



Volume 163 · Number 1 · May 2010

CONCEPTS, REVIEWS, AND SYNTHESSES

Interactive effects of elevated carbon dioxide and environmental stresses on root mass fraction in plants: a meta-analytical synthesis using pairwise techniques.  
X. Wang · D.R. Tsubo 1

Physiological Ecology

Remobilizing nitrogen to control plant invasion  
L.G. Perry · D.M. Blumenthal · T.A. Monaco · M.W. Paschke · E.F. Redente 13

Physiological Ecology

Functional and evolutionary correlations of steep leaf angles in the mesic shrubland  
A. Valiente-Banuet · M. Verú · F. Valladares · P. García-Fayos 25

Levels of antioxidants in rural and urban birds and their consequences  
A.P. Møller · J. Entzow · F. Karadzic 35

Transition zones between vegetation patches in a heterogeneous Arctic landscape: how plant growth and photosynthesis change with abundance at small scales  
B.J. Fitzchar · M.C. Press · R. Baxter · G.K. Phoenix 47

Molecular keys unlock the mysteries of variable survival responses of blue crabs to hypoxia  
G.W. Bell · D.B. Eggleston · E.J. Noga 57

BEHAVIORAL ECOLOGY

Should I stay or should I go? predator- and conspecific-induced hatching in a marine snail  
B.G. Miner · D.A. Donovon · K.E. Andrews 69

Order of invasion affects the spatial distribution of a reciprocal intraguild predator  
T. von der Hammen · A.M. de Roos · M.W. Sabelis · A. Janzen 79

POPULATION ECOLOGY

High red deer density depresses body mass of roe deer fawns  
E. Rönald · J.M. Gaillard · S. Sævi · J.L. Hänninen · F. Kruuk 91

Linking environmental and demographic data to predict future population viability of a perennial herb  
P. Tringali · J. Ehrlén · J. Ågren 99

Priority effects and habitat complexity affect the strength of competition  
S.W. George · A.C. Sher 111

Disjunct performance and distribution in the sedge *Carex prasina*  
K.M. Finn · M.J. Watkinson · M.J. Lechowicz 119

PLANT-ANIMAL INTERACTIONS

Gardening by the psychomyiid caddisfly *Troctes waeneri*: evidence from stable isotopes  
N.L. Ings · A.G. Hodson · J. Gray 127

Delayed response in a plant-pollinator system to experimental grassland fragmentation  
H.-P. Rusterholz · B. Ebnar 141

Does human-induced habitat transformation modify pollinator-mediated selection? A case study in *Viola portulacastris* (Violaceae)  
M. Munoz · C. Espinosa · R. Bustamante · M. Marsh · R. Møller 153

Ecological engineering by a native leaf-cutting ant increases the performance of exotic plant species  
A.G. Faj-Benier · N. Lescano · L. Ghemardi 163

COMMUNITY ECOLOGY

Effects of resource availability on seedling recruitment in a fire-maintained savanna  
G.D. Iason · L.K. Kokman · E.M. Burns 171

Abiotic stress mediates top-down and bottom-up control in a Southwestern Atlantic salt marsh  
J. Albert · A. Moxter · C. Caserio · P. Daleo · E. Farjál · B. Silman · M. Borries · O. Inchausti 181

Reciprocity in predator-prey interactions: exposure to defended prey and predation risk affects intermediate predator life history and morphology  
E. Hamill · A.P. Bockerman 193

Impact of plant architecture versus leaf quality on attack by leaf-feeding caterpillars on five oak species  
R.J. Marquis · J.T. Liu 203

ECOSYSTEM ECOLOGY

Spatial and temporal variability of plant-available water in calcium carbonate-cemented soils and consequences for soil ecosystem resilience  
M.C. Dunaway · J.E. Herrick · H.C. Monger 215

Bias and uncertainty of  $\delta^{13}\text{C}_{\text{org}}$  isotopic mixing models  
Z.E. Kayler · L. Gano · M. Haux · T.G. Pysker · E.W. Sutherland · A.C. Mix · B.J. Bond 227

Linking calcification by exotic snails to stream inorganic carbon cycling  
E.R. Hochberg · R.D. Hall Jr. 235

Erratum 245

GLOBAL CHANGE ECOLOGY

The importance of biotic factors in predicting global change effects on decomposition of temperate forest leaf litter  
S. Bourde · T.T. Hovda · J.F. David · S. Hättenschüller 247

Elevated  $\text{CO}_2$  increases plant uptake of organic and inorganic N in the desert shrub *Larrea tridentata*  
V.L. Jin · R.D. Evans 257

Summer freezing resistance decreased in high-elevation plants exposed to experimental warming in the central Chilean Andes  
A. Sierra-Almeida · L.A. Cavalerio 267

ERRATUM

Erratum to: Herbivore impacts to the moss layer determine tundra ecosystem response to grazing and warming  
J.L. Gornall · S.J. Woodin · I.S. Jónsdóttir · R. Van der Wal 277



This article was published in the above mentioned Springer issue. The material, including all portions thereof, is protected by copyright; all rights are held exclusively by Springer Science + Business Media. The material is for personal use only; commercial use is not permitted. Unauthorized reproduction, transfer and/or use may be a violation of criminal as well as civil law.

# Effects of resource availability on seedling recruitment in a fire-maintained savanna

Gwenllian D. Iacona · L. Katherine Kirkman ·  
Emilio M. Bruna

Received: 21 November 2008 / Accepted: 27 October 2009 / Published online: 17 November 2009  
© Springer-Verlag 2009

**Abstract** The herbaceous ground cover of the longleaf pine ecosystem harbors the highest plant species richness in North America, with up to 50 species per square meter, but the mechanisms that regulate this diversity are not well understood. In this system, variability in seedling recruitment events may best explain the extremely high small-scale species richness and its relationship to soil moisture and system net primary productivity. To understand the potential mechanistic controls on species richness, we used a long-term resource manipulation study across a natural soil moisture gradient to assess environmental controls on seedling recruitment. We considered the availability of resources to be an indicator of seedling safe-site supply, and also manipulated seed availability to examine the relative importance of recruitment limitations on seedling diversity. We found that water availability regulated the number of species in the seedling community regardless of the underlying natural moisture gradient, and that this effect

may result from differential responses of seedling guilds to resource availability. Water supply was more important than seed supply in determining seedling establishment, suggesting that appropriate sites for regeneration are a factor limiting seedling success. This is the first study that shows that the episodic supply of microsites for recruitment could influence species richness in the highly threatened and biodiverse longleaf pine savanna.

**Keywords** Longleaf pine savanna · Recruitment limitation · Resource limitation · Safe-site limitation · Seed limitation

## Introduction

Seedling recruitment can have major effects on the dynamics of plant populations (Grubb 1977; Tilman 1993; Bruna 2003), and is generally thought to be regulated by two fundamental factors. The first of these is “seed limitation,” whereby seedling establishment may be constrained by the availability of propagules (Turnbull et al. 2000). Propagule supply, in turn, can be influenced by factors such as species pool composition, plant fecundity, and herbivory (Eriksson and Ehrlen 1992; Ehrlen et al. 2006; Clark et al. 2007). However, even if viable seeds are present, seedling recruitment will not occur if there are no “safe-sites” available that can provide the necessary resources for germination and establishment (Fowler 1988; Eriksson and Ehrlen 1992; Caspersen and Sapruff 2005). The presence of these safe-sites is often a stochastic process influenced by disturbance (Harper et al. 1965), productivity (Stevens et al. 2004), or plant–plant interactions (Valientebanuet and Ezcurra 1991), as well as physical features such as topography and climate (Pollock et al. 1998).

---

Communicated by Meelis Partel.

**Electronic supplementary material** The online version of this article (doi:10.1007/s00442-009-1502-y) contains supplementary material, which is available to authorized users.

---

G. D. Iacona · E. M. Bruna  
Department of Wildlife Ecology and Conservation,  
University of Florida, P.O. Box 110430,  
Gainesville, FL 32611-0430, USA

G. D. Iacona (✉) · L. K. Kirkman  
J. W. Jones Ecological Research Center,  
3988 Jones Center Drive, Newton, GA 39870, USA  
e-mail: gdiacona@gmail.com

E. M. Bruna  
Center for Latin American Studies, University of Florida,  
P.O. Box 115530, Gainesville, FL 32611-5530, USA

In addition to population-level effects, seed and safe-site limitation can influence community composition (Grubb 1977; Foster and Tilman 2003; Zeiter et al. 2006). For instance, niche heterogeneity theory proposes that physical and environmental variability results in recruitment of safe-sites that are uniquely suited to each species' competitive strategy (Grubb 1977). Examples of safe-site properties that can influence species diversity at a site include water supply (Silvertown et al. 1999; Lundholm and Larson 2003), rates of N mineralization (Smith and Huston 1989; Gundale et al. 2006), light availability (Hubbell et al. 1999), space for recruitment (Huston 1979), or soil properties such as cation availability and texture (Freestone and Inouye 2006). However, many studies suggest that seed limitation is ubiquitous and that species diversity at a site is more strongly controlled by seed availability than by other factors (Foster and Tilman 2003; Ehrlén et al. 2006; Clark et al. 2007; but see Dickson and Foster 2008). Interestingly, in highly diverse ecosystems such as tropical forests, no one factor may have primacy in driving patterns of community composition. Instead, the interaction between seed supply and safe-site availability may be the best explanation for the observed community composition (Hubbell et al. 1999; Busing and Brokaw 2002). Despite the potential for such synergisms between safe-site and seed limitation, few studies have simultaneously manipulated both safe-site availability and seed supply across environmental gradients to examine their relative impact on patterns of seedling recruitment and richness (Hubbell et al. 1999; Xiong et al. 2003; Houseman and Gross 2006; Poulsen et al. 2007; Dickson and Foster 2008).

The longleaf pine savanna is an ideal model for examining the relative importance of different factors influencing seedling community composition in a species-rich ecosystem. The grass-dominated groundcover (defined as the herbaceous understory that is characteristic of this system) of the longleaf pine ecosystem harbors the highest small-scale levels of species richness in North America with up to 50 species per square meter (Peet and Allard 1993; Kirkman et al. 2001). This system is characterized by numerous environmental stresses—deep sandy soils and high summer temperatures often result in severe water limitation, and N availability is very low (Wilson et al. 1999). In addition, disturbance by fire is ubiquitous, with return intervals as frequent as 1–3 years (Robins and Myers 1992). The primarily perennial grasses, forbs, and legumes that compose the groundcover are thought to be resilient to frequent disturbance and resource limitation, and may be long lived once established (Clewell 1989). However, the environmental stressors in this system have the potential to limit seedling recruitment with implications for seedling diversity.

A positive linear relationship between soil moisture and species richness in the longleaf pine savanna suggests that

soil moisture may regulate species richness by affecting seedling recruitment rates (Kirkman et al. 2001). However, no studies to date have evaluated the relative importance of safe-site limitation via nutrient availability and moisture stress for seedling recruitment in this system, or how resource availability interacts with seed limitation to influence community composition.

Here, we test the hypothesis that the availability of recruitment safe-sites is the key factor driving species richness in the longleaf pine savanna. To do so, we used a long-term experiment in which water and nitrogen (N) availability—and hence the availability of putative safe-sites—has been manipulated in a fully factorial experimental design for the past 5 years. Specifically, we asked:

1. Does N or water availability influence seedling recruitment rates across the natural moisture gradient (i.e., what is the intensity of safe-site limitation)?
2. How does seedling species richness relate to resource availability and seedling recruitment rate?
3. How does seed supply influence patterns of recruitment (i.e., can seed addition affect seedling success indicating that seed limitation is in effect)?

## Materials and methods

### Study site

Ichauway (31°13'N, 84°29'W) is an 11,300-ha property of the J. W. Jones Ecological Research Center that includes 7,500 ha of natural stands of longleaf pine (*Pinus palustris* Mill.) savanna. A wiregrass (*Aristida stricta* Michx.)-dominated ground cover that is characterized by notably high levels of fine-scale species richness (Drew et al. 1998) has been managed under a 2–5 year prescribed fire return interval for at least the past 90 years. This site is located in the Lower Coastal Plain and Flatwoods section of southwest Georgia, USA (McNab and Avers 1994). Average yearly temperatures range from 5–17°C in the winter to 21–34°C in the summer with about 131 cm of precipitation evenly distributed throughout the year (Goebel et al. 1997). Across its range, the longleaf pine–wiregrass savanna occupies a wide moisture gradient that extends from extremely mesic locations with saturated soils and a perched water table, to extremely xeric locations along deep sand ridges. At Ichauway, this moisture gradient occurs naturally, with seasonally saturated Aquic Arenic Paleudult soils characterizing the mesic regions, while Typic Quartzipsamment soils are found in the xeric regions (Goebel et al. 1997). Within this moisture gradient the local species pool has been characterized with about  $64 \pm 2.8$  species in the xeric sites and  $159 \pm 4.2$  species in the mesic sites with low floristic

similarity between sites (26 species in common; Kirkman et al. 2001).

### Resource manipulation experiment

Our experiments take advantage of an on-going and long-term resource manipulation study established at Ichauway in 2002. The overarching goal of the long-term study is to examine the effect of the addition of N and water on system net primary productivity and plant community diversity. N and water are manipulated in a  $2 \times 2$  factorial design conducted at both the mesic and xeric ends of the natural soil moisture gradient (mean percent volumetric soil moisture  $8 \pm 0.68$  in xeric, and  $15 \pm 1.04$  in mesic). Due to the inherently clustered spatial distribution of appropriate soil types and disturbance history, the xeric sites were closer to each other than the mesic sites. For each of the soil moisture extremes, 16 plots ( $50 \text{ m} \times 50 \text{ m}$ ) were established and four factor-level combinations were randomly assigned (water only, N only, water + N, control), with four replications of each treatment. Irrigation with approximately 825 mm water year<sup>-1</sup> (~65% increase in yearly precipitation) maintained the water treatment plots at close to 40% field capacity. The level of water addition was determined by engineering limitations but was a substantial amount for the system (R. Mitchell, personal communication). Irrigation was applied for 24 h once every 8 days unless sufficient rain fell to reach the target field capacity. Water was treated with reverse osmosis to minimize cation accumulation in the soil. N fertilizer ( $\text{NH}_4\text{NO}_3$ , 34-0-0) was applied by hand 3 times a year at a rate of  $50 \text{ kg ha}^{-1} \text{ year}^{-1}$  (natural N mineralization:  $10\text{--}20 \text{ kg ha}^{-1} \text{ year}^{-1}$ ). The percent of the yearly total fertilizer that was distributed at each application varied to mirror natural N mineralization rates in the system, with 23% applied in January, 60% added in May, and 17% in September. All sites were burned every 2 years as part of the prescribed fire management regime at Ichauway.

In 2003, we installed 13 polyvinylchloride (PVC) rings (30 cm diameter  $\times$  10 cm deep) in each of the 16 plots. These rings served as seedling recruitment manipulation sites and also allowed us to ensure that observed seedlings were recruits from seed rather than vegetative resprouts. To install the PVC, we first hammered a 30-cm steel ring into the soil to sever the plant roots and then extracted this ring with as little soil disturbance as possible. The PVC rings were inserted into the pre-cut trench so that the top edge of the ring was flush with the ground. In 2005, we removed the existing vegetation in each ring with two treatments of glyphosate, applied in summer and fall. All sites were burned in February 2004 and 2006.

### Microsite limitation and seedling species richness

To examine the effect of water and N availability on seedling recruitment rate, we censused all naturally occurring seedling recruits in five PVC rings per plot in May and November 2006 and 2007. We classified each newly recruited seedling into a functional group (graminoid, forb, and legume) and identified it to species. In cases where seedling identification was not possible (e.g., small size, death before maturity), we recorded the functional group only. Species concepts followed Wunderlin and Hansen (2003), except *Dicanthelium* which followed Freckmann and Lelong (1993).

### Seed limitation

To determine how seed availability influenced seedling recruitment across the experimental moisture gradient, we documented patterns of seed rain, described the composition of the soil seed bank, and experimentally manipulated seed supply rates. Seed rain was collected monthly from August 2006–August 2007 in nine funnel traps per plot. We used funnel traps constructed from a 10-cm-diameter plastic funnel fitted inside a 10-cm PVC tube that was recessed to ground level so that the mouth of the funnel was level with the soil surface (Page et al. 2002). Any matter that fell into a funnel was caught in an attached micromesh bag. The traps were grouped into arrays of three, with each array surrounding three randomly chosen “natural recruitment” PVC rings per plot (3 funnels  $\times$  3 arrays = 9 traps per plot). The contents of the three funnels at each array were combined for each 2-month sampling interval. Each composited sample was sifted into a pot containing potting mix (0.07 m<sup>3</sup> Miracle Grow potting mix: 22.7 kg sand). All experimental pots were maintained in a climate-controlled greenhouse and any seedling recruits were removed biweekly upon identification.

To estimate soil seed bank composition, we retained a 10-cm  $\times$  10-cm cylinder of soil that was removed during the installation of one of the seed traps in each plot. Soil samples were stored at 4°C for 4 months and then sieved to remove large debris and rhizomes. One liter of soil from each soil core was spread on top of potting mix in a tray. The trays were maintained in the greenhouse and the seedlings removed upon identification as above.

To manipulate seed supply, we planted seeds of common ground cover species in a subset of the PVC rings for 2 consecutive years. In each PVC ring, seeds of one of three species were sown at high or low density (250 or 50 seeds), for a total of six PVC rings per plot (3 species  $\times$  2 seed densities). We chose species that were representative of three major functional groups found in the ground cover of the longleaf pine savanna: a common legume [*Desmodium*

*ciliare* (Muhl. ex Willd.) DC.], a widespread forb (*Rudbeckia hirta* L.), and a ubiquitous grass species [*Sorghastrum secundum* (Elliott) Nash]. All seeds were obtained from the native plant research garden at Ichauway. The percent viability of each species was determined in 2007 using petri dish germination or tetrazolium tests (*R. hirta*, 75% viable; *S. secundum*, 40% viable; *D. ciliare*, 90% viable). In 2006, seeds were sown in April. Based on low germination success of *R. hirta* in 2006, presumably due to the late planting date, we re-seeded all species in early March the following year. An 8-cm-tall ring of aluminum window screen was fitted around each PVC ring to reduce loss of seeds due to wind. We counted all seedlings in May and November 2006 and 2007.

### Statistical analyses

We used a mixed model analysis (PROC MIXED, version 9.1; SAS Institute) to determine mean recruitment parameters for each of the study questions (individual analyses explained more fully below). In all of the mixed model analyses, the independent variables (resource treatment, gradient location, and plot) were the same, but the dependent variable (mean value per plot) varied according to research question. We modeled resource treatment (e.g., water only, N only, water + N, control), collection period (May 2006, November 2006, May 2007, and November 2007), and gradient location (mesic, xeric) as fixed factors, and plot ( $n = 32$ ) as a random factor. The model was fit using a Gaussian distribution because the use of mean response values allowed for the assumption of a normal distribution. In addition, examination of the model residuals indicated that the robustness of the model allowed for adequate model fit (M. Brennan, personal communication). We used a value of  $\alpha = 0.05$  to assess statistical significance.

We used a mixed model (as described above) to examine the mean number of naturally recruiting seedlings per ring in each plot and the mean species richness per ring in each plot (response variable = mean of five rings per plot). The objective of these analyses was to determine if there were differences in seedling recruitment rate and species distribution across resource treatments, and gradient locations. Variation due to collection period was also modeled, but since an increase in the number of seedlings over time was assumed we were primarily interested in the interaction between time and resource availability. We also determined if differences in mean seedling recruitment by functional group (graminoid, forb, and legume) occurred as a result of the resource treatments or gradient locations (response variable = mean number of seedlings per functional group, based on five rings per plot).

To determine the relative importance of seed availability (seed limitation) and resource availability (safe-site limitation)

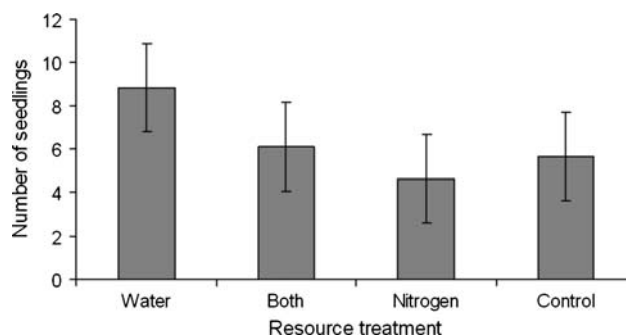
on recruitment rate, we compared the mean number of seedlings emerging in our seed-addition rings across seeding densities and resource treatments. For this analysis, we used a mixed model as explained above, but included a split-plot design with resource treatment as the whole plot factor, and seeding density (high or low) as the split-plot factor. We also compared cumulative species counts of seedlings per plot in the field (actual recruitment) to cumulative species per plot from seed rain (potential recruitment), using a mixed model as above, to determine if the number of species in the field was limited by seed availability. Potential recruits were obtained from 1 year of seed rain collection, while actual seedling recruitment was based on seedlings present in November 2006 (1 year of field recruitment).

## Results

### Microsite limitation and seedling species richness

There were no differences in recruitment due to resource treatment or moisture gradient location (Fig. 1; Table 1). Although there were more seedlings in the fall of each year, and over time, there was no interaction indicating that resource availability effects were time dependent. However, after 2 years, legume recruitment was greatest in the watered only plots, yet, there was no increase in the water plus N plots, regardless of gradient location (Fig. 2; Table 2).

In contrast to patterns of seedling recruitment density, species richness differed significantly between treatments. The mean number of species in watered plots was greater than in control plots regardless of gradient location (Table 1; Fig. 3). However, the mean number of species per treatment did not differ between xeric and mesic locations ( $P > 0.05$ ), and although the number of species increased over time there was no interaction between resource availability and collection period (Table 1).

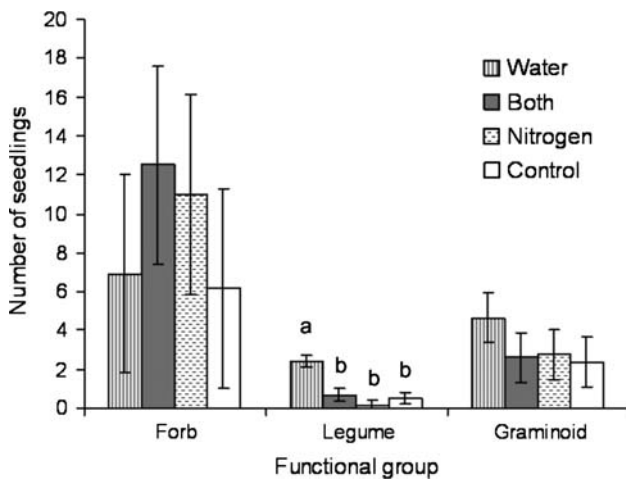


**Fig. 1** Seedling recruitment in longleaf pine savanna sites in response to experimental treatments [water addition (*Water*), water + N (*Both*), N addition (*Nitrogen*), control]. Values are means  $\pm$  SE ( $n = 32$ )

**Table 1** Results from mixed model ANOVA for mean seedling recruitment across four resource treatments [water addition (*W*), N addition (*N*), water + N (*B*), control (*C*)] and two locations (mesic, xeric), over four data collection events (May 2006, November 2006, May 2007, November 2007)

	<i>F</i>	<i>ndf</i>	<i>ddf</i>	<i>P</i>
Seedlings per treatment (no. of natural recruits per ring: fixed effects)				
Resource treatment	0.78	3	24	0.51
Location	0.23	1	24	0.64
Collection	4.24	3	84	0.01
Resource × Collection	1.19	9	84	0.31
Resource × Location interaction	1.20	3	24	0.33
Species per treatment (no. per ring; fixed effects)				
Resource treatment	8.26	3	24	0.001
Location	0.18	1	24	0.67
Collection	16.03	3	84	<0.0001
Resource × Collection	1.59	9	84	0.13
Resource × Location interaction	0.18	3	24	0.91
	<i>t</i>			<i>P</i>
Differences of least squares means (species per ring, <i>ddf</i> = 24)				
B–C	1.45			0.16
B–N	2.48			0.02
B–W	–2.21			0.04
C–N	1.03			0.31
C–W	–3.66			0.001
N–W	–4.69			<0.0001
Xeric–Mesic	1.95			0.06

Results considered significant at  $\alpha = 0.05$   
*ndf* numerator *df*, *ddf* denominator *df*



**Fig. 2** Numbers of seedling recruits from three functional groups in response to experimental treatments. Values are mean  $\pm$  SE ( $n = 32$ ), letters indicate differences at  $P = 0.05$ . For treatments, see Fig. 1

Seed limitation

There was no difference in seedling recruitment from seed rain across resource treatments or gradient locations (all  $P > 0.05$ ). Species richness in seed rain recruits varied across resource treatments, but the effect was opposite that

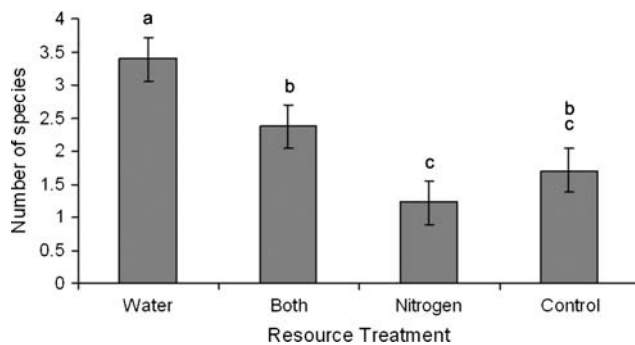
observed in the field with more species in the seed rain from plots without added water ( $F_{3,24} = 3.4, P = 0.034$ ). Overall, the species richness of seedlings in the field was higher with 112 species observed, than that of recruits from seed rain with 82 species observed. There were 52 species observed in both pools (S1). In addition, the cumulative number of species per plot germinating from seed rain collections was about 8 times higher than the cumulative number of species for recruits in the field, regardless of resource treatment or gradient location (mesic,  $F_{1,15} = 98.73, P < 0.0001$ ; xeric,  $F_{1,15} = 52.38, P < 0.0001$ ; Fig. 4). We saw no effect of resource treatment or gradient location on seedling recruitment rate or species richness in germinants from the seed bank in the 32 samples that we obtained; however, we acknowledge that the few replicates limit inference (all  $P > 0.05$ ). Of the 53 species of seedlings that were observed from the seed bank, 45 species were also observed in the field (S1).

The relationship between seeding density and recruitment rate was dependent on gradient location and water availability (Fig. 5). To simplify the discussion of these results, we present the final analysis of this study component with separate analyses for mesic and xeric locations to allow for the interpretation of significant interaction terms (Table 3). After 2 years, the high density seed amendment resulted in greater seedling recruitment than

**Table 2** Results from ANOVA for mean seedling recruitment by functional group across four resource treatments (W, N, B, C) and two moisture gradient locations (mesic, xeric) after 2 years of recruitment

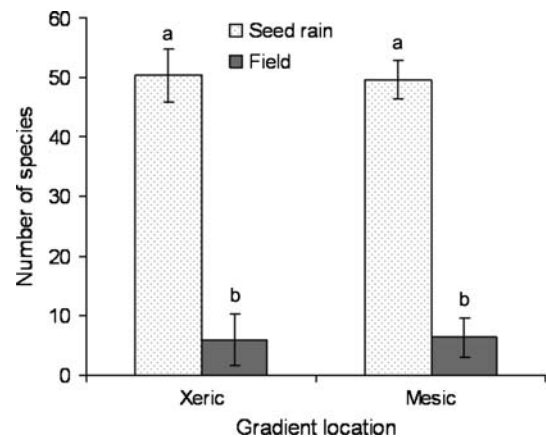
	<i>F</i>	<i>ndf</i>	<i>ddf</i>	<i>P</i>
<i>Seedlings per functional group (no. of seedlings per ring per group)</i>				
Forbs				
Test of fixed effects				
Resource treatment	0.36	3	24	0.78
Location	0.36	1	24	0.55
Resource × Location interaction	0.67	3	24	0.58
Graminoids				
Test of fixed effects				
Resource manipulation	0.65	3	24	0.59
Location	2.41	1	24	0.13
Resource × Location interaction	0.27	3	24	0.85
Legumes				
Test of fixed effects				
Resource manipulation	10.51	3	24	0.0001
Location	4.10	1	24	0.05
Resource × Location interaction	2.44	3	24	0.09
	<i>t</i>			<i>P</i>
<i>Species per treatment (no. per ring; least square means, ddf = 24)</i>				
B–C	0.42			0.68
B–N	1.27			0.22
B–W	−3.9			0.001
C–N	0.85			0.41
C–W	−4.32			0.0002
N–W	−5.17			<0.0001
Xeric–Mesic	−2.03			0.05

Significant at  $\alpha = 0.05$ . For abbreviations, see Table 1



**Fig. 3** Values are species richness of seedling recruits in longleaf pine savanna sites in response to experimental treatments. Letters indicate differences at  $P = 0.05$ . For treatments, see Fig. 1

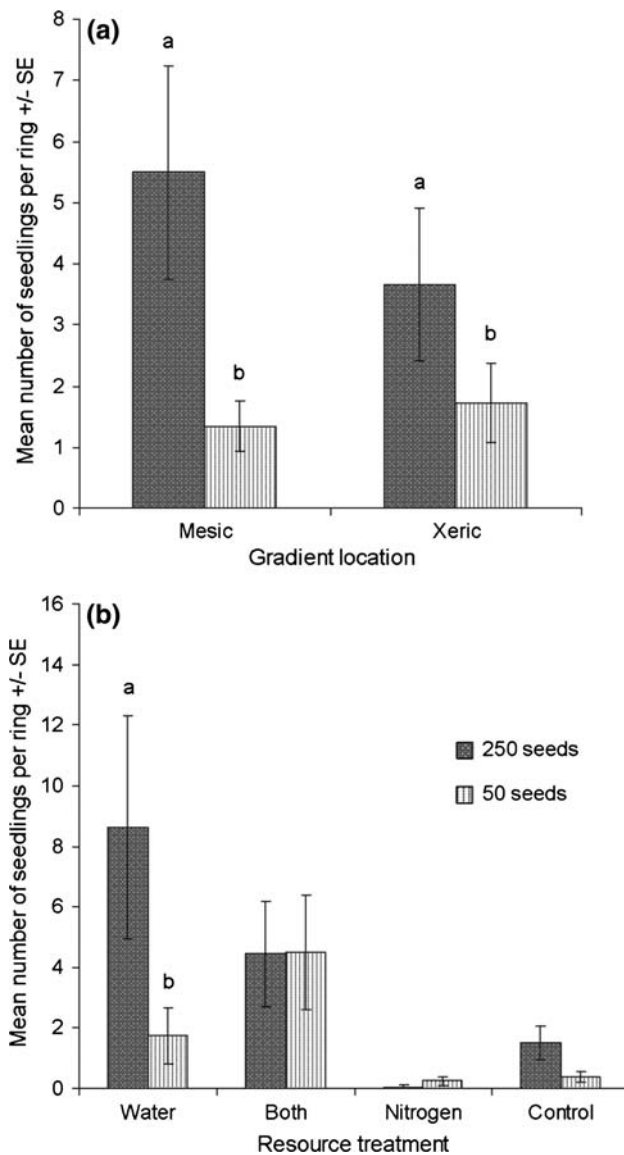
the low density seed treatment in the mesic location. However, in the xeric location, water availability regulated the impact of seed density on seedling recruitment rate with a fivefold increase in recruitment in the high seed density treatments in response to water addition. However, for all other resource treatments, no difference in mean seedling recruitment occurred between the two seed densities (all  $P > 0.05$ ).



**Fig. 4** Values are potential (seed rain) versus actual (field) seedling species richness observed in mesic and xeric sites. Letters indicate differences ( $P = 0.05$ ) between potential and observed richness at each site

## Discussion

We found that seed limitation can influence seedling recruitment of ground cover species in the longleaf pine savanna. However, the availability of water—and thus



**Fig. 5** Values are seedling recruitment rate (50 vs. 250 seeds added) in response to **a** gradient location and **b** resource manipulation in the xeric sites. Letters indicate differences at  $P = 0.05$ . For treatments, see Fig. 1

safe-sites—appears relatively more important in driving recruitment success, particularly in xeric sites, and also influences seedling species richness across the gradient. We conclude that moisture-limited recruitment is an important mechanism promoting the high levels of species diversity found in this system.

#### Microsite limitation, species richness, and seed limitation

We found that safe-site availability drove seedling species diversity during the 2 years of our study. However, the overall pattern of seedling recruitment was not a simple relationship between resource availability and seedling

number. Instead, it appeared that some aspect of safe-site (water) availability directly influenced seedling species richness and may be particularly important under xeric conditions where it can even override the effects of seed limitation. In addition, although N mineralization is extremely low in this system (Mitchell et al. 1999), its availability does not appear to be closely linked to patterns of community composition in the ground cover species.

One potential explanation for how resource availability may influence the number of species observed in the seedling community—regardless of the overall number of seedlings—is that safe-site limitations are guild specific. For instance, the increase in legume seedling recruitment that occurred with water addition may drive the increase in seedling species richness that we observed in water treatments. A recent study on seedling recruitment in grassland plants observed a similar result whereby variation in seedling survival was correlated with seedling life form (Lauenroth and Adler 2008), and legume species are an important contribution to species diversity in the longleaf pine ecosystem (Hains et al. 1999).

The effect of resource availability on species richness could alternatively be explained by seasonal differences in seedling mortality across the gradient (Price and Morgan 2007). Elsewhere we compared recruits resulting from seeds experimentally sown pre- and post-summer, and found no difference in survival that could be attributed to resource availability (Iacona 2008). This result was consistent regardless of species, gradient location, or year. However, we did not measure seedling mortality directly, which limits any conclusions we could draw about establishment success.

The importance of seed availability was dependent on location along the moisture gradient, which provides additional evidence that safe-site availability is of particular importance in this system. At the xeric location, recruitment was only dependent on seed density if supplemental water was added. The fact that many more species germinated from seed rain than were present in natural recruitment further supports our contention that the species composition of the seedling recruits is not solely limited by seed supply in this system.

#### Implications for community composition

One explanation for the observed patterns of species diversity is that the heterogeneity of recruitment safe-sites, instead of merely their presence, could influence seedling community composition. If the establishment of species is limited by interspecific variation in minimum moisture levels, watered plots would have a greater amplitude of safe-sites; they would thus support a greater diversity of seedling recruits (Silvertown et al. 1999). However, we cannot



**Table 3** Results of split-plot ANOVA for mean of experimentally seeded recruitment at two levels of seed density (high = 250 seeds, low = 50 seeds), four resource treatments (W, N, B, C) at two gradient locations (mesic, xeric) after 2 years of recruitment

	Mesic				Xeric			
	<i>F</i>	<i>ndf</i>	<i>ddf</i>	<i>P</i>	<i>F</i>	<i>ndf</i>	<i>ddf</i>	<i>P</i>
<i>Seedlings per treatment (no. of natural recruits per ring)</i>								
Test of fixed effects								
Resource treatment (whole plot factor)	1.95	3	12	0.17	2.96	3	12	0.08
Seeding level (split-plot factor)	9.41	1	12	0.01	7.12	1	12	0.02
Resource × Level interaction	1.50	3	12	0.26	5.28	3	12	0.01
Differences of least squares means for interaction between resource treatment and seeding level in the xeric location								
Treatment comparison	High		Low		High		Low	
	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>
Comparison of resource treatments within seeding levels								
B–C	1.28	0.23	1.79	0.1				
B–N	1.90	0.08	1.84	0.09				
B–W	–1.81	0.10	1.19	0.26				
C–N	0.62	0.54	0.05	0.96				
C–W	–3.09	0.01	–0.60	0.56				
N–W	–3.71	0.003	–0.65	0.53				
Level comparison	Resource treatment							
	Water		Both		N		Control	
	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>	<i>t</i>	<i>P</i>
Comparison of seeding levels within resource treatments								
Low–High	4.73	0.0005	–0.03	0.97	–0.13	0.9	0.77	0.45

Significant at  $\alpha = 0.05$ . For abbreviations, see Table 1

rule out lottery-based seed supply (Hurt and Pacala 1995) as a driver of high species diversity in this system. For some extremely diverse ecosystems, the apparent niche overlap between co-occurring species precludes niche heterogeneity as the most plausible explanation for the observed patterns of diversity. In these cases, species richness depends on a lottery strategy that is regulated by chance and the composition of the regional species pool (Sale 1977). However, according to Sale (1977), several assumptions must be met before this strategy can be invoked to explain high levels of diversity. First, the availability of microhabitats for recruitment must be the limiting factor that determines propagule success. Second, the chance of a propagule reaching an available safe-site must not be closely related to the density of adults in the community (i.e., not seed limited).

Our results suggest that recruitment microsites are limiting in this system and that seed limitations are less important. Under these conditions lottery recruitment strategies could be occurring across the resource treatments and may provide an explanation for the observed patterns of species richness in the seedling recruits.

#### Gradient effects

Although the results of the mixed models suggest that gradient effects are limited, the inference in our study is limited by the spatial relationships of the plot locations since the xeric sites are closer to each other than the mesic sites are. It is also important to note that there were extreme drought conditions during the study period that permitted us to study the effects of resource availability under particularly harsh conditions. This climatic factor was favorable for our study because we were able to manipulate water extremes. However, in such dry and hot conditions, water limitation may have imposed similar constraints on seedling survival in mesic as in xeric locations, potentially masking an effect of the natural moisture gradient.

Several other aspects of our study design may also have influenced the results. We artificially limited the competitive effect of surrounding vegetation on the seedling recruits by installing PVC tubes as study sites. Although other work in this system suggests that competitive effects are minimal (Keddy et al. 2006), we did not control for this factor in this study. There is also likely to

be a dormant seed bank, especially of legume species, that we were unable to germinate in the seed bank, whose effect may be important in explaining overall patterns of community diversity (Coffey and Kirkman 2006). Finally, fire may act in synergy with seed availability, soil moisture, and N levels to influence seedling establishment in this system. Although we did not directly test for the effects of fire, the schedule of prescribed fires at Ichauway resulted in our plots being burned the spring prior to the start of the study and again 2 years later (e.g., midway through the study). Consequently, our treatments were imposed on a landscape with a historically accurate fire return interval.

## Conclusion

Based on these patterns of recruitment, heterogeneity of water availability appears to be an important driver of species richness in the seedling recruits, which may explain the notably high levels of small-scale species richness that occur at the mesic end of the naturally occurring moisture gradient. Seed limitation was observed throughout the naturally occurring moisture gradient, but its relative importance was also related to water availability. This study demonstrates that resource-mediated limitations on seedling recruitment can provide a mechanism for the maintenance of species richness within a resource-limited, yet diverse ecosystem.

**Acknowledgments** We would like to thank many members of the Ichauway plant lab for assistance in the field. These people include M. Kaeser, M. Creech, K. Stuble, S. Wiggers, K. Coffey, and many others. Assistance with statistical analyses was provided by M. Brennan with the University of Florida Institute of Food and Agricultural Science Statistics Department. W. Cropper and M. Creech provided helpful comments on an early version of this manuscript. The suggestions of R. Mitchell, H. Stevens, and an anonymous reviewer significantly improved the final version and pointed out some relevant studies that had been overlooked. The University of Florida's Department of Wildlife Ecology and Conservation and the J. W. Jones Ecological Research Center provided logistical support throughout the project. Funding was provided by the J. W. Jones Ecological Research Center, The Robert Woodruff Foundation, and The University of Florida College of Agriculture and Life Sciences. All experiments and field work comply with the current laws of the USA.

## References

- Bruna EM (2003) Are plant populations in fragmented habitats recruitment limited? Tests with an amazonian herb. *Ecology* 84:923–947
- Busing RT, Brokaw N (2002) Tree species diversity in temperate and tropical forest gaps: the role of lottery recruitment. *Folia Geobot* 37:33–43
- Caspersen JP, Sapruff M (2005) Seedling recruitment in a northern temperate forest: the relative importance of supply and establishment limitation. *Can J For Resour* 35:978–989
- Clark CJ, Poulsen JR, Levey DJ, Osenberg CW (2007) Are plant populations seed limited? A critique and meta analysis of seed addition experiments. *Am Nat* 170:128–142
- Clewell AF (1989) Natural history of wiregrass (*Aristida stricta* Michx., Gramineae). *Nat Areas J* 9:223–233
- Coffey KL, Kirkman LK (2006) Seed germination strategies of species with restoration potential in a fire-maintained pine savanna. *Nat Areas J* 26:289–299
- Dickson TL, Foster BL (2008) The relative importance of the species pool, productivity and disturbance in regulating grassland plant species richness: a field experiment. *J Ecol* 96:937–946
- Drew MB, Kirkman LK, Gholson A (1998) The vascular flora of Ichauway, Baker County, Georgia: a remnant longleaf pine/wiregrass ecosystem. *Castanea* 63:1–24
- Ehrlen J, Munzbergova Z, Diekmann M, Eriksson O (2006) Long-term assessment of seed limitation in plants: results from an 11-year experiment. *J Ecol* 94:1224–1232
- Eriksson O, Ehrlen J (1992) Seed and microsite limitation of recruitment in plant populations. *Oecologia* 91:360–364
- Foster BL, Tilman D (2003) Seed limitation and the regulation of community structure in oak savanna grassland. *J Ecol* 91:999–1007
- Fowler NL (1988) What is a safe site? Neighbor, litter, germination date, and patch effects. *Ecology* 69:947–961
- Freckmann RW, Lelong MC (1993) *Dichanthelium*. In: Editorial committee (eds) *Flora of North America*. Oxford University Press, New York, pp 406–450
- Freestone AL, Inouye BD (2006) Dispersal limitation and environmental heterogeneity shape scale-dependent diversity patterns in plant communities. *Ecology* 87:2425–2432
- Goebel PC, Palik BJ, Kirkman LK, West L (1997) Field guide: landscape ecosystem types of Ichauway. Report number 97-1. Joseph W. Jones Ecological Research Center at Ichauway, Newton
- Grubb PJ (1977) The maintenance of species-richness in plant communities: the importance of the regeneration niche. *Biol Rev* 52:107–145
- Gundale MJ, Metlen KL, Fiedler CE, De Luca TH (2006) Nitrogen spatial heterogeneity influences diversity following restoration in a ponderosa pine forest, Montana. *Ecol Appl* 16:479–489
- Hains MJ, Mitchell RJ, Palik BJ, Boring LR, Gjerstad DH (1999) Distribution of native legumes (Leguminosae) in frequently burned longleaf pine (Pinaceae)-wiregrass (Poaceae) ecosystems. *Am J Bot* 86:1606–1614
- Harper JL, Williams JT, Sagar GR (1965) The behavior of seeds in soil: I. The heterogeneity of soil surfaces and its role in determining the establishment of plants from seed. *J Ecol* 53:273–286
- Houseman GR, Gross KL (2006) Does ecological filtering across a productivity gradient explain variation in species pool-richness relationships? *Oikos* 115:148–154
- Hubbell SP, Foster RB, O'Brien ST, Harms KE, Condit R, Wechsler B, Wright SJ, Loo de Lao S (1999) Light-gap disturbances, recruitment limitation, and tree diversity in a Neotropical forest. *Science* 283:554–557
- Hurt GC, Pacala SW (1995) The consequences of recruitment limitation: reconciling chance, history and competitive differences between plants. *J Theor Biol* 176:1–12
- Huston M (1979) A general hypothesis of species diversity. *Am Nat* 113:81–101
- Iacona G (2008) Seedling recruitment as a driver of species richness in the understory of the longleaf pine savanna, M.S. thesis. University of Florida, Gainesville
- Keddy PA, Smith L, Campbell DR, Clark M, Montz G (2006) Patterns of herbaceous plant diversity in southeastern Louisiana pine savannas. *Appl Veg Sci* 9:17–26
- Kirkman LK, Mitchell RJ, Helton RC, Drew MB (2001) Productivity and species richness across an environmental gradient in a fire-dependent ecosystem. *Am J Bot* 88:2119–2128

- Lauenroth WK, Adler PB (2008) Demography of perennial grassland plants: survival, life expectancy and life span. *J Ecol* 96:1023–1032
- Lundholm JT, Larson DW (2003) Temporal variability in water supply controls seedling diversity in limestone pavement microcosms. *J Ecol* 91:966–975
- McNab WH, Avers PE (1994) Ecological sub-regions of the United States: section descriptions Administrative publication WO-WSA-5. USDA Forest Service Administration, Washington, DC
- Mitchell RJ, Kirkman LK, Pecot SD, Wilson CA, Palik BJ, Boring LR (1999) Patterns and controls of ecosystem function in longleaf pine - wiregrass savannas. I. Aboveground net primary productivity. *Can J For Res Rev Can Rech For* 29:743–751
- Page MJ, Newlands L, Eales J (2002) Effectiveness of three seed-trap designs. *Aust J Bot* 50:587–594
- Peet RK, Allard DJ (1993) Longleaf pine vegetation of the southern atlantic and eastern gulf coast regions: a preliminary classification. In: Proceedings of the 18th Tall Timbers Fire Ecology Conference, Tallahassee
- Pollock MM, Naiman RJ, Hanley TA (1998) Plant species richness in riparian wetlands—a test of biodiversity theory. *Ecology* 79:94–105
- Poulsen JR, Osenberg CW, Clark CJ, Levey DJ, Bolker BM (2007) Plants as reef fish: fitting the functional form of seedling recruitment. *Am Nat* 170:167–183
- Price JN, Morgan JW (2007) Vegetation dynamics following resource manipulations in herb-rich woodland. *Plant Ecol* 188:29–37
- Robins LE, Myers RL (1992) Seasonal effects of prescribed burning in Florida. Misc. publ. no. 8. Tall Timbers Research, Tallahassee
- Sale PF (1977) Maintenance of high diversity in coral reef fish communities. *Am Nat* 111:337–359
- Silvertown J, Dodd ME, Gowing D, Mountford JO (1999) Hydrologically defined niches reveal a basis for species richness in plant communities. *Nature* 400:61–63
- Smith TM, Huston MA (1989) A theory of the spatial and temporal dynamics of plant communities. *Vegetation* 83:49–69
- Stevens MH, Bunker DE, Schnitzer SA, Carson WP (2004) Establishment limitation reduces species recruitment and species richness as soil resources rise. *J Ecol* 92:339–349
- Tilman D (1993) Species richness of experimental productivity gradients: how important is colonization limitation? *Ecology* 74:2179–2191
- Turnbull LA, Crawley MJ, Rees M (2000) Are plant populations seed-limited? A review of seed sowing experiments. *Oikos* 88:225–238
- Valientebanuet A, Ezcurra E (1991) Shade as a cause of the association between the cactus *Neobuxbaumia-Tetetzto* and the nurse plant *Mimosa-Luisana* in the Tehuacan Valley, Mexico. *J Ecol* 79:961–971
- Wilson CA, Mitchell RJ, Hendricks JJ, Boring LR (1999) Patterns and controls of ecosystem function in longleaf pine–wiregrass savannas. II. Nitrogen dynamics. *Can J For Res Revue Canadienne De Recherche Forestiere* 29:752–760
- Wunderlin RP, Hansen BF (2003) Guide to the vascular plants of Florida. University Press of Florida, Gainesville
- Xiong S, Johansson ME, Hughes F, Hayes A, Richards KS, Nilsson C (2003) Interactive effects of soil moisture, vegetation canopy, plant litter and seed addition on plant diversity in a wetland community. *J Ecol* 91:976–986
- Zeiter M, Stampfli A, Newbery DM (2006) Recruitment limitation constrains local species richness and productivity in dry grassland. *Ecology* 87:942–951