN and DIN/SRP (Fig. 12). For respiration and chlorophyll *a*, there were only two statistically-significant regression models: mean respiration rate declined exponentially with increasing mean stream temperature and chlorophyll *a* standing stocks increased asymptotically with increasing stream DIN levels (Fig. 12). There was no evidence for differences among species types for both of these models ($p > 0.30$) (Appendix F). There also was no evidence for species-specific responses to any explanatory variable for all models ($p>0.10$) (Appendix F).

Experiment 2

Decomposition of substrates during the 313-d study on Mack Creek had significant differences among species type, N treatments and incubation time (Fig. 13 – 16). Overall, red alder showed the fastest breakdown rates (red alder: $k = 0.0024$ (ambient) – 0.0036 (+N); Douglas-fir: $k = 0.0004$ (ambient) – 0.0008 (+N)), higher respiration rates, and greater extracellular enzyme activity (Fig. 13 and 14). Highest standing stock of chlorophyll *a* occurred on Douglas-fir +N substrates in September (2.02 ± 0.78 μg cm^{-2}) while the lowest were on red alder +N in May (0.05 ± 0.01 μg cm^{-2}). Chlorophyll *a* standard errors overlapped for all species by N treatments (Fig. 13A).

Overall breakdown rates were significantly faster for red alder than Douglas-fir ($p = 0.001$), with a nonsignificant trend for faster breakdown of N enriched substrates (Fig. 13B). Average half-life (time to 50% disappearance; Harmon et al. 1986) was: red alder, ambient = 286 d; red alder +N = 202 d; Douglas-fir, ambient = 1,824 d; Douglas-fir +N = 867 d. Accrual of AFDM occurred for Douglas-fir +N between August and September and for ambient red alder between September and November (Fig. 13B). These accruals corresponded to maximum chlorophyll *a* standing stocks and respiration rates on a per area basis (Fig. 13A and 13C).

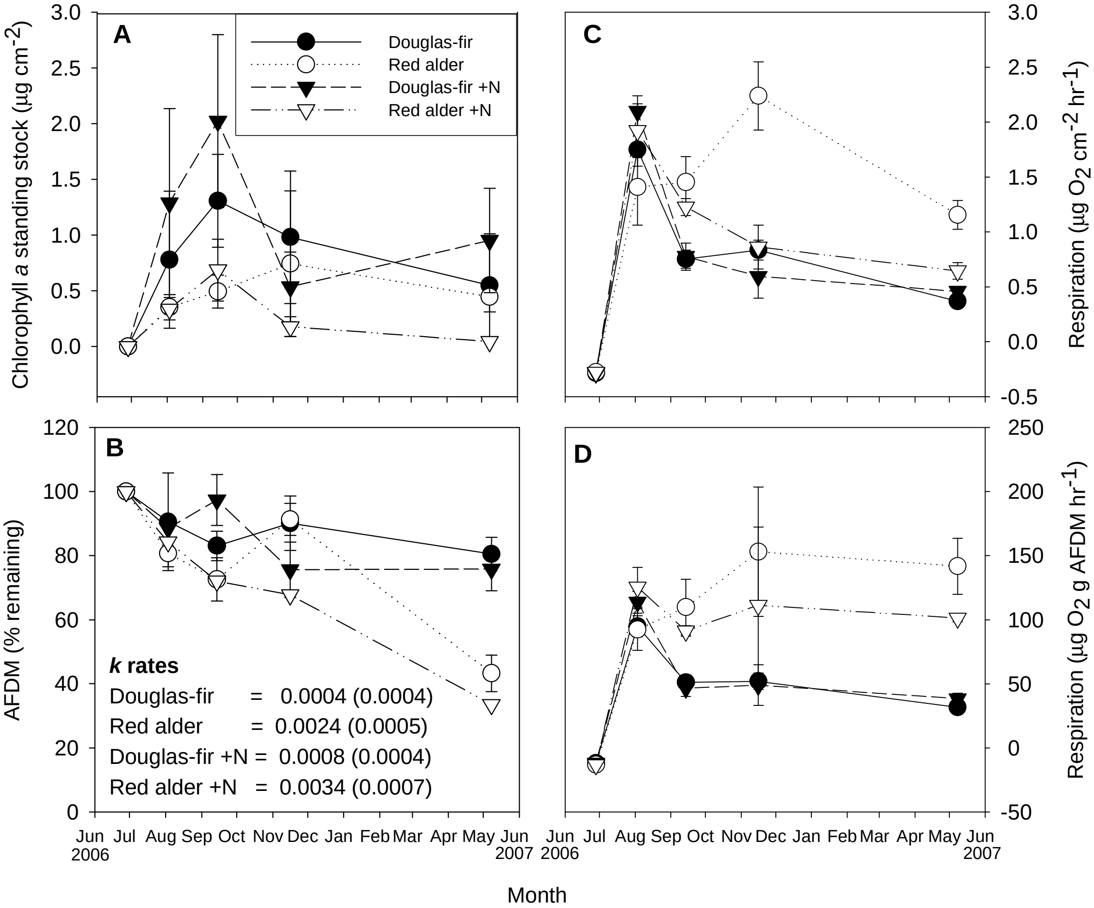


Fig. 13. (A) Chlorophyll *a*, (B) ash-free dry mass (AFDM), (C) respiration rate per surface area, and (D) respiration per AFDM for the four sampling periods on Mack Creek. All respiration trials were conducted at 15ºC. Bars represent one standard error. For panel (B), values in parentheses are one standard error; units of *k* are d^{-1}.

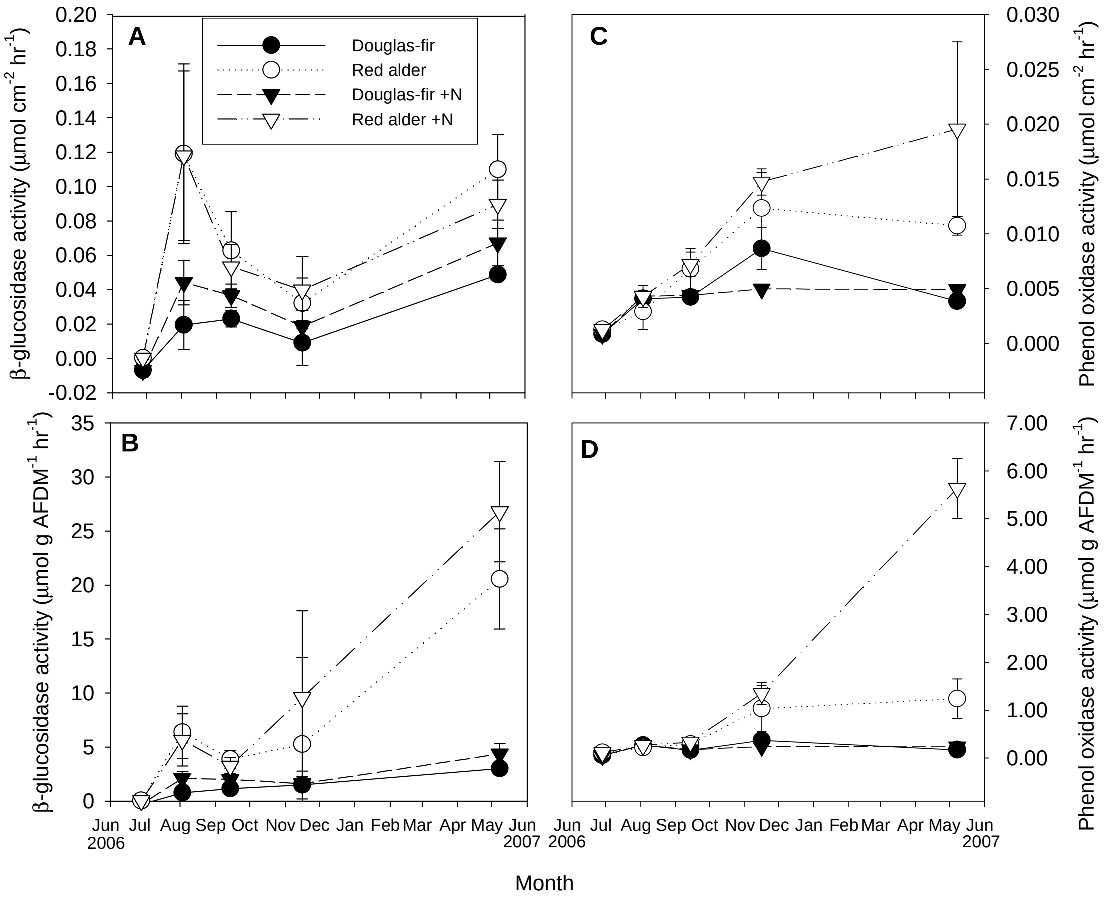


Fig. 14. Activity of the extracellular enzymes (A, B) β-glucosidase and (C, D) phenol oxidase expressed (A,C) per area or (B,D) per mass for the four sampling periods on Mack Creek. All enzyme trials were conducted at 15ºC. Bars represent one standard error. For panel (B), values in parentheses are one standard error

Respiration per area for all species and N treatments—except ambient red alder—had similar trajectories through time, with peak respiration observed during August followed by an exponential decline (Fig. 13C). Ambient red alder, on the other hand, exhibited consistently higher per area respiration than both ambient and +N Douglas-fir and red alder +N during September, November and May (Fig. 13C). Trends were similar for respiration per mass (Fig. 13D). Both ambient and +N Douglas-fir continued to exhibit a one month respiration peak followed by an exponential decline in subsequent months and ambient red alder continued to show highest overall respiration rates for September through May (Fig. 13D). The main difference was that respiration per mass for red alder +N tracked more closely to, but was still lower than, ambient red alder (Fig. 13D).

β-glucosidase activity per area showed an initial peak in August, an exponential decline from August through November, and an increase from November to May (Fig. 14A). In contrast, activity per mass showed a general increase (except September) over time through May (Fig. 14B). Standard errors for β-glucosidase activity per area overlapped among species and N treatments for each incubation time (Fig. 14A). However, red alder (ambient and +N) showed a widening gap to Douglas-fir substrates over time (Fig. 14A and 14B). Activity of phenol oxidase showed different patterns over time depending on whether expressed as per area or per mass (Fig. 14C and 14D). Activity on red alder was consistently greater than activity on Douglas-fir (Fig. 14C and 14D). But when expressed per mass, phenol oxidase activity on red alder +N diverged from Douglas-fir and ambient red alder in May (Fig. 14D).

Differences in breakdown rate and biofilm characteristics between species and N treatments depended on incubation time in the stream reach (Tables 10 and 11; Fig. 15 and 16). For relative ratios of red alder to Douglas-fir (R/D) versus the relative ratios of N enriched substrates (R_N/D_N), four ANOVA models (respiration per mass, mass loss, phenol oxidase, and assimilatory efficiency index) showed significant effects of incubation time

($p<0.03$), but not overall differences between the two ratios when corrected for time ($p>0.10$) (Table 10). Only ANOVA models for chlorophyll *a* and β-glucosidase did not show significant differences for incubation time ($p>0.16$) (Table 10). The trend of the four significant ANOVA models was for increased differences between red alder and Douglas-fir in both ambient and N-treated conditions over time (Fig. 15 and 16). Differences between mass loss (regardless of N treatment) showed that red alder had less AFDM remaining than Douglas-fir at the end of the experiment (May). Respiration rates, phenol oxidase, and the assimilatory efficiency index all showed significant trends for greater activity on red alder than Douglas-fir over time, with a non-significant trend for larger differences between +N and ambient for phenol oxidase and assimilatory efficiency index (Fig. 16).

There was only one significant ANOVA model ($p=0.002$) for N-treated versus ambient substrates (D_N/D versus R_N/R) (Table 11; Fig. 15 and 16). Ratios of mass loss were significantly different between August and September (Table 11). However, there also was non-significant trend for red alder over time for respiration, phenol oxidase activity, and assimilatory efficiency index specifically (Fig. 16). The difference between N enriched and ambient red alder increased with incubation time for all three of these metrics, with differences in respiration decreasing on N enriched substrates while differences for phenol oxidase and assimilatory efficiency index increasing with incubation time (Fig. 15 and 16).

Table 10. Summary of two-way analysis of variance models for log-transformed ratios between red alder (R) and Douglas-fir (D) wood substrates for ambient and N enrichment (denoted with subscript N) at sites in the study reach on Mack Creek over time.

	Model summary				*p*-values		
Model	Model d.f.	Remaining d.f.	*F*	r^2	R/D vs. R_N/D_N	Date	Overall
Respiration	4	23	7.51	0.49	0.55	<0.001	<0.001
Mass Loss	4	23	11.44	0.61	0.11	<0.001	<0.001
Chlorophyll *a*	4	23	0.95	<0.01	0.08	0.95	0.45
β-glucosidase	4	23	1.84	0.11	0.20	0.16	0.16
Phenol oxidase	4	23	13.92	0.66	0.10	<0.001	<0.001
Assimilatory Index	4	23	2.95	0.22	0.33	0.03	0.04

Table 11. Summary of two-way analysis of variance models for log-transformed ratios N-enriched (denoted with subscript N) wood substrates relative to ambient substrates for red alder (R) and Douglas-fir (D) at sites in the study reach in Mack Creek over time.

	Overall model summary				*p*-values		
Model	Model d.f.	Remaining d.f.	*F*	r^2	D_N/D vs. R_N/R	Date	Overall
Respiration	4	23	2.53	0.18	0.58	0.04	0.07
Mass Loss	4	23	5.87	0.42	0.09	0.002	0.002
Chlorophyll *a*	4	23	1.12	0.02	0.12	0.62	0.37
β-glucosidase	4	23	1.58	0.08	0.12	0.31	0.21
Phenol oxidase	4	23	2.02	0.13	0.06	0.26	0.13
Assimilatory Index	4	23	1.90	0.12	0.30	0.12	0.14

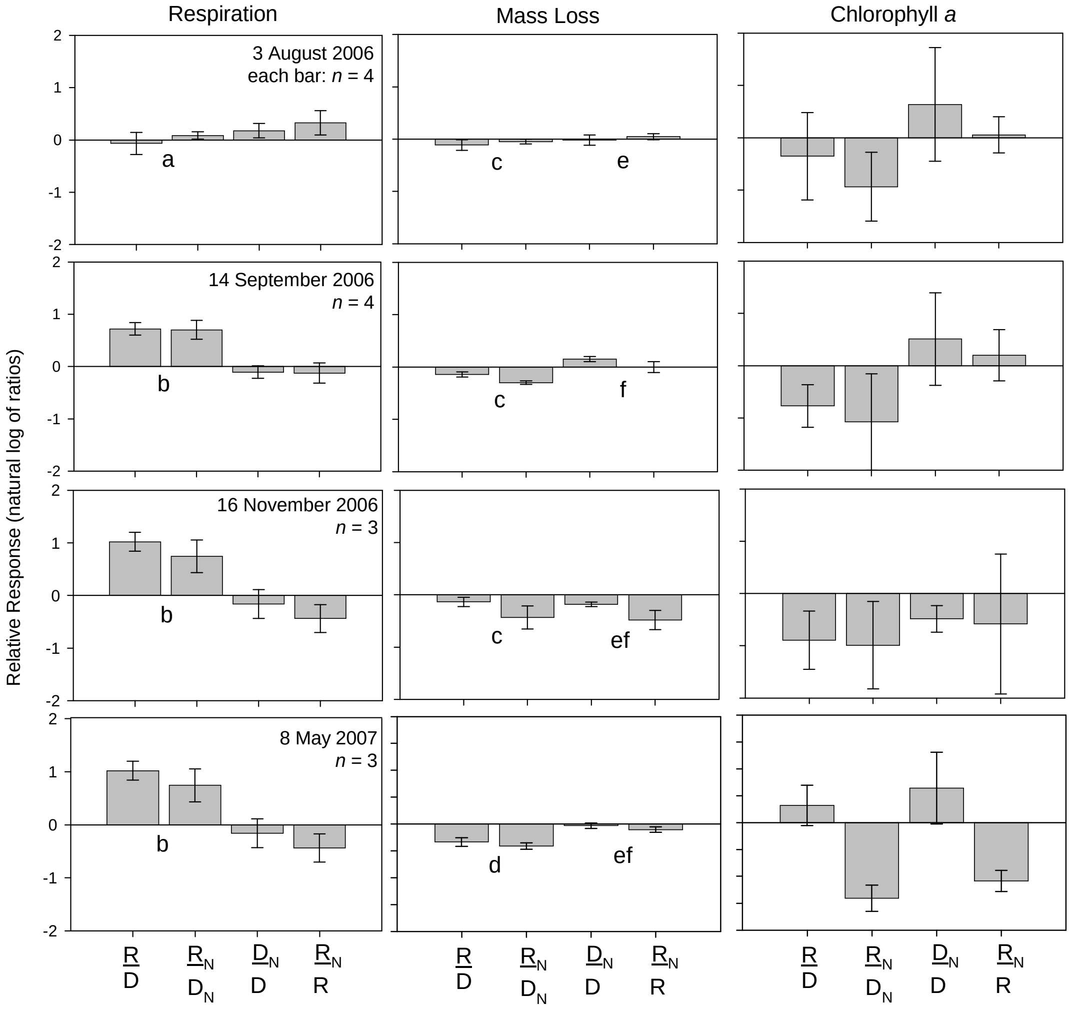


Fig. 15. Relative responses of differences in species (R = red alder; D = Douglas-fir) or N enrichment (denoted with subscript N) at sites within the study reach on Mack Creek for biofilm respiration (µg O_2 g AFDM hr^{-1}), mass loss (% AFDM remaining), and chlorophyll *a* (µg chl a cm^{-2}) for the four sampling dates. Bars are standard errors. Positive values indicate the numerator had a greater response than the denominator; vice versa for negative values. Letters denote significant ($p<0.05$ adjusted for Tukey's HSD) for differences among sampling periods for the comparison of R/D versus R_N/D_N or D_N/D versus R_N/R.

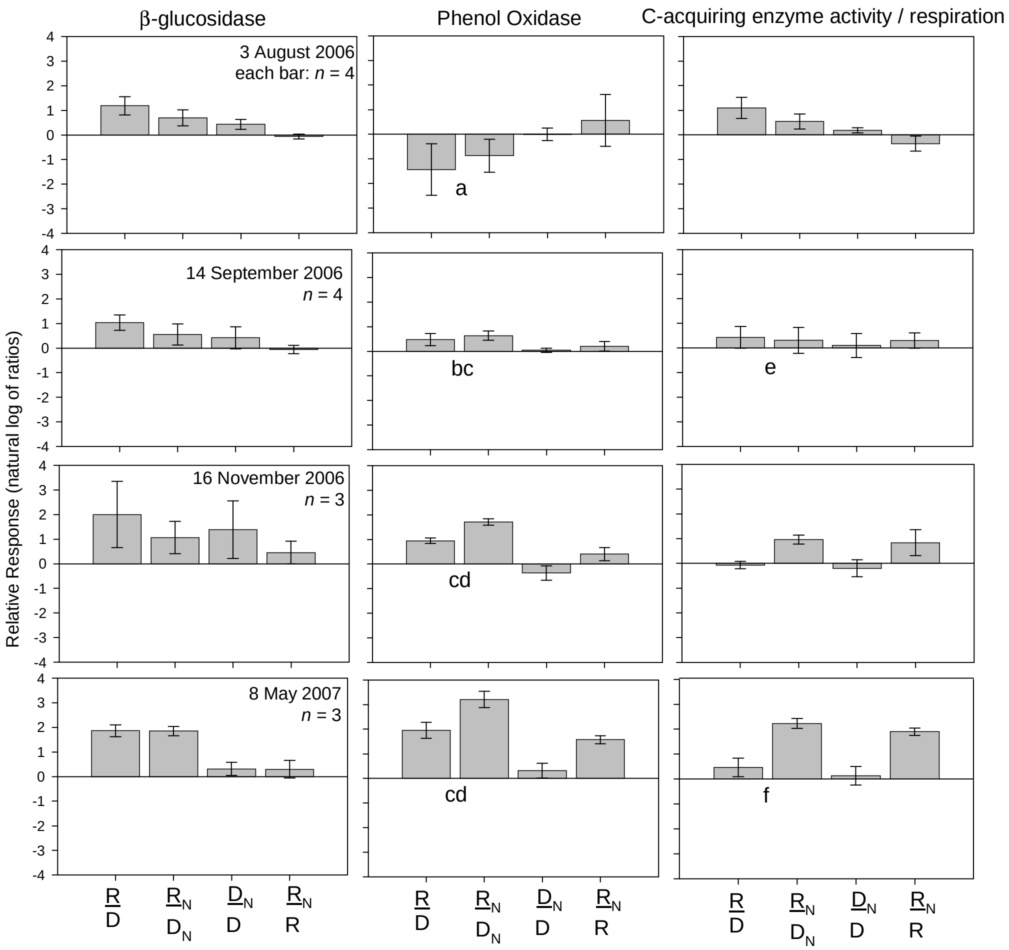


Fig. 16. Relative responses of differences in species type (R = red alder; D = Douglas-fir) or N enrichment (denoted with subscript N) of sites within the study reach on Mack Creek for the extracellular enzymes β-glucosidase and phenol oxidase and an index of assimilation efficiency (AE) on the four sampling dates. The AE index is the ratio of C-acquiring extracellular enzymes (β-glucosidase and phenol oxidase) to respiration (assuming a respiratory quotient of 1 and 75% of respiration by heterotrophs) per ash-free dry mass. Bars are standard errors. Positive values indicate the numerator had a greater response than the denominator; vice versa for negative values. Letters denote significant (p<0.05 adjusted for Tukey's HSD) for differences among sampling periods for the comparison of R/D versus R_N/D_N.

DISCUSSION

Previous field experiments (Gulis et al. 2004) and laboratory microcosm studies (Aumen et al. 1985) have shown almost immediate and long-lasting effects of N enrichment (especially > 10 mg N L^{-1}) on both mass loss and microbial biofilm activity on wood in streams. Yet we found evidence for different effects of increasing N availability on wood decomposition depending upon incubation time (up to 313 d). One main difference, however, was our placement of un-colonized substrates in the study streams whereas previous studies added N following in-stream conditioning. We found little evidence in both our six stream survey and the manipulative field experiment on Mack Creek to support the hypothesis that increasing inorganic N availability increases wood breakdown rate over short (< two month) incubation times in streams. This result is similar to reviews on the effects of external N supply on plant litter decomposition (Fog et al. 1988; Knorr 2005), which have found little to no response in the initial (five month) phase of decomposition for eight plant litter types. Instead, effects of N enrichment in this study became apparent nearly four months after initial substrate placement, suggesting that N availability influenced wood breakdown rates only when significant biological degradation of woody tissues began.

Short-term wood breakdown and DIN concentration

At first glance, correlative evidence from the short-term study across the six small streams (plus one additional set on Mack Creek) supported the hypothesis that the wood breakdown rates increased with higher concentrations of inorganic N (Fig. 11). However, this also highlights a major problem with correlative analyses: mean breakdown rates also were strongly correlated with mean temperature and, surprisingly (Melillo et al. 1984; Golloday and Webster 1988), the direction of the correlation was negative (Fig. 11). In fact, the strong inverse correlation (r = -0.70) between mean temperature and inorganic N confounded the interpretation of the relationship of breakdown rate with DIN and molar DIN/SRP ratio, which indicates degree of N-limitation (< 15:1, N-limited) (Redfield 1958; Gregory 1980).

Previous studies suggest that wood breakdown rate should increase with degree of human land use activity in the surrounding catchment and thus could be used as indicator of human impact on stream ecosystem structure and function (Spänoff and Meyer 2004). There are three inter-related explanations for this hypothesis: (1) the composition of wood recruited to human-impacted streams should shift from slow-growing species with poor nutritional quality tissues to faster growing species with higher nutritional quality wood (Harmon et al. 1986); (2) surface area to volume ratio of wood recruited to streams should increase relative to un-disturbed systems (Harmon et al. 1986); and (3) wood breakdown should accelerate as human land use activities increase loading of nutrients to streams (Golloday and Webster

1988). However, we observed the opposite pattern across our six study streams for the first month of breakdown. In the two urban streams, which possessed the highest overall concentrations of N and high mean temperature, the breakdown rates for both red alder and Douglas-fir were generally lower than those observed for forest or agricultural streams, all of which had substantially lower TDN concentrations and lower temperatures. The majority of dissolved N at urban sites was in organic form (>99%) and may have been less available for biological use than inorganic N, which comprised > one-third of total dissolved N at the remaining streams (Table 2). Additionally, urban sites were devoid of wood (Table 2) and other coarse particulate organic matter (see Chapter 2), suggesting that initial breakdown of wood in these streams could have partially been limited by colonization rate of wood degrading fungi and bacteria. Moreover, substrates from one urban site (Periwinkle) showed signs of an anoxic environment, with black ferric sulfide visually coating both substrates. It should be noted, however, that had we incubated wood for a longer time period (> two months) over multiple seasons on each stream, we may have seen different trends in breakdown as substrates progressed from initial phases of leaching of soluble organics to mineralization of lignocellulose (Melillo et al. 1984) and proportion of inorganic to organic N concentrations in the stream environment shifted with precipitation patterns (Poor and McDonnell 2007).

Temporal trends in wood breakdown

The composition of wood biofilms on incubated substrates in the second experiment likely included algae and bacteria that acquire organic C from exudates in the biofilm matrix or from the stream water column. In fact, a previous study on Mack Creek indicated that up to 50% of observed wood biofilm respiration is attributable to a complex algal community (Sabater et al. 1998). Our results also suggest an initial colonization phase that included organisms not directly associated with wood breakdown (Fig. 13A). However, results show significant activity of wood degrading fungi and bacteria on incubated substrates. Wood-degrading microorganisms initially colonizing wood substrates may have utilized simpler C compounds including soluble sugars and other relatively labile forms of C (Baker et al. 1983), with assimilation of more complex woody tissue constituents increasing over time (Fig. 14). This trend is consistent with a study of the same two enzymes on milled wood substrates (popsicle sticks) incubated for a seven month period in a northern New York stream (Sinsabaugh et al. 1992) and in a study of stick and twig breakdown in a southern Appalachian headwater stream (Tank et al. 1998).

A complex interaction between physical and biological processes is evident from a comparison between measured amount of C lost from substrates and estimated C losses based on biofilm respiration rates (Fig. 17). To make these calculations, we assumed that proportional C content of each substrate remained similar to their original C content (see methods). We also assumed that microbial biomass constituted an insignificant fraction of total substrate biomass. For C lost to respiration, we assumed that 25% (50% of substrate surface exposed to sunlight) of total respiration was attributable to algal metabolism among all sampling dates (Sabater et al. 1998). For simplicity, we also assumed that the remaining respiration was solely attributable to mineralization of C from woody tissues. Uptake of DOC from the water column likely contributed to heterotrophic respiration (Fischer 2003), but we assumed this proportion was small and constant across species and N treatments. To correct for temperature, we assumed a Q_{10} relationship by halving of respiration for every 10ºC decrease in temperature (Schmidt-Neilson 1996). Finally, we assumed a respiratory quotient of 0.85 moles of organic C converted to CO_2 for every mole of O_2 consumed (Wetzel 2000). We estimated C lost based on AFDM measurements and C lost to respiration for each of the four intervals between sampling dates (June-August, August-September, September-November, and November-May), by integrating the area under each curve for the estimates of mass loss (Fig. 13B) or respiration on per mass basis (Fig. 13D) between sampling dates.

According to this model, an average of 0 to 1.6% of total C lost on each substrate regardless of N treatment could be directly accounted for by respiration (Fig. 17). Both Douglas-fir and red alder substrates incubated without N enrichment had slightly higher percentages of C lost attributable to respiration (Douglas-fir = 1.6 ± 0.4%, red alder = 1.5 ± 0.6%) versus N-enriched substrates (Douglas-fir +N = 0 ± 0.9%; red alder +N = 1.1 ± 0.5%),

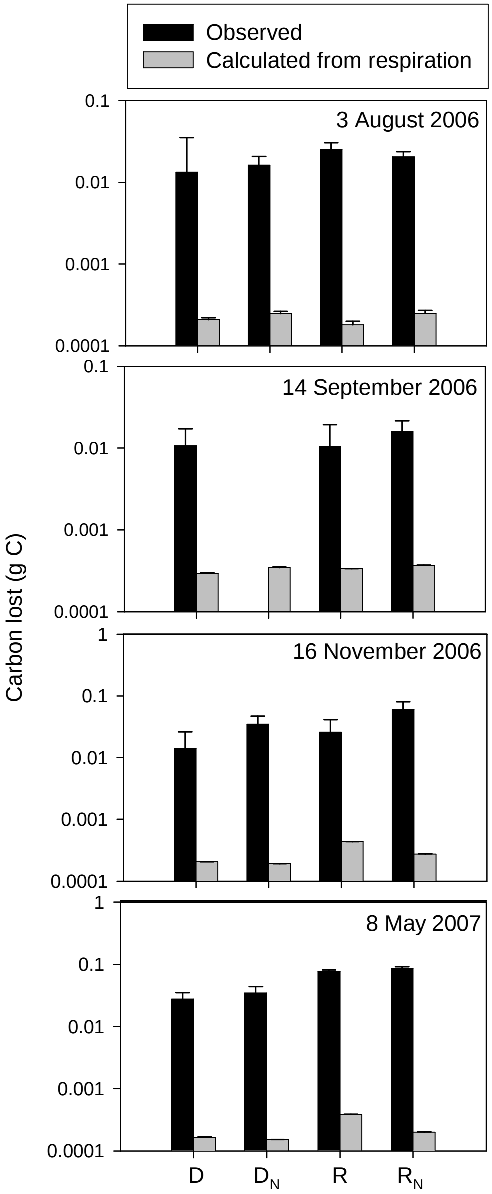


Fig. 17. Loss of carbon and calculated carbon respired from wood substrates for each species (R = red alder; D = Douglas-fir) or N enrichment (denoted with subscript N) between each sampling date. See text for details on calculations. Bars are standard errors.

but 95% confidence intervals for these estimates overlapped among species and N treatments. Even if 100% of biofilm respiration is attributed to wood-degrading fungi and bacteria, only 3.2% of mass loss for all substrates can be accounted for by respiration. While chamber effects (i.e., nutrient depletion from the surrounding water) may have induced lower rates of respiration in the laboratory, the short time period of incubation (hours) relative to observations of N turnover in wood biofims (> 30 d; Ashkenas et al. 2004) suggest these effects do not account for such large differences. Thus, this model provides a compelling insight on the nature of wood mass loss in these systems: the majority C appears to be lost either through physical fragmentation or leaching. Previous laboratory and field studies have indicated that leaching of relatively soluble constituents can account for up to 25% of mass loss in plant litter breakdown, especially within the first few weeks after entering the stream (Melillo et al. 1984; Suberkropp et al. 2001). However, separate laboratory incubations of un-colonized Douglas-fir and red alder substrates in sterile distilled water yield leaching losses <5% for both substrates over one month (on a shaker table at 80 rpm), though these incubations do not account for physical abrasion due to abrasion for suspended sediments. While leaching in field settings and the laboratory may be considerably different because of physical and chemical differences, we do not believe they are enough to account for the 97 to 99% of missing C. Previous studies indicate the majority of physical leaching is completed within one week to one month of stream entry (Melillo et al. 1984; Suberkropp et al. 2001); yet we apparently saw substantial leaching losses over ten months after stream placement. Stream water pH also is an important factor for physical leaching of plant litter (Suberkropp et al. 2001). However, this factor cannot account for the missing

C in our calculations: leaching is expected to increase as pH decreases; yet the pH range for Mack Creek was slightly basic (7.3 – 7.5).

Instead of pure physical leaching/fragmentation processes, one hypothesis is that a major portion of C released from woody tissues by actions of lignocellulose enzymes was lost as dissolved organic carbon to downstream stream. This fraction of C lost to downstream transport appeared to be consistent across substrate types and N treatment levels. However, the addition of N stimulated the loss of mass for at least red alder, which was consistent with the two enzyme activities, especially phenol oxidase (Fig. 14 and 16). Correspondingly, our index of assimilatory efficiency (enzyme activity / hetrotrophic respiration) also showed a response to N addition, suggesting that fungi and bacteria may have been devoting a greater proportion of acquired C to synthesis of new biomass rather than maintenance (Fig. 16). Another possibility, however, is that the missing C had been assimilated into xylophagous macroinvertebrates (Anderson et al. 1978). Several aquatic insect taxa, including *Lara avara* (Coleoptera), *Lipsopthorix* spp. (Diptera), Trichoptera, Ephemeroptera and Chironomidae (Diptera), are known to consume wood or biofilms colonizing wood surfaces (Anderson et al. 1978).

Implications

Riparian forests vary naturally (Sarr and Hibbs 2007) and according to legacy effects of land use (Kennedy and Spies 2004) within narrow geographic areas. Our study suggests turnover time of wood in headwater streams can be strongly related to riparian forest composition. While we used commercially-made substrates that may not characterize natural rates of wood breakdown (Spänoff and Meyer 2004), the qualitative differences between the substrate types are still useful for describing conceptual differences. Both Douglas-fir and red alder are native components of riparian forests bordering small streams in the Pacific Northwest (Waring and Franklin 1979; Nierenberg and Hibbs 2001) and both contribute substantial amounts of wood to adjacent streams (Harmon et al. 1986; Keim et al. 2000). Red alder also is a N_2-fixer and can increase loading of inorganic N to adjacent streams by over an order of magnitude, especially where red alder is distributed throughout riparian and hillslope areas (Compton et al. 2003).

Our study suggests a stimulation of organic C release from wood of high nutritional quality (red alder) in streams resulting from increases to inorganic N loading. In contrast, wood of low nutritional quality showed little sign of faster breakdown rates in response to elevated N levels. Therefore, turnover of wood should be rapid in streams with red alder riparian forests with high quality wood and high loading rates of inorganic N. Likewise, wood recruited from riparian forests composed of species with lignin-rich, N poor wood (i.e., Douglas-fir) should have relatively long residence times—regardless of N loading to the system—and have a longer-lasting geomorphic influence in the system (Harmon et al. 1986). Implications for organic carbon supply to aquatic food webs are less clear. If standing stocks of stream wood are similar and reflect the composition of the surrounding riparian forest, then a greater proportion of wood-derived organic C should be available for aquatic food webs in streams bordered by red alder riparian forests than for streams bordered by coniferous riparian forests. However, streams bordered by deciduous riparian forest in the Pacific Northwest often contain standing stocks of wood nearly an order of magnitude lower than those bordered by coniferous forests (Harmon et al. 1986; Keim et al. 2000). Therefore, contribution of C from wood to aquatic food webs could be nearly equivalent in streams with red alder or Douglas-fir riparian forests, despite large difference in overall wood standing stocks.

CHAPTER 4

LAND USE, CHANNEL AND RIPARIAN VEGETATION CHARACTERISTICS, AND NITRATE UPTAKE IN STREAMS ACROSS NORTH AMERICA

Daniel J. Sobota, Stan V. Gregory, Sherri L. Johnson, Linda R. Ashkenas,
and P.J. Mulholland and other LINX authors yet to be determined

ABSTRACT

We examined relationships between land use (forest, grassland, agriculture or urban development) and attributes of channel morphology, sediment composition, and riparian vegetation for 72 streams from eight major North American biomes. We also related channel, sediment, and riparian characteristics to aspects of nitrate (NO_3^-) uptake in these streams. Agricultural and urban streams exhibited simplified stream channel structure, with lower width-to-depth ratio, smaller coefficient of variation (CV) of stream depth, and higher stream banks than forest or grassland streams. Agricultural and urban streams also had significantly smaller median sediment diameters (D_{50}) and percent of sediments composed by silt than in forest or grassland streams. Riparian canopy cover was lowest in agricultural settings but not statistically different between forest or grassland streams and urban streams. After correcting areal NO_3^- uptake (U) for region, stream discharge, and N concentration, U decreased with increasing riparian canopy cover but increased with percent of silt in benthic sediments, suggesting responses to in-stream primary production and availability of habitats suitable for denitrification. A multiple regression model for the fraction of U by denitrification (f_{den}) versus channel, sediment, and riparian characteristics further supports the concept that silt and fine-sediments were important for denitrification. These results demonstrate that physical features of streams and adjacent riparian zones associated with different forms of adjacent land use practices are linked to the capacity of streams to process N.

INTRODUCTION

Agricultural and urban land use practices generally increase nitrogen (N) and phosphorus (P) input to stream ecosystems above natural levels (Vitousek et al. 1997; Wollheim et al. 2005; Dumont et al. 2005; Harrison et al. 2005). These increased inputs often lead to eutrophication of downstream water bodies and shifts in aquatic community structure (Townsend et al. 2003). Land use practices also change the physical environment of stream-riparian systems leading to multiple yet predictable shifts in the structure of these ecosystems (Paul and Meyer 2001; Sweeney et al. 2004). Stream channel structures generally are simplified in human-dominated landscapes, resulting from active removal of large organic material (Sedell et al. 1988), alteration of flow regimes (Poff et al. 2007), and incision, which disconnects active channels from adjacent floodplains (Paul and Meyer 2001). Stream bed sediments generally are finer and less diverse in agricultural and urban streams relative to forest or grassland streams due to increased delivery of surface fine sediment from overland or channelized runoff (Waters 1995), scouring of bed sediments from increased flow magnitudes in agricultural and urban settings (Paul and Meyer 2001), and their general basin position, which tend to be in lowland valleys (Townsend et al. 1997).

Alterations to riparian vegetation composition and structure caused by agricultural and urban land use practices also change stream biological communities, especially in small (< 3rd order) streams (Vannote et al. 1980;

Paul and Meyer 2001). Riparian vegetation influences productivity of aquatic foodwebs by regulating input of photosynthetically active radiation (PAR) available for primary producers and input of allochthonous organic matter in the form of vascular plant tissue, terrestrial macroinvertebrates, and soil solution (Fisher and Likens 1973; Mulholland et al. 2006). Forestry, agricultural, and urban land use practices often encroach into riparian areas and significantly alter or completely remove riparian vegetation, depending on regulations in place to protect riparian areas (Gregory 1997; Young et al. 2000). These riparian alterations cascade to adjacent stream ecosystems, potentially increasing autotrophic production while at the same time decreasing the standing stock of detrital organic matter.

While the consequences of land use practices on stream-riparian ecosystems have been well-defined conceptually (Paul and Meyer 2001; Grimm et al. 2005) and studied intensively in specific areas (e.g., Groffman et al. 2005; Hale and Groffman 2006), fewer studies have analyzed patterns of channel and riparian characteristics of streams in a broad range of vegetation types, climatic zones, geophysical features, and land uses. The Lotic Intersite Nitrogen eXperiment, phase II (LINX II) provided an opportunity to compare headwater streams surrounded by forest or grassland, agricultural, and urban land uses in eight biomes across North America and Puerto Rico. Biomes ranged from humid tropic rainforest to arid desert and included diverse land use practices.

Our objective in this study was to identify cross-region commonalities and region-specific features of riparian zones and channel structure for streams in forests or grasslands, agricultural landscapes, or urban areas and relate these attributes with whole-stream nitrate (NO_3^-) processing. Our hypotheses were: measures of channel complexity would reflect simpler and incised channel structures for agricultural and urban streams than for forest or grassland streams regardless of region; sediment size would be smaller in agricultural and urban streams relative to forest or grassland streams across all regions, reflecting landscape position of these streams (valley bottoms) and increased delivery of fine sediments in human-dominated landscapes; differences in riparian vegetation cover (overstory riparian channel cover, overstory riparian zone cover, and understory riparian vegetation cover) among the three land use categories would depend on biome. Specifically, riparian vegetation cover will be highest for streams in forested regions, intermediate in agricultural streams, and lowest in urban streams. In non-forested regions, we predicted that metrics of riparian vegetation cover would not be related to land use. In our last set of hypotheses, we thought whole-stream uptake of NO_3^- (U) would increase with decreases in overstory riparian channel cover, reflecting increases in whole-stream primary production. U and fraction of U attributable to denitrification were expected to decrease as channel complexity decreased, reflecting losses of available habitats for denitrification (anaerobic respiration that removes of NO_3^- from ecosystem cycling).

SITE DESCRIPTIONS

Seventy-two streams in eight biomes across North America and Puerto Rico were selected to represent forest or grassland, agricultural, and urban land uses (Table 12). The eight biomes included (region in parentheses): Northeast hardwood forest (Massachusetts), Great Lakes hardwood forest (Michigan/Indiana), Southern Appalachian hardwood forest (North Carolina), Atlantic neotropical rainforest (Puerto Rico), tall grass prairie (Kansas), semiarid montane coniferous forest (Wyoming), Sonohoran-Chihuahuan desert (New Mexico/Arizona) and Pacific Northwest temperate coniferous rainforest (Oregon) (Fig. 18). All studies were conducted in the summers of 2003 – 2005, except for Puerto Rican streams (late winter from 2004 – 2006). Catchments ranged from 3 to 1,601,970 ha (median = 339 ha), reach lengths ranged from 120 to 1,800 m (median = 493 m), stream discharges ranged from 0.2 to 267.8 L s^{-1} (median = 18.4 L s^{-1}), and stream NO_3^- concentration ranged from 0.1 to 21,162 μg N L^{-1} (median = 101 μg N L^{-1}) (Appendix G). Field-based land use classification generally agreed with remotely-sensed catchment land use cover data (2001 National Land Cover Database, Multi-Resolution Land Characteristics Consortium, US EPA, Washington, D.C.), though two field-designated agricultural streams in New Mexico and Arizona (southwest) did not have agricultural land cover (Table 12). We continued to classify these streams as agricultural because immediately adjacent land use may have not been captured in the resolution (30-m pixels) of NLCD data.

Table 12. Catchment land use cover for study sites the 72 study streams across eight regions in North America and Puerto Rico. Values are medians with minimum and maximum in parentheses.

Region	Biome	Land Use	Native vegetation (%)	Agriculture (%)	Urban (%)
Massachusetts	Northeast deciduous forest	FGV	90 (85 – 91)	3 (1 – 3)	9 (4 – 12)
		AGR	33 (10 – 73)	63 (19 – 90)	4 (0 – 7)
		URB	12 (9 – 48)	1 (1 – 5)	86 (45 – 91)
Michigan/ Indiana	Great Lakes deciduous forest	FGV	64 (50 – 93)	17 (0 – 38)	12 (5 – 13)
		AGR	31 (8 – 38)	60 (51 – 87)	9 (5 – 9)
		URB	17 (13 – 42)	17 (8 – 51)	40 (31 – 79)
North Carolina	Southern Appalachian deciduous forest	FGV	100 (100 – 100)	0 (0 – 0)	0 (0 – 0)
		AGR	72 (55 – 79)	17 (14 – 30)	11 (7 – 15)
		URB	76 (61 – 99)	6 (0 – 11)	18 (1 – 29)
Puerto Rico	Atlantic neotropical rainforest	FGV	100 (98 – 100)	1 (0 – 1)	0 (0 – 1)
		AGR	61 (25 – 70)	30 (26 – 74)	52 (1 – 56)
		URB	43 (7 – 45)	2 (0 – 3)	56 (52 – 93)
Kansas	Tallgrass prairie	FGV	97 (96 – 99)	0 (0 – 0)	3 (1 – 4)
		AGR	29 (23 – 98)	17 (0 – 28)	42 (2 – 59)
		URB	6 (3 – 76)	2 (0 – 8)	86 (23 – 95)
New Mexico/ Arizona	Sonoran – Chihuahuan desert	FGV	99 (99 – 100)	0 (0 – 0)	1 (0 – 1)
		AGR	94 (26 – 99)	0 (0 – 67)	6 (1 – 7)
		URB	15 (14 – 19)	10 (1 – 64)	71 (22 – 85)
Wyoming	Semiarid coniferous forest	FGV	100 (92 – 100)	0 (0 – 1)	0 (0 – 0)
		AGR	45 (18 – 92)	50 (2 – 78)	4 (1 – 4)
		URB	76 (0 – 99)	0 (0 – 10)	7 (1 – 100)
Oregon	Temperate coniferous forest	FGV	100 (97 – 100)	0 (0 – 1)	0 (0 – 2)
		AGR	75 (74 – 98)	17 (2 – 25)	0 (0 – 8)
		URB	31 (1 – 71)	18 (0 – 70)	30 (11 – 69)

FGV = forest or grassland vegetation; AGR = agriculture; URB = urban
Land cover data from the NLCD (2001) except for Puerto Rico (NLCD 1991)

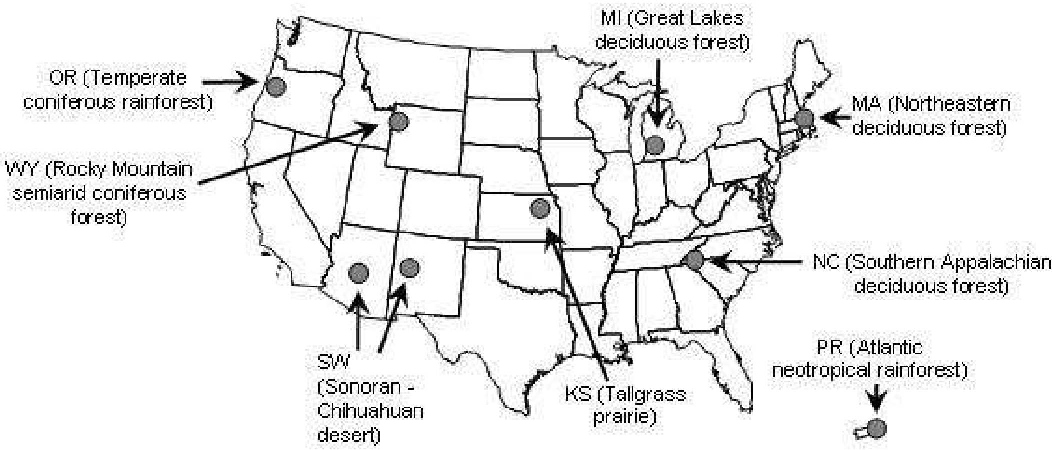


Fig. 18. The eight regions across North America and Puerto Rico in this study with biome in parentheses. Nine streams were located in each region (three each in forest or grassland, agricultural, or urban settings). Figure was created by P.J. Mulholland, Oak Ridge National Laboratory, Tennessee.

METHODS

Field studies

Data from each region were collected according to standardized protocols developed for the LINX study (Mulholland et al., *in submission*). Channel morphology, benthic sediments, and riparian vegetation were assessed immediately prior to or immediately following a $^{15}NO_3^-$ isotope release on each reach. Channel morphology, sediment, and riparian vegetation were assessed using protocols developed for the U.S. Environmental Protection Agency Environmental Monitoring and Assessment Program (EMAP) (http://www.epa.gov/emap2/index.html). Channel transects (10 to 15 depending on spatial dimensions of individual reaches) were sampled at each stream. At each transect, we measured wetted width and 10 evenly-spaced depth measurements, average diameter of 10 sediments (Wolman 1954), and vertical bank height at 2.5 m and 5 m horizontal distance from the wetted channel on both banks. Sediments were classified as clay, silt (< 1 mm diameter), sand (1 – 2 mm diameter), gravel (2 – 128 mm diameter), or cobble (> 128 mm diameter). Bank height was taken as the average of vertical bank heights measured at horizontal distances of 2.5 m and 5 m from the wetted channel on both banks. Riparian canopy cover was quantified with a concave spherical densitometer at the midpoint of the wetted channel (overstory riparian channel cover) and at 12.5 and 25 m lateral distance (overstory riparian zone cover) from the wetted channel in the riparian zone on both banks. Percent cover of understory riparian vegetation was measured in five cells (5 by 2 m) extending 25 m lateral distance from the channel on both banks at transects. Percent of stream bank modified was quantified as the proportion of both stream bank (5 m horizontal distance from channel) in between transects that had rip rap, hardened, or was cleared of vegetation.

Statistical Analyses

We first tested for differences in channel morphology, benthic sediment, and riparian vegetation among different forest or grassland, agricultural, and urban land use types and among regions. We next selected channel, sediment, and riparian vegetation characteristics that correlated with whole-stream NO_3^- uptake (U) and fraction of U by denitrification (f_{den}). All percentage variables were converted to fractions and arcsine-square root transformed to improve normality for statistical analyses, except for coefficient of variation (CV) measures since this measure can have fractional values > 1. All other variables were natural-log transformed to improve normality (McCune and Grace 2002).

In the first analysis, we conducted univariate and multivariate statistical tests on channel, sediment, and riparian vegetation characteristics. Channel characteristics included: wetted width / stream depth ratio, CV of wetted width, CV of stream depth, and average bank height. Sediment characteristics included median sediment diameter (D_{50}) and percent of sediments composed by clay, silt, sand, gravel, and cobble. Riparian vegetation characteristics included % of riparian canopy cover directly over the stream (overstory riparian channel cover), % of riparian canopy cover in the riparian zone (overstory riparian zone cover), % of understory riparian vegetation cover, and percent of the stream bank modified. For univariate analyses, we analyzed individual variables with a two-way analysis of variance (ANOVA) without interaction terms to test for effects of land use type after taking region into account and region after taking land use type into account (Ramsey and Schafer 1999). Model terms were considered significant if $p < 0.05$. Following ANOVAs, we used Tukey's multiple comparison procedure to make family-wise adjustments to 95% confidence intervals for comparisons among land use after correcting for region and region after correcting for land use (Ramsey and Schafer 1999). Tukey's correction adjusts confidence intervals to reduce the random chance of detecting differences among groups (Ramsey and Schafer 1999).

We also performed a two-way multivariate analysis of variance (MANOVA) grouped by land use and blocked by region (Ramsey and Schafer 1999) on arcsine-square root transformed sediment fractions of clay, silt, sand, gravel, and cobble since we were interested in the distribution of sediment types among stream types. Following the MANOVA, we performed individual ANOVAs followed by Tukey's multiple comparison procedure to analyze differences of sediments among land uses and regions.

In the second analysis, we constructed multiple regression models relating whole-stream NO_3^- uptake (U) and fraction of U by denitrification (f_{den}) to channel, sediment, and riparian vegetation characteristics that showed significant differences among land uses. Both U and f_{den} were calculated using standard field protocols on each study stream (Mulholland et al., *submitted*) (data in Appendix G). We also included stream discharge (Q) and water column concentrations of NO_3^- and NH_4^+ in initial models because these parameters have been shown to influence NO_3^- uptake (Wollheim et al. 2001; Peterson et al. 2001; O'Brien et al. 2007). U, Q, NO_3^- and NH_4^+ were natural log transformed while f_{den} was arcsine-square root transformed. We selected multiple regression models for each of U and f_{den} models according to a stepwise selection procedure using small sample size Akiake's Information Criterion (AIC_c) to balance variance explained and the number of explanatory variables included in the model (Burnham and Anderson 2006).

We used AIC_c instead of AIC because the ratio of stream sample size (72) to number of explanatory variables possible (including intercept and error term) was < 40 (Burnham and Anderson 2006). We initially constructed a full model for U and f_{den} that included an indicator variable for region, Q, NO_3^-, NH_4^+, and channel, sediment, and riparian characteristics significantly different among land use (no interactions). Explanatory variables could be dropped or added to minimize AIC_c value. However, no terms were added for both models; thus the selection procedure reflected backwards selection. Statistical analyses were performed in R v.2.4.0 (R Development Core Team 2006).

RESULTS

Channel morphology

Ratio of wetted width-to-depth varied significantly across land use type after being corrected for regional differences (*F* = 7.09 on 2, 62 d.f.; *p* = 0.002; natural-log transformed data) (Table 13) (Fig. 19A). Specifically, forest or grassland streams had greater width-to-depth ratio than agricultural or urban streams after corrections for region (Fig. 19A). Forest or grassland stream had a median width-to-depth ratio 7.76 units (95% CI = 3.60 – 25.79) greater than agricultural streams and 5.10 units (95% CI = 2.77 – 13.60) greater than urban streams.

Variation in stream channel width (CV) was neither correlated with land use category or region (overall *p* = 0.38) (Table 13). However, CV of stream depth exhibited significant influence of land use and region (Table 13 and Fig. 19B), CV stream depth for forest or grassland stream channels averaged 18.9% (95% C.I. = 5.6 – 32.3%) greater than in regional-corrected urban streams. Forest or grassland streams also averaged 11.8% greater than regional-corrected agricultural streams, but adjusted 95% CI for this comparison overlapped with zero (-1.6 – 25.6%). CV stream depth also significantly differed among regions after land use correction (*p* = 0.009), but only Puerto Rico and Wyoming significantly differed (*p* = 0.002).

Table 13. Summary of two-way ANOVA models for selected attributes of channel morphology across land use types and regions.

	Model summary				*p*-values		
Model	Model d.f.	remaining d.f.	*F*	r^2 †	Land use	Region	Overall
ln(Width / Depth)	9	62	3.25	0.22	0.002	0.051	0.003
CV Width	9	62	1.01	0.01	0.46	0.32	0.38
CV Depth	9	62	3.65	0.25	0.004	0.009	0.001
Bank height	9	62	4.85	0.33	0.016	<0.001	<0.001

† Adjusted r^2

Table 14. Summary of two-way ANOVA models for selected attributes of riparian vegetation across land use types and regions. All response values have been arcsine-square root transformed.

	Model summary				*p*-values		
Model	Model d.f.	remaining d.f.	*F*	r^2 †	Land use	Region	Overall
% overstory riparian channel cover	9	62	8.38	0.48	<0.001	<0.001	<0.001
% overstory riparian zone cover	9	62	5.41	0.36	<0.001	0.002	<0.001
% riparian zone vegetated	9	62	3.83	0.26	0.004	0.006	<0.001
% stream bank modified	9	62	4.24	0.29	<0.001	0.03	0.002

† Adjusted r^2

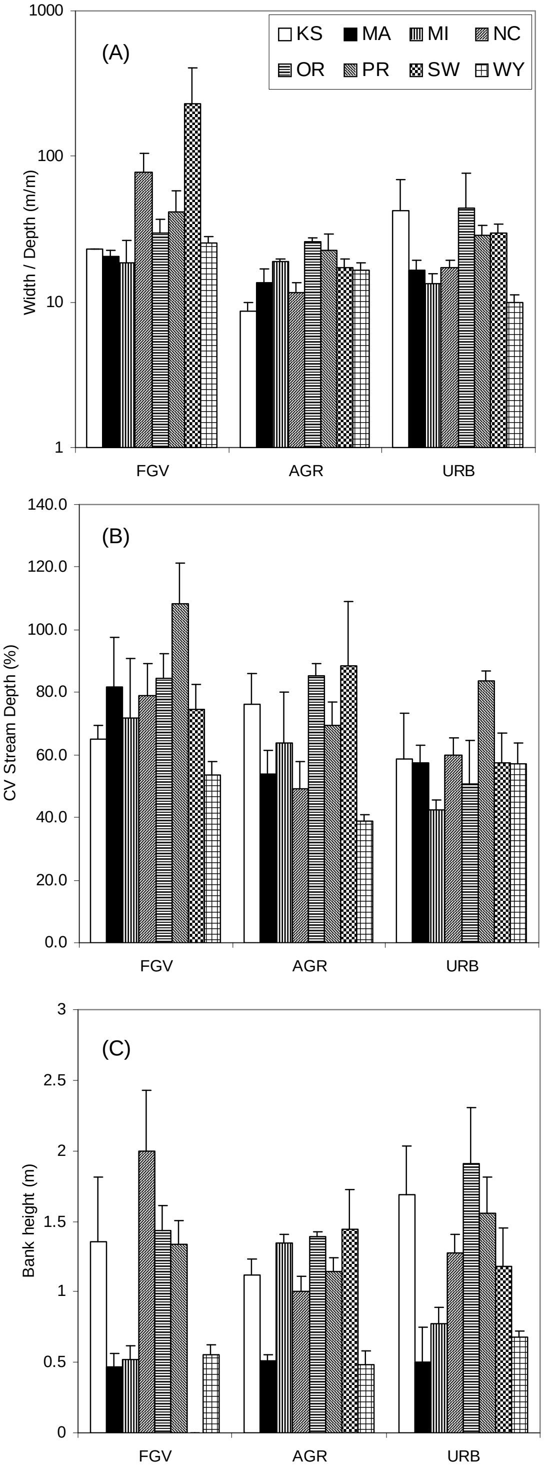


Fig. 19. (A) Width-to-depth ratio, (B) CV stream depth, and (C) bank height of study sites grouped by land use type and blocked by region. FGV = forest or grassland; AGR = agricultural; URB = urban. Bars are one standard error. See Table 14 for ANOVA results.

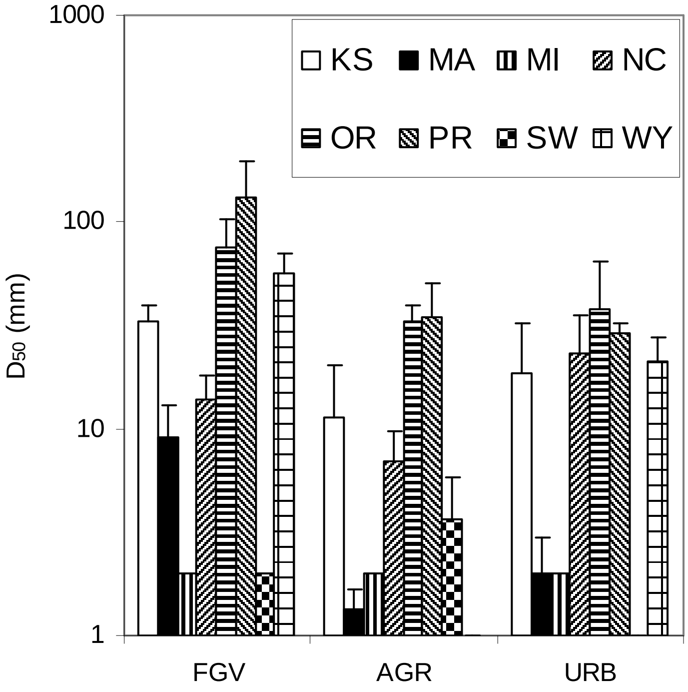


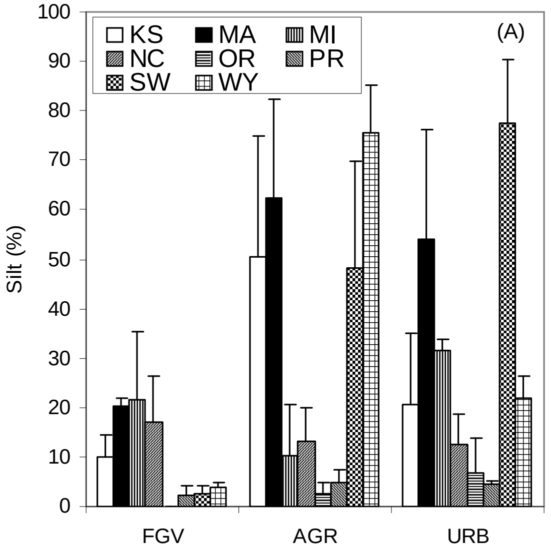
Fig. 20. Median substratum diameter (D_{50}) of study sites grouped by land use type and blocked by region. FGV = forest or grassland; AGR = agricultural; URB = urban. Bars are one standard error. See text for ANOVA results.

Bank height also varied significantly among land uses and regions (Table 14) (Fig. 19C). Corrected for region, agricultural streams had a median bank height 0.62 m (95% CI = 0.02 – 1.23 m) higher than forest or grassland streams while urban streams had a median bank height 0.68 m (95% CI = 0.07 – 1.29 m) higher than forest or grassland streams; bank heights did not significantly differ between agricultural and urban streams (*p* = 0.97). Corrected for land use, only southwest sites were significantly shallower than a subset of other regions (Kansas, Puerto Rico, Oregon, and North Carolina) (*p* < 0.004).

Benthic sediments

Median sediment diameter (D_{50}) significantly differed among land use and regions (*F* = 4.24; p<0.001; 9, 62 d.f.; natural log transformed data) (Fig. 20). However, after applying the post hoc comparison correction, there were no significant (p>0.06) differences among regions after correcting for land use type. D_{50} tended to be smaller in agricultural and urban streams than in forest or grassland streams after corrections for regional differences. D_{50} was a median of 1.5 mm (Tukey's 95% CI = 1.2 – 1.9) smaller for agricultural and urban streams than for forest or grassland streams. D_{50} did not significantly differ between agricultural and urban streams (*p* = 0.92).

The MANOVA of percent of clay, sand, silt, gravel, and cobble sediments showed significant differences among land uses (Pillai-Bartlett statistic = 0.32; approximate *F* = 2.27; p = 0.02; 10, 118 d.f.; arcsine-squareroot transformed fraction data) and region (Pillai-Bartlett statistic = 1.51; approximate *F* = 3.82; p < 0.001; 35, 310 d.f.). However, when each sediment percent was analyzed individually, only silt and cobble exhibited significant effects of both land use (*F* > 4.54 on 2, 62 d.f.; p < 0.014) and region (*F* > 3.00 on 7, 62 d.f.; *p* < 0.009) (Fig. 21). Silt in regional-corrected forest or grassland streams was an average of 6.9% (95% CI = 0.2 – 21.9%) lower than in agricultural streams and 8.6% (95% CI = 0.6 – 24.5%) lower than in urban streams (Fig. 21A). After corrections for land use and post hoc comparisons, significant differences in sediment size percents among regions existed for Oregon (lower silt sediment percents) versus Wyoming, southwest, Massachusetts,



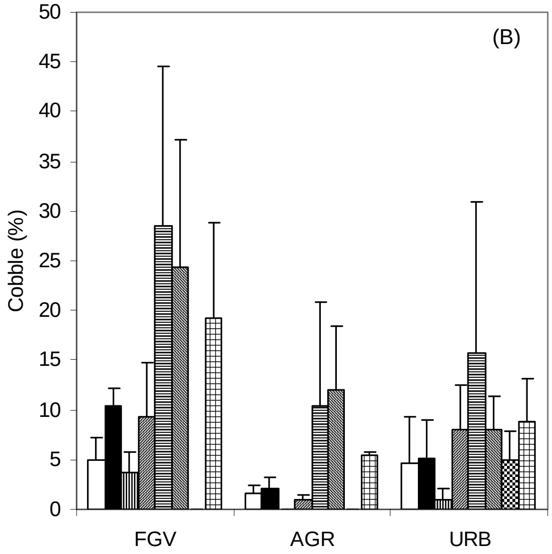


Fig. 21. Percent of (A) silt and (B) cobble sediments for study sites grouped by land use type and blocked by region. FGV = forest or grassland; AGR = agricultural; URB = urban. Bars are one standard error.

and Puerto Rico (higher silt percent) ($p < 0.03$) and Puerto Rico (lower silt percent) versus the southwest and Massachusetts (higher silt fraction) ($p < 0.02$). Correspondingly, cobble in forest or grassland streams (corrected for region) was an average of 2.4% (95% CI = 0.1 – 8.6%) greater than in agricultural streams. Unlike silt, no significant differences were detected for cobble percent between forest or grassland streams and urban streams (p = 0.21) (Fig. 21B). After correcting for land use, only one significant (p = 0.05) difference was detected among regions: Oregon streams tended to have a greater percent of cobble than Puerto Rico streams. The remaining sediments exhibited significant, but inconsistent, regional differences after being corrected for land use ($F > 4.51$ on 7, 62 d.f.; $p < 0.001$), but no evidence for a significant differences among land use after correcting for region ($F < 2.26$ on 2, 62 d.f.; $p > 0.11$).

Riparian zone attributes

Riparian canopy cover over directly over the stream and in the riparian zone differed significantly among land use and regions ($p < 0.001$) (Table 14; Fig. 22A and 22B). After corrections for regional differences, riparian canopy cover over the channel for forest or grassland streams was an average of 16.9% (95% CI: 4.1 – 37.6%) greater than agricultural streams; likewise while canopy cover in the riparian zone at forest or grassland streams was 23.2% (95% CI: 6.2 – 45.3%) greater than agricultural streams. Channel canopy cover for urban streams was an average of 8.6% (95% CI: 0.4 – 24.5%) greater than agricultural streams while riparian zone canopy cover for urban streams was an average of 7.0% (95% CI: 0.1 – 24.5%) greater than agricultural streams. No significant differences between canopy covers were detected between urban and forest or grassland streams ($p > 0.09$).

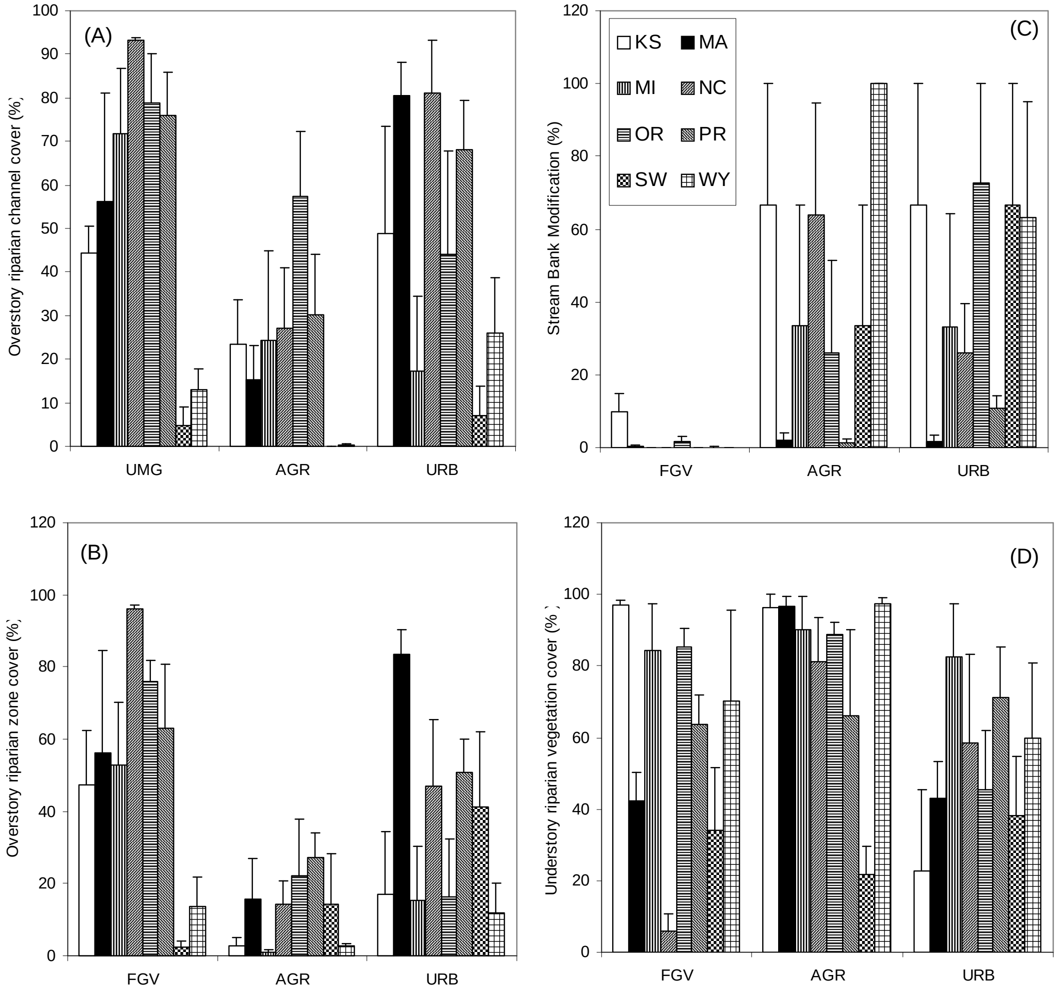


Fig. 22. (A) Channel overstory cover; (B) Riparian overstory; (C) arcsine-squareroot transformed fraction of stream bank modified by human activities; and (D) understory riparian vegetation cover for study sites grouped by land use type and blocked by region. FGV = forest or grassland; AGR = agricultural; URB = urban. Bars are one standard error. See Table 14 for ANOVA results.

After correcting for land use and for post hoc comparisons, sites in the southwest and in Wyoming had significantly lower channel canopy cover (but not riparian cover) than five other regions ($p < 0.05$) (Fig. 22A). The only non-significant difference was with Michigan/Indiana sites ($p > 0.08$) (Fig. 22A).

There was a significant difference in percent of stream bank modified and understory riparian vegetation cover (extending 25 m lateral distance from the stream) among land use types ($p<0.001$) (Table 14) (Fig. 22C). While there also were significant differences among regions ($p < 0.03$), these differences may be a statistical artifact since regions were not significantly different ($p > 0.06$) after applying the post hoc correction. For understory vegetation cover, only southwest sites were significantly different ($p < 0.02$) than Michigan/Indiana and Wyoming sites (Fig. 22D). Both agricultural and urban streams had a significantly greater proportion of modified stream bank (averages of 31.6% and 37.6%, respectively; 95% CIs of 4.8 – 68.4% and 7.8 – 74.1%, respectively) than forest or grassland streams after correcting for region. Agricultural streams had a significantly greater proportion of understory vegetation cover than both forest or grassland streams and urban streams after correcting for region, with agricultural streams having an average of 7.0% (95% CI: 0.1 – 25.9%) greater than forest or grassland streams and 12.5% (95% CI: 1.2 – 36.5%) greater than urban streams.

Multiple regression models for NO_3^- uptake

NO_3^- uptake rate (U) was correlated with stream discharge, NO_3^- concentration, and NH_4^+ concentration ($p < 0.01$). Among models evaluated in the stepwise selection, the top model (Model 8 in Table 15) was 7.3 times more likely than the third-best model (Model 6) and 2.8 times more likely than the second-best model (Model 7) as indicated by evidence ratios (Burnham and Anderson 2006). Region also was strongly correlated with U (*p*<0.001). In addition to including region, Q, NO_3^- and NH_4^+ in the top model (Model 8), overstory riparian channel cover and the percent of silt and cobble sediments were selected (Tables 15 and 16). When all other variables were held constant, the median of U decreased by 0.06 $\mu g\ m^{-2}\ min^{-1}$ (95% CI = 0.02 – 0.10) for every 10% increase in overstory riparian channel cover. Likewise, the median of U increased by 0.52 $\mu g\ m^{-2}\ min^{-1}$ (95% CI = 0.24 – 1.16) for every 10% increase in silt sediment composition. Lastly, median of U decreased by 0.04 $\mu g\ m^{-2}\ min^{-1}$ (95% CI = 0.01 – 0.82) for every 10% increase in cobble sediment composition. In fact, the top model for fraction of U by denitrification (f_{den}) (Model 10 in Table 17) included silt sediment composition (along with NO_3^-, CV stream depth, % stream bank modified, and % understory riparian vegetation cover). f_{den} showed increased by an average of 0.0020 (95% CI = 0.00004 – 0.0078) for every 10% increase in silt sediment composition (Table 18). Moreover, the third-best model for f_{den} (only 1.7 times less likely than the top model given available data) included a negative correlation with D_{50}, showing that proportion of U accounted for by denitrification decreased as sediment size increased (Table 16).

Table 15. Summary of multiple regression models of NO_3^- uptake rate (U). Potential explanatory variables included region (RE; indicator), discharge (Q; L s^{-1}), NO_3^- (µg N L^{-1}), NH_4^+ (µg N L^{-1}), CV stream depth (CVD; %), width/depth (WD; m/m), bank height (BH; m), modified stream bank (mod; %), channel canopy cover (chan; %), riparian canopy cover (rip; %), understory vegetation cover (UV; %), D_{50} (m), silt (S; %), and cobble (C; %). Percents were arcsine squareroot transformed (except for CV stream depth); other variables were natural log transformed. *K* = number of model terms (including ε); AIC_c = small sample size Akaike's Information Criterion; $L(g_i \mid x)$ = likelihood of model given data; w_i = model probability (Akaike weight); Evidence ratio = evidence for model *i* versus the final model.

Model†	*K*	AIC_c	$\Delta\ AIC_c$	$L(g_i \mid x)$	w_i	Evidence ratio
1	16	-19.9	18.6	0.0001	0.0001	11035.2
2	15	-23.2	15.3	0.0005	0.0003	2098.8
3	14	-26.4	12.1	0.0023	0.0015	433.8
4	13	-29.4	9.2	0.0103	0.0066	97.2
5	12	-32.2	6.3	0.0423	0.0272	23.6
6	11	-34.6	4.0	0.1376	0.0884	7.3
7	10	-36.5	2.0	0.3636	0.2336	2.8
8	9	-38.5	0.0	1.0000	0.6424	1.0

† Model 1: U ~ RE+Q+NO_3+NH_4+CVD+WD+BH+mod+chan+rip+UV+D_{50}+S+C
Model 2: U ~ RE+Q+NO_3+NH_4+CVD+BH+mod+chan+rip+UV+ D_{50}+S+C
Model 3: U ~ RE+Q+NO_3+NH_4+CVD+BH+mod+chan+UV+ D_{50}+S+C
Model 4: U ~ RE+Q+NO_3+NH_4+CVD+BH+mod+chan+ D_{50}+S+C
Model 5: U ~ RE+Q+NO_3+NH_4+BH+mod+chan+ D_{50}+S+C
Model 6: U ~ RE+Q+NO_3+NH_4+mod+chan+ D_{50}+S+C
Model 7: U ~ RE+Q+NO_3+NH_4+mod+chan+S+C
Model 8: U ~ RE+Q+NO_3+NH_4+chan+S+C

Table 16. Variables selected by AIC_c criteria (Model 8 in Table 15) for NO_3^- uptake rate (U). Variable codes are described in Table 15. *F* = 26.25 on 13 and 58 d.f.; p <0.001; adjusted r^2 = 0.82.

Variable	coefficient	SE	*t*	*p*
RE - KS (Intercept)	1.57	0.41	3.87	<0.001
RE - MA	-0.64	0.39	-1.65	0.11
RE - MI	0.23	0.37	0.62	0.54
RE - NC	-1.16	0.39	-3.00	0.004
RE - OR	-0.53	0.36	-1.45	0.15
RE - PR	0.22	0.39	0.57	0.57
RE - SW	-1.23	0.38	-3.27	0.002
RE - WY	-0.68	0.42	-1.64	0.11
Q	0.38	0.07	5.03	<0.001
NO_3^-	0.56	0.05	12.38	<0.001
NH_4^-	-0.19	0.07	-2.59	0.01
chan	-1.35	0.37	-3.65	0.001
S	0.95	0.41	2.32	0.02
C	-1.44	0.72	-2.01	0.05

Table 17. Summary of multiple regression models of fractional NO_3^-uptake by denitrification (f_{den}). Potential explanatory variables included region (RE; indicator), discharge (Q; L s^{-1}), NO_3^- (μg N L^{-1}), NH_4^+ (μg N L^{-1}), CV stream depth (CVD; %), width/depth (WD; m/m), bank height (BH; m), modified stream bank (mod; %), channel canopy cover (chan; %), riparian canopy cover (rip; %), understory vegetation cover (UV; %), (D_{50} (m), silt (S; %), and cobble (C; %). Percents were arcsine-square root-transformed (except for CV stream depth); remaining variables were natural log transformed. See caption of Table 15 for header term descriptions.

Model†	*K*	AIC_c	Δ AIC_c	$L(g_i \mid x)$	w_i	Evidence ratio
1	16	-194.6	22.3	0.0000	0.0000	69418.2
2	15	-203.6	13.2	0.0013	0.0005	748.6
3	14	-206.8	10.1	0.0064	0.0022	155.5
4	13	-209.3	7.6	0.0225	0.0078	44.5
5	12	-211.2	5.7	0.0592	0.0204	16.9
6	11	-213.0	3.8	0.1477	0.0510	6.8
7	10	-214.5	2.3	0.3148	0.1086	3.2
8	9	-215.8	1.0	0.6041	0.2084	1.7
9	8	-216.3	0.6	0.7421	0.2561	1.3
10	7	-216.9	0.0	1.0000	0.3450	1.0

† Model 1: f_{den} ~ RE+Q+NO_3+NH_4+CVD+WD+BH+mod+chan+rip+UV+D50+S+C
Model 2: f_{den} ~ Q+NO_3+NH_4+CVD+WD+BH+mod+chan+rip+UV+D50+S+C
Model 3: f_{den} ~ Q+NO_3+NH_4+CVD+WD+BH+mod+chan+UV+D50+S+C
Model 4: f_{den} ~ Q+NO_3+NH_4+CVD+BH+mod+chan+UV+D50+S+C
Model 5: f_{den} ~ Q+NO_3+NH_4+CVD+BH+mod+UV+D50+S+C
Model 6: f_{den} ~ Q+NO_3+NH_4+CVD+mod+UV+D50+S+C
Model 7: f_{den} ~ Q+NO_3+NH_4+CVD+mod+UV+D50+S
Model 8: f_{den} ~ NO_3+NH_4+CVD+mod+UV+D50+S
Model 9: f_{den} ~ NO_3+NH_4+CVD+mod+UV+S
Model 10:f_{den} ~ NO_3+CVD+mod+UV+S

Table 18. Coefficients for variables selected according to AIC_c criteria (Model 10 in Table 17) for fractional NO_3^-uptake by denitrification (f_{den}). Variable codes are listed Table 17. *F* = 6.80 on 5 and 66 d.f.; p <0.001; adjusted r^2 = 0.29.

Variable	coefficient	SE	*t*	*p*
(Intercept)	0.35	0.12	2.83	0.006
NO_3	0.05	0.01	4.29	<0.001
CVD	0.01	0.005	-2.00	0.05
mod	-0.26	0.07	-3.72	<0.001
UV	-0.21	0.09	-2.32	0.02
S	0.22	0.10	2.10	0.04

Like the AIC$_c$-selected multiple regression model for U, NO_3^- concentration also was significantly correlated to f_{den} (Table 19). But unlike U, region, discharge, and NH_4^+ were not included based on selection criteria (Table 18). When other variables were held constant, average ASR-transformed f_{den} decreased by 0.0010 (95% CI = 0.0002 – 0.006) for every 10% increase in CV of stream depth. Average f_{den} decreased by 0.0044 (95% CI = 0.0005 to 0.0079) for every 10% increase in stream bank modification. Lastly, f_{den} decreased by 0.002 (95% CI = 0.0005 to 0.0079) for every 10% increase in understory riparian vegetation cover (Table 19).

DISCUSSION

Channel morphology

Our observations of simplified channel morphology in agricultural and urban landscapes relative forest and grassland streams suggest several critical influences of adjacent land use practices on stream ecosystems (Paul and Meyer 2001; Sweeney et al. 2004). High width-to-depth ratio, high variability in stream depth, and lower bank heights observed in forest or grassland streams likely reflect greater interaction between stream channels and adjacent floodplains (Gregory et al. 1991). This interaction strongly influences capacity for streams to store detrital organic matter (Sweeney et al. 2004), exchange of surface water into hyporheic zone sediments (Fisher et al. 1998), and contact between the nutrients transported in the water column and biologically reactive substrates (Valett et al. 2006). In contrast, channel morphology of agricultural and urban streams were simplified and incised, likely disrupting interactions between channels and adjacent floodplains and reducing the capacity for organic matter storage, hyporheic exchange, and nutrient uptake and transformation (Paul and Meyer 2001; Grimm et al. 2005; Wollheim et al. 2006).

Benthic sediments

Despite tremendous variation in geologies, reach position within drainage basins, and flow regimes, sediments in forest or grassland streams tended to be larger and have less silt than agricultural and urban streams, regardless of region. These results are consistent with the pattern of increased loading of fine sediments to stream channels modified by human activities (Waters 1995). Increased fine sediment loading often results from concurrent increases in overland flow, particularly during storm events, and decreases in terrestrial retention structures such as riparian vegetation and plant detritus (Malanson 1993).

Significant regional differences in stream sediment characteristics after accounting for land use likely reflect interactions of local geology and climate. Sites in Wyoming were located in a glaciated, depositional alluvial valley of the upper Snake River and, for at least forest or grassland streams, exhibit an annual hydrograph dominated by late spring/early summer snowmelt (Poff and Ward 1988). Southwest sites were located in a sedimentary desert environment and can experience drastic variation in stream flow due to delivery of overland flow in flash-flood events (Dent and Grimm 1998). Massachusetts streams drain a glaciated landscape extenuated by a relatively long history (400 yrs) of intense European agricultural land use (Harvard Forest LTER, unpublished data). Overall, these sites would be expected to naturally contain a higher percentage of fine-grained sediments. In comparison, streams in both Oregon and Puerto Rico drain mountainous landscapes with relatively recent volcanic geologies and experience high annual rates of precipitation primarily in the form of rain (Taylor and Hannan 1999; Daly et al. 2003). Thus, these regions would be expected to contain higher proportion of large-grained sediments and boulders.

Riparian zone attributes

Contrary to our initial hypothesis, results suggested common patterns of riparian vegetation structure according to surrounding land use practice across diverse regions. Two attributes in particular, overstory riparian channel cover and overstory riparian zone cover, showed consistent patterns across land use types from all regions despite diverse natural vegetation cover that included forest, grassland, and desert regions. The lower percent of channel and riparian canopy cover in agricultural streams is consistent with higher rates of gross primary production (GPP) observed in these streams (Bernot et al., in preparation). However, channel and riparian canopy cover were similar between forest or grassland and urban streams, a pattern that has previously been observed in region-specific studies (Groffman et al.

2003; Poor and McDonnell 2007). This similarity may result from the presence of buildings or planting of ornamental trees and shrubs (Poor and McDonnell 2007). Therefore, while urban development often alters composition and structure of riparian vegetation, specific functional components of riparian zones can be maintained in these modified landscapes.

While we observed a common pattern of channel and riparian canopy cover among forest or grassland, agricultural, and urban streams, the magnitude of canopy cover among different regions reflected differences in local vegetation. Specifically, arid and semiarid regions (Kansas, Wyoming, the southwest) typically had less channel and riparian canopy cover than most forested regions (Massachusetts, North Carolina, Puerto Rico, and Oregon). However, there was one exception: channel and riparian canopy cover for Michigan/Indiana sites were more similar to the arid/semiarid streams than to other forested sites. Since the Michigan/Indiana sites were in an area long-dominated by intense agriculture (Bernot et al. 2006), the overall landscape probably was more similar to the semiarid and arid landscapes of the southwest and Wyoming than other forested regions.

The majority of sites across all biomes had experienced at least a century of European land use modification in the surrounding catchment. The land use legacy on ecosystem structure and function can last for decades to centuries (Foster et al. 2003). For example, a biodiversity survey of forested southern Appalachian streams with mixed land use histories showed that agricultural practices 50 years prior had reduced aquatic macroinvertebrate diversity to the present day (Harding et al. 1995). Only one stream in this analysis (Mack Creek, Oregon) had never experienced substantial influence of Euro-American land use activities (surrounded by a 500-yr old coniferous forest; F. Swanson, H.J. Andrews Experimental Forest, personal communication). All other forest or grassland streams in this study had been modified by timber harvest, agricultural practices, or other land use practices in the past two centuries.

Relation with NO_3^- uptake

Positive relationships between N uptake rate (either NO_3^- or NH_4^+) with stream discharge (Wollheim et al. 2001; Peterson et al. 2001; Ensign and Doyle 2006; Mulholland et al., in submission) and stream dissolved inorganic N concentration are well-demonstrated (Mulholland et al. 2002; O'Brien et al. 2007). The relationship with discharge can reflect changes in U in response to the physical stream environment, biological community, or a combination of both (Wollheim et al. 2001). Because all variables were natural log transformed, the positive correlation between U and NO_3^- suggests a saturating response of U to increasing NO_3^- concentration (O'Brien et al. 2007), though the exact curvilinear nature of the response (Michaelis-Menton vs. power law) was not ascertained. Since U was estimated using a ^{15}N tracer, the negative correlation of U with NH_4^+ suggests an inhibition of NO_3^-

uptake by availability of NH_4^+, which is less energetically-expensive for assimilatory uptake than NO_3^- (Schlesinger 1997).

Two factors may account for significant effect of region on U: (1) degree of N-limitation (Grimm et al. 2003) or (2) the overall N availability in the stream, which may lead to different biological community structure and associated N requirements (O'Brien et al. 2007). Sites in eastern North America (Massachusetts, Michigan/Indiana, North Carolina, Kansas, Puerto Rico) may have been more likely to be phosphorus-limited than western sites (Oregon, southwest, Wyoming) because of high deposition rates of inorganic N in the eastern US relative to the western US, high degrees of weathering in the east and Puerto Rico, or greater abundance of P leached from relatively young volcanic geologies in the west (Gregory et al. 1987; Grimm et al. 2003). However, P concentrations did not show consistent regional or land use patterns (Mulholland et al., submitted). Overall N availability appears a more plausible explanation for regional differences; intercept adjustments in regression models showed that the highest U was in regions with high N concentrations (Kansas, Massachusetts, Michigan/Indiana, Puerto Rico).

A strong relationship between whole-stream NO_3^- uptake or biological demand for N and GPP has been found in many regions of North America, including montane streams in New Mexico (Fellows et al. 2006), southern Appalachian forest streams (Fellows et al. 2006; Mulholland et al. 2006), and Pacific Northwest streams (Gregory 1980; Chapter 2). While we did not directly calculate the correlation of U with GPP, the negative correlation with overstory riparian channel cover agreed with the concept of autotrophic control on whole-system U. Furthermore, the final model for fractional U by denitrification (f_{den}) selected the percent of understory riparian vegetation cover, which was highest in agricultural streams where overstory channel cover was the least. While channel or riparian zone cover was not selected for in final f_{den} model, the selection of understory vegetation cover may represent an increased role of assimilatory NO_3^- uptake by primary producers. In more overstory-shaded forest or grassland streams and urban streams, U likely is more distributed among assimilatory uptake and denitrification.

While U and f_{den} correlated with environmental factors reflecting proportional shifts of autotrophic NO_3^-uptake, our results also suggest an important role for denitrification in whole-stream U. Final regression models for U and f_{den} both showed positive correlations with proportional fraction of silt in benthic sediments. All else being equal, hydraulic conductivity of stream water infiltrating through fine benthic sediments should by lower than through larger sediments (Boulton et al. 1998; Dahm et al. 2006). Given adequate supply of organic carbon, heterotrophic respiration in fine-grained benthic sediments can create anoxic environmental conditions (Findlay 1995; Hill et al. 2000). Both U and f_{den} of NO_3^- should be positively related to extent of anoxic environments in the stream channel since NO_3^- is the terminal electron acceptor most similar to oxygen in terms of energy yield per mole reduced (Hedin et al. 1998). Thus, our results suggest a relationship between the capacity of streams to denitrify and sediment composition.

Conclusions

This study shows that stream channel morphology, benthic sediment diversity, and riparian vegetation among diverse biomes had similar patterns among three common land use designations. These effects in turn influenced capacity of NO_3^- processing in the stream ecosystem by influencing factors that control in-stream primary production, contact time between water column nutrients and biologically reactive substrates, and environments conducive for denitrification. Canopy cover directly over the channel—lowest in agricultural streams—likely influenced U by limiting photosynthetically active radiation available for use by primary producers. Abundance of silt in benthic sediments likely reflected the extent of benthic areas suitable for denitrification (anoxic environments) and had positive correlations with both U and f_{den}. Therefore, U and f_{den} were highest in streams surrounded by agricultural and urban land uses. However, because higher concentrations of NO_3^- generally were observed in human-altered streams and the asymptotic relationship between U and NO_3^- concentration (Table 16), N uptake and transformation in agricultural and urban streams accounted for a lower fraction of total N in stream transport. This study demonstrates that channel morphology, sediments, and riparian vegetation and associated stream nutrient dynamics among three different land uses are comparable across multiple regions despite diverse climates, and geologies, natural vegetation composition and structure, and forms of land use practices.

Chapter 5

CONCLUSIONS

Riparian zones play key roles in regulating transfer of energy and nutrients between terrestrial and aquatic ecosystems (Gregory et al. 1991; Young and Huryn 1999). In the case of bio-available nitrogen (N), plant uptake and microbial processing in the complex physiochemical environment of riparian soils reduces the flux of N from terrestrial sources to adjacent streams by as much as 50-100% (e.g., Hedin et al. 1998; Cey et al. 1999; Wigington et al. 2003). This filtering function is an important management tool for reducing terrestrial export of N to downstream water bodies (Cey et al. 1999; Wigington et al. 2003). But another less-recognized influence of riparian zones on attenuation of terrestrial N export is their control on organic matter dynamics in the adjacent stream ecosystem. Riparian zones are the dominant allochthonous source of organic matter—primarily in the form of leaves and wood—for many small streams, particularly those forested landscapes (Fisher and Likens 1973; Vannote et al. 1980). Because the is carbon source often is deficient in N relative to C, heterotrophic microbes decomposing these substrates must rely on N supplied from the surrounding stream water environment (Tank and Webster 1998; Gulis et al. 2003). Also, plant detritus not only provides an important organic carbon source for aquatic food webs, but it also has strong influences on stream channel complexity (Bilby and Likens 1981) and stream sediment composition (Valett et al. 2002). Riparian zones also limit in-stream photosynthesis through shading (Vannote et al. 1980; Mulholland et al. 2006).

In Chapter 2 of this dissertation, I quantified shifts in uptake and transformation of NO_3^- in naturally N-limited stream ecosystems in the Willamette River basin in response to human alteration of riparian zones and N loading. Ambient uptake of NO_3^- was dominated by assimilation into autotrophic and heterotrophic biomass and, correspondingly, denitrification of tracer $^{15}NO_3^-$ was a small proportion of fate in the majority of study reaches. Highest demand for NO_3^- (V_f; Hall and Tank 2003) was observed in agricultural and urban reaches with high rates of GPP, likely due to combined influence of lower riparian canopy cover and high sustained supply of N (indicated by TDN concentrations). However, whole-stream uptake rates of NO_3^- did not show clear trend with GPP, N concentration (both NO_3^- and TDN), or stream-riparian biophysical characteristics like detrital organic matter standing stock and stream discharge. Instead, uptake by autotrophic and detrital benthic biomass changed proportionally relative to surrounding biophysical conditions. Therefore, modifications to biophysical characteristics of riparian zones by human land use in western Oregon altered N uptake and transformation in streams by altering pathways of uptake by detrital and autotrophic stream biomass compartments.

Results from Chapter 3 showed the response of wood breakdown in streams of western Oregon to NO_3^- availability in the stream environment. Wood is an important component of Pacific Northwest streams and recruitment is closely linked with dynamics of riparian forests in the region (Meleason et al. 2003). Little evidence for a positive relationship between short-term (< two month) wood breakdown rate and NO_3^- availability was found in a survey of six Willamette River basin streams and the manipulative field experiment on a stream surrounded by 500-yr old coniferous forest in the Oregon Cascades. However, I did observe effects of NO_3^- availability on wood substrates with different nutritional qualities (red alder and Douglas-fir) after four months of incubation. This pattern likely reflected differential responses of wood biofilm microbial communities that utilized more labile components of woody tissues (early decomposition phase) versus one that actively decomposed cellulose and lignin constituents (later decomposition phases) (Mellilo et al. 1984). Red alder substrates (high quality wood) showed a greater response (i.e., biofilm respiration and activity of two extra-cellular enzymes) to NO_3^- enrichment after four months of incubations, suggesting a stimulation of organic C release from high quality wood substrates by increased NO_3^- loading. Therefore, turnover of wood should be rapid in streams with red alder riparian forests with high quality wood inputs and high loading rates of NO_3^-. On the other hand, wood recruited from coniferous riparian forests with lignin rich, poor nutritional quality wood inputs should have relatively long residence times regardless of NO_3^- loading since there is low relative abundance of labile carbon in the stream ecosystem.

The analysis of streams from eight regions in North America and Puerto Rico presented in Chapter 4 showed several widely observed influences of human land use activities on riparian zones and stream channel morphology. This analysis also suggested links between surrounding land use and the capacity of stream ecosystems to take up and transform NO_3^-. The abundance of fine-grained benthic sediments (silt) was positively associated with the fractional stream NO_3^- uptake by denitrification regardless of region, likely reflecting positive relationships between fine sediments and extent of anoxic environments in the stream benthos. Canopy cover provided by riparian vegetation directly over the stream, which was lowest in agricultural streams and similar between forest and grassland streams and urban streams, appeared to influence whole-system NO_3^- uptake by limiting the amount light available for aquatic photosynthesis. Therefore, rates of NO_3^- uptake and fractional stream denitrification rates were highest in streams surrounded by agricultural and urban land uses. However, NO_3^- uptake and transformation in agricultural and urban streams accounted for a lower fraction of total N in stream transport because of the asymptotic relationship between uptake and NO_3^- concentration, which generally was highest in streams surrounded by agricultural and urban land use practices. This analysis demonstrated that diverse forms and intensities of agricultural and urban land use practices can have similar influences on stream NO_3^- dynamics across a broad range of biomes by having similar influences on stream channel structure and riparian zone vegetation.

In summary, I demonstrated that attributes of riparian zone structure and vegetation composition can strongly influence NO_3^- uptake and transformation in stream ecosystems. Based on my results and previous studies, riparian zones bordering stream ecosystems directly influence primary production through light-limitation (Chapters 2 and 4), nutrient dynamics (Fisher et al. 1998; Ashkenas et al. 2004), and input of allochthonous organic matter (Young and Huryn 1999; Chapter 2). I also have shown that land use effects on riparian zone – stream ecosystems interactions must be considered in concert with other factors, particularly increased nutrient loading, alteration of channel physical structure, and basin position of the stream. Land use activities generally increase supply of nutrients to streams, potentially altering biological community structure (O'Brien et al. 2007) and components of stream ecosystem metabolism (Young and Huryn 1999; Gulis et al. 2003; Fellows et al. 2006; Chapters 2 and 3 in this dissertation). Land use activities also have a direct impact on stream channel structure (Chapter 4). These direct impacts included removal of organic matter, particularly wood (Sedell et al. 1988), channelization (Paul and Meyer 2001), and increased rates of channel incision (Paul and Meyer 2001; Booth 2005). However, streams that experience effects of agricultural and urban land use activities often are located at lower catchment elevations than streams surrounded by forests or native grasslands (Goldstein et al. 1997). Consequently, background climatic, geological, and biological processes structuring riparian zones and streams (Montgomery 1999) in agricultural and urban land uses differ from those structuring higher elevation streams currently bordered by forests or native grasslands. Thus, future studies should be designed to disentangle specific effects of land use on riparian zones and stream ecosystems versus natural variation along the stream - river continuum.

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APPENDIX A. SITE PHOTOGRAPHS FOR CHAPTER 2

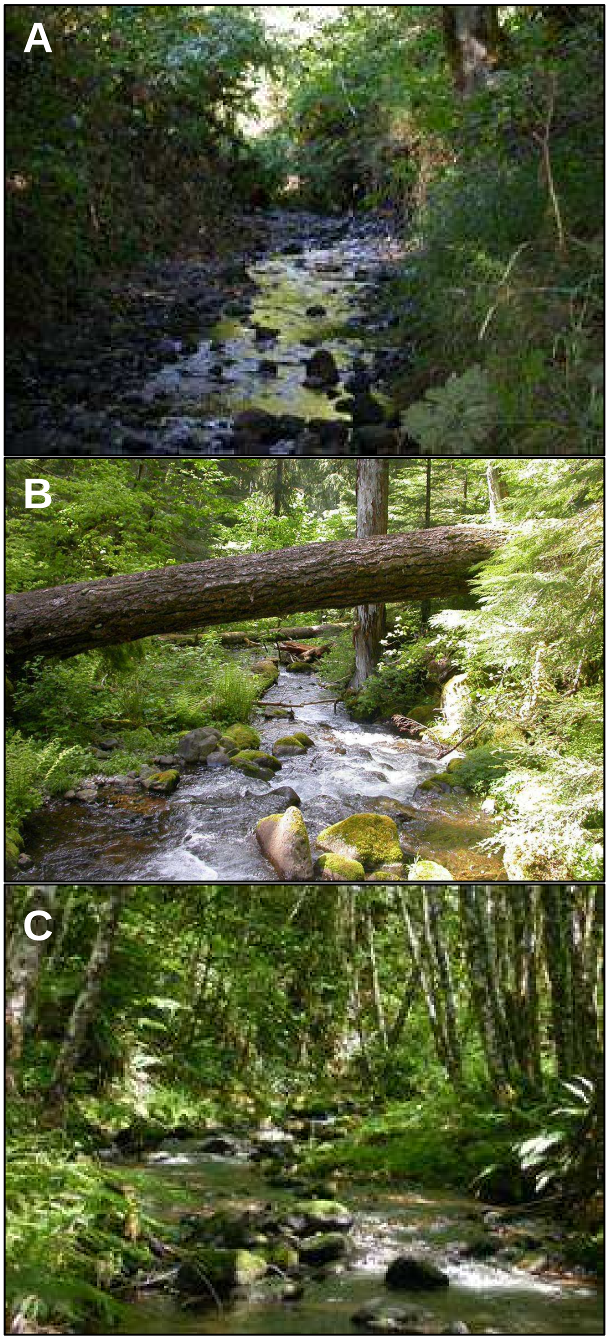

Plate A1. The forest stream reaches used in this study. Oak Creek-upper, second-growth mixed deciduous-conifer forest (A); Mack Creek, old-growth conifer forest (B); Potts Creek, second-growth mixed deciduous-conifer forest (C). All photographs taken by Sherri Johnson except (B) (taken by Roy Haggerty).

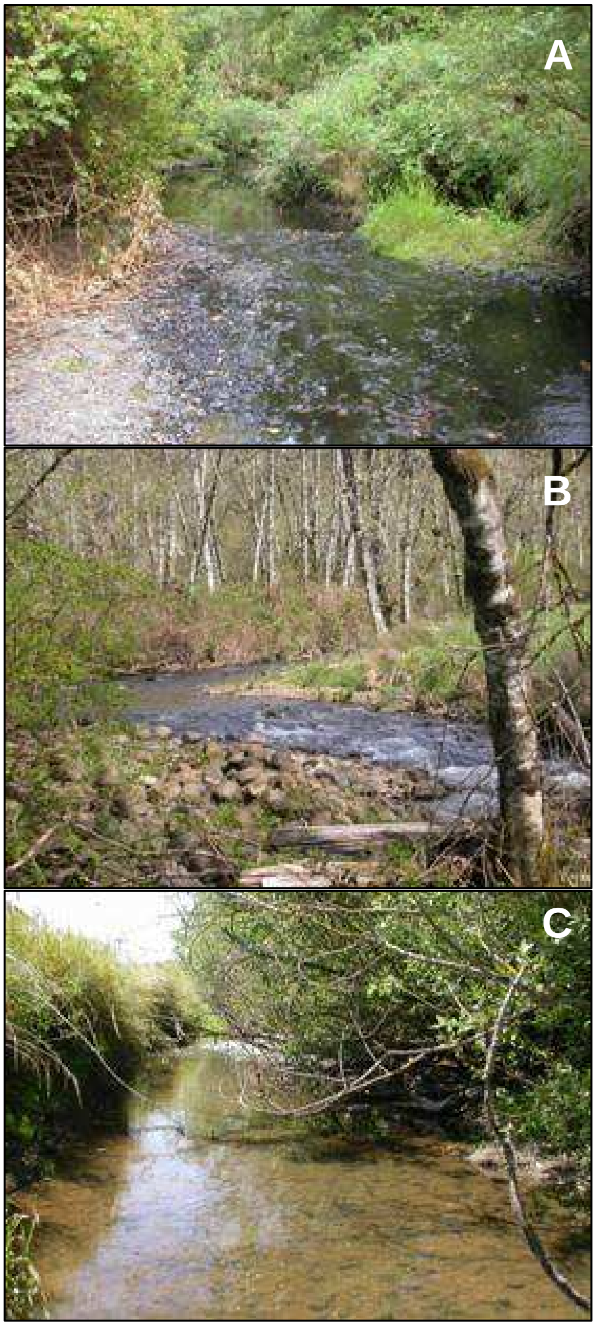

Plate A2. The agricultural stream reaches used in this study. Oak Creek-middle, adjacent grass seed fields with livestock (A); Camp Creek, adjacent pasture with livestock (B); Courtney Creek, adjacent grass seed fields with livestock (C). All photographs taken by Sherri Johnson.

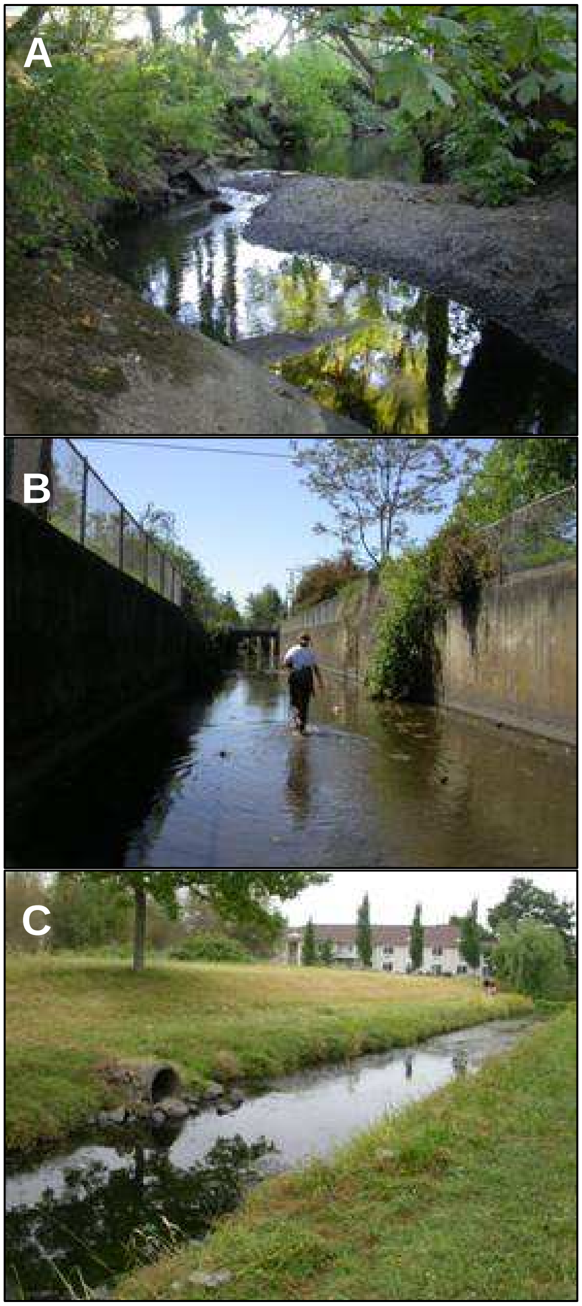

Plate A3. The urban stream reaches used in this study. Oak Creek-lower, adjacent university campus (A); Amazon Creek, adjacent residential and commercial property (B); Periwinkle Creek, adjacent residential neighborhood (C). All photographs taken by Sherri Johnson.

APPENDIX B. STREAM BENTHIC ORGANIC MATTER NO_3^- UPTAKE FOR CHAPTER 2

Table B1. Uptake rates of NO_3^- by stream biomass compartments calculated from ^{15}N tracer label 48 hr after end of the isotope release for the nine reaches. Standard errors based on six sampling points in the reach are in parentheses.

	Forest			Agriculture			Urban		
Uptake (mg N m^{-2} d^{-1})	Oak -upper	Mack	Potts	Oak -mid	Camp	Courtney	Oak -lower	Amazon	Periwinkle
FBOM	2.79 (0.64)	1.47 (0.16)	3.24 (0.44)	12.75 (1.56)	2.50 (0.60)	113.59 (24.59)	35.38 (6.86)	4.32 (2.29)	49.53 (23.84)
Leaves	0.88 (0.13)	--	10.67 (1.60)	1.34 (0.22)	2.18 (0.66)	3.74 (1.17)	2.06 (0.82)	0.004 (0.001)	--
Needles	--	0.01 (0.003)	--	--	--	--	--	--	--
Small wood	0.46 (0.19)	0.98 (0.23)	8.96 (1.42)	7.50 (2.15)	5.49 (1.47)	2.00 (0.34)	0.62 (0.13)	--	--
Large wood	1.84 (0.80)	0.33 (0.11)	0.31 (0.08)	0.15 (0.03)	0.69 (0.20)	1.67 (0.32)	0.99 (0.24)	--	--
Total detrital:	5.97 (0.24)	2.78 (0.32)	23.17 (2.36)	21.73 (2.86)	10.86 (1.81)	120.99 (24.84)	39.05 (6.79)	4.32 (2.29)	49.53 (23.84)
Epilithon	1.81 (0.66)	6.98 (1.16)	5.40 (0.38)	6.18 (0.67)	4.99 (0.55)	26.22 (6.97)	2.52 (0.98)	0.53 (0.21)	--
Filamentous algae	--	--	--	57.29 (18.68)	--	1.33 (0.70)	0.64 (0.22)	12.67 (3.19)	59.37 (52.88)
Algal fines	--	--	--	--	--	--	--	3.35 (0.75)	--
Macrophytes	--	--	--	--	--	--	--	--	29.20 (7.01)
Aquatic bryophytes	--	9.06 (1.18)	1.91 (0.94)	--	--	--	--	--	--
Total autotrophic:	1.81 (0.66)	16.05 (3.09)	7.30 (1.30)	63.48 (18.63)	4.99 (0.55)	27.56 (7.46)	3.17 (0.99)	16.55 (3.50)	88.56 (47.56)
Total uptake rate	7.78 (0.50)	18.83 (3.16)	30.48 (2.68)	85.21 (19.01)	15.85 (1.86)	148.55 (29.37)	42.21 (7.72)	20.87 (5.55)	138.09 (45.05)

APPENDIX C. ORIGINAL ^{15}N MASS BALANCE FOR CHAPTER 2

Table C1. Original mass balances of $^{15}NO_3^-$ tracer in the nine reaches based on weighted mean abundance of stream biomass types, ^{15}N tracer in benthic biomass 48 hrs after the release end, and whole-system ^{15}N spiraling metrics.

Distribution of ^{15}N recovered (%)	Forest			Agriculture			Urban		
	Oak -upper	Mack	Potts	Oak -mid	Camp	Courtney	Oak -lower	Amazon	Periwinkle
Retention									
FBOM	2.7	1.2	1.3	12.4	1.0	8.1	21.1	50.2	41.6
Leaves/Needles	1.4	<0.01	4.5	1.3	1.1	0.2	1.8	<0.01	--
Small wood	1.2	0.5	3.4	6.2	2.3	0.2	0.4	--	--
Large wood	0.9	0.6	1.2	1.1	0.3	0.0	0.7	--	--
Detrital retention	6.1	2.3	10.4	20.9	4.8	8.5	24.0	50.3	41.6
Epilithon	1.9	5.7	2.0	6.1	2.1	1.4	2.1	7.0	--
Filamentous algae	--	--	--	63.3	--	0.1	1.0	61.5	33.4
Algal fines	--	--	--	--	--	--	--	12.3	--
Macrophytes	--	--	--	--	--	--	--	--	22.5
Aquatic bryophytes	--	6.9	0.8	0.0	--	--	--	--	--
Autotrophic retention	1.9	12.5	2.9	69.3	2.1	1.6	3.1	80.8	55.9
Total retention	8.0	14.8	13.3	90.3	6.8	10.1	27.0	131.1	97.5
Denitrification	--	--	--	1.2	2.6	6.1	6.8	--	--
Export									
NO_3^-	60.4	77.0	76.1	55.9	89.7	69.7	75.2	<0.01	0.8
NH_4^+	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
DON	?	?	?	?	?	?	?	?	?
SPON	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Total export	60.4	77.0	76.1	55.9	89.7	85.9	75.2	<0.01	0.8
Total account	68.4	91.8	89.4	147.3	99.2	71.1	109.1	131.1	98.3

APPENDIX D. NH_4^+ AND SPON CALCULATIONS FOR CHAPTER 2

Table D1. Rates of net ammonification, gross NH_4^+ uptake, SPON generation, and SPON uptake estimated from incorporation of tracer ^{15}N into NH_4^+ and SPON. Error in estimates of gross NO_3^- uptake, NH_4^+ and SPON concentration, and stream discharge is not accounted for.

	Forest			Agriculture			Urban		
Parameter	Oak -upper	Mack†	Potts	Oak -mid	Camp	Courtney	Oak -lower	Amazon	Periwinkle
NH_4^+ dynamics									
Net ammonification (s^{-1})	2.3E-07	n.d.	2.5E-07	1.3E-07	3.6E-07	n.d.	n.d.	n.d.	n.d.
Sw NH_4^+ (m)	83	n.d.	133	34	449	n.d.	n.d.	n.d.	n.d.
U NH_4^+ (μg N m^{-2} min^{-1})	3.4	n.d.	11.7	30.7	15.8	n.d.	n.d.	n.d.	n.d.
V_f NH_4^+ (mm min^{-1})	2.6	n.d.	2.9	3.7	2.6	n.d.	n.d.	n.d.	n.d.
SPON dynamics									
SPON (μg N L^{-1})	57	11	21	31	50	20	47	90	137
Net SPON generation (s^{-1})	2.56E-10	n.d.	n.d.	1.0E-10	6.1E-10	n.d.	n.d.	n.d.	n.d.
Sw SPON (m)	100000	n.d.	n.d.	100.0	449	n.d.	n.d.	n.d.	n.d.
U SPON (μg N m^{-2} min^{-1})	0.1	n.d.	n.d.	38.9	128.7	n.d.	n.d.	n.d.	n.d.
V_f SPON (mm min^{-1})	0.002	n.d.	n.d.	1.3	2.6	n.d.	n.d.	n.d.	n.d.

† n.d. = not detected according to inability to adequately fit model.

APPENDIX E: STUDY DESIGN FOR CHAPTER 3

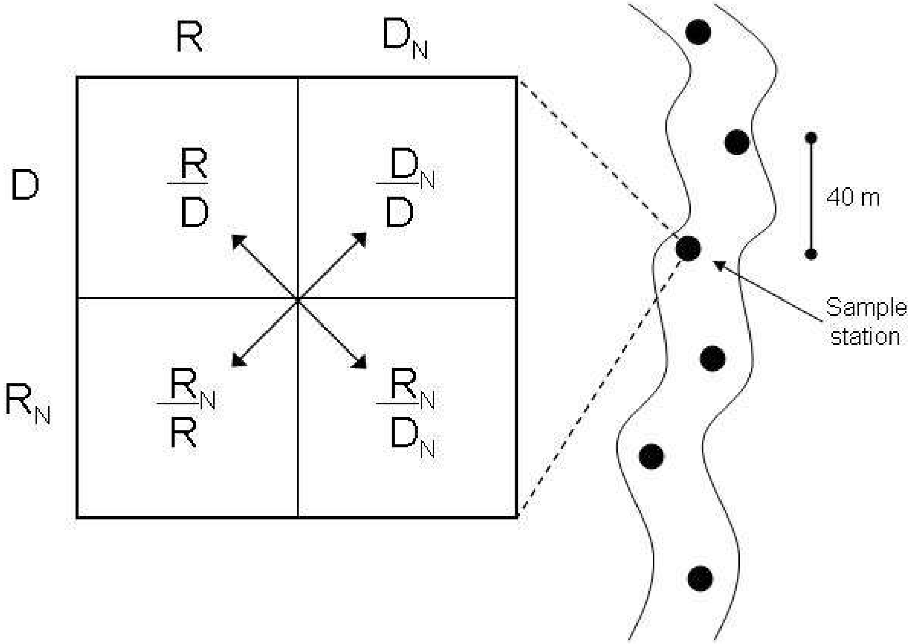

Fig. E1. Schematic of sampling design for the experiment of decomposition of Douglas-fir (D) and red alder (R) wood according to ambient N levels or N enrichment (denoted with the subscript N). Ratios of N enriched substrates to ambient substrates represent values measured of microbial biofilm characteristics (respiration, chlorophyll a, or enzyme activities). Sample stations consisted of four replicate wood substrates of each species for each N treatment level (ambient vs. enriched). For August and September 2006, four sample stations were randomly selected and one replicate of each species * treatment were collected from each station. As a consequence of losses during high flow events, only three sample stations were randomly selected for November 2006 and May 2007 collections.

APPENDIX F: ANOVA TABLE FOR CHAPTER 3

Table F1. Summaries of full multiple regression models for the correlation of short-term decay rates (*k*), respiration rates (resp), and chlorophyll *a* (chla) standing stocks to the interaction of mean temperature (temp), dissolved inorganic N (DIN), or molar DIN/SRP (N.P) with species type (spp) on the six study streams in 2004 – 2005. Values in parentheses are standard errors. d.f. = degrees of freedom; SSQ = sum-of-squares.

Model	Variable	d.f.	SSQ	*F*	*p*
k~temp*spp					
	temp	1	2.81E-05	8.36	0.02
	spp	1	6.95E-05	20.65	< 0.01
	temp*spp	1	1.30E-06	0.39	0.55
	residuals	10	3.37E-05		
k~ln(DIN)*spp					
	ln(DIN)	1	2.06E-05	5.94	0.04
	spp	1	6.95E-05	20.03	< 0.01
	ln(DIN)*spp	1	7.77E-06	2.24	0.17
	residuals	10	3.47E-05		
k~ln(N.P)*spp					
	ln(N.P)	1	2.02E-05	6.23	0.03
	spp	1	6.95E-05	21.40	< 0.01
	ln(N.P)*spp	1	1.04E-05	3.19	0.10
	residuals	10	3.25E-05		
resp~temp*spp					
	temp	1	3109	4.22	0.07
	spp	1	880	1.20	0.30
	temp*spp	1	9	0.01	0.92
	residuals	10	7364		
resp~ln(DIN)*spp					
	ln(DIN)	1	1764	2.04	0.18
	spp	1	880	1.02	0.34
	ln(DIN)*spp	1	60	0.07	0.80
	residuals	10	8657		
resp~ln(N.P)*spp					
	ln(N.P)	1	1141	1.22	0.29
	spp	1	880	0.94	0.35
	ln(N.P)*spp	1	21	0.02	0.88
	residuals	10	9320		

Table F1. Continued.

Model	Variable	d.f.	SSQ	*F*	*p*
chla~temp*spp					
	temp	1	0.93	0.79	0.39
	spp	1	0.58	0.50	0.50
	temp*spp	1	0.69	0.59	0.46
	residuals	10	11.70		
chla~ln(DIN)*spp					
	ln(DIN)	1	4.63	5.36	0.04
	spp	1	0.58	0.68	0.43
	ln(DIN)*spp	1	0.04	0.04	0.84
	residuals	10	8.65		
chla~ln(N.P)*spp					
	ln(N.P)	1	3.42	3.47	0.09
	spp	1	0.58	0.59	0.46
	ln(N.P)*spp	1	0.03	0.03	0.86
	residuals	10	9.86		

APPENDIX G. DATA ON INDIVIDUAL SITES IN CHAPTER 4

Table G1. NO_3^- uptake rates (U), fraction of NO_3^- uptake attributable to denitrification (f_{den}), discharge (Q), and NO_3^- and NH_4^+ concentrations for all LINX 2 streams. UMG = forest and grassland; AGR = agriculture; URB = urban.

Region	Stream	Land use	U (mg N/m/min)	f_{den} (%)	Q (L/s)	NO_3^- (μg N/L)	NH_4^+ (μg N/L)
MA	Cart Creek	UMG	5.1	0	4.8	15.3	293.2
MA	IS_104	URB	87.0	61	2.1	1336.2	121.2
MA	Boxford	UMG	18.0	27	12.2	52.8	13.3
MA	Runaway Brook	AGR	82.0	25	0.7	1164.2	80.2
MA	Long Meadow Brook	AGR	68.5	97	2.4	989.3	63.1
MA	Gravelly Brook	UMG	11.5	0	2.0	112.2	435.4
MA	Black Brook	AGR	37.2	26	120.1	50.3	30.5
MA	Sawmill Brook	URB	28.2	64	4.9	1024.7	39.2
MA	IS_118	URB	43.4	100	11.3	512.8	253.8
NC	Hugh White Creek	UMG	5.3	0	19.4	7.3	3.2
NC	Hoglot Branch	AGR	71.0	0	52.7	154.5	17.1
NC	Crawford Branch	URB	27.0	0	45.0	102.5	15.4
NC	Big Hurricane Branch	UMG	5.9	43	12.2	240.7	5.6
NC	Jerry Branch	AGR	19.1	57	26.5	405.7	108.2
NC	Mud Creek	URB	29.2	4	51.8	139.5	6.0
NC	Cunningham Creek	UMG	13.1	74	49.3	10.1	2.7
NC	Blacks Branch	AGR	122.9	10	189.4	172.6	8.5
NC	Sugarloaf Creek	URB	22.4	54	79.8	54.2	2.6
MI	Sand Creek	UMG	79.0	15	4.9	282.6	54.8
MI	STEINKE DRAIN	AGR	866.7	1	1.7	4158.3	29.4
MI	DORR	URB	326.7	16	35.0	1100.2	127.7
MI	Bullet	UMG	46.3	89	6.5	384.5	11.0
MI	Buskirk	AGR	71.5	4	6.0	81.5	20.7
MI	Wayland	URB	168.9	19	11.7	694.6	74.3
MI	Honeysuckle	UMG	62.7	0	99.4	4.2	21.1
MI	Bellingham	AGR	187.8	5	22.9	1452.9	27.9
MI	Arcadia	URB	515.9	6	110.1	273.5	32.0
KS	Kings Creek N4D	UMG	17.8	5	13.4	8.6	0.3
KS	Agnorth	AGR	4.0	0	0.2	34.8	31.7
KS	Campus Creek	URB	256.8	2	2.9	2942.0	7.8
KS	K2A	UMG	4.7	0	26.3	0.9	6.7
KS	Natalie Creek	AGR	5.6	0	1.3	6.0	3.1
KS	Walmart Ditch	URB	87.0	38	1.6	277.4	28.3
KS	Shane Creek	UMG	6.5	0	4.4	1.2	4.7
KS	Swine	AGR	7593.9	2	5.4	21162.3	3.4
KS	Little Kitten	URB	73.2	13	20.1	167.6	24.2
WY	Ditch	UMG	0.3	0	55.7	0.1	1.7
WY	Giltner	AGR	458.5	0	158.5	49.7	3.0
WY	Golf	URB	10.3	0	110.0	0.8	1.0
WY	Two Oceans	UMG	50.8	9	64.5	18.9	3.8
WY	Headquarters	AGR	12.2	48	131.1	0.7	2.9

Table G1. Continued.

Region	Stream	Land use	U (mg N/m/min)	f_{den} (%)	Q (L/s)	NO_3^- (µg N/L)	NH_4^+ (µg N/L)
WY	Teton Pines	URB	63.2	2	9.5	152.1	1.0
WY	Spread	UMG	16.4	0	267.8	2.8	2.2
WY	Kimball	AGR	176.7	3	153.8	27.9	1.1
WY	FISH	URB	241.6	7	102.9	234.7	4.3
SW	Agua Fria	UMG	2.5	41	11.9	0.4	1.5
SW	Bernalillo drain	AGR	9.9	0	23.5	1.5	1.8
SW	Rio Rancho	URB	32.0	0	17.8	12.5	3.4
SW	Rio Salado	UMG	1.5	0	5.8	3.8	3.6
SW	Rio Puerco	AGR	8.5	1	2.5	3.6	3.9
SW	San Pedro	AGR	129.8	18	4.0	297.2	4.4
SW	Sycamore Ck	UMG	104.9	2	21.3	57.5	2.0
SW	Indian Bend Wash	URB	6.8	0	28.4	99.2	65.2
SW	Tempe Town lake	URB	7.8	2	18.0	4.3	9.8
OR	Oak	UMG	18.9	0	7.5	70.5	1.3
OR	Oak	AGR	18.0	4	5.5	96.0	8.4
OR	Oak	URB	15.6	18	5.6	162.7	19.3
OR	Mack	UMG	11.5	0	30.7	62.7	5.9
OR	Camp	AGR	14.8	25	113.4	54.2	6.1
OR	Amazon	URB	5.6	0	25.0	2.4	4.8
OR	Potts	UMG	12.4	0	19.0	69.3	4.0
OR	Courtney	AGR	109.8	13	34.7	96.6	10.6
OR	Periwinkle	URB	15.0	0	2.7	7.7	4.4
PR	Bisley	UMG	41.3	36	12.5	170.7	2.6
PR	Grande	AGR	151.0	3	12.3	275.8	11.1
PR	Petunia	URB	126.5	37	4.7	997.1	15.1
PR	RIT	UMG	26.8	7	20.0	130.8	7.2
PR	Maizales	AGR	194.0	2	25.0	205.6	7.1
PR	Mtrib	URB	103.2	97	23.2	174.3	2204.3
PR	Pared	UMG	37.4	1	5.2	104.7	2.5
PR	Vaca	AGR	180.1	73	111.9	445.7	2.6
PR	Ceiba	URB	251.1	40	49.5	511.7	50.3