Impact of nitrate enrichment on wetland and dryland seed germination and early seedling development

Jere A. Boudell & Juliet C. Stromberg

Boudell, J.A. (corresponding author, jboudell@clayton.edu)[1],
Stromberg, J.C. (jstrom@asu.edu)[2]

[1]Department of Natural Sciences, Clayton State University, Morrow, GA 30260, USA;
[2]School of Life Sciences, Arizona State University, Tempe, AZ 85287, USA

Abstract

Questions: Atmospheric and agricultural inputs of nitrogen have increased significantly. Because riparian zones act as buffers for nutrient transfer and treated municipal effluent and untreated agricultural run-off are released into streams, and because desert riparian ecosystems contain a wide range of functional groups with respect to water relations, we asked if wetland and dryland species respond differently to increasing levels of soil nitrate. Specifically, we asked if seed germination rate, total percentage germination and seedling development varied by species and functional group in response to increasing nitrate levels.

Location: Southwestern US.

Methods: We subjected seeds of common wetland and dryland species to distilled water (control), 0.025 M (low), 0.05 M (intermediate) and 0.075 M (high) KNO_3 treatments for 30 days. We determined seed germination rates, total percentage germination and seedling development for each species. Generalized linear mixed models tested for differences between treatments.

Results: Seeds of wetland species germinated rapidly and in high percentages, and developed into seedlings regardless of nitrate treatments. Dryland species had unique responses to nitrate treatments, with some species exhibiting high germination rates in control treatments and others showing strong germination responses at low to intermediate nitrate levels. A larger number of dryland germinants developed into seedlings under control to low nitrate treatments.

Conclusions: Riparian plant community dynamics arising from germination responses could change under increased nitrogen inputs, as some species are seemingly unaffected and others significantly impacted. Based on our limited species pool and treatment conditions, the functional group 'wetland species' produces a consistent germination and early seedling response regardless of nitrate concentration, while 'dryland species' responses are variable and species-specific. Species known to be nitrophiles can be inhibited by high nitrogen concentration as germinants, highlighting the importance of investigating plant community response to changing conditions at a variety of life stages.

Introduction

Since the industrial and green revolutions, inputs of nitrogen (N) into aquatic and terrestrial ecosystems have increased substantially. While atmospheric N inputs have declined in the US due to tighter controls on industry and transportation; agricultural inputs, due to demands to feed and power a growing population, continue to increase (Davidson et al. 2012). Excess N migrates through the watershed into riparian zones, and ultimately into rivers, lakes and oceans, resulting in eutrophication, coastal dead zones and increased nitrous oxide production (Beaulieu et al. 2011; Davidson et al. 2012). The release of treated municipal effluent into streams further adds to N loading (Carey & Migliaccio 2009). Because N is a limiting factor for many plants, increasing N availability can alter plant community dynamics (Davidson et al. 2012).

Riparian buffer zones, linear zones of riparian vegetation and integrated wetlands that parallel stream channels, remove excess N principally through denitrification, but

also by adhesion to soil particles and uptake by plants and microbes (Peterjohn & Correll 1984; Gregory et al. 1991; Naiman et al. 2005). Microbial denitrification typically occurs under anaerobic conditions and releases nitrous oxide, a greenhouse gas (Beaulieu et al. 2011). Plant uptake and growth are usually stimulated by N in the form of ammonium and nitrate, but the response is species-specific and is influenced by life-history traits such as age, growth rate moderated by adaptations to low nutrient levels and N use efficiency (Chapin et al. 1986; Berendse & Aerts 1987; Del Pozo et al. 2000).

Seed germination is typically stimulated by N in the form of nitrate at concentrations between 0 and 0.05 M, which is a common range for many soils (Hilhorst & Karssen 2000). However, nitrate interacts with other factors that stimulate germination, such as water and temperature (Vincent & Roberts 1977; Bewley & Black 1982). Light interactions with nitrate have been proposed as a gap detection mechanism (Pons 1989) and as an indicator of flood cessation (Mollard & Insausti 2009), ultimately triggering germination when conditions become favourable. Increased N availability in riparian zones may change community dynamics, as some species will germinate earlier and/or have a higher percentage of successful germinants, not germinate or have decreased germination in response to nitrate levels within or beyond a species' physiological tolerance range.

In addition to affecting germination, nitrate impacts seedling growth and development (Farnsworth 2008), although the response is species-specific (Reich et al. 2003). As a seed germinates, it must produce roots to adhere to surfaces and absorb water and minerals. High soil nitrate tends to restrict lateral root development, while low concentrations stimulate root growth. Nitrate also increases cytokinin production in roots which, when nitrate is readily available, curtails nitrate uptake (Sakakibara et al. 2006; Farnsworth 2008). Seedlings must also incorporate N for photosynthesis and for growth and development of stems and leaves. A high relative growth rate (RGR) in response to increased nitrate has been found for many species (Reich et al. 2003; Thomas et al. 2010; Davidson et al. 2012), including several North American riparian tree species (Marler et al. 2001) and European Mediterranean grasses (Huenneke et al. 1990; Link et al. 1995; Brooks 2003; MacKown et al. 2009).

Despite the increasing availability of atmospheric and terrestrial N, with the exception of studies focusing on non-native vs native responses (Huenneke et al. 1990; Brooks 2003; MacKown et al. 2009), few studies have examined functional or trait-based germination and seedling responses to varying nitrate levels. Reich et al. (2003) examined the RGR of C_3 and C_4 grass, forb, oak and legume seedlings in response to N and found varying

results within and among the functional groups, indicating that the individual functional groups did not have a unified response to nitrate addition. In riparian ecosystems, species can be categorized by their dependence on water availability into the broad functional groupings of wetland and dryland species (Gitay & Noble 1997). Wetland species are restricted to sites with high water availability (Penfound 1952), and dryland species can tolerate soils with varying degrees of soil moisture, in part due to larger seed size (Leishman et al. 2000; Moles & Westoby 2004; Stromberg & Boudell 2013). Wetland and dryland functional groups are strongly correlated with hydro-gradients in desert riparian ecosystems. Wetland species populate areas near active channels and riparian wetlands where water availability is high, and dryland species colonize the rest of the floodplain where water availability increases in response to rainfall and periodic flooding.

In the arid Southwest US, N levels in the floodplain vary. Dryland *Prosopis* spp. forests (Mesquite Bosque) are common at the outer edge of riparian ecosystems. *Prosopis* spp., members of the Fabaceae, form a symbiotic relationship with *Rhizobium*, a N-fixing bacterium (Jenkins et al. 1987). Consequently, N is abundant under the canopy of *Prosopis* spp. trees (Virginia & Jarrell 1983; Schade & Hobbie 2005). Several herbaceous dryland species, such as *Bromus diandrus*, establish large populations in the understorey of Mesquite forests (Wolden & Stromberg 1997). Along stream channels, in contrast, N levels often can be low because of uptake by riparian trees and high sand content, which reduce nutrient retention (Lewis et al. 2009). Nutrient levels along the stream can vary widely through time, however, depending on the time since flooding and traits of particular flood events (Adair et al. 2004; Green et al. 2009).

Because treated municipal effluent and untreated agricultural run-off are released into streams, and riparian zones act as buffers for nutrient transfer, and because plant communities in desert riparian ecosystems are populated by species categorized into wetland and dryland functional groups, we asked if wetland and dryland species respond differently to increasing levels of soil nitrate. Specifically we asked if seed germination rate, total percentage germination and seedling development varied by species and functional group in response to increasing nitrate levels. We expected dryland species to have higher germination rates, total percentage germination and more germinants that develop into seedlings in comparison to wetland species. Understanding how increasing levels of nitrate impact seed germination and early seedling development of wetland and dryland species may help managers predict how riparian plant communities may change under increased atmospheric and terrestrial N deposition.

Methods

Study sites and species

Southwestern riparian ecosystems located in the Sonoran Desert in central Arizona, US, experience a mean annual low and high temperature of ca. 1 °C and 39 °C respectively. Precipitation falls in a bimodal pattern, with Pacific frontal and monsoon storms occurring during the winter and summer months, respectively. Mean annual precipitation ranges from 29 to 36 cm among riparian sites selected for study. Riparian soils are typically sandy loams. The bimodal precipitation pattern supports a diverse array of floodplain plant species.

Four dominant plant communities, active channel bar, *Populus–Salix* forest, *Hymenoclea* shrubland and *Prosopis* forest, reflect the range of moisture and flood disturbance conditions (both high to low) that are found across a declining hydro-gradient that occurs with increasing distance from the channel in southwestern riparian ecosystems. As a result of periodic flooding and drought, the four communities are dominated by seed-banking annual and short-lived perennial species (Wolden et al. 1994; Bagstad et al. 2005; Boudell & Stromberg 2008).

We selected species regularly found in southwestern riparian ecosystems for this study. *Mimulus guttatus, Nasturtium officinale, Polypogon monspeliensis* and *Veronica anagallis-aquatica* are common cool-season species in wetlands and active channel communities (Wolden et al. 1994). *M. guttatus, N. officinale* and *V. anagallis-aquatica* are short-lived perennial forbs. *P. monspeliensis*, frequently a dominant species in active channel communities, is an annual grass. *Bromus diandrus* and *Hordeum murinum*, both grasses, and *Sisymbrium irio*, a forb, are common cool/warm season annual species in the understorey of *Populus–Salix* and *Prosopis* forests (Wolden et al. 1994; Wolden & Stromberg 1997). *B. diandrus* and *S. irio* are frequently dominant species in *Prosopis* forests. All of the aforementioned species, with the exception of *M. guttatus* and *V. anagallis-aquatica*, originate in Eurasia and/or the Mediterranean region (Table 1). All species produce seeds that are viable for at least 1 yr (Howard & Lyon 1952; Popay & Sanders 1974; Harradine 1986; Boudell 2004).

Species were placed into the broad functional groups of wetland and dryland species based on their Wetland Indicator Status (WIS) for the arid west (obtained from http://plants.usda.gov; Wolden et al. 1994; Table 1). We use the term functional group to refer to species that share a similar ability to establish under varying degrees of water availability, as indicated by their WIS (Gitay & Noble 1997). The obligate wetland species *M. guttatus, N. officinale, V. anagallis-aquatica* and facultative wetland species *P. monspeliensis* were categorized as wetland species. These

Table 1. Species origin, distribution, and functional group classification (USDA Plants Database).

Species	Origin	Distribution in US
Wetland		
Mimulus guttatus	Native US	Western
Nasturtium officinale	Eurasia	Most
Polypogon monspeliensis	S. Europe	Most
Veronica anagallis-aquatica	Native US	Most
Dryland		
Bromus diandrus	Mediterranean	South, E & W coasts
Hordeum murinum	Eurasia, Mediterranean	West, E coast
Sisymbrium irio	S. Europe, Mediterranean	South, spotty NE, FL

wetland species establish in riparian wetlands and active channel communities. The non-wetland species *B. diandrus* and *S. irio* and the facultative dryland species *H. murinum* were categorized as dryland species. These dryland herbaceous species are drought-intolerant, do not require a persistent source of water to thrive, and are rarely found in active channel bars and wetlands of riparian ecosystems. They are drought evaders that germinate in response to the increased moisture availability that occurs through periodic rainfall and flooding in southwestern riparian ecosystems (Went 1949; Monson & Smith 1982; Freas & Kemp 1983). This strategy allows the selected herbaceous dryland species to populate the drier communities (*Populus–Salix* forest, *Hymenoclea* shrubland and *Prosopis* forest) located along the declining riparian hydro-gradient.

Treatments

Mature seeds from multiple populations and individuals were collected in May 2010 from sites along Cienega Creek, Hassayampa River and San Pedro River in Arizona, US (Table 2). After collection, seeds were cold-stored at 3 °C in paper bags. Prior to treatment application in September 2011, seeds were excised from fruits if needed and long awns (*B. diandrus*) removed. For each species, 25 seeds from multiple individuals (ten to 56 per species) and populations (one to five per species) were placed in the same orientation on pre-wetted filter paper in 9-cm plastic Petri dishes (Table 2). Groups of six Petri dishes were treated with 1.5 mL distilled water (control) or 0.025 M (low), 0.05 M (intermediate) or 0.075 M (high) potassium nitrate (KNO_3) solution for a total of 150 seeds per treatment per species. Excess solution was carefully drained off. Petri dishes were wrapped with clear wrap and placed in a walk-in environmental growth chamber using a randomized block design with shelf as the block. Seeds were subjected to a 12-h photoperiod and 10 h of high temperature

Table 2. Total number of sites, populations and individuals sampled by species.

Species	Sites	Populations	Individuals
Wetland			
Mimulus guttatus	3	3	11
Nasturtium officinale	1	1	10
Polypogon monspeliensis	1	1	10
Veronica anagallis-aquatica	1	1	14
Dryland			
Bromus diandrus	5	5	54
Hordeum murinum	5	5	56
Sisymbrium irio	2	2	12

at 22.8 °C and 14 h at 3.9 °C for 30 days. These conditions approximated spring conditions in the Sonoran Desert. Every 5 days, germinants were counted, mouldy seeds rinsed, mouldy filter paper replaced and treatments reapplied.

We selected our N concentrations for the following reasons. Nitrate typically stimulates germination (Bewley & Black 1982; Hilhorst & Karssen 2000) at concentrations between 0 and 0.05 M, which is a common range found in soils (Hilhorst & Karssen 2000). Soil nitrate concentration in *Prosopis* spp. understorey is high relative to surrounding communities (Virginia & Jarrell 1983; Schade et al. 2003; Schade & Hobbie 2005) and ranges from 1.36 ± 0.8 g^{-2} to 1.68 g NO$_3$- kg of soil (Virginia & Jarrell 1983; Stromberg et al. 1996). The wetland species used in this study populate stream edges where soil nitrate has been found to range from 2.7 ± 2.4 g^{-3} to 4.6 ± 2.6 g^{-3} NO$_3$- kg of soil (Stromberg et al. 1996). Our treatments ranged from 0 to 7.6 g of KNO$_3$ kg of water, which captured the low to high range of nitrate found in soils of desert riparian *Prosopis* spp. understorey and stream edges, as well as the reported range of nitrate required to stimulate germination (Hilhorst & Karssen 2000).

At the end of the 30-day trial, ungerminated seeds were cold-stored at 3 °C, and 10 days later tetrazolium viability tests were conducted on the ungerminated seeds. Ungerminated seeds were treated with 4.5 mL 1% 2,3,5-triphenyltetrazolium chloride in a pH 7 phosphate buffer solution, and incubated at room temperature in the dark for 24 h. Viable seeds contained pink-stained embryos, whereas dead seeds were unstained or unevenly stained; 95–100% of the ungerminated *B. diandrus, H. murinum, N. officinale, P. monspeliensis* and *V. anagallis-aquatica* seeds were viable, as were 60% and 32% of *M. guttatus*, and *S. irio* seeds, respectively.

Qualitative and quantitative assessments

At the end of the study, all seeds were recorded as ungerminated or germinated, and the life cycle stages of germinated seeds were designated as either germinant or seedling. Germinants were determined based on the presence or absence of a radicle, and seedlings as germinants that produced primary leaves. The proportion of germinants vs seedlings in Petri dishes was quantified using classes (i.e. <1/3, 1/3–2/3, >2/3). This distinction allowed us to determine if seedling development of a given species, in general, halted at the germinant stage or proceeded to the seedling stage. Additionally, germinants were classified as having high root:shoot ratios if they had noticeably more root branching or longer roots than shoots.

For each species, germination data adjusted for viability were modelled using generalized linear mixed models (GLMMs) based on the binomial distribution of the dependent variable (Bolker et al. 2009; Zuur et al. 2009). Models were fitted using LaPlace approximation. Nitrate treatment was the fixed effect and growth chamber shelf location (block) and Petri dish were random effects. These random effects were selected because heterogeneity is inherent in growth chambers (Potvin et al. 1990) and seeds in Petri dishes are not independent. Likelihood ratio tests (LRT) were conducted to select the best models ($\alpha = 0.05$). Shelf location was not a significant random effect and was removed from the models. Pair-wise comparisons were conducted by resetting the reference group in the best GLMM model and obtaining z-test scores and P-values ($\alpha = 0.05$) (Sala et al. 2012). Modelling was conducted using R software (R Foundation for Statistical Computing, Vienna, AT) and the GLMM lme4 package.

Seeds of one of the study species germinated after the end of the trial (following incubation in the dark for the tetrazolium testing), these germination results were analysed. Differences between total percentage germination before and after incubation *within* each treatment group were analysed with Wilcoxon signed-rank tests ($\alpha = 0.05$) using R software. To determine if germination responses differed *between* treatment groups post-incubation, as opposed to pre-incubation, total percentage germination data for this species were modelled a second time as previously described using GLMMs ($\alpha = 0.05$).

Results

Wetland species

All wetland species responded similarly to all nitrate treatments. There were no significant differences between models or treatments (LRT and multiple comparisons all $P > 0.05$; Fig. 1, Table 3). Almost all (92–100%) *M. guttatus, N. officinale, P. monspeliensis* and *V. anagallis-aquatica* seeds germinated within 5–10 days regardless of treatment (Fig. 2). *M. guttatus* seeds subjected to control treatments had a slightly lower total percentage germination (88%)
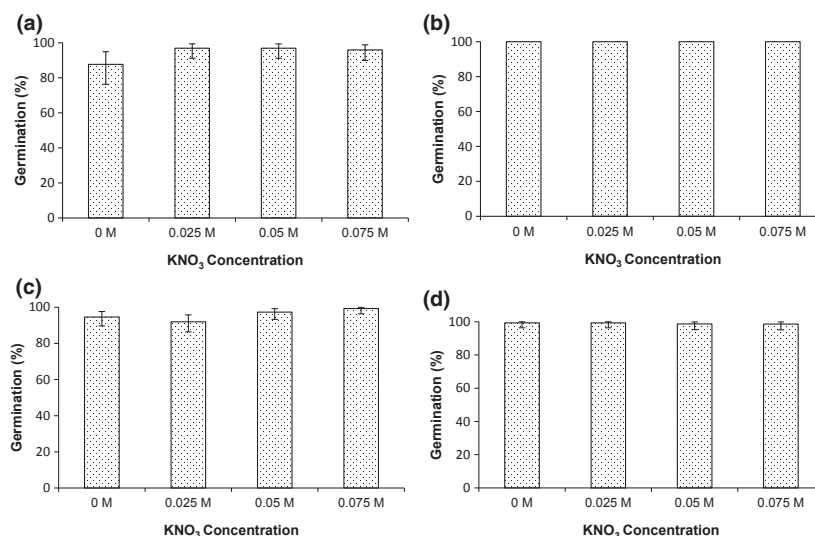
Fig. 1. (**a**–**d**) Total percentage germination, ±95% binomial confidence interval, by KNO$_3$ concentration for wetland species. (**a**) *Mimulus guttatus*, (**b**) *Nasturtium officinale*, (**c**) *Polypogon monspeliensis* and (**d**) *Veronica anagallis-aquatica*.

and a slightly delayed germination response (10–15 days; Fig. 2a). Germinants of all wetland species developed into seedlings under all treatments (Table 4). *N. officinale* and *V. anagallis-aquatica* had high root:shoot ratios in response to control treatments, with little noticeable difference in root:shoot ratios under nitrate treatments (Table 4).

Dryland species

Dryland species had varying responses to nitrate treatments. Models were significantly better than null models

Table 3. Model vs null model comparisons for species in response to nitrate treatment.

Species	Model	AIC	Loglink	P-value
Wetland				
Mimulus guttatus	Null	34.524	−15.262	
	Model	34.132	−12.066	0.094
Nasturtium officinale	Null	4	−3.6984e-10	
	Model	10	−3.6984e-10	1
Polypogon monspeliensis	Null	4	−3.6961e-10	
	Model	10	−3.6961e-10	1
Veronica	Null	4	−1.9357e-10	1
anagallis-aquatica	Model	10	−1.9357e-10	1
Dryland				
Bromus diandrus	Null	70.288	−33.144	
	Model	58.545	−24.272	0.0005*
Hordeum murinum	Null	64.966	−30.483	
	Model	59.794	−24.897	0.011*
Sisymbrium irio	Null	71.166	−33.583	
	Model	51.915	−20.958	1.368e-05*

*Models significantly different from null models.

(LRT all $P < 0.01$; Table 3). *B. diandrus* had a delayed germination response under all treatments, waiting until days 15–20 to germinate to higher percentages (Fig. 3a), and had significantly higher germination in response to low and intermediate nitrate solutions, with a total percentage germination of 97% for both treatments (all comparisons to other treatments $P < 0.005$; Fig. 4a, Table 5). There was greater variability in germination in response to control and high nitrate treatments (69% and 76%, respectively; Fig. 4a). Additionally, development was arrested at the germinant stage under high nitrate treatments, while a mixture of germinants and seedlings was produced in response to control treatments. Most germinants proceeded to the seedling stage under low and intermediate levels of nitrate (Table 4).
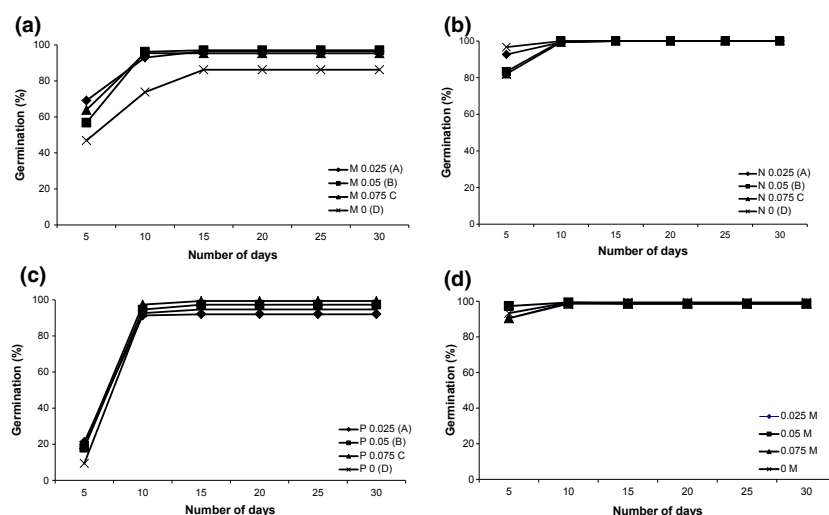
Hordeum murinum also had a delayed germination response, with peaks occurring at days 25–30 (Fig. 3b). Although there was generally low germination (16–46%) in response to all treatments, the largest total percentage germination occurred under the low nitrate treatment (46%; all comparisons to other treatments $P < 0.04$; Fig. 4b). Germinants typically developed into seedlings, except those exposed to high nitrate treatments, which produced a mixture of germinants and seedlings (Table 4).

During tetrazolium tests, when seeds were treated with 4.5 mL 1% tetrazolium solution and incubated in the dark for 24 h, *H. murinum* experienced a second flush of germination (Fig. 5). After incubation, the total percentage germination increased significantly for seeds initially treated with distilled water (72% vs 27%) and for those in the high nitrate treatment (58% vs 21%; both $P = 0.03$). The

Fig. 2. (a–d) Total percentage germination over time by KNO_3 concentration for wetland species. (**a**) *Mimulus guttatus*, (**b**) *Nasturtium officinale*, (**c**) *Polypogon monspeliensis* and (**d**) *Veronica anagallis-aquatica*.

Table 4. Final life-cycle stage reached for all species by treatment. Hundred percent of all germinated seeds reached the final stage listed unless otherwise indicated.

| Species	Distilled Water (0)	0.025 M KNO_3 (low)	0.05 M KNO_3 (intermediate)	0.075 M KNO_3 (high)
Wetland				
Mimulus guttatus	Seedlings	Seedlings	Seedlings	Seedlings
Nasturtium officinale	Seedlings*	Seedlings	Seedlings	Seedlings
Polypogon monspeliensis	Seedlings	Seedlings	Seedlings	Seedlings
Veronica anagallis-aquatica	Seedlings*	Seedlings	Seedlings	Seedlings
Dryland				
Bromus diandrus	1/3–2/3 mix germinants, seedlings	Seedlings	>2/3 seedlings	>2/3 germinants
Hordeum murinum	Seedlings	Seedlings	Seedlings	1/3–2/3 mix germinants, seedlings
Sisymbrium irio	Seedlings*	Seedlings	Germinants	Germinants

*High root:shoot ratio.

difference in treatment response that was present before tetrazolium tests disappeared after the tests (LRT $P = 0.16$).

Sisymbrium irio responded with a significantly larger total percentage germination (98%) when treated with the control solution (all comparisons to other treatments $P < 0.009$; Fig. 4c, Table 5). The response to low nitrate treatments (57%) was significantly better than intermediate (15%; $P = 0.049$) and high treatments (11%; $P = 0.016$; Fig. 4c, Table 5). Germination of *S. irio* seeds peaked at days 5–10 for all treatments, except the high nitrate (Fig. 3c). *S. irio* development was arrested at the germinant stage under intermediate and high nitrate treatments, and germinants developed into seedlings under control and low nitrate treatments (Table 4). *S. irio* produced a high root:shoot ratio under control treatments (Table 4).

Discussion

The results from our investigation suggest that wetland and dryland herbaceous species may respond differently to varying levels of nitrate in riparian ecosystems subjected to N enrichment. All four wetland species quickly germinated to high percentages regardless of nitrate treatment, indicating that nitrate was not a critical germination requirement. Each dryland species responded uniquely to varying levels of nitrate, which supports prior findings of variability within functional groups in response to N (Reich et al. 2003). Although the three Mediterranean dryland species are considered nitrophilic (Huenneke et al. 1990; Link et al. 1995; Brooks 2003; MacKown et al. 2009), they did not have increased germination, or a larger number of germinants transition to the seedling stage under high nitrate conditions.
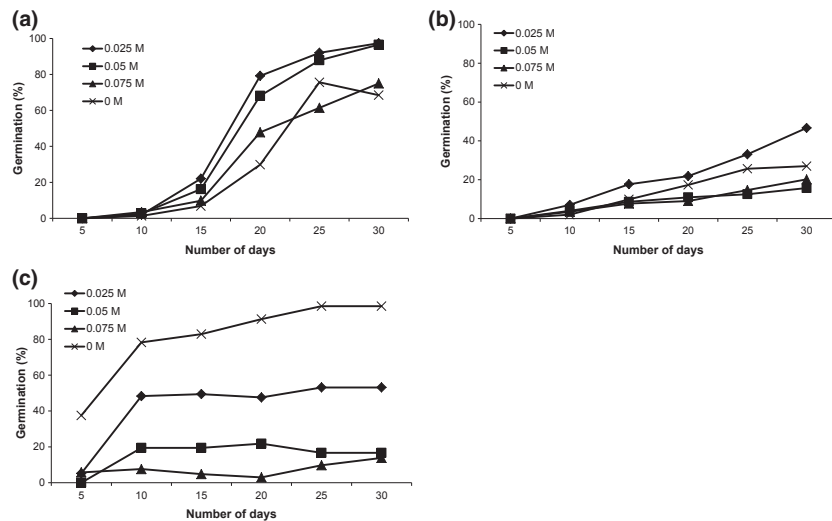
Fig. 3. (a–c) Total percentage germination over time by KNO_3 concentration for dryland species. (a) *Bromus diandrus*, (b) *Hordeum murinum* and (c) *Sisymbrium irio*.
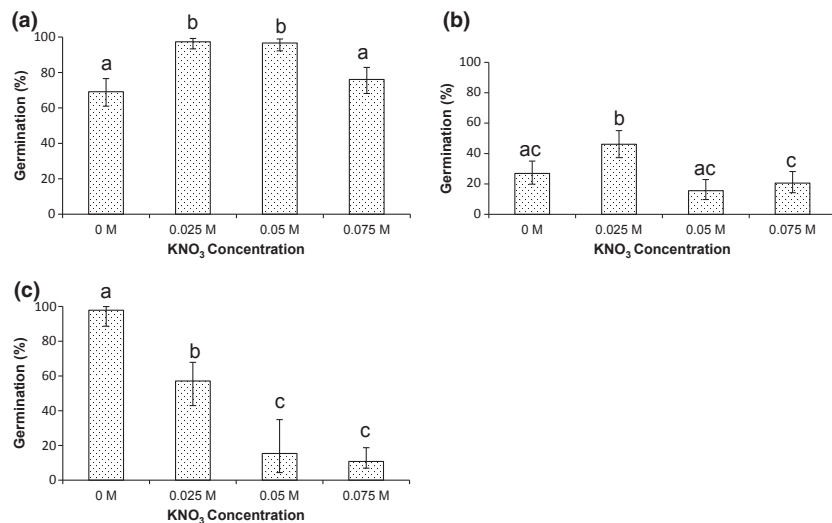
Fig. 4. (a–c) Total percentage germination, ±95% binomial confidence interval, by KNO_3 concentration for dryland species. (a) *Bromus diandrus*, (b) *Hordeum murinum* and (c) *Sisymbrium irio*. Different letters indicate significant differences between treatment groups.

Together, these findings suggest that riparian plant community dynamics arising from germination dynamics could change under increased N inputs, as some species are seemingly unaffected and others significantly impacted. It also suggests, based on our limited species pool and treatment conditions, that the functional group 'wetland species' produces dependable germination and early seedling response regardless of nitrate concentration, while 'dryland species' responses are variable and species-specific.

## Functional groups and species responses

Wetland species

That a critical germination trigger for our group of wetland species is water, and not nitrate, is not surprising (Leck et al. 1989). Our pool of wetland species successfully establish in active channel and wetland communities where water availability is high, and not in the drier communities within the floodplain. Wetland species require persistent and reliable water availability to successfully establish and

Table 5. GLMM model pair-wise comparisons by treatment for dryland species. 0 (distilled water), low (0.025 M), intermediate (0.05 M) or high (0.075 M) KNO_3 solution.

Treatment Comparisons	Bromus diandrus		Hordeum murinum		Sisymbrium irio	
	z-value	P-value	z-value	P-value	z-value	P-value
0-low	−3.472	<0.001*	−2.052	0.040*	2.621	0.009*
0-intermediate	−3.350	<0.001*	1.475	0.140	3.806	<0.001*
0-high	−0.608	0.543	0.735	0.463	4.138	<0.001*
Low-intermediate	0.207	0.836	−3.437	<0.001*	−1.966	0.049*
Low-high	−2.96	0.003*	−2.769	0.006*	−2.415	0.016*
Intermediate-high	−2.824	0.005*	0.760	0.447	−0.151	0.880

*Significant difference.
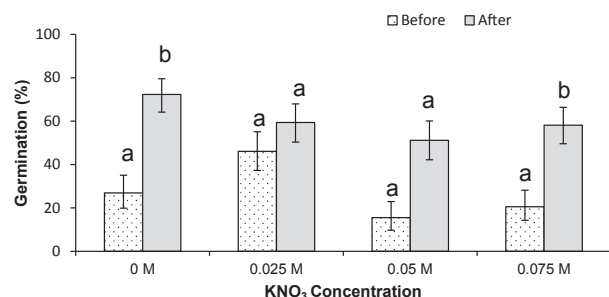
Fig. 5. Total percentage germination, ±95% binomial confidence interval, of *Hordeum murinum* seeds by nitrate treatment before and after tetrazolium tests. Different letters indicate significant differences within treatment groups.

thrive (Penfound 1952), and aquatic species typically require enough water to prevent water stress in order to germinate (Baskin & Baskin 2014), although some wetland species may require the additional trigger of alternating temperatures to germinate (Carta et al. 2013).

The wetland species used in this study are native to the US (*M. guttatus*, *V. anagallis-aquatica*), Eurasia (*N. officinale*) or Southern Europe (*P. monspeliensis*), are common in the US and all produce small seeds. As typical small-seeded species (Stromberg & Boudell 2013), they do not have the maternal resources necessary to support growth and establishment in resource-stressed environments (Leishman et al. 2000; Westoby et al. 2002). However, what is surprising is that increased N availability had no impact on germination rates or total germination, considering that wetland species produce small seeds which lack resources, and given some research suggesting that larger-seeded species have an advantage over smaller-seeded species under poor nutrient conditions (Jurado & Westoby 1992; Leishman et al. 2000; Westoby et al. 2002). Within our pool of wetland species, all germinants transitioned to seedlings under all nitrate treatments. While increased N availability may not impact the earliest life stages, it may be that wetland species, once past the early seedling stage, respond

positively to increased N. *N. officinale* and *V. anagallis-aquatica* had high root:shoot ratios under control treatments, but not under nitrate treatments, indicating resource optimization by allocating fewer resources to roots and more to shoot development (Bloom et al. 1985).

Dryland species

The dryland species in our study are drought-evaders that germinate in response to the increased moisture availability caused by floods and rainfall events (Went 1949; Monson & Smith 1982; Freas & Kemp 1983). As was exhibited by *B. diandrus* and *H. murinum*, some dryland species have a delayed germination response. It has been proposed that this response prevents germination triggered by irregular rainfall events and delays germination until later in the season to coincide with seasonal rainfall and with seeds germinating rapidly when triggered (Chapman et al.1999). Dryland species typically produce larger seeds (Stromberg & Boudell 2013), which support seedling establishment in drier conditions (Baker 1972; Moles & Westoby 2004; Guerrero-Campo & Fitter 2011). Large seed size has been suggested as a mechanism that supports seedling establishment in resource-stressed environments (Leishman et al. 2000; Westoby et al. 2002). Our dryland species, although capable of forming dense populations in the N-rich *Prosopis* spp. understorey, had varied responses to nitrate treatments and likely would have varying germination responses to soil nitrate enrichment.

Bromus diandrus, a large-seeded Mediterranean grass, produced seeds that had the highest germination, and a majority of germinants transitioned to seedlings, under low and moderate levels of nitrate. A congener species, *Bromus tectorum*, has high N productivity (Del Pozo et al. 2000), and *B. diandrus* is a common dominant species in N-enriched soils (Maron & Connors 1996; Hoopes & Hall 2002), suggesting that *B. diandrus* should respond positively to increased nitrate availability as a nitrophilic species. In our study, *B. diandrus*, although nitrophilic, had

reduced germination in response to the high nitrate treatment, and germinants did not develop into seedlings. It is not uncommon for high soil nitrate to inhibit germination (Hilhorst & Karssen 2000; Baskin & Baskin 2014).

Hordeum murinum, another large-seeded Mediterranean grass, responded most positively to the low nitrate treatment. Other studies have found *H. murinum* is a nitrophilic species requiring high soil fertility (Davison 1971) and becoming a dominant species under the highest soil N levels (Groves et al. 2003). Our study found a higher total percentage germination under low nitrate levels, and a decrease in the number of germinated seeds that progressed to the seedling stage at the highest nitrate treatment. Like the wetland species, perhaps the positive response by *B. diandrus* and *H. murinum* to increased nitrate availability is expressed once seedlings have successfully established.

Hordeum murinum responded differently than the other species included in this trial. The lower total percentage germination at the end of the nitrate trial under all treatments, and the flush of germination in the dark during tetrazolium tests, suggests that *H. murinum* may require a dark treatment for maximum germination. While uncommon, some species germinate to higher percentages in response to darkness (Baskin & Baskin 2014). The lower total percentage germination during the trial could also be due to missed germination requirements, bet hedging or, more likely, seed age (Baskin & Baskin 2014). Although the seeds used in this study were cold-stored, some seeds can vary in their germination responses and lose viability as they age in storage (Baskin & Baskin 2014).

Germination of *S. irio*, a species native to Eurasia and the Mediterranean, was suppressed under nitrate treatments. Additionally, germinants only developed into seedlings in response to distilled water and low nitrate treatments. Like the wetland species *N. officinale* and *V. anagallis-aquatica*, *S. irio* under control treatments produced a high root:shoot ratio, likely due to resource optimization in response to nitrate treatments. We found the negative response to the high nutrient concentration surprising, given that germination of *Sisymbrium officinale*, a congener of *S. irio*, is stimulated by nitrate in combination with optimal light conditions (Hilhorst 1990).

Community dynamics

With increasing N inputs from atmospheric and aquatic sources, and with buffer zones used as mechanisms to absorb excess N, it is important to predict how riparian community dynamics will likely respond to N enrichment. Species that germinate earlier rather than later have better access to resources, unless species germinate into highly competitive environments. For species that use nitrate as a gap detection mechanism (Pons 1989), N-enriched soil may confound the germination response, as high nitrate signals a low competitive environment, which could result in species inadvertently germinating into a more competitive environment. Mollard & Insausti (2009) found that the germination requirement of high nitrate and daily pulses of red light, which triggered germination of *Setaria parviflora* seeds in nonflooded soils, also prevented seeds from germinating in flooded soils when levels of these factors were low or non-existent. Other riparian species with the same germination requirements may germinate under flooded conditions when shallow flood waters are N-enriched. After germination, community dynamics may change as species take advantage of high N availability through increased growth rates and biomass (Farnsworth 2008), unless the species are adapted to low N availability, which results in lower N saturation points and lower N use efficiency (Shaver & Melillo 1984; Parsari et al. 2011).

All species in this trial are common in the US, and the species are often dominant or abundant in the annual-dominated southwestern riparian communities. Our results suggest that at the germination and early seedling stages, increased N availability may not significantly impact wetland community dynamics. However, for our dryland species, several of which have been reported to strongly respond to increased N availability, community dynamics may change when flood waters, municipal effluent and untreated agricultural run-off flow into riparian communities. *Bromus diandrus*, a species known to favour N-rich environments (Maron & Connors 1996; Hoopes & Hall 2002), does have a high germination response to low and moderate levels of N. These traits point to *B. diandrus*-dominated communities in areas subjected to moderate N enrichment, as long as N levels remain below supra-optimal (Hilhorst & Karssen 2000). Under comparable conditions, germination of *H. murinum* may also respond similarly, although further studies are needed to determine additional possibly interacting germination requirements. *S. irio*, while capable of establishing successful populations in N-rich soils in *Prosopis* spp. forests, has suppressed germination under N enrichment, and thus may form small or sink populations in areas of the floodplain that have supra-optimal N levels. Further studies are needed to examine effects of nitrate addition on freshly collected seeds, relative growth rates, biomass and fecundity of seedlings and adults of these species, all of which may alter community dynamics. Additionally, germination response may vary under alternating temperatures. Greenhouse and field-based experiments, which better replicate light conditions, also are needed at a variety of life stages.

Conclusion

In riparian ecosystems subjected to both atmospheric and aquatic inputs via treated effluent, N enrichment may impact community dynamics in a somewhat predictable manner. Management approaches that recognize the fundamental environmental changes to which some species respond, will allow for the presence of some, likely dominant, non-native species such as *B. diandrus*. If not, management will be engaged in perhaps an epic battle where seed dispersal and seed bank dynamics cannot be manipulated to rid communities of so called 'pest species'. This management issue will be further compounded as increasing CO_2 will interact with increased N availability; this could either level the playing field, as non-nitrophilic species respond to increased CO_2, or reinforce the position of dominant species.

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