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Quantifying targets to manage invasion risk: light gradients dominate the early regeneration niche of naturalized and pre-commercial *Miscanthus* populations

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Abstract Prospective bioenergy crops have caused concern about their invasive potential because they often share characteristics with known invasive species. Studies that examine the factors that limit regeneration of these crop species will be crucial for identifying vulnerable habitats and devising management strategies to reduce the likelihood of escape from cultivation. Using a response surface design, we investigated the influence of light availability, soil moisture, and litter cover on recruitment and establishment of a potential biofuel cultivar of *Miscanthus* as well as an invasive congener. Responses were similar for the two plant types. Light availability had a strong influence on seedling success at both stages, though light limitation prevented establishment only at the lowest light level. Although variation in recruitment rates was low within plant types, establishment varied extensively in response to different light conditions. Low variation in *Miscanthus* seedling recruitment that led to establishment of a seedling bank under a range of light intensities may facilitate a "sit and wait" situation that raises the likelihood of successful escapes. Therefore, management efforts that restrict seed movement and increase light competition for seedlings will be important for lowering invasion risk. As deliberate introductions of bioenergy crops increase, ecological studies that quantify conditions required for successful escapes will be key to helping agronomists and managers mitigate the risk of unintended invasions.

Keywords *Miscanthus* · Biofuels · Regeneration niche · Cultivation · Response surface

Introduction

Plans for broad scale cultivation of herbaceous perennial bioenergy crops have raised concern about potential escapes and subsequent invasions (Barney et al. 2012; DiTomaso et al. 2010; Raghu et al. 2006). The wide geographic distribution and temporal population stability of agricultural crops can generate relentless propagule pressure, potentially increasing the likelihood of establishment outside plantings (Mack 2000). Several candidate crops also display characters associated with invaders; therefore, understanding the ecological conditions and traits that influence spread and establishment success is crucial for predicting and mitigating invasion risk (Davis et al. 2010; DiTomaso et al. 2010; Raghu et al. 2011). However, identifying traits or conditions that consistently predict invasiveness is difficult in

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general, as different traits favor invasion in different habitats (Alpert et al. 2000; Richards et al. 2006). Also, there has been little opportunity to track and quantify invasions from their inception, meaning evaluation of any predictions rely on post hoc analyses rather than experiments (Mack et al. 2000). The recent increase in the production of perennial bioenergy crops provides a rare opportunity for agronomists and ecologists to work together in designing management strategies for crop introductions that minimize invasions (Davis et al. 2010; DiTomaso et al. 2010). Experiments that provide information on critical stages of the escape process can facilitate planning to lower the risk of agricultural escapes or identify conditions potentially vulnerable to invasion.

Management strategies to reduce invasions from crop introductions should be multifaceted to increase chances of success. Important components of such strategies include monitoring at multiple levels, from germplasm and demographic screening to proper siting, and plantation and landscape management to reduce escapes that includes scouting and control (Davis et al. 2010). Focusing efforts to reduce successful crop escapes can be aided by considering how cultivation can foster invasions. Agricultural husbandry provides a buffer against the demographic and environmental stochasticity that limits the success of a majority of plant introductions (Mack 2000). Successful invasions are the result of iterative population introductions filtered through sequential stochastic processes including survival, reproduction, dispersal, recruitment and establishment. Under non-cultivated conditions, the chance of successful establishment is the product of the independent probabilities of each of these stages, which results in a low probability of establishing successfully. In contrast, cultivation sets the probability of the first three steps close to 1, greatly increasing the overall possibility of invasive spread from a cultivated situation. Because cultivation buffers these initial stages against stochastic effects, we can focus on characterizing the plant requirements and environmental conditions influencing the steps that occur after dispersal when quantifying risk factors for invasive spread of bioenergy crops. Therefore, we examined how the combined effects of key environmental factors influenced the recruitment and establishment, two stages of the early regeneration niche, of a potential biofuel cultivar of *Miscanthus* as well as an invasive congener. Identifying environmental conditions that best characterize the regeneration niche can allow us to better design buffer areas to contain escapes from plantations, and to focus detection and containment of escapes during ongoing monitoring efforts.

The regeneration niche is composed of the requirements that make it likely a species will persist in a community for more than one generation, and requires identifying how the relative influence of ecological factors at successive life cycle stages contributes to a species' regeneration potential (Grubb 1977). This concept is useful in invasion ecology, as it provides a connection between combined and varying effects of habitat conditions that influence plant success and the likelihood of a species' establishment and persistence within a community, particularly if habitat limitations change over time or with plant life stage (e.g., "ontogenetic niche shifts", Parish and Bazzaz 1985). For instance, mature plant success can be a poor predictor of both short and long term invasion likelihood as factors favoring an adult in an established population differ from those affecting newly colonizing and early establishment plant stages (Gabler and Siemann 2012), particularly in sensitivity to abiotic conditions and competition (Grubb 1977; Hötzel 2005). For example, germination in American ginseng (*Panax quinquefolius* L.) relates negatively to the frequency and duration of sunflecks, contrary to positive adult plant responses (Wagner and McGraw 2013). Additionally, although most demographic rates of the exotic annual *Erodium brachycarpum* are positively correlated, emergence patterns are negatively correlated with all other stages and strongly associated with small scale variation (Latimer and Jacobs 2012). Within-individual conflicts may arise when conditions that facilitate one developmental stage are disadvantageous at another, leading to impacts on population dynamics through combined effects on regeneration patterns (Schupp 1995). Further, variation in plant response that differs from one stage to the next can provide insight into the range of potential habitat breadth, and identify whether particular stages are more vulnerable to fluctuations in the environment. Thus, we asked: (1) what is the relative importance of abiotic factors (e.g. light, soil moisture, litter cover) for success at two early stages of the regeneration niche, (2) do the important abiotic factors differ between stages, and (3) is the variation in recruitment success similar to that of establishment?

We tested the combined relative influence of three environmental factors expected to be key determinants of early seedling success: litter (plant residue) cover, light availability, and soil moisture. Litter cover affects light interception, can have varying effects on soil temperature and moisture fluctuation, and presents a physical barrier to seedling emergence (Facelli and Pickett 1991; Kuhman et al. 2013; Ruprecht et al. 2010). Light availability has been extensively tied to invasion success (Parendes and Jones 2000; Theoharides and Dukes 2007), and *Miscanthus sinensis* adults have been shown to maintain high photosynthetic capacity in a range of light conditions (Horton et al. 2010; Matlaga et al. 2012a; Quinn et al. 2012). Soil moisture is critical for survival at early stages, as desiccation is an important source of seedling mortality (Warren et al. 2013), and *Miscanthus* benefits from well-watered, though well drained, soils (Waggy 2011). We looked at this combination of factors in a response surface design, as interactions among environmental factors can produce nonlinear impacts on invasions (Eschtruth and Battles 2009) and potentially obscure relationships that might drive invasions (Warren et al. 2013). Previous studies suggest *Miscanthus* experiences high mortality early and high survivorship post establishment (Quinn et al. 2010), but information on seedling establishment and growth is limited (Waggy 2011). We expected the combined effects of the three factors to influence *Miscanthus* recruitment and establishment, with potential variation in plant sensitivity to each factor between the two life stages studied.

Methods

Seed selection

Considering escapes from cultivation allowed us to focus on post-dispersal stages of plant invasion (i.e. recruitment and establishment), with particular emphasis on the fate of the F1 generation. Therefore, we utilized three different sources of F1 seeds from two types of *Miscanthus*. The first of these were field collected seeds of *Miscanthus sinensis* Andress. from escaped populations that have persisted for decades in Daniel Boone National Forest, KY, USA. *Miscanthus sinensis* is a seed fertile species introduced from Japan for horticultural use in the 1800s that became naturalized in the eastern U.S. by the mid-twentieth century, and has formed large escaped populations in some areas (Quinn et al. 2010). These seeds provided an indication of the early requirements of a closely related species that has already escaped cultivation.

The second and third were two different stages of the same pre-release commercial *Miscanthus* × *giganteus* variety. Much of the *Miscanthus* currently planted for biofuel production, such as the 'Illinois clone' of *Miscanthus* × *giganteus*, are seed sterile varieties in which rhizome fragmentation is the primary means of dispersal and cultivation (Jørgensen 2011; Quinn et al. 2010). However, because mortality of early rhizome plantings is high and conversions to the machinery necessary for large-scale cultivation of a rhizome-established species is costly (Jørgensen 2011), the bioenergy seed industry has been working to develop seed fertile cultivars. Therefore, to examine the regeneration niche of the seed that would be planted by farmers, as well as the potential seed issue from plantations, we included seeds from a pre-release biofuel crop variety of *Miscanthus* × *giganteus* (hereafter, *M* × *g*: G1), as well as seeds obtained from plants grown from seeds of this type (hereafter, *M* × *g*: G2). For G1, we used seeds of the pre-release cultivar provided by Mendel Bioenergy Seeds, a subsidiary of Mendel Biotechnology, Inc. (Hayward, CA, USA). For G2, seeds were field collected and provided by Mendel from their *M* × *g*: G1 plantations in Tifton, Georgia, USA. Testing the consequences of our abiotic gradients on different seed types allowed us to examine the consistency of our results given different invasion sources.

Experimental design

We performed a greenhouse experiment to characterize the success of *Miscanthus* seedlings in relation to abiotic factors critical to seedling establishment and persistence. We used a central composite response surface design (CCD) to identify the effects of a combination of factors (light, soil moisture, and residue cover) simultaneously along broad gradients while avoiding an unfeasible number of experimental units. CCDs are two-level full or fractional factorial designs that include additional treatments to estimate second-order effects used to approximate curvature in the response surface (Neter et al. 1996). Each factor

Table 1 Treatment values for the three factors in the response surface design. "LL" and "L" represent the low star value and low base value, "HH" and "H" represent the high star and high base values, and "M" represents the center value in the design

Factors	LL	L	M	H	HH
Residue cover (% area covered)	19	33	54	75	89
Soil moisture (% gravimetric content)	11.6	15	20	25	28
PAR (μmol m^{-2} s^{-1})	6	63	204	257	441

had a high and low base (corner) treatment value with a center treatment value in between, such that (high or low − center)/[0.5 × (high − low)] = 1 (high) or −1 (low). These treatments provided estimation of linear main effects and two-factor interactions in the response surface. Additional ("star") treatments greater and less than the corner values at a distance of α from the center were also included to allow for second order curvature effects. For a detailed explanation of a central composite design, see Neter et al. (1996); Myers and Montgomery (2002). Our design had k = 3 factors, and 2 base levels of each factor, for a total of 8 corner points and 6 star points (Table 1). The center point was replicated 4 times, and α was 1.6818 (for rotatability, see Neter et al. 1996). We performed two independent runs of the experiment. Therefore, with 18 experimental units per each of the three seed types, and 54 units total per run, we had 108 total experimental units. Hereafter, "L" and "H" will refer to the low and high base level values, "M" will refer to the center value, and "LL" and "HH" will refer to the low and high star values, respectively.

We planted seeds in 12.7 × 12.7 cm square pots filled 2 cm deep with locally collected field soil spread over 5 cm deep synthetic potting mix (510 Metro-Mix, Sun Gro Horticulture, Bellevue, WA 98008). Overall, pots were kept under ambient light conditions between 7 a.m. and 9 p.m., except if irradiance levels fell below approximately 3,200 μmol m^{-2} s^{-1} and high pressure sodium vapor lamps (1,000 W) located approximately 2.5 m above the benches maintained light for growing conditions. Specific light and watering regimes per pot were dictated by treatments (see below). Pots were placed in the greenhouse for approximately 10 weeks in two runs (run 1: 27 March–1 June 2012; run 2: 17 April–4 June 2012) and the number of *Miscanthus* seedlings per pots was censused every 3 days. Seedlings were destructively sampled at the end of the experiment (run 1: June 6; run 2: June 14).

To control for the possibility that differences in germination potential among seed accessions might confound comparisons of treatment effects among species, we performed germination tests to ascertain the initial seed numbers required for each species to produce similar potential seedling numbers. Targeting an initial seedling number, rather than seed number, also allowed us to standardize the influence of density on seedling success among the different species. Based on initial germination tests, flats were sown with either 55 (*M. sinensis*), 25 (*M* × *g*: G1), or 110 (*M* × *g*: G2) seeds, for a target population of 20 seedlings.

Abiotic conditions

We measured soil moisture as gravimetric moisture content, with low (L) and high (H) soil moisture values ranging from 25 % gravimetric content (field capacity, −33 kPA) to 15 % gravimetric content (−100 kPA) (Table 1). Experimental pots were brought to the target soil moisture at the outset of the experiment and watered every 3 days to maintain the requisite weight associated with the target soil moisture value. Initially, we established the desired gravimetric content by weighing our pots with dry soil, and calculating the water weight in grams required to reach the target value using the equation: [(wet soil − dry soil)/dry soil] × 100 (Juma 2001).

The influence of litter was manipulated by varying the percent pot soil surface area covered by plant residue. We collected *Miscanthus* biomass from adult plants kept in the University of Illinois Urbana-Champaign Plant Care Facility. Grass tillers were clipped into 3–6 cm long pieces, and laid out to dry on greenhouse benches for 2 weeks to eliminate the possibility of exudates from fresh vegetation affecting experimental outcomes (Facelli and Pickett 1991). To standardize cover percentages, we determined the regression relationship ($\%cover = 0.0343 \times biomass - 0.2002$, $r^2 = 0.947$) between residue (litter) biomass and percent of the pot soil surface covered by the residue. This was accomplished by creating 33 residue samples that varied in weight from 0.1 to 3.0 g and estimating the percent of a 12.7 × 12.7 cm area (dimensions of the pots used in the experiment) covered by the residue using digital photography and the software package ImageJ (Schneider et al. 2012). The regression relationship was used to determine the residue biomass required to attain the desired percent litter cover (Table 1).

We manipulated light by constructing shade cloth covers of different materials that filtered light to different intensities. For each treatment: LL had two layers of 70 % shade cloth, L—one layer of 70 % shade cloth, M—one layer of 50 % shade cloth, H—one layer of 30 % shade cloth, and HH had an open canopy (without shade cloth). Shade cloth was supported on PVC structures above the pots (79 × 79 × 56 cm tall), and light level per treatment was quantified as instantaneous photosynthetically active radiation (PAR, μmol m^{-2} s^{-1}) using the external sensor on a linear ceptometer (LP-80 Accu-PAR, Decagon Devices, Court Pullman, WA 99163). Due to logistical limitations, we were not able to closely approximate target PAR values for a fully rotatable RSM design. However, we did succeed in varying light over a range consistent with potential field conditions, and similar to that of the other two variables; thus, results should be indicative of the qualitative effects of varying light treatments, allowing us to examine its relative importance among the three factors.

Data analysis

Relative importance of environmental factors for recruitment and establishment

We analyzed treatment effects on seedling recruitment and establishment. We quantified seedling recruitment as the maximum number of seedlings counted over the census period (peak in seedling abundance within plots). This response represents recruitment potential from a known initial input, but does not represent total germination, as it was tracked as the total number of seedlings in each pot per census period. Seedling establishment was the final number of seedlings harvested at the end of the experiment. This response represents the number of recruited seedlings left after self-thinning.

We analyzed the three seed accessions separately for effects of variation in the three abiotic factors. Data were coded as: (value-M)/[0.5 × ($H - L$)], to better examine the relative shape of the response surface unaffected by differences in the magnitude of treatment values (Myers and Montgomery 2002; Neter et al. 1996). Thus, for soil moisture and residue cover, variables were coded as: (−1.68, −1, 0, 1, 1.68) for (LL, L, M, H, HH) values. Light was coded as: (−2, −1.5, 0, 0.5, 2.4). As there was no effect of run on treatment effects (all run*treatment $p > 0.05$), data were pooled from the two runs for analysis.

All analyses were performed in R v 2.15.2 (R Core Team 2012). To examine the treatment effects on seedling recruitment and establishment, we compared the relative strength of multiple models using AICc, and to examine the relative contribution of the three factors included, determined the importance of factors based on Akaike weights (Burnham and Anderson 2002) using the *MuMIn* package. Initial global models included first and second order effects as well as two-way interactions, and all second order terms appeared only in the presence of their corresponding first order terms. We considered all models within <1 AICc value of the highest ranked model to be functionally similar, as model weights dropped substantially after this cutoff, and it is within the rule of thumb suggested by Burnham and Anderson (2002). As we were interested in the combined contribution of light, litter cover, and soil moisture, we retained the model that included the most terms out of this suite of top models. Therefore, "best" models were the most inclusive models within <1 AICc value of the highest ranked model. We then recorded the statistical significance and lack of fit (LOF: non-significant values indicate an acceptable fit) of this best model (*rsm* package). Because our best models were the most inclusive of functionally similar models, it was possible that this model be tied with the intercept-only model, and thus, despite an acceptable fit (LOI > 0.05), not statistically significant. As we were interested in the relative contributions of the 3 environmental factors, in this case, we reported a LOF value for the most inclusive model, but did not include a p value for significance.

Variation in response between recruitment and establishment

We also examined the variance in the early seedling stages of recruitment and establishment. This comparison allowed us to examine the relative sensitivity of each stage to the imposed environmental gradient. If variance was similar across stages, we expected environmental factors to exert a similar pressure in each stage. However, higher variance in one stage compared to the other across the same range of environmental conditions could indicate a relatively stronger response to habitat variation. We used a bootstrap analysis to compare the variance in recruitment versus establishment, using the proportion of seedlings present at each stage as the response variable to standardize the comparison. The proportion recruited was quantified as the maximum number of seedlings (recruitment)/initial number of seeds planted. The proportion of seedlings established was represented by the final number of seedlings (establishment)/maximum number of seedlings (recruitment). We sampled these proportions with replacement 2,000 times, and calculated the variance for each iteration. We then took the standard deviation of these 2,000 variances per stage, and multiplied that by 1.96 to obtain the 95 % confidence intervals (Dixon 2001).

Results

Light was central to both recruitment and establishment for all three seed accessions, and was retained as a factor in all but one best model (Table 2). Generally, greater light availability increased both the maximum and final number of seedlings, though this effect tapered off in establishment at high light levels (Fig. 1, represented by negative L^2 effects in Table 2). Both cover and moisture also had positive effects (Fig. 1), although, overall, their importance values were low compared to light (Table 2).

Environmental effects on seedling success

For the invasive *M. sinensis*, light and moisture increased seedling success in both stages of the regeneration niche (+L and +M, Table 2). However, the effect of increasing light was tempered at high light levels in the establishment stage ($-L^2$ values), and soil moisture increased seedling success regardless of light value at both stages (Fig. 1A: $p_R = 0.03$, LOF = 0.6; $p_E = 0.001$, LOF = 0.4). Effects on precommercial *M* × *g* success differed between seed sources. *M* × *g*: G1 recruitment was relatively insensitive to all three experimental factors. The intercept-only model had the strongest support, though moisture, rather than light, played a key role—this time, with negative effects (−M and $-M^2$, Table 2: LOF = 0.5). However, *M* x *g*: G1 establishment was solely related to light (+L and $-L^2$, Table 2: $p_E = 0.005$, LOF = 0.3). *M* × *g*: G2 models were similar for both stages. Light and litter cover increased seedling recruitment as well as establishment, although light-related increases were lessened at the high light values in the establishment stage (Fig. 1B; Table 2: $p_R = 0.04$, LOF = 0.9; $p_E < 0.001$, LOF = 0.2). *M* × *g*: G2 seedling recruitment and establishment were relatively lower at intermediate values compared to the highest and lowest values for litter cover (Fig. 1B), particularly in recruitment.

Table 2 Importance values based on Akaike weights of all potential model terms. The direction of relationship (+, −) of terms are included for models within Δ2 AICc values of the "best" model, which is indicated by the bolded terms. Light (L) had substantially high importance values for all three species, and the importance of both residue cover (C) and soil moisture (M) varied among species

	First order			Second order			Interactions		
	L	C	M	L^2	C^2	M^2	C*L	C*M	L*M
M. sinensis									
Recruitment	**(+) 0.88**	(−) 0.51	**(+) 0.61**	(−) 0.28	0.13	0.12	0.11	0.06	0.1
Establishment	**(+) 1**	(+) 0.52	**(+) 0.55**	(−) **0.98**	0.19	0.19	(+) 0.3	0.05	0.09
M × *g*: G1									
Recruitment	(+) 0.44	(+) 0.42	(−) **0.61**	0.09	0.1	(−) **0.33**	0.04	0.06	0.07
Establishment	**(+) 1**	0.31	0.37	(−) **0.44**	0.08	0.07	0.06	0.02	0.15
M × *g*: G2									
Recruitment	**(+) 0.86**	**(+) 0.57**	(+) 0.53	**(+) 0.36**	**(+) 0.4**	0.1	0.09	0.11	0.11
Establishment	**(+) 1**	**(+) 0.79**	0.4	(−) **0.8**	**(+) 0.72**	0.08	0.13	0.12	0.15

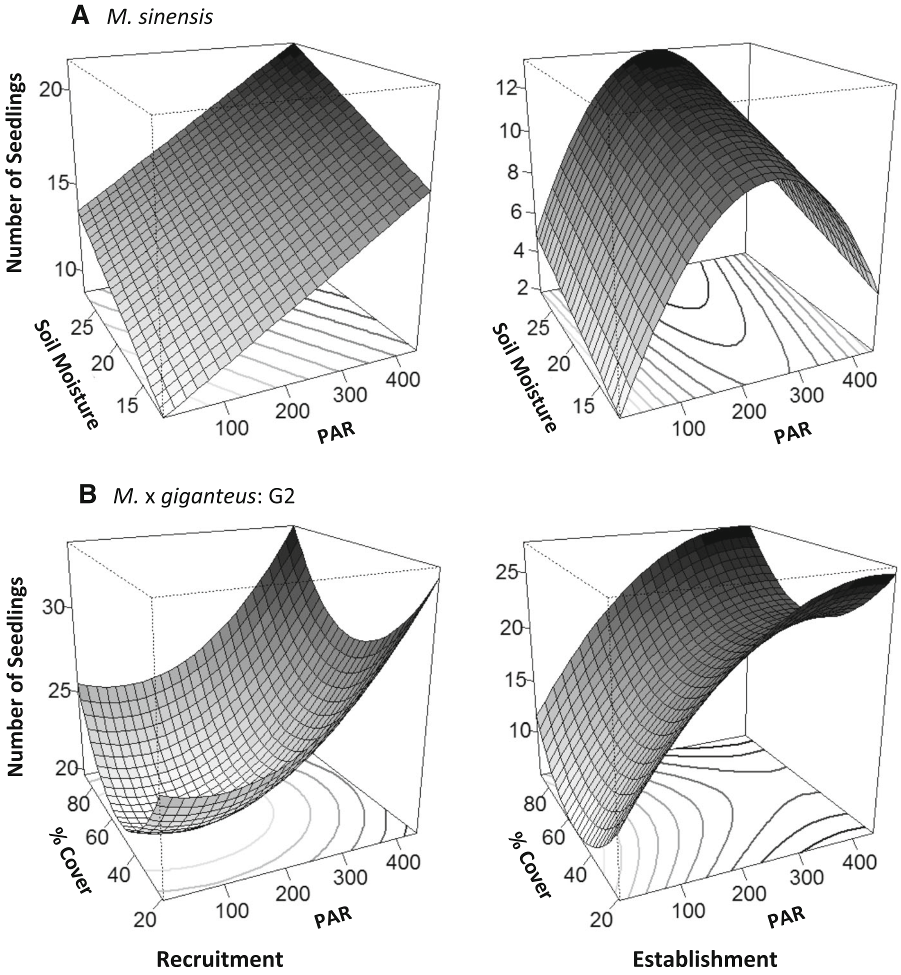

Fig. 1 Response surface plots displaying variable effects on the recruitment and establishment of *M. sinensis* (**A**) and *M.* × *giganteus*: G2 seedlings (**B**). Increasing PAR had a positive effect on both the recruitment and establishment of seedlings in both species, though soil moisture and residue cover modified this effect at the extremes of the measurement scale. *M.* × *giganteus*: G1 had a single factor response, and so is not shown (see Table 2)

Recruitment versus establishment

Overall, we found substantially lower variance among environments in recruitment compared to establishment (Fig. 2). For *M. sinensis* and *M* × *g*: G2, this difference in variance was significant (non-overlapping confidence intervals; mean variance ± CI (all values multiplied by 10^{-3}): *M. sinensis* = 8.71 ± 3.58 versus 55.09 ± 35.05; *M* × *g*: G2 = 1.51 ± 0.91 vs. 48.15 ± 42.88). However, *M* × *g*: G1 had substantial variance in establishment that overlapped with recruitment, resulting in no significant difference (39.08 ± 22.47 versus 70.90 ± 45.12). As light was the factor of highest relative importance, data are displayed to illustrate how this pattern is manifest among light treatments (Fig. 2).

Discussion

Examining the regeneration niche provides insight into how the interactions among different life stages and the relative importance of habitat conditions affect the likelihood of a species colonizing and persisting within a community. In cultivated systems, this framework allows us to focus on how factors that facilitate or limit invasion in post-dispersal stages contribute to establishment as well as persistence outside of agricultural plantings. Identifying environmental conditions that best characterize the regeneration niche can allow us to better design buffer areas to contain escapes from plantations, and to focus detection and containment of escapes during ongoing monitoring efforts.

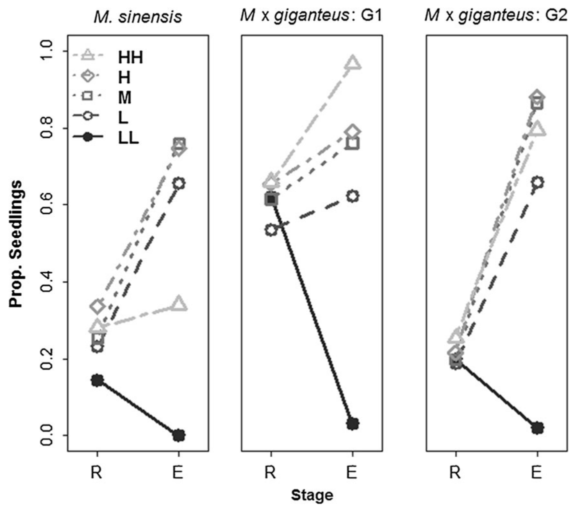

Fig. 2 Light treatment effects on seedling recruitment (R) versus seedling establishment (E). The recruitment to seedlings from seeds was relatively lower than establishment of those recruited seedlings, and variance in seedling recruitment was lower than seedling establishment. Therefore, though recruitment was on average less than 0.5 for two of the seed types, a high proportion of those that recruited survived to establishment across light treatments. Recruitment, or the maximum number of seedlings censused over the course of the experiment, is standardized as a proportion of the initial number of seeds planted. Establishment, or the number of seedlings present at the end of the experiment, is standardized as a proportion of the maximum seedling numbers. “HH” and “H” are the high star and high light treatments, “M” is the center treatment, and “LL” and “L” are the low star and low light treatments, respectively

The shape of the response surface as well as the variation in seedling response to environmental factors varied between stages for all three seed types. Light increased seedling success, but the shape of the light response differed by stage and in combination with other environmental factors. The inclusion of soil moisture variation (*M. sinensis*) and litter cover (*M* × *g*: G2) revealed local optima for improved seedling success at low light levels and further increased seedling numbers at high light levels in both stages. This result highlights the importance of considering multiple factors simultaneously, as release from other limitations may affect the potential consequences of fluctuations in key drivers such as light (Warren et al. 2013). For instance, litter is generally associated with negative effects due to suppression of light and moisture penetration; however, it can also facilitate seedling success when present in low quantities because it insulates plants from temperature fluctuation, desiccation, and microsite competition (Baker and Murray 2010; Bliss and Smith 1985; Eckstein et al. 2012). Additionally, increased soil moisture may allow greater light penetration through leaf litter and soil due to greater light transmittance and scattering, and may reduce high seedling mortality due to desiccation under deep leaf litter (Bliss and Smith 1985; Ciani et al. 2005; Facelli and Pickett 1991; Warren et al. 2013). In our system, it appears light availability drove seedling success, but litter cover and soil moisture relaxed limitation at either end of the light gradient.

That variation in seedling numbers was higher in establishment compared to recruitment suggests that the same range of environmental variation can give rise to different consequences in the recruitment versus establishment stage. It is likely, for many species, that conditions for establishment are more sensitive than those for germination (Turnbull et al. 2000), and niche breadth can vary between different stages of plant development (Parish and Bazzaz 1985). Therefore, though temporary increases in resource availability may accelerate or facilitate the invasion process, species tolerance that allows recruitment and persistence at the earliest stages may be a relatively important trait contributing to invasion potential for these *Miscanthus* species (e.g., Martin et al. 2008; Richards et al. 2006).

Plant growth and recruitment can be expected to increase with light availability (Parendes and Jones 2000; Theoharides and Dukes 2007), and in our study, light availability was an important driver of seedling abundance in both stages considered. Light increased seedling success, but low light was limiting only at the lowest values in the establishment stage (0 % establishment, Fig. 2). This result is consistent with findings that suggest seedlings benefit from greater light levels, though they can tolerate a wide range of light conditions (Matlaga et al. 2012a; Quinn et al. 2012). Although *M. sinensis* does not express plasticity in many characters associated with acclimation to low light (Horton et al. 2010; Matlaga et al. 2012a), slow stomatal closing during low light periods and relatively rapid stomatal opening during photoinduction may predispose *Miscanthus* to survival in the ephemeral light availability of understory or shaded environments (Horton et al. 2010). However, that very few seedlings survived to establishment in the lowest light, compared to an increased proportion surviving under all other light treatments (positive slopes in Fig. 2) suggests a probable threshold requirement in light availability at early plant stages.

Low variation in *Miscanthus* seedling recruitment that led to establishment of a seedling bank under a range of light intensities, combined with high survivorship after establishment, may provide a “sit and wait” strategy. Shade-tolerant exotics are likely to be problematic in areas that are sheltered from frequent disturbance because they are persistent, or even increase, during succession (Martin et al. 2008). The invasive liana *Celastrus orbiculatus* Thunb. (Oriental bittersweet) establishes under closed canopy forest conditions and persists until it is released by a disturbance that creates conditions optimal for rapid growth (Greenberg et al. 2001). Similarly, the spatial distribution and success of American ginseng (*Panax quinquefolius* L.) can be related to the frequency and duration of sunflecks, and may be facilitated by increased light from canopy disturbance (Wagner and McGraw 2013). Our findings, combined with evidence that reproductive output is only slightly reduced in low light (Horton et al. 2010), and that seeds can germinate and seedlings can grow in low light conditions (Matlaga et al. 2012a), suggests factors that increase light availability, such as canopy disturbance, should increase the likelihood of early establishment, but that low light may not prevent these species from establishing outside cultivation. Further studies that quantify the degree to which low light availability affects long term persistence would aid in prioritizing time frames for monitoring and control.

Invasion potential can be difficult to predict because it depends on interacting processes that occur intermittently and on different temporal scales (Davis et al. 2000). For instance, though gaps may create localized temporal release from competition, existing litter layers can reinforce or prolong interference by established vegetation (Facelli and Pickett 1991; Hötzel 2005). Also, relatively high light and moisture availability commonly enhance community invasion, but within communities, these factors do not exert a consistent pattern of influence (Clarke and Davison 2004; Theoharides and Dukes 2007). If *Miscanthus* can recruit seedlings into a wide range of light, moisture, and cover conditions, then invasion may be facilitated by temporal variation in abiotic conditions (“windows of opportunity”) that permit establishment in what would appear to be less suitable habitat (Alpert et al. 2000; Davis et al. 2000; Eriksson and Fröborg 1996; Gabler and Siemann 2012). When species have a wide habitat tolerance, variation in these conditions may affect the rate more than the likelihood of invasion (Martin et al. 2008; Richards et al. 2006).

Mechanisms that control exotic plant invasion remain poorly understood, and little is known about how propagule pressure interacts with various mechanisms of ecological resistance to determine invasion success (Eschtruth and Battles 2009; D’Antonio et al. 2001). In cultivated systems, a stable protected propagule source would be expected to reduce the influence of stochastic environmental factors that limit population growth (Mack 2000). Future studies that examine the degree to which the ecological tolerance displayed in our experiment translates to greater possibility of establishment in the field could focus on how opportunity windows may provide a link between increased propagule pressure and habitat filters. Our study indicates that, for a given propagule pressure, seedling establishment more than recruitment should be sensitive to environmental factors permitting footholds in the landscape.

Habitat choice is filtered first through unequal seed dispersal across patches in the landscape, and second by environmental variation that favors plant survival and establishment in some patches more than others (Martin et al. 2008; Schupp 1995). *Miscanthus* seeds have been shown to travel long distances (Quinn et al. 2011); this in combination with the persistent seed source provided by perennial agricultural production of *Miscanthus* species can be expected to weaken this first, dispersal based, filter, and place pressure on the second, environmental, aspect of establishment limitation. For instance, that the two cultivated seed sources (*M* × *g*: G1 and G2) had similar requirements and success to that of *M. sinensis* seeds from an invasive population warrants further examination of situations or requirements that will mitigate the risk of *Miscanthus* escapes. That both *M. sinensis* and *M* × *g*: G2 required a higher seeding volume to realize the same potential for recruitment compared to the seed provided for growers (*M* × *g*: G1) suggests that mechanisms that provide further limitation might aide management. For example, light availability was a critical factor for both *Miscanthus* species. Thick undergrowth can preempt ground level light, and keep seeds from reaching the soil surface. Therefore, creating buffer zones of perennial vegetation, or of sterile *Miscanthus* varieties, around plantations might present a physical barrier to dispersing seed and limit seedling establishment through light competition that further lowers that probability of successful escapes. We lack basic information on the success or fate of seed sterile varieties such as the Illinois cultivar of *M.* × *giganteus*, as well as seeded varieties that have yet to be commercially available, outside of cultivation (Barney et al. 2012; Matlaga et al. 2012b). Although *M.* × *giganteus* has been considered to have low invasion risk due to clonal propagation and low early survival (Jørgensen 2011), rhizomes placed in riparian zones establish even in the presence of competing vegetation (Barney et al. 2012). *Miscanthus sinensis* has escaped horticultural plantings, and has been considered as a parent species for seed fertile varieties (Quinn et al. 2010). With the additional pressure of continued ornamental use and potential cultivation for bioenergy, its distribution is likely to increase (Quinn et al. 2012). As deliberate introductions of bioenergy crops increase, ecological studies that quantify conditions required for successful escapes will be key to helping agronomists mitigate the risk of unintended invasions.

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