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High Potential Nitrate Removal by Urban Accidental Wetlands in a Desert City: Limitations and Spatiotemporal Patterns

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Abstract

Urban areas are typically considered to be net exporters of reactive nitrogen. As a result, much effort has gone into creating or restoring areas supporting microbial denitrification, which permanently removes nitrate from urban ecosystems. However, denitrification is a facultative process, with complex spatiotemporal drivers and limitations, making it difficult to predict where or when denitrification will occur. This is particularly true in urban systems, where drivers and limitations can differ greatly from those of native systems. In this study, we examine novel urban ecosystems in a unique geographic setting, investigating limitations and spatiotemporal drivers of denitrification in accidental wetlands (AW) located in a desert city (Phoenix, AZ). These wetlands were unintentionally created by runoff generated in Phoenix and exiting storm pipes into a dry riverbed. Previous work in native, nonurban Arizona wetlands (NW) found that monsoon floods and plant patches are important spatiotemporal drivers of denitrification. While we found that AW had high potential to process nitrate, denitrification patterns in AW exhibit different drivers from NW. As predicted, denitrification potential in AW was greater under plant patches, but surprisingly, this was not only due to the plants alleviating carbon limitation as both vegetated and unvegetated patches were not carbon limited. Contrary to predictions, monsoon floods did not increase denitrification potential, and perennially inundated AW had the highest denitrification potential, suggesting less temporal variation in denitrification in AW than in NW. Together, these findings offer novel insights into the complex interactions shaping spatiotemporal patterns of nitrate processing in arid urban regions.

Key words: Denitrification; Urban; Accidental wetlands; Desert; Baseflow; Nitrate; Monsoon.

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Author's Contribution AKS conceived and designed the study, performed the research, analyzed the data, and led the writing of the manuscript. MMP and DLC provided inputs on study design and provided edits for the manuscript. JCS provided edits for the manuscript.

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Highlights

- Accidental wetlands are understudied systems that reduce nitrate via denitrification.
- Inundation patterns, rather than plant patches, affect what limits denitrification.
- Drivers of denitrification in accidental wetlands differed from native wetlands.

Introduction

Urban areas are a common source of anthropogenically derived nitrate (NO_3^-) to downstream ecosystems due to inputs from atmospheric deposition, fertilizer use, and municipal effluent discharges (Baker and others 2001; Kaushal and others 2011; Hale and others 2014a). In high concentrations, NO_3^- can promote ecologically devastating algal blooms and can be harmful to human health (Vitousek and others 1997; Sinha and others 2017). Consequently, much research has been devoted to understanding how to mitigate NO_3^- export from cities, often using combinations of engineered green infrastructure and remnant ecosystems that promote ecological processes that reduce NO_3^- concentrations (Passeport and others 2012; Koch and others 2014); one such process is microbial denitrification, an anaerobic form of metabolism where a diverse group of microorganisms can convert NO_3^- to inert N_2 gas. An understudied feature of urban landscapes that could also reduce NO_3^- concentrations via denitrification is *accidental urban wetlands*. Accidental urban wetlands are neither remnant wetlands in urban landscapes, nor are they constructed or engineered. Rather, they are wetlands that result from human activities but are not designed nor managed for any specific purpose and are often relatively unnoticed (Palta and others 2017). In this study, we examined accidental urban wetlands that have formed at storm drain outfalls in a dry riverbed in Phoenix, Arizona, to uncover potentially important drivers of spatiotemporal denitrification patterns (plant patches and monsoon floods).

At the most basic level, denitrification requires three conditions: suboxic (< 0.2 mg L^{-1} O_2) soils, which is promoted by inundation, and high concentrations of both NO_3^- and labile carbon (Seitzinger and others 2006). In theory, spatiotemporal patterns of high denitrification rates are associated with locations or times where these three conditions co-occur (McClain and others 2003). However, because denitrification is a facultative process, the environmental drivers of denitrification can vary greatly depending on setting, making rates and limitations difficult to predict (Groffman and others 2009). The urban environment can further alter environmental drivers of denitrification as both hydrology and resources (NO_3^- and labile carbon) are augmented relative to the surrounding native environment (Ehrenfeld 2000; Paul and Meyer 2001). However, the magnitude and direction of change are not the same for all urban environments and are affected by choices in urban infrastructure and city-specific policies, as well as geographic and climatic setting (Hopkins and others 2015; Hale and others 2016). For example, the inclusion of green infrastructure can alter resource inputs and reduce flashy hydrographs often associated with urbanization, thus altering inundation patterns of urban wetlands (Hale and others 2014b; Johnson and others 2014). In addition, fertilizer inputs via runoff and fossil fuel combustion inputs of NO_3^- to urban wetlands are often much higher than in native wetlands, and labile carbon inputs can change due to changes in plant community composition in urban watersheds and wetlands (Ehrenfeld 2003; White and Stromberg 2011; Newcomer and others 2012; Hale and others 2014a). However, local (city and/or county) policies such as fertilizer bans, regional patterns of nitrogen deposition, and climatic constraints on plant communities all make generalization of the effects of urbanization on and predicting patterns of denitrification challenging.

In desert systems, which are water and resource poor, patterns of denitrification are heavily tied to the spatiotemporal characteristics of water and resource (NO_3^-, labile carbon) availability. Notably, however, desert cities are associated with an increase in surface waters relative to native areas, while temperate cities are associated with a decrease (Steele and Heffernan 2013), which has implications for how the desert urban environment may affect patterns of denitrification relative to temperate city. For example, temperate urban riparian wetlands can become disconnected from groundwater sources due to increased stream flow velocity and subsequent channel incision; the result is drier wetlands with lower denitrification rates compared to native riparian wetlands in the same region (Groffman and others 2002). However, in drier regions, where wetland inundation is often seasonal rather than perennial, the inclusion of ''dry weather'' urban runoff and wastewater inputs (also called ''urban baseflow'') to urban wetlands can decouple inundation from seasonal precipitation patterns (Stromberg and others 2007;

Bateman and others 2015). The result is more frequent inundation and potentially higher denitrification rates compared to native desert systems. These contextual and structural differences between urban wetlands and native wetlands may create a radical difference in what drives denitrification within a particular geographic region, depending on whether the wetlands are located inside or outside of an urban area.

Accidental wetlands have been little studied, and the drivers and rates of functions they support are little understood. However, because they are not directly designed or managed for desirable ecosystem functions, accidental urban wetlands offer a unique opportunity for examining how urban infrastructure and activities may change the nature of ecosystem function regulation. Actions such as planting native species or controlling inundation regimes in restored floodplains are two examples of how drivers of ecosystem processes are being directly managed by people. Such management actions are taken to both to promote particular ecosystem processes and to counteract negative effects of the built environment on said processes (Zhu and others 2005; Hernandez and Mitsch 2007; Roach and Grimm 2011). In contrast, accidental urban wetlands are recipients and integrators of many decisions made in an urban watershed that can affect everything from hydroperiod to species assembly to water quality (Ehrenfeld 2000). Further, accidental wetlands are typically younger and in an earlier successional stage than urban remnant wetlands, because unlike remnant wetlands they do not predate human presence and activities in the landscape (Palta and others 2017). Thus, accidental urban wetlands allow for an examination of the combined effects of both human-induced and nonhuman, geographic drivers on ecosystem processes.

Previous studies in native desert riverine wetlands have found two important drivers of denitrification patterns: (1) Plant patches, which supply the carbon necessary for denitrification, and (2) monsoon floods, which provide a temporal delivery of water, carbon, and nitrogen (Schade and others 2001; Harms and others 2009; Harms and Grimm 2010; Heffernan and Fisher 2012). For this study, we used accidental wetlands in a desert city to examine whether these nonhuman, geographic drivers of denitrification patterns in wetlands remained important in an urban context. Because these accidental wetlands are located on a formerly dry riverbed and have a largely unidirectional flow of water, from upstream (pipe outfall) to downstream, these wetlands were compared to riverine wetlands studied in undeveloped watersheds in Arizona (hereafter ''native desert wetlands''). Both native and urban wetland systems have reaches that are both ephemerally and perennially flooded. Notably, however, these systems differ in groundwater inputs as native desert wetlands have reaches with gaining groundwater hydrology (Harms and others 2009), whereas the accidental urban wetlands in this study have only surface water inputs from urban baseflow or precipitation.

The presence of plants has been shown to typically increase denitrification rates (Alldred and Baines 2016) through a variety of mechanisms such as changes in soil carbon (Hume and others 2002; Zhai and others 2013), nitrogen (Windham and Ehrenfeld 2003), and oxygen concentrations (Armstrong and Armstrong 1990). Plants may also physically alter the soil environment to be more favorable to denitrification, as roots can penetrate compact soils, increasing infiltration of substrates from surface waters into sediments; roots also trap fine sediments more favorable to microbial colonization (Angers and Caron 1998). In addition, species of plants differ in their specific physiological and anatomical characteristics, thus differentially altering the soil environment and ultimately denitrification (Windham and Ehrenfeld 2003). In resource-poor desert systems, plant patches are an important spatial organizer of where carbon and nutrients accumulate (Schlesinger and others 1996); the few studies of the mechanistic relationship between plants and denitrification in native desert wetlands show that plants alleviate carbon limitation for denitrifiers by providing organic carbon via both litter deposition and root exudates (Schade and others 2001; Heffernan and Fisher 2012). However, urban ecosystems have additional inputs of carbon and NO_3^- that differ from nonurban areas, subsequently altering patterns of resource availability and resource limitations (Hall and others 2009; Newcomer and others 2012). Thus, the importance of plant patches as a driver of spatial patterns in denitrification in urban accidental wetlands may be reduced.

Rainfall occurs during two distinct seasons in the Southwestern United States: summer monsoon and winter frontal seasons. The effects of winter frontal rains on denitrification have been little studied, but lower temperatures during this season may result in lower denitrification rates than other seasons (Stanford and others 1975). Monsoon floods, however, have been shown to affect patterns of denitrification both temporally and spatially. Specifically, monsoon floods seasonally inundate wetlands that are dry for the majority of the year, and deliver necessary carbon or NO_3^- to perennially flooded wetlands, while also spatially homogenizing the patch pattern of denitrification by both delivering allochthonous resources to wetlands and by scouring and redistributing autochthonous resources among wetland plant patches (Harms and Grimm 2008, 2010; Harms and others 2009). The result of these temporal and spatial influences is a seasonal increase in denitrification and a spatial homogenization (that is, a reduction in variability) of soil resources and denitrification after monsoon floods (Harms and others 2009; Harms and Grimm 2010).

In desert urban areas, river regulation and stormwater management infrastructure, such as retention basins, typically decrease the magnitude of monsoon floods, potentially minimizing the scouring effect that monsoon floods have on soil resources (Hale and others 2014b). Thus, a reduction in flood magnitude could result in less spatial homogenization of soil resources after monsoon floods in accidental wetlands. In addition, urban baseflows are a source of nutrient- and carbon-enriched water to accidental wetlands (Palta and others 2017). Where wetland systems are receiving continual inputs of carbon or NO_3^- via urban baseflow (that is, in perennially inundated systems), we might expect to see no seasonal increase in denitrification after monsoon floods.

A defining characteristic of native desert wetlands is a lack of water permanence, and similarly, accidental urban wetlands can experience frequent changes in inundation, since runoff from urban watersheds is a result of human decisions and activities. Generally, greater denitrification is associated with locations experiencing longer inundation periods or during times of flooding (Baker and Vervier 2004; Harms and others 2009; Roach and Grimm 2011). This is due not only to suboxic soil conditions, but also to water delivering the resources necessary for denitrification as discussed above. In accidental urban wetlands, wetlands with shorter inundation periods (that is, drier wetlands) likely have lower exogenous inputs of resources (carbon and NO_3^-) due to a lack of urban baseflow and lower soil moisture (and associated soil anoxia) compared to permanently inundated wetlands. As a result, plant patches may act as ''islands of fertility'' and thus would be a more significant spatial organizer of denitrification patterns (Schlesinger and others 1996) and monsoon floods an important temporal driver of denitrification, in drier wetlands compared to wetlands that are permanently inundated.

The objective of this study was to test the assumption that common drivers of spatial and temporal patterns of denitrification in native desert wetlands also drive denitrification patterns in accidental wetlands in a desert city. If we assume the drivers of denitrification are the same between native and accidental wetlands, then we can make the following specific predictions about accidental wetlands:

- (P1) Potential denitrification will be (a) greater under plant patches compared to unvegetated patches, and (b) this increase will be due to an alleviation of carbon limitation observed in unvegetated patches.
- (P2) Potential denitrification will vary by plant patch type.
- (P3) Potential denitrification will be highest after seasonal monsoon flooding.
- (P4) Seasonal monsoon flooding will homogenize (that is, reduce variability) potential denitrification and soil resources among patches.
- (P5) Drier wetlands will be (a) more spatially heterogenous with respect to soil resources and denitrification, and (b) the effect of plant patches and monsoon floods on denitrification will be proportionally greater in these drier wetlands.

Although seasonal and spatial differences in water temperature, water inputs, plant productivity, and soil characteristics exist in desert wetlands, few studies have examined how these patterns interact to influence denitrification. Fewer, if any, studies examined how drivers of denitrification patterns in desert wetlands may be altered within an urban context, where temperature, hydrology, and plant communities differ greatly from native ecosystems. We utilized field and laboratory studies to examine the effects of season, hydrology, plant presence/absence, and plant community composition on potential denitrification rates and drivers.

Methods

Study Area

This study was conducted along a 30 km reach of the Salt River in the Phoenix metropolitan area, AZ, USA (Figure 1). The Salt River is a historically perennial river that has been mostly dry as it bisects the Phoenix metropolitan area since 1938, when the last of seven upstream dams was completed. The floodplain has been highly modified for flood management. Dozens of storm drains discharge urban runoff into the riverbed during storms. However, during dry periods, relatively continuous urban baseflow is conveyed through a subset of

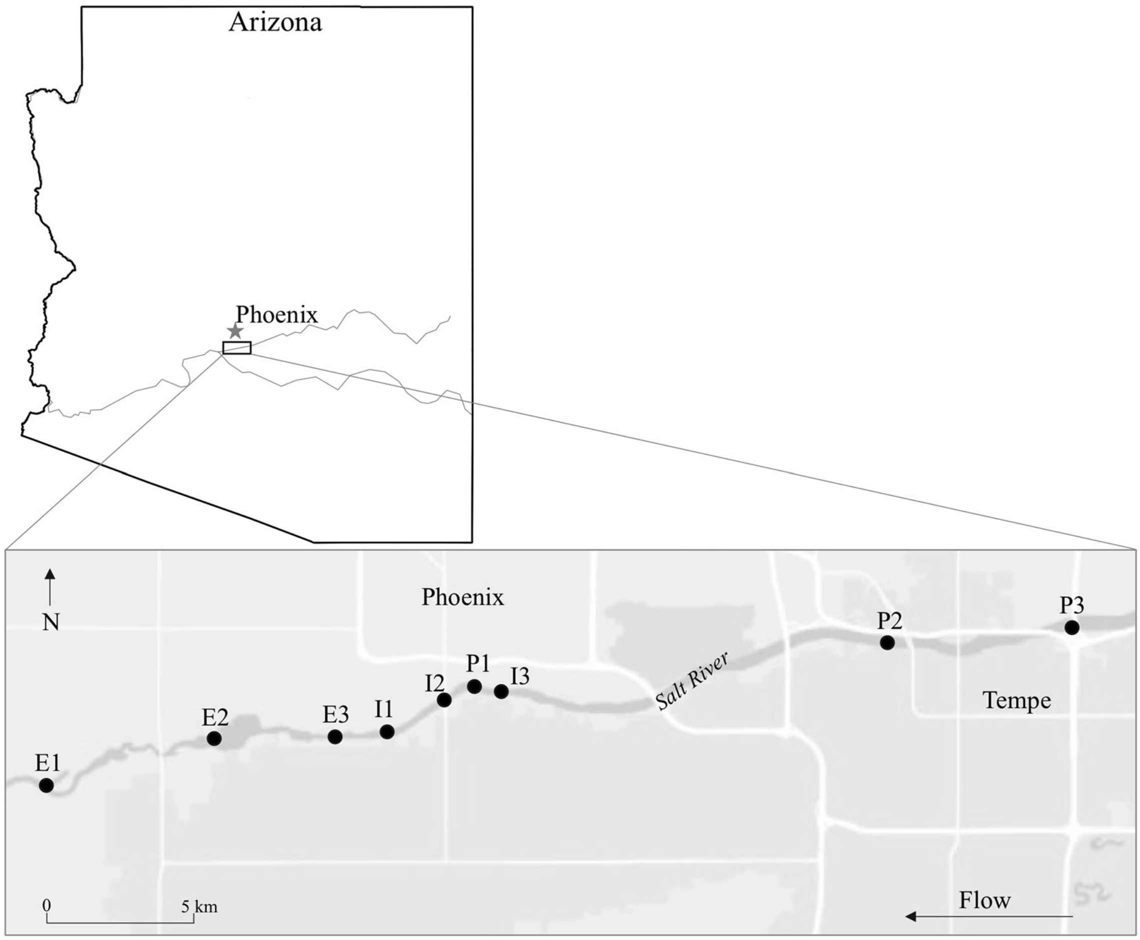

Figure 1. Map of field sites. E = ephemeral, I = intermittent, P = perennial wetlands.

these drains. The exact source of the baseflow is unknown but does not include treated wastewater, which is transported through a separate pipe system and discharged to groundwater or agricultural fields outside Phoenix. Rather, it is likely a mix of various other sources that may include runoff from flood irrigation of lawns, or other activities such as car washing or pool maintenance. We have observed elevated levels of NO_3^- in the baseflow relative to native desert wetlands and variability in water chemistry among the drains (M. Palta and A. Suchy, unpublished data). Wetlands have formed at many of these drains and vary in size and vegetation composition based on the quantity of urban baseflow each drain supplies (White and Stromberg 2011; Bateman and others 2015). The result is a patchwork of wet and dry locations along the bed of the Salt River that flood and may become connected by flow during large rain events. We sampled nine of these accidental wetlands that differed in the timing, frequency, and volume of their storm drain discharges. The wetlands were grouped into three different inundation patterns (hereafter, ''wetland type''): ephemeral, intermittent, and perennial. Ephemeral wetlands received minimal urban baseflow and flooded largely in response to precipitation; they were inundated for 10–30% of the year. Intermittent wetlands received urban base flow during dry periods and were inundated for 50–85% of the year. Perennial wetlands received enough continuous urban base flow to remain inundated for more than 90% of the year.

Phoenix is in an arid climate zone and receives an average of 19 cm of rain annually divided between two seasons: the summer monsoon season and the winter frontal season (www.wrcc.dri.edu). On average, half of the rain falls during the monsoon season, which runs from mid-June to mid-September. Precipitation during this period typically occurs as intense, localized events that result in flashy urban runoff, and sometimes substantial floods. The winter frontal season runs from November to April. Winter rains are the result of Pacific frontal storms that produce more gentle, sustained rains, usually resulting in less intense flooding.

Each wetland type had different seasonal inundation patterns. Ephemeral wetlands did not remain inundated for the entire monsoon or winter rainy season, but rather had shorter inundation responses to individual rain events (Figure S1). Intermittent wetlands had the most variable inundation pattern but were typically inundated during the pre-monsoon season and drier during the winter rainy season, with variable inundation patterns during the monsoon season (Figure S1). Perennial wetlands were generally inundated in all seasons (Figure S1).

Sampling Design

In each study wetland, we identified two to four dominant plant patch types including one patch without vegetation designated as ''open'' (Table S1). We had a total of seven different patch types among all nine wetlands: open, *Amaranthus* spp. (AMSP), grass (various spp.), *Ludwigia peploides* (LUPE, floating primrose-willow), *Rumex dentatus* (RUDE, toothed dock), *Tribulus terrestris* (TRTE, puncture vine), and *Typha* spp. (TYSP). Grass patches were either *Cynodon dactylon* (Bermuda grass), *Paspalum distichum* (knotgrass) or *Schismus* sp. (Mediterranean grass). Patches designated *Amaranthus* spp. were *Amaranthus palmeri* (carelessweed), *Amaranthus albus* (prostrate pigweed), or a mix of both. Patches designated *Typha* spp. were either *Typha domingensis* (southern cattail), *Typha latifolia* (broadleaf cattail), or a mix of both. Wetlands were sampled three times between June 2013 and March 2014 to capture differences in seasonal precipitation and urban runoff that affected wetland inundation. The pre-monsoon sampling was conducted in June 2013, the post-monsoon sampling period was conducted in October 2013, and winter sampling was conducted in February and March 2014. During winter sampling period, only six of the nine sites were sampled due to unforeseen sampling constraints (Table S1).

Inundation Measurements

We estimated wetland inundation using iButton® temperature sensors placed in waterproof casings and deployed on the soil surface to record temperature every hour (Maximum Integrated Products). The presence or absence of water in each wetland was estimated by manually comparing the temperature record from iButtons® to the temperature record from local weather sensors (http://www.fcd.maricopa.gov/Weather/Rainfall/ALERT/ssdata.aspx). Periods with dampened daily temperature oscillations indicated wetland inundation (Palta and others 2012). Those periods were compared to air temperature records to determine whether the dampened temperature oscillations were due to inundation or to changes in air temperature. The iButton® data interpretations were further verified with field observations of inundation.

Soils

We collected two soil cores from each patch during pre-monsoon sampling period, and we collected four soil cores from each patch during the post-monsoon and winter sampling period ($N = 246$). Soil cores of the same patch types were taken from distinct patches when they existed. If a site had only a single large contiguous plant patch, cores were taken at least 5 meters apart. Soil cores were taken to a depth of 10 cm to include the most microbially active soil layer (Groffman and others 1999). Cores were stored on ice in the field and then stored at 4°C in the laboratory until processing, typically within 24 h.

Soil cores were homogenized and analyzed for moisture content, organic matter, NO_3^-concentrations, texture, and potential denitrification rates. Soil moisture was determined gravimetrically by drying soils for 48 h at 105°C. Soil organic matter was determined by mass loss on ignition for 4 h at 550°C. Soil NO_3^- was extracted by shaking 10 g of sample with 50 mL 2 M KCl for 1 h and then filtering through pre-leached Whatman 42 ashless filters. Extracts were collected and frozen until analyzed colorimetrically on a Lachat QC8000 flow injection analyzer. Forty grams of 2 mm sieved soils was shaken overnight in 100 mL of a sodium hexametaphosphate solution, and percent sand, silt, and clay were determined using the hydrometer method (Robertson and others 1999). Soils samples with greater than 10% organic matter were processed to remove organic matter using the hydrogen peroxide extraction method before determining soil texture (Robertson and others 1999).

Denitrification Measurements

Denitrification potential rates (DNP) were measured using denitrification enzyme assays (Groffman and others 1999). Fifty grams of soil was placed into 125 ml Wheaton bottles, and 50 ml of one of the following media was added. To measure DNP rates (conditions in which no factor is limiting denitrification), we added media amended with

NO_3^- (100 mg NO_3^-—N kg soil^{-1} as KNO_3) and carbon (40 mg glucose-C kg soil^{-1} as glucose) to the samples (Groffman and others 1999; Roach and Grimm 2011). To measure limitation effects of carbon and NO_3^-, samples were amended with only glucose (hereafter ''C''), only NO_3^-, both glucose and NO_3^- (hereafter ''C + NO_3^-''), or received neither (that is, distilled water only, hereafter ''DI''). Headspace of samples was evacuated and then purged with N_2 gas five times to create anaerobic conditions, and 10 ml of acetylene gas was added to block the reduction of N_2O to N_2 (Groffman and others 1999). Samples were incubated at approximately 20°C and shaken at 140 rpm for 4 h. Gas samples were collected at 30 min and 4 h and analyzed on a Varian 3800 gas chromatograph for N_2O concentration.

Statistical Analyses

All tests were run using Stata 14 (StataCorp 2015). Statistical tests were run separately for each wetland type because data were highly skewed due to low DNP rates in ephemeral wetlands, and assumptions of normality and equal variance could not be met with grouped data. The effect of wetland type on DNP was examined using a nonparametric Kruskal–Wallis H test. Pairwise comparisons between wetland types were made using a Bonferroni-corrected Mann–Whitney U test.

To determine whether the presence of plant patches affected DNP (P1a), we conducted an independent *t* test comparing vegetated patches (all species together) with open patches (no vegetation) for each wetland type. To adhere to assumptions of normality and equal variance, data were log-transformed for only intermittent and perennial wetland types. Data for ephemeral wetlands adhered to assumptions of normality and equal variance without transformation.

To determine whether plant patches alleviated carbon limitation on denitrification (P1b), we conducted a Kruskal–Wallis H test for patches with and without vegetation within each wetland. Post hoc analysis was completed using Dunn's test to compare between limitation treatments (DI, C, NO_3^-, C + NO_3^-).

We used a two-way ANOVA to simultaneously compare the main effects of (a) season and (b) plant patch type, as well as potential for an interaction between season and plant patch type, on DNP (P2 and P3). A separate two-way ANOVA was run for each wetland type. Data for intermittent and perennial wetland types were log-transformed so model residuals would conform to assumptions of normality and equal variance. Tukey's HSD post hoc tests were used to further determine pairwise significance levels when necessary. If the interaction between season and plant patch type was not significant, it was not included in the reported model.

We used coefficients of variation (CVs; standard deviation/mean × 100) to assess spatial variability of soil characteristics (DNP, soil organic matter, soil texture, and soil extractable NO_3^-) within each wetland site (P4 and P5a). The CVs for each of the three wetlands in each wetland type were averaged for an overall CV of the wetland type. A CV higher than 100% indicated high spatial variability (Harms and others 2009). To determine whether monsoon floods homogenized DNP and soil resources (P4), we conducted a paired t test using pre- and post-monsoon CVs as the dependent variable.

The effect sizes of season and plant patches on DNP were also calculated to examine whether the magnitude of effect differed among wetland types (P5b). We calculated effect sizes of pre- versus post-monsoon season and unvegetated versus vegetated patches for each wetland type. Effect sizes were calculated using Hedge's g to account for different sample sizes among groups.

Results

Plant Presence/Absence and Nutrient Limitations (P1)

Results of the t test comparing DNP in unvegetated and vegetated patches in each wetland type

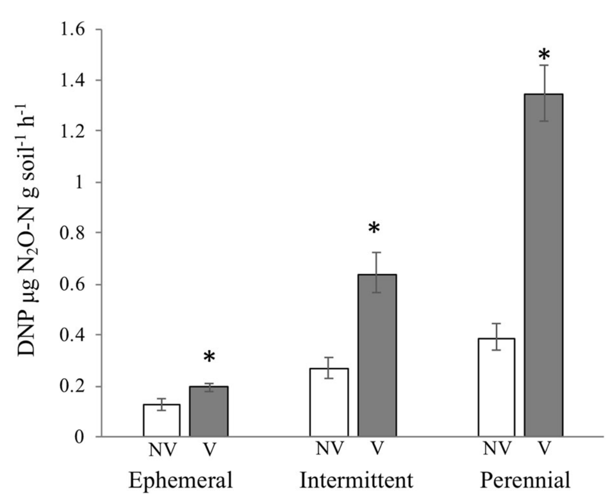

Figure 2. Effect of vegetation on DNP for each wetland type. NV = patches with no vegetation; V = patches with vegetation. Asterisks denote significant differences within a wetland type at $P < 0.05$. Error bars indicate ± 1 SE.

showed that DNP was significantly greater ($P < 0.05$) when vegetation was present than when it was absent for all wetland types (Figure 2; ephemeral, $t = 2.42$, df = 70; intermittent, $t = 4.06$, df = 88; perennial, $t = 6.45$, df = 82).

Kruskal–Wallis H test found significant ($P < 0.05$) differences among limitation treatments for all wetland types (ephemeral unvegetated, χ^2 (3) = 9.70; ephemeral vegetated, χ^2 (3) = 44.32; intermittent unvegetated, χ^2 (3) = 60.40; intermittent vegetated, χ^2 (3) = 158.41; perennial unvegetated, χ^2 (3) = 75.06; and perennial vegetated, χ^2 (3) = 160.75). However, Dunn's post hoc comparisons revealed within each wetland type there was no difference in what substrate was limiting denitrification between the unvegetated and vegetated patches (Figure 3). Specifically, ephemeral wetlands showed evidence of colimitation by carbon and NO_3^- in both unvegetated and vegetated patches, whereas intermittent and perennial wetlands showed evidence of NO_3^- limitation in both unvegetated and vegetated patches (Figure 3).

Plant Patch Type and Seasonal Floods (P2, P3)

For each of the wetland types, a two-way ANOVA predicting DNP using plant patch type, season, and plant patch type x season found significant differences in DNP between plant patch types (Table 1). The identity of these plant patches and the DNP of particular plant patches relative to open patches differed by wetland type. In ephemeral wetlands, DNP was significantly higher in AMSP and TRTE patches relative to open patches ($P < 0.05$; Figure 4; Table S3). In intermittent wetlands, DNP was higher in LUPE patches than in TYSP, grass, or open patches ($P < 0.001$; Figure 4; Table S3). TYSP patches also had significantly higher DNP compared with open patches ($P = 0.04$; Figure 4, Table S3).

Significant differences among seasons were also found in this analysis (Table 1), but seasonal differences varied by wetland type. In ephemeral wetlands, DNP was significantly lower during the post-monsoon season than in the winter season ($P = 0.04$; Figure 4; Table S3). Intermittent wetlands showed the opposite pattern, with DNP significantly higher during the post-monsoon season compared with the winter season ($P = 0.02$; Figure 4; Table S3). Neither ephemeral nor intermittent wetlands showed significant interaction effects between patch type and season (Table 1).

Perennial wetlands demonstrated a significant interaction effect between patch type and season on DNP (Table 1). During the pre-monsoon season, LUPE patches had significantly higher DNP than open and TYSP patches ($P < 0.05$; Figure 4). During the post-monsoon season, LUPE, grass, and TYSP had significantly higher DNP than in open patches, but they were not significantly different from each other ($P < 0.05$; Figure 4). During the winter season, TYSP patches had significantly higher DNP than open patches ($P < 0.001$; Figure 4).

Homogenization of DNP and Soil Resources (P4)

Paired t tests comparing CV for DNP, soil organic matter, soil texture, and soil extractable NO_3^- with each wetland type in the pre- versus post-monsoon season found no significant differences in CV between these seasons for any soil characteristic.

Wetland Type Effects on Patterns of DNP and Soil Resources (P5)

A Kruskal–Wallis H test found that DNP increased significantly from ephemeral to intermittent to perennial wetlands (χ^2 (2) = 99.385, $P < 0.001$; Table 2). However, spatial variability was not high (as defined by CV greater than 100%) in DNP or any soil characteristic potentially predicting DNP in any wetland type, with the exception of soil extractable NO_3^- at intermittent (CV = 123.3) and perennial (CV = 139.6) sites in the post-monsoon season (Table 2).

In general, ephemeral wetlands exhibited less spatial variation in DNP and soil characteristics than intermittent or perennial wetlands (Table 2). Relatedly, the Hedge's g effect size of vegetated patches compared to unvegetated patches showed an increasing effect of vegetation presence on DNP from ephemeral to intermittent to perennial wetlands (Figure 5). However, we found no clear pattern related to the effect size of temporal variation (that is, monsoon floods) on DNP across wetland types (Figure 5). Using the Cohen (1988) interpretation of effect sizes, the presence of vegetation had a medium effect (defined as 0.5) on DNP at ephemeral sites, while it had a large effect (defined as 0.8) on DNP at intermittent and perennial sites. Monsoon floods had a small effect (defined as 0.2) on DNP at all sites.

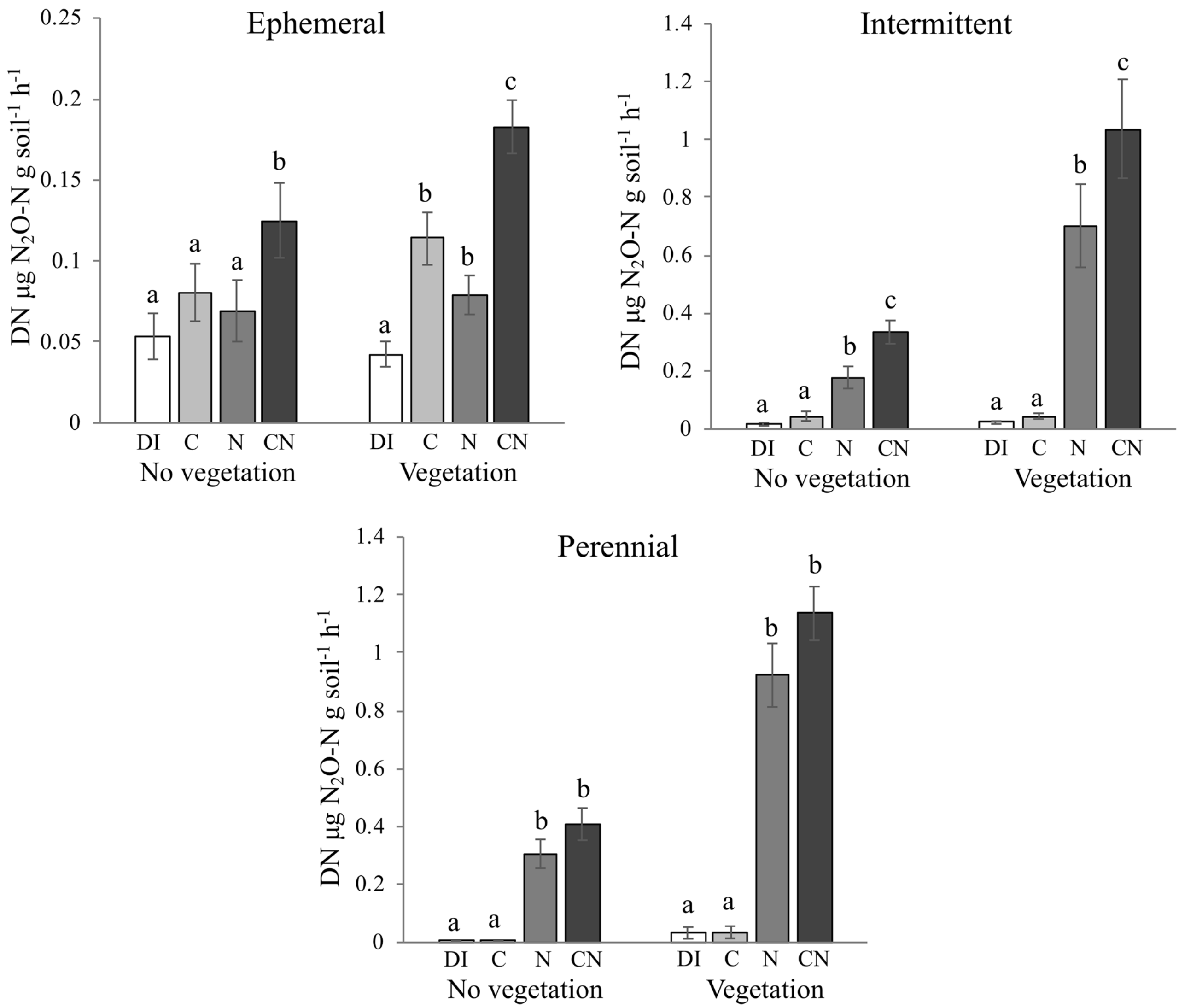

Figure 3. Mean denitrification (DN) rates for limitation treatments in patches with and without vegetation for each wetland type. DI = distilled water addition; C = carbon addition; N = NO_3^- addition; and CN = carbon and NO_3^- addition. Different letters denote significant differences from nonparametric Dunn's pairwise comparison tests at $P < 0.05$. Error bars indicate ± 1 SE.

Table 1. Two-way ANOVA: Effect of Season and Plant Patch on DNP

Wetland type	Independent variable	*df*	MS	*F*	*p*
Ephemeral	Season	2,65	0.03	3.26	0.04
	Patch	4,65	0.04	4.20	0.004
Intermittent	Season	2,84	2.43	3.93	0.02
	Patch	3,84	11.89	19.24	<0.001
Perennial	Season	2,72	0.86	2.75	0.07
	Patch	3,72	4.94	15.89	<0.001
	Season X patch	6,72	1.38	4.43	<0.001

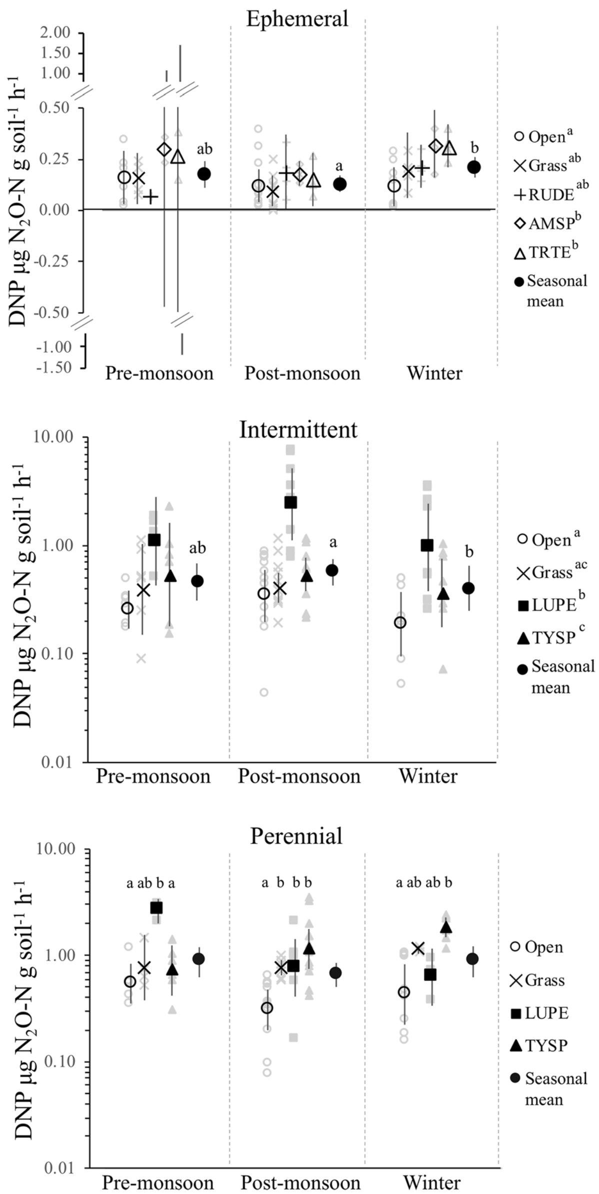

Figure 4. Means of DNP for plant patches in different seasons by wetland type. Symbols in black represent means of the given plant patch in that season for given wetland type. Symbols in light gray represent individual data points. Bars represent 95% confidence interval. Lowercase letters represent significant pairwise comparisons for Tukey post hoc tests. For ephemeral and intermittent wetlands, only comparisons for main effects of season or plant patch are represented as corresponding to ANOVA results. For perennial wetlands, differences among plant patches within each season are represented as ANOVA revealed significant interaction between season and plant patch. Open = unvegetated; Grass = mixed species; RUDE = *Rumex dentatus*; AMSP = *Amaranthuss* spp.; TRTE = *Tribulus terrestris*; LUPE = *Ludwigia peploides*; TYSP = *Typha* spp. Note log scale on intermittent and perennial graphs.

Discussion

Wetlands have repeatedly been shown to be effective sinks for NO_3^- due to high denitrification rates. However, urban environments may alter drivers of ecosystem function, challenging previously held assumptions about where and when important ecosystem processes, such as denitrification, occur (Groffman and others 2002; Stander and Ehrenfeld 2008; Palta and others 2014). The present study examined whether drivers of patterns of denitrification (that is, those drivers found to be important in nonurban systems) were also important in shaping patterns of denitrification in accidental urban wetlands. Key findings from this study are:

(1) The presence of vegetation increased DNP, but not by alleviating carbon limitation as in native desert wetlands.
(2) Unlike native desert wetlands, monsoon floods had limited spatial or temporal effects on patterns of denitrification, but inundation patterns (that is, wetland type) did influence the rates, limitations, and spatial patterns of denitrification.
(3) Accidental urban wetlands had high DNP, and thus a high capacity to reduce NO_3^- in urban waterways. Together these results suggest that the urban environment likely alters drivers of denitrification patterns in accidental urban wetlands and the mechanisms behind those changes warrant further investigation.

Plant Patches Drive Spatial Heterogeneity in Denitrification but Not Patterns of Limitation

As predicted, the presence of vegetation increased DNP, in line with findings of several studies (reviewed in Alldred and Baines 2016); however, in contrast to our predictions, our findings demonstrate that the increase in DNP was not due to plants alleviating carbon limitation of denitrifiers as there were no differences in limiting substrates between the vegetated and unvegetated patches in any wetland type. In addition, denitrifiers in ephemeral wetlands showed evidence of colimitation by carbon and NO_3^-, whereas NO_3^- limitation became stronger from intermittent to perennial wetlands. This is not entirely surprising as intermittent and perennial wetlands have greater plant productivity than ephemeral wetlands, which is associated with greater NO_3^- uptake. Further, more continuous inundation due to urban base-

Table 2. Summary of Soil Characteristics Among Seasons

Season	Variable	Ephemeral				Intermittent				Perennial			
		Med	Min	Max	[d]CV	Med	Min	Max	[d]CV	Med	Min	Max	[d]CV
Pre	[a]DNP	0.18	0.02	0.38	39.2	0.46	0.08	2.13	60.2	0.68	0.31	2.98	71.6
	[b]SM%	2	0	24	–	–	–	–	–	–	–	–	–
	cm	–	–	–	–	14	0	71	–	22	0	50	–
	OM%	3	1	8	29.4	6	1	18	55.2	6	1	27	90.9
	Si/Cl%	57	10	87	24.0	39	7	79	44.5	25	5	72	92.5
	[c]NO_3^-	5.28	0.34	28.6	68.9	0.43	0.12	11.1	66.8	0.46	0.04	10.6	93.4
Post	[a]DNP	0.12	0.01	0.39	81.0	0.53	0.04	6.90	87.3	0.72	0.07	3.41	72.2
	[b]SM%	14	3	24	–	–	–	–	–	–	–	–	–
	cm	–	–	–	–	6.5	0	108	–	25.5	0	65	–
	OM%	2	1	7	37.3	7	0	32	70.4	8	0	26	92.2
	Si/Cl%	60	13	96	19.5	32	3	86	53.7	28	3	81	80.0
	[c]NO_3^-	12.2	0.87	50.1	64.1	0.24	0.01	15.2	123.3	0.07	0.02	1.38	139.6
Winter	[a]DNP	0.22	0.01	0.40	53.5	0.40	0.05	3.18	83.8	1.04	0.16	2.34	62.3
	[b]SM%	8	2	22	–	–	–	–	–	–	–	–	–
	cm	–	–	–	–	0	0	24	–	17.5	0	56	–
	OM%	2	1	6	48.9	7	1	25	68.6	6	0	17	81.7
	Si/Cl%	62	16	71	20.8	31	5	83	67.0	23	1	80	70.9
	[c]NO_3^-	5.39	0.33	36.1	78.8	1.43	0.18	8.99	76.4	0.68	0.10	3.37	77.5

DNP denitrification potential, SM soil moisture, OM soil organic matter, Si/Cl% silt clay fraction of soil, NO_3^- soil extractable NO_3^-, CV coefficient of variation, Med median.
[a]*DNP units are μg-N g soil^{-1} h^{-1}.*
[b]*SM results are expressed as a percent when sites do not have standing water. If sites had standing water, depth in centimeters is reported.*
[c]*NO_3^- units are mg N kg soil^{-1}.*
[d]*Reported CV is average of three CVs for each study wetland in given wetland type to account for changes in within wetland variability.*

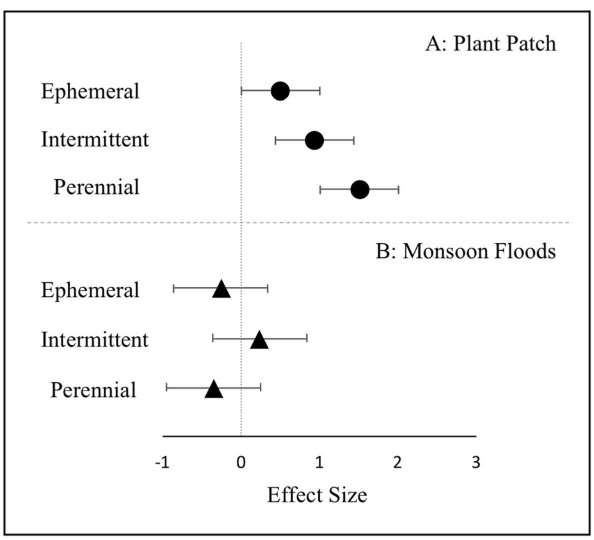

Figure 5. Hedge's g effect sizes of (A) plant patches relative to unvegetated patches on DNP and of (B) monsoon floods relative to the pre-monsoon season on DNP. Error bars indicate 95% confidence interval.

flow inputs (and associated low-oxygen conditions) could both lessen the amount of NO_3^- production via nitrification and increase competition for NO_3^- with other anaerobic microbes undertaking dissimilatory nitrate reduction to ammonium (Rysgaard and others 1994; Rütting and others 2011). It is surprising, however, that denitrifiers in unvegetated patches are not carbon limited in any wetland type, suggesting there must be alternate sources of either autochthonous or allochthonous labile carbon. These latter sources could also generally increase microbial metabolic activity, leading to more permanent anoxia and thus greater competition for and limitation of NO_3^- at the study sites (Mallin and others 2009). One possible source of autochthonous labile carbon is algal and biofilm growth promoted by increased water permanence and reactive nitrogen delivered during baseflow at more frequently inundated wetlands. However, some of these wetlands experienced shading from overpasses, and we did not observe algal or biofilm growth at these sites.

Alternatively, the intermittent and perennial accidental wetlands in this study could be receiving allochthonous labile carbon sources from urban baseflow. Studies in the Phoenix metropolitan area have found inputs of dissolved organic carbon during both baseflow and stormflow in the perennially flooded wetlands in this study (M. Palta, unpublished data). Further, studies from temperate regions have shown that carbon inputs associated with suburban catchments, such as grass clippings (Newcomer and others 2012), or agricultural catchments (Williams and others 2010) are more labile than carbon derived from forested catchments and thus can increase denitrification. Desert cities may be susceptible to similar changes in organic carbon quantity and quality in runoff relative to native desert ecosystem. Residential areas in desert cities have plant communities that more closely resemble those found in temperate cities than those in the surrounding native desert, and this likely changes the quantity and quality of carbon inputs to urban baseflow and stormwater runoff (Wheeler and others 2017).

Although differences in carbon and NO_3^- limitation did not explain why we observed higher DNP under vegetated versus unvegetated patches, it is likely that the plant patches are altering the soil conditions to be more favorable to denitrifiers via several potential mechanisms. Under plant patches at intermittent and perennial wetlands, we observed soils with a greater percentage of silt and clay (relative to sand) and higher soil organic matter (Table S2). Plant roots and fine sediments in these patches can provide greater surface area for microbial colonization, and greater soil organic matter content can support a larger microbial community (Groffman and others 1996; Schade and others 2001; Hernandez and Mitsch 2007; Heffernan and others 2008). Plant roots can also facilitate infiltration of surface water substrates to sediments, potentially increasing denitrification under plant patches relative to unvegetated patches (Angers and Caron 1998). In addition, wetland plant roots, well adapted to low-oxygen conditions, can aerate surrounding sediments, resulting in spatially adjacent aerobic and anaerobic microsites at the root–sediment interface. The result is increased dentification via coupling with the aerobic process of nitrification (Reddy and others 1989). In such a case, however, we might expect an alleviation of NO_3^- limitation under plant patches, which was not observed in this study. Further research is needed to determine what mechanisms are driving increased denitrification under plant patches in these accidental urban wetlands.

Inundation Duration Drives Magnitude of Denitrification Under Plant Patches

Plant patches interacted with wetland inundation duration to create spatial and temporal patterns in DNP, and this was in part driven by the species of a plant patch in a given wetland type (P2, P5). We predicted plant patches would act as ''islands of fertility,'' as they do in native desert wetlands, by providing more resources that stimulated microbial processes compared to the surrounding matrix; thus, plant patches would have a greater effect on DNP at ephemeral wetlands due to low soil fertility, low soil moisture, and infrequent exogenous inputs of carbon and NO_3^- from runoff relative to intermittent and perennial wetlands (Schlesinger and others 1996; Schade and Hobbie 2005). Instead, the presence of vegetation had larger effects in intermittent and perennial wetlands. One possible explanation is that plant patches in ephemeral wetlands may be less resilient to flood events, resulting in patches that are less permanent and thus do not have the time to build ''island'' resources. Frequent inundation from baseflow in intermittent or perennial wetlands allows wetland plants to become established to the point that these plant patches have greater biomass and are more resilient to flood events. In the ephemeral wetlands, we often observed annual (in contrast to perennial) plant species that are not typically associated with wetlands; these annuals are likely not well adapted to floods and thus likely do not facilitate denitrification via the same mechanisms as wetland-adapted plants in the intermittent and perennial wetlands discussed above (for example, AMSP, TRTE, and the grass patch species *Schismus* sp. and *Cynodon dactylon*; plants.usda.gov). We anecdotally observed dead patches of AMSP and TRTE buried by up to 30 cm of sediment after monsoon floods in ephemeral wetlands, further supporting the idea that plant patches established in ephemeral wetlands may not have sufficient longevity to develop islands of fertility. Further, plants in resource-poor environments often produce litter of lower quality than in resource-rich environments potentially further limiting denitrification activity relative to intermittent or perennial wetlands (Hobbie 1992). Some combination of these factors may explain why plant patches had little influence on soil resources and DNP in ephemeral wetlands.

In intermittent and perennial wetlands, plant patch identity also affected DNP. At intermittent wetlands, LUPE patches consistently had the highest DNP relative to other plant patches and had the highest DNP of all wetlands. At perennial wetlands, there was seasonal variability with LUPE having higher DNP in the pre-monsoon season and TYSP having the highest DNP in the winter season. These patterns are likely due to an interaction between how plant patches alter resources and the disturbance level at the different wetland types. Plants of difference species have been shown to alter denitrification based on their carbon and nitrogen inputs (Windham and Ehrenfeld 2003; Hernandez and Mitsch 2007). LUPE has tissue with low C:N ratios and is likely the best source of labile carbon compared to the other plant patches in this study, explaining why DNP would be greatest under those patches (Suchy 2016). However, LUPE patches in perennial wetlands were also routinely smaller and were often scoured during monsoon floods (personal observation), compared to intermittent wetlands, which could explain the decline in DNP in LUPE patches during the post-monsoon and winter seasons.

Seasonal Monsoon Flooding has Little Effect on Denitrification

In contrast to native desert wetlands, monsoon floods did universally not increase DNP nor decrease spatial heterogeneity of DNP or soil resources (P3, P4). One of the more surprising results of this study was the decline in DNP in ephemeral wetlands following the monsoon season and increase in DNP during the winter season despite cooler temperatures. In ephemeral native desert wetlands, monsoon floods had been shown to increase DNP by orders of magnitude due to the creation of saturated conditions and delivery of carbon and NO_3^- (Harms and others 2009). The mechanism behind this decline is unclear but may be due to the sediment deposits observed after monsoon floods discussed above. These sediment depositions result from a buildup in storm drains during the dry season which get washed out during monsoon rains; thus, they may be low in labile carbon, or even lack a robust microbial community. This could explain the increase in DNP during the winter season, after which the denitrifier community had time to recolonize those sediments. However, this is speculative and should be investigated further by characterizing the sediment being deposited and changes in microbial biomass in the freshly deposited sediments.

The diminished effect of monsoon floods compared with native desert wetlands may be a common attribute in urban rivers and wetlands of desert cities. Urban hydrology is greatly controlled, and in desert cities a dominant feature of this control is to reduce flooding and retain stormwater within the city resulting in smaller floods. In addition, if urban baseflow delivers allochthonous resources year-round to intermittent and perennial wetlands, the episodic delivery of allochthonous resources during monsoon floods may become less important for stimulating DNP in the study wetlands.

Urban Accidental Wetlands Can Significantly Mitigate NO_3^- Pollution in a Desert City

Accidental urban wetlands also had considerable potential to remove NO_3^-. DNP in these systems was comparable to rates observed in lawns, anthropogenic lakes, and stormwater control structures in Phoenix, AZ (M = 2.6, 1.75, 0.7 µg NO_3^--N g soil^{-1} h^{-1}, respectively; Zhu and others 2005; Hall and others 2009; Roach and Grimm 2011). Further, DNP in accidental urban wetlands was higher than rates observed in native desert wetlands. This pattern was particularly pronounced in ephemeral wetlands, where DNP in accidental urban wetlands was several orders of magnitude higher than DNP in ephemeral native wetlands (Harms and others 2009).

We also found that intermittent and perennial sites had much higher DNP than ephemeral sites. Although this finding is unsurprising alone, it has implications for the Phoenix watershed nitrogen budget (Baker and others 2001), because the accidental wetlands in the Salt River may increasingly shift from perennial or intermittent wetlands to ephemeral wetlands under increased water conservation practices implemented in Phoenix (Gober and others 2010). Palta and others 2017 found the perennial wetlands in this study are effective as reducing NO_3^- during both baseflow and storm events. If the study wetlands shifted from functioning as intermittent or perennial wetland types to functioning as ephemeral wetland types, we project up to a fourfold reduction of NO_3^- removal by these systems when they flood during storms. This is an important consequence as these wetlands currently receive high NO_3^- loads during storms and they are the last point of potential processing before water infiltrates into groundwater. Groundwater in Arizona and the Phoenix metropolitan area can have very high concentrations of NO_3^- (Power and Schepers 1989); thus, reducing NO_3^- concentrations in infiltrating water is necessary for maintaining water safe for human consumption (Townsend and others 2003).

Conclusions

This study demonstrated that accidental urban wetlands are another feature of the urban landscape that can help reduce nitrogen export, with the added benefit of having minimal management investments. While management is certainly not necessary to see benefits from accidental urban wetlands, our findings are informative about where to invest time and money to maximize NO_3^- removal if desired. For example, planting vegetation at ephemeral sites to increase soil resources for denitrification might not be effective, whereas distributing urban baseflow to maintain continual inundation would be effective. In addition, the use of accidental urban wetlands in this study allowed us to parse out the drivers of patterns of denitrification that are affected by urbanization in a desert city and suggest interesting avenues for future research to further understand the mechanisms creating these patterns.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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