MINIREVIEW

Nitrogen-cycling process rates across urban ecosystems

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One sentence summary: Urban watersheds are made up of different types of ecosystems, with each ecosystem capable of decreasing nitrogen pollution via different microbial transformations that we reviewed across different urban ecosystem types.

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ABSTRACT

Nitrogen (N) pollution of freshwater, estuarine and marine ecosystems is widespread and has numerous environmental and economic impacts. A portion of this excess N comes from urban watersheds comprised of natural and engineered ecosystems that can alter downstream N export. Studies of urban N cycling have focused on either specific ecosystems or on watershed-scale mass balances. Comparisons of specific N transformations across ecosystems are required to contextualize rates from individual studies. Here we reviewed urban N cycling in terrestrial, aquatic and engineered ecosystems, and compared N processing in these urban ecosystem types to native reference ecosystems. We found that net N mineralization and net nitrification rates were enhanced in urban forests and riparian zones relative to reference ecosystems. Denitrification was highly variable across urban ecosystem types, but no significant differences were found between urban and reference denitrification rates. When focusing on urban streams, ammonium uptake was more rapid than nitrate uptake in urban streams. Additionally, reduction of stormwater runoff coupled with potential decreases in N concentration suggests that green infrastructure may reduce downstream N export. Despite multiple environmental stressors in urban environments, ecosystems within urban watersheds can process and transform N at rates similar to or higher than reference ecosystems.

Keywords: urban; mineralization; nitrification; denitrification; nitrogen spiraling; green infrastructure

INTRODUCTION

The majority of the global human population currently dwell in cities (Grimm *et al.* 2008), and the proportion of people living in cities is expected to rise for the foreseeable future (United Nations 2010; Pickett *et al.* 2011). A consequence of urban expansion is the homogenization of urban ecosystems (Groffman *et al.* 2014). More specifically, urban ecosystems across the USA appear to be more similar to each other than to nearby native ecosystems (e.g. residential yards in Baltimore and Phoenix are more similar to each other than to either forests in Maryland or deserts in Arizona). Urban homogenization is evident in reduced floral (Knapp *et al.* 2012) and faunal biodiversity (Faeth, Bang and Saari 2011), similarities in surface-water distributions across metropolitan areas (Steele *et al.* 2014) and converging biogeochemical cycles in urban ecosystems (Kaye *et al.* 2006).

Urbanization has a particularly large effect on the nitrogen (N) cycle (Kaye *et al.* 2006; Bettez *et al.* 2015), reducing watershed N retention and increasing downstream N export. Excess N export leads to eutrophication and periodic hypoxia in coastal ecosystems globally (Diaz and Rosenberg 2008), with major environmental, economic and human health consequences (Dodds *et al.* 2009). Although a large portion of excess N export at a regional scale comes from agricultural areas (Alexander *et al.* 2008), urban ecosystems can be major contributors of point and non-point source N (Shields *et al.* 2008). Sources of N to urban watersheds include atmospheric deposition, wastewater effluent, lawn fertilizer application and leaky sewage infrastructure (Anisfeld *et al.* 2007; Kaushal *et al.* 2011; Hale *et al.* 2014). These sources differ from agricultural ecosystems where fertilizer is the dominant source of N. Differences in sources and forms of N pollution may affect microbial transformations of N or N retention at the watershed scale (Groffman *et al.* 2004).

Urban N dynamics at the watershed scale are influenced by the various sources of N to the watershed as well as N processing within the watershed. Using the watershed approach to balance inputs and outputs of urban watersheds has been, and will continue to be, a powerful tool showing that urban watershed N retention is not nearly as efficient as in reference watersheds (Dietz and Clausen 2008; Bettez *et al.* 2015). However, the blackbox view of urban ecosystems provided by the watershed mass balance approach can lack the detailed focus on processes necessary to understand and manage N export.

Urban watersheds are comprised of multiple ecosystem types that have undergone various transitions over the course of urbanization. Examples of these include native forests, grasslands and former agricultural lands transitioning to urban forests and lawns, streams exhibiting symptoms of the 'urban stream syndrome' (Walsh *et al.* 2005) and the creation of novel ecosystems via green infrastructure development (Passeport *et al.* 2013; Koch *et al.* 2014). Each of these urban ecosystem types is capable of retaining and transforming N via the same pathways as native ecosystems, but rates of N transformations and mechanisms controlling these rates likely differ between urban and native ecosystems.

For the most part, N cycle processes are carried out by diverse microbial communities necessitating a focus on environmental factors regulating these processes rather than on the specifics of community composition (Fierer *et al.* 2009a,b; Wallenstein and Hall 2012; Graham *et al.* 2014). Mineralization and immobilization of N are byproducts of basic C metabolism and take place under a wide range of environmental conditions (Schimel and Bennett 2004; Ramirez, Craine and Fierer 2012; Fujita, Witte and van Bodegom 2014; Manzoni *et al.* 2014). Denitrification is a widely distributed form of facultative anaerobic respiration that is strongly driven by soil redox and carbon flux (Wallenstein *et al.* 2006; Philippot, Hallin and Schloter 2007). Nitrification is carried out by a more restricted group of organisms and has been shown to be sensitive to variation in community composition but is still strongly driven by soil environmental conditions, especially ammonium availability (De Boer and Kowalchuk 2001; Yao *et al.* 2011). Specific aspects and recent developments of the microbes involved in N cycling are not the focus of our analysis, but have been recently reviewed (Isobe and Ohte 2014). The widely distributed nature of N-cycle processes allows us to examine N-process rates from a range of ecosystem types with different environmental conditions within urban watersheds and to make comparisons with reference ecosystems (i.e. less human-dominated native ecosystems). This approach shows that urban ecosystems are microbially and biogeochemically active despite poor ecological condition in the traditional sense.

APPROACH

We performed an extensive literature search in April 2015 for studies focused on N dynamics in urban ecosystems using Web of Science (https://apps.webofknowledge.com) and Google Scholar (https://scholar.google.com) to identify potential studies to include in this review. We searched iteratively for different nitrogen-transformation processes and urban ecosystem types using various keywords, including 'urban', 'nitrogen', ecosystem type (e.g. 'lawn', 'stream', 'green roof') and nitrogen transformation (e.g. 'denitrification'), and all potential combinations of these keywords (see supporting information for comprehensive list). For green infrastructure, we performed an initial search using the terms 'urban green infrastructure nitrogen'. Based upon this search, we focused primarily on bioretention cells (rain gardens + detention/retention basins/cells) and green roofs after the preliminary search revealed these two engineering options had a large number of N dynamics studies. Based on the literature available, we focused our review on urban forests, lawns, wetlands, riparian zones, streams, bioretention cells (rain gardens + detention/retention basins/cells) and green roofs. For all ecosystem types other than green infrastructure, we focused on process rates of specific N transformations. The majority of green infrastructure studies did not measure/report process rates and we therefore focus on overall N removal (%) measured via changes in concentrations between influent and effluent.

After identifying all potential studies to include in this review, we extracted relevant data from each study. We only included studies that used standard methods (NH_4^+ production for mineralization, NO_3^- production for nitrification, denitrification enzyme activity assays, etc.). We initially attempted to only include studies that measured N dynamics in both urban and reference ecosystems, to allow us to calculate the effect size (see below) for urban land use effects on N dynamics, but many of the studies we found did not include reference ecosystems. Therefore, we performed two separate analyses. First, we compared process rates across urban ecosystem types from all applicable studies. Next, we analyzed the subset of studies that included both urban and reference sites, allowing us to calculate an urban effect size for each individual study. We define a 'reference ecosystem' as one dominated by mature native vegetation with minimal management by humans, and defer to the original study if site classification was unclear. If studies with reference sites used a paired approach, we calculated effect sizes for each pair. However, for studies that included reference sites but not in a paired fashion, we calculated average process rates from reference sites to calculate effect sizes for each urban site. We also note that many studies compared various treatments or environmental factors within an urban setting (e.g. % impervious surface cover in the watershed). We were not able to include the detailed nuances of each specific paper, and therefore categorized each ecosystem as either urban (which includes suburban) or reference based upon the definition provided by the authors of each respective study, but we ignored other factors (e.g. % impervious surface cover). We did not include exurban sites/studies in the analysis.

Certain studies also focused on comparing various levels of management effects on N dynamics. For example, McMillan *et al.* (2014) compared stream N spiraling across varying ages of stream restorations and unrestored streams. Reviewing stream restorations or other management practices nested within different urban ecosystems is beyond the scope of our study. Therefore, we included these management practices within the ecosystem type to which they are related (e.g. restored streams are grouped with other urban streams; constructed wetlands are

grouped with other wetlands). Studies that specifically identified ecosystems as 'green infrastructure', or used terms commonly associated with ecological engineering (e.g. bioretention cell, green roof, rain garden, stormwater control measure), were grouped together for analysis. Overall we found 85 studies representing 358 unique urban ecosystems spanning Canada, China, Estonia, Japan, Korea, Spain, Sweden, the USA and Vietnam.

We extracted data (mean, standard deviation/error, *n*) from each study by reading data directly from tables or in-text reporting; if data were not directly reported, we extracted data from relevant figures using PlotDigitizer (https://plotdigitizer.sourceforge.net), an open-source program that allowed us to digitize and extract data directly from manuscript figures. Where possible, we extracted data on individual sites included in each study, but when data were only reported as averages across sites we used these averages. For certain studies, multiple dates were compared within the same site. For any repeated sampling of the same sites, we averaged all measurements to provide one datum per site due to a lack of independence from repeated sampling of the same site. We also attempted to limit specific studies from skewing our analysis by including only regional (Mulholland *et al.* 2008) or land-use (Zhu *et al.* 2006) averages from two specific studies with large sample sizes.

After extracting data, we assigned each site to an ecosystem type and converted all relevant N-process rates into common units for that specific ecosystem-process combination. Potential ecosystem types included forest, lawn, riparian, stream and wetland for 'natural' ecosystems and bioretention cells and rain gardens for green infrastructure. After converting specific processes to the same units, we calculated an effect size (Cohen's *d*; Cohen 1988) for each measurement with an accompanying reference site as:

$$d = \frac{M_{\text{urb}} - M_{\text{ref}}}{S_{\text{p}}} \tag{1}$$

where M_{urb} and M_{ref} are mean process rates from urban and reference sites, respectively, and S_{p} is the pooled standard deviation. We selected Cohen's *d* as our effect size metric as it is commonly used in meta-analyses, it incorporates both means and variation, which is important for highly variable N-cycling processes, and it can accommodate different signs (+ or –) between urban and reference rates, which was common for certain N-process rates (net N mineralization, net nitrification).

We visualized process rate and effect size results using box plots created in R (version 3.2.3; R Development Core Team 2015). Any points exceeding 1.5× the inner quartile range were excluded from the box plots for visualization purposes, but these points were included in statistical analyses. In addition to visualizing the data using box plots, we statistically tested whether process rates differed across ecosystem types and if effect sizes differed from zero. We first compared process rates across urban ecosystem types using separate one-way ANOVAs for each N-process metric. Response variables were the process rate, and ecosystem type was the factor. We log transformed denitrification and stream spiraling metrics prior to analysis to satisfy ANOVA assumptions. We analyzed whether effect sizes differed from zero for each ecosystem type-process metric combination using Wilcoxon signed-rank test (WSR; non-parametric t-test). For green infrastructure, we used WSR to quantify whether N removal by bioretention cells or green roofs differed from zero, and Kruskal–Wallis rank sum test (non-parametric ANOVA) to test whether N removal differed between bioretention cells and

green roofs. We used non-parametric tests for effect sizes and green infrastructure comparisons because simple transformations of the data were unable to satisfy parametric assumptions.

NITROGEN TRANSFORMATIONS THROUGHOUT URBAN WATERSHEDS

A multitude of N-cycling processes occur in urban and natural environments, and N-cycling microbes appear to be more functionally and taxonomically diverse than previously thought. Novel N-transformation pathways have been recently discovered in a range of environments including environments commonly found in urban areas, such as wastewater sludge and sewage (Isobe and Ohte 2014). Despite a wide range of possible N transformations, the most commonly measured processes are net N mineralization, net nitrification and denitrification. Other processes such as anaerobic ammonium oxidation (anammox) and dissimilatory nitrate reduction to ammonium (DNRA) may be important in certain ecosystems (particularly freshwaters; Burgin and Hamilton 2008), but here we focused on the most commonly measured aspects of the N cycle.

Net N mineralization refers to the conversion of organic N to NH_4^+. Although mineralization is typically measured as the production of NH_4^+ in soils or sediments, it is actually the culmination of multiple physiological processes (Isobe and Ohte 2014). In soils and sediments, N mineralization is driven by microbial degradation of proteins and other N-containing compounds in organic matter produced in either terrestrial or aquatic environments. The microorganisms that degrade these compounds use the carbon (C) to produce biomass or to support metabolism and either take up N to make new proteins (immobilization), or release it to the environment (mineralization). Given the general needs of heterotrophic microbes for C and N, mineralization and immobilization are very widely distributed processes carried out by a wide range of organisms under a wide range of environmental (including anaerobic) conditions. Rates of mineralization have been shown to be controlled by temperature, moisture and pH (Schimel and Bennett 2004; Paul 2007), and the balance between mineralization and immobilization is regulated by the N content of the substrate (Paul 2007).

Nitrification refers to the chemoautotrophic conversion of NH_4^+ to nitrite (NO_2^-), followed by the conversion of NO_2^- to nitrate (NO_3^-). Nitrification is of great interest in urban watersheds due to the susceptibility of NO_3^- to hydrologic loss and the responsiveness of this process to N additions. The NO_3^- produced by nitrifiers also drives denitrification and thus facilities gaseous removal of N from the ecosystem. Variable amounts of nitrous oxide (N_2O), a potent greenhouse gas, are generated during both nitrification and denitrification (Ostrom *et al.* 2010; Beaulieu *et al.* 2011). The factors regulating this N_2O production are complex and include pH, oxygen levels and NO_3^- concentration (Firestone and Davidson 1989). While our focus here is on rates of nitrification, there has been great recent interest in the organisms that carry out nitrification. While much past research has focused on a few genera of bacteria (e.g. *Nitrosomonas* for NH_4^+ oxidation and *Nitrobacter* for NO_2^- oxidation), recent work has shown that there are many other genera of NH_4^+- and NO_2^--oxidizing bacteria, as well as newly discovered NH_4^+-oxidizing archaea (Liu *et al.* 2013).

Denitrification refers to (facultative) anaerobic respiration that results in the conversion of NO_3^- to N_2O and dinitrogen (N_2). The ability to denitrify is widely distributed and includes

both heterotrophic and chemoautotrophic organisms. Denitrification is of great interest in urban watersheds to the extent that wet soils and sediments can become significant sinks for NO_3^- moving across the landscape, ultimately transporting N from terrestrial and aquatic ecosystems to the atmosphere as either N_2O or N_2. Denitrification requires anoxic conditions, and is often limited by the availability of either NO_3^- or labile C (Tiedje *et al.* 1984; Seitzinger *et al.* 2006). A wide range of bacteria and archaea are capable of denitrification, likely due to the presence of a common ancestor that existed prior to the split between the two prokaryotic domains (Knowles 1982; Zumft and Kroneck 2006; Jones *et al.* 2008).

Even though mineralization, nitrification and denitrification are commonly measured, there are a variety of methodological approaches to quantifying these processes. We only included studies that used similar methods and excluded highly specific or novel methodologies, such as isotopic approaches or trace gas emissions. Our analysis is thus dominated by measurements of potential net N mineralization and net nitrification made on mixed soil and sediment samples in short-term (10–30 day) incubations in the laboratory and measurements of denitrification potential based on the short-term (e.g. 90 min), anaerobic laboratory denitrification enzyme activity assay. These methods were commonly used across terrestrial, riparian and aquatic environ-

ments (Fig. 1). We note that the process rates from streams included in Fig. 1 are from within stream habitat-specific studies focused on N-process rates across pools, riffles and debris dams.

Both net N mineralization (Fig. 1a) and nitrification (Fig. 1c) exhibited little variation despite representing a wide range of climatic, geographic and social regions. For net N mineralization, this may reflect the diverse microbial taxa that contribute to mineralization, whereas nitrification is often assumed to be phylogenetically constrained (Isobe and Ohte 2014). However, more taxa of bacteria and archaea may be capable of nitrification than previously thought (Liu *et al.* 2013). Net N mineralization differed across urban ecosystem types (ANOVA; df = 4, 53, F = 3.22, P = 0.02), with urban lawns exhibiting higher net N mineralization than urban streams (Tukey HSD; P < 0.05; Fig. 1a), but no other differences were found across ecosystem types. Effect sizes for net N mineralization were significantly greater than zero for urban forests and riparian zones (WSR; P < 0.05 for both; Fig. 1b), but not for lawns or streams. There were no differences among ecosystem types for net nitrification process rates (ANOVA; df = 4, 59, F = 1.65, P > 0.05; Fig. 1c), but effect sizes for net nitrification were greater than zero for urban forests, riparian zones and streams (WSR; P < 0.05 for each; Fig. 1d).

Although process rate comparisons suggest little difference across urban ecosystem types, positive effect sizes for net N
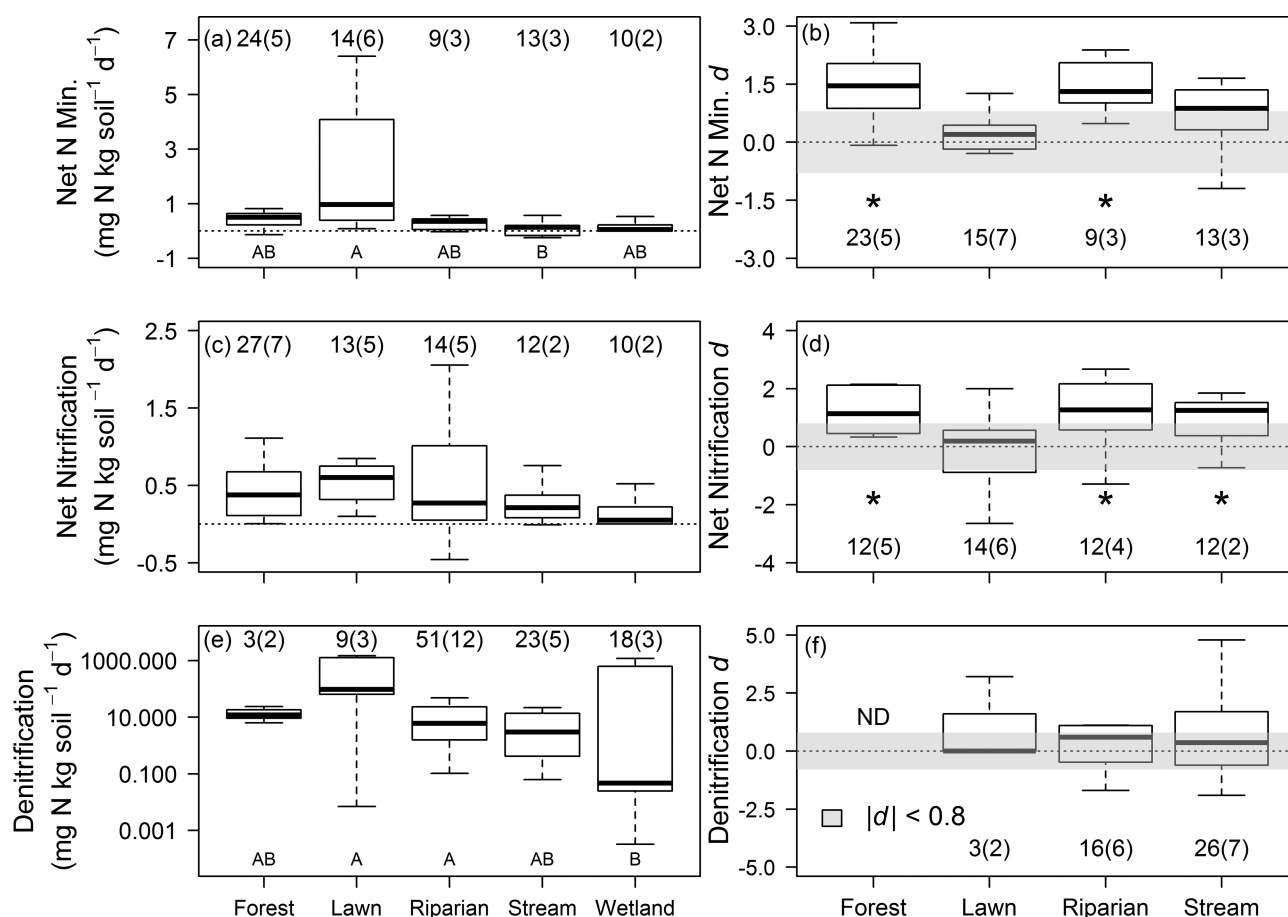

Figure 1. Box plots showing process rates (**a, c, e**) and effect sizes (Cohen's *d*; **b, d, f**) of net nitrogen (N) mineralization (a, b), net nitrification (c, d) and denitrification (e, f) from various urban ecosystem types. Boxes represent the 25th and 75th percentiles, the solid line represents the median and whiskers represent +/− ×1.5 the inner quartile range. Note the logarithmic scale for denitrification process rates (e). Any datum greater than ×1.5 the inner quartile range was excluded from the plots. Numbers above or below each box represent the number of unique sites (and unique citations) used to make each box. The dashed line represents y = 0 for each plot and the shaded region denotes effect sizes <0.8. Effect sizes >0.8 have been interpreted as a large effect (Cohen 1988). Letters below rate comparisons indicate significant differences between urban ecosystem types (Tukey's HSD; P < 0.05). Asterisks denote effect sizes that significantly differ from zero (WSR; P < 0.05). ND = no data.

mineralization and net nitrification suggest that these processes are enhanced in urban environments. In particular, effect sizes > 0.8 have previously been identified as 'large effects' (Cohen 1988). The combination of multiple effect sizes >0.8, and effect sizes differing significantly from zero both support that urbanization increases net N mineralization and net nitrification. Increases in net N mineralization and net nitrification would lead to more N leaving the system, as both processes increase N mobility in the environment. Urban forests and riparian zones had higher rates of N mineralization and nitrification than reference sites (Fig. 1b and d). The riparian zone is a particularly important ecosystem type for N cycling in urban watersheds (Groffman and Crawford 2003), and increased net N mineralization and net nitrification could cause increased N export from urban streams.

While the majority of these studies quantified N transformations on a mass (mgN kg soil^{-1} d^{-1}) basis, a smaller number of studies expressed N cycling on an areal (mgN m^{-2} d^{-1}) basis, and have provided more variable results. For example, N mineralization in urban lawns in Baltimore (123.5 mgN m^{-2} d^{-1}; Raciti *et al.* 2011) was much higher (and of a different direction) than in residential soils in Phoenix (–1.9 mgN m^{-2} d^{-1}; Zhu *et al.* 2006), whereas mineralization in urban forests fell between these two extremes (Zhu, Dillard and Grimm 2004; Enloe *et al.* 2015). Areal nitrification in urban lawns also appears to differ regionally, with Baltimore lawns having higher nitrification than comparable residential soils in Phoenix (Zhu *et al.* 2006; Raciti *et al.* 2011).

In contrast to mineralization and nitrification, denitrification was highly variable both within and across urban ecosystems (Fig. 1 e and f). This variability is unsurprising given that denitrification is a notoriously patchy process, particularly in terrestrial environments (McClain *et al.* 2003; Groffman *et al.* 2006, 2009). It is also possible that larger variability and higher magnitude of denitrification compared to mineralization or nitrification may be due to methodological approaches. In order for denitrification to occur, each of the requisite factors (available NO$_3^-$ and labile C, low oxygen, microbial taxa capable of denitrifying) must occur at the same place and time. The transient nature of these factors leads to hot spots and hot moments of denitrification, especially in urban environments (Groffman *et al.* 2009). The commonly used denitrification-potential method that we included in this analysis alleviates some, if not all, of the limiting factors of denitrification. These highly variable denitrification rates, particularly in lawns and wetlands, demonstrate that management of these environments may allow for enhanced N removal prior to N export from the watershed (Raciti *et al.* 2011). Despite high variability, denitrification was higher in urban lawns and riparian zones than wetlands (ANOVA on log-transformed data; df = 4, 99, F = 3.82, P < 0.05; Fig. 1e), but effect sizes did not differ from zero in lawns, riparian zones or streams (Fig. 1f).

Quantifying and managing urban watershed denitrification is of particular interest as denitrification represents a permanent removal of N. Being an anaerobic process, favorable conditions for denitrification are particularly prevalent in riparian, wetland and stream ecosystems. Urban denitrification could be enhanced by improved management techniques. For example, stream restoration occurs throughout the USA (Bernhardt *et al.* 2005), but the focus in urban streams has been primarily on restoring channel form, protecting infrastructure (e.g. sewage lines, bridges) and increasing social use of streams, rather than attempting to restore biodiversity or other ecological components of streams (Bernhardt and Palmer 2007; Kenney *et al.* 2012). If these restorations enhance stream habitats known to be hot spots for denitrification such as debris dams (Groffman, Dorsey

and Mayer 2005; Waters *et al.* 2014), or improve reach-scale denitrification and reduce N concentrations within streams (Kaushal *et al.* 2008; Sivirichi *et al.* 2011; Newcomer Johnson *et al.* 2014), then N removal may be enhanced. However, the actual efficacy of restorations on enhancing habitat-specific denitrification remains unclear (Tuttle *et al.* 2014). Urban areas also offer opportunities for enhancing connectivity between N sources and sink areas such as constructed wetlands and stormwater detention features (Bettez and Groffman 2012).

URBAN STREAM NITROGEN SPIRALING

Similar to terrestrial ecosystems, urban stream N dynamics are comprised of a multitude of biotic and abiotic transformations, including assimilatory and dissimilatory processes. Assimilation of N by the multitude of primary producers (e.g. algae, macrophytes) and heterotrophic microbial communities typically represents the majority of N processing in stream ecosystems (Mulholland *et al.* 2008). Denitrification and nitrification are also important processes both within the sediment and at the whole-stream scale (Peterson *et al.* 2001; Mulholland *et al.* 2008; Findlay *et al.* 2011), whereas denitrification in the water column may occur but is less ubiquitous across streams (Reisinger *et al.* 2016). Other N transformations may occur within urban streams (e.g. DNRA, anammox; Burgin and Hamilton 2008), but these transformations are understudied and likely important only in specific circumstances.

Nutrient spiraling is a concept used to describe nutrient dynamics at the reach-scale in stream ecosystems. Simply put, the cycling of a nutrient between organic and inorganic phases is extended into a spiral due to the unidirectional flow of stream water (Webster and Patten 1979; Newbold *et al.* 1981). Reach-scale tracer additions of N are performed to understand the cumulative effect of these N transformations on N spiraling (Stream Solute Workshop 1990). Spiraling theory provides three key metrics to represent nutrient dynamics in streams: nutrient uptake length (S$_w$) represents the distance downstream a molecule of nutrient travels in dissolved form before being removed from the water column, nutrient uptake velocity (V$_f$) represents the speed at which a molecule of nutrient is moving towards the process that is taking it up and is generally thought of as a vertical velocity towards the stream bottom where most nutrient uptake occurs and areal nutrient uptake (U) represents the actual amount of nutrient being removed from the water column per unit area of stream bed (Stream Solute Workshop 1990). Uptake length is most commonly reported but is highly influenced by hydrological and biological processes, whereas uptake velocity accounts for differences in stream size and represents the biological demand for a nutrient, and areal uptake is strongly influenced by background nutrient concentrations. The concept of nutrient spiraling has been applied throughout a wide range of streams and rivers, representing a wide variety of watershed land uses (Ensign and Doyle 2006; Tank *et al.* 2008; Hall *et al.* 2013), but N spiraling in urban streams has only been studied for approximately the past decade (Grimm *et al.* 2005; Meyer, Paul and Taulbee 2005).

Nutrient spiraling is typically measured using a tracer addition. This tracer addition can either be a short-term plateau addition in which a concentrated solution of nutrient is added to a stream until a new plateau concentration is formed (Tank, Bernot and Rosi-Marshall 2006), or an isotopic tracer addition that uses a similar approach but a much lower amount of isotopically enriched ^{15}N is added to the stream over a longer

time period than the short-term plateau addition (Tank, Bernot and Rosi-Marshall 2006; Mulholland *et al.* 2008). Both of these approaches allow for the calculation of spiraling metrics, but due to methodological discrepancies we have separated plateau and isotopic approaches in our analysis. We have also separated studies of ammonium (NH_4^+) and NO_3^- spiraling. Typically, NH_4^+ is assumed to be preferred by biological uptake over NO_3^- (Dortch 1990), but NO_3^- is the dominant form of dissolved inorganic N in most urban environments. Due to the differences in biological preference (NH_4^+ preferred over NO_3^-) and environmental abundance (NO_3^- more abundant than NH_4^+), changes in NH_4^+ uptake in urban streams may be more informative for how urbanization is affecting biological processes, whereas changes in NO_3^- uptake are more indicative of how biological processes may affect urban N export.

In urban streams, N-uptake length (S_w) did not differ among N forms (ANOVA on log-transformed data; df = 2, 78, F = 2.52, $P > 0.05$; Fig. 2a), but uptake velocity (V_f) was highest for NH_4^+, and unlabeled NO_3^- also exhibited higher uptake velocity than ^{15}N-NO_3^- (ANOVA on log-transformed data, df = 2, 65, F = 22.13, $P < 0.05$; Tukey's HSD $P < 0.05$ for post-hoc comparisons; Fig. 2c). Preference for NH_4^+ over NO_3^- has been shown repeatedly across land uses for small streams (Tank *et al.* 2008), and is unsurprising given the energetic preference for NH_4^+ over

NO_3^- (Dortch 1990). Areal uptake (U) was higher for unlabeled NO_3^- than ^{15}N-NO_3^-, but NH_4^+ U did not differ from other solutes (ANOVA on log-transformed data, df = 2, 49, F = 6.49, $P < 0.05$; Tukey's HSD $P < 0.05$ for significant post-hoc comparisons; Fig. 2e). Although there are solute-specific differences in N spiraling within urban streams, urban spiraling metrics were similar to reference streams (Fig. 2b, d and f) with no effect sizes significantly differing from zero. Despite the lack of significant differences, the magnitudes of these effect sizes were suggestive of large effects (Cohen 1988). Yet the low sample size ($n = 2$–10, depending upon solute and spiraling metric) led to non-significant effect sizes (Fig. 2b, d and f). Overall, these results suggest a need for more research conducted on urban stream N spiraling.

Although N spiraling metrics between urban and reference streams were more similar than might be expected, the mechanisms driving N uptake in urban streams are necessarily altered by urbanization (Meyer, Paul and Taulbee 2005; Walsh *et al.* 2005). Urbanization alters the hydrology, geomorphology, light, temperature and water chemistry of urban streams (Walsh *et al.* 2005; Wenger *et al.* 2009) and each of these physicochemical factors may differentially affect stream N dynamics. For example, increased light availability in an urban stream coupled with an increase in NO_3^- availability may result in increased N
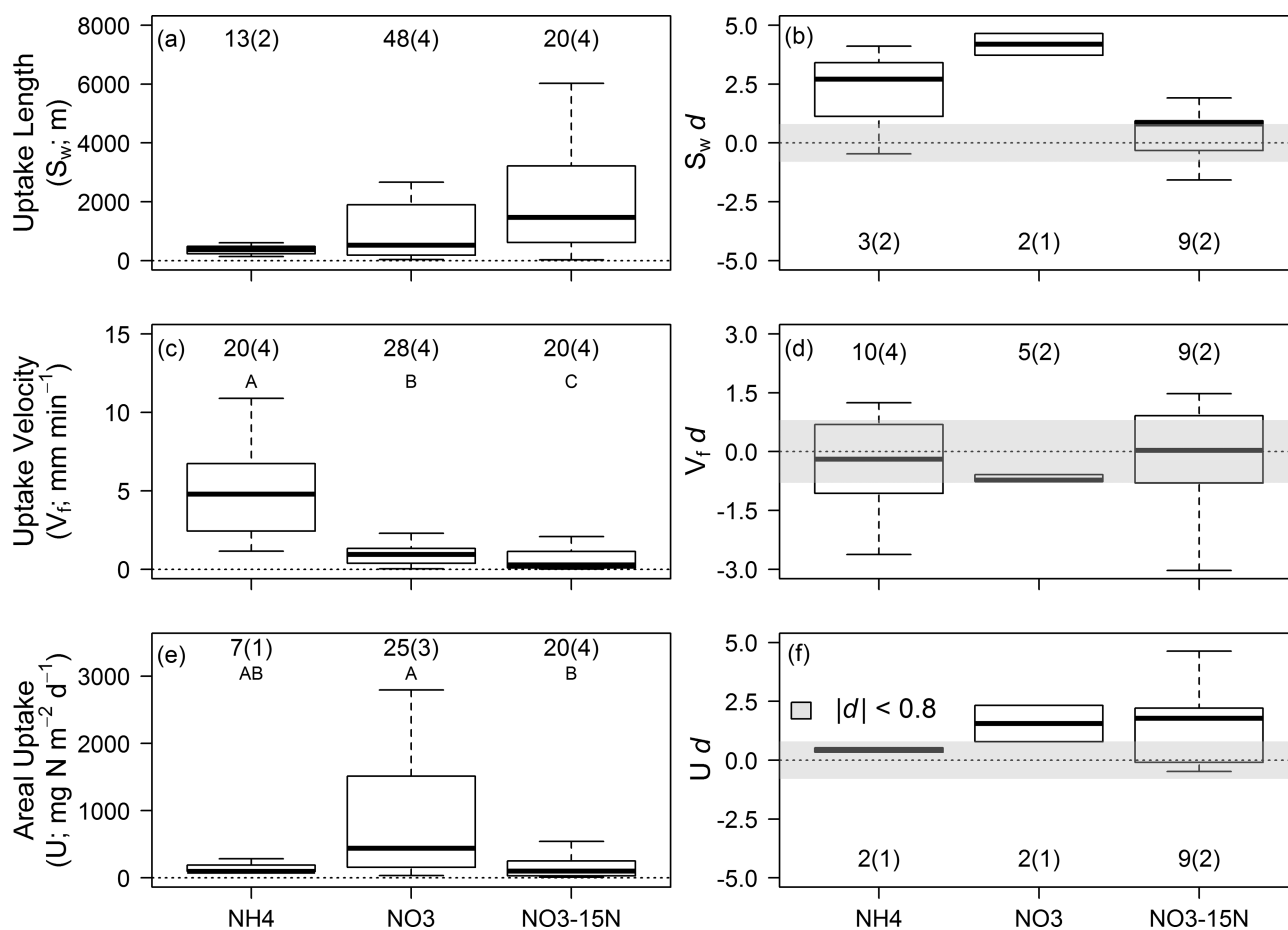
Figure 2. Box plots showing spiraling metrics (**a, c, e**) and effect sizes (Cohen's *d*; **b, d, f**) of nitrogen (N) uptake length (S_w; a, b), uptake velocity (V_f; c, d) and areal uptake (U; e, f) from tracer releases of ammonium (NH_4), nitrate (NO_3) and isotopically enriched nitrate (NO3-15N). Boxes represent the 25th and 75th percentiles, the solid line represents the median and whiskers represent +/− ×1.5 the inner quartile range. Any datum greater than ×1.5 the inner quartile range was excluded from the plots. Numbers above or below each box represent the number of unique sites (and unique citations) used to make each box. The dashed line represents y = 0 for each plot and the shaded region denotes effect sizes <0.8. Effect sizes >0.8 have been interpreted as a large effect (Cohen 1988). Letters above spiraling metric comparisons indicate significant differences between solutes (Tukey's HSD; $P < 0.05$). No effect sizes significantly differed from zero (WSR; $P > 0.05$).

assimilation and areal uptake, but reduced uptake velocity. Future studies of mechanisms controlling urban stream N spiraling, and specific processes (e.g. assimilation or denitrification) will greatly improve our understanding and potential management for altered N export across a range of urban streams (Kaushal and Belt 2012).

Similar to the previous section focused on specific N transformations, the number of N-spiraling studies that included reference ecosystems was relatively low and it is therefore difficult to draw any firm conclusions from the effect sizes. However, a large study of N spiraling across the USA in reference, agricultural and urban streams allows for more direct comparisons to be made (Mulholland *et al.* 2008, 2009; Hall *et al.* 2009). Of the 72 streams studied in eight different ecoregions, agricultural and urban streams had higher areal uptake rates than reference streams, and urban streams exhibited the highest areal denitrification rates based upon the spiraling approach (Mulholland *et al.* 2008). Results from this previous study and the current review suggest that urban streams are biogeochemically active, but this biogeochemical activity does not significantly reduce N export from urban watersheds as N-removal efficiency decreases with increasing concentration (Mulholland *et al.* 2008) and urban watersheds export a large amount of N despite biogeochemical activity throughout the watershed (Bettez *et al.* 2015).

Although urban streams may exhibit similar N uptake in comparison to reference ecosystems, N-spiraling metrics, and N dynamics in urban streams in general, may be enhanced by stream restoration. For example, stream restoration using the natural channel design approach (Rosgen 2007) increased V_f of NO_3^- in urban North Carolina streams relative to unrestored reaches (McMillan *et al.* 2014), and had variable effects on habitat-specific denitrification (Tuttle *et al.* 2014). Another approach to urban stream restoration includes 'daylighting' buried streams (Wild *et al.* 2011). Buried streams have greatly reduced nutrient uptake as shown by increased S_w in Baltimore and Ohio streams (Beaulieu *et al.* 2014; Pennino *et al.* 2014), suggesting daylighting (i.e. uncovering) buried streams has the potential to reduce N export from urban watersheds (Beaulieu *et al.* 2015).

GREEN INFRASTRUCTURE EFFECTS ON NITROGEN EXPORT

In addition to restoration of natural ecosystems, the installation of green infrastructure using novel ecological engineering approaches can affect N export from urban watersheds (Passeport *et al.* 2013; Koch *et al.* 2014). Most of the studies available for green infrastructure effects on N transformations do not measure process rates; rather they measure differences between influent and effluent N (but see Zhu, Dillard and Grimm 2004; Bettez and Groffman 2012). This focus on N retention rather than process rates led us to focus on % N removed by various green infrastructure approaches. Additionally, although there are a wide variety of different green infrastructure approaches currently employed, we focused primarily on bioretention cells and green roofs for our analysis due to data availability (Fig. 3). We note
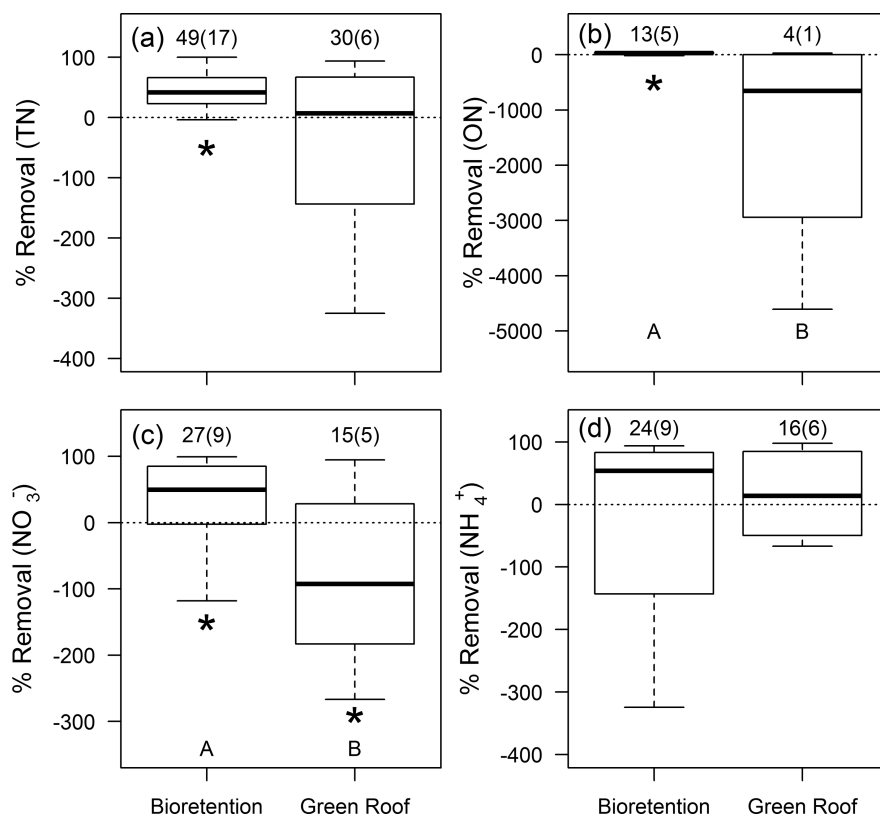
Figure 3. Box plots showing % removal of total nitrogen (TN; **a**), organic nitrogen (ON; **b**), nitrate (NO_3^-; **c**) and ammonium (NH_4^+; **d**) from bioretention cells (bioretention) and green roofs (green roof). Boxes represent the 25th and 75th percentiles, the solid line represents the median and whiskers represent +/– ×1.5 the inner quartile range. Any datum greater than ×1.5 the inner quartile range was excluded from the plots. Numbers above each box represent the number of unique sites (and unique citations) used to make each box. The dashed line represents $y = 0$ for each plot. Letters below boxes denote significant differences between bioretention cells and green roofs (Kruskal–Wallis rank sum; $P < 0.05$). Asterisks below boxes denote % removal significantly differing from zero (WSR; $P < 0.05$). Positive values indicate net removal of N, whereas negative values indicate net production of N.

that we included data in previous sections from other ecological engineering approaches, such as stream restoration or riparian buffer installation; these approaches, and green infrastructure in general, are covered more thoroughly in previous reviews (e.g. Dietz 2007; Passeport *et al.* 2013; Koch *et al.* 2014).

Bioretention cells and green roofs are designed primarily to retain stormwater (Dietz 2007), reducing the hydrologic flashiness commonly associated with the urban stream syndrome (Walsh *et al.* 2005). Improvements in the quality of water draining green infrastructure are often thought of as an added benefit of these best management practices. Therefore, even if N concentrations are higher in effluent draining green infrastructure relative to the influent, the reduction in total runoff and buffering of the timing of runoff can both reduce the mass of N moving through urban watersheds and can improve N retention in receiving ecosystems by increasing residence time of water and N. Of the studies we analyzed, bioretention cells exhibited greater removal of organic N (ON; Kruskal–Wallis rank sum test; df = 1, chi-squared = 4.15, $P < 0.05$) and NO_3^- (Kruskal–Wallis rank sum test; df = 1, chi-squared = 6.57, $P < 0.05$) than green roofs. Overall, bioretention cells tended to be a net sink for N (e.g. outflow N concentration < inflow N concentration), with % removal of TN, ON and NO_3^- all being significantly higher than zero (WSR; $P < 0.05$ for each; Fig. 3a–c). Retention of NH_4^+ in bioretention cells, however, did not differ from zero ($P > 0.05$). In contrast to bioretention cells, green roofs appear equally likely to be sources or sinks of TN and NH_4^+ (Fig. 3a and d), and were sources of ON and NO_3^- (Fig. 3b and c).

Due to the performance of both bioretention cells and green roofs for stormwater retention (Dietz 2007), any increases in N concentration may be due to a reduction in water export rather than an increase in N mass, therefore positive % removal based upon concentration changes is strong evidence for a reduction in N export. A formal evaluation of different bioretention cell and green roof designs is beyond the scope of this review and has been performed elsewhere (e.g. Berndtsson 2010; Passeport *et al.* 2013; Buffam and Mitchell 2015), but the combination of reduced stormwater export (Dietz 2007) and the potential for reducing outflow N concentrations under certain scenarios suggests that both of these techniques can reduce N export in urban watersheds.

While most green infrastructure studies we analyzed were focused on mass balance approaches, Bettez and Groffman (2012) found that various types of riparian stormwater control measures in Baltimore, MD, exhibited higher potential denitrification than riparian areas in reference or urban ecosystems. In contrast, Zhu, Dillard and Grimm (2004) found no differences in net N mineralization, net nitrification or denitrification between retention basins and non-retention city parks in Phoenix, AZ. To more accurately understand N cycling and the ultimate fate of N retained in green infrastructure, studies focused on specific N-process rates within green infrastructure are needed.

The utilization of multiple ecological engineering techniques, coupled with the biogeochemical activity of urban forests, lawns and streams, shows that urban watersheds are capable of reducing N export. Unfortunately, these ecosystems are not able to keep up with N inputs coming from multiple different natural and anthropogenic sources. The installation of low-impact development strategies (permeable pavement, rain gardens, detention ponds, etc.) throughout an entire watershed can minimize stormwater runoff and pollutant export traditionally accompanying urbanization while still allowing for residential development (Dietz and Clausen 2008). Indeed, an experimental low-impact development watershed exhibited reduced N mass export relative to traditional development due to decreases in stormwater runoff (despite increases in runoff concentration; Bedan and Clausen 2009). Although the application of these green infrastructure approaches is expensive, particularly at the whole-watershed scale, the technologies do exist to reduce N export from urban watersheds. These new technologies are difficult to apply to areas already urbanized, and retrofitting urban ecosystems with other green infrastructure alternatives (e.g. Sansalone *et al.* 2013) is possible but unfortunately is expensive. However, traditional 'grey' infrastructure approaches are also difficult and expensive to implement. Thorough cost–benefit analyses that include non-economic benefits (Vineyard *et al.* 2015) such as enhanced N cycling are needed to evaluate the economics of green and grey infrastructure.

CONCLUSIONS

Urban watersheds are comprised of a wide variety of ecosystems, from forests and lawns to streams and green roofs. These various ecosystem types are capable of processing N and other contaminants at rates similar to, or higher than reference ecosystems (Figs 1 and 2). Indeed, we did not find a single process that was significantly lower in urban ecosystems relative to reference ecosystems. Net N mineralization and net nitrification were similar across a range of ecosystem types and a wide geographic range, but certain other N-cycling processes (e.g. denitrification) exhibit high variability. Variability in biogeochemical processes does not discount the urban homogenization theory, as this variability is unsurprising given the 'hot spots and hot moments' known to be associated with denitrification and other biogeochemical processes within and across ecosystems (McClain *et al.* 2003; Groffman *et al.* 2009).

While urban ecosystems are biogeochemically active environments, the process rates are outpaced by human alterations to N inputs and other anthropogenic changes (Bettez *et al.* 2015). Implementing low-impact development techniques may allow for development without increasing N export (Dietz and Clausen 2008; Bedan and Clausen 2009). Although these techniques are expensive and difficult to apply as retrofits to already urbanized areas, environmental benefits may outweigh costs. Although the economics of low-impact development need to be considered more fully, these technologies have the potential to reduce N export from urban watersheds. Focusing efforts on optimizing N-cycling processes in environments with large potential impacts (e.g. urban lawns, buried streams, green infrastructure) while minimizing economic costs must be a priority for scientists, managers and policy makers. In order to improve these efforts, an increased focus on urban ecology and biogeochemistry research is needed. Ultimately, reducing N export from urban ecosystems will require both controlling the amounts and sources of N inputs into urban watersheds and optimizing N removal in both natural and novel ecosystems within urban environments.

SUPPLEMENTARY DATA

Supplementary data are available at FEMSEC online.

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