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Suburbanization alters small pond ecosystems: Shifts in nitrogen and food web dynamics

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1 **Title:** Suburbanization alters small pond ecosystems: Shifts in nitrogen and
2 food web dynamics

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ABSTRACT

Small ponds often survive the transition from forested to suburban land cover and provide habitat for many species, yet little is known about how suburbanization affects pond ecosystems. We surveyed 18 small ponds across a forest-to-suburban land cover gradient and compared how physical and chemical changes altered biological and ecosystem properties, such as nutrient and food web dynamics. Suburbanization decreased canopy cover, increased water temperatures, and increased periphyton chlorophyll *a*, but was associated with only weak increases in total nutrients. Yet, stable isotope analysis indicated that suburbanization altered nitrogen dynamics and resource use in the food web. We observed increases in $\delta^{15}\text{N}$ in algae, biofilm, and frog larvae across the suburban gradient, indicative of wastewater intrusion. Suburbanization also shifted the energy and nutrient source of a dominant consumer (*Rana sylvatica*) from leaf litter to algae. Overall, we identified cryptic changes to suburban pond ecosystems, highlighting that suburbanization can profoundly impact nutrients and food web resources. As residential land use increases globally, we may expect substantial shifts in nutrient dynamics and food web pathways.

Keywords: food web, land use, nutrient, septic system

INTRODUCTION

Suburbanization is rapidly expanding worldwide (Seto et al. 2012). In the United States alone, suburban land use increased from 5% to 25% between 1950 and 2000 (Brown et al. 2005), concurrent with the proportion of the population living in the suburbs more than doubling (Mather et al. 2011). With this demographic and land-use transition comes the potential to transform ecosystem functioning, such as nutrient pathways, carbon cycling, and trophic dynamics (Grimm et al. 2008; Hutyra et al. 2011; McDonnell and Hahs 2008). Physically, suburban habitats replace native ecosystems with buildings, lawns, gardens, roads, and relictual patches of native vegetation. This physical change is coupled with chemical changes as humans add nutrients and contaminants to the environment, from sources such as lawn runoff or on-site septic systems. However, the implications of such physical and chemical shifts on suburban ecosystem processes remain unknown.

In this study, we explored the effects of suburbanization on the ecosystem dynamics of small freshwater ponds. Small ponds frequently survive the transition from forested to suburban land cover and provide critical habitat for a range of species, including amphibians and macroinvertebrates often found nowhere else (Paton 2005; Scheffer et al. 2006). Because ponds persist across the landscape from intact forests to backyards, they can act as harbingers of environmental change at a small spatial scale. We selected study ponds across a land-use gradient with landscapes composed of between 2.5% and 74.7% suburban land cover within a 200 m buffer around the pond (Fig. 1, Table A1). The ponds were selected to bracket typical US suburban residential housing densities (Irwin and Bockstael 2007), with 0.1 to 5.1 (mean = 1.3) households ha⁻¹ within the 200 m buffer (Table A1). For each pond across this suburbanization gradient, we analyzed physical (e.g., surface area, canopy cover), chemical (e.g., temperature,

nutrients), and biological parameters (e.g., algal biomass, carbon and nitrogen stable isotopes of pond organisms).

We were particularly interested in studying how the physical and chemical changes associated with suburbanization cascaded to biological and ecosystem changes in the pond. Specifically, food web dynamics are central to many ecological patterns, such as species diversity, ecosystem stability and resilience, and biogeochemical cycles (Polis and Strong 1996; Rooney and McCann 2012; Schmitz 2010). Small pond food webs are supported by two ultimate energy sources: external terrestrial resources (e.g., leaf litter) and internal primary producers (e.g., algae and macrophytes). Algae is a higher quality resource, but terrestrial resources are often of high quantity in small ponds (Holgerson et al. 2016). The relative importance of these two resources is mediated by factors influencing algal productivity and terrestrial inputs, such as light levels and surrounding land use (Tanentzap et al. 2017). Therefore, we measured algal biomass (periphyton and phytoplankton chlorophyll *a*) and used stable isotope analysis of dominant pond resources and consumers to understand if suburbanization is associated with changes to the food web.

We used stable isotope analysis to first assess if the nutrient source to the food web changed along the land use gradient. Nutrient inputs into ponds in undeveloped forested landscapes come from terrestrial runoff, groundwater, and leaf fall, with microbial decomposition of leaf litter mineralizing nitrogen (N) to the water column (Cheever et al. 2013; Holgerson et al. 2016). Suburbanization can enhance nutrient loading in runoff and groundwater, altering the source and amount of nutrients available to the food web (Carey et al. 2013; Harrison et al. 2012; Withers et al. 2011). In this study, we compared N stable isotopes ($\delta^{15}\text{N}$) in resources (biofilm, periphyton, and seston) and consumers (zooplankton and frog

larvae) across the suburbanization gradient. The $\delta^{15}\text{N}$ signatures are associated with different N sources, with atmospheric N and synthetic fertilizers having lower $\delta^{15}\text{N}$ signatures than human and animal waste (see Methods; Jankowski et al. 2012; Moore et al. 2014). As agricultural land cover near our study ponds was negligible (<5% within one pond's buffer), an increase in $\delta^{15}\text{N}$ would indicate human wastewater incursion. However, this pattern could be obscured by high use of synthetic fertilizers.

Secondly, we evaluated whether suburbanization impacted the relative importance of algal vs. terrestrial resources to a dominant pond consumer, larval *Rana sylvatica* (= *Lithobates sylvaticus*, the wood frog). *Rana sylvatica* is a common pond-breeding amphibian in the northern and eastern United States that can comprise significant animal biomass in small ponds (Werner et al. 2007). As a dominant consumer, *R. sylvatica* is critical to pond nutrient processing, and this species can be an important conduit of nutrient exchange between freshwater and terrestrial ecosystems when juveniles metamorphose and leave the ponds (Capps et al. 2015). Larval *R. sylvatica* filter feed and graze on leaf litter particles and biofilm; in forested ponds, their nutrient source comes almost equally from leaf litter and algal resources (directly or indirectly) (Holgerson et al. 2016). However, larvae may shift their diet and resource use with changing land cover; Schiesari et al. (2009) found that the gut contents of *R. sylvatica* larvae shifted from a dominance of algae to detritus in open- versus closed-canopy ponds, respectively. Therefore, we hypothesized that larval *R. sylvatica* would become more reliant upon algal resources as suburbanization increased.

Overall, we hypothesized that with increasing suburbanization we would observe decreases in canopy cover, increases in water temperature and total nutrients, and greater algal biomass, which would cascade to different sources of N and resource use by consumers.

MATERIALS AND METHODS

Study Sites

We surveyed eighteen ponds along a suburbanization gradient in Madison, Connecticut, USA (41.296, -72.574; Tables A1 and A2). The town of Madison is completely reliant upon septic systems for wastewater management. All study ponds were ephemeral and dried either annually or every few years, which prohibits the establishment of fish predators that can decimate amphibian populations. The study ponds were used for breeding by *R. sylvatica* as evidenced by egg masses on 15-Apr-2015, except for SHLD Pond where larvae were later observed.

To classify land cover types around each pond, we used a free, high-resolution (0.3 m) global satellite base map (downloaded 04-Jun-2015; satellite imagery credited to ESRI World Imagery) in ArcMap 10.1. The sub-meter resolution of this map allowed for easy detection of different landscape variables. In ArcCatalog 10.1, we created new polygonal shapefiles for each pond at the 200 m buffer size, which is a commonly used buffer for small pond amphibian studies (Lambert et al. 2015; Skelly et al. 2010). When deriving land cover shapes, we created Forest, Tidal Wetland, Building, Paved Surfaces, Lawn, and Yard categories. Buildings are mostly houses but occasionally include other commercial buildings. Paved Surfaces are predominantly surface streets but also include highways, driveways, and parking lots. Yard is predominantly gardens and landscaping vegetation (not lawn grasses) but occasionally includes swimming pools and hot tubs. In ArcMap, we edited each shapefile, manually digitizing the landscape by creating polygons around the perimeter of each of the above-mentioned land cover types. To ensure all polygons fit together tightly, we used the snapping function. For each pond, we “dissolved” each 200 m buffer by the land cover type and calculated the surface area of each land cover category using the “calculate geometry” function. These methods have been

employed previously (Lambert et al. 2016). Here, we focus on the percentage of the landscape that is “Suburban” which includes the area of the landscape covered by Building, Paved Surfaces, Lawn, and Yard.

Environmental Sampling

Ponds were sampled between 30-May-2015 and 03-Jun-2015. We measured pH and conductivity (Waterproof Multiparameter PCS Testr 35, Oakton Instruments, Vernon Hills, Illinois, USA) and collected surface water with reach samples near the center of each pond, or as deep as wading would allow in deeper ponds (~1 m). In instances where wading stirred up loose sediments, we reached out past the disturbed area of water to collect our water sample. Water samples were pre-filtered through 150- μ m mesh to remove most zooplankton and debris, and a known volume was filtered through pre-combusted Whatman GF/F filters (Whatman, Brentford, UK). Filters were frozen for phytoplankton chlorophyll *a* analysis, while filtrate was frozen prior to analysis of dissolved organic carbon (DOC) using a Shimadzu TOC 5000 auto-analyzer (Shimadzu Corporation, Kyoto, Japan). Total nitrogen (TN) and total phosphorus (TP) concentrations were determined from pre-filtered (150- μ m mesh) water samples using a persulfate digestion and colorimetric standard methods (Clesceri et al. 1998) in conjunction with an auto-analyzer (Astoria2 Analyzer, Astoria- Pacific, Clackamas, Oregon, USA) following Environmental Protection Agency Method 365.4.

Periphyton chlorophyll *a* was sampled from glass slides that were deployed for two weeks directly below the water surface near the pond center or as close as wading would permit (Periphyton Sampler, Wildco, Yulee, Florida, USA). Glass slides were scraped, rinsed with water, and the resulting periphyton-water slurry was filtered onto pre-combusted Whatman GF/F filters and frozen until analysis. Periphyton and phytoplankton chlorophyll *a* were measured

using a fluorometer (TD-700 Fluorometer, Turner Designs, Sunnyvale, California, USA) following Environmental Protection Agency Method 445.0. Water temperatures were recorded every thirty minutes for two weeks (18-May-2015 to 31-May-2015) using HOBO pendant loggers (Onset Computer Corporation, Bourne, Massachusetts, USA) placed 10 cm below the pond surface. Canopy cover was estimated using a convex spherical densitometer (Forestry Suppliers, Jackson, Mississippi, USA) at five locations within the pond basin (N shoreline, E shoreline, S shoreline, W shoreline, and center) and averaged. Proportion of light penetrating the pond surface was taken as the difference between light measurements immediately above and below the water surface.

Stable Isotope Sampling and Analysis

The dominant types of natural leaf litter were collected throughout the basin of each pond. Biofilm (representing algae, bacteria, fungi, and detritus) was gently rinsed from representative leaf samples into glass scintillation vials, and leaf litter was then rinsed with DI water and sorted by species. Periphyton was scraped from glass slides into glass scintillation vials. Seston samples were taken from GF/F filters collected in the same manner as phytoplankton chlorophyll *a*. Periphyton and phytoplankton samples were predominantly comprised of algae; however, some microbes and/or detritus were likely included. Zooplankton was collected with an 80- μ m, 20-cm diameter net (Wildco, Yulee, Florida, USA) towed horizontally throughout the pond to sample littoral, pelagic, and benthic waters. Zooplankton samples were frozen on dry ice in the field and later hand-sorted to separate from detritus. Larval *R. sylvatica* were collected with a dipnet, euthanized in the field with tricaine methanesulfonate (MS-222), which does not alter *R. sylvatica* $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ values (Atwood 2013), rinsed, and frozen on dry ice in the field.

All samples were dried, homogenized to a fine powder, and loaded into pressed tin capsules (Costech Analytical Technologies, Valencia, CA, USA) for stable isotope analysis. For seston, which was collected on filters, we took representative hole punches from the filters to load into capsules. For periphyton, seston, biofilm, and zooplankton, one composite sample was analyzed per pond. For *R. sylvatica* larvae, we analyzed whole organisms minus their guts, which can comprise high animal biomass, but include food items that are not necessarily assimilated. Three *R. sylvatica* were analyzed per pond, with the exception of COLL Pond where only one individual was recovered.

Samples were analyzed for percent carbon (C), percent nitrogen (N), $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ values in a combustion elemental analyzer (ESC 4010; Costech Analytical Technologies) coupled to an isotope ratio mass spectrometer (Delta Plus Advantage; ThermoFinnigan, Bremen, Germany). Measurements were performed at the Yale Analytical and Stable Isotope Center. The stable isotope ratio results are presented in typical delta (δ) notation in units of per mil (‰) reported relative to the international standards Vienna Pee Dee Belemnite ($\delta^{13}\text{C}$) and atmospheric air ($\delta^{15}\text{N}$ values). The δ -values were computed as $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \times 1000$, where R is equal to $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$. Each run included three standards: United States Geological Survey (USGS) glutamic acid 40, USGS glutamic acid 41, and an internal standard of beech leaves (plant samples) or trout tissue (animal samples). The precision for each run was $\leq 0.2\text{‰}$ for $\delta^{13}\text{C}$ and 0.1‰ for $\delta^{15}\text{N}$ based off of the standard deviation for internal replicate standards. Because consumer $\delta^{13}\text{C}$ values are affected by lipid content, values were lipid corrected based on the consumer's C:N ratio following Equation 3 in Post et al. (Post et al. 2007).

Statistical Analysis

We conducted all statistical analyses with R (version 3.1.2, R Core Team). We first assessed the extent to which suburbanization explained the pond physical, chemical, and biological (chlorophyll *a* only) parameters using linear regression models. Environmental variables were natural-log transformed if the model residuals were not normally distributed (variables transformed: TN, TP, surface area, canopy cover, periphyton chlorophyll *a*, and phytoplankton chlorophyll *a*, Table 1). We also used similar linear regression models to evaluate the extent to which $\delta^{15}\text{N}$ changed over the suburban land cover gradient, with suburban land cover as the predictor variable.

We used multiple linear regression to evaluate the impact of physical and chemical parameters on algal biomass, as measured by periphyton and phytoplankton chlorophyll *a*. Models were first fit with all environmental variables (transformed as above) with periphyton chlorophyll *a* included in the phytoplankton model, and vice versa, in order to account for possible competitive effects. Models were assessed for collinearity by calculating the variation inflation factor (VIF) and removing variables with a VIF > 5, which included TP, DOC, and percent canopy cover. Because TP and DOC were highly correlated with TN, they are still represented in the models. Similarly, canopy cover was highly correlated with water temperature, which was also included in the models. We iteratively built models by removing one variable at a time using AICc model selection (Burnham and Anderson 2002). Once the top models were identified, we built the models back up adding one variable at a time to ensure we evaluated all possible best models. Top models are reported within two AICc of the best model (lowest AICc). As we were also interested in effects solely due to suburbanization, we include model results for when percent suburban cover was used as the only predictor (it could not be included in the multivariate model due to collinearity).

We estimated the proportion of *R. sylvatica* biomass derived from algae versus leaf litter using $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ isotope mixing models from the “SIAR 4.0” package (Parnell and Jackson 2013). For this analysis, we used 13 of the 18 sampled ponds because we did not recover *R. sylvatica* larvae from two ponds (HSPD Pond, MGNS Pond), we did not have algae samples from two ponds (COLL Pond, OVBK Pond), and the *R. sylvatica* $\delta^{13}\text{C}$ was more depleted than the food resources in one pond (COMN Pond). The leaf litter end-member was averaged from the dominant leaf litter types collected from the pond basin. The algae end-member was averaged between periphyton and seston, as they did not differ in $\delta^{13}\text{C}$ (paired t-test, $t = 0.83$, $df = 11$, $p = 0.42$), a pattern also seen in other small temporary ponds (Holgerson et al. 2016). There were three ponds where the $\delta^{13}\text{C}$ from seston was unreliable (due to small samples); for these ponds, we used the periphyton $\delta^{13}\text{C}$ with an SD of 2.0‰. There were an additional three ponds that only had one dominant leaf type; for these ponds we also used an SD of 2.0‰. Trophic enrichment factors (TEF) for consumers were assumed to be $0.4 \pm 1.2\text{‰}$ ($\pm$ SD) for $\delta^{13}\text{C}$ and $2.3 \pm 1.6\text{‰}$ ($\pm$ SD) for $\delta^{15}\text{N}$ based on values for aquatic animals (McCutchan et al. 2003). We then evaluated the effect of suburbanization and environmental variables on the proportion of *R. sylvatica* biomass derived from algae using multiple linear regression in the same way as described above for algal biomass.

RESULTS

Pond Environment

Increased suburbanization was associated with increases in pond temperature and pH, and decreases in canopy cover and proportion of light penetration (Table 1, Table A2). There was a weak positive relationship between the degree of suburbanization and TN and TP (Table 1, Fig. A1). We did not detect any influence of suburbanization on conductivity, dissolved organic

carbon (DOC), or pond surface area (Table 1). We found that periphyton chlorophyll *a* increased along the suburban gradient; however, there was no relationship between suburbanization and phytoplankton chlorophyll *a* (Table 1). When we evaluated the effects of the physical and chemical variables on algal biomass, we found that increased periphyton chlorophyll *a* was best predicted by a model that included warmer water temperatures, lower light penetration, and smaller pond surface areas ($R^2 = 0.73$, Table 2). None of the chemical or physical variables measured well predicted phytoplankton chlorophyll *a*.

Patterns in $\delta^{15}\text{N}$

There was a dramatic increase in $\delta^{15}\text{N}$ with increasing suburban land cover in four of the five compartments of the food web we studied (Fig. 2, Table A4). For instance, algal $\delta^{15}\text{N}$ ranged from -3.8 to -2.0‰ in the most forested pond (BLIN) up to 6.7 to 7.6‰ in the most suburban pond (SHLD; Table A3). The $\delta^{15}\text{N}$ in biofilm, periphyton, seston, and larval *R. sylvatica* increased by 1.1 to 1.2‰ with every 10% increase in suburban land cover (Fig. 2, Table A4). Zooplankton $\delta^{15}\text{N}$ was unchanged along the suburban gradient (Fig. 2, Table A4).

Consumer Diet

Across a subset of 13 study ponds, the proportion of larval *R. sylvatica* biomass derived from algae ranged from as low as 15% to as high as 83%. The degree of suburbanization explained 37% of this variation, with *R. sylvatica* diet shifting from predominantly leaf litter in forested ponds to algae in the most suburbanized ponds (Fig. 3, Table A5). The proportion of *R. sylvatica* biomass derived from algae was predicted by two top models: periphyton chlorophyll *a* ($R^2 = 0.54$) and periphyton chlorophyll *a* and phytoplankton chlorophyll *a* ($R^2 = 0.61$, Table A5).

DISCUSSION

Pond Environment

Increased suburbanization dramatically changed the physical and chemical properties of small pond ecosystems, and ultimately changed nutrient dynamics and resource use in the food web. The most notable changes included decreased canopy cover, decreased light penetration, and increased temperature (Table 1). A reduction in canopy cover was expected as suburban landscapes typically replace native forest cover with yards (Milesia et al. 2005). As the canopy thins, temperatures are expected to rise (Skelly et al. 2002). The most suburban ponds had the least light penetration, which might appear counterintuitive, but this was driven by duckweed (*Lemna* sp.) colonizing the surface of the most suburban ponds (e.g., duckweed covered the majority of water surface in ESTY and MGNS).

Surprisingly, we observed only a weak positive relationship between suburbanization and total nutrients (Table 1, Figure A1). While it is possible that land development did not increase nutrient inputs to the ponds, it is more likely that we could not detect added nutrients due to small ponds having variable nutrient concentrations across the landscape (Holgerson 2015) and / or nutrient uptake by primary producers, such as algae or duckweed. Duckweed, present only in the more suburban ponds, grows rapidly and efficiently removes both nitrogen and phosphorus from the water column; duckweed can also absorb multiple forms of N, including nitrate and ammonium, the latter of which dominates the N pool in small ponds (Capps et al. 2014; Cedergreen and Madsen 2002). While most of the ponds were bordered by forest, three of the more suburban ponds had landscaped vegetation at their perimeter (e.g., reeds and/or tall grasses in EMSN, ESTY, and MGNS) that may have taken up anthropogenic nutrients before they entered the pond. Because uptake by primary producers can obscure nutrient patterns, algal biomass or composition can be a better detector of eutrophication (Porter et al. 2008).

We did observe increases in periphyton chlorophyll *a* along the suburban gradient (Table 1), but no significant changes in phytoplankton chlorophyll *a*. Increases in algal biomass generally result from increased light (i.e., decreased canopy cover), temperature, nutrients, or a combination thereof (Hansson 1992; Larned 2010). Our multivariate analysis indicated that warmer waters, lower light penetration, and smaller ponds had higher periphyton chlorophyll *a*; however, none of the environmental variables predicted phytoplankton chlorophyll *a* (Table 2). It is likely that because our periphyton racks were located right below the surface of the pond, and displaced some of the duckweed, light penetration did not have the same effect on periphyton and phytoplankton. For phytoplankton, shading from duckweed may have countered any positive effects from temperature, light, and nutrients. Additionally, our analysis does not incorporate grazing effects. Periphyton racks were open to the water column, but may have been difficult for tadpoles to access and we did not observe snails on them. Future studies should consider how phytoplankton grazing pressures (e.g., zooplankton assemblages and densities) change across the land-use gradient. It would also be beneficial to explore temporal variability as we only sampled the ponds at one point in time.

Patterns in $\delta^{15}\text{N}$

While there was only a weak observed effect of suburbanization on total nutrient concentrations, stable isotope analysis provided strong evidence that nitrogen dynamics differed along the suburban gradient. Suburbanization was associated with increased $\delta^{15}\text{N}$ in biofilm, periphyton, seston, and larval *R. sylvatica*, but not zooplankton (Fig. 2, Table A4). Increases in $\delta^{15}\text{N}$ can be caused by changes in the N source or in N cycling. While natural sources of N (e.g., atmospheric N with uptake by terrestrial or aquatic plants) will dominate in forested ponds, suburban ponds may receive nutrient inputs from human waste, animal waste, or fertilizer. Agricultural land

cover was negligible near the study ponds (Table A1), but both human waste and fertilizers could impact $\delta^{15}\text{N}$. We expect that direct fertilizer inputs were minimal because all but two study ponds had a vegetated buffer (e.g., trees, shrubs, and emergent vegetation) between the lawn and the entire pond shoreline. Additionally, synthetic fertilizer has a similar or lower $\delta^{15}\text{N}$ signature to natural sources (Aravena et al. 1993; Heaton 1986), meaning that our observed increases in $\delta^{15}\text{N}$ cannot be attributed to synthetic fertilizer (i.e., inputs of synthetic fertilizer would have lowered $\delta^{15}\text{N}$). Therefore, it is likely that our observed increase in $\delta^{15}\text{N}$ resulted from wastewater incursion from septic systems.

Septic systems are the dominant waste management strategy in suburban landscapes due to their cost-effectiveness over installing a public sewer system (Newburn and Berck 2011). However, septic systems are designed to leach nitrogen to the environment, amounting to 5 – 10 kg N year⁻¹ from a typical household (Reay 2004). The nitrogen cycle in septic systems begins with organic N that enters the septic tank, which is transformed to ammonium under anoxic conditions, and then leaves the septic tank and enters the leach field, where the presence of oxygen transforms it to nitrate (Kaplan 1991). Nitrate can undergo denitrification or it may enter ground and surface waters, including well water (Verstraeten et al. 2005), streams (Moore et al. 2014), lakes (Moore et al. 2003), and estuaries (McClelland et al. 1997).

To assess the relative importance of background N versus septic wastewater N for pond organisms, we ran stable isotope mixing models to estimate the proportion of an organism's diet derived from each N source (Appendix Text A1). The models assumed that wastewater inputs are the only source of $\delta^{15}\text{N}$ enrichment, although denitrification may also play a role (see below). Overall, our models estimated that the proportion of producer and consumer N derived from wastewater was extremely high; reaching 62% to 76% among the food web compartments we

studied (Table A4). The relationships between suburbanization and the proportion of an organism's N derived from septic wastewater were significant for all organisms except zooplankton, and were strongest for biofilm and *R. sylvatica* larvae (Table A4).

Both the $\delta^{15}\text{N}$ and model results indicate that different compartments of the food web utilize N sources differently. Suburbanization had a stronger effect on biofilm N ($\delta^{15}\text{N}$ $R^2 = 0.70$, wastewater N $R^2 = 0.69$) than on seston and periphyton N ($\delta^{15}\text{N}$ and wastewater N $R^2 = 0.37 - 0.45$). As biofilm incorporates microbial assemblages whereas seston and periphyton are predominantly algal in origin, our results may suggest that bacteria and fungi use wastewater $\delta^{15}\text{N}$ to a greater extent than algae. An increased dependence of wastewater N by microbes could be due to spatial heterogeneity of N sources in the pond (i.e., more wastewater N in the benthos) or due to preferential uptake. As zooplankton depend on algae to a greater extent than microbial food web pathways (Holgerson et al. 2016), this may explain why zooplankton were less affected by wastewater N. It is also worth noting that periphyton slides were located at the pond surface; however, periphyton naturally occurs as part of the biofilm. Therefore, our sampling design may underestimate the importance of anthropogenic N to the periphyton community.

In addition to altering the N source (i.e., natural vs. wastewater) in ponds, suburbanization may also affect the N cycle. Specifically, septic systems release nitrate to the environment, whereas the dominant N form in forested ponds is ammonium (Capps et al. 2014). As low nitrate concentrations may limit denitrification rates in forested ponds (Capps et al. 2014), suburban nitrate additions may increase denitrification. As denitrification increases $\delta^{15}\text{N}$, this may help explain our observed pattern of increasing $\delta^{15}\text{N}$ with suburban land cover. While we did not directly measure denitrification, we measured N_2O concentrations and all ponds had

concentrations close to atmospheric conditions, indicating low levels of denitrification (Holgerson unpublished data). Future research should measure nitrate, ammonium, and *in situ* denitrification rates across the suburban land cover gradient, which could be accomplished using dual stable isotope analysis with $\delta^{15}\text{N}$ and $\delta^{18}\text{O}$ (Kendall et al. 2007), and also across multiple points in time.

Overall, our study provides clear evidence that suburbanization alters nitrogen dynamics in small ponds, and that wastewater N likely subsidizes the N pool. While this pattern has also been seen in lakes and rivers (Jankowski et al. 2012; Kaushal et al. 2006; Vander Zanden et al. 2005), our study is unique in that we show that anthropogenic N can have a big impact at a small spatial scale, where individual septic systems act as point sources of pollution (Moore et al. 2014; Steffy and Kilham 2004). Wastewater intrusion from septic systems could indicate the potential for long-term eutrophication, and likely means that other solutes also reach suburban ponds, such as pharmaceutical and personal care products (PPCPs). While the extent to which PPCPs affect aquatic organisms and ecosystems is not well studied, recent studies report endocrine disruption in amphibians (Lambert et al. 2016) and reduction to growth and development in mussels (Peters and Granek 2016) and benthic invertebrates (Hoppe et al. 2012), processes that could alter ecosystem properties such as biogeochemical cycling and population dynamics (Rosi-Marshall and Royer 2012). Additionally, wastewater nutrients may be exported to the terrestrial environment when pond consumers, such as *R. sylvatica*, metamorphose and leave the ponds. The implications of such nutrient translocation for terrestrial ecosystems remain unknown.

Consumer Diet

Suburbanization also changed the food web, as evidenced by shifts in the diet of a dominant pond consumer. Larval *R. sylvatica* are a dominant consumer in small ponds, and persist across the suburban gradient. As *R. sylvatica* can both filter feed and graze, they can derive carbon and nutrients from algae, microbes, and leaf detritus (Holgerson et al. 2016; Schiesari et al. 2009). We found that *R. sylvatica* shifted their carbon and nitrogen source from one ultimately derived from leaf litter in forested ponds to one based on algae in more suburban ponds. Specifically, mixing models estimated that larval biomass was derived from only 15% algae in the least suburban ponds and up to 83% algae in the more suburban ponds (Fig. 3).

The dietary shift from leaf litter to algae with increased suburbanization was best explained by increasing periphyton chlorophyll *a*, with increasing phytoplankton chlorophyll *a* providing some additional support to the model. As discussed above, periphyton chlorophyll *a* increased along the suburban gradient with increasing temperatures, decreased light penetration, and smaller ponds (Table 2). While we measured periphyton chlorophyll *a* from glass slides at the ponds surface, natural benthic periphyton growth could be light limited in ponds with duckweed. However, we still observed a more algal dietary signature in tadpoles from ponds with duckweed, indicating that light does not appear to limit algae (although we cannot determine whether tadpoles consumed benthic periphyton or water-column phytoplankton).

Although there were no significant relationship between algal biomass and nutrients, nutrient uptake by algae could boost biomass while obscuring increases in nutrient concentrations. Other studies have also found that increased nutrient loading and algal biomass in lakes (Carpenter et al. 2005; Cole et al. 2006) or increased sunlight in streams (England and Rosemond 2004; Hill et al. 2001) boosts the importance of algae in food webs. Therefore, it is

likely that both septic wastewater intrusion and reduced forest cover increased the importance of algae to larval *R. sylvatica*.

The ecological consequences of this shift from a leaf litter to algal energy source needs further study. Because algae has a lower C:N ratio than leaf litter, it is considered higher quality. Therefore, it is possible that larval *R. sylvatica* may have increased growth rates and metamorphose larger and earlier when consuming more algae. Yet, in a forested pond with no apparent algae or leaf litter limitation, *R. sylvatica* biomass was derived from about equal amounts of algae and leaf litter, indicating that both food sources may be important (Holgerson et al. 2016). Because suburban ponds had less canopy cover, they likely had fewer terrestrial resources available; the extent to which availability of terrestrial resources influenced energy pathways was not evaluated, but could be important. Future work should conduct feeding trials on *R. sylvatica* to evaluate food preferences and larval performance. Another factor that could contribute to changes in the food web across the suburban gradient is the macroinvertebrate community. As shredders likely mobilize leaf nutrients to pond consumers, changes in their abundance or composition could limit the availability of leaf nutrients to tadpoles and other consumers (Holgerson et al. 2016).

To conclude, our study emphasizes that land use change can have pervasive, and often cryptic, effects on native ecosystem functioning. We provide clear evidence that converting forests to suburban landscapes changes the physical, chemical, and biological parameters of pond ecosystems. Importantly, we show changes to the $\delta^{15}\text{N}$ signature of pond organisms, likely indicating wastewater incursion, as well as shifting resource use by a dominant consumer. Stable isotopes provided a critical tool for observing these ecosystem changes, which would have gone unnoticed if only monitoring for total nutrient concentrations or the presence / abundance of

dominant vertebrate species. An important next step is definitively linking wastewater incursion to suburban ponds, assessing if wastewater also influences denitrification, and tracing how these processes cascade to changes in amphibian and invertebrate populations. As we are currently undergoing a global increase in suburbanization (Seto et al. 2012) and decline in suburban water quality (Harrison et al. 2012), there is a critical need to monitor freshwater habitats, improve wastewater management, and establish or enforce buffer zones to better protect our freshwater ecosystems.

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REFERENCES

- Aravena, R., Evans, M.L., and Cherry, J.A. 1993. Stable isotopes of oxygen and nitrogen in source identification of nitrate from septic systems. *Ground Water* **31**(2): 180-186.
- Atwood, M.A. 2013. Effects of euthanasia method on stable-carbon and stable-nitrogen isotope analysis for an ectothermic vertebrate. *Rapid Communications in Mass Spectrometry* **27**(8): 909-913.

- 438 Brown, D.G., Johnson, K.M., Loveland, T.R., and Theobald, D.M. 2005. Rural land-use trends
439 in the conterminous United States, 1950-2000. *Ecological Applications* **15**(6): 1851-
440 1863.
- 441 Burnham, K.P., and Anderson, D.R. 2002. Model selection and multimodel inference: A
442 practical information-theoretic approach. Springer, New York, New York, USA.
- 443 Capps, K.A., Berven, K.A., and Tiegs, S.D. 2015. Modelling nutrient transport and
444 transformation by pool-breeding amphibians in forested landscapes using a 21-year
445 dataset. *Freshwater Biology* **60**: 500-511.
- 446 Capps, K.A., Rancatti, R., Tomczyk, N., Parr, T.B., Calhoun, A.J.K., and Hunter, M. 2014.
447 Biogeochemical hotspots in forested landscapes: The role of vernal pools in
448 denitrification and organic matter processing. *Ecosystems* **17**(8): 1455-1468.
- 449 Carey, R.O., Hochmuth, G.J., Martinez, C.J., Boyer, T.H., Dukes, M.D., Toor, G.S., and Cisar,
450 J.L. 2013. Evaluating nutrient impacts in urban watersheds: Challenges and research
451 opportunities. *Environmental Pollution* **173**: 138-149.
- 452 Carpenter, S.R., Cole, J.J., Pace, M.L., Van de Bogert, M., Bade, D.L., Bastviken, D., Gille,
453 C.M., Hodgson, J.R., Kitchell, J.F., and Kritzberg, E.S. 2005. Ecosystem subsidies:
454 Terrestrial support of aquatic food webs from ^{13}C addition to contrasting lakes. *Ecology*
455 **86**(10): 2737-2750.
- 456 Cedergreen, N., and Madsen, T.V. 2002. Nitrogen uptake by the floating macrophyte *Lemna*
457 *minor*. *New Phytologist* **155**(2): 285-292.
- 458 Cheever, B.M., Webster, J.R., Bilger, E.E., and Thomas, S.A. 2013. The relative importance of
459 exogenous and substrate-derived nitrogen for microbial growth during leaf
460 decomposition. *Ecology* **94**(7): 1614-1625.

- 461 Clesceri, L.S., Greenberg, A.E., and Eaton, A.D. 1998. Standard methods for the examination of
462 water and wastewater. American Public Health Association, Water Environment
463 Federation, Washington D.C., USA.
- 464 Cole, J.J., Carpenter, S.R., Pace, M.L., Van de Bogert, M.C., Kitchell, J.L., and Hodgson, J.R.
465 2006. Differential support of lake food webs by three types of terrestrial organic carbon.
466 Ecology Letters **9**(5): 558-568.
- 467 England, L.E., and Rosemond, A.D. 2004. Small reductions in forest cover weaken terrestrial-
468 aquatic linkages in headwater streams. Freshwater Biology **49**(6): 721-734.
- 469 Grimm, N.B., Foster, D., Groffman, P., Grove, J.M., Hopkinson, C.S., Nadelhoffer, K.J., Pataki,
470 D.E., and Peters, D.P.C. 2008. The changing landscape: ecosystem responses to
471 urbanization and pollution across climatic and societal gradients. Frontiers in Ecology
472 and the Environment **6**(5): 264-272.
- 473 Hansson, L.-A. 1992. Factors regulating periphytic algal biomass. Limnology and Oceanography
474 **37**(2): 322-328.
- 475 Harrison, M., Stanwyck, E., Beckingham, B., Starry, O., Hanlon, B., and Newcomer, J. 2012.
476 Smart growth and the septic tank: Wastewater treatment and growth management in the
477 Baltimore region. Land Use Policy **29**(3): 483-492.
- 478 Heaton, T.H.E. 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere:
479 A review. Chemical Geology: Isotope Geoscience section **59**: 87-102.
- 480 Hill, W.R., Mulholland, P.J., and Marzolf, E.R. 2001. Stream ecosystem responses to forest leaf
481 emergence in spring. Ecology **82**(8): 2306-2319.
- 482 Holgerson, M.A. 2015. Drivers of carbon dioxide and methane supersaturation in small,
483 temporary ponds. Biogeochemistry **124**: 305-318.

- 484 Holgerson, M.A., Post, D.M., and Skelly, D.K. 2016. Reconciling the role of terrestrial leaves in
485 pond food webs: A whole-ecosystem experiment. *Ecology* **97**: 1771-1782.
- 486 Hoppe, P.D., Rosi-Marshall, E.J., and Bechtold, H.A. 2012. The antihistamine cimetidine alters
487 invertebrate growth and population dynamics in artificial streams. *Freshwater Science*
488 **31**(2): 379-388.
- 489 Hutyra, L.R., Yoon, B., and Alberti, M. 2011. Terrestrial carbon stocks across a gradient of
490 urbanization: a study of the Seattle, WA region. *Global Change Biology* **17**(2): 783-797.
- 491 Irwin, E.G., and Bockstael, N.E. 2007. The evolution of urban sprawl: Evidence of spatial
492 heterogeneity and increasing land fragmentation. *Proceedings of the National Academy*
493 *of Sciences* **104**(52): 20672-20677.
- 494 Jankowski, K., Schindler, D.E., and Holtgrieve, G.W. 2012. Assessing nonpoint-source nitrogen
495 loading and nitrogen fixation in lakes using $\delta^{15}\text{N}$ and nutrient stoichiometry.
496 *Limnology and Oceanography* **57**(3): 671-683.
- 497 Kaplan, O.B. 1991. *Septic Systems Handbook*. Lewis Publishers, Inc., Chelsea, Michigan, USA.
- 498 Kaushal, S.S., Lewis Jr, W.M., and McCutchan Jr, J.H. 2006. Land use change and nitrogen
499 enrichment of a Rocky Mountain watershed. *Ecological Applications* **16**(1): 299-312.
- 500 Kendall, C., Elliott, E.M., and Wankel, S.D. 2007. Tracing anthropogenic inputs of nitrogen to
501 ecosystems. *In* *Stable Isotopes in Ecology and Environmental Science*. *Edited by* R.H.
502 Michener and K. Lajtha. Blackwell Publishing. pp. 375-449.
- 503 Lambert, M.R., Giller, G.S.J., Barber, L.B., Fitzgerald, K.C., and Skelly, D.K. 2015.
504 Suburbanization, estrogen contamination, and sex ratio in wild amphibian populations.
505 *Proceedings of the National Academy of Sciences* **112**(38): 11881-11886.

- 506 Lambert, M.R., Giller, G.S.J., Skelly, D.K., and Bribiescas, R.G. 2016. Septic systems, but not
507 sanitary sewer lines, are associated with elevated estradiol in male frog metamorphs from
508 suburban ponds. *General and Comparative Endocrinology*.
- 509 Larned, S.T. 2010. A prospectus for periphyton: recent and future ecological research. *Journal of*
510 *the North American Benthological Society* **29**(1): 182-206.
- 511 Mather, M., Pollard, K., and Jacobsen, L.A. 2011. Reports on America: First results from the
512 2010 census. Population Reference Bureau, Washington D.C., USA.
- 513 McClelland, J.W., Valiela, I., and Michener, R.H. 1997. Nitrogen-stable isotope signatures in
514 estuarine food webs: A record of increasing urbanization in coastal watersheds.
515 *Limnology and Oceanography* **42**(5): 930-937.
- 516 McCutchan, J.H., Lewis, W.M., Kendall, C., and McGrath, C.C. 2003. Variation in trophic shift
517 for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* **102**(2): 378-390.
- 518 McDonnell, M., and Hahs, A. 2008. The use of gradient analysis studies in advancing our
519 understanding of the ecology of urbanizing landscapes: Current status and future
520 directions. *Landscape Ecology* **23**(10): 1143-1155.
- 521 Milesia, C., Elvidge, C., Dietz, J., Tuttle, B., Nemani, R., and Running, S. 2005. A strategy for
522 mapping and modeling the ecological effects of US lawns, Tempe, AZ, USA, March 14–
523 16 2005.
- 524 Moore, J.W., Lambert, T.D., Heady, W.N., Honig, S.E., Osterback, A.-M.K., Phillis, C.C.,
525 Quiros, A.L., Retford, N.A., and Herbst, D.B. 2014. Anthropogenic land-use signals
526 propagate through stream food webs in a California, USA, watershed. *Limnologia -*
527 *Ecology and Management of Inland Waters* **46**: 124-130.

- 528 Moore, J.W., Schindler, D.E., Scheuerell, M.D., Smith, D., and Frodge, J. 2003. Lake
529 eutrophication at the urban fringe, Seattle Region, USA. *AMBIO: A Journal of the*
530 *Human Environment* **32**(1): 13-18.
- 531 Newburn, D., and Berck, P. 2011. Exurban development. *Journal of Environmental Economics*
532 *and Management* **62**(3): 323-336.
- 533 Parnell, A.C., and Jackson, A.L. 2013. Stable isotope analysis in R. R package version 4.2.
- 534 Paton, P.W.C. 2005. A review of vertebrate community composition in seasonal forest pools of
535 the northeastern United States. *Wetlands Ecology and Management* **13**(3): 235-246.
- 536 Peters, J.R., and Granek, E.F. 2016. Long-term exposure to fluoxetine reduces growth and
537 reproductive potential in the dominant rocky intertidal mussel, *Mytilus californianus*.
538 *Science of The Total Environment* **545–546**: 621-628.
- 539 Polis, G.A., and Strong, D.R. 1996. Food web complexity and community dynamics. *The*
540 *American Naturalist* **147**(5): 813-846.
- 541 Porter, S.D., Mueller, D.K., Spahr, N.E., Munn, M.D., and Dubrovsky, N.M. 2008. Efficacy of
542 algal metrics for assessing nutrient and organic enrichment in flowing waters. *Freshwater*
543 *Biology* **53**(5): 1036-1054.
- 544 Post, D., Layman, C., Arrington, D., Takimoto, G., Quattrochi, J., and Montaña, C. 2007. Getting
545 to the fat of the matter: Models, methods and assumptions for dealing with lipids in stable
546 isotope analyses. *Oecologia* **152**(1): 179-189.
- 547 Reay, W.G. 2004. Septic tank impacts on ground water quality and nearshore sediment nutrient
548 flux. *Ground Water* **42**(7): 1079-1089.
- 549 Rooney, N., and McCann, K.S. 2012. Integrating food web diversity, structure and stability.
550 *Trends in Ecology & Evolution* **27**(1): 40-46.

- 551 Rosi-Marshall, E.J., and Royer, T.V. 2012. Pharmaceutical compounds and ecosystem function:
552 An emerging research challenge for aquatic ecologists. *Ecosystems* **15**(6): 867-880.
- 553 Scheffer, M., Geest, G.J.v., Zimmer, K., Jeppesen, E., Søndergaard, M., Butler, M.G., Hanson,
554 M.A., Declerck, S., Meester, L.d., and Persson, L. 2006. Small habitat size and isolation
555 can promote species richness: Second-order effects on biodiversity in shallow lakes and
556 ponds. *Oikos* **112**(1): 227-231.
- 557 Schiesari, L., Werner, E.E., and Kling, G.W. 2009. Carnivory and resource-based niche
558 differentiation in anuran larvae: Implications for food web and experimental ecology.
559 *Freshwater Biology* **54**(3): 572-586.
- 560 Schmitz, O.J. 2010. Resolving ecosystem complexity. Princeton University Press, Princeton,
561 New Jersey, USA.
- 562 Seto, K.C., Güneralp, B., and Hutyra, L.R. 2012. Global forecasts of urban expansion to 2030
563 and direct impacts on biodiversity and carbon pools. *Proceedings of the National*
564 *Academy of Sciences* **109**(40): 16083-16088.
- 565 Skelly, D.K., Bolden, S.R., and Dion, K.B. 2010. Intersex frogs concentrated in suburban and
566 urban landscapes. *EcoHealth* **7**(3): 374-379.
- 567 Skelly, D.K., Freidenburg, L.K., and Kiesecker, J.M. 2002. Forest canopy and the performance
568 of larval amphibians. *Ecology* **83**(4): 983-992.
- 569 Steffy, L.Y., and Kilham, S.S. 2004. Elevated δ ¹⁵N in stream biota in areas with septic tank
570 systems in an urban watershed. *Ecological Applications* **14**(3): 637-641.
- 571 Tanentzap, A.J., Kielstra, B.W., Wilkinson, G.M., Berggren, M., Craig, N., del Giorgio, P.A.,
572 Grey, J., Gunn, J.M., Jones, S.E., Karlsson, J., Solomon, C.T., and Pace, M.L. 2017.

- 573 Terrestrial support of lake food webs: Synthesis reveals controls over cross-ecosystem
574 resource use. *Science Advances* **3**(3).
- 575 Vander Zanden, M.J., Vadeboncoeur, Y., Diebel, M.W., and Jeppesen, E. 2005. Primary
576 consumer stable nitrogen isotopes as indicators of nutrient source. *Environmental Science*
577 *& Technology* **39**(19): 7509-7515.
- 578 Verstraeten, I.M., Fetterman, G.S., Meyer, M.T., Bullen, T., and Sebree, S.K. 2005. Use of
579 tracers and isotopes to evaluate vulnerability of water in domestic wells to septic waste.
580 *Ground Water Monitoring & Remediation* **25**(2): 107-117.
- 581 Werner, E.E., Skelly, D.K., Relyea, R.A., and Yurewicz, K.L. 2007. Amphibian species richness
582 across environmental gradients. *Oikos* **116**(10): 1697-1712.
- 583 Withers, P.J.A., Jarvie, H.P., and Stoate, C. 2011. Quantifying the impact of septic tank systems
584 on eutrophication risk in rural headwaters. *Environment International* **37**(3): 644-653.
- 585

Table 1. Environmental variables sampled across the 18 study ponds, including the range of values and the variance explained by suburbanization (classified as the percent of suburban land at a 200 m buffer).

Variable	Range	Sign	R ²	p
Temperature (average of mean daily temp, 2 weeks)	14.2 – 23.4	+	0.46	0.004
Temperature (maximum daily temp, 2 weeks)	18.0 – 33.6	+	0.60	<0.001
pH	5.31 – 7.28	+	0.52	<0.001
Conductivity ($\mu\text{S cm}^{-1}$)	43.7 – 575		0.08	0.26
Phytoplankton chlorophyll a ($\mu\text{g L}^{-1}$) (LN)	0.8 – 153.1		0.01	0.71
Periphyton chlorophyll a ($\mu\text{g cm}^{-2}$) (LN)	2.7 – 239.6	+	0.40	0.008
TN (mg L^{-1}) (LN)	0.4 – 15.6		0.16	0.10
TP ($\mu\text{g L}^{-1}$) (LN)	20.4 – 1,738.2		0.20	0.06
DOC (LN)	3.1 – 36.9		0.14	0.13
Surface area (m^2) (LN)	34 – 11,529		0.03	0.46
Open canopy (%) (LN)	5.8 – 88.2%	+	0.36	0.008
Proportion of light penetrating water surface	0.001 – 0.85	-	0.51	<0.001

Table 2. Multiple linear regression models predicting periphyton and phytoplankton chlorophyll *a*. Estimates include SE in parentheses. Full models included average daily temperature over two weeks, proportion light penetration, periphyton chlorophyll *a*, phytoplankton chlorophyll *a*, TN, and surface area. Transformations as in Table 1.

Model	k	AICc	Δ AICc	Est. Intercept	Est. 1	Est. 2	Est. 3	R ²	p
<i>Periphyton</i>									
Temperature + proportion light + area (LN)	3	49.67	0	3.60 (2.50)	0.41 (0.13)	-2.61 (1.24)	-0.96 (0.21)	0.73	0.002
Temperature + area (LN)	2	50.07	-0.04	-0.06 (2.03)	0.53 (0.13)	-0.90 (0.23)		0.62	0.003
Null	0	60.02	-10.36	3.55 (0.35)					
<i>Phytoplankton</i>									
Null	0	63.81	0	1.94 (0.30)					
Periphyton (LN)	1	64.51	-0.70	0.76 (0.87)	0.33 (0.23)			0.12	0.17
Temperature + area (LN)	2	64.62	-0.81	0.45 (2.42)	0.30 (0.16)	-0.58 (0.27)		0.26	0.10
Area (LN)	1	65.12	-1.31	3.95 (1.68)	-0.31 (0.25)			0.09	0.24

FIGURE LEGENDS

Figure 1. An example portion of the suburban gradient studied here. The ponds are colored in light blue in the center of each image. From left-to-right, the ponds are surrounded by 2.5% (BLIN Pond), 25.4% (COLL Pond), 40.3% (STHL Pond), and 74.7% (SHLD Pond) suburban cover within a 200 m radius of the pond edge. Suburban neighborhoods are composed of a mixture of buildings, paved surfaces, lawns, and yard. Natural land cover types include forests and tidal wetlands.

Figure 2. The relationship between suburban land cover and $\delta^{15}\text{N}$ of pond organisms (biofilm $n = 18$, $R^2 = 0.70$, $p < 0.001$; periphyton $n = 16$, $R^2 = 0.40$, $p = 0.008$; seston $n = 18$, $R^2 = 0.38$, $p = 0.006$; zooplankton $n = 16$, $R^2 = 0.14$, $p = 0.16$; *R. sylvatica*, $n = 16$, $R^2 = 0.65$, $p < 0.001$). Shaded area indicates 95% confidence interval.

Figure 3. The modeled proportion (mean $\pm$ SE) of larval *R. sylvatica* diet coming from algae in relation to (A) suburban land cover ($R^2 = 0.37$, $p = 0.03$) and (B) periphyton chlorophyll *a* ($R^2 = 0.54$, $p = 0.004$) ($n = 13$ ponds). Shaded area indicates 95% confidence interval.

APPENDIX FOR

Suburbanization alters small pond ecosystems: Shifts in nitrogen and food web dynamics

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Text A1. Details for estimating the wastewater N influence to pond organisms.

We created stable isotope mixing models with $\delta^{15}\text{N}$ using the “SIAR 4.0” package (Parnell and Jackson 2013) to evaluate the contribution of two N sources (background vs. wastewater N) to organisms. The model explicitly ignores denitrification occurring within the pond (see main text). The $\delta^{15}\text{N}$ used for end-members were $-1.25 (\pm 1.08 \text{ SD}) \text{ ‰}$ for background N and $+13.0 (\pm 1.67 \text{ SD}) \text{ ‰}$ for wastewater-derived N, which were selected to encompass the mean and range of $\delta^{15}\text{N}$ observed in the literature. Background values of $\delta^{15}\text{N}$ range from -4.5 to $+2.0 \text{ ‰}$ (Jankowski et al. 2012, Kaushal et al. 2006, Moore et al. 2014), which includes the range of $\delta^{15}\text{N}$ we observed in primary producers from our least developed pond (BLIN Pond, 97.5% forested at a 200 m buffer). Values for wastewater-derived N were based off septic system effluent, where $\delta^{15}\text{N}$ ranges from $+8$ to $+18 \text{ ‰}$ (Aravena et al. 1993, Kaushal et al. 2006, Komor and Anderson 1993). These estimates of septic $\delta^{15}\text{N}$ account for N transformations that occur within the septic tank (ammonification) and within the leach field (nitrification, denitrification). The range for both background and wastewater $\delta^{15}\text{N}$ end-members are relatively wide, and uncertainty could be reduced by directly measuring groundwater $\delta^{15}\text{N}$ near the ponds in both septic and natural landscapes. Models also included trophic enrichment factors (TEF) for consumers, which were assumed to be $2.3 \pm 1.6 \text{ ‰}$ ($\pm \text{SD}$) for $\delta^{15}\text{N}$ based on values for aquatic animals (McCutchan et al. 2003).

After using the mixing models to calculate the proportion of N derived from wastewater, we used linear regression methods to test the relationship between suburban land use and the proportion of N derived from wastewater in the five pond organisms studied. No data transformations were required for these analyses.

Table A1. Land-use surrounding the 18 study ponds in Madison, CT. Values represent a percentage of 200 m buffer around each pond classified by each land type. Suburban land cover represents the sum of buildings, yards, lawns, and pavement.

Pond	Building %	Yard %	Lawn %	Pavement %	Suburban %	Houses ha ⁻¹	Forest %	Agriculture %
BART	3.1	2.8	12.4	8.0	26.4	1.23	73.6	0.0
BLIN	0.3	0.3	1.6	0.3	2.5	0.12	97.5	0.0
BLKN	5.0	1.4	8.8	13.8	28.9	0.84	71.1	0.0
CNCD	2.4	2.0	6.3	6.8	17.5	1.03	82.5	0.0
COLL	2.6	1.6	10.9	10.2	25.4	0.77	74.6	0.0
COMN	2.1	0.3	10.0	5.7	18.1	0.89	75.2	0.0
EMSN	2.7	5.2	20.6	5.5	33.9	1.03	66.1	0.0
ESTY	7.3	3.6	22.5	28.0	61.4	2.03	38.3	0.0
FIPT	3.0	3.3	4.5	10.8	21.5	0.61	78.5	0.0
FLHS	3.6	3.5	27.9	7.0	42.0	1.09	50.8	4.9
HSPD	3.6	5.6	24.3	10.3	43.9	1.51	56.1	0.0
MGNS	7.8	3.5	35.0	10.0	56.3	3.66	22.9	0.0
OVBK	2.5	4.6	4.5	9.6	21.3	0.84	78.7	0.0
RDGE	0.9	0.2	5.9	5.2	12.3	0.43	86.9	0.0
SHLD	10.4	3.6	48.7	11.9	74.7	5.10	18.6	0.0
STHL	4.0	4.7	21.1	10.4	40.3	1.68	59.7	0.0
SUHL	1.2	2.8	4.9	3.2	12.1	0.35	84.0	0.0
WNHL	2.9	1.7	18.4	7.2	30.3	1.46	69.7	0.0

Table A2. Physical and chemical characteristics of the 18 study ponds in Madison, CT. Temperature represents the mean daily temperature, with the range (minimum and maximum) over a two-week period.

Pond	Surface Area (m ²)	Water Temp. (° C)	Canopy Cover %	Prop. Light Penetration	Periphyton chl. <i>a</i> (µg cm ⁻²)	Phyto. chl. <i>a</i> (µg L ⁻¹)	TN (mg L ⁻¹)	TP (µg L ⁻¹)
BART	1150	17.0 (10.9 – 24.0)	93.3	0.50	5.7	1.9	4.49	365.2
BLIN	1527	16.8 (12.2 – 21.9)	84.8	0.66	5.3	10.1	1.10	69.4
BLKN	1033	17.1 (11.7 – 22.5)	91.1	0.45	23.9	4.8	8.38	847.0
CNCD	152	16.5 (12.4 – 20.7)	89.2	0.79	20.7	153.1	1.81	209.5
COLL	818		80.2	0.49		15.9	1.14	62.9
COMN	449	14.2 (11.1 – 18.0)	75.3	0.43	2.7	0.8	0.44	20.9
EMSN	1154		23.5	0.85	38.4	7.3	0.46	27.2
ESTY	1181	18.4 (13.1 – 24.3)	40.3	0.03	145.2	8.3	2.28	234.6
FIPT	34	15.9 (10.5 – 22.5)	90.6	0.56	108.7	4.5	1.53	126.2
FLHS	258	19.7 (14.0 – 27.5)	63.0	0.49	239.6	6.3	1.40	118.1
HSPD	11529	23.4 (17.9 – 31.8)	11.8	0.25	20.1	3.7	0.57	20.4
MGNS	696	19.0 (10.6 – 27.0)	21.8	0.00	179.2	8.8	1.46	117.5
OVBK	833	15.9 (11.9 – 20.2)	89.2	0.75		1.1	1.13	32.0
RDGE	1659	16.6 (12.1 – 21.5)	83.2	0.46	9.3	2.5	1.18	95.1
SHLD	770	21.0	52.0	0.16	91.2	25.4	8.80	1738.2

		(13.3 – 33.6)						
		16.6						
STHL	756	(11.4 – 21.9)	94.2	0.56	48.1	3	15.56	1279.9
		16.9						
SUHL	386	(13.4 – 20.1)	90.4	0.53	30.8	35	0.64	47.0
		16.9						
WNHL	206	(12.9 – 21.7)	60.7	0.51	181	18.4	1.16	91.4

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Table A3. Organismal $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope analysis from the 18 study ponds in Madison, CT. Biofilm, periphyton, and seston values represent one composite sample from each pond; *Rana sylvatica* larvae represent an average of three individuals, except for COLL where only one individual was recovered.

Pond	Biofilm		Periphyton		Seston		Zooplankton		<i>Rana sylvatica</i>	
	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
BART	-30.1	4.6	-29.6	-1.4	-30.1	0.3	-27.1	2.0	-28.4	3.2
BLIN	-30.7	1.2	-32.2	-2.0	-37.6	-3.8	-32.0	2.7	-28.7	3.4
BLKN	-29.9	0.5	-35.3	-4.2		-3.5			-29.6	2.2
CNCD	-30.0	1.7	-34.9	1.3	-40.5	0.5	-37.8	4.1	-34.7	3.0
COLL	-31.3	2.5				1.0	-32.9	5.7	-30.9	5.1
COMN	-32.9	2.2	-29.1	1.6	-31.9	2.0	-34.7	6.6	-36.0	5.6
EMSN	-33.9	3.2	-33.0	1.8	-37.6	4.2	-38.2	3.1	-35.0	4.2
ESTY	-32.3	7.2	-33.0	7.3	-34.3	5.9	-39.3	8.4	-34.6	10.1
FIPT	-31.5	2.4	-35.6	2.3	-30	4.8	-30.8	7.2	-30.7	6.2
FLHS	-29.0	4.6	-31.6	7.4	-32.2	9.6	-32.7	12.0	-31.9	10.0
HSPD	-28.9	1.3	-31.0	0.2	-30.8	-0.2	-30.1	1.6		
MGNS	-31.5	6.0	-29.3	2.7		4.9	-35.6	8.6		
OVBK	-31.4	2.2				-0.7	-30.1	2.0	-34.2	4.6
RDGE	-29.9	-0.4	-35.2	-3.0	-29.5	-0.4	-26.1	3.9	-27.9	3.5
SHLD	-30.4	8.5	-30.9	7.6		6.7			-30.0	11.4
STHL	-28.7	5.9	-32.5	-2.1		1.9	-28.2	1.6	-28.8	5.4
SUHL	-30.8	2.1	-33.6	2.1	-35.4	3.5	-35.4	5.5	-33.2	4.6
WNHL	-30.5	3.7	-35.4	-1.1	-34.9	0.3	-35.9	3.0	-32.7	4.0

Table A4. The relationship between suburban land cover (at a 200 m buffer) and observed range of $\delta^{15}\text{N}$ and the modeled range of the proportion of N derived from septic wastewater in study organisms.

Organism	n	Range	Intercept	Slope	R ²	p
$\delta^{15}\text{N}$						
Biofilm	18	-0.4 – 8.5	-0.08	0.11	0.70	< 0.001
Periphyton	16	-4.2 – 7.6	-2.59	0.12	0.40	0.008
Seston	18	-3.8 – 9.6	-1.58	0.11	0.38	0.006
Zooplankton	16	1.6 – 12.0	2.88	0.07	0.14	0.16
<i>Rana sylvatica</i>	16	2.2 – 11.4	1.91	0.12	0.65	< 0.001
Wastewater N (<i>modeled</i>)						
Biofilm	18	0.09 – 0.68	0.096	0.007	0.69	< 0.001
Periphyton	16	0.02 – 0.62	-0.012	0.007	0.45	0.005
Seston	18	0.02 – 0.76	0.047	0.007	0.37	0.007
Zooplankton	16	0.08 – 0.76	0.172	0.004	0.15	0.14
<i>Rana sylvatica</i>	16	0.11 – 0.70	0.121	0.007	0.65	< 0.001

Table A5. Linear regression models for predicting the proportion of wood frog biomass derived from algae. Estimates include SE in parentheses. Full model included average daily temperature over two weeks, proportion light penetration, periphyton chlorophyll *a*, phytoplankton chlorophyll *a*, TN, and surface area. Transformations as in Table 1. Percent suburban at 200 m was not included in the model due to issues with collinearity; we include the results of a model with only % suburban for comparison.

Model	k	AICc	Δ AICc	Estimate Intercept	Estimate 1	Estimate 2	R ²	p
Periphyton (LN)	1	-7.84	0	0.11 (0.12)	0.11 (0.03)		0.54	0.004
Periphyton chl. <i>a</i> (LN) + phytoplankton chl. <i>a</i> (LN)	2	-5.55	-2.30	0.04 (0.13)	0.11 (0.03)	0.04 (0.03)	0.61	0.009
Null	0	-1.12	--	0.52 (0.05)				
Suburban % at 200 m	1	-3.66	-4.18	0.34 (0.09)	0.006 (0.002)		0.37	0.03

Figure A1. The relationship between suburban land use and (A) TN ($R^2 = 0.16$, $p = 0.10$) and (B) TP ($R^2 = 0.20$, $p = 0.06$) ($n=18$ ponds). Nutrient concentrations are natural-log transformed.

Appendix Reference List

- Aravena, R., Evans, M.L., and Cherry, J.A. 1993. Stable isotopes of oxygen and nitrogen in source identification of nitrate from septic systems. *Ground Water* **31**(2): 180-186.
- Jankowski, K., Schindler, D.E., and Holtgrieve, G.W. 2012. Assessing nonpoint-source nitrogen loading and nitrogen fixation in lakes using $\delta^{15}\text{N}$ and nutrient stoichiometry. *Limnology and Oceanography* **57**(3): 671-683.
- Kaushal, S.S., Lewis Jr, W.M., and McCutchan Jr, J.H. 2006. Land use change and nitrogen enrichment of a Rocky Mountain watershed. *Ecological Applications* **16**(1): 299-312.
- Komor, S.C., and Anderson, H.W. 1993. Nitrogen isotopes as indicators of nitrate sources in Minnesota sand-plain aquifers. *Ground Water* **31**(2): 260-270.
- McCutchan, J.H., Lewis, W.M., Kendall, C., and McGrath, C.C. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* **102**(2): 378-390.
- Moore, J.W., Lambert, T.D., Heady, W.N., Honig, S.E., Osterback, A.-M.K., Phillis, C.C., Quiros, A.L., Retford, N.A., and Herbst, D.B. 2014. Anthropogenic land-use signals propagate through stream food webs in a California, USA, watershed. *Limnologia - Ecology and Management of Inland Waters* **46**: 124-130.
- Parnell, A.C., and Jackson, A.L. 2013. Stable isotope analysis in R. R package version 4.2.