Denitrification in Accidental Urban Wetlands:

Exploring the Roles of Water Flows and Plant Patches

by

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ABSTRACT

Cities can be sources of nitrate to downstream ecosystems resulting in eutrophication, harmful algal blooms, and hypoxia that can have negative impacts on economies and human health. One potential solution to this problem is to increase nitrate removal in cities by providing locations where denitrification— a microbial process in which nitrate is reduced to N_2 gas permanently removing nitrate from systems— can occur. Accidental urban wetlands– wetlands that results from human activities, but are not designed or managed for any specific outcome– are one such feature in the urban landscape that could help mitigate nitrate pollution through denitrification.

The overarching question of this dissertation is: how do hydrology, soil conditions, and plant patches affect patterns of denitrification in accidental urban wetlands? To answer this question, I took a three-pronged approach using a combination of field and greenhouse studies. First, I examined drivers of broad patterns of denitrification in accidental urban wetlands. Second, I used a field study to test if plant traits influence denitrification indirectly by modifying soil resources. Finally, I examined how species richness and interactions between species influence nitrate retention and patterns of denitrification using both a field study and greenhouse experiment.

Hydroperiod of accidental urban wetlands mediated patterns of denitrification in response to monsoon floods and plant patches. Specifically, ephemeral wetlands had patterns of denitrification that were largely unexplained by monsoon floods or plant patches, which are common drivers of patterns of denitrification in non-urban wetlands. Several plant traits including belowground biomass, above- and belowground tissue chemistry and rooting depth influenced denitrification indirectly by changing soil organic

matter or soil nitrate. However, several other plant traits also had significant direct relationships with denitrification, (i.e. not through the hypothesized indirect relationships through soil organic matter or soil nitrate). This means these plant traits were affecting another aspect of soil conditions not included in the analysis, highlighting the need to improve our understanding of how plant traits influence denitrification. Finally, increasing species richness did not increase nitrate retention or denitrification, but rather individual species had the greatest effects on nitrate retention and denitrification.

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Chapter 1

INTRODUCTION

Urban areas cover less than 3% of the land, yet have global impacts on ecosystem structure and function (Grimm et al. 2008). Urbanization can change biodiversity, biogeochemical cycles, resources, hydrology, and disturbances relative to non-urban systems (McDonnell and Pickett 1990; Kaye et al. 2006). Understanding how these changes affect ecosystem functions can be especially challenging given that human decision-making has both direct and indirect effects on ecosystem structure and function.

The Southwest is one of the most rapidly populating and urbanizing regions in the United States, leading to dramatic changes in the distribution of water in the landscape (Fitzhugh and Richter 2004; Gober et al. 2010). In desert cities, large amounts of water are imported for human consumption and use (Sabo et al. 2010). Irrigation of urban landscapes results in a general shift from xeric to mesic environments, and also in local loss of stream and wetland habitat (Roach et al. 2008; Steele et al. 2014). Therefore, historically perennial rivers become more dry, and engineered canals and lakes increase in number (Larson and Grimm 2012).

Water availability and biogeochemical cycling are tightly coupled (McClain et al. 2003; Belnap et al. 2005). Changing where and when water is present in urban watersheds affects the transformations of nutrients and pollutants such as nitrate, a common pollutant in urban areas (Zhu et al. 2005; Roach et al. 2008). High nitrate levels are commonly found in urban waters due to runoff from over-fertilized landscaping, leaky septic tanks, and wastewater discharges (Paul and Meyer 2001). High nitrate in water has deleterious consequences for human health (Nolan et al. 1997; Townsend et al.

2003) and can cause ecologically harmful algal blooms (Vitousek et al. 1997; Carpenter et al. 1998). Nitrate levels in groundwater used for human consumption are high in significant portions of the Southwest (Rosen and Kropf 2009). Consequently, mitigation of nitrate inputs is critical in the region and is intimately tied to water availability.

Aquatic ecosystems such as rivers and wetlands are important for the transport and processing of nitrogen in watersheds. The convergence of water and nutrients supports high primary production, and saturated conditions promote the anaerobic microbial process of denitrification (Seitzinger et al. 2006). Much of the research examining the processes and environmental conditions that promote denitrification in urban wetlands has been conducted on wetlands constructed and managed specifically for nitrate removal (Kadlec et al. 2000). However, wetlands in urban landscapes can occur anywhere that water is present, such as at storm drain outfalls or in brownfields (White and Stromberg 2011; Palta et al. 2014; Bateman et al. 2015). These wetlands result indirectly from human decisions and design, but, contrary to many urban features, are largely unmanaged. I will refer to these unplanned and unmanaged urban wetlands as "accidental wetlands." These accidental wetlands are largely unstudied, but have high potential for processing nitrate in urban ecosystems through denitrification.

Processes and characteristics unique to urban wetlands may affect the frequency with which key substrates (labile carbon and nitrate) and anaerobic conditions necessary for denitrification co-occur. Relative to non-urban counterparts, some accidental urban wetlands have altered hydrology, low groundwater levels, low soil organic carbon, and compacted soils, all of which tend to reduce denitrification rates (Ehrenfeld 2000). Changes in the frequency and duration of inundation and surface-groundwater

interactions determine whether nitrification or denitrification occurs by changing soil redox conditions (Hernandez and Mitsch 2007a). In remnant urban riparian zones, decreases in groundwater levels and increased stream incision disconnect riparian floodplains from nitrate-rich groundwater. This results in riparian floodplains with aerobic soils, which promote the aerobic process of nitrification and that may act as a source rather than a sink of nitrate (Groffman et al. 2002). Previous industrial or commercial land use can result in depletion of carbon from urban soils (Gift et al. 2010; Marcotullio 2011). Consequently, the shortage of organic carbon may limit denitrification even in the presence of ample nitrate. Further, even if nitrate and labile carbon are plentiful, certain soil characteristics may interfere with denitrification. For example, urban soils can be compacted due to previous industrial use (Marcotullio 2011). This reduces infiltration of water carrying nitrate and carbon to deep soils, resulting in low denitrification rates (Myrold and Tiedje 1985). Palta et al. (2014) found that soil properties that typically promote denitrification (small particle size and high organic matter) supported lower denitrification in urban brownfields because of nitrate limitation. Finally, changes in vegetation in urban wetlands, and how the vegetation modifies the soil environment have the potential to affect nitrogen cycling (Ehrenfeld 2003).

In addition to characteristics unique to urban wetlands that can influence denitrification, we also do not have a clear understanding of how plant traits or species richness influences denitrification generally (Cardinale et al. 2011; Sutton-Grier et al. 2012; Alldred and Baines 2016). Understanding the mechanistic link between plant species and denitrification via plant traits, and how species interactions resulting from

increased richness affects denitrification could be of particular importance in degraded or urban wetlands where improving ecosystem functions is often a desired outcome.

The overarching question for this dissertation is: How do hydrology, soil conditions, and plant patches affect patterns of denitrification in accidental urban wetlands? To answer this question, I took a three-pronged approach. First, I examined broad patterns and drivers of denitrification in accidental urban wetlands. Second, I tested if plant traits influence denitrification indirectly by modifying soil resources. Finally, I examined how species richness and interactions between species influence nitrate retention and patterns of denitrification using both a field and greenhouse study.

STRUCTURE OF THIS DISSERTATION

In Chapter 2, I describe patterns of potential denitrification in accidental urban wetlands in the Salt River in Phoenix, Arizona. Little is known about patterns of denitrification in accidental urban wetlands, and this is the first study to look for broad scale patterns and drivers of denitrification in the accidental urban wetlands of the Salt River. To identify general patterns, I took a patch-based sampling approach and measured potential denitrification from nine wetlands with different hydroperiods. I sampled from three to four dominant plant patches in each wetland during three different seasons. My objective for Chapter 2 is to examine the relative importance of hydrology (as a large-scale driver) and plant patches (as a small-scale driver) for shaping spatial and temporal patterns of potential denitrification in accidental urban wetlands.

For Chapter 3, I examine how plant traits affect denitrification by modifying soil resources. Many studies on denitrification focus on the effects of different species, as I

did in Chapter 2 (Groffman et al. 1996; Groffman et al. 1996; Windham and Ehrenfeld 2003; Hernandez and Mitsch 2007b; Alldred and Baines 2016). However, plants can affect denitrification by modifying the soil environment that denitrifying microbes experience. Because the hypothesized effect of plant traits on denitrification is indirect (i.e., mediated by soil conditions), traditional statistical techniques, such as linear regression, only test if there is a relationship between plant traits and denitrification. Structural Equation Modeling (SEM) allows for the testing of specific hypothesized relationships, including direct and indirect relationships, because models are specified *a priori*. For Chapter 3, I use SEM to examine if plant traits influence denitrification indirectly via commonly hypothesized effects on soil conditions.

For Chapter 4, I examine the relationship between species diversity and nitrogen retention in a greenhouse experiment. I also conducted a field study to examine if the interaction of plant patches generates spatial variation in denitrification in a field study. How ecosystem function relates to species diversity has been a central debate in ecology for the past two decades (Díaz and Cabido 2001). However, despite the hundreds of studies in grasslands, relatively few have been conducted in wetlands (Cardinale et al. 2011), and even fewer have examined how species diversity affects denitrification (Engelhardt and Ritchie 2001; Callaway et al. 2003; Bouchard et al. 2007; McGill et al. 2010). Wetlands plants often occur in monotypic clonal patches (Spence 1982), a configuration different than species mixtures typical of grasslands. My first objective for Chapter 4 is to determine if increasing species richness increases nitrate retention and denitrification when wetland species are grown in patches rather than mixtures. This objective is addressed using a greenhouse experiment, in which wetland mesocosms were

planted with one, two or three species patches. My second objective is to determine if species interactions at wetland plant patch edges increase denitrification. This objective is addressed by: (1) determining if there was spatial variation between plant-patch edges and centers in a field study; and (2) determining if plant edge interactions were the cause of variation between patch edges and centers in a greenhouse experiment.

In Chapter 5, I summarize the main findings of this dissertation. Additionally, I discuss the implications of the findings for other systems, and how the findings may be useful for city managers.

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Chapter 2

SPATIAL AND TEMPORAL PATTERNS OF POTENTIAL DENITRIFICATION IN ACCIDENTAL URBAN WETLANDS IN PHOENIX, AZ

ABSTRACT

Denitrification is an ecosystem process that permanently removes reactive nitrogen from systems. Much research on denitrification has occurred in non-urban or highly managed urban wetlands. However, in urban landscapes nitrogen-rich water is often discharged into areas not designed or managed to reduce nitrate loads. "Accidental" wetlands resulting from these discharges may have the capacity to remove nitrate, but are subject to unique hydrologic and soil conditions that could create unexpected spatial and temporal patterns of denitrification. My objective for this study was to examine the relative importance of hydrologic regime as a large-scale driver, and plant patches as small-scale driver of spatial and temporal patterns of denitrification in accidental urban wetlands.

I measured potential denitrification (DNP) on soils from nine wetlands forming at storm drain outfalls in Phoenix, AZ. Wetland sites were categorized into three hydroperiods: perennial, intermittent, and ephemeral (inundated >90%, 50 to 85%, and 10 to 30% of the year respectively). To assess spatial variation of DNP, I collected samples from two to four dominant vegetation patch types within each wetland. To assess temporal variation of DNP, samples were also collected during three seasons differing in rainfall pattern.

I found small- and large-scale spatiotemporal patterns in DNP that have important implications for management of urban wetlands for stormwater quality. DNP increased from ephemeral to intermittent to perennial wetlands. The presence of plants increased DNP compared to unvegetated patches at wetlands of all hydroperiods. Further, plants did not alleviate carbon limitation at wetlands of any hydroperiod; rather, DNP became more nitrate limited as hydroperiod increased. I found a range of responses in DNP among wetlands to seasonal monsoon floods, which interacted with wetland hydroperiod. In perennial wetlands, monsoon floods equalized DNP among plant patches; however, coefficients of variation did not decrease in individual wetlands after monsoon floods suggesting that monsoon floods were not homogenizing resources or denitrification in wetlands. At ephemeral wetlands, overall DNP unexpectedly decreased after monsoon floods possibly due to deposition of fresh sediment. Together, these findings offer novel insights into the complex interactions in *accidental urban wetlands* among plant patches, monsoon floods, and hydroperiod.

INTRODUCTION

Urban watersheds are often sources of nitrogen (N) to downstream systems (Shields et al. 2008; Kaushal et al. 2011). However, features in the urban landscape have the potential to reduce N exports (Baker et al. 2001; Passeport et al. 2012; Koch et al. 2014). Such studied features, including stormwater detention/retention basins, treatment wetlands, swales, and restored floodplains, are often designed, engineered, and managed (Zhu et al. 2005; Bettez and Groffman 2012; Hale et al. 2014; Johnson et al. 2014). An understudied feature of urban landscapes that could affect N export from cities are

accidental urban wetlands. Accidental urban wetlands are neither remnant wetlands in urban landscapes, nor are they constructed/engineered wetlands. Rather, they are wetlands that result from human activities, but that are not designed or managed for any specific purpose, such as N removal.

Wetlands are effective removers of N because of soil conditions that promote high rates of denitrification (Seitzinger et al. 2006). Denitrification, a microbial process that reduces nitrate (NO_3^-) to nitrogen gas (N_2), requires three conditions to occur: soil anoxia, high nitrate, and high labile carbon (McClain et al. 2003; Seitzinger et al. 2006; Wallenstein et al. 2006; Groffman et al. 2009). Urban wetlands can have substantially altered hydrology and biota potentially influencing soil conditions and the effectiveness of urban wetlands to remove nitrate via denitrification (Ehrenfeld 2000; Groffman and Crawford 2003; Palta et al. 2014). For example, lower groundwater tables in urban riparian areas reduce saturation of soils and decrease denitrification (Groffman et al. 2003). Previous industrial or commercial land use can deplete carbon from soil reducing carbon available to denitrifiers (Gift et al. 2010; Marcotullio 2011). Further, changes in vegetation can affect soil carbon and nitrate available for denitrification (Ehrenfeld 2000; Windham and Ehrenfeld 2003).

The few studies on urban wetlands have been conducted in mesic cities (Groffman and Crawford 2003; Ehrenfeld 2008; Stander and Ehrenfeld 2008; Gift et al. 2010; Harrison et al. 2011; Palta et al. 2014). Urban wetlands can also occur in desert cities (White and Stromberg 2011; Bateman et al. 2015), where the urban environment may have different effects on denitrification than in urban wetlands in mesic environments relative to their non-urban counterparts (Hale et al. 2016). To our

knowledge, this is the first study to examine denitrification in accidental urban wetlands in a desert city. Denitrification in wetlands is highly variable, both spatially and temporally. This study therefore contributes to understanding the drivers of denitrification in accidental urban wetlands, and how urbanization may affect these drivers. Specifically, this study examines how the hydrologic regime—a large-scale driver—and variation in plant patches—a small-scale driver via their effect on soil environmental conditions—affect spatial and temporal patterns of denitrification.

Hydrologic regime and soil environmental conditions (e.g. soil carbon, redox potential, soil texture) are important drivers of denitrification in wetlands (Seitzinger et al. 2006; Wallenstein et al. 2006; Groffman et al. 2009). Hydrologic regime is an important large-scale driver of wetland ecosystem functions, such as denitrification, as it affects the local soil environment (e.g. soil anoxia) and biota (Brinson 1993; Mitsch and Gosselink 2007) that can affect denitrification. However, urban wetlands can have hydrologic regimes that are dramatically altered compared to non-urban counterparts, thus affecting ecosystem functions (Ehrenfeld 2000; Groffman and Crawford 2003; Stander and Ehrenfeld 2008). In non-urban desert rivers, where riparian areas and riverine wetlands are important sites of denitrification, hydrologic regimes can affect denitrification through both water and resource availability (Harms and Grimm 2010). Surface flow is not always present in these rivers, and floods create saturated, anoxic soil conditions necessary for denitrification. The timing, frequency, and location of floods affects patterns of where and when denitrification occurs in desert rivers (Harms et al. 2009). Further, non-urban desert rivers are subject to intense seasonal flooding which have the capability of both delivering resources from the landscape and homogenizing

local soil resources and denitrification in associated riparian areas (Harms et al. 2009; Harms and Grimm 2010).

Urbanization can affect hydrologic regimes of desert riverine wetlands and riparian areas by changing the amount of water available and the magnitude of seasonal floods. Urbanization can decrease water available to urban rivers and wetlands owing to groundwater pumping and upstream impoundment (Roach et al. 2008). However, water availability may be increased by engineered water features in the landscape and runoff resulting from urban water use (i.e. urban baseflow) in other parts of the urban environment (Roach and Grimm 2011; Larson and Grimm 2012; Steele et al. 2014). Further, the magnitude of seasonal floods and resource delivery can be dampened by retention basins and other flood control features in desert cities (Hale et al. 2014) resulting in urban wetlands that would not see increases in denitrification, or homogenization of denitrification after monsoon floods observed in non-urban desert riverine wetlands.

In addition to hydrologic regime as a large-scale driver, plant patches are important small-scale drivers of patterns of denitrification. Plants can affect denitrification by influencing the soil environment that microbes experience, such as carbon availability, redox potential, infiltration, soil moisture, and soil texture (Brady and Weil 2008). Generally, plants in wetlands have been shown to increase denitrification compared to unvegetated areas (Alldred and Baines 2016), and different plant communities have been shown to differentially affect denitrification through changes they induce in soil resources (Windham and Ehrenfeld 2003; Hernandez and Mitsch 2007a). In desert rivers, plants alleviate carbon limitation for denitrifiers by providing

organic carbon as litter deposition and root exudates (Schade et al. 2001; Heffernan and Fisher 2012). However, urbanization can also mask the effects of plants on soil resources because resource deposition (external inputs of resources) is often high in urban environments (Hall et al. 2009).

The central question of this study was: What is the relative importance of plant patches and seasonal flooding for explaining patterns of denitrification in accidental urban wetlands with different hydroperiods? The research objectives of this study were to: (1) Determine the effects of hydroperiod on patterns of denitrification; (2) determine the effects of plant patches on patterns of denitrification; and (3) determine the effects of monsoon floods on patterns of denitrification by examining interactions between seasonal floods and plant patches on denitrification. Assuming urban wetlands function as their non-urban counterparts with respect to denitrification, I hypothesized that hydrologic regimes will affect denitrification in accidental urban wetlands in two ways: (1a) Denitrification will be greater in accidental urban wetlands that are more frequently inundated, which creates conditions more conducive to denitrification (i.e., soil anoxia); and (1b) seasonal monsoon floods will increase denitrification due to delivery of resources (e.g. nitrate, carbon) and generation of soil anoxia necessary for denitrification. I hypothesized that plant patches will affect denitrification in two ways: (2a) denitrification will be higher under plant patches compared to unvegetated patches; and (2b) plants will enhance denitrification by alleviating carbon limitation on denitrifiers. Finally, I hypothesized that: (3) denitrification rates will be more similar among plant patches after seasonal monsoon floods due to scouring and redistribution of resources (i.e. monsoon floods will have a homogenizing effect on denitrification).

METHODS

Study area

The historically perennial Salt River has been mostly dry as it bisects downtown Phoenix since 1938. In this reach of the Salt River, the floodplain has been highly modified and engineered for flood management, and in places it is mined for gravel. The study was conducted in a 30-km reach of the Salt River in Phoenix, Arizona.

Dozens of storm drains discharge urban runoff into the Salt River during storms. However, during dry periods, the Salt River also receives relatively continuous urban baseflow through a subset of these storm drains that serve large urban watersheds, and wetlands have formed at many of these outfalls (White and Stromberg 2011; Bateman et al. 2015). I identified nine accidental wetlands that were characterized by storm-drain discharges differing in their timing and frequency of discharges. I categorized the wetlands into three different hydroperiod groups: ephemeral, intermittent, and perennial. Ephemeral wetlands flooded largely in response to precipitation and remained inundated for 10 to 30% of the year. Intermittent wetlands received urban base flow during dry periods and remained inundated for 50 to 85% of the year. Perennial wetlands received enough continuous urban base flow to be inundated for more 90% of the year (Figure 2.1 andFigure 2.2).

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 through mid-September in the form of intense, localized rainfall that results in flashy urban runoff, and sometimes substantial floods.

The winter rainy season runs from November through April. Winter rains are the result of Pacific frontal storms that generate more gentle, sustained rains, usually resulting in less intense flooding.

Sampling design

In each study wetland, two to four dominant plant patches were identified including one patch without vegetation designated "open" (Table 2.1). At ephemeral sites, patches designated "grass" were either *Schismus sp.* or *Cynodon dactylon*. While these species are from different genera, they are both from the *Poaceae* family, which share several unique characteristics. Patches designated *Amaranthus sp.* were *Amaranthus palmeri, Amaranthus albus,* or both. At the intermittent and perennial sites, patches designated "grass" were *Paspalum distichum*. Patches designated *Typha sp*. were either *Typha domingensis*, *Typha latifolia*, or both. Wetlands were sampled three times between June 2013 and March 2014 to capture differences in seasonal precipitation and urban runoff. The pre-monsoon sampling was conducted in June 2013 before monsoon rains began. The post-monsoon sampling period was conducted in October 2013 after the monsoon rains ended. The winter sampling period was conducted from February to March 2014. During winter sampling period only six of the nine sites were sampled due to time and sampling constraints (Table 2.1; Figure 2.2).

Hydroperiod measurements

Estimations of wetland hydroperiod were determined using ibutton temperature sensors (DS1921G; https://www.maximintegrated.com). Ibuttons were placed in waterproof casings (DS9107; https://www.maximintegrated.com) and deployed on the soil surface to record temperature every hour. 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 that could indicate inundation of wetlands were identified. Those periods were compared to air temperature records to determine if the dampened temperature oscillations were due to inundation or due to changes in air temperature. Categorization of presence/absence of water from ibutton data was also compared to field observations of inundation to verify the accuracy of categorization.

Soils

During the pre-monsoon season, two soil cores were taken from each patch, during the other two seasons four soil cores were taken from each patch (n = 246). Soil cores of the same patch type were taken from different patches when possible. If only a single large contiguous plant patch occurred at a site, cores were taken at least 5 meters apart. Soil cores were taken to a maximum depth of 10 cm to encompass the most active soil layer (Groffman et al. 1999). Cores were stored on ice in the field and then stored at 4 °C in the lab until processing, which typically occurred within 24 hours.

Soil cores were homogenized and analyzed for moisture content, organic matter, nitrate, texture, and potential denitrification. Soil moisture was determined gravimetrically by drying soils for 48 hours at 105°C. Soil organic matter was determined by mass loss on ignition for 4 hours at 550°C. Soil nitrate (NO_3^-) was extracted by shaking 10 grams of sample with 50 mL 2M KCl for 1 hour and then filtering through pre-leached Whatman number 42 ashless filters. Extracts were collected and frozen until analyzed colorimetrically on a Lachat QC8000 flow-injection analyzer. Soils were dried

and sieved through a 2-mm sieve to determine gravel fraction of soil. Forty grams of sieved soils were shaken overnight in 100 mL of a sodium hexametaphosphate solution and percent sand, silt and clay were determined using the hydrometer method (Robertson et al. 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 et al. 1999).

Denitrification was measured using denitrification enzymes assays (Groffman et al. 1999). Fifty grams of soil were placed into 125 ml Wheaton bottles and 50 ml of one of the following media was added. To measure denitrification potential (conditions in which no factor is limiting denitrification) I 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 et al. 1999; Roach and Grimm 2011). To measure limitation effects of carbon and nitrate, samples were amended with only NO_3^-, only glucose, or received neither. The resulting four treatments for each soil core were: distilled water only (DI), NO_3^- only (N), carbon only (C), and NO_3^- and carbon (N+C). Headspace of samples was replaced with N_2 gas to create anaerobic conditions and 10 ml of acetylene gas to block the reduction of N_2O to N_2 (Yoshinari and Knowles 1976; Groffman et al. 1999). Samples were incubated at room temperature and shaken at 140 rpm for 4 hours. Gas samples were taken at 30 minutes and 4 hours and analyzed on a Varian 3800 gas chromatograph for N_2O concentration.

Statistical Analyses

Soil moisture, soil organic matter, soil NO_3^-, and soil texture (%silt/clay) were compared for each hydroperiod across seasons and across plant patches using two-way

ANOVA with Tukey's HSD post hoc tests if main effects were significant. Soil moisture, soil organic matter, and soil texture data were arcsine transformed and soil NO_3^- data were log transformed to conform to rules of normality.

Hypotheses 1a, 1b, 2a, and 3a were tested using ANOVA with Tukey's HSD post hoc tests that compared the effects of season (i.e. effects of monsoon floods), hydroperiod, and plant patches on denitrification potentials. Notably, ephemeral wetlands had a different data distribution than intermittent and perennial wetlands. Data for ephemeral wetlands were normally distributed, while data for intermittent and perennial wetlands were log-normally distributed. Therefore, the analyses for ephemeral wetlands, and for intermittent and perennial wetlands were run separately. I ran a two-way ANOVA to examine effects of season and plant patches at ephemeral wetlands, and a three-way ANOVA to examine effects of season, hydroperiod, and plant patches for intermittent and perennial wetlands. Type 4 sum of squares was used to account for unbalanced cell sizes and cells with missing data as a result of smaller sampling effort during winter season.

To further examine if monsoon floods had a homogenizing effect on denitrification in wetlands (hypothesis 3a), coefficients of variation (CV) were calculated for log-transformed data for each of the nine sites in the pre- and post-monsoon season. Then a paired t-test was used to determine if the CV changed between these two seasons. The winter season was excluded from this analysis due to the reduced sampling effort during that season.

To determine whether NO_3^- or carbon limited denitrification (hypothesis 2c), the results of limitation experiments were normalized to the control treatment (DI) to determine the relative response (RR) of each treatment:

$$RR\text{-}X = DNP_X / DNP_{DI}$$

DNP is denitrification potential and X is the treatment of interest (N, C, or N+C). The closer an RR is to one the closer that factor is to the control (DI).

Relative responses of N to C treatments (RR-N/RR-C) indicate the relative strength of N or C limitation. RR-N/RR-C was calculated as:

$$(RR\text{-}N/RR\text{-}C) = DNP_N / DNP_C$$

For RR-N/RR-C, a result greater than one indicated stronger NO_3^- limitation, while a result less than one indicated stronger carbon limitation. These relative responses were tested for effects of plant patch and hydroperiod using two-way ANOVA. All statistical analyses were conducted in SPSS version 19.

RESULTS

Hydroperiod, season (monsoon floods), and plant patch type, along with significant interactions among the three, significantly affected denitrification. In the following section, I first describe general patterns of soil conditions across hydroperiods, season, and plant patches. I then describe the results for each of the hypotheses.

Soil conditions

In general, across the different hydroperiods, soil moisture, organic matter, NO_3^-, and texture there were not different among seasons, but differed among plant patches (Figure 2.3Figure 2.4). Soil organic matter and %silt/clay did not differ significantly

across seasons for wetlands of any hydroperiod. Soil moisture was significantly different among seasons only for ephemeral wetlands. Soil NO_3^- was significantly different among seasons for all wetlands (Figure 2.3). There were significant differences among plant patches for all soil conditions except soil organic matter at ephemeral wetlands, and soil NO_3^- at intermittent and perennial wetlands (see Figure 2.4 for details).

Effects of hydroperiod and seasonal monsoon floods (hypotheses 1a and 1b)

Across all wetlands, I found a significant main effect of hydroperiod with increasing potential denitrification (μg N_2O g $soil^{-1}$ h^{-1}) from ephemeral to intermittent to perennial sites (ANOVA: $F_{(2,208)} = 31.29$, $P < 0.001$; Table 2.2; Figure 2.5a). However, there was no significant main effect of season on potential denitrification (ANOVA: $F_{(2,208)} = 1.76$, $P = 0.17$; Table 2.2; Figure 2.5b) suggesting that monsoon floods did not uniformly increase potential denitrification in the study reach.

Effects of plant patches (hypothesis 2a)

For each hydroperiod, the presence of vegetation significantly increased potential denitrification (t-test: ephemeral, t =-2.05, df = 70, P = 0.04; intermittent, t = -2.75, df = 88, P = 0.007; perennial, t = -5.67, df = 82, $P < 0.001$; Figure 2.6). The effect of plant patch type was also significant (i.e., species; ANOVA: $F_{(6,208)} = 2.84$, $P = 0.01$; Table 2.2); however, there was a significant interaction among plant patch type and hydroperiod. For this reason, I discuss specific plant patch type effects in more detail under section "Effects of seasonal monsoon floods on patterns of denitrification."

Limiting substrates (hypothesis 2b)

The main effect of plant patch type on substrate limitation was not significant (P = 0.20). There was a significant effect of hydroperiod ($P < 0.001$) and a significant

interaction between plant patch type and hydroperiod ($P < 0.001$). Because of this significant interaction, the effect of plant patches will be discussed separately for each hydroperiod. For simplicity, plant patch effects will be discussed for the relative response of N treatment to C treatment (RR-N/RR-C) as this provides a general summary of the whether the patch was NO_3^- limited (RR-N/RR-C > 1), carbon limited (RR-N/RR-C < 1), or co-limited (RR-N/RR-C = 1). For ephemeral wetlands, none of the vegetated patches were significantly different from the open patch (Figure 2.7a). Further, the average RR-N/RR-C for ephemeral wetlands was not significantly different than one ($P = 0.13$; Figure 2.8d). Together, these results showed that ephemeral wetlands, generally, were not limited by either carbon or NO_3^-, and vegetation did not alleviate carbon limitation. At intermittent wetlands, the average RR-N/RR-C for all patch types, including unvegetated patches, was significantly greater than one ($P < 0.001$). Only patches of *Ludwigia peploides* had greater NO_3^- limitation than open patches ($P = 0.001$; Figure 2.7b). Together, these results showed that intermittent wetlands were consistently NO_3^- limited regardless of patch type (Figure 2.8d). In perennial wetlands, the average RR-N/RR-C for all patches, including unvegetated patches, was significantly greater one ($P < 0.001$). Only *Typha sp.* patches were more NO_3^- limited than open patches ($P = 0.02$; Figure 2.7c). Together, these results showed that denitrification in perennial wetlands, similar to intermittent wetlands, was NO_3^- limited regardless of patch type.

There was a significant effect of hydroperiod on substrate limitation in accidental urban wetlands. Average relative response (RR) of denitrification to N additions was significantly different across hydroperiods, increasing from ephemeral to intermittent to perennial wetlands (ANOVA: $F_{(2,233)} = 55.24$, $P < 0.001$; Figure 2.8a) suggesting

increasing NO_3^- limitation as wetlands were more frequently inundated. The average relative responses of denitrification to C additions were significantly different among hydroperiods, with ephemeral sites having an overall greater response than intermittent and perennial sites (ANOVA: $F_{(2,233)} = 6.95$, $P < 0.01$; Figure 2.8b). Average relative response of denitrification to N+C additions showed results similar to those with N additions, with slightly greater overall responses (ANOVA: $F_{(2,233)} = 55.33$, $P < 0.001$; Figure 2.8c). The average RR-N/RR-C, which reveals the relative strength of NO_3^- or carbon limitation, also showed increasing NO_3^- limitation from ephemeral to intermittent to perennial sites (ANOVA: $F_{(2,233)} = 55.10$, $P < 0.001$; Figure 2.8d). As discussed above, RR-N/RR-C values at ephemeral wetlands were not significantly different from one, suggesting denitrification in ephemeral wetlands was not limited by either NO_3^- or carbon.

RR-N/RR-C of intermittent and perennial wetlands were significantly greater than one, suggesting denitrification in these wetlands was NO_3^- limited. Together these results suggested that NO_3^- limitation of denitrification increased as wetlands were inundated more frequently. To confirm this, I examined how RR-N/RR-C changed with hydroperiod values for individual wetlands (percent flooded days per year rather than hydroperiod categories). Wetlands that were inundated for less than 45% of the year did not have RR-N/RR-C values different from each other, and did not have values significantly greater than one demonstrating neither carbon nor NO_3^- limitation at these wetlands (Figure 2.9). Whereas, wetlands inundated for more than 45% of the year also had RR-N/RR-C values that were not significantly different from each other, but were significantly different than wetlands that were inundated less frequently. Further, RR-

N/RR-C values for wetlands were significantly greater than one, suggesting NO_3^- limitation of denitrification in these wetlands (Figure 2.9).

Effects of seasonal monsoon floods on patterns of denitrification (hypothesis 3a)

To determine if seasonal monsoon floods changed patterns of denitrification, I examined interactions among season and plant patch type. The analysis of hypothesis 3a was divided into two separate analyses: ephemeral wetlands, and intermittent plus perennial wetlands. For the ephemeral wetlands, two-way ANOVA revealed that season and plant patch had significant effects on potential denitrification ($F_{(2,57)} = 3.77$, $P = 0.03$; $F_{(4,57)} = 3.29$, $P = 0.02$ respectively; Table 2.2), but there was no interaction between the two, indicating that monsoon floods controlled the magnitude of potential denitrification, but not the patterns. Potential denitrification was not significantly different in pre- versus post-monsoon season, but post-monsoon was significantly lower than the winter season despite similar soil moisture during those two seasons (Figure 2.3and 2.10a). While there was a significant main effect of plant patch (Table 2.2), pairwise comparisons revealed only one significant relationship: *Amaranthus sp.* had higher potential denitrification than open patches (P=0.04; Figure 2.10b).

For intermittent and perennial wetlands, three-way ANOVA revealed significant effects of hydroperiod and plant patch type ($F_{(1,151)} = 6.74$, $P = 0.01$; $F_{(3,151)} = 16.06$, $P <$ 0.001 respectively), and a three way interaction among season, hydroperiod, and plant patch type ($F_{(5,151)} = 2.80$, $P = 0.02$; Table 2.2). Comparing hydroperiods across seasons, potential denitrification at intermittent and perennial wetlands was significantly different in pre-monsoon and winter seasons, but became more similar to each other (and not significantly different) in the post-monsoon season (Figure 2.11a). To further deconstruct

this pattern and examine significant interactions, I looked at potential denitrification in plant patches across seasons at intermittent and perennial sites separately. At perennial sites, plant patches were significantly different in the pre-monsoon and winter season, but there were no significant differences among plant patches during the post-monsoon season (Figure 2.11b). Specifically, during the pre-monsoon season, potential denitrification in *Ludwigia peploides* patches was significantly higher than potential denitrification in all other patches. During the post-monsoon season, vegetated patches had significantly higher potential denitrification than in open patches, but did not differ from one another. During the winter season, grass and *Typha sp,* patches had significantly higher potential denitrification compared with open and *Ludwigia peploides* patches. At the intermittent sites, the increase in potential denitrification during the post-monsoon season was driven by an increase in one plant patch, *Ludwigia peploides* (Figure 2.11c). Specifically, during the pre-monsoon season, potential denitrification in *Typha sp*. and *Ludwigia peploides* patches was significantly higher than open patches while potential denitrification in *Ludwigia peploides* patches was significantly higher than in grass patches. During the post-monsoon season, potential denitrification in *Ludwigia peploides* patches was significantly higher than in all other patches, and potential denitrification in *Typha sp.* patches was significantly higher than potential denitrification in grass patches. During the winter season, only potential denitrification in *Ludwigia peploides* patches was significantly higher than all other patches.

I examined coefficients of variation (CV) to determine if monsoon floods had a homogenization effect (i.e. if monsoon floods reduced variation) on potential denitrification at individual wetlands. If monsoon floods homogenized potential

denitrification, then one would expect to see a decrease in CV in the post-monsoon season. There was a marginally significant increase in CV from the pre-monsoon to the post-monsoon season suggesting monsoon floods did not have a homogenizing effect on individual wetlands (0.81 vs 1.32; t-test: $t = -2.12$, df = 8, $P = 0.06$; Figure 2.12).

DISCUSSION

Findings from this study revealed that accidental urban wetlands in the Salt River, in Phoenix AZ, do have the capacity for denitrification. However, patterns of potential denitrification were not necessarily similar to patterns observed in nearby non-urban desert riverine wetlands. I first discuss notable findings in relation to the hypotheses. Then I discuss denitrification in accidental wetlands from this study compared to similar studies from non-urban wetlands. Finally, I discuss why accidental wetlands should be considered within the context of city planning and management.

Clear hydroperiod, but no seasonal variation at the reach scale (hypotheses 1a and 1b)

Accidental urban wetlands showed no seasonal variation in denitrification potential across the 30-km study reach, refuting hypothesis 1a. This was unexpected, as previous researchers have found that monsoon floods increased potential denitrification in nearby mesic and xeric desert river riparian areas (Harms et al. 2009). Despite the lack of seasonal effects on potential denitrification, wetlands may still experience reduced capacity to remove NO_3^- in the winter season as lower temperatures slow microbial processes and thus reduce denitrification rates (Pfenning and McMahon 1997; Hernandez and Mitsch 2007b). While Phoenix air temperature rarely falls below freezing, the winter-season sampling period did have lower average temperature compared to the pre- and

post-monsoon sampling season (18°C, 34.8°C and 23.6°C respectively; http://w2.weather.gov). However, I did not measure denitrification in the field and cannot conclusively say denitrification rate in wetlands was lower during the winter season.

My findings indicated that hydroperiod did affect potential denitrification in accidental urban wetlands. Hypothesis 1b was therefore supported, as potential denitrification increased with increased hydroperiod. This finding is similar to other studies in non-urban and constructed wetlands, where researchers have generally found that wetlands with permanent standing water had higher denitrification rates compared with wetlands that dry out occasionally (Koch et al. 1992; Johnston et al. 2001; Hernandez and Mitsch 2007b). Lower potential denitrification in ephemeral wetlands is likely due to persistent low soil moisture that creates a soil environment not conducive for denitrification (Peterjohn and Schlesinger 1991; Austin et al. 2004). However, in intermittent wetlands, lower potential denitrification may be due to greater oxygen availability compared to perennial wetlands. Oxygen inhibits the production of the enzymes that reduce NO_3^- to N_2 gas (Smith and Tiedje 1979; Körner and Zumft 1989).

Plant patch type affected potential denitrification (hypothesis 2a)

Similar to many studies, my results showed that the presence of vegetation enhanced potential denitrification (see Alldred and Baines 2016 for a recent meta-analysis). Plants have the capacity to alter the soil environment through various mechanisms, including increased soil organic matter, the trapping of sediments, and increased infiltration (Angers and Caron 1998; Brady and Weil 2008). In the intermittent and perennial wetlands, vegetated patches typically had more soil organic matter and a greater percentage of silt/clay, both of which can contribute to higher potential

denitrification (Groffman et al. 1996; Hernandez and Mitsch 2007a; Attard et al. 2011). This suggests that plant patches are modifying the soil environment making it more favorable for denitrification. *Typha sp.* and *Ludwigia peploides* patches showed the highest potential denitrification (Figure 2.11), but these plants are also considered by some city managers to be nuisance species (City of Phoenix, personal communication); both are listed by the USDA as weedy or invasive (http://plants.usda.gov). However, these species are beneficial for promoting denitrification in the accidental wetlands of the Salt River. I argue that this is an important consideration for city managers, particularly in Phoenix where NO_3^- removal is a valued ecosystem service.

Hydroperiod, not plant patch type, controlled limiting factors for denitrification (hypothesis 2b)

Hydroperiod, rather than plant patch type, appeared to control whether carbon or NO_3^- limited denitrification in accidental urban wetlands. I hypothesized that plant patches would alleviate carbon limitation; however, there were no differences in limiting substrates between open and vegetated patches in wetlands with the same hydroperiod. Ephemeral wetlands exhibited no limitation from carbon or NO_3^-, and intermittent and perennial wetlands exhibited strong NO_3^- limitation. An apparent "threshold" appears at ~45% days of inundation per year, at which point the wetlands switched from not being limited by either carbon or NO_3^-, to NO_3^- limitation. This is an interesting observation that warrants further investigation, as it is not clear if this pattern is driven by characteristics within the wetlands, or characteristics of the surrounding urban watersheds. Within-wetland characteristics could explain this pattern, as autochthonous carbon sources may be higher in wetlands with greater inundation duration and thus with

greater plant and algal growth that could alleviate carbon limitation in even open areas. However, watershed characteristics may also explain this pattern as land use can change the quantity and quality of allochthonous carbon inputs that enters accidental wetlands via urban stormwater baseflow and during floods. Newcomer et al. (2012) found that urbanized watersheds in Baltimore, Maryland had higher concentrations of dissolved organic carbon (DOC). Further, they showed that DOC derived from sources such as lawn clippings stimulated denitrification rates in urban streams (Newcomer et al. 2012). These findings have direct applications to this study, as the wetlands with more than 45% days of inundation per year received urban baseflow from watersheds that were largely residential, while the wetlands with less than 45% inundated days per year received urban baseflow from more industrial or agricultural watersheds (unpublished data). Therefore, wetlands that flood more frequently are not only getting DOC delivered more frequently, but also could be receiving higher concentrations of and better quality DOC due to the land-use difference among the storm watersheds. These differences could affect carbon limitation in urban wetlands.

Unexpected effect of monsoon floods and plant patches at ephemeral sites (hypothesis 1b and 3a)

Monsoon floods have been shown to increase potential denitrification at xeric sites in nearby desert rivers by orders of magnitude due to the creation of saturated conditions and delivery of NO_3^- and carbon (Table 2.3; Harms et al. 2009). Potential denitrification at ephemeral wetlands in this study did not increase after monsoon floods, opposite of what I hypothesized (1b). Potential denitrification did increase during the winter season, despite similar soil moisture conditions between the post-monsoon and

winter seasons. One reason for this may be the difference in the magnitude and timing of flood disturbance between those two seasons. Monsoon floods are typically more intense than winter floods and can flush out sediment that builds up in storm drains during the dry period prior to monsoon season (City of Phoenix Stormwater Services, personal communication). After the monsoon floods, I observed between 10 and 30 centimeters of new sediment deposited at the ephemeral wetlands. It is possible that this newly deposited sediment did not have many denitrifying microbes, which would lower potential denitrification in the recipient wetlands. However, this is speculative and should be investigated further by quantifying changes in microbial biomass in sediments.

Plant patch type also was not important for explaining patterns of potential denitrification in the ephemeral wetlands. Further, soil organic matter content was not significantly different among plant patches in the ephemeral wetlands (Figure 2.4). This suggests that plants may not serve as important "islands of fertility" for these systems, as they do in other resource-poor environments (Schlesinger et al. 1996). Taken together, accidental ephemeral wetlands did not show predictable patterns of potential denitrification as a result of either monsoon floods or plant patch type.

Unclear effect of reach-scale homogenization after monsoon floods (hypothesis 3a)

Monsoon floods had no discernable effects on denitrification in ephemeral wetlands (i.e., no significant interactions between season and plant patch type). However, monsoon floods did appear to affect the magnitude of potential denitrification, as discussed in the previous section. Potential denitrification in intermittent and perennial wetlands, however, did have a significant interaction among season and plant patches, suggesting an influence of monsoon floods on patterns of denitrification. Potential

denitrification patterns in perennial wetlands suggested that monsoon floods could have a homogenizing effect as potential denitrification was more similar among plant patches after monsoon floods (Figure 2.11b). To check if this pattern was due to homogenization, I examined the variation within individual wetlands. If monsoon floods were homogenizing potential denitrification, the variance within each individual wetland should decrease after the monsoon season as well. However, I found that eight of the nine wetlands showed an increase in intra-site CV after monsoon floods. This suggested that the observed pattern of homogenization in perennial wetlands may be an anomaly and therefore not a result of homogenization. Conducting a follow-up study across multiple monsoon seasons would more clearly elucidate the effect of monsoon floods on patterns of potential denitrification. Future studies should also consider measuring the magnitude of floods in individual wetlands. Storm drain networks break up large urban watersheds into smaller storm pipe-sheds that differ greatly in size and incorporate different stormwater control infrastructures (Hale et al. 2014). Both of these characteristics will affect the magnitude of floods experienced by recipient accidental wetlands. Consequently, individual wetlands within the same river reach experience floods of widely different magnitudes during the same rain event, which should differentially influence patterns of denitrification in downstream wetlands.

Potential denitrification in accidental urban wetlands compared with other locations

My results have shown that accidental urban wetlands have the capacity for denitrification, but this capacity does not appear to be greater than has been found in other green infrastructures in Phoenix, Arizona (Table 2.3). For example, retention basins are features of urban landscapes intended to capture water during storms, which

subsequently remove nitrogen from stormwater (Hale et al. 2014). Retention basins are typically not flooded between storms, but they have potential rates of denitrification that are similar to the perennial wetlands in my study (Zhu et al. 2005). Indian Bend Wash is another designed green flood-management feature in Scottsdale, Arizona. Rather than retention basins, Indian Bend Wash is designed with a series of permanently flooded shallow lakes and streams surrounded by grassy floodplains, more similar to perennial wetlands in this study. However, potential denitrification in these lakes is more than four times higher than potential denitrification in the accidental perennial wetlands downstream of the Wash (Roach and Grimm 2011). The one advantage of accidental urban wetlands is that they do not require design or maintenance investments, saving time and money for city managers.

I also found that potential denitrification in accidental urban wetlands was higher than has been reported for nearby non-urban desert river wetlands (Table 2.3). Interestingly, despite the fact that potential denitrification in accidental urban ephemeral wetlands did not increase after monsoon floods, these wetlands had higher post-flood potential denitrification than nearby desert riverine wetlands (Harms et al. 2009). This suggests that urban ephemeral wetlands have an increased capacity for denitrification, possibly due to resource subsidies of organic carbon and nitrogen supply from the urban environment. Other studies have found that nitrogen deposition from urban areas can modify substrate limitation and increase denitrification in lakes (Elser et al. 2009; McCrackin and Elser 2010), and can increase microbial processes, such as soil respiration, in deserts (McCrackin et al. 2008).

CONCLUSION

Urbanization is often associated with increases in downstream N export (Shields et al. 2008; Kaushal et al. 2011); however, certain landscape features have been shown to mediate this downstream N supply (Passeport et al. 2012; Koch et al. 2014). 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. However, accidental wetlands are also at risk of disappearing because their "unmanaged" status largely precludes them from consideration in city planning and management schemes. As Phoenix (and many cities) implements policies to become more efficient with water use (Gober et al. 2010), less urban baseflow will likely be generated. This will almost certainly reduce the area and number of accidental wetlands in the Salt River. While reducing urban baseflow is generally considered to be positive from a sustainability standpoint, the results of my study suggest an unexpected trade-off: the subsequent reduction in accidental wetland area, which may have negative consequences for NO_3^- removal from the surface waters of Phoenix. Excess NO_3^- could then contribute to downstream pollution or infiltrate groundwater posing risks for human health and the health of downstream ecosystems (Nolan et al. 1997; Vitousek et al. 1997; Townsend et al. 2003). The findings in this study suggest that if accidental urban wetlands are inundated less frequently (i.e. shift from functioning as intermittent and perennial wetlands to functioning as ephemeral wetlands), NO_3^- removal during storm events will likely decrease.

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FIGURES AND TABLES

Table 2.1. Season and plant patches sampled at each site. Bolded plant patches indicate patches that were not sampled during winter sampling effort.

Site ID (abbreviation)	Season sampled	Plant patches sampled
Ephemeral 1 (E1)	Pre-monsoon Post-monsoon Winter	Open *Rumex dentatus* Grass *(Cynodon dactylon)*
Ephemeral 2 (E2)	Pre-monsoon Post-monsoon Winter	Open *Amaranthus sp.* *Tribulus terrestris*
Ephemeral 3 (E3)	Pre-monsoon Post-monsoon	Open Grass *(Schismus sp.)*
Intermittent 1 (I1)	Pre-monsoon Post-monsoon Winter	Open *Ludwigia peploides* *Typha sp.* **Grass *(Paspalum distichum)***
Intermittent 2 (I2)	Pre-monsoon Post-monsoon Winter	Open *Ludwigia peploides* *Typha sp.* **Grass *(Paspalum distichum)***
Intermittent 3 (I3)	Pre-monsoon Post-monsoon	Open *Typha sp.* Grass *(Paspalum distichum)*
Perennial 1 (P1)	Pre-monsoon Post-monsoon Winter	Open *Typha sp.* Grass *(Paspalum distichum)*
Perennial 2 (P2)	Pre-monsoon Post-monsoon Winter	Open *Ludwigia peploides* *Typha sp.*
Perennial 3 (P3)	Pre-monsoon Post-monsoon	Open *Ludwigia peploides* *Typha sp.* Grass *(Paspalum distichum)*

Table 2.2. F statistic for ANOVA comparisons of potential denitrification rates. Degrees of freedom are in parentheses. Interactions include hydroperiod * season (H*S), hydroperiod * patch (H*P), season * patch (S*P) and hydroperiod * season * patch (H*S*P). Dashes indicate factors or interactions not included in model.

	Hydro-period	Season	Patch	H*S	H*P	S*P	H*S*P
Three-way ANOVA for all sites	31.29** (2,208)	1.76 (2,208)	2.84** (6,208)	2.13 (4,208)	2.64* (4,208)	1.47 (12,208)	1.89 (7,208)
Two-way ANOVA for ephemeral sites	--	3.77* (2,57)	3.28* (4,57)	--	--	0.88 (8,57)	--
Three-way ANOVA for "wet" sites	6.74* (1,151)	0.17 (2,151)	16.06** (3,151)	4.03* (2,151)	4.22* (3,151)	0.91 (6,151)	2.80 (5,151)

* $P \leq 0.05$
** $P \leq 0.001$

Table 2.3. Comparisons of literature values of potential denitrification. All measurements of potential denitrification are by denitrification enzyme activity (DEA).

Ecosystem type Location	DEAs ($\mu g\ N_2O\text{-}N\ g\ soil^{-1}\ h^{-1}$)	Reference
Urban features in Phoenix, Arizona		
Accidental urban wetlands; Phoenix, Arizona	Ephemeral: 0.10 Intermittent: 0.44 Perennial: 0.65	This study
Modified urban stream; Phoenix, Arizona	Lake: 4.77 Stream: 1.07 Floodplain: 1.54	Roach and Grimm 2011
Retention basins; Phoenix, Arizona	0.73	Zhu et al. 2005
Residential yards; Phoenix, Arizona	Xeriscaped yards: 1.5 Lawns: 2.7	Hall et al. 2009
Deserts and streams in Arizona		
Sonoran desert; Phoenix, Arizona	Inter-plant spaces: 0.04 Under plants: 0.20	Hall et al. 2009
Desert stream; Sycamore Creek; Arizona	Parafluvial: 0.015 River bank: 0.06 – 0.08	Holmes et al. 1996
Desert stream riparian area; San Pedro River, Arizona	Xeric pre-monsoon: 0.00045 Xeric post-monsoon: 0.0045 Mesic pre-monsoon: 0.014 Mesic post-monsoon: 0.25	Harms et al. 2009
Urban or agriculture wetlands in other regions		
Restored urban riparian area; Baltimore, Maryland	0.28, 0.77 (two different restored sites)	Gift et al. 2010
Constructed wetland; Columbus, Ohio	Emergent macrophytes: ~0.07 Open water: ~0.05 Forested edge: ~0.02	Hernandez and Mitsch 2007a
Restored agriculture ditch; Shatto Ditch, Indiana	Vegetation removed: ~0.5 Vegetated: ~0.7	Roley et al. 2012
Riparian area; Baltimore, Maryland	Forested reference: 0.46 Urban: 2.2	Groffman and Crawford 2003

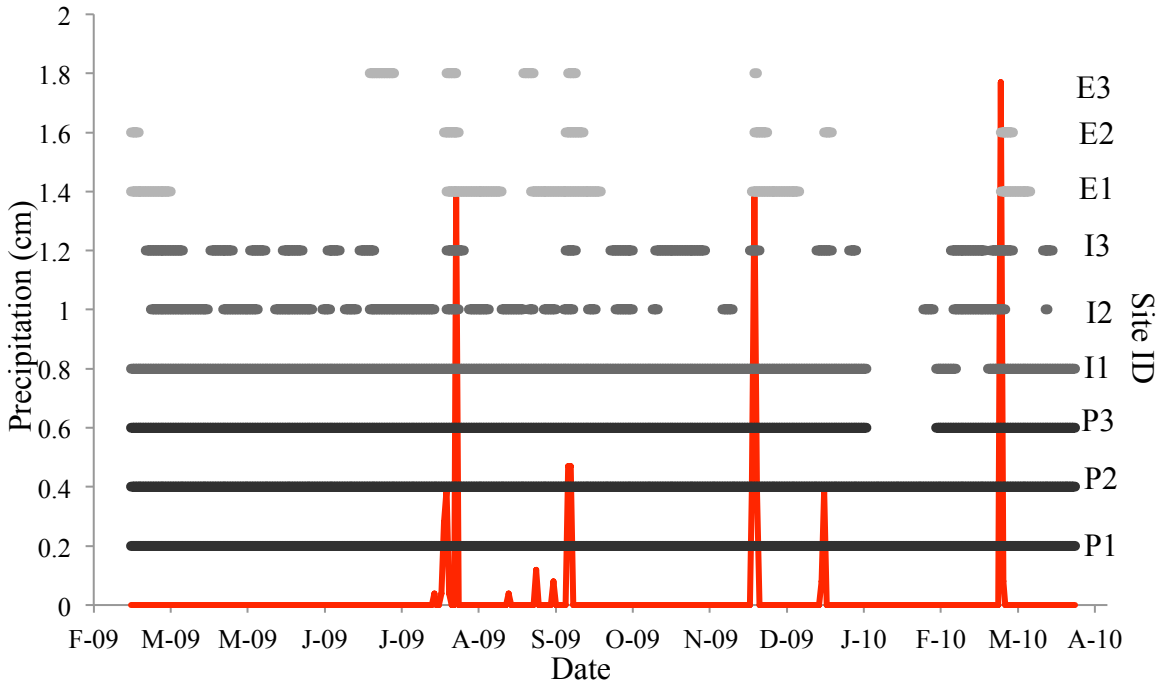

Figure 2.1. Presence of inundated conditions and precipitation record at nine sites. Precipitation record for gage at site P2 during sampling period (http://www.fcd.maricopa.gov/Weather/Rainfall/ALERT/ssdata.aspx). P=perennial wetlands, I=intermittent wetlands, E= ephemeral wetlands. Red line represents the precipitation record. Presence of gray line represents inundation at give date.

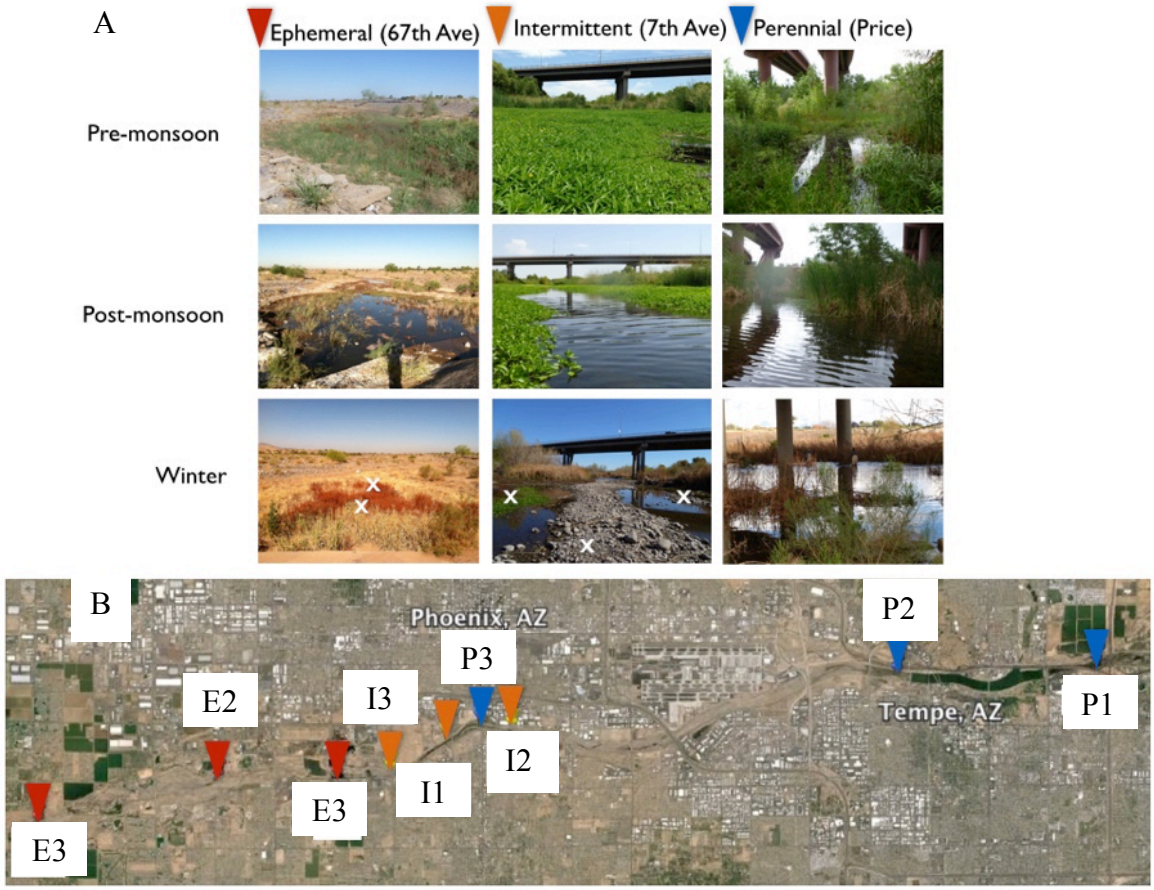

Figure 2.2. Images of site locations. (A) Images of wetlands of different hydroperiods across seasons. (B) Map of nine selected sites along Salt River. Red triangles represent ephemeral sits, orange triangles represent intermittent sites, and blue triangles represent perennial sites. White x's represent examples of patch types.

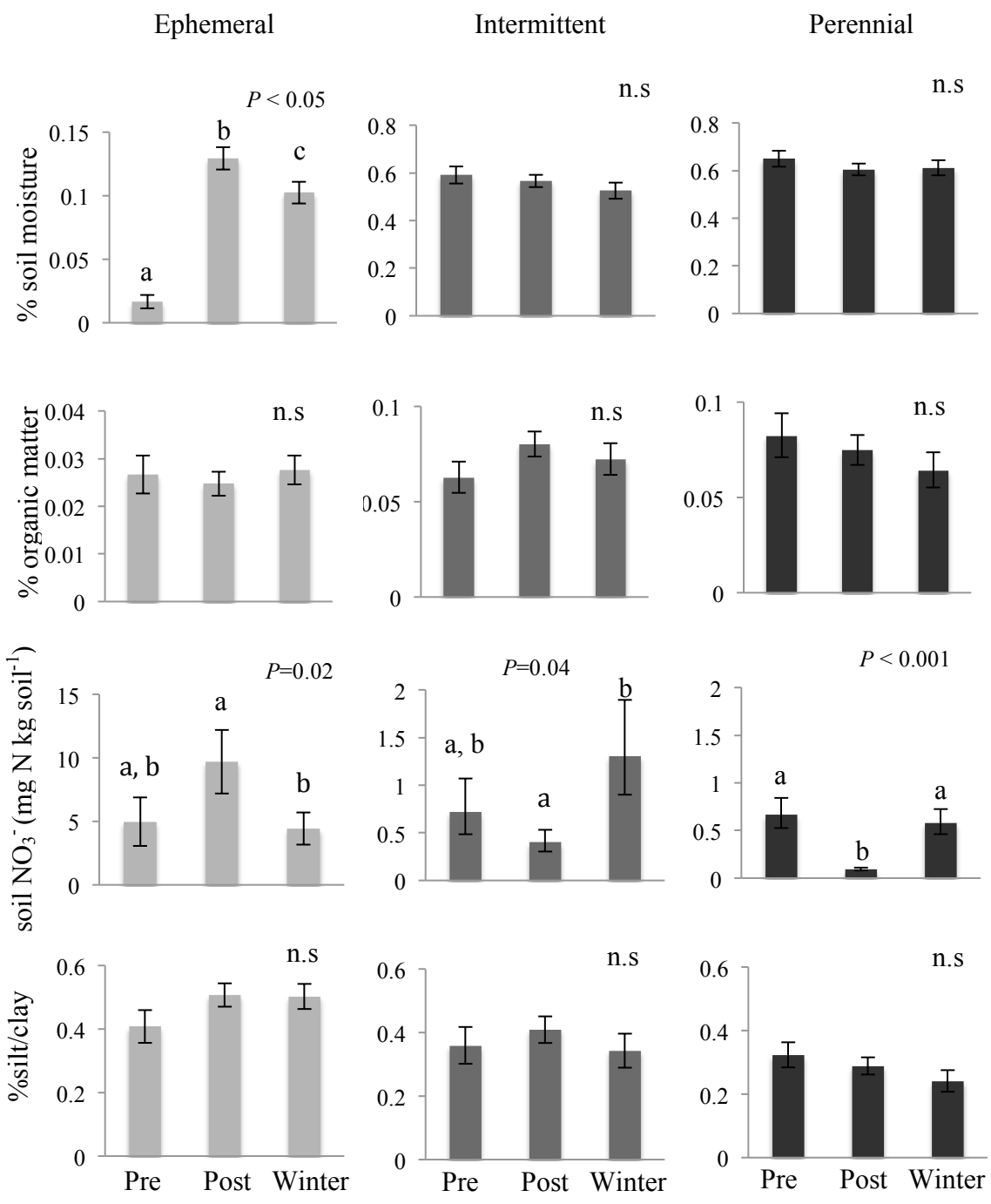

Figure 2.3. Arithmetic means of soil variables for ephemeral, intermittent, and perennial wetlands across seasons. Pre indicates pre-monsoon, and post indicates post-monsoon season. Note scale differences. Error bars indicate ±1 SE.

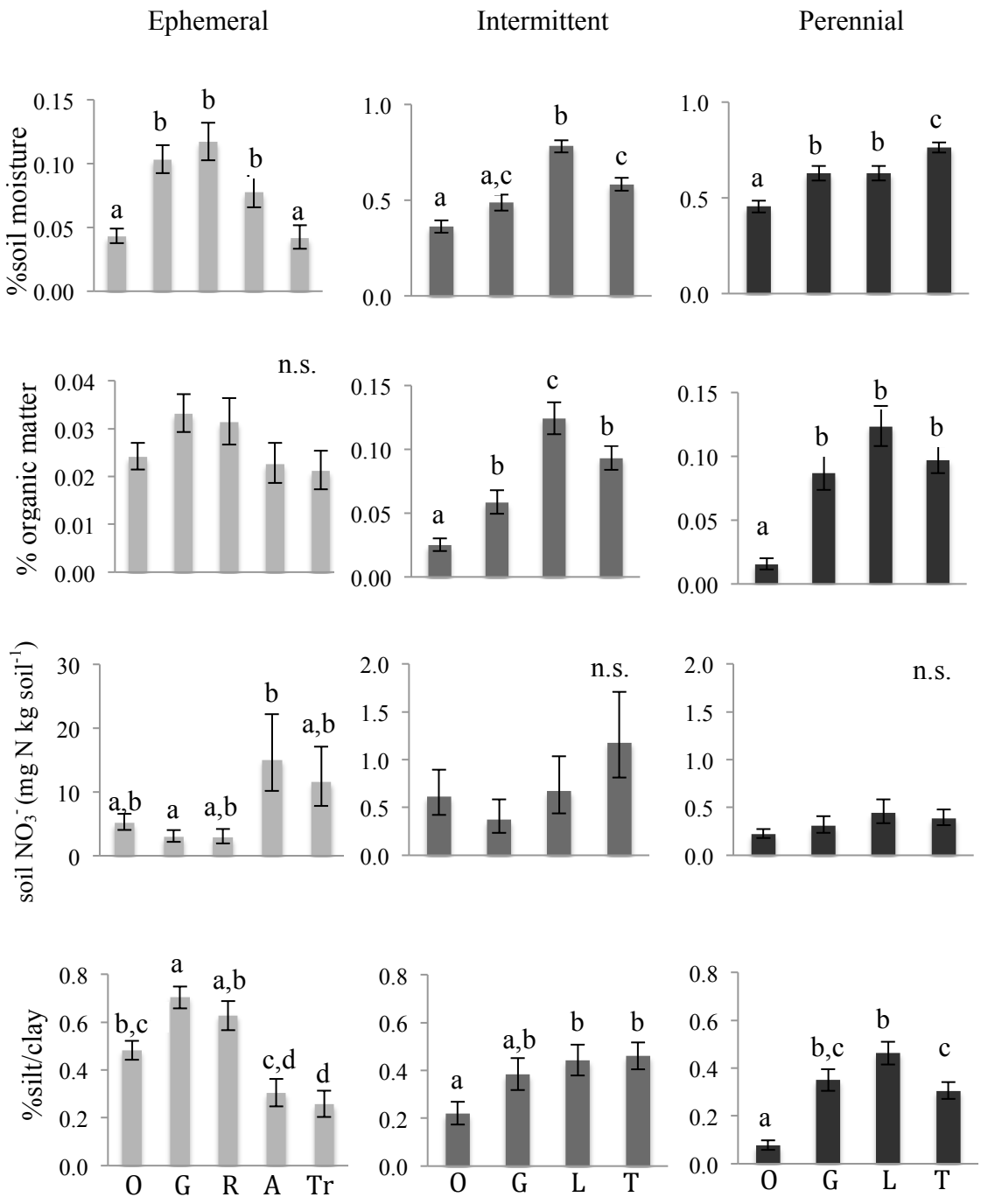

Figure 2.4. Arithmetic means of soil variables for plant patches at ephemeral, intermittent, and perennial wetlands. O=open, G=grass, R=*Rumex dentatus,* A=*Amaranthus sp*,, Tr=*Tribulus terrestris*; L=*Ludwigia peploides*, T=*Typha sp*. Note scale differences. Significance levels are $P < 0.05$. Error bars indicate ±1 SE.

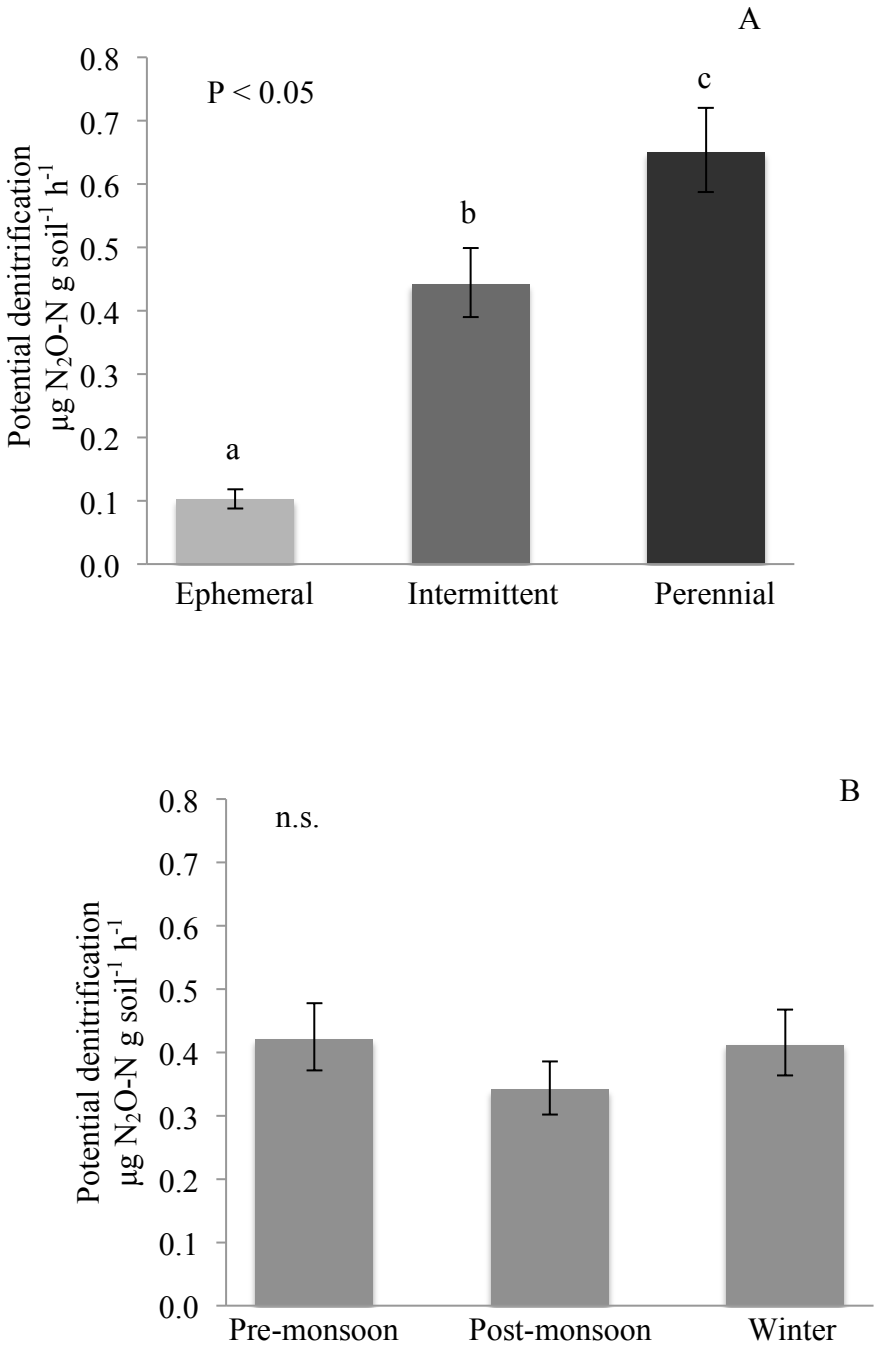

Figure 2.5. Estimated marginal means of three-way ANOVA comparing potential denitrification (DNP) for season and hydroperiod for all sites. (A) DNP across seasons; (B) DNP across hydroperiods. Error bars indicate ±1 SE.

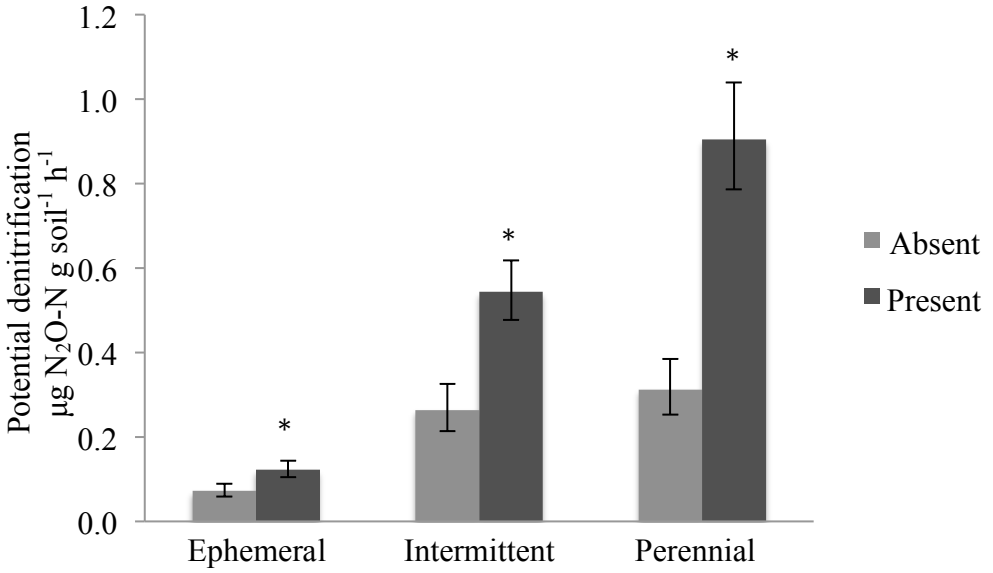

Figure 2.6. Effect of the presence and absence of vegetation on potential denitrification for each hydroperiod. Asterisks denote significant differences within a hydroperiod at $P < 0.05$. Error bars indicate ±1 SE.

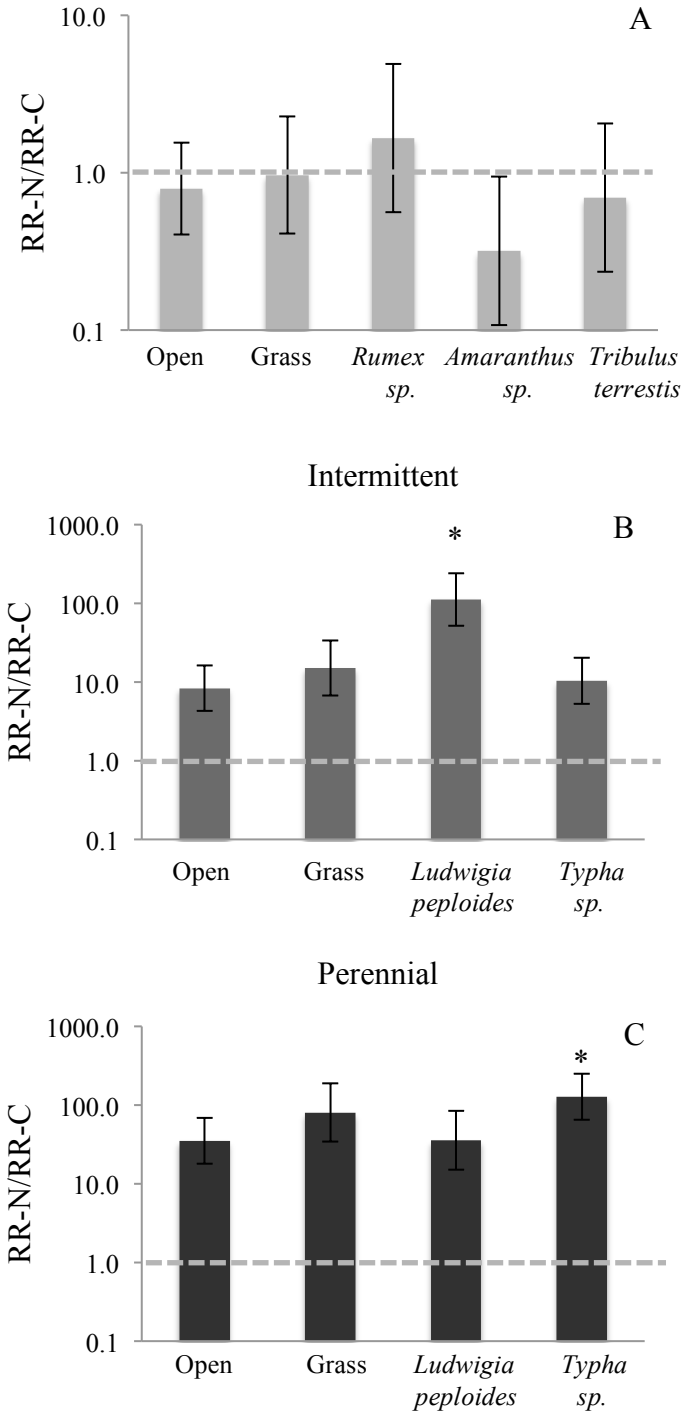

Figure 2.7. Average RR-N/RR-C for plant patches at different hydroperiods. Asterisks denote plant patches significantly different than open patches at $P < 0.05$. Values above one indication greater nitrate limitation, values below one indication greater carbon limitation. Note scale differences. Bars represent 95% confidence interval.

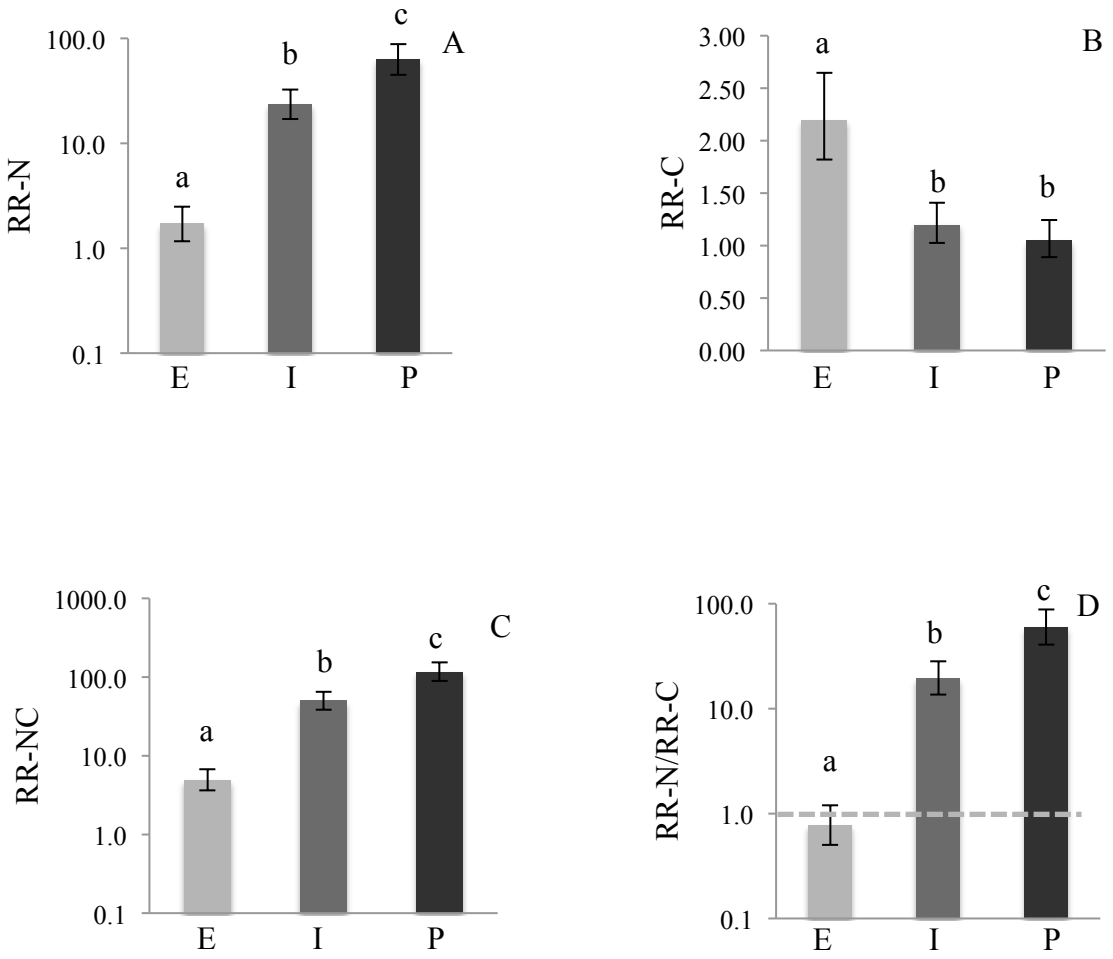

Figure 2.8. Average relative responses (RR) of denitrification limitation experiments. (A) additions of nitrate; (B) additions of carbon; and (C) additions of nitrate and carbon. (D) Relative response of nitrate additions to carbon additions (RR-N/RR-C). Values above one indication greater nitrate limitation, values below one indication greater carbon limitation. Note scale differences: graphs A,C,D are log scale, graph B is not. E= ephemeral; I= intermittent; P= perennial. Bars represent 95% confidence interval.

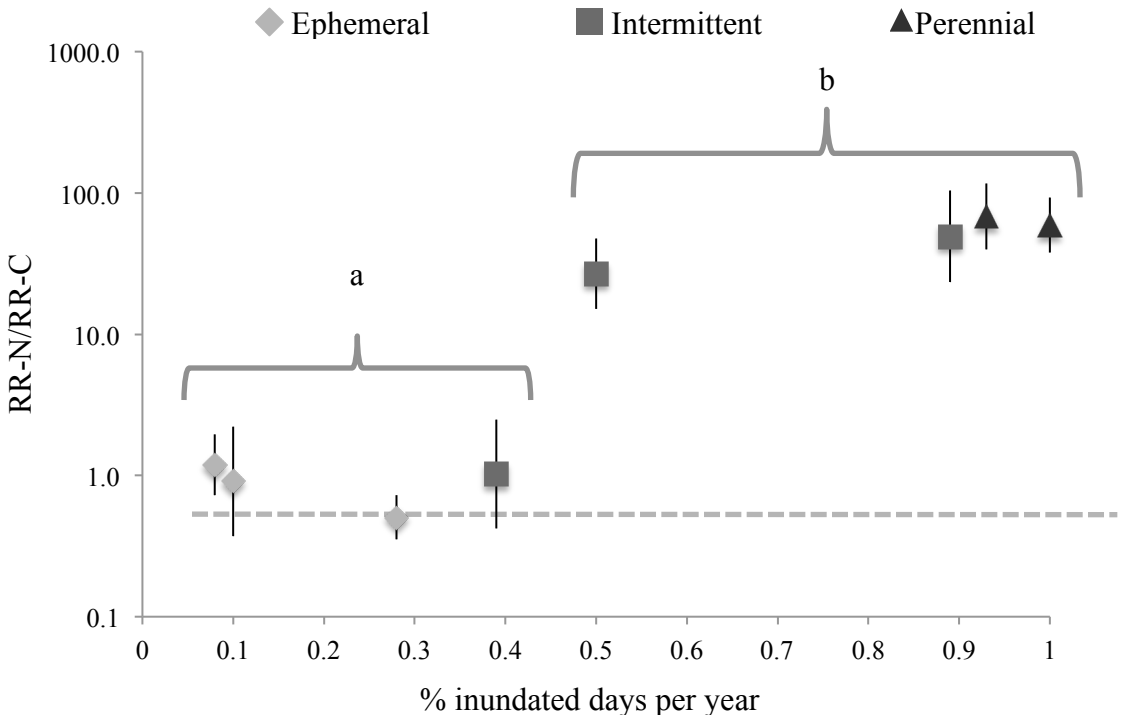

Figure 2.9. Changes in response ration (RR) of nitrogen relative to carbon (RR-N/RR-C) as a function of hydroperiod (%inundated days per year). Values greater than one indication greater nitrate limitation, values below one indication greater carbon limitation. Perennial sites show only two markers because two of the three perennial sites were inundated for the same period of time—100% of the year. Y-axis is log scale. Bars represent 95% confidence interval.

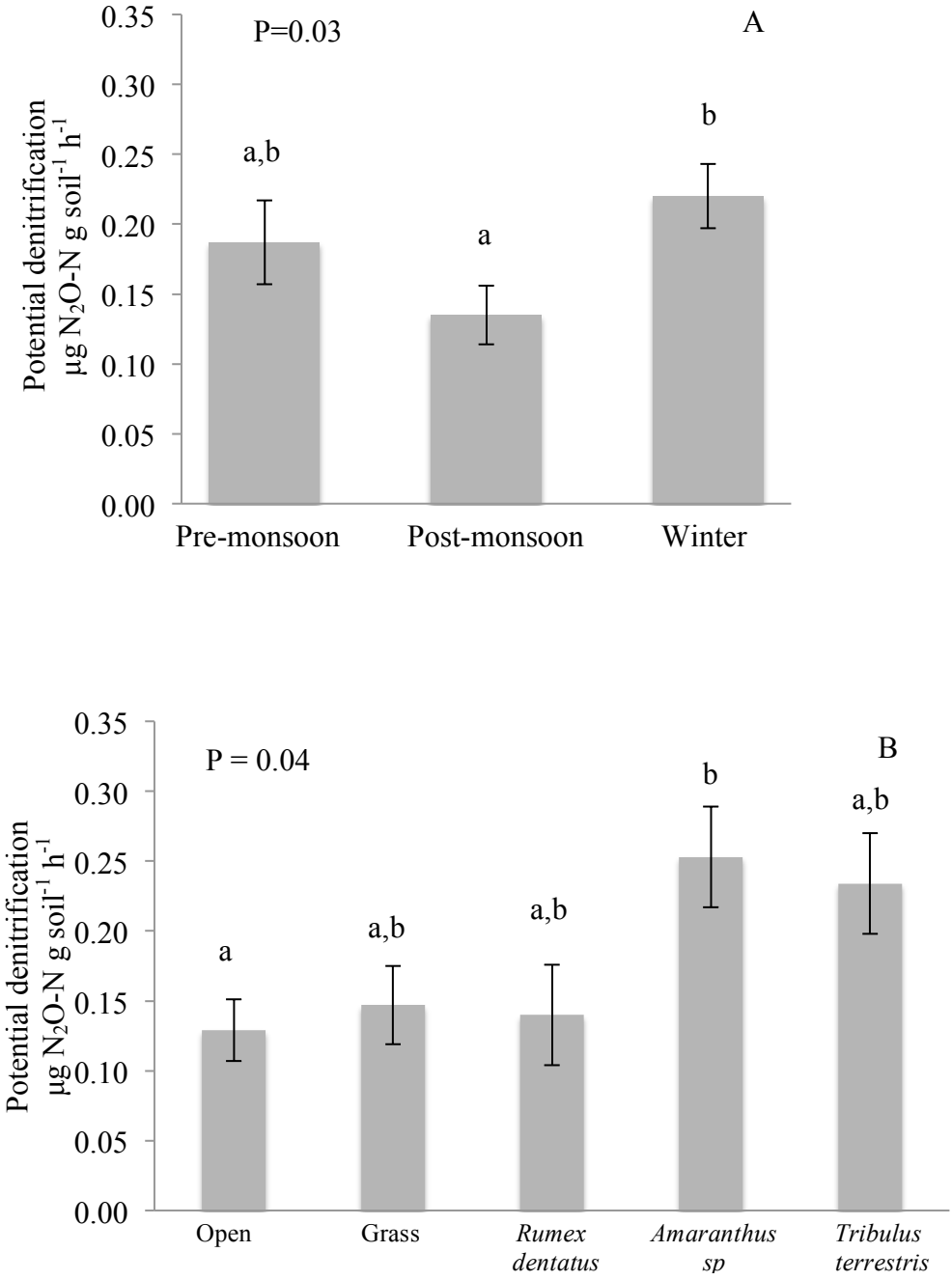

Figure 2.10. Estimated marginal means of potential denitrification (DNP) for main effects for two-way ANOVA for ephemeral sites. (A) DNP across seasons; (B) DNP among plant patches. Error bars indicate ±1 SE.

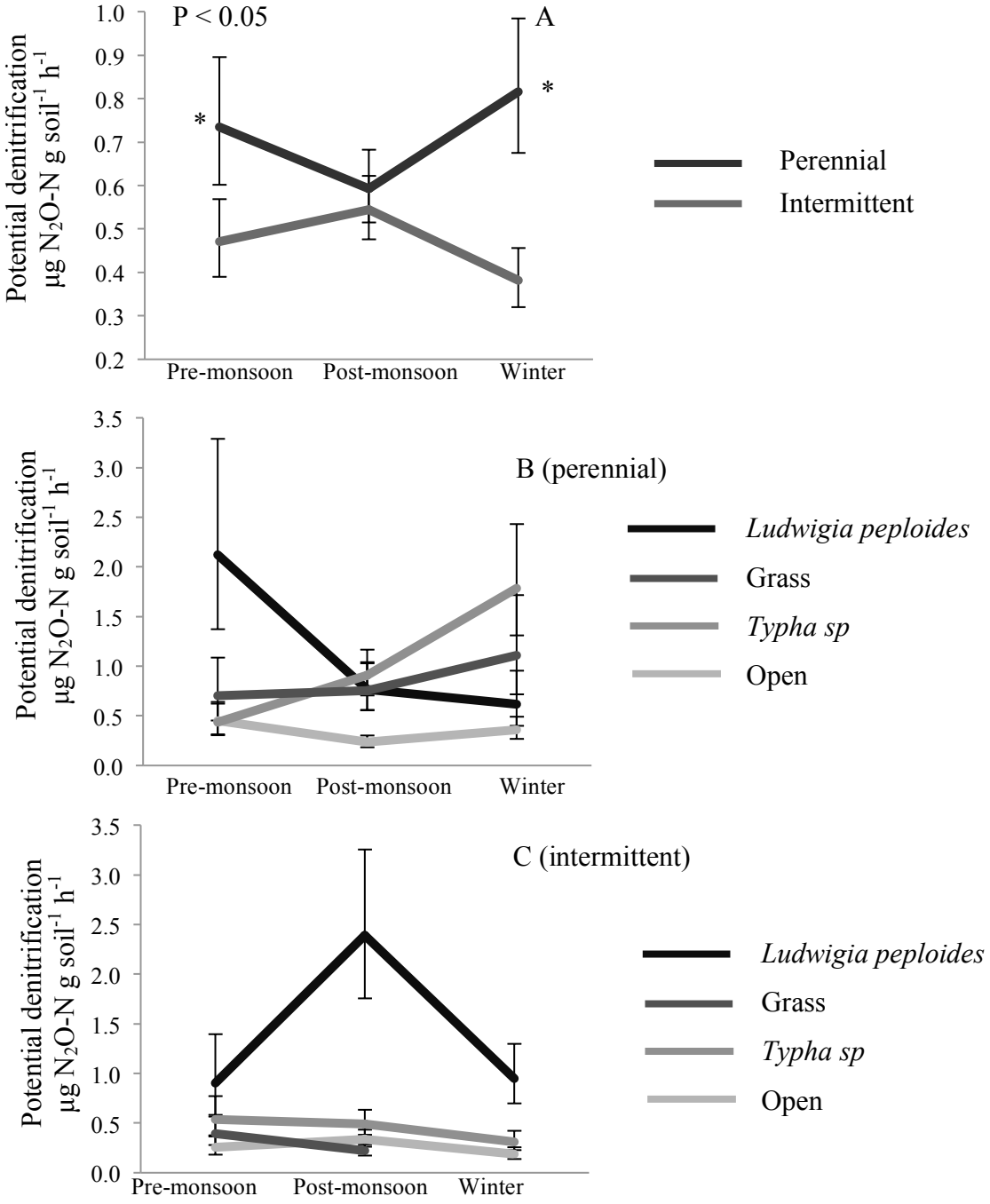

Figure 2.11. Estimated marginal means of three-way ANOVA for intermittent and perennial wetlands. (A) Interaction plot between hydroperiod and season; (B) Interaction plot between plant patches and season for perennial wetlands; (C) Interaction plot between plant patches and season for intermittent wetlands. Note scale differences. Error bars indicate ±1 SE.

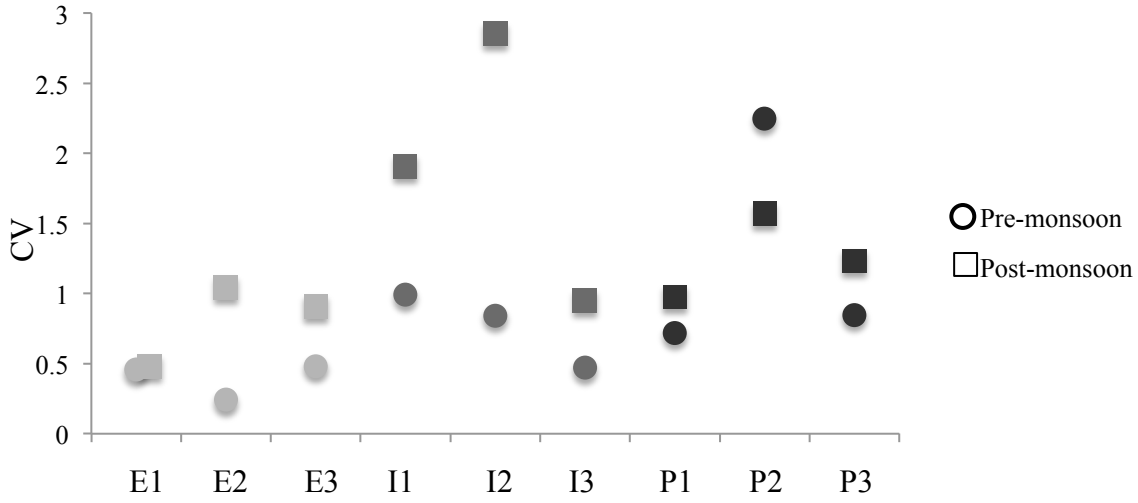

Figure 2.12. Coefficients of variation (CV) for each site. Circles represent pre-monsoon CVs. Squares represent post-monsoon CVs. Different shades of gray represent hydroperiod categories. $P = 0.06$ for difference between pre- and post-monsoon.

Chapter 3

DIRECT AND INDIRECT EFFECTS OF PLANT TRAITS ON POTENTIAL DENITRIFICATION

ABSTRACT

In wetlands, denitrification is an important ecosystem process that permanently removes reactive nitrogen from systems. Much research has examined how vegetation and different plant species affect denitrification in wetlands; however, few studies address the mechanisms by which plants affect denitrification. Plant functional traits are one method to mechanistically link plant species to ecosystem functions. In this study, I tested hypothesized indirect relationships between plant traits and denitrification through their effect on soil organic matter and soil nitrate.

I collected plant trait data and soils from nine urban wetlands located at storm drain outfalls in Phoenix, AZ. Wetlands were inundated between 10% and 100% of the year. Samples were taken from two to three plant patch types within each wetland. Potential denitrification (DNP), soil organic matter, and extractable nitrate were measured on soil samples. Measured plant traits were above- and belowground biomass, above- and belowground C:N ratios, and rooting depth. Hypothesized relationships between plant traits and DNP were modeled *a priori* using two different models: one model including only direct relationships between plant traits and DNP, and another model including indirect relationships between plant traits and DNP through soil conditions. Structural equation modeling was used to determine which model better fit the data and what plant traits significantly predicated DNP.

Modeling indirect effects of plant traits on DNP better fit the data than modeling plant traits as having direct effects only. Soil organic matter had greatest total effect on denitrification (0.67). Belowground biomass, belowground C:N ratio, and rooting depth had significant indirect relationships with DNP through soil organic matter. While above- and belowground C:N ratios and rooting depth had significant indirect relationships with DNP through soil nitrate. The model also identified residual direct paths from aboveground biomass, rooting depth, and aboveground C:N ratios to DNP. Residual direct paths indicate that the mechanisms by which these plant traits affected DNP were not identified in the model. Overall, this study shows that while we have an understanding how some plant traits, such as belowground biomass, increase denitrification, our overall knowledge about the mechanistic links between plant traits and DNP needs improvement.

INTRODUCTION

Wetlands are widely recognized as key ecosystems for mitigating widespread anthropogenic nitrogen pollution largely because wetlands are important sites of denitrification (Vitousek et al. 1997; Schlesinger 2009). Denitrification is a microbial process in which nitrate is reduced to N_2 gas and consequently is permanently removed from ecosystems. Wetland soils are effective sites for denitrification because inputs of carbon and nitrate provide necessary substrates for denitrifying bacteria, and because flooded soils produce anoxic conditions that are necessary for denitrification (Seitzinger et al. 2006). Wetlands also are characterized by hydrologic regimes that cycle wetlands between wet and dry stages, which can couple the aerobic process of nitrification

(oxidation of NH_4 to NO_3^-) to the anaerobic process of denitrification (Seitzinger et al. 2006), creating conditions that remove both NH_4 and NO_3^-.

Denitrification studies often focus on how soil resources and conditions, such as labile carbon, nitrate, and moisture, affect denitrification (Drury et al. 1991; Seitzinger 1994; Groffman et al. 1996; Ettema et al. 1999; Pinay et al. 2000; Lowrance and Hubbard 2001; Groffman and Crawford 2003). Vegetation can modify the soil resources that affect denitrification (Hobbie 1992; Eviner and Chapin 2003). Specific plant traits (e.g., rooting depth, biomass) and processes (e.g. maintenance of an oxic microrhizosphere) can alter the soil environment through litter deposition, sediment trapping, oxygen availability, and connectivity between surface and soil waters (Chapin et al. 2000; Lavorel and Garnier 2002; Wardle et al. 2004; Zedler and Kercher 2004). A comparison of plant functional traits has been proposed as a way to mechanistically ascertain how plants may affect ecosystem processes such as denitrification (Diaz et al. 2004; Wardle et al. 2004). For this study, I asked: Do plant traits affect denitrification indirectly through changes in soil conditions?

Very few studies have examined the relative importance of functional plant traits and environmental variables for predicting denitrification (see but Sutton-Grier et al. 2012). One challenge in disentangling the effects of plant traits and soil environmental variables is that such variables tend to be correlated (Diaz et al. 2004). Structural equation modeling (SEM) is an analytic technique that accounts for correlation among variables. Further, SEM not only tests relationships among variables, but also allows for the testing of hypotheses about *how* those variables are related by specifying a model *a priori*. It also allows for the modeling of indirect relationships among variables.

Therefore, one can test not only which plant traits and soil conditions are important for predicting denitrification (evaluating model parameterization), but also whether hypothesized relationships among plant traits, soil conditions, and denitrification are correct (evaluating model structure).

The importance of labile soil carbon, nitrate, and anoxic conditions for denitrification are well documented (Drury et al. 1991; Ettema et al. 1999; Pinay et al. 2000; Lowrance and Hubbard 2001; Groffman and Crawford 2003), but how plants control denitrification by modifying soil conditions is not well understood. Previously, studies examining how plants affect denitrification focused on either the presence/absence of vegetation, or specific species effects. A recent meta-analysis of 419 studies examining the effects of wetland vegetation on denitrification found that, on average, the presence of plants increased denitrification by 55% (Alldred and Baines 2016). Other studies suggest that the presence of vegetation can alleviate carbon limitation on denitrifying bacteria in carbon-poor environments (Schade et al. 2001; Heffernan and Fisher 2012). Contradictory results have been obtained from studies of effects of various plant species on denitrification: some have shown an effect of plant species identity (Windham and Ehrenfeld 2003; Hernandez and Mitsch 2007a; Pinay et al. 2007), while others have not (Groffman et al. 1996; Otto et al. 1999; Clément et al. 2002; Roley et al. 2012; Song et al. 2014). Species comparisons do not inherently take into account the mechanisms by which different species might affect denitrification, potentially explaining the contradictory findings.

Plant traits have the potential to provide mechanistic explanations for how different species affect denitrification. Traits such as biomass, tissue chemistry, and

rooting depth can modify soil conditions consequently affecting denitrification. Plants provide organic carbon sources through plant litter (Gift et al. 2010) and root exudation (Farrar et al. 2003; Bais et al. 2006). Further, the stoichiometry of plant litter or root exudates (C:N of tissues) affects decomposition rates and nitrogen mineralization, thus affecting the availability of soil carbon and nitrate (Craine et al. 2002; Cornwell et al. 2008). Roots can loosen soils, increasing infiltration and extending the depth to which denitrification occurs (Angers and Caron 1998; Rotkin-Ellman et al. 2004; Gift et al. 2010). Further, roots deliver oxygen to the soil altering redox conditions, at least locally around the roots, and affecting denitrification in one of two ways. (1) Denitrification could increase by creating aerobic zones that promote nitrification increasing nitrate available to denitrifiers (Reddy et al. 1989). (2) Denitrification could decrease because oxygen inhibits denitrification, an outcome more likely when nitrate is not a limiting substrate for denitrification (Hernandez and Mitsch 2007b).

For this study, I selected five plant traits that I hypothesized were most likely to affect denitrification in urban wetlands and used SEM to examine the relative importance of plant traits and soil conditions for explaining potential denitrification. Specifically, I compared two different models: one with direct paths between individual plant traits or soil conditions and potential denitrification, and one where plant traits were indirectly linked to denitrification via soil conditions (Figure 3.1). I determined which model better fit the data and then asked the following questions: (1) What plant traits are important for predicting potential denitrification? (2) Are plant traits or soil conditions more important for predicting potential denitrification?

METHODS

Study Area

This study was conducted in *accidental urban wetlands* located in a 30 kilometer reach of the Salt River in Phoenix, Arizona USA. Because these wetlands were not designed or planned by the City of Phoenix, and are not managed, I refer to them as "accidental." The historically perennial Salt River is now a mostly dry riverbed as it bisects downtown Phoenix. In this reach of the Salt River, the floodplain has been highly modified and engineered for flood management, and in places it is mined for gravel. However, these accidental urban wetlands have formed at storm drain outfalls. These outfalls not only discharge stormwater during storm events, but some also discharge urban baseflow during dry periods into the Salt River bed. I identified nine study wetlands receiving storm drain discharges that differed in their hydroperiod ranging from inundation from 10% to 100% of the year. A large range of hydroperiods were included in this study to identify plant traits and soil conditions that promote denitrification over the full range of flooding conditions experienced by *accidental urban wetlands* of the Salt River.

Plant trait selection

I selected five plant traits that I hypothesized could affect denitrification via their effect on soil conditions known to affect denitrification (soil organic matter and soil nitrate; Seitzinger et al. 2006): (1) aboveground biomass (AG); (2) belowground biomass (BG); (3) aboveground C:N ratio of plant tissues (CNAG); (4) belowground C:N ratio of plant tissues (CNBG); and (5) rooting depth (RD; Table 3.1). I expected that aboveground biomass and belowground biomass would affect potential denitrification by

affecting the quantity of carbon available to denitrifiers, with increases in biomass resulting in increases in soil organic matter (Ehrenfeld 2003; Wardle et al. 2004; Gift et al. 2010). I expected C:N ratios of above- and belowground biomass would affect both soil organic matter and soil nitrate. The C:N ratio of plant tissues affects how easily plant tissues are decomposed and how much nitrogen is mineralized (Craine et al. 2002; Wardle et al. 2004; Cornwell et al. 2008; Cotrufo et al. 2013). Lower C:N ratios denote "higher quality" tissues that are more easily decomposed. I expected decreasing C:N ratios to result in increases in soil organic matter and soil nitrate. Rooting depth could also affect potential denitrification by increasing the depth that nitrate was able to infiltrate into the soil. Roots have the potential to loosen soils, increasing infiltration; therefore, I expected increases in rooting depth result in increases in soil nitrate.

Plant trait measurements

One to three dominant plant patches were identified in each study wetland, vegetated by a total of eight different species and species groups (Table 3.2). The two species groups were patches of *Typha sp*. and *Amaranthus sp.* Patches of *Typha sp.* contained either *Typha latifolia* or *Typha domingensis*. Patches of *Amaranthus sp*. contained either *Amaranthus palmeri* or *Amaranthus albus*. In general, these species did not occur in mixtures, but rather in monotypic patches as are commonly found in wetlands (Spence 1982).

Measurements of plant traits were made in the field three times between June 2013 and March 2014 to capture seasonal variation. Notably, only six of the nine sites were sampled during the third sampling period because of sampling constraints. In each patch, aboveground and belowground biomass was harvested from three 100 cm^2

quadrats. Additionally, rooting depth was measured in each quadrat. Biomass samples were brought to the lab, rinsed to remove soil, dried at 60 °C for a week, and weighed. After weighing, plants were ground using a Wiley mill and a subsample was analyzed to determine C:N ratios of plant tissues using a PE2400 CHN elemental analyzer. The triplicate quadrat data from each patch were averaged to designate the trait measurements for each particular patch, at each site (n=9), and in each season (n=3). These patch, site, and season-specific trait measurements allowed me to more accurately assess links between plant traits and denitrification, as plant traits vary with season and in response to a variety of environmental conditions (Callaway et al. 2003).

Soil sampling

Soil cores were also collected three times between June 2013 and March 2014. I collected two to four soil cores for each plant patch type at each site (n = 164). Soil cores of the same patch type were taken from different patches when possible. If only a single large contiguous plant patch occurred at a site, cores were taken at least 5 meters apart. Soil cores were taken to a maximum depth of 10 cm to encompass the most active part of the soil (Groffman et al. 1999). Cores were stored on ice in the field and then refrigerated in the lab until processing (usually within 24 hours). For this processing, soil cores were homogenized and analyzed for soil organic matter, soil nitrate, and potential denitrification. Soil organic matter was determined by mass loss on ignition at 550°C for 4 hours. Soil nitrate was extracted by shaking 10 grams of sample with 50mL 2M KCl for 1 hour, filtering through pre-leached Whatman 42 ashless filters, and analyzed on a Lachat QC8000 flow-injection analyzer.

Denitrification was measured in the lab as potential denitrification. Potential denitrification is a measurement of denitrification under optimal conditions (excess labile carbon, excess nitrate, and anaerobic soils; Groffman et al. 1999). While potential denitrification does not give actual rates of denitrification, it reflects activity of soil denitrifier microorganisms, is related to long term patterns of environmental conditions such as carbon availability, and allows for the processing of many samples necessary for complex statistical models (Groffman et al. 2006). Potential denitrification was measured using denitrification enzymes assays (Groffman et al. 1999). Fifty grams of soil and 50 mL of media were added into a 125 ml Wheaton bottle. To ensure neither nitrate nor carbon were limiting, the media was 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). Headspace in the bottles was replaced with N_2 gas to ensure anaerobic conditions, and 10ml of acetylene gas was added to inhibit the reduction of N_2O to N_2. Samples were incubated at room temperature and shaken at 140 rpm for 4 hours. Gas samples were taken from the headspace at 30 minutes and 4 hours, and analyzed on a Varian 3800 gas chromatograph for N_2O concentration.

Statistical analyses

In this study, I proposed 2 models to compare whether a more parsimonious direct effects model or a model that included hypothesized indirect effects (see Table 3.1) better fit the data. In Model 1, plant traits and soil condition variables (soil organic matter and soil nitrate) were included with only direct paths to potential denitrification (Figure 3.1a). In Model 1, soil organic matter and soil nitrate were modeled as correlated, and plant traits were modeled as correlated. In Model 2, hypothesized relationships among

plant traits, soil conditions, and potential denitrification were modeled with indirect paths to potential denitrification through soil conditions (Figure 3.1b).

I used structural equation modeling (SEM) to test if Model 1 or Model 2 better fit the data and to test for how plant traits are related to denitrification. Bivariate plots were examined for deviations from linearity. Potential denitrification and soil nitrate data were log transformed to conform to assumptions of linearity (Kline 2015). To test if the proposed model fit the data, chi-square goodness of fit was used. For SEM, a non-significant p-value means that the proposed model and the data were not significantly different (i.e. a non-significant model indicates good fit). If the proposed model was not a good fit, STATA suggested modification indices, which are paths or correlations that would improve model fit if added. Suggested modification indices were included to respecify the proposed model. Modification indices were only included if they were theoretically justified, and not for the sole purpose of improving model fit (Kline 2015). In addition to chi-square, I report three other indices of model fit: Comparative Fit Index (CFI); Standardized Root Mean Square Residual (SRMR); and Root Mean Square Error of Approximation (RMSEA). Good-fitting models have CFI ≥ 0.95, SRMR ≤ 0.08, and RMSEA ≤ 0.05 (Kline 2015). After a final model was selected, Satorra-Bentler correction for chi-square and other model fit indices was applied to account for potential non-normality of data. All analyses were completed in Stata v14.

RESULTS

The model including indirect effects of plant traits on potential denitrification (Figure 3.1b) fit the data better than the model including only direct paths (Figure 3.1a).

In the following section, I provide descriptive statistics of the plant trait data for each species, the bivariate relationships among variables included in the model, and results of Model 1 and Model 2.

Plant traits

Plant traits, soil conditions, and potential denitrification measurements encompassed a wide range of values. This suggests that the eight species patches I selected represented a variety of plant traits and soil conditions experienced by denitrifiers (See Table 3.2).

Bivariate relationships

Potential denitrification (DNP) showed significant correlations with soil organic matter, soil nitrate, aboveground biomass, and belowground biomass ($r = 0.71$, $P < 0.001$; $r = -0.34$, $P < 0.001$; $r = 0.23$, $P = 0.003$; $r = 0.23$, $P = 0.003$ respectively; Figure 3.2). Rooting depth, aboveground C:N ratios, and belowground C:N ratios were not significantly correlated with DNP. However, all three were significantly correlated with soil organic matter (r = -0.16, $P = 0.04$; $r = 0.18$, $P = 0.02$; $r = -0.15$, $P = 0.05$ respectively), and above- and belowground C:N ratios were significantly correlated with soil nitrate ($r = -0.23$, $P = 0.003$; $r = -0.15$, $P = 0.05$ respectively). This justifies their inclusion in the proposed models.

Model 1: direct effects only

Several modification indices (pathways or correlations that would improve the fit of the proposed model to the data) were identified using the *estat mindices* command in STATA. Suggested modification indices were included in the final Model 1 compared to

the proposed model (Figure 3.1a and Figure 3.3), and consisted of correlations between soil conditions and different plant traits (Table 3.4).

After including the suggested correlations in Model 1, the structure of Model 1 still was not a good fit to the data (χ^2 = 15.44, df = 5, P = 0.009; CFI = 0.947, SRMR=0.073, RMSEA = 0.113). Significant predictors of DNP were soil organic matter (β = 0.68, P < 0.001), soil nitrate (β = -0.21, P < 0.001), aboveground biomass (β = 0.29; P < 0.001), C:N ratio of aboveground biomass (β = -0.20, P < 0.001), C:N ratio of belowground biomass (β = 0.15, P < 0.03), and rooting depth (β = -0.22, P < 0.005) (Table 3.3, Figure 3.3). Many correlations between predictor variables were significant (Table 3.4). Specifically, all plant traits were positively correlated with each other. Soil organic matter was correlated with aboveground biomass, belowground biomass, and C:N ratio of aboveground biomass. Further, soil nitrate was negatively correlated with C:N ratio of aboveground biomass, and positively correlated with rooting depth.

Model 2:indirect effects included

Several suggested modification indices were included in the final Model 2 compared to the proposed model (Figure 3.1b and Figure 3.4), and consisted of direct paths from aboveground biomass, C:N ratio of aboveground biomass, and rooting depth to DNP, and a path from rooting depth to soil organic matter.

After including these modification indices in Model 2, the model structure was a good fit for the data and had better model fit than Model 1 (χ^2 = 10.09, df = 5, P = 0.07; CFI = 0.979, SRMR=0.033, RMSEA = 0.079). In Model 2, I found three types of significant relationships ($P \leq 0.05$) among predictor variables and DNP: (1) predictors related to DNP via direct paths only; (2) predictors related to DNP via indirect paths

only; and (3) predictors related to DNP via both direct and indirect paths (Figure 3.4). Variables with only direct paths to DNP were soil organic matter (positive predictor), soil nitrate (negative predictor), and aboveground biomass (positive predictor). Belowground biomass and belowground C:N ratios affected DNP via indirect paths only. Belowground biomass positively predicted soil organic matter resulting in a positive total effect (sum of all paths) on DNP. Belowground C:N ratios negatively predicted soil organic matter and soil nitrate resulting in a overall negative effect on DNP. Variables with both indirect and direct paths to DNP were rooting depth and aboveground C:N ratios. Rooting depth had a negative effect on soil organic matter, a positive effect on soil nitrate, and a negative direct effect on DNP. The total effect of rooting depth on DNP was negative. Aboveground C:N ratio had a negative effect on soil nitrate, and a negative direct effect on DNP.

By examining the total effect of variables on DNP (sum of all path coefficients to DNP), I found soil organic matter and rooting depth had the strongest influence on DNP (0.67, -0.38 respectively). Aboveground biomass (0.29), belowground biomass (0.27) and soil nitrate (-0.23) had moderate influences on DNP. Aboveground C:N (-0.06), and belowground C:N ratios (-0.08) had only minor influences DNP (Table 3.3).

DISCUSSION

Plant traits were significantly related to DNP, particularly when indirect paths between plant traits and DNP were modeled. While many of the hypothesized relationships among plant traits, soil conditions and DNP were supported, many plant traits also retained significant direct paths to DNP (i.e. residual direct paths;Figure 3.4).

Residual direct paths reveal that the final model is missing important relationships among these variables. In the following section, I discuss substantive findings regarding direct and indirect paths between plant traits and denitrification, and other important considerations when examining the relationship among plant traits, soil conditions, and denitrification.

Importance of modeling indirect paths

I found that Model 1 (direct paths) did not adequately fit the structure of the data. Model 1 does include several correlations among soil variables and plant traits similar to significant indirect paths in Model 2. This suggests correlations can convey important information about relationships among plant traits and soil variables (Sutton-Grier et al. 2012). However, explicitly modeling indirect paths better fit the data. This is not surprising as the effect of plant traits on DNP is not often discussed as direct effects, but rather as mediated by soil conditions (Sutton-Grier et al. 2012; Alldred and Baines 2016). The importance of modeling hypothesized indirect effects is highlighted in the change in the significance of the relationship between belowground biomass and DNP in Model 1 versus Model 2. In Model 1, belowground biomass showed no significant relationship with DNP, but Model 2 showed a significant indirect relationship between belowground biomass and DNP through soil organic matter- as hypothesized. Given that soil organic matter is typically derived from root litter, this indirect relationship is expected (Puget and Drinkwater 2001; Eviner and Chapin 2003; Gift et al. 2010). However, if only Model 1 was used to determine which plant traits were important for increasing DNP, belowground biomass would have been excluded, ignoring an important mechanism by which plants can increase DNP.

Effect of soil conditions on DNP

Soil conditions are important predictors of denitrification (Boyer et al. 2006; Seitzinger et al. 2006; Wallenstein et al. 2006). In line with these studies, I hypothesized that DNP would increase as soil organic matter and soil nitrate (substrates necessary for denitrification) increased. These hypotheses were partially supported: I found that DNP did increase with increasing soil organic matter; however, DNP decreased with increasing soil nitrate. This unexpected relationship could be due to the range of inundation durations experienced by the study wetlands. On one hand, drier wetlands have lower potential denitrification, but have more extractable soil nitrate due to aerobic conditions that promote nitrification (Groffman and Crawford 2003). Permanently flooded wetlands, on the other hand, have lower soil nitrate as anaerobic conditions promote denitrification. Hernandez and Mitsch (2007a) found similar results: permanently flooded wetlands had lower soil nitrate concentrations, but higher denitrification rates than wetlands that dried out periodically. When examining wetlands that range in their inundation durations, a more comprehensive measurement such as total N loading would likely be more appropriate (Seitzinger et al. 2006)

Effect of plant traits on DNP

Several hypothesized plant traits were important predictors of DNP via their relationship with soil conditions. Similar to other studies on the effects of plant traits on microbial processes such as decomposition (Chapin 2003; Cornwell et al. 2008) and nitrogen mineralization (Wedin and Tilman 1990; Orwin et al. 2010), I found that plant biomass and tissue chemistry were related to DNP by influencing soil organic matter and soil nitrate. Increases in belowground biomass resulted in increased DNP via increased

soil organic matter. This is similar to a study by Gift et al. (2010), which did not model indirect relationships, but found root biomass was positively correlated to soil organic matter, and soil organic matter was positively correlated to DNP. Interestingly, Sutton-Grier et al. (2012) found belowground biomass did not significantly affect DNP, but was positively correlated to soil organic matter when using an SEM model with direct paths only (similar to Model 1). These studies, in combination with this study, provides evidence that belowground biomass is an important plant trait for increasing DNP; however, it also highlights the importance of including indirect effects in models as discussed in the previous section.

Belowground C:N was negatively related to DNP through soil organic matter, supporting my hypothesis that more decomposable tissues (i.e. lower C:N) will increase soil organic matter and consequently increase DNP. However, this relationship warrants further investigation since the increase in soil organic matter associated with lower C:N may by a byproduct of decomposition processes and not the primary mechanism increasing denitrification. Similar to this study, previous denitrification studies have found that denitrification increases when litter has lower C:N due to increased decomposability of tissues (Hume et al. 2002). However, previous decomposition studies have found that plant tissues with lower C:N have greater fractions that do not decompose and remain as soil organic matter (Berg 2000; Cotrufo et al. 2013). Thus, increases in denitrification associated with lower C:N may be driven by increases in the labile carbon pool rather than increases in soil organic matter itself.

I also found support for hypothesized relationships between plant traits and DNP via soil nitrate. However, the direction of these relationships should be considered

cautiously given that inundation duration is likely influencing the relationship between soil nitrate and DNP as discussed previously. Inundation duration may be overwhelming the relationship of plant traits to DNP via soil nitrate, but it is still reasonable to examine plant-trait effects on soil nitrate. As predicted, increases in rooting depth were related to increased soil nitrate. I hypothesized that this would be due to increased infiltration of nitrate into soils. While depth of nitrate infiltration was not measured, this finding offers support for increased infiltration as a potential mechanism. I also found that increases in soil nitrate were associated with decreases in C:N of above- and belowground tissues, supporting my hypothesis. This relationship is likely driven by increases in N mineralization in tissues with lower C:N (Bragazza et al. 2007; Orwin et al. 2010)

In addition to the relationships among plant traits and DNP that supported my hypotheses, rooting depth and aboveground biomass had unexpected associations with DNP (Figure 3.4). Rooting depth was also related to DNP not only via the indirect path through soil nitrate discussed above, but also via a negative indirect relationship with soil organic matter, and a residual negative direct path to DNP. The negative relationship between soil organic matter and rooting depth was surprising, as I would typically expect increases in rooting depth to lead to increased soil organic matter (Gift et al. 2010). Further, rooting depth was negatively associated with DNP via a residual direct path. Residual direct paths reveal that there is a relationship between two variables, but the model does not explain the mechanism by which those variables are related (Grace and Keeley 2006). Therefore, rooting depth is negatively related to DNP, but the mechanism of this effect is not clear. It is also possible that rooting depth is correlated to another, unmeasured plant trait that is decreasing DNP. For example, roots can exude oxygen,

increasing the redox potential of soils (Ehrenfeld et al. 2005). While this can increase denitrification in nitrate limited systems via coupling of nitrification to denitrification, urban wetlands receive water with high nitrate concentrations that could result in soils where coupled nitrification denitrification is not as important for increasing denitrification (Hernandez and Mitsch 2007b). Consequently, increased oxygenation of soils could decrease denitrification. It is possible that rooting depth was a proxy measurement for the amount of oxygen reaching soils. However, if this were the case, I would also expect belowground biomass to perhaps be negatively related to DNP as well. Notably, rooting depth has been suggested as a potential plant trait important for predicting DNP (Alldred and Baines 2016); however, these results suggest that either rooting depth is a poor choice for a plant trait or there needs to be further research on the mechanism by which rooting depth affects denitrification.

Another curious residual direct path was modeled between aboveground biomass and DNP (Figure 3.4). This is of particular note, as there was not a significant path from aboveground biomass to soil organic matter as I hypothesized. Less aboveground biomass is typically incorporated into soil organic matter than belowground biomass so aboveground biomass likely is affecting denitrification through another mechanism (Puget and Drinkwater 2001). Aboveground biomass can contribute to available dissolved organic carbon (Park and Matzner 2003) and the labile carbon pool (Wedin and Pastor 1993; Eviner and Chapin 2003). The residual direct path from aboveground biomass to DNP in addition to the residual direct path from C:N for aboveground biomass to DNP suggest that aboveground plant traits could be affecting DNP through

dissolved organic carbon or labile carbon pools that were not measured in this study (Duan et al. 2014).

Relative importance of plant traits and soil conditions

Both soil conditions and plant traits were important predictors of DNP and many of the variables have similar total effects on DNP (Table 3.3). Soil organic matter had by far the strongest effect on DNP. This is similar to many studies that have found soil organic matter to be an important predictor of DNP (Hernandez and Mitsch 2007a; Gift et al. 2010; Sutton-Grier et al. 2012). Rooting depth, aboveground biomass, belowground biomass, and soil nitrate all had moderate effects on DNP. While soil organic matter retained the strongest effect on DNP, several plant traits had significant total effects on DNP. Overall, I found stronger effect of plant traits on DNP than Sutton-Grier et al. (2012) who found that soil characteristics were much more important than plant traits for explaining DNP. This could be because they modeled plant traits as directly affecting DNP; however, it could also result from differences between study sites or methods. For example, I measured plant traits in the field, compared to traits measured on plants grown in a greenhouse, which could result in a stronger association between plant traits and DNP.

Other considerations

Environmental variables such as inundation duration or nitrate loading could potentially affect how plant traits affect denitrification. For example, wetlands that do not flood frequently, or have high nitrate loads, may rely less on coupled nitrification–denitrification in plant rhizospheres for increasing denitrification. Consequently, plant traits linked to oxygen exudation my not be important in wetlands with these

environmental conditions (Sutton-Grier et al. 2012). In the study wetlands, hydroperiod likely influenced how plant traits affected DNP. This is highlighted by the negative relationship between soil nitrate and DNP that was likely due to the inclusion of wetlands with a variety of flooding conditions. Varying hydroperiods are characteristic of wetland ecosystems and can greatly influence nitrogen cycling due to oscillations between anaerobic and aerobic conditions. Therefore, when examining how plant traits affect denitrification it will be important to consider to what extent hydrology is also affecting these relationships. For this study, I was limited by sample size from parsing out sites by hydroperiod and exploring relationship among plant traits, soil conditions, and DNP at these sites separately.

CONCLUSION

As we face a world of rapid environmental change and shifting species distributions and abundances, plant traits may be an essential way for us to predict how changing community compositions could affect ecosystem processes. Despite the call for using plant traits to examine the relationship among plants and ecosystem processes, I am aware of only one other study that examines the effect of plant traits on denitrification (Sutton-Grier et al. 2012). Further, this is the only study I am aware of to explicitly model *indirect effects* of plant traits on denitrification. Overall, this study provides evidence that plant traits indirectly affect denitrification through soil conditions. However, several unexplained residual paths highlight our lack of a mechanistic understanding of how these plant traits affect denitrification, despite their inclusion in lists of plant traits that could affect denitrification (Sutton-Grier et al. 2012; Alldred and Baines 2016). This

study also highlights the challenge of disentangling whether plant traits or environmental conditions are driving ecosystem processes, a unique challenge in wetlands that are characterized by fluctuating inundation levels. Effectively linking plant traits to ecosystem functions, such as denitrification, could inform the restoration of degraded wetlands, or management of wetlands in urban landscapes where specific services, such as nitrate removal, may be desired.

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TABLES AND FIGURES

Table 3.1. Hypothesized independent variables that could affect potential denitrification (DNP). Measured variables could affect potential denitrification either directly (soil conditions), or indirectly (plant traits) by affecting soil conditions.

Variables	Justification and predicted effect
Soil conditions	
Soil organic matter (SOM)	Carbon is a required substrate for denitrification (Seitzinger et al. 2006; Wallenstein et al. 2006); therefore, increases in organic matter will increase carbon sources for denitrifiers.
Soil NO_3^- (NO_3^-)	Nitrate is a required substrate for denitrification (Seitzinger et al. 2006; Wallenstein et al. 2006); therefore, increases in available nitrate will increase potential denitrification.
Plant traits	
Aboveground biomass (AG)	The more aboveground biomass a plant generates the more plant litter will enter the soil (Ehrenfeld 2003; Wardle et al. 2004); therefore, increases in aboveground biomass will result in increases in soil organic matter.
Belowground biomass (BG)	The more belowground biomass a plant generates more carbon will be available through root turnover (Eviner and Chapin 2003; Gift et al. 2010); therefore, increases in aboveground biomass will result in increases in soil organic matter.
Aboveground C:N (CNAG)	Plant litter with lower C:N ratios is more easily decomposed by microbes increasing available carbon for microbes and nitrogen mineralization (Craine et al. 2002; Wardle et al. 2004); therefore, decreases in aboveground C:N ratios will result in increases in soil organic matter and soil nitrate.
Belowground C:N (CNBG)	Plant roots with lower C:N ratios are more easily decomposed by microbes increasing available carbon for microbes and nitrogen mineralization (Craine et al. 2002; Wardle et al. 2004);therefore, decreases in belowground C:N ratios will result in increases in soil organic matter and soil nitrate.
Rooting depth (RD)	Roots loosen compacted soils resulting in increased infiltration of water and substrates into soil (Gift et al. 2010); therefore, increases rooting depth will result in increases in soil nitrate.

Table 3.2. Average plant trait values, soil conditions, and potential denitrification for each species. Parentheses denote minimum/maximum. AG and BG reported as g/100 cm2. RD reported as cm. CNAG and CNBG reported as ratios of C:N. SOM reported as percent. NO_3^- reported as mg-N kg^{-1} soil. DNP reported as ug N_2O-N g^{-1} soil hr^{-1}.

Species	AG	BG	RD	CNAG	CNBG	SOM	NO_3^-*	DNP*
Amaranthus sp.	13.16 (2.9/41.40)	1.63 (0/5.60)	5.6 (0/25.33)	35.82 (29.48/40.89)	12.32 (0/24.47)	0.03 (0.01/0.06)	17.08 (5.01/36.06	0.24 (0.14/0.40)
Cynodon dactylon	4.43 (0.56/10.73	4.33 (1.66/9.20)	5.5 (2.89/7.06)	28.88 (18.94/47.79)	31.30 (24.26/43.91)	0.07 (0.02/0.26)	0.56 (0.02/5.11)	0.33 (0.01/1.44)
Ludwigia peploides	9.89 (2.87/24.24)	3.49 (0/13.27)	3.7 (0/10.56)	38.4 (17.26/93.16)	19.2 (0/44.89)	0.14 (0.01/0.32)	0.48 (0.03/10.64)	1.21 (0.16/6.90)
Paspalum distichum	3.31 (1.3/8.87)	4.43 (1.93/8.57)	6.7 (4.78/9.11)	34.16 (18.42/61.54)	34.36 (10.47/77.11)	0.06 (0.04/0.10)	0.35 (0.02/11.08)	0.4 (0.08/1.08)
Rumex dentatus	20.65 (5.76/34.60)	4.51 (1.41/8.90)	11.0 (4.22/20.56)	53.75 (24.11/79.72)	43.61 (29.48/70.49)	0.03 (0.02/0.04)	2.95 (1.04/19.13)	0.15 (0.06/0.33)
Schismus sp	0.59 (0.35/1.07)	2.1 (1.16/4.00)	5.1 (1.89/6.67)	21.53 (16.57/24.01)	24.95 (24.92/25.03)	0.04 (0.02/0.06)	19.67 (4.27/50.07)	0.06 (0.01/0.24)
Tribulus terrestris	3.1 (0.56/7.80)	2.33 (1.26/3.37)	8.0 (2.44/21.67)	20.79 (19.41/23.55)	28.41 (19.93/37.90)	0.02 (0.01/0.03)	11.53 (7.23/23.56)	0.21 (0.07/0.40)
Typha sp	43.01 (14.24/90.75)	23.59 (4.22/31.10)	11.1 (4.67/24.00)	63.24 (22.03/138.32)	47.19 (9.65/122.56)	0.11 (0.01/0.28)	0.57 (0.03/15.16)	0.76 (0.07/3.41)
Full data	19.04 (0.35/90.75)	9.90 (0/37.10)	7.51 (0/25.33)	44.34 (16.57/138.32)	33.20 (0/122.56)	0.09 (0.01/0.32)	0.97 (0.02/50.07)	0.50 (0.01/6.90)

*These variables were natural log transformed for analysis. Averages are back transformations of average for log transformed data. Zero values for BG and CNBG indicate plots in which there was no belowground biomass.

Table 3.3. Standardized and unstandardized path coefficients for Model 1 (direct effects only) and Model 2 (indirect effects included). For Model 2, path coefficients reported are for total effects. Bolded values are significant at $P < 0.05$.

	Standardized path coefficients		Unstandardized path coefficients	
Paths	Model 1	Model 2	Model 1	Model 2
SOM → DNP	**0.68**	**0.67**	**11.32**	**10.96**
NO_3^- → DNP	**-0.21**	**-0.23**	**-0.12**	**-0.13**
AG → DNP	**0.29**	**0.29**	**0.02**	**0.02**
BG → DNP	-0.05	**0.28**	-0.005	**0.03**
CNAG → DNP	**-0.20**	-0.06	**-0.008**	-0.002
CNBG → DNP	**0.15**	-0.08	**0.008**	-0.004
RD → DNP	**-0.22**	**-0.38**	**-0.04**	**-0.79**

Table 3.4. Standardized correlations and unstandardized covariates for Model 1. All values are significant at $P < 0.05$, except italicize result for SOM → NO_3^-.

Paths	Standardized correlations Model 1	Unstandardized covariates Model 1
SOM → NO_3^-	*-0.10*	*-0.01*
$^+$SOM → AG	0.21	0.32
$^+$SOM → BG	0.29	0.23
$^+$SOM → CNAG	0.23	0.42
$^+NO_3^-$ → CNAG	-0.14	-7.33
$^+NO_3^-$ → RD	0.11	1.21
AG→ BG	0.81	203.89
AG → CNAG	0.45	267.62
AG → CNBG	0.55	267.70
AG → RD	0.60	73.65
BG → CNAG	0.44	134.38
BG→ CNBG	0.57	142.98
BG → RD	0.60	38.26
CNAG → CNBG	0.25	149.30
CNAG → RD	0.32	48.09
CNBG → RD	0.70	85.39

$^+$ Indicate correlations not present in the proposed model.

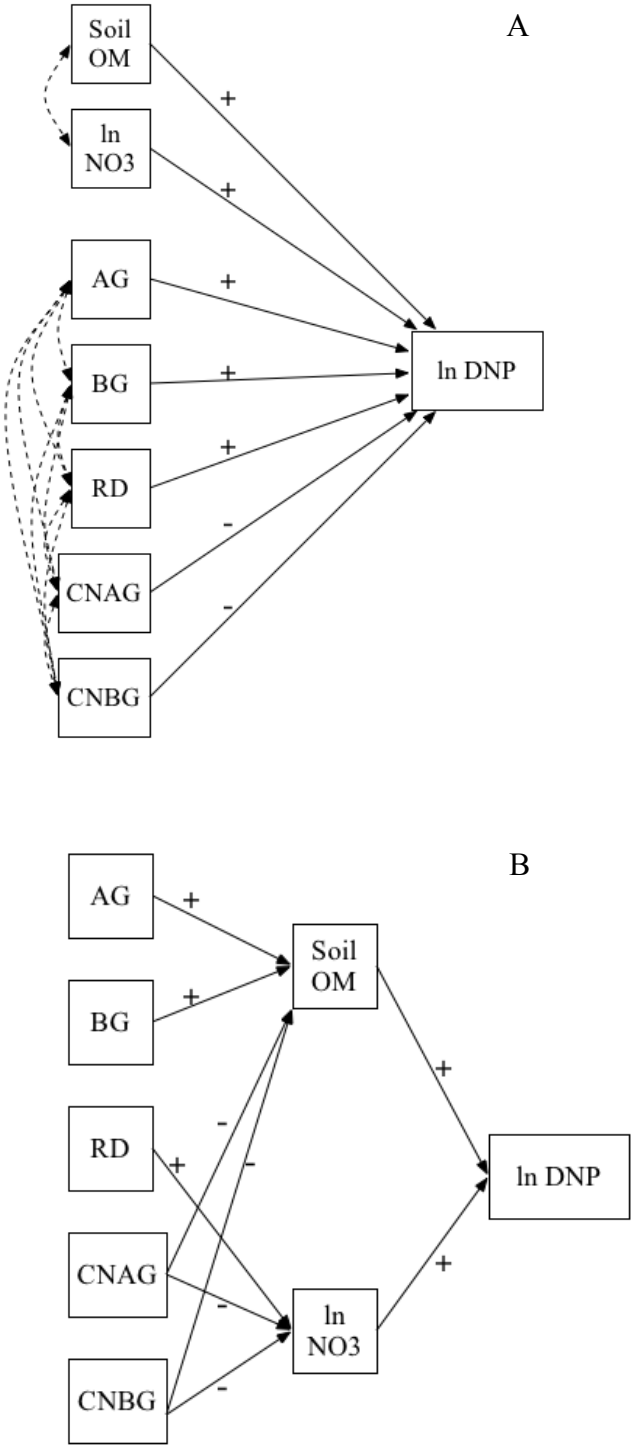

Figure 3.1. Proposed models for SEM. (A) Model 1 with direct paths and correlations only, and (B) Model 2 with indirect paths. Solid lines represent direct paths and dashed lines represent correlations. Pluses (+) and minuses (-) represent hypothesized direction of effects.

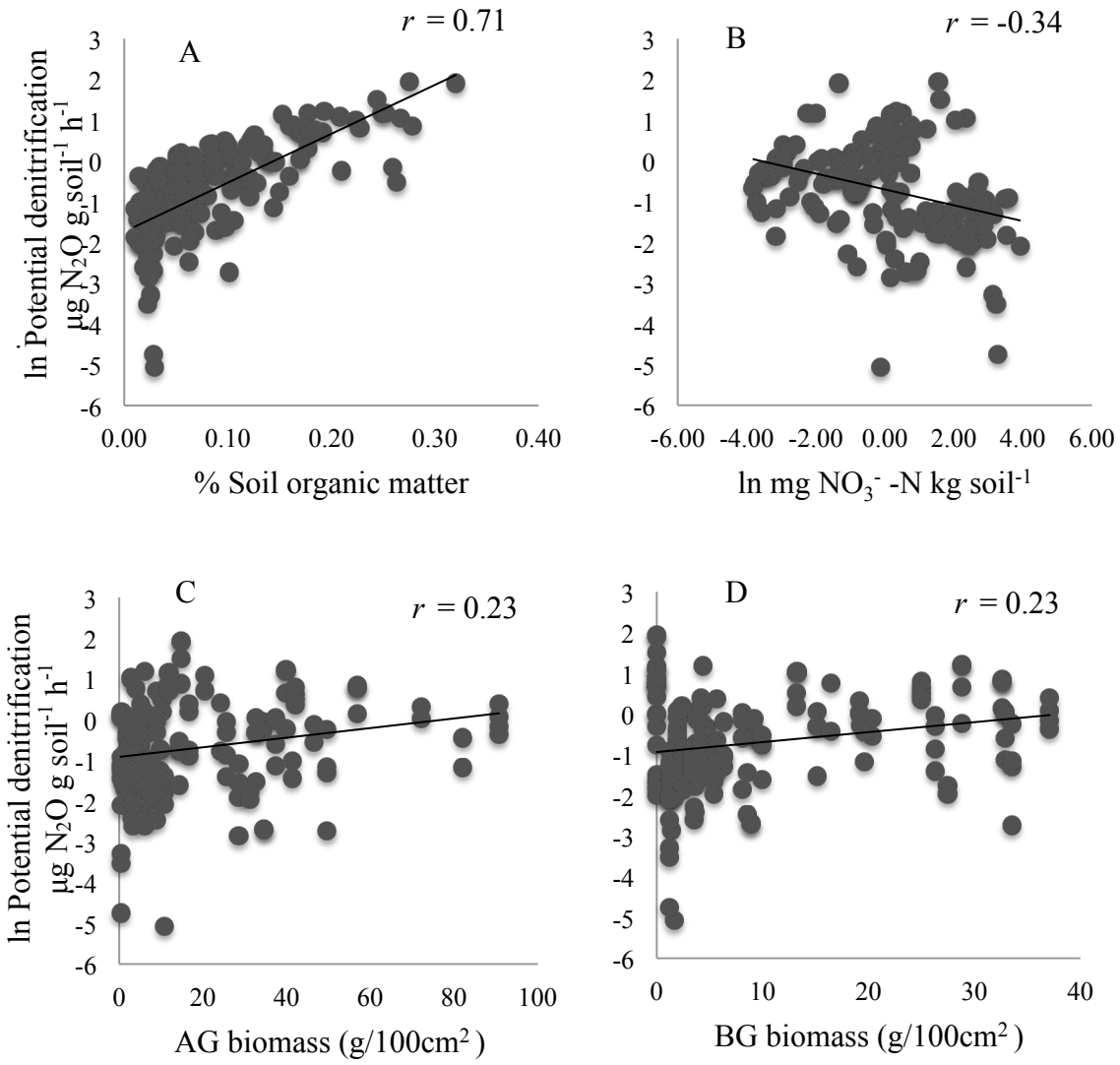

Figure 3.2. Bivariate plots of DNP, and soil conditions and plant traits. Note DNP is natural log transformed. AG is aboveground and BG is belowground.

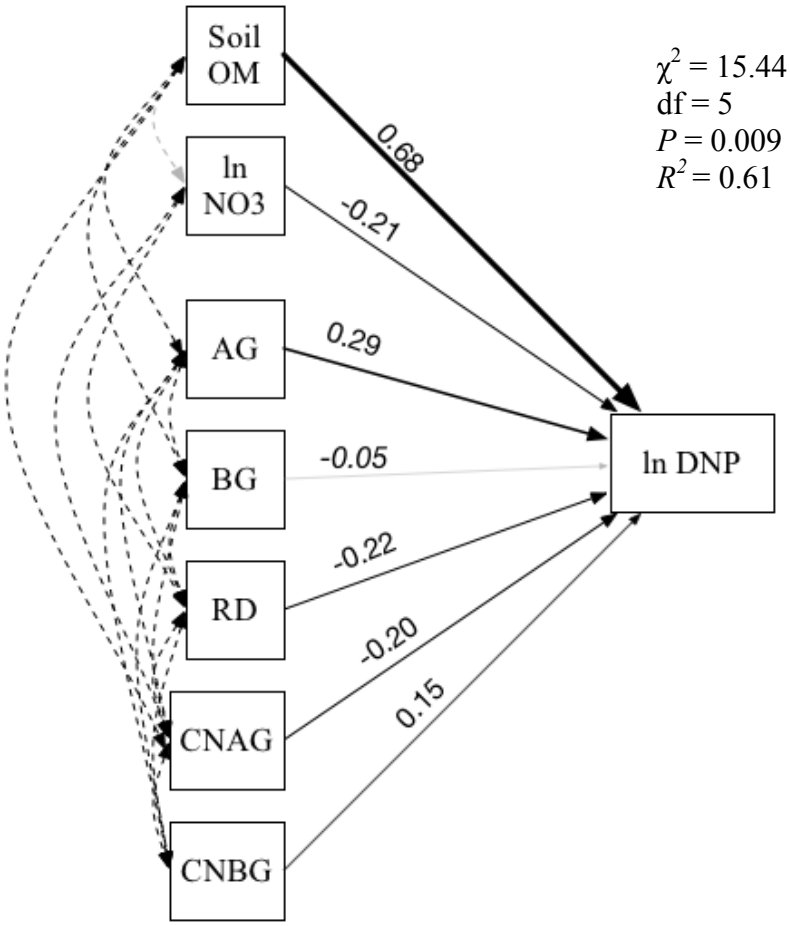

Figure 3.3. Final model structure and standardized path coefficients for Model 1. Solid lines represent direct paths and dashed lines represent correlations. Thickness of lines denotes each parameter's relative influence with thicker lines indicating stronger influence. Non-significant paths are indicated by gray lines and italicized coefficients.

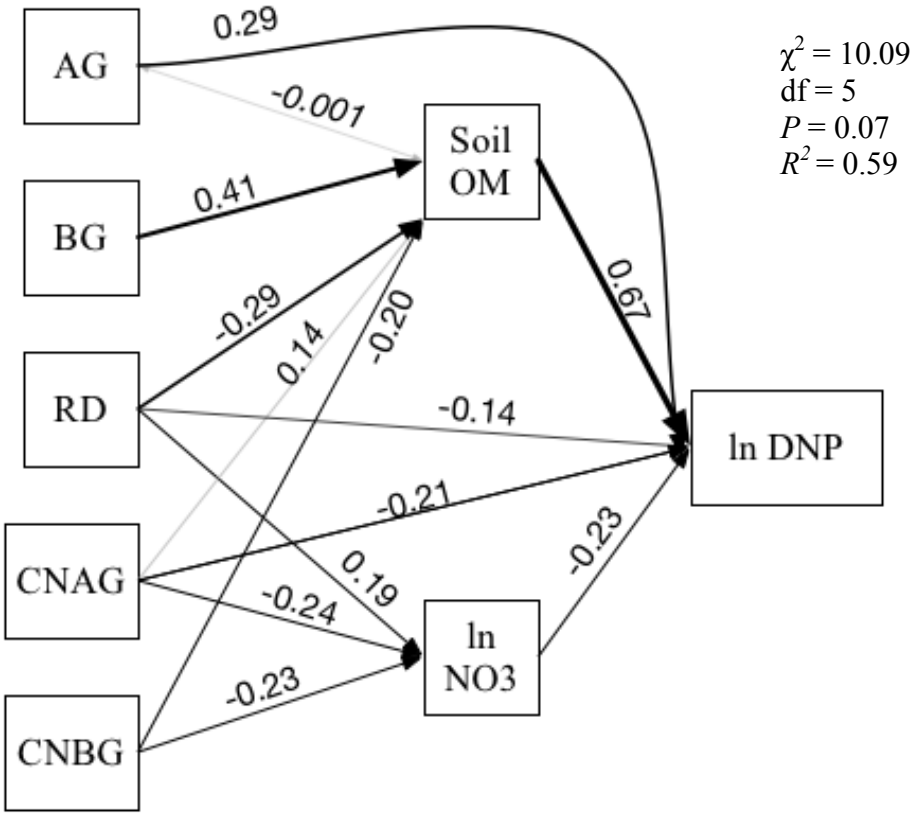

Figure 3.4. Final model structure and standardized path coefficients for Model 2. Thickness of lines denotes each parameter's relative influence with thicker lines indicating stronger influence. Non-significant paths are indicated by gray lines and italicized coefficients.

Chapter 4

NITRATE RETENTION AND DENITRIFICATION AS AFFECTED BY WETLAND PLANT PATCH RICHNESS AND EDGE INTERACTIONS

ABSTRACT

How ecosystem function relates to species richness has been a central debate in ecology for the past two decades. However, despite the hundreds of studies in grasslands, relatively few have been conducted in wetlands and even fewer have examined how species richness in wetlands affects denitrification. Wetland plants often grow in monotypic patches, a configuration very different than mixtures typical of grasslands. Consequently, wetland plants may only interact at plant patch edges, which could result edge interactions being important for increasing ecosystem functions such as denitrification. The objectives of this study were to (1) determine if increasing species richness increases nitrate retention and denitrification when wetland plants are configured as patches and (2) determine if belowground interactions between species at patch edges increase nitrate retention and denitrification.

To test how plant patch richness affects denitrification, I conducted a greenhouse experiment with 45 wetland mesocosms planted with one, two or three different patches of wetland plants. I measured whole mesocosm nitrate retention, *in situ* denitrification rates and potential denitrification. To test if belowground interactions at plant patch edges affected mesocosm nitrate retention and denitrification, an additional 12 mesocosms planted with 3 species had a barrier inserted between plant patches to prevent

belowground interactions. A field study was conducted to determine if there was spatial variation in denitrification between plant patch edges and centers under field conditions.

I found that increased plant patch richness did not increase whole mesocosm nitrate retention, *in situ* denitrification rates, or potential denitrification; rather, increased species richness decreased aboveground biomass. The presence of *Typha domingensis* increased whole mesocosm nitrate retention, *in situ* denitrification rates and potential denitrification. When *Paspalum distichum* was present in a mesocosm, nitrate retention and *in situ* denitrification rates were lower than when it was absent. The field study revealed spatial variation in plant patches where plant patch edges had higher potential denitrification than plant patch centers. Interestingly, the greenhouse study did not reveal similar patterns of denitrification between plant patch centers and edges. The addition of a barrier to block belowground interactions between plant patches increased belowground biomass and *in situ* denitrification rates.

INTRODUCTION

How ecosystem functions relate to species richness has been a central debate in ecology for the past two decades (Díaz and Cabido 2001; Balvanera et al. 2006; Cardinale et al. 2011). Many studies in grasslands have shown that as plant richness increases so do certain functions, such as nutrient retention and primary productivity (Loreau et al. 2001; Balvanera et al. 2006). Wetlands provide many ecosystem services, such as nutrient removal, that could be improved by understanding how plant richness affects wetland ecosystem functions. Only a few studies, however, have been conducted to assess how species richness affects ecosystem function in wetlands (Engelhardt and

Ritchie 2001; Callaway et al. 2003; Bouchard et al. 2007; McGill et al. 2010) compared to the hundreds conducted in grasslands (Balvanera et al. 2006; Cardinale et al. 2011). Wetlands are often dominated by large monotypic patches of vegetation resulting in a configuration of plant patches different than species mixtures and distributions seen in grasslands (Spence 1982), resulting in patches of plants that only interact with other species at the patch edges. If increased species interactions are driving increased ecosystem functions, as work in grasslands suggest (Cardinale et al. 2007), then the different configuration of wetland plants could have two consequences for the relationship between plant richness and ecosystem function: (1) plant richness may not increase ecosystem functions because species are not interacting; and (2) edges of plant patches where different species interact could be "hotspots" of microbial processes that drive certain ecosystem functions such as denitrification. For this study, I examined if wetland patch richness and edge interactions increase nitrate retention and denitrification. I used a multicomponent greenhouse experiment to examine how wetland patch richness affects wetland mesocosm nitrogen retention and denitrification, and a field study, in addition to the greenhouse experiment, to examine how wetland patch edge interactions affect denitrification.

Several hypotheses have been put forward regarding the mechanisms by which species richness affects ecosystem functions (Hooper et al. 2005). Three of the most common hypotheses are the sampling effect, the insurance effect, and the complementarity effect (Tilman 1999; Yachi and Loreau 1999). The sampling effect and insurance effect explain the observed relationship between species richness and ecosystem function largely as an artifact of the number of species present (Tilman 1999).

Specifically, the sampling effect suggests that with greater plant richness the odds increase that a functionally superior species will occur in the mixture. The insurance effect states species that differ in phenology or responses to environmental variables will maintain ecosystem functions at different times (Yachi and Loreau 1999). In contrast, the complementarity effect attributes the increase in ecosystem function with increased species richness to the synergistic effect of species interactions where species perform better when in mixtures than when in monocultures. Complementarity effects can occur as the result of either niche differentiation (Tilman 1999) or facilitation (Brooker et al. 2008), but these processes are difficult to disentangle and are often considered together (Fornara and Tilman 2008).

Plants affect nitrogen retention in wetlands via two pathways: (1) directly, by assimilating nitrogen into tissues; and (2) indirectly, by affecting soil conditions through leaf litter, root exudates, dead roots, and oxygen exudation (which couples nitrification to denitrification) that promote denitrification (Reddy et al. 1989; Callaway et al. 2003; Rotkin-Ellman et al. 2004; Wardle et al. 2004; Bais et al. 2006; Gift et al. 2010). Much research has been conducted on how species richness affects primary production in grasslands (Tilman 2000; Hooper et al. 2005; Cardinale et al. 2011); concluding that the complementarity effect is largely driving the observed increase in primary production with increased species richness (Cardinale et al. 2007). Increased primary production affects the amount of nitrogen retain in systems because as biomass increases, more nitrogen is sequestered into tissues of plants (Callaway et al. 2003). Therefore, if the complementarity effect drives the relationship among species richness and ecosystem functions, I would expect that increased species richness would not affect nitrogen

retention via assimilation into plant tissues in wetlands dominated by monotypic patches due to the lack of interspecific interactions among monotypic patches.

Plant richness has also been shown to affect belowground microbial processes and consequently could affect denitrification (Hooper et al. 2000; Zak et al. 2003). Denitrification is a microbial process that reduces NO_3^- into N_2 gas, permanently removing biologically reactive nitrate from ecosystems. Denitrification occurs when three conditions occur, including low oxygen, available labile carbon, and available nitrate (Boyer et al. 2006; Seitzinger et al. 2006). Increasing plant richness has also been shown to increase N mineralization, which could increase the amount of nitrate available for denitrifiers (Zak et al. 2003). Also, increased biomass associated with increased plant richness could increase the availability of soil organic matter and thus available soil carbon for denitrifiers (Hooper et al. 2000; Fornara and Tilman 2008; Steinauer et al. 2015). Further, mixed litter and greater chemical diversity of litter has been shown to increased decomposition (Bardgett and Shine 1999; Gartner and Cardon 2004; Meier and Bowman 2008; Stoler et al. 2016), potentially increasing denitrification. For wetlands dominated by monotypic plant patches, this means that denitrification could be greater at edges of plant patches where different species are interacting and different litter types or belowground carbon sources are available to denitrifying bacteria (Bais et al. 2006).

For this study, I asked: How do wetland plant patch richness and edge interactions between plant patches affect nitrate retention and denitrification? To address this question, I conducted both a greenhouse experiment and a field study. To determine how wetland plant patch richness affects nitrogen retention and denitrification, I conducted a

greenhouse experiment where I planted mesocosms with one, two, or three species planted as monotypic patches rather than mixtures. The objectives of this study were:

1) Determine if increasing wetland patch richness increased whole mesocosm biomass, nitrate retention and denitrification.

2) Determine if specific wetland species are associated with increased whole mesocosm nitrate retention and denitrification.

3) Determine if interactions between certain species at edges of plant patches either increase or decrease whole mesocosm nitrate retention and denitrification.

To determine how species interactions between wetland plant patches affect nitrogen retention and denitrification, I conducted a field study, in addition to the greenhouse experiment, with the following objectives:

4) Determine if there is any spatial variation in denitrification between plant patch centers and edges in both study wetlands and greenhouse mesocosms.

5) Determine if interactions among belowground resources increased nitrogen removal and denitrification in greenhouse experiment by interrupting belowground interactions.

This will be the first study to test if increasing the richness in wetlands increases ecosystem functions, when wetland plants are configured in patches as observed in most wetlands. This study further addresses if changes in ecosystem functions are explained by interactions between species by looking for spatial variation within patches at study wetlands, and by physically preventing belowground interactions between species in a greenhouse experiment.

METHODS

Greenhouse: experimental design

I conducted a greenhouse experiment in a closed greenhouse on the Arizona State University campus in Tempe, Arizona from July 2015 to May 2016. I filled 57 plastic bins (87.9 cm x 47.6 cm x 32.1 cm) with 15 cm of a mixture of 90% washed sand and 10% silt. The soil mixture mimicked unvegetated soils of local wetlands and was low in soil organic matter (< 0.001%) to better assess how the addition of plants affected denitrification. Bins were outfitted with a drip irrigation system that maintained a constant flow through of water with a target flood depth of 4 cm and target water residence time of 36 hours (Figure 4.2). The concentration of the inflow of nutrients was the same for all mesocosms (Table 4.1) and was generated by dosing DI water with a solution of salts in similar concentrations to the Salt River wetlands where the field study was conducted (See "Field study" for description of Salt River wetlands). A target concentration of 1ppm NO_3^- - N was chosen as this represents concentrations typical of baseflows into the Salt River wetlands (Palta, unpublished).

I chose four emergent macrophytes commonly found in monotypic patches in wetlands around Phoenix, Arizona: *Typha domingensis, Ludwigia peploides, Paspalum distichum, and Schoenoplectus americanus*. All four species are perennial, emergent macrophytes that reproduce vegetatively and are found growing in shallow water of wetlands around Phoenix, Arizona. However, they differ in morphologies that could affect nitrate retention and denitrification. *Typha domingensis* is a high-biomass, erect emergent macrophyte and has greater rooting depth than the other three species (Grace and Wetzel 1982; Boutin and Keddy 1993). *Ludwigia peploides* is a creeping emergent

macrophyte that is shallowly rooted, can quickly grow into dense mats of vegetation in the water column, and decomposes rapidly (Rejmánková 1992). *Paspalum distichum* is a creeping, wetland grass with similar biomass to *Ludwigia peploides*. *Schoenoplectus americanus* is another erect emergent macrophyte, but generates less biomass than *Typha domingensis* (Boyd 1970). Mesocosms were planted with grown plants of similar size between June 22nd and July 10th, 2015. *Typha domingensis* and *Ludwigia peploides* were collected from wetlands in the Salt River in Phoenix, Arizona (33°25'16.44"N, 112° 3'56.76"W). *Schoenoplectus americanus* and *Paspalum distichum* were collected from Sycamore Creek, a desert stream near Phoenix, Arizona (33°43'54.08"N, 111°30'52.65"W).

Mesocosms were planted with one, two, or three species with three replicates for each species combination. Species were not planted in mixture, but rather in distinct patches to more closely represent how these species are typically configured in emergent wetlands (Figure 4.2Figure 4.3). Amount of individuals planted was adjusted for the treatment so the density of plants in a patch was the same across treatments. I also maintained three control mesocosms with no plantings throughout the course of the experiment for a total of 45 mesocosms. To test whether belowground interactions between plant patches affected denitrification, an additional 12 mesocosms planted with three species had a plastic belowground barrier inserted into the soil between plant patches to prevent belowground interactions (Figure 4.3).

In addition to measurements of nitrate retention and denitrification discussed below, aboveground and belowground biomass was harvested at the end of the

experiment in June 2016. Biomass samples were brought to the lab, rinsed to remove soil, dried at 60°C for a week, and weighed.

Greenhouse: whole mesocosm nitrate retention

Whole mesocosms nitrate retention was measured on three occasions: once at the end of the fall in October 2015 (T1), and twice during the spring in April 2016 (T2) and May 2016 (T3). Measurements were not taken between these times because plants were either senesced or in early stages of growth. To measure whole system nitrate retention, inflows and outflows were closed and each mesocosms was spiked with nitrate for a target concentration of 1 mg NO_3^- - N l^{-1} in fall and 3 mg NO_3^- - N l^{-1} in spring. Samples were taken at 1 and 18 hours after addition of spike for the fall sampling. In the spring, samples were taken at 1 and 6 hours after the addition of the nitrate spike. The spike was increased and sampling period shortened in the spring due to increased rates of nitrate uptake, likely due to increases in denitrifying bacteria (discussed below). Surface water samples were collected with a syringe, filtered through GF/F Whatman filter, and frozen until analyzed colorimetrically for nitrate on a Lachat QC8000 flow-injection analyzer.

Greenhouse: denitrification

I measured denitrification at the end of the study in May 2016 using two methods: *in situ* measurements of denitrification rates and laboratory measurements of denitrification potentials. I measured denitrification only once at the end of the study as early tests revealed undetectable denitrification in during October 2015 sampling. Denitrification was not detected until March 2016 and the microbial community was left to develop until the end of the study. To test for the effect of species interactions I took denitrification samples from the center and the edges of the patches (Figure 4.4).

Denitrification rates were measured *in situ* using the acetylene block technique (Groffman et al. 2006). Acetylene inhibits the reduction of N_2O to N_2. When this step is inhibited, the final product of denitrification becomes the trace gas N_2O, which is more easily measured than changes in N_2, which is abundant in the atmosphere (Groffman et al. 2006). A PVC pipe, 3 cm in diameter, was inserted fully into the mesocosms soil and allowed to rest for one hour. The water in the pipe was spiked with nitrate to bring the background concentration to at least 1 mg NO_3^- - N l^{-1}. I did this to prevent nitrate limitation that could result from the pipe blocking the diffusion of nitrate into the soils. I then injected 15 ml acetylene (10% of the headspace) directly into the surface water to facilitate diffusion into the soils (Ryden and Dawson 1982; Hernandez and Mitsch 2007) and then capped the pipe with a well cap fitted with a septum. Gas samples were taken from the headspace immediately after capping and after 20 hours, and analyzed on a Varian 3800 gas chromatograph for N_2O concentration.

Potential denitrification was measured in the lab using denitrification enzymes assays (DEAs; Groffman et al. 1999). Potential denitrification is a measurement of denitrification under optimal conditions (excess carbon, excess nitrate, and anaerobic soils). While potential denitrification does not give rate measurements, it may allow for clearer comparisons among treatments, as potential denitrification is sensitive to changes in environmental conditions (Groffman et al. 2006). For DEAs, 50 grams of soil and 50 ml of media were added into a 125 ml Wheaton bottle. To ensure neither nitrate nor carbon were limiting, media was amended with NO_3^- (100 mg NO_3-N kg^{-1} soil as KNO_3) and carbon (40 mg glucose-C kg^{-1} soil as glucose). Headspace of samples was replaced with N_2 gas to create anaerobic conditions, and 10ml of acetylene gas was added to

inhibit the reduction of N_2O to N_2. Samples were incubated at room temperature and shaken at 140 rpm for 4 hours. Gas samples were taken from the headspace at 30 minutes and 4 hours, and analyzed on a Varian 3800 gas chromatograph for N_2O concentration. Whole mesocosm denitrification estimates were calculated by scaling *in situ* or DEA measurements by patch area.

Field study

To examine if denitrification differed spatially between the edge and centers of wetlands plant patches, soil and water samples were collected from different plant patches in two wetlands located in the Salt River in Phoenix, Arizona in April 2016. These wetlands received water from storm drains that discharge urban baseflow during dry periods and consequently remained inundated for much of the year. The wetlands are dominated by patches of *Typha domingensis* and *Ludwigia peploides* as well as open, unvegetated areas (Figure 4.2). At each wetland, I established transects (four at one wetland, seven at the other) perpendicular to bank that crossed different plant patches and open areas. Along each transect, I collected soil cores, surface water samples, and water depth measurements at patch edges and at patch centers (Figure 4.5). Soil cores were taken to a maximum depth of 10 cm to encompass the most active part of the soil (Groffman et al. 1999). Soil cores were stored on ice in the field and then stored at 4 °C in the lab until processed (within 24 hours). Surface water, when present, was filtered through a GF/F Whatman filter, and frozen until analyzed colorimetrically for nitrate and ammonium on a Lachat QC8000 flow-injection analyzer.

Soil cores were homogenized and analyzed for soil moisture, soil organic matter, soil texture, and denitrification potential. Soil moisture was determined gravimetrically

by drying soils for 48 hours at 105 °C. Soil organic matter was determined by mass loss on ignition for 4 hours at 550 °C. Denitrification was measured using denitrification enzymes assays, the same method described in the section "Greenhouse: denitrification" (Groffman et al. 1999).

Statistical analyses

Linear regression was used determine if diversity treatment (number of species) affected final above- and belowground biomass, whole mesocosm nitrate retention, and whole mesocosm denitrification (objective 1). Control mesocosms were excluded from regression to ensure results were comparisons of different richness treatments and not planted versus unplanted treatments. Multiple regression was also used to assess if the presence/absence of a species or of a particular species interaction affected mesocosm nitrate retention and denitrification (objective 2 and 3). For whole mesocosm nitrate retention, the starting concentration of nitrate in each mesocosm was included in the regression as a covariate, because the starting concentration of nitrate in each mesocosm was not exactly the same. Multiple regressions were run for each sampling date separately. *In situ* denitrification rates and potential denitrification were log transformed to conform to rules of normality for regressions.

To determine if species interactions affected denitrification in the greenhouse experiment, paired t-tests were use to test if there were differences between the centers and edges of patches for mesocosms planted with two or three species (objective 4). ANOVA with Tukey's HSD post hoc tests was use to test if particular species interactions affected the magnitude of difference between patch centers and edges (calculated as center - edge).

For the field study, ANOVA with Tukey's HSD post hoc tests were used to test if there were between patch differences and spatial variability within patches of potential denitrification. Sampling locations within a patch were put into three categories: patch centers, patch edges, and patch bank. Patch bank was a sampling location in a plant patch at the outer edge of the wetland. These sampling locations were different than patch edges because at bank locations plants patches were not clearly interacting with another wetland patch type (Figure 4.5).

To test if belowground interactions affected mesocosm nitrate retention, denitrification, and above- and belowground biomass (objective 5), paired t-tests were used to compare bins with and without barriers. Test bins were "paired" in the greenhouse such that they were on the same irrigation line and spatially adjacent to account for any environmental factors (e.g. duration or timing of sun exposure) in the greenhouse that could affect plant growth. All statistical analyses were conducted in SPSS v19.

RESULTS

Overall, the greenhouse study revealed species richness was less important for explaining nitrate retention and denitrification than the presence of particular species in a mesocosm. Further, field study revealed patch edges had higher potential denitrification than patch centers, but this pattern was not reflected in the greenhouse experiment. The following section describes the specific results for each study objective of the greenhouse experiment and field study.

Objective 1: Effect of plant patch richness on biomass, nitrate retention and denitrification

Plant patch richness significantly decreased aboveground biomass (β =- 0.36, P = 0.02; Figure 4.6a), and did not have a significant effect on belowground biomass (Figure 4.6b). Plant patch richness also did not affect whole mesocosm nitrate uptake in any sampling period (Figure 4.7), *in situ* denitrification rate (Figure 4.8a), or potential denitrification (Figure 4.8b). When mesocosms were lumped as monotypic mesocosm versus multiple patch mesocosms, there was a significant increase in whole mesocosm nitrate retention in October 2015 (T1; t-test: t = -2.20, df = 40, P = 0.03), but not in April or May 2016 (T2 and T3; t-test: t = 1.36, df = 40, P = 0.18; t = -0.94, df = 40, P = 0.35 respectively).

Objective 2:Effect of individual species on whole mesocosm nitrate retention and denitrification

To better understand how plant patch richness affected ecosystem function, I used multiple linear regression to examine the individual effect of each of the four species on mesocosm nitrate retention and denitrification.

The presence of certain plant species affected whole mesocosms nitrate retention, but which species affected nitrate retention changed across sampling periods. In October 2015 (T1), no individual species affected nitrate retention. In April 2016 (T2), nitrate retention was reduced by 33% in mesocosms with *Paspalum distichum* compared to when it was absent (P = 0.009; Table 4.2). This trend continued into May 2016 (T3), where nitrate retention was reduced by 51% in mesocosms with *Paspalum distichum* compared to when it was absent (P = 0.02; 4.2). Further, during T3, mesocosms with

Typha domingensis removed 77% more nitrate than mesocosms without it ($P = 0.02$; Table 4.2).

The presence of *Paspalum distichum* and *Typha domingensis* also affected denitrification measurements, but the presence of the other two species did not. *In situ* denitrification rates were reduced by 60% in mesocosms with *Paspalum distichum* compared to mesocosms without it ($P = 0.003$; Table 4.3). In mesocosms with *Typha domingensis*, *in situ* denitrification rates doubled ($P = 0.02$; Table 4.3). *Paspalum distichum* did not have an effect on potential denitrification (DEAs), but mesocosms with *Typha domingensis* had 60% greater potential denitrification than mesocosms in which it was absent ($P = 0.03$; Table 4.3).

Objective 3: Effect of species edge interactions on mesocosm nitrate retention and denitrification

Species interactions could also affect nitrate retention and denitrification, so I used multiple linear regression to examine the effect of the presence of particular species interactions. Species interactions are defined as two patches that are adjacent to each other.

Species interactions did not affect mesocosm nitrate retention in October 2015 (T1). In April 2016 (T2), two species interactions decreased mesocosm nitrate retention. Interactions between *Ludwigia peploides/Paspalum distichum* (LP) and *Paspalum distichum/Schoenoplectus americanus* (PS) reduced mesocosm nitrate uptake by 37% and 42% respectively compared to mesocosms without those interactions ($P = 0.01$, $P = 0.03$ respectively; Table 4.4). In May 2016 (T3), LP remained significant, reducing mesocosm nitrate retention by 19% ($P = 0.05$; Table 4.4). *Ludwigia peploides/Typha domingensis*

(LT) interaction significantly increased nitrate retention in mesocosms by 61% ($P = 0.05$; Table 4.4).

Similar to whole mesocosm nitrate retention, LP interaction decreased *in situ* mesocosm denitrification rates by 50% ($P = 0.02$; Table 4.5). Species interactions did not affect mesocosm potential denitrification (Table 4.5).

Objective 4: Spatial variation between plant patch centers and edges

In the field study, I examined how potential denitrification varied within patches by sampling in different locations: patch centers, patch edges, and bank locations (Figure 4.5). Location had a significant effect (i.e. center, edge, bank; ANOVA: $F_{2,78} = 5.92$, $P = 0.004$) with potential denitrification at patch edges being significantly greater than patch centers and bank locations (Figure 4.9a). Patch type (i.e. *Ludwigia peploides*, *Typha domingensis*, or open) also significantly affected potential denitrification (ANOVA: $F_{2,78} = 7.30$, $P = 0.001$) with *Ludwigia peploides* patches having the greatest potential denitrification (Figure 4.9b). Potential denitrification was not different between patch centers of different species (ANOVA: $F_{2,30} = 2.43$, $P = 0.115$; Figure 4.10), but was different among different types of edge locations (ANOVA: $F_{6,40} = 5.17$, $P = 0.001$; Figure 4.10). This suggests the differences observed among patch types were driven by differences only at edge locations. There also were not differences among soil organic matter, soil moisture, surface water nitrate concentrations, or %silt/clay among patch centers, edges and banks (ANOVA: $F_{2,78} = 1.74$, $P = 0.07$; $F_{2,78} = 1.36$, $P = 0.26$; $F_{2,78} = 0.18$, $P = 0.83$; $F_{2,78} = 1.23$, $P = 0.30$ respectively). These soil and water conditions are often important predictors of denitrification (Boyer et al. 2006; Seitzinger et al. 2006).

Consequently, this suggests that spatial variation in these soil and water conditions are not explaining the observed spatial variation in potential denitrification at these wetlands.

In the greenhouse study, there was no spatial variability within patches (i.e. no differences between plant patch centers and edges) for both *in situ* rates of denitrification (paired t-test: $t = 1.5$, df = 83, $P = 0.14$) and potential denitrification (paired t-test: $t = 0.90$, df = 83, $P = 0.37$). Further, there was no effect of type of edge interaction on the difference between plant patch edges and centers for *in situ* rates (ANOVA: $F_{5,78} = 0.41$, $P = 0.84$) and potential denitrification (ANOVA: $F_{5,78} = 0.29$, $P = 0.92$).

Objective 5: Effect of belowground interactions on whole mesocosm nitrate removal and denitrification

To test if belowground interactions affected nitrate retention and denitrification, belowground barriers were added into some mesocosms to prevent belowground interactions. The addition of belowground barriers significantly increased total mesocosm belowground biomass, total mesocosm nitrate retention during April 2016 (T2) sampling period, and *in situ* denitrification rates (paired t-test: $t = -2.61$, df = 11, $P = 0.02$; $t = -3.37$, df = 11, $P = 0.006$; and $t = -2.34$, df = 11, $P = 0.04$ respectively; Figure 4.11). The increase in total mesocosm belowground biomass in mesocosms with barriers was reflected in the increase in total belowground biomass of *Typha domingensis* only (paired t-test: $t = -2.90$, df = 8, $P = 0.02$). The addition of belowground barriers also increased the difference between center and edges of patches for *in situ* rates of denitrification (paired t-test: $t = -2.53$, df = 44, $P = 0.02$; Figure 4.12). This difference was driven by a significant increase of *in situ* rates of denitrification in patch centers in barrier treatments (t-test: $t = -2.89$, df = 94, $P = 0.005$; Figure 4.13).

DISCUSSION

Five main conclusions can be drawn from the results. (1) Species richness affects biomass, but (2) does not affect nitrate retention or denitrification. (3) Individual species, rather than species richness, are more important for explaining nitrate retention and denitrification. (4) Wetlands in the field show spatial variation in potential denitrification in plant patches, but this pattern is not reflected in greenhouse experiment. (5) Blocking belowground interactions between species increases belowground biomass and *in situ* denitrification rates.

Species richness and biomass

In this study, aboveground biomass decreased with increasing richness, while belowground biomass remained unchanged. Previous studies have found different relationships among species richness and biomass. Of the few studies manipulating species richness in wetlands, researchers have found both no effect on above- or belowground biomass with increasing species richness (Engelhardt and Ritchie 2001), and increasing total and belowground biomass with increasing richness (Callaway et al. 2003; Bouchard et al. 2007). Notably, these studies examined different wetland community types. Engelhardt and Ritchie (2001) examined submerged clonal macrophytes that often grow in monotypic patches but were planted in mixtures for the experiment. Bouchard et al. (2007) specifically excluded clonal dominants from their study (they were included in my study) and Callaway et al. (2003) examined a wetland halophyte community. Further, in these studies species were grown in mixtures, a configuration similar to grassland studies. Field studies have also found either a lack of correlation between species richness and biomass in wetlands, or higher biomass

associated with fewer species (Moore and Keddy 1989; García et al. 1993). Taken together, this suggests the relationship between species richness and productivity in wetlands is not as clear as it is in grasslands (Cardinale et al. 2011) and more studies are necessary to elucidate the relationship between the two. One key difference between wetlands and grasslands is wetlands are characterized by fluctuations in inundation. How frequently wetlands inundate partially dictates the zonation of wetland functional groups and these different groups may respond differently to the presence of other species (van der Valk 1981; Boutin and Keddy 1993). Therefore, it is possible the direction of the relationship between species richness and biomass could be dependent on the environmental context, such as inundation duration, in wetlands (Casanova and Brock 2000).

Whole mesocosm nitrate removal and denitrification did not increase with increasing species richness

Increasing species richness had no effect on whole mesocosm nitrate retention or denitrification. Again, the studies examining this relationship in wetlands are limited, and produced mixed results. Callaway et al. (2003) found increasing species richness increased retention of nitrogen in plant biomass, but did not measure microbial processes such as denitrification. Similar to this study, Engelhardt and Ritchie (2001) found no effect of species richness in whole mesocosm nitrogen retention. Of the two studies examining the relationship between species richness and potential denitrification in wetlands, no relationship was found (Bouchard et al. 2007; McGill et al. 2010). It is clear that more studies need to be conducted on how species richness affects nitrogen retention in wetlands before any generalizations can be made. One important consideration in

future studies should be distinguishing between the two mechanisms of nitrogen retention in wetlands: assimilation into plant tissues and denitrification. These might be affected differently by species richness, muddling correlations between species richness and whole system nitrogen retention in wetlands. In grasslands, increases in nitrogen retention with increases in species diversity are attributed to increased assimilation of nitrogen into plant tissues. Because grasslands are not frequently saturated, conditions are not conducive for denitrification and plant uptake accounts for more nitrogen retention than denitrification (Scherer-Lorenzen et al. 2003; Palmborg et al. 2005). However, wetland soils are frequently inundated, creating conditions more conducive to denitrification. This results in a situation where both plant uptake and denitrification are contributing to nitrogen retention. Increased species richness could still result in wetland plant tissues assimilating more nitrate (Callaway et al. 2003), but denitrifiers could use remaining available soil nitrate, masking the effect of plant uptake on whole mesocosm nitrogen retention experiments (Bachand and Horne 1999). My study did find patterns suggestive of such a masking effect. In October 2015 sampling (T1), I could not detect denitrification with DEAs suggesting the first sampling period was representative of only plant assimilation of nitrate. At T1, there was a significant increase in whole mesocosm nitrate retention in mesocosm planted with multiple species, versus mesocosms planted with one species. After the denitrifying microbes established, there no longer were such differences between mesocosms with single and multiple species. This certainly is not conclusive, as I did not intend to test the effects of plant species richness on nitrate retention before and after denitrifying microbes were established. However, it does suggest that studies parsing out the effects of plant assimilation and denitrification could

offer better insight into the mechanisms by which species richness affects nitrate retention in wetlands.

Individual species are more important for nitrate retention and denitrification

The results of the greenhouse experiment suggest that the presence of certain species were more important for whole mesocosm nitrate retention and denitrification than species richness. *Typha domingensis* and *Paspalum distichum* had the greatest, but opposite, effects on nitrate retention and denitrification. Similar to other studies, the presence of *Typha domingensis* increased both whole mesocosm nitrate retention and denitrification (Davis 1991; Bachand and Horne 1999; Maltais-Landry et al. 2009). *Typha domingensis* also has the greatest biomass of the four experimental species so it is likely to accumulate more nitrogen in plant tissues contributing to greater mesocosm nitrate retention (Tanner 1996). Further, increasing plant biomass often increases denitrification likely due to increased availability of organic matter; another mechanism by which *Typha domingensis* could affect nitrate retention and denitrification (Sutton-Grier et al. 2012). Interestingly, the presence of *Paspalum distichum* decreased nitrate retention and *in situ* denitrification rates compared to mesocosms in which it was absent, but did not affect potential denitrification rates. It is not clear why this is the case. *Paspalum distichum* did not have the lowest biomass of the four species so it likely another unmeasured plant effect on soil conditions, such as oxygenation of the rhizosphere or labile carbon availability (Reddy et al. 1989), limited denitrification *in situ* but did not affect potential denitrification measurements which are taken under optimal conditions for denitrifiers (low oxygen, high carbon, and high nitrate; Alldred and Baines 2016). Plants affect denitrification by changing the soil environment that denitrifiers

experience (Zak et al. 2003; Wardle et al. 2004). How plants change the soil environment will depend on the specific morphology and physiology (i.e plant traits) of the species. Before we can understand how species richness affects denitrification, we need a better understanding of the mechanistic link between plant species and denitrification.

Spatial variation

The field study revealed spatial variation within plant patches such that edge locations within plant patches were higher than centers. I hypothesized that patch edges that were interacting with other plant patches would have higher potential denitrification due to diversity of litter or belowground carbon sources available at those locations (Meier and Bowman 2008); however, even edges adjacent to open (unvegetated areas) had higher potential denitrification than patch centers. This result could still be due to increased diversity of carbon sources, as algae often colonize open areas at these particular study sites; however, this pattern could also be the result of another, unmeasured mechanism, such as hydrodynamics, because other common predictors of denitrification (soil organic matter, soil texture) did not show spatial variation within patches.

The hypothesis that plant interactions at edges of patches would increase denitrification was further tested in the greenhouse experiment. Interestingly, plant patches in the greenhouse lacked of spatial variation. Given the short duration of the experiment (less than one year), it is possible greenhouse mesocosms were not given enough time to develop substantial interactions among patches that could affect denitrification. Barriers were placed in a subset of greenhouse mesocosms to further test if diversity of belowground carbon sources would increase denitrification. If this

hypothesis is correct, then denitrification would decrease in the presence of barriers, and edges and centers would be more similar to each other. Instead, the presence of a belowground barrier increased belowground biomass and *in situ* denitrification rates, but did not increase potential denitrification. This seems to imply that interacting edges of patches were inhibiting *in situ* denitrification rates resulting in higher rates in mesocosms with barriers. Interestingly, this was not the case as there were no differences between centers and edges of patches in mesocosms without barriers. Rather, the addition of barriers created a significant gradient between centers and edges of patches that was largely driven by an increase in *in situ* denitrification rates at patch centers. It is possible that the increase in denitrification rates was driven by an increase in biomass in mesocosms with barriers; however, it is not clear why this was not reflected in potential denitrification as well.

Synthesis

This study was the first to examine the relationships between species richness and ecosystem function using the patch configurations of clonal plants typical of many wetlands. Only a few studies have examined how species diversity affects ecosystem functions in wetlands, and each has been conducted in wetlands with different plant communities and with different hydroperiods as discussed earlier. The varying results from these studies, including this one, suggests that wetlands cannot be lumped into one category, but rather plant community type, hydroperiod, and configuration of plants in wetlands need to be considered when examining relationships among biodiversity and ecosystem functions. This study further highlights the need to parse out the effects of plant assimilation and denitrification in nitrogen retention, an important distinction as

denitrification often accounts for a significant portion of nitrate removal in wetlands (Bachand and Horne 1999).

Notably, greenhouse experiment and field study results were not in agreement. Different species have the highest potential denitrification in greenhouse experiment and field study (*Typha domingensis* and *Ludwigia peploides* respectively). Field study also shows strong positive effect of edge interactions, while greenhouse experiment suggests no effect of edge interactions on denitrification. The short duration of this experiment could explain some disagreement between studies as greenhouse mesocosms were newly established and denitrifying microbes took several months to develop. Running a multiyear greenhouse experiment could reveal changes in patterns of denitrification as wetlands develop.

Belowground interspecific interactions among plants result in competition that can lead to changes in biomass (Casper and Jackson 1997; Bais et al. 2006). The combined effect of increased belowground biomass in mesocosms with barriers, and increased aboveground biomass in mesocosm with one species, suggest that competition among these wetland species decrease biomass when more than one species is present, rather than increase biomass as commonly observed in grasslands.

CONCLUSION

Generalizable relationships between species richness and ecosystem functions may be challenging in wetlands given the various hydrologic environments (e.g. standing water vs no standing water) and growth forms of plants (e.g. submerged vs emergent; clonal vs. not clonal) that can influence ecosystem functions (van der Valk 1981; Boutin

and Keddy 1993). For example, denitrification could be an important pathway of loss for nitrate in permanently saturated wetlands, where as plant uptake could be more important in wetlands with fluctuating water levels (Hernandez and Mitsch 2007). Given the diversity of wetland types, a mechanistic understanding of what plant traits increase nitrogen assimilation and denitrification is needed to fully understand how changing hydrology could mediate the relationship between species richness and nitrate retention in wetlands.

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TABLES AND FIGURES

Table 4.1. Target concentrations of water used in greenhouse experiment.

Solute	Added as	Target concentration
NO_3^--N	KNO_3	1 mg-N l^{-1}
PO_4-P	NaH_2PO_4	1 mg-P l^{-1}
K^+	Instant ocean and KNO_3	10 mg l^{-1}
Cl^-	Instant ocean	306 mg l^{-1}
Na^+	Instant ocean	250 mg l^{-1}
Fe^{2+}	$FeSO_4$	0.01 mg l^{-1}
Mg^{2+}	Instant ocean	27 mg l^{-1}
Mn^{2+}	Instant ocean	0.003 mg l^{-1}
SO_4^{2-}	Instant ocean	30 mg l^{-1}
Ca^{2+}	Instant ocean and $CaCl_2$	17 mg l^{-1}
Si	Instant ocean	5 mg l^{-1}
DOC	Glucose	7 mg l^{-1}

Table 4.2. Mean mesocosm nitrate removal (mg NO_3^--N h^{-1}) for the presence and absence of each species. Parentheses contain 95% confidence interval. Results of multiple regression for each sampling period presented as standardized beta coefficient and *P-value*. Significant results are bolded. L = *Ludwigia peploides*; P = *Paspalum distichum*; S = *Schoenoplectus americanus*; T = *Typha domingensis*.

	Present	Absent	Std beta coefficient	*P*
T1 (October 2015)				
L	0.1 (0.1, 0.2)	0.1 (0.0, 0.2)	0.21	0.11
P	0.1 (0.1, 0.2)	0.1 (0.0, 0.1)	0.13	0.32
S	0.1 (0.1, 0.2)	0.1 (0.1, 0.2)	-0.02	0.91
T	0.1 (0.1, 0.2)	0.1 (0.1, 0.1)	0.009	0.95
starting conc			0.635	<0.001
T2 (April 2016)				
L	2.4 (1.8, 2.9)	2.6 (2.1, 3.2)	-0.15	0.32
P	**2.0 (1.5, 2.5)**	**3.0 (2.5, 3.5)**	**-0.42**	**0.009**
S	2.4 (1.9, 2.8)	2.6 (2.0, 3.2)	-0.15	0.33
T	2.9 (2.3, 3.4)	2.1 (1.6, 2.7)	0.18	0.24
starting conc			0.05	0.81
T3 (May 2016)				
L	4.0 (2.9, 5.0)	2.1 (1.0, 3.3)	0.02	0.87
P	**2.0 (1.0, 3.0)**	**4.1 (2.9, 5.3)**	**-0.23**	**0.02**
S	2.8 (1.7, 3.8)	3.4 (2.1, 4.6)	-0.14	0.16
T	**3.9 (2.8, 5.0)**	**2.2 (1.1, 3.4)**	**0.22**	**0.02**
starting conc			0.70	<0.001

Table 4.3. Mean mesocosm in situ denitrification rates (mg N_2O-N m^{-2} h^{-1}) and potential denitrification (mg N_2O-N m^{-2} h^{-1}) the presence and absence of each species. Parentheses contain 95% confidence interval. Results of multiple regression for each sampling period presented as standardized beta coefficient and *P*-value. Significant results are bolded. L = *Ludwigia peploides*; P = *Paspalum distichum*; S = *Schoenoplectus americanus*; T = *Typha domingensis*.

	Present	Absent	Std beta coefficient	*P*
In situ rates				
L	0.3 (0.2, 0.5)	0.3 (0.2, 0.5)	-0.08	0.60
P	**0.2 (0.2, 0.3)**	**0.5 (0.3, 0.7)**	**-0.46**	**0.003**
S	0.3 (0.2, 0.4)	0.3 (0.2, 0.5)	-0.13	0.35
T	**0.4 (0.3, 0.6)**	**0.2 (0.2, 0.4)**	**0.25**	**0.02**
Potential denitrification				
L	5.1 (3.6, 7.3)	6.9 (5.6, 8.4)	-0.15	0.32
P	5.3(4.3, 6.5)	5.3 (4.3, 6.5)	-0.11	0.47
S	6.9 (5.5, 8.6)	5.1(3.7, 7.2)	0.23	0.14
T	**7.5 (6.0, 9.4)**	**4.7 (3.4, 6.4)**	**0.35**	**0.03**

Table 4.4. Mean mesocosm nitrate removal (mg NO_3^--N h^{-1}) for the presence or absence of species interactions. Parentheses contain 95% confidence interval. Results of multiple regression for each sampling period presented as standardized beta coefficient and *P-value*. Significant results are bolded. LP = *Ludwigia peploides*/*Paspalum distichum*; LS = *Ludwigia peploides/Schoenoplectus americanus*; LT = *Ludwigia peploides*/*Typha domingensis*; PS = *Paspalum distichum/ Schoenoplectus americanus*; PT = *Paspalum distichum/ Typha domingensis*; ST = *Schoenoplectus americanus/Typha domingensis.*

	Present	Absent	Std beta coefficient	*P*
T1 (October 2015)				
LP	0.1 (0.1, 0.2)	0.1 (0.1, 0.1)	0.15	0.31
LS	0.1 (0.1, 0.1)	0.1 (0.1, 0.2)	0.14	0.33
LT	0.1 (0.1, 0.2)	0.1 (0.1, 0.1)	0.06	0.69
PS	0.2 (0.0, 0.3)	0.1 (0.1, 0.1)	-0.02	0.90
PT	0.2 (0.1, 0.4)	0.1 (0.1, 0.1)	0.14	0.38
ST	0.1 (0.0, 0.1)	0.1 (0.1, 0.2)	-0.17	0.24
starting conc			0.61	<0.001
T2 (April 2016)				
LP	**1.7 (0.9, 2.6)**	**2.7 (2.3, 3.1)**	**-0.45**	**0.01**
LS	2.6 (2.1, 3.2)	2.4 (2.0, 2.9)	0.11	0.51
LT	2.8 (1.7, 3.9)	2.4 (2.0, 2.9)	0.18	0.27
PS	**1.5 (0.5, 2.6)**	**2.6 (2.2, 3.0)**	**-0.38**	**0.03**
PT	2.3 (1.8, 2.8)	2.5 (2.1, 3.0)	0.06	0.73
ST	2.8 (1.9, 3.7)	2.4 (2.0, 2.9)	-0.05	0.78
starting conc			0.08	0.60
T3 (May 2016)				
LP	**2.6 (1.4, 3.8)**	**3.2 (2.2, 4.2)**	**-0.22**	**0.05**
LS	3.7 (2.5, 4.9)	2.9 (1.9, 3.9)	-0.02	0.90
LT	**4.5 (2.5, 6.5)**	**2.8 (1.9, 3.7)**	**0.22**	**0.05**
PS	1.3 (-0.2, 2.9)	3.3 (2.4, 4.2)	-0.14	0.21
PT	2.5 (0.2, 4.8)	3.2 (2.3, 4.0)	0.07	0.54
ST	5.0 (3.0, 6.9)	2.7 (1.9, 3.6)	0.05	0.67
starting conc			0.74	<0.001

Table 4.5. Mean mesocosm *in situ* denitrification rates (mg N_2O-N m^{-2} h^{-1}) and potential denitrification (mg N_2O-N m^{-2} h^{-1}) for the presence and absence of species interactions. Parentheses contain 95% confidence interval. Results of multiple regression for each sampling period presented as standardized beta coefficient and *P-value*. Significant results are bolded. LP = *Ludwigia peploides*/*Paspalum distichum*; LS = *Ludwigia peploides/Schoenoplectus americanus*; LT = *Ludwigia peploides*/*Typha domingensis*; PS = *Paspalum distichum/ Schoenoplectus americanus*; PT = *Paspalum distichum/ Typha domingensis*; ST = *Schoenoplectus americanus/Typha domingensis.*

	Present	Absent	Std beta coefficient	*P*
In situ rates				
LP	**0.2 (0.2, 0.3)**	**0.4 (0.3, 0.5)**	**-0.40**	**0.02**
LS	0.3 (0.2, 0.5)	0.3 (0.2, 0.4)	-0.04	0.82
LT	0.5 (0.3, 0.8)	0.3 (0.2, 0.4)	0.26	0.12
PS	0.2 (0.1, 0.3)	0.3 (0.3, 0.5)	-0.25	0.14
PT	0.3 (0.1, 0.5)	0.3 (0.3, 0.4)	-0.02	0.90
ST	0.5 (0.3, 0.9)	0.3 (0.2, 0.4)	0.16	0.32
Potential denitrification				
LP	4.6 (3.2, 6.7)	6.4 (5.0, 8.1)	-0.29	0.11
LS	7.9 (5.4, 11.4)	5.5 (4.3, 7.0)	0.32	0.07
LT	6.8 (4.1, 11.1)	5.8 (4.6, 7.3)	0.25	0.15
PS	5.1 (3.7, 7.0)	6.1 (4.8, 7.7)	-0.12	0.51
PT	6.8 (4.8, 9.7)	5.8 (4.6, 7.3)	0.19	0.28
ST	7.7 (4.6, 12.7)	5.7 (4.5, 7.1)	0.06	0.73

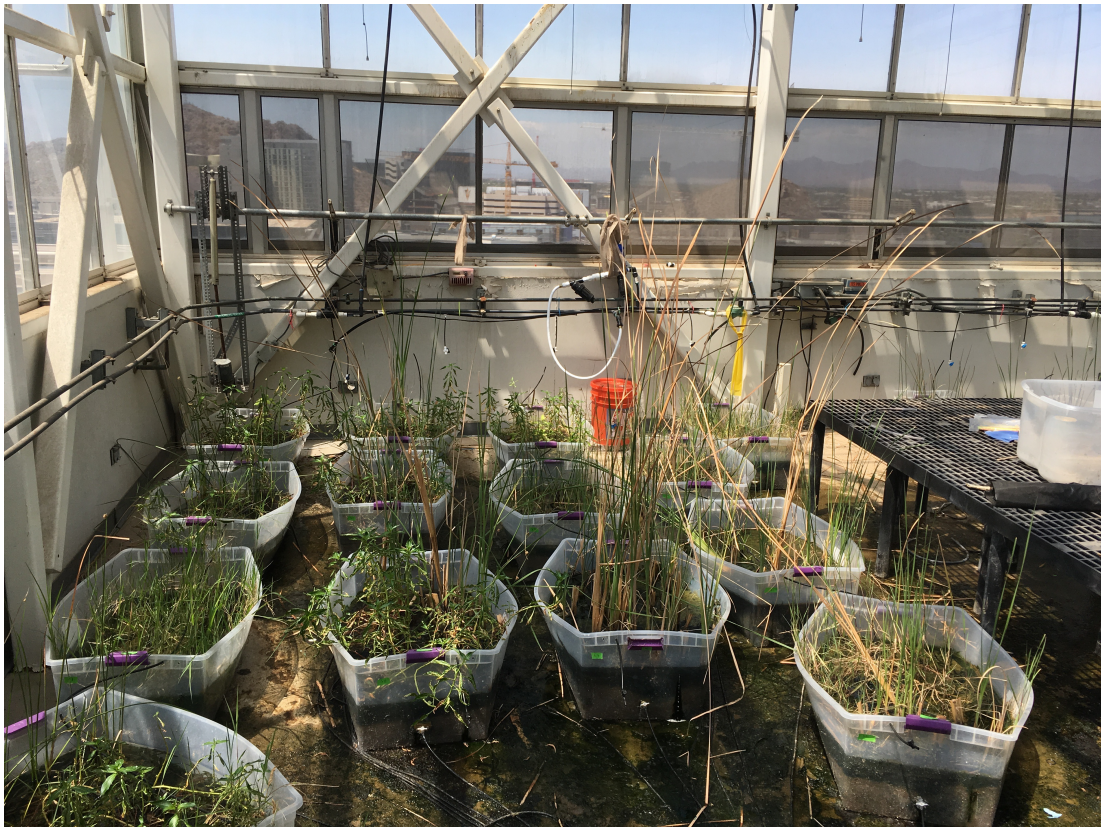

Figure 4.1. Image of greenhouse experiment setup.

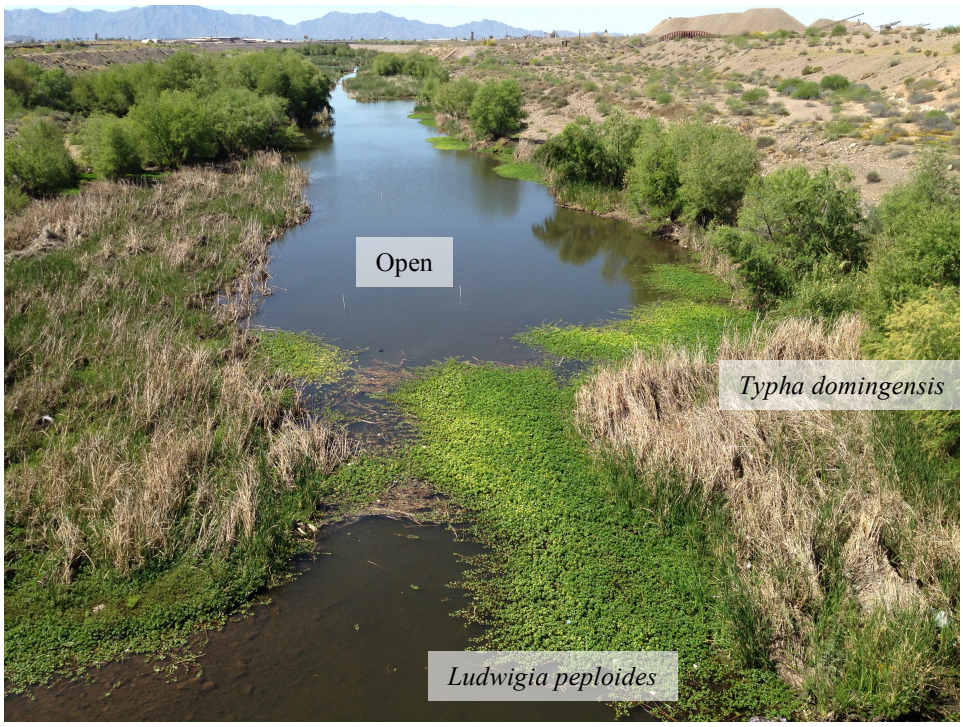

Figure 4.2. Wetland in Salt River highlighting monotypic patches typical of emergent wetlands.

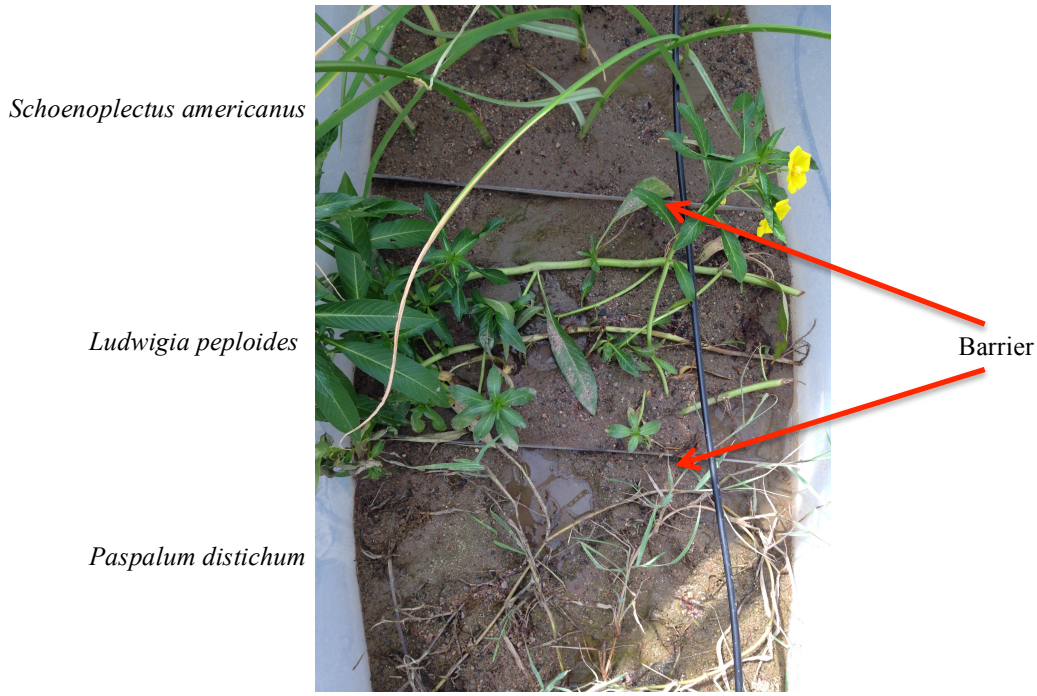

Figure 4.3. Image of barriers in mesocosm planted with three patches.

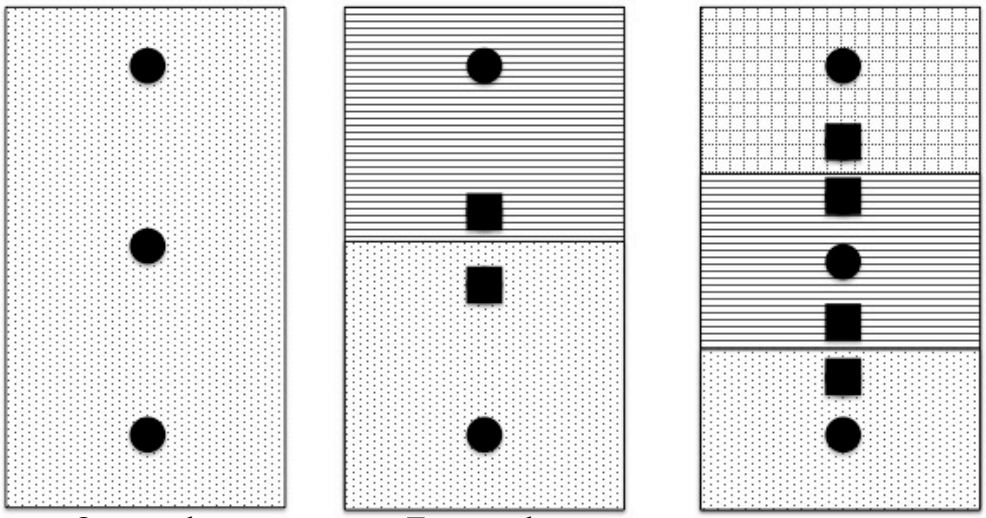

Figure 4.4. Denitrification sampling locations in mesocosms for each richness treatment. Different patterns represent different patch type. Solid circles represent center of patch sampling locations. Solid squares represent edge of patch sampling locations.

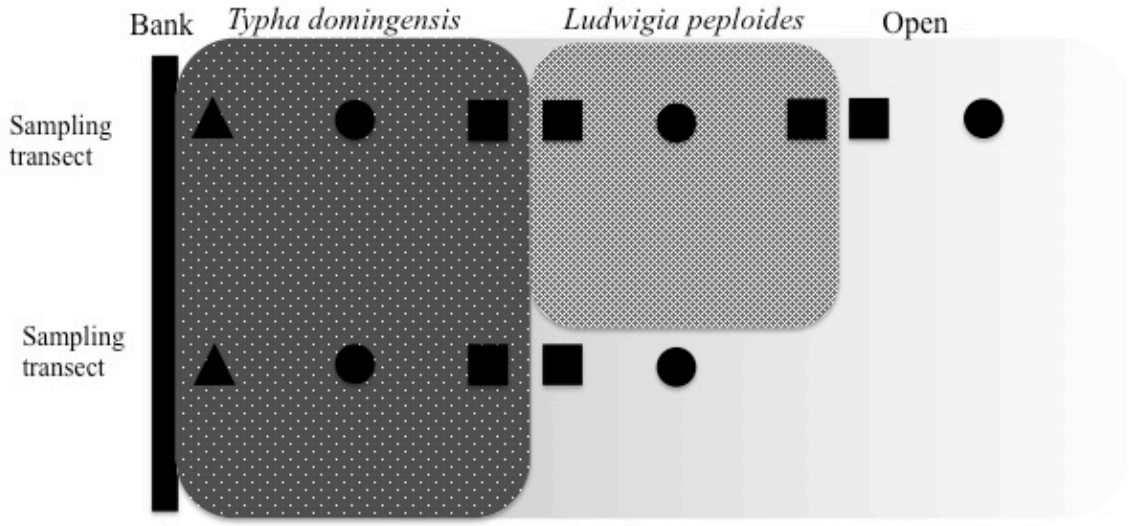

Figure 4.5. Field sampling locations. Different patterns represent different patch types. Solid circles represent center of patch sampling locations. Solid squares represent edge of patch sampling locations. Triangles represent bank sampling locations.

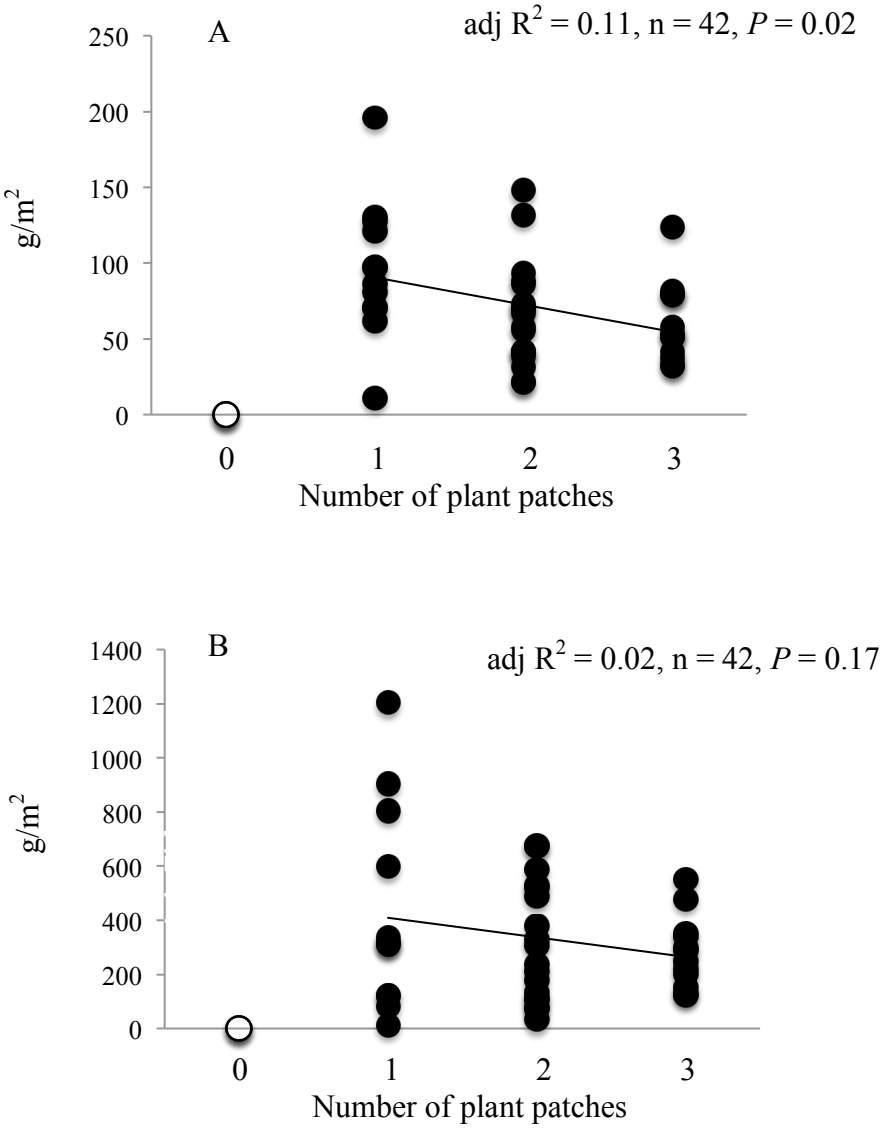

Figure 4.6. Effects of mesocosm plant patch richness on biomass. (A) Aboveground biomass, and (B) belowground biomass. Solid circles represent treatments included in regression. Line represents simple regression. Open circles represent unplanted control mesocosms not included in regression.

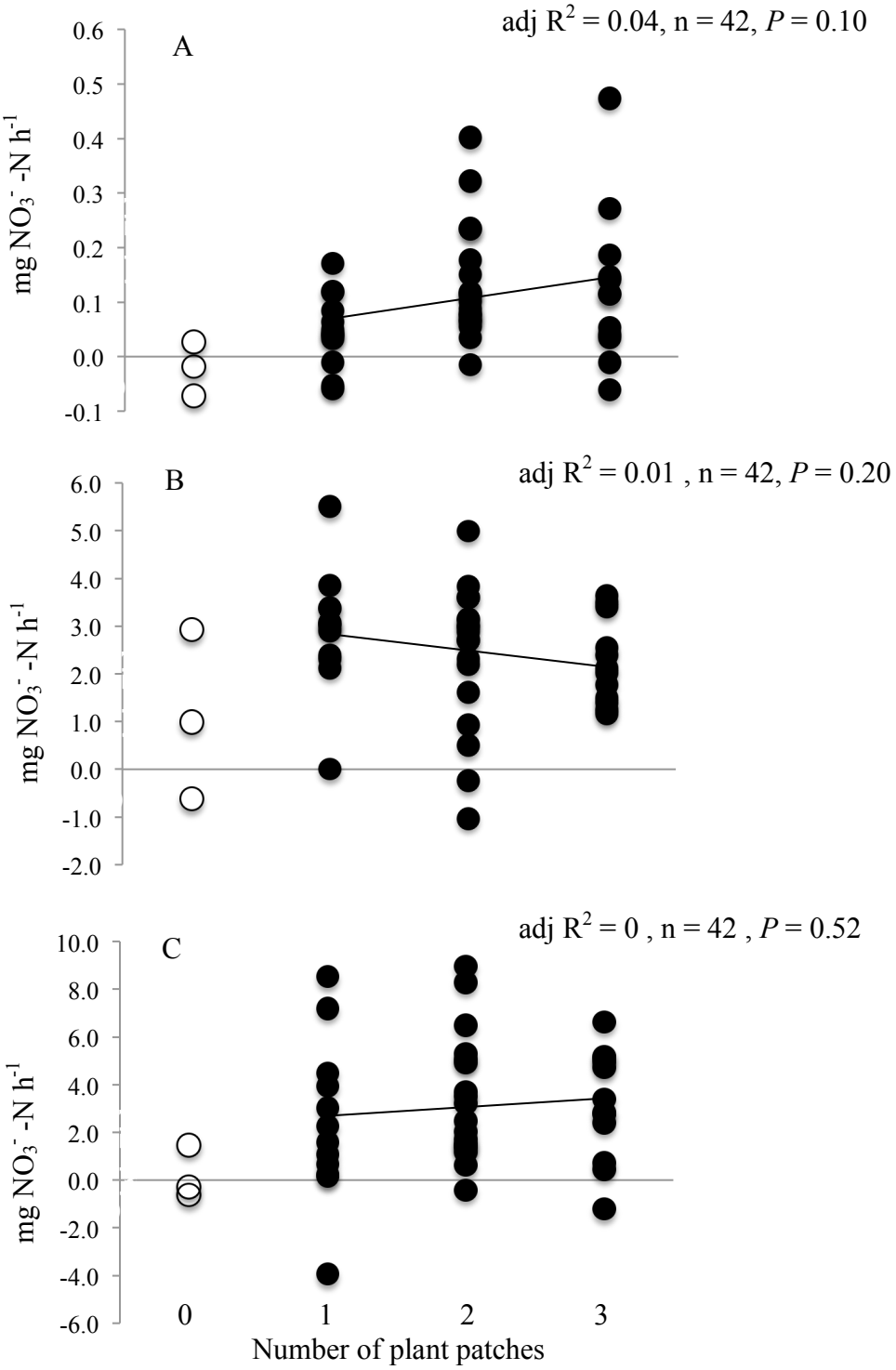

Figure 4.7. Effects of mesocosm plant patch richness on whole mesocosm nitrate removal. Sampling periods are (A) T1 in October 2015, (B) T2 in April 2016, and (C) T3 in May 2016. Solid circles represent treatments included in regression. Line represents simple regression. Open circles represent unplanted control mesocosms not included in regression. Note scale differences.

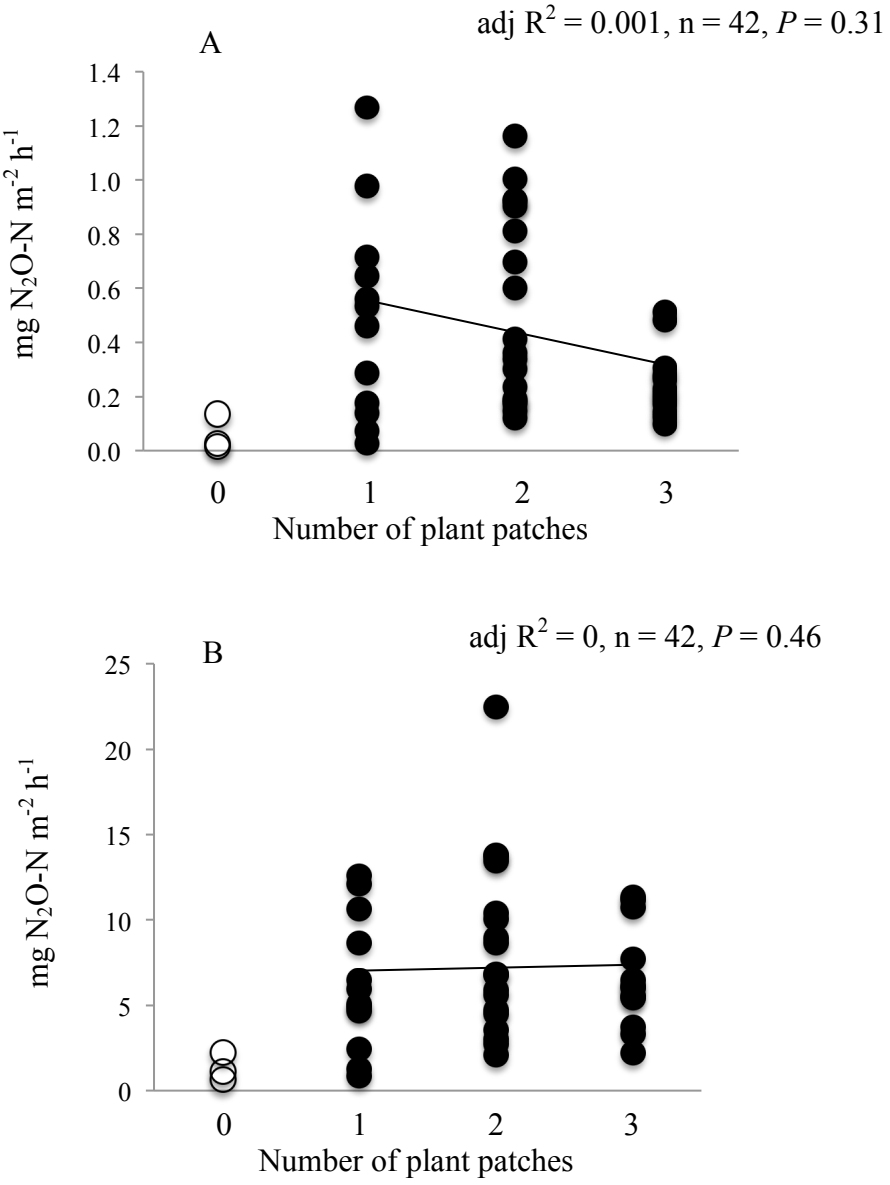

Figure 4.8. Effects of mesocosm plant patch richness on denitrification. (A) *In situ* denitrification rates, and (B) denitrification potential. Solid circles represent treatments included in regression. Line represents simple regression. Open circles represent unplanted control mesocosms not included in regression.

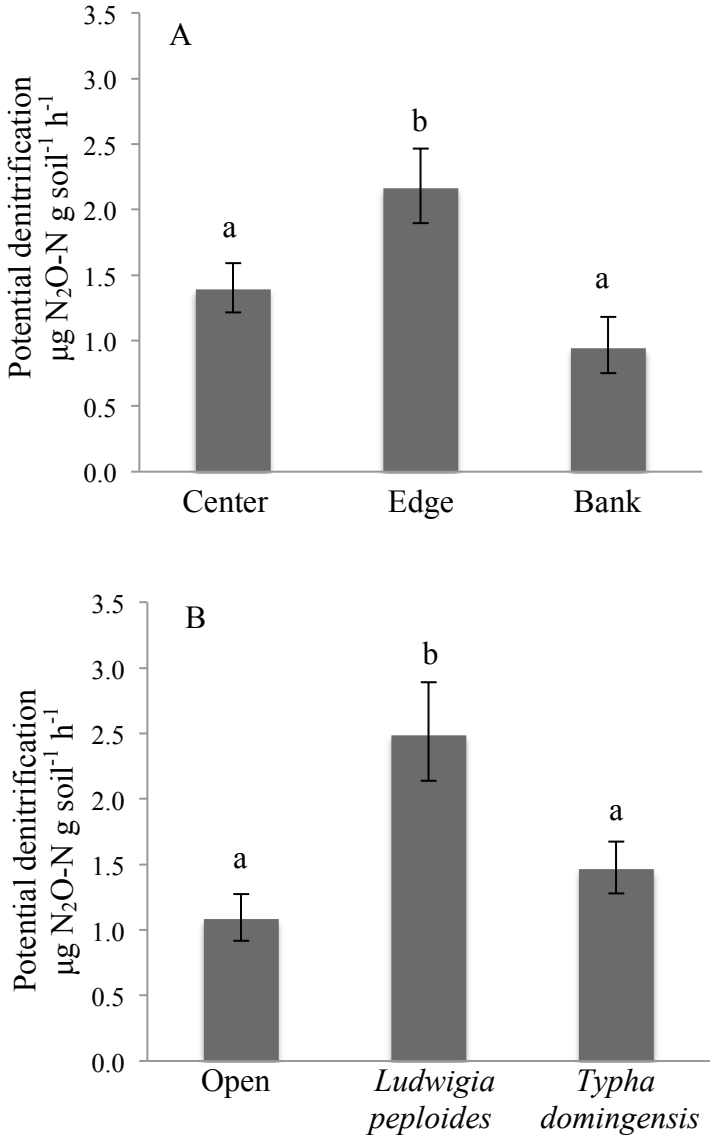

Figure 4.9. Mean potential denitrification (DNP) of different sampling locations in field study. (A) Patch centers, edges, and banks, and (B) patch types. Significance level $P < 0.05$. Bars represent ±1 SE.

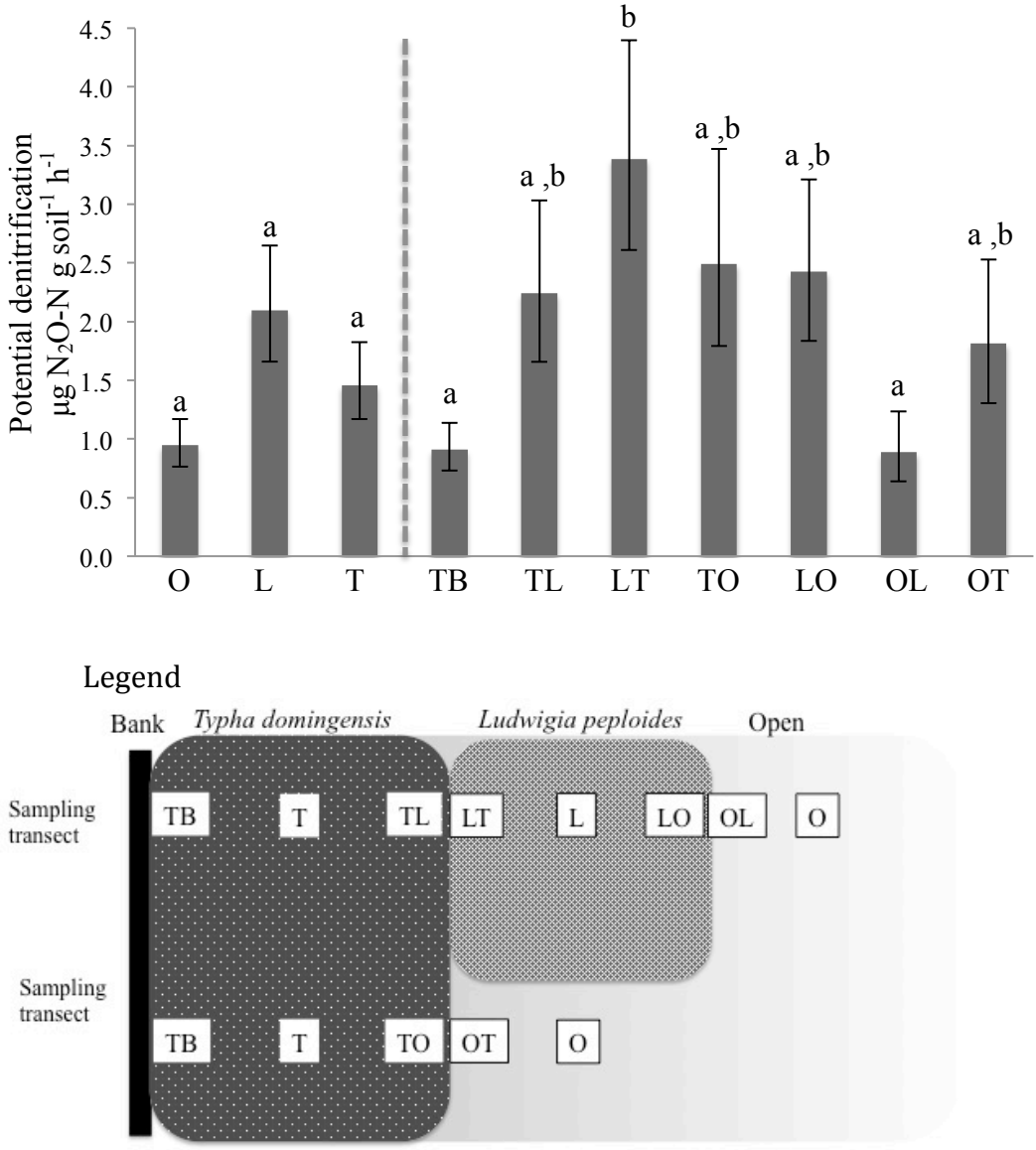

Figure 4.10. Mean potential denitrification (DNP) of different patch interactions in field study. Center locations are: O=open (unvegetated) patches; L=*Ludwigia peploides*; T=*Typha domingensis*. Edge locations use two letter codes using the same patch codes plus B=bank. The first letter indicates the patch the sample was taken in and the second letter indicate the adjacent patch. Therefore, LT indicates a sample collected at the edge of a *Ludwigia peploides* patch that was adjacent to a *Typha domingensis* patch. While TL indicates a samples take in a *Typha domingensis* patch that was adjacent to a *Ludwigia peploides* patch. See legend for visual depiction of sampling locations. Center locations compared with ANOVA and edge locations compared with ANOVA. Significance level $P < 0.05$. Bars represent ±1 SE.

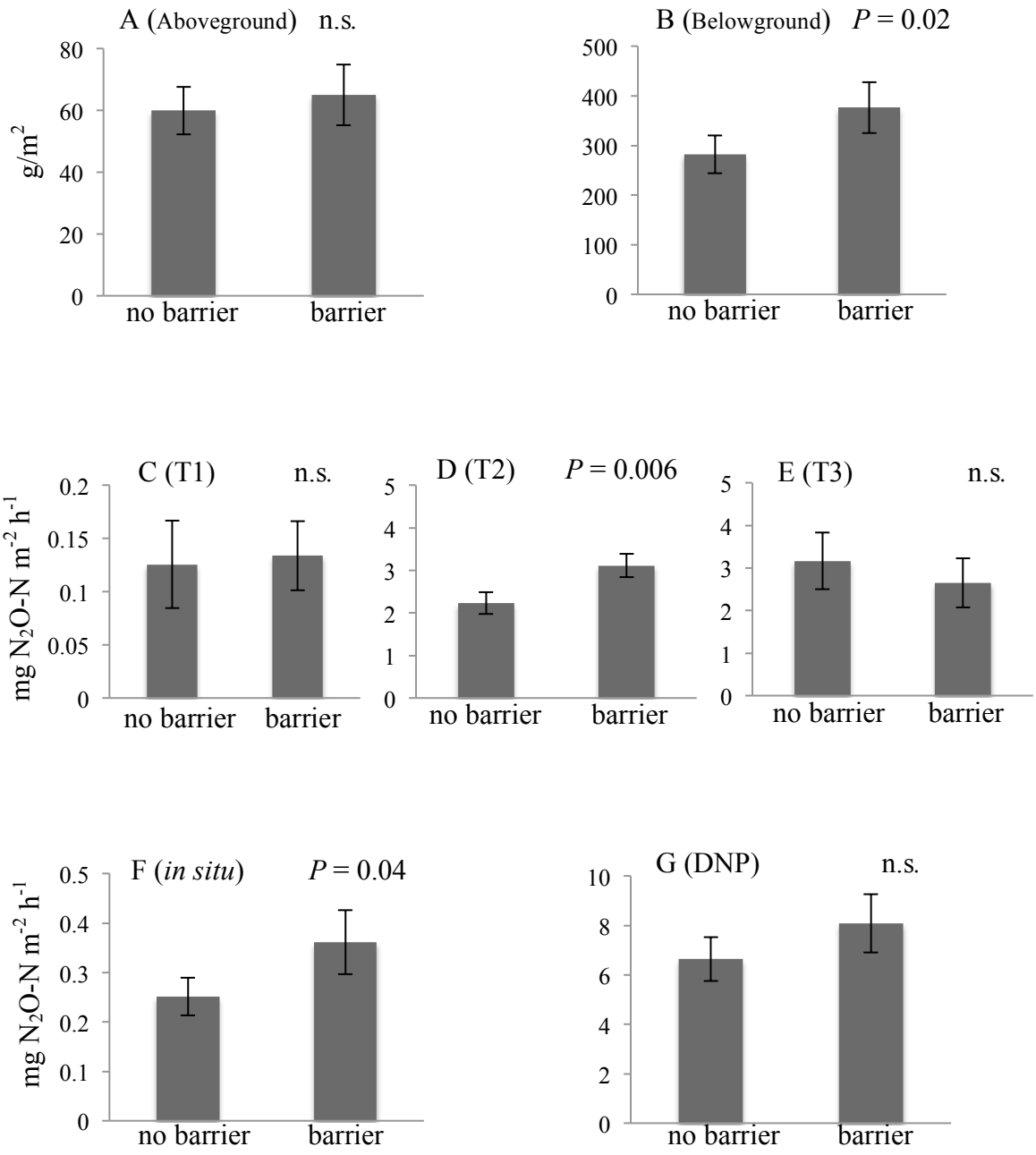

Figure 4.11. Mean differences between mesocosms without and with a belowground barrier. (A) Aboveground biomass, (B) belowground biomass, whole mesocosm nitrate uptake at (C) T1, (D) T2, and (E) T3, (F) *in situ* denitrification rates, and (G) potential denitrification. Bars represent ±1 SE. Note scale differences.

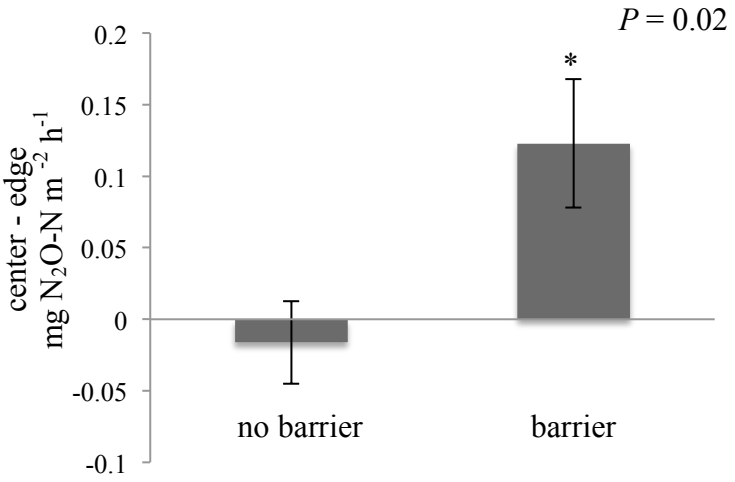

Figure 4.12. Difference between *in situ* denitrification rates at patch centers and edges (calculates as center – edge) for treatments without and with a barrier. A value below zero represents a gradient where denitrification is lower at patch centers than edges. A value equal to zero represents no gradient where patch centers and edges are equal. A value above zero represents a gradient where denitrification is higher at patch centers than edges.

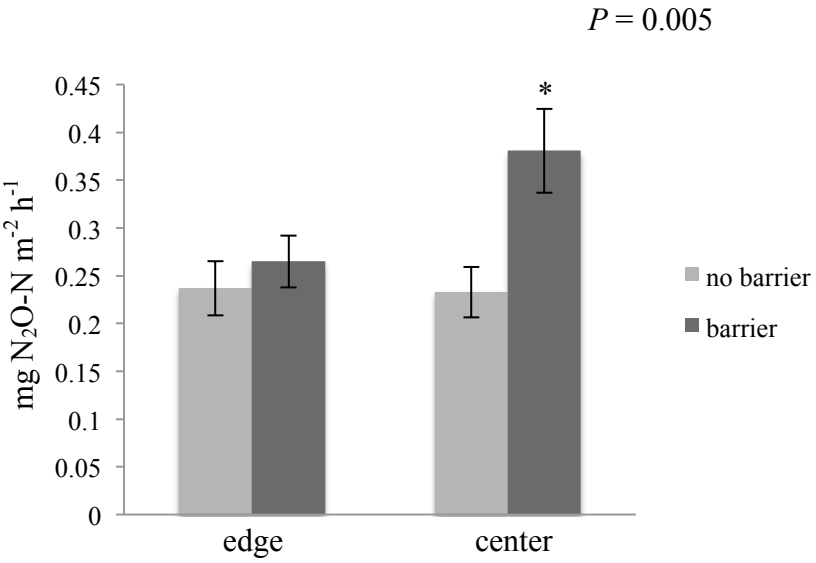

Figure 4.13. Mean *in situ* denitrification rates for patch edges in treatments without and with a barrier, and patch centers with and without a barrier.

Chapter 5

SYNTHESIS

Humans have greatly altered the nitrogen cycle by nearly doubling the amount of available biologically reactive nitrogen (NO_3^-, NH_4; Galloway et al. 2004). Excess nitrate can cause eutrophication, harmful algal blooms, and hypoxia, negatively affecting economies and human health (Vitousek et al. 1997; Townsend et al. 2003; Howarth et al. 2011). Cities are often sources of nitrate to downstream ecosystems; therefore, several solutions have been proposed to mitigate nitrate pollution, such as incorporating stormwater retention basins, swales, and wetlands into urban landscapes (Zhu et al. 2005; Shields et al. 2008; Kaushal et al. 2011; Bettez and Groffman 2012; Hale et al. 2014). In addition to these features, the urban environment has several features that are not managed, but have the potential to reduce nitrate loads. One such feature is accidental urban wetlands–wetlands that results from human activities, but are not designed or managed for any specific outcome.

To effectively reduce nitrate loads, these systems need to permanently remove nitrate via the microbial process of denitrification. Great strides have been made in understanding what abiotic conditions promote the process of denitrification, yet clear links between community structure and denitrification have not been established (Hooper et al. 2005; Boyer et al. 2006; Seitzinger et al. 2006). Additionally, understanding what biotic and abiotic conditions promote denitrification is further complicated in urban environments as hydrology, soils, and biota can be dramatically altered relative to non-urban counterparts (Ehrenfeld 2000; Windham and Ehrenfeld 2003; Stander and Ehrenfeld 2008).

In this dissertation, I used an understudied feature of urban landscapes–accidental urban wetlands–to examine if frequently identified drivers of denitrification remain important for these wetlands. Further, data collected from accidental urban wetlands were used to advance our understanding of how plants affect denitrification, addressing two ecological questions: (1) What is the relationship between plant traits and denitrification? (2) What is the relationship between species richness and denitrification? Here, I summarize the key findings from these studies and discuss the implications of this research for management of urban wetlands.

Unexpected patterns of denitrification in accidental wetlands (Chapter 2)

Accidental urban wetlands have the capacity to remove nitrate via denitrification, but that the urban landscape was likely influencing drivers of patterns of denitrification in these wetlands. Hydroperiod was an important driver of denitrification for all wetlands as increases in hydroperiod increased potential denitrification. Interestingly, hydroperiod also was related to whether nitrate or carbon limited denitrification. Wetlands that flooded for more than 45% of the year exhibited nitrate limitation of denitrification, while wetlands that flooded less than 45% of the year showed no clear limitation. Interestingly, plants did not alleviate carbon limitation of denitrification as they do in nearby desert streams. This result could be explained by differences in land-use of the stormwater pipe-sheds that supplied water to each wetland. Wetlands that flooded for more than 45% of year had pipe-sheds that consisted of more commercial and residential land, while wetlands that flooded less than 45% of the year had pipe-sheds with more industrial and agricultural land use. Differences between these land-use types could affect the amount of nitrate, and amount and quality of dissolved organic carbon that enter wetlands,

potentially affecting what limits denitrification (Newcomer et al. 2012). This study, however, did not examine differences in water quality from pipe-sheds with different land-uses, an important question to help us better understand drivers of denitrification in accidental urban wetlands.

Monsoon floods, important for increasing denitrification at non-urban desert riverine wetlands, were not an important driver of denitrification in accidental urban wetlands. Ephemeral wetlands should have had the greatest proportional increase in denitrification after monsoon floods because of the dramatic increase in soil moisture from the pre- to post-monsoon season. However, I found no significant difference between the two, and a trend of decreasing denitrification after monsoon floods. This could have negative implications for nitrate removal, as the monsoon season is when ephemeral wetlands receive the most water with high nitrate concentrations. If monsoon flooding does not increase denitrification, then the nitrate in this water will end up in downstream ecosystems, or filter into the groundwater that already has high nitrate concentrations (Rosen and Kropf 2009). Denitrification in ephemeral wetlands could be affected by a build-up of dust in storm drains during dry season preceding monsoon rains–a dynamic unique to desert cities (City of Phoenix Stormwater Services, personal communication). This dust is flushed out with storms and creates a layer of fresh sediment in ephemeral wetlands that may not harbor a substantial community of denitrifying microbes. This explanation for my results is speculative and future research should therefore examine what mechanisms are causing reduced denitrification at ephemeral wetlands after monsoon floods.

Plants also influence denitrification across many wetland types (Alldred and Baines 2016). The presence of plants increased denitrification at intermittent and perennial wetlands, but did not have as substantial an effect at ephemeral wetlands. This is interesting, as ephemeral wetlands had low organic matter, and plants are often important for providing resources, such as carbon, in resource poor environments such as ephemeral wetlands. In sum, it is not clear what is driving patterns of denitrification in ephemeral urban wetlands. Future studies of accidental urban wetlands should explicitly examine links between stormwater pipe-shed land use, flood magnitudes, water chemistry, and denitrification to identify specific aspects of the urban landscape affecting denitrification.

Challenges in linking plants to denitrification

Identifying how ecosystem structure relates to ecosystem function has been a goal of ecologists for decades (Hooper et al. 2005). Despite the recognition that plants can have considerable influence on denitrification by changing abiotic soil conditions, such as labile carbon and redox potential, little effort has been made to incorporate plants into models of denitrification (Boyer et al. 2006). This could be because generalities about mechanistic links between plants and denitrification remain elusive. While there is ample research on how plant traits and species diversity are related to ecosystem functions, relatively few studies have been conducted in wetlands, particularly on denitrification.

Plant traits (Chapter 3)

Two important findings emerged from the examination of plant traits related to denitrification: (1) plant traits were more effectively modeled as indirectly influencing denitrification through soil conditions; and (2) several plant traits were related to greater potential denitrification. The few previous studies examining how plant traits relate to denitrification used statistical methods that accounted for only a direct relationship between plant traits and denitrification. However, my study demonstrated that modeling only direct relationships excludes plant traits that strongly influence denitrification indirectly by changing soil conditions. I found that belowground biomass, rooting depth, and tissue chemistry indirectly influenced denitrification through either soil organic matter or soil nitrate. The model also revealed several direct paths between plant traits (aboveground biomass, aboveground C:N ratios, and rooting depth) and denitrification, but not through the hypothesized indirect paths through soil organic matter or soil nitrate. This means these plant traits are affecting another aspect of soil conditions not included in the model. For example, aboveground biomass and tissue chemistry did not affect potential denitrification through organic matter. Rather, these plant traits may affect the quantity or quality of dissolved organic carbon, soil conditions not included in the model, available to denitrifiers.

Species richness (Chapter 4)

Previous studies examining the relationship between species richness and ecosystem functions in wetlands grew plant species in mixtures, similar to studies conducted in grasslands (Engelhardt and Ritchie 2002; Callaway et al. 2003; Bouchard et

al. 2007). However, several wetland species grow in monotypic patches that interact with other species only at edge of patches. When accounting for this configuration of wetland plants in my study, species richness did not affect nitrate retention or denitrification, but rather individual species had a greater effect on nitrate retention and denitrification. Further, I found that patch edges had greater potential denitrification than patch centers in urban wetlands, but this pattern was not replicated in the greenhouse. Consequently, it is unclear what drives the increase in potential denitrification at edges of patches observed in the study wetlands.

Future challenges

A key challenge for understanding how plant traits and richness affects denitrification is how they interact with environmental controls of denitrification (Hooper et al. 2005; Alldred and Baines 2016). In wetlands, hydrology not only affects what community of plants will be present, but also whether aerobic (e.g, nitrification) or anaerobic (e.g. denitrification) microbial processes will occur (van der Valk 1981; Boutin and Keddy 1993; Mitsch and Gosselink 2007). Consequently, plant traits and species richness may affect denitrification differently in wetlands that are permanently inundated compared to wetlands that are more frequently dry.

Implications for nitrate management

The Southwest is rapidly growing and therefore confronted with providing a sustainable water supply for future populations; consequently, cities are implementing policies to increase water use efficiency. While increasing efficiency of water use is positive from a sustainability standpoint, such policies could, unintentionally, reduce

wetland areas in urban environments such as in the Salt River. These wetlands provide much needed habitat to various wetland species (White and Stromberg 2011; Bateman et al. 2015), and also mitigate nitrate pollution. If accidental urban wetlands are inundated less frequently (i.e. shift from functioning as intermittent and perennial wetlands to functioning as ephemeral wetlands), nitrate removal during storm events will likely decrease. Accidental urban wetlands are unique features of the urban landscape that can mitigate nitrate pollution, but also are vulnerable to changes in policy as their "unmanaged" status largely precludes them from consideration in city planning and management schemes. Recognition of the value of accidental urban wetlands by city planners could help maintain wetland habitat within cities and mitigate nitrate export to downstream ecosystems.

Further, elucidating how plants improve denitrification or other ecosystem functions is important not only for accidental urban wetlands, but also for any projects seeking to improve the functioning of restored or degraded wetlands. Linking plant traits to denitrification can guide decisions about what plants to include in wetlands to optimize nitrate removal. Understanding how species diversity affects nitrate retention via plant assimilation and denitrification will also inform managers on what type of plant community may best suit their restoration or management goals.

Overall, the results of this dissertation suggest that accidental urban wetlands can effectively remove nitrate via denitrification; however, the urban environment alters drivers of this process. Further, plant traits offer a mechanism by which we can understand how plant communities affect denitrification, but out knowledge of these

mechanisms need improvement before they can be effectively applied in management scenarios to improve denitrification.

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