

Insect emergence as a nitrogen flux in Neotropical streams: comparisons with microbial denitrification across a stream phosphorus gradient

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Abstract. Research on how N is retained and removed from stream networks has focused on microbial metabolic pathways, such as denitrification. An alternative pathway for N to escape streams is in the form of emerging aquatic insects, and unlike denitrification, previous studies suggest that this pathway may be stimulated by increased availability of P. We tested the hypothesis that the flux of N exported from streams through insect emergence increases relative to flux through denitrification with increasing levels of stream P. We measured emergence and denitrification rates at 7 lowland stream sites in Costa Rica that differ in dissolved P levels and substrate characteristics. Emergence N flux ranged from 2.0 to 16.3% of measured denitrification rates and was not related to any measured physical or chemical variables. Sediment redox conditions were the best predictor of denitrification rates. Our emergence results suggest that most invertebrate biomass production in these streams is consumed by in-stream predators, keeping this N in the stream ecosystem. Nevertheless, our findings indicate that in streams with low denitrification rates, emergence should be considered as an additional pathway of N removal from stream ecosystems.

Key words: emergence, denitrification, nitrogen, phosphorus, tropical stream.

Human-caused alteration of the global N cycle has led to widespread elevation of N in aquatic ecosystems with detrimental effects in coastal waters (Diaz and Rosenberg 1995, Vitousek et al. 1997, Smith and Schindler 2009). In recent years, extensive research effort has focused on quantifying the capacity of streams to retain and remove dissolved N before it reaches coastal areas (Alexander et al. 2000, Mulholland et al. 2008). Microbial denitrification (along with alternative metabolic pathways; Burgin and Hamilton 2007) can create important sinks of N in freshwater ecosystems by transforming dissolved NO_3^- to inert N_2 gas and effectively removing N from the ecosystem (Seitzinger 1988). However, a recent interbiome study of stream denitrification found that an average of 84% of NO_3^- that was removed from stream water was not immediately denitrified (Mulholland et al.

2008). The fate of this remaining NO_3^- , once assimilated by benthic microbes and algae (as organic N), is poorly understood, but has important implications for N budgets at the river-network scale.

Another pathway by which N is removed from stream ecosystems is insect emergence. Many aquatic insects have terrestrial adult stages, and only a small fraction of emergence biomass returns to streams (Jackson and Fisher 1986, Werneke and Zwick 1992), so emergence represents a net flux of biomass and associated nutrients out of the stream ecosystem. Insect emergence is an important food subsidy for riparian predators including bats, birds, spiders, and lizards (reviewed by Baxter et al. 2005). Emergence has received less attention as a nutrient flux (but see Vallentyne 1952, Webster and Patten 1979), although it has been included in several whole-stream N budgets (Naiman and Melillo 1984, Triska et al. 1984, Grimm 1987, Francis et al. 2006). Up to 67% of dissolved N that is taken up by the benthic ecosystem can go into primary consumer biomass (Dodds et al. 2000), and much of this biomass can be exported to the terrestrial ecosystem through emergence (Nakano

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and Murakami 2001, Sanzone et al. 2003). Even if aquatic insects feed on terrestrial material, their emerging biomass represents a flux of N to the terrestrial ecosystem that would otherwise be mineralized and transported downstream. Direct comparisons of the N-flux from emergence and denitrification across streams are needed to improve our understanding of N removal at the stream-network scale.

Both denitrification and insect emergence rates have been quantified in only a few streams (and never simultaneously, to our knowledge), but limited data suggest that N flux from these very different pathways could be similar in magnitude in some streams. In Sycamore Creek (Arizona, USA), denitrification removes N at a rate of $3.0 \text{ mg N m}^{-2} \text{ d}^{-1}$ (Mulholland et al. 2008), and insect emergence rates range from 0.3 to $8.2 \text{ mg N m}^{-2} \text{ d}^{-1}$ (Grimm 1987). Kings Creek (Kansas, USA) loses N through denitrification at a rate of $1.3 \text{ mg N m}^{-2} \text{ d}^{-1}$ (Mulholland et al. 2008), and, in the summer, exports N through emergence at a rate of $\sim 2.3 \text{ mg N m}^{-2} \text{ d}^{-1}$ (Gray 1989, assuming emergence biomass is 10% N). Denitrification rates for a pair of streams at Coweeta Hydrologic Laboratory (North Carolina, USA) were 3.7 and $13.9 \text{ mg N m}^{-2} \text{ d}^{-1}$ (Mulholland et al. 2008), and a nearby 1st-order stream had summertime emergence rates of $1.4 \text{ mg N m}^{-2} \text{ d}^{-1}$ (Davis et al. 2011). These few examples from desert, grassland, and forest streams suggest that insect emergence may be an important N flux in stream ecosystems.

Denitrification and insect emergence both remove N from stream ecosystems, but they are controlled by different factors. Denitrification, in which NO_3^- acts as an electron acceptor for the mineralization of organic compounds, requires anoxic conditions, labile organic C, and NO_3^- (Piña-Ochoa and Álvarez-Cobelas 2006, Mulholland et al. 2008). Increased P typically does not increase denitrification rates (McCrackin and Elser 2010) and potentially could be an inhibitor of denitrification (Piña-Ochoa and Álvarez-Cobelas 2006). In contrast, many benthic invertebrates are limited by P (Frost et al. 2006), and P-enriched streams can have elevated levels of benthic secondary production (Cross et al. 2006, Davis et al. 2010) and emergence (Davis et al. 2011). The fraction of benthic invertebrate secondary production that emerges also depends on the efficiency of in-stream predators (Jackson and Fisher 1986). The controls on denitrification and insect emergence differ, so the relative importance of these 2 processes for removing N has the potential to vary greatly among streams, especially with respect to P availability.

To understand better the relative importance of insect emergence and denitrification as N-removal

pathways and how stream characteristics may affect the relative importance of these fluxes, we measured rates of emergence and denitrification in 7 stream sites in a lowland tropical rainforest that differed widely in dissolved P levels and in substrate characteristics. We tested the hypothesis that the flux of N exported through insect emergence relative to flux via denitrification increases with stream P levels.

Methods

Site description

La Selva Biological Station (lat $10^\circ 26' \text{N}$, long $84^\circ 01' \text{W}$) is on the Caribbean Slope of Costa Rica at the gradient break between the central mountain range and coastal plain. La Selva receives almost 4000 mm of rainfall annually, with a dry season from February–April ($< 200 \text{ mm/mo}$) and a wet season from May–December ($> 320 \text{ mm/mo}$) (Sanford et al. 1994). Geomorphological features of the La Selva landscape cause some streams to receive groundwater inputs with high concentrations of P and other solutes (Na^+ , Cl^- , HCO_3^- ; Pringle et al. 1993). Streams that do not receive inputs from regional groundwater sources have low dissolved P, and rates of microbial respiration and organic matter processing are P limited in these streams (Rosemond et al. 2002, Ramírez et al. 2003).

We chose 7 stream sites that differed widely in average soluble reactive P (SRP) concentration (2–135 $\mu\text{g/L}$) because of differential inputs of solute-rich groundwater (Table 1). NO_3^- levels across these study streams ranged from 50 to 254 $\mu\text{g NO}_3\text{-N/L}$, resulting in a range of NO_3^- :SRP molar ratios from 2 to 280. These sites were 2nd- to 3rd-order, within close proximity ($< 2 \text{ km}$ apart), and surrounded by dense forest. Channel widths ranged from 1 to $\sim 8 \text{ m}$, and the dominant substrata were detritus, silt, and clay, with boulders present at some sites. The study sites are a subset of sites used in a long-term study of the physicochemistry of La Selva streams (Pringle and Triska 1991, Triska et al. 2006), for which continuous monthly data from 1997 to the present are available. Invertebrate biomass in the study streams ranges from 50 to 1000 mg/m and varies seasonally, with higher levels found during the dry season in some streams (Ramírez et al. 2006). Sites used in our study are named using the watershed name followed by a number indicating the approximate elevation (m asl).

Insect emergence

We used 4- m^2 aquatic insect emergence traps (BioQuip Products, Rancho Dominguez, California)

TABLE 1. Physicochemical variables at the 7 study sites. DO = dissolved O₂.

Site	SRP ($\mu\text{g/L}$)	NO ₃ -N ($\mu\text{g/L}$)	NH ₄ -N ($\mu\text{g/L}$)	DO (mg/L)	pH	Conductivity ($\mu\text{S/cm}$)	Temperature ($^{\circ}\text{C}$)	Width (m)	Discharge (m^3/s)	Boulders (% area)
Arboleda-30	135	142	2	6.98	6.66	278.0	23.5	5.4	0.17	0
Sura-30	56	125	2	7.06	6.57	108.1	23.6	7.9	0.61	0
Saltito-60	12	135	4	7.36	6.74	89.9	23.6	4.6	0.11	10
Carapa-60	2	50	8	7.48	5.22	15.3	23.6	1.1	0.02	20
Saltito-100	2	108	4	7.44	5.88	17.6	21.9	1.0	0.02	0
Piper-30	2	145	18	7.51	5.92	20.8	23.1	2.9	0.03	10
Sura-60	2	254	2	8.53	6.45	16.6	23.0	6.2	0.19	75

to make quantitative estimates of emergence in June and July 2006 and 2007, for a total of 209 trap nights (range 19–49 trap nights/stream; Table 2). We removed insects from traps every 2–4 d with a modified handheld vacuum. Emergence can vary greatly between pools and riffles (Iwata 2007), so we attempted to account for spatial heterogeneity by deploying 2 traps simultaneously over a 20-m length of stream and repositioning traps within the same 20-m length every 3 d. We froze insect samples, sorted them to remove terrestrial taxa, dried them at 50°C for 48 h, and weighed the bulk sample. We homogenized samples collected in 2006 from each site and measured total N content with a Carlo Erba NA 1500 CHN analyzer (Carlo Erba, Milan, Italy) at the University of Georgia Analytical Chemistry Laboratory. We used poplar leaves as an external N standard.

To gain a better understanding of seasonal variation in insect emergence at our study sites, we also collected monthly emergence samples from high-P Arboleda-30 and low-P Sura-60 from January 2008 to February 2009 (8 trap nights stream⁻¹ mo⁻¹). For these monthly samples, we set emergence traps at the same 2 locations in each stream for a 4-d sampling period. We collected and processed samples as described above.

Denitrification assays

We measured denitrification at the same 7 sites in early March 2009 with the acetylene (C₂H₂)-block technique (Balderston et al. 1976, Yoshinari and Knowles 1976). These measurements do not account for possible seasonal differences in denitrification, but we note that stream temperature, organic-matter inputs, and nutrient concentrations are relatively uniform across seasons in these streams. We collected 10 sediment samples from the upper 3 cm at each site in an effort to represent the sediment types along a 10-m stream length. We began incubations within 2–3 h of collection. We added 12 cm³ of wet sediment and

40 mL of stream water to 125-mL glass media bottles (Inwood et al. 2005). We amended the water added to 10 bottles from each site with 0.5 mL of NO₃⁻ and glucose (1 mM final concentration per substrate) to measure maximum potential denitrification rates (Smith and Tiedje 1979, Duff et al. 1996), and we used unamended stream water in a 2nd set of 10 bottles to measure endogenous denitrification rate potentials. To prevent the de novo synthesis of nitrate reductase, we added 5 mL of a chloramphenicol-water solution to all incubation media to a final concentration of 0.15 mg/mL (Richardson et al. 2004). We sealed media bottles with gas-tight caps fitted with butyl rubber septa and flushed them for 8 min with O₂-free He. We added acetylene (10 mL) generated from calcium carbide-saturated water by syringe to the headspace of each bottle. We shook the bottles vigorously before collecting an initial headspace sample (3 mL), incubated them on a shaker table at 25°C, and collected 3 to 4 additional 3-mL gas samples over a 4- to 6-h period. We report denitrification rates relative to sediment dry mass.

We collected surface and porewater samples corresponding to sediment samples from all study sites. High porewater NH₄-N concentrations indicate reducing conditions in the sediment. We filtered water samples (0.45- μm pore size) and analyzed them for SRP (ascorbic acid method), NO₃-N (Cd-reduction method), and NH₄-N (phenate method) (APHA 1998). We dried 12-mL subsamples of sediment (48 h at 80°C) and weighed, combusted (4 h at 500°C), and reweighed them to measure % organic matter. We also measured exchangeable NH₄-N by extracting sediment with 3M KCl. Last, we analyzed C and N content of the sediment samples. We used linear regression to evaluate the effects of stream chemistry and sediment characteristics on endogenous and nutrient-amended denitrification rates.

We used the average N₂O accumulation in unamended sediment slurries ($n = 10$) measured per cm³ of sediment over a depth of 3 cm (our samples integrated sediment over that depth) to estimate areal

TABLE 2. Emergence biomass and N-flux at the 7 study sites, including the monthly samples at Arboleda-30 and Sura-60. Benthic macroinvertebrate biomass values for these sites from Ramírez et al. (2006) are included for comparison.

Site	Trap nights	Emergence biomass flux ($\text{mg N m}^{-2} \text{d}^{-1}$)	Biomass (mg/m^2)	Biomass turnover from emergence (d^{-1})	N-flux ($\text{mg N m}^{-2} \text{d}^{-1}$)
Arboleda-30	142	3.69 ± 0.45	504	0.007	0.40
Sura-30	29	4.18 ± 0.88	1702	0.002	0.46
Saltito-60	26	11.45 ± 3.52	470	0.024	1.25
Carapa-60	28	10.34 ± 3.74	221	0.047	1.13
Saltito-100	19	6.61 ± 1.54	277	0.024	0.72
Piper-30	49	3.74 ± 1.10	176	0.021	0.41
Sura-60	128	4.36 ± 0.51	757	0.006	0.48

denitrification rates for each stream site. We assumed that no additional denitrification occurs below 3 cm because of lack of NO_3^- availability in these highly reducing sediments. We adjusted reach-length areal denitrification estimates for % cover by bedrock or large boulders in each site (Table 1), based on the assumption that only exposed sediment contributed to denitrification.

We used linear regression to test potential relationships between emergence biomass flux and stream physical and chemical variables (SRP, NO_3^- , discharge, and stream width), between emergence N-flux and SRP, and between potential and endogenous denitrification and sediment characteristics (porewater $\text{NO}_3\text{-N}$, $\text{NH}_4\text{-N}$, and SRP, KCl-extractable NH_4^+ , sediment organic content, and sediment C:N). We did not correct for multiple comparisons. We used Spearman's ρ to assess the correlation between emergence biomass flux and endogenous denitrification.

Results

Emergence biomass flux ranged from ~ 3.7 to $\sim 11.5 \text{ mg N m}^{-2} \text{d}^{-1}$ across the 7 study sites in June and July 2006 and 2007 (Table 2). Because of spatial and temporal heterogeneity, variance was high among samples within sites (coefficients of variation [CVs] ranged from 0.48 to 1.24 across study sites). Percent N of bulk emergence samples showed little variation across sites ($\text{CV} = 0.07$), so we used an overall mean of 10.9% N to calculate N fluxes from emergence. N flux ranged from 0.40 to $1.25 \text{ mg N m}^{-2} \text{d}^{-1}$ (Table 2). Emergence was not related to any of the stream physical or chemical variables we measured ($p > 0.05$). N flux from emergence was not related to stream SRP across the study sites ($F_{1,5} = 1.11$, $p = 0.34$). The highest emergence rates were measured in the low-P Carapa-60 and in the high-P Saltito-60 (Table 2).

Monthly emergence rates in 2008 ranged from 0.09 to $0.55 \text{ mg N m}^{-2} \text{d}^{-1}$ in the high-P Arboleda-30 and from 0.09 to $0.78 \text{ mg N m}^{-2} \text{d}^{-1}$ in the low-P Sura-60.

The lowest emergence rates were observed from November to January during the height of the wet season, and the highest rates were observed from March to July (Fig. 1A, B). Mean emergence biomass in June and July 2006 and 2007 was higher than annual mean emergence biomass from the monthly samples at Sura-60 (4.4 vs $3.1 \text{ mg m}^{-2} \text{d}^{-1}$) and Arboleda (3.7 vs $3.4 \text{ mg m}^{-2} \text{d}^{-1}$) in 2008.

Endogenous denitrification rates ranged from 0.07 to $0.96 \mu\text{g N g}^{-1} \text{h}^{-1}$ (Table 3). Denitrification rates across sites were negatively related to sediment $\text{NH}_4\text{-N}$ ($r^2 = 0.67$, $p = 0.02$), but were not related to the other variables measured ($p > 0.05$). Potential denitrification rates, from samples amended with NO_3^- and glucose, ranged from 1.47 to $7.75 \mu\text{g N g}^{-1} \text{h}^{-1}$. Potential denitrification rates showed a weak negative relationship with sediment organic matter

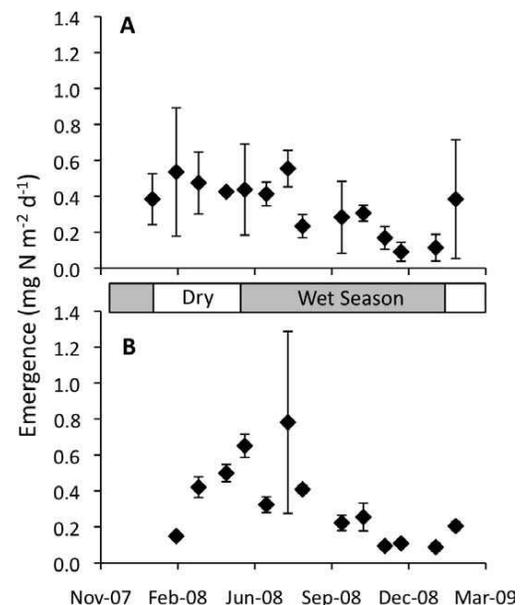


FIG. 1. Mean (± 1 SE) monthly N flux from insect emergence in high-P Arboleda-30 (A) and low-P Sura-60 (B). Dates are formatted mo-yy.

TABLE 3. Sediment characteristics and denitrification rates. Porewater soluble reactive P (SRP) values for Saltito-100 and Piper-30 were below the analytical detection limit (bdl).

Site	Sediment characteristics						Denitrification rates	
	Porewater chemistry				Organic content (%)	Sediment C:N (molar)	Endogenous ($\mu\text{g N g}^{-1} \text{h}^{-1}$)	Amended ($\mu\text{g N g}^{-1} \text{h}^{-1}$)
	NO ₃ -N ($\mu\text{g/L}$)	NH ₄ -N ($\mu\text{g/L}$)	SRP ($\mu\text{g/L}$)	KCl-extractable NH ₄ -N ($\mu\text{g/L}$)				
Arboleda-30	13	76	3	435	11.4	15.0	0.38 \pm 0.06	1.47 \pm 0.60
Sura-30	19	144	11	106	9.5	14.6	0.63 \pm 0.09	3.87 \pm 0.86
Saltito-60	8	225	2	82	11.3	18.2	0.96 \pm 0.17	7.02 \pm 1.45
Carapa-60	14	228	1	148	4.8	17.7	0.51 \pm 0.17	7.75 \pm 1.96
Saltito-100	20	549	bdl	560	4.5	18.7	0.39 \pm 0.09	7.51 \pm 2.32
Piper-30	18	1584	bdl	920	10.0	20.2	0.07 \pm 0.04	5.65 \pm 1.13
Sura-60	61	76	1	147	17.1	17.9	0.41 \pm 0.13	2.52 \pm 1.04

content ($r^2 = 0.53$, $p = 0.06$). Endogenous denitrification rates ranged from 1 to 25% of potential rates.

Areal denitrification rates ranged from 1.6 to 24.0 $\text{mg N m}^{-2} \text{d}^{-1}$ across the 7 study sites, with an overall mean of 12.0 $\text{mg N g}^{-1} \text{d}^{-1}$. Emergence N flux ranged from 2.4 to 25.4% of N flux through denitrification (Fig. 2). Emergence and denitrification rates were weakly positively correlated ($\rho = 0.071$, $p = 0.07$), but the relative importance of emergence (measured as emergence:denitrification ratios) was higher in streams in which denitrification was low (Fig. 2).

Discussion

As our results indicate, in some of these study streams, insect emergence may constitute a potentially important N sink; emergence N-flux ranges from 2.4 to 25.4% of the N flux from denitrification. Our emergence N-flux and denitrification values are

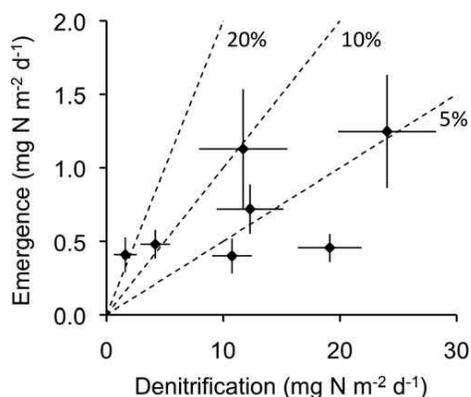


FIG. 2. Mean (\pm SE) emergence N-flux vs areal denitrification in the 7 study streams. Dashed lines represent emergence N-flux values that are 5, 10, and 20% of the magnitude of denitrification values.

comparable to values reported in the literature for both fluxes (Fig. 3). Emergence and denitrification rates were variable across study streams, and, in contrast to our hypothesis, neither the magnitude of emergence flux nor the relative importance of emergence as an N-removal pathway was directly related to stream P levels.

Detritus-feeding benthic insects may often be P limited (Frost et al. 2006). P does increase insect growth rates in our streams (Rosemond et al. 2001, Ramírez and Pringle 2006), but streams with elevated P did not have higher insect emergence rates. The absence of a relationship between emergence rates and stream P levels in our landscape-scale study contrasts with results from a controlled nutrient addition experiment in a temperate detritus-based headwater stream. In a multiyear nutrient (N+P) addition to a 1st-order stream, insect production increased (Cross et al. 2006, Davis et al. 2010), and corresponding increases in emergence biomass were documented because of increased prevalence of larger-bodied taxa (Davis et al. 2011). Growth rates of some common invertebrate taxa in our study streams have been linked to stream P levels, but variability in invertebrate biomass among habitats obscured any relationship between dissolved P and invertebrate secondary production (Ramírez and Pringle 2006). Other factors, such as substrate, leaf-litter standing stock, algal production, and the presence of in-stream predators (Power et al. 2009) probably influence insect biomass and, therefore, emergence rates. Interspecific interactions, such as competition and predation, could counteract the effects of nutrient enrichment on emergence (Nakano et al. 1999).

We can make some comparisons of our emergence data with previously measured production and biomass values from the same study streams. Assemblage-level

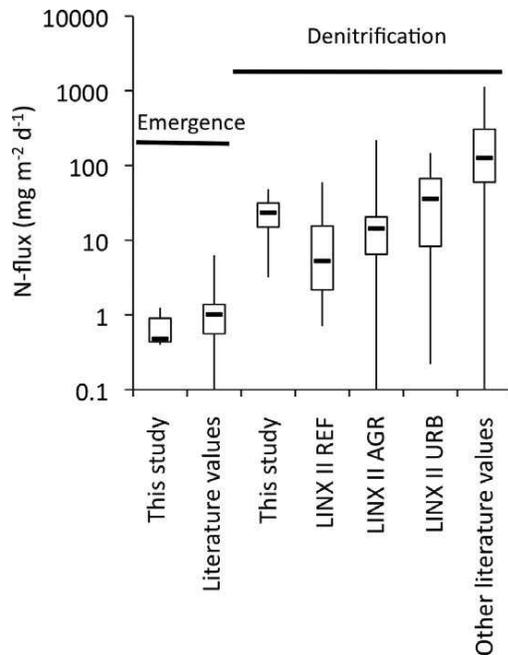


FIG. 3. N-flux from insect emergence and microbial denitrification from this study and other values reported in the literature. Box-and-whisker plots indicate minimum, 1st quartile, median, 3rd quartile, and maximum values. Literature values for emergence come from papers by Jackson and Fisher (1986) (and values therein), Naiman and Melillo (1984), Triska et al. (1984), Gray (1989), and Davis et al. (2011). Where emergence values were reported in terms of dry mass, we calculated N-fluxes assuming N-content of 10%. Lotic Interstream Nitrogen Experiment reference (LINX-II REF), agriculture (LINX-II AGR), and urban (LINX-II URB) denitrification values are from Mulholland et al. (2008). Other denitrification values come from the review by Piña-Ochoa and Álvarez-Cobelas (2006).

estimates of secondary production are not available for these study streams, but Ramírez and Pringle (2006) measured rates of secondary production for chironomid larvae in these sites that ranged from 3 to 25 $\text{mg ash-free dry mass (AFDM) m}^{-2} \text{d}^{-1}$, similar to the range of total emergence biomass flux (of which chironomids made up a relatively low proportion because of their small size) of 3.7 to 11.5 $\text{mg m}^{-2} \text{d}^{-1}$ that we observed. Combining emergence rates reported here with invertebrate biomass values from these study streams reported by Ramírez et al. (2006) indicates that, on average, 0.4% of invertebrate biomass is removed each day through emergence (Table 2). The mean production:biomass ratio of 18/y reported by Ramírez and Pringle (1998) for aquatic invertebrates from another La Selva stream suggests that ~5% of insect biomass is replaced each day. Observed emergence rates in the 4 smaller sites were between 2 and 5% of standing

biomass (i.e., emergence may have accounted for much of this biomass turnover), whereas emergence rates in the 3 larger sites were <1% of biomass (Table 2).

The lower emergence rates in larger streams suggest that much of the organic N from benthic invertebrates is consumed by in-stream predators, such as fish, and remains in the stream. At one of the larger study sites, Sura-60, where fish biomass is ~30 g wet mass/m^2 , excretion by the fish assemblage recycles $\geq 15 \text{ mg N m}^{-2} \text{d}^{-1}$ derived from aquatic insects (Small et al. 2011), >30× the magnitude of emergence N flux in this stream. In contrast, in the smallest of our study streams (Carapa-60 and Saltito-100) where fish biomass is ~3 g wet mass/m^2 , fish excretion derived from aquatic insects is only 0.8 $\text{mg N m}^{-2} \text{d}^{-1}$ (Small et al. 2012), similar to or slightly less than the N flux through insect emergence from these streams. All of our study streams contain insectivorous fish, but these data suggest that higher rates of predation in the largest streams may decrease N fluxes via emergence. Among our study sites, the highest N flux from emergence occurred in Saltito-60, which had the lowest discharge among the high-P sites, a result suggesting that effects of P on N flux via emergence may be greatest in low-predation environments. Downstream drift is another potentially important fate of invertebrate biomass but has not been quantified in these streams.

Our emergence sampling was designed to integrate spatial and temporal heterogeneity, but assessing the accuracy of average emergence fluxes for each stream, which were based on a series of short-term observations over relatively small areas, is difficult. Trap placement affects emergence results, especially for taxa that emerge from stream banks rather than the water column (Malison et al. 2010). In our smaller streams, the emergence traps included the entire cross section of the stream, but in the larger streams, a smaller fraction of stream area was represented. The monthly samples at the Arboleda-30 and Sura-60 sites suggest that average annual emergence rates may be slightly lower than values measured during our intensive summer surveys, but the temporal sampling did not account for spatial heterogeneity, so it is unclear which number is more accurate. Future investigators should consider quantifying spatial and temporal heterogeneity specifically to improve annual estimates. Nevertheless, the values that we report seem to align with previously reported values for invertebrate biomass and secondary production. Moreover, consumption of aquatic invertebrates by insectivorous fishes, described above, supports their use in this comparative analysis.

We found no relationship between stream nutrient levels and denitrification rates. This result may have

been caused partially by the relatively high but uniform range of NO_3^- concentrations across our study streams. In addition, although denitrification increases in high- NO_3^- streams (Piña-Ochoa and Álvarez-Cobelas 2006, Mulholland et al. 2008), even the highest values we observed were much lower than some of the agriculturally affected streams from which the highest denitrification rates have been documented. We found no evidence of inhibition of denitrification by high P as suggested by Piña-Ochoa and Álvarez-Cobelas (2006).

Our data strongly suggest that sediment redox conditions control denitrification rates in these study streams. The lowest endogenous rates were measured at Piper-30, which had highest sediment and water-column NH_4^+ concentrations. Denitrification rates appear to be limited by sediment NO_3^- availability under strongly reducing conditions, which preclude nitrification. The 80 \times increase in potential denitrification relative to endogenous values for this stream, combined with the lack of a relationship between sediment organic C and denitrification rates across the study streams, suggests that NO_3^- supply limits denitrification rates. Consistent with this finding, Duff et al. (1996) separately amended samples from other La Selva streams with NO_3^- and glucose, but found a response only to NO_3^- additions. We also found low areal denitrification rates at Sura-60. We measured intermediate denitrification rates g^{-1} of sediment (Table 2), but the study reach had numerous boulders, so the small area covered by sediment led to low areal rates.

The limitations of the C_2H_2 -block method for measuring denitrification have been addressed in detail elsewhere (Seitzinger et al. 1993, Lohse et al. 1996). Perhaps most significant is the potential to underestimate denitrification by depleting sediment NO_3^- supplies because C_2H_2 inhibits sediment nitrification (Hynes and Knowles 1978). However, we think our rates are reasonable estimates because of strong reducing conditions in the sediment and linear N_2O production rates during the 6-h incubations. Our endogenous denitrification estimates were similar to values reported from the Lotic Interstream Nitrogen Experiment (LINX-II) study (Mulholland et al. 2008), in which denitrification of isotopically labeled NO_3^- was measured in the water column of a variety of streams, but lower than many values reported from other streams and rivers (Fig. 3).

Some generalizations are possible, even though we did not observe a simple relationship between stream chemistry and the relative importance of emergence as an N-removal pathway. Denitrification was 1 to 2 orders of magnitude larger than emergence rates, but

also was more variable across sites. Endogenous denitrification rates varied $>13\times$ across streams (Table 3) compared to only a $3\times$ variation in emergence among sites (Table 2). As a result, the relative importance of emergence as a N removal pathway is highest in streams with low denitrification rates. We do not expect denitrification rates to change seasonally in these study streams, but substantial temporal variability would further support this conclusion. Our results are consistent with other literature values, which indicate that, across streams, emergence N flux may typically represent 1 to 10% of N flux from denitrification (Fig. 3). Denitrification and emergence are not controlled by the same factors, so the magnitude of emergence N flux relative to denitrification should be highest in streams where conditions for denitrification are unfavorable—i.e., low NO_3^- availability, aerobic conditions in the hyporheic zone, with substrate dominated by boulders or cobbles, and low organic sediment C and where conditions for insect emergence are optimized, with high secondary production rates and a small fraction of this production going to in-stream predators. N-limited, highly productive streams (e.g., Grimm 1987) are likely to have the highest N flux in emergence relative to denitrification, whereas agricultural and urban streams with high NO_3^- concentrations (Mulholland et al. 2008) are likely to have denitrification rates far greater than emergence N flux.

The movement of some aquatic organisms is an important mechanism for translocating nutrients in some streams, particularly via fish migrations (Wipfli et al. 1998, Winemiller and Jepsen 2004). Like fish migrations, insect emergence can provide an important source of nutrients to recipient ecosystems (Sabo and Power 2002, Sanzone et al. 2003) and is a sink for N from the stream ecosystem. Gauging the relative magnitudes of these nutrient fluxes in animal biomass has been hindered by the paucity of microbial nutrient-flux measurements quantified in the same streams. Our results show that the export of organic N from streams via emergence could be an important pathway of N removal and that emergence should be considered when calculating N removal at the scale of stream networks.

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