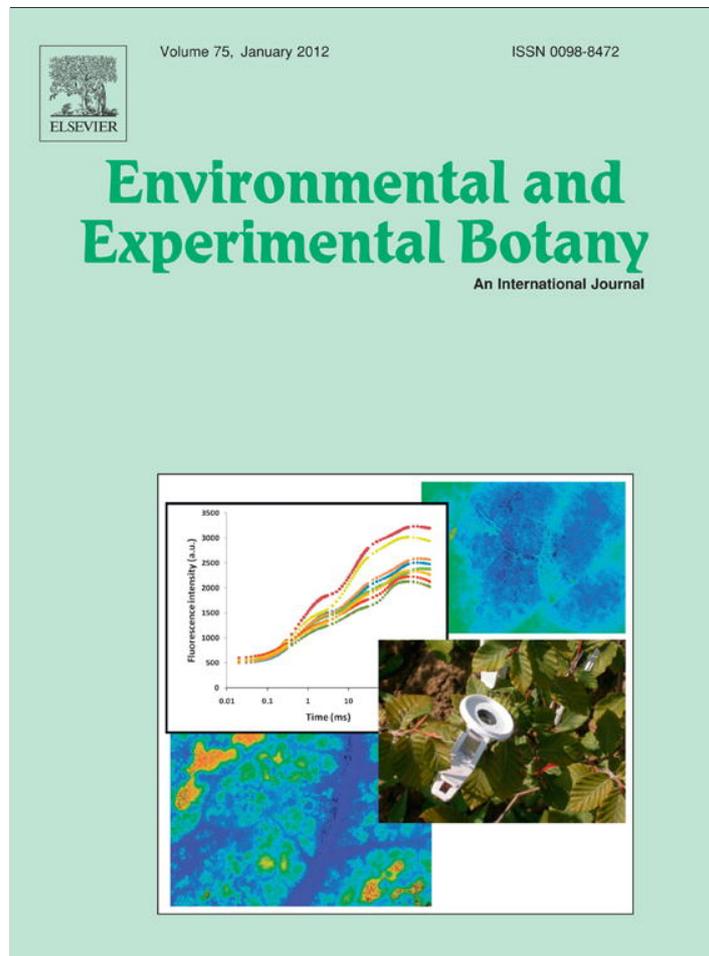


Provided for non-commercial research and education use.
Not for reproduction, distribution or commercial use.



(This is a sample cover image for this issue. The actual cover is not yet available at this time.)

This article appeared in a journal published by Elsevier. The attached copy is furnished to the author for internal non-commercial research and education use, including for instruction at the authors institution and sharing with colleagues.

Other uses, including reproduction and distribution, or selling or licensing copies, or posting to personal, institutional or third party websites are prohibited.

In most cases authors are permitted to post their version of the article (e.g. in Word or Tex form) to their personal website or institutional repository. Authors requiring further information regarding Elsevier's archiving and manuscript policies are encouraged to visit:

<http://www.elsevier.com/copyright>



Contents lists available at SciVerse ScienceDirect

Environmental and Experimental Botany

journal homepage: www.elsevier.com/locate/envexpbot

Highly plastic response in morphological and physiological traits to light, soil-N and moisture in the model invasive plant, *Phalaris arundinacea*

Jason P. Martina*, Carl N. von Ende

Department of Biological Sciences, Northern Illinois University, DeKalb 60115, USA

ARTICLE INFO

Article history:

Received 30 July 2011

Received in revised form 26 March 2012

Accepted 27 March 2012

Keywords:

Phenotypic plasticity

Invasion ecology

Phalaris arundinacea

Resource allocation

Three-way interaction

Reed canarygrass

ABSTRACT

The ability of an introduced species to thrive is often influenced by its capacity to cope with disturbance and resource fluctuation, and one way to cope is by being phenotypically plastic. The biomass and resource allocation of the invasive plant species, *Phalaris arundinacea* (reed canarygrass), to contrasting levels of light, soil-N and moisture were evaluated. We predicted that *P. arundinacea* would show a highly plastic response in important growth and physiological traits to treatment conditions (presence of three-way interactions and large phenotypic plasticity index (PI) values) because of its ability to persist in variable environments. MANOVA tests showed significant three-way interactions for each of the three groups of plant traits (aboveground (AGB) and belowground biomass (BGB), shoot C/N and root C/N ratios, leaf chlorophyll and soluble protein), demonstrating the complex correlated response to the treatment effects by pairs of response variables. There were significant three-way interactions for seven of nine plant traits (univariate analyses), including AGB and BGB, AGB per tiller, shoot/root ratio, shoot C/N ratio, root C/N ratio and leaf chlorophyll content. Total plasticity values, which represented the greatest possible plasticity for each plant trait, were larger than any of the PI values for the main effects. Understanding which traits show plasticity, as well as the magnitude of response expressed in common invasive species is an important area of research because aspects of their aggressive behavior may be explained by how they grow and allocate resources under variable environmental conditions, which in turn can be important when seeking to make predictions about the probability and degree of invasion success with species-specific invasion models.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

The negative ecological and economic effects of invasive plant species (Pimentel et al., 2000, 2005) have generated a keen interest in knowing plant traits that identify potentially invasive species (Moles et al., 2008; Schlaepfer et al., 2010; van Kleunen et al., 2010, 2011). Plant traits that could enhance a species' ability to invade include a large seed set, rapid clonal growth, high resource-use efficiency, broad abiotic tolerance, allelopathy and the ability to escape natural enemies (Zedler and Kercher, 2004). Ecosystem identity probably plays a large role in determining which plant traits will increase a species' chance of invading. In a constantly fluctuating ecosystem, such as a wetland, where resources, hydrology and

disturbance are dynamic, a species' ability to cope with environmental variation will likely increase its chance of success (Sher and Hyatt, 1999; Davis et al., 2000).

Phenotypic plasticity, environmentally induced variation in the growth and development of an organism (Scheiner, 1993), enable introduced species to survive and thrive in variable environments by adjusting both their morphology and physiology to match conditions (Richards et al., 2006). Detailed studies are needed to determine if some of the most successful invasive species have traits that show high levels of plasticity. Recent evidence suggests invasives are more plastic than natives. A meta-analysis by Davidson et al. (2011) showed that among species pairs, invasive species were more phenotypically plastic than non-invasive species, while non-invasives were more able to maintain fitness homeostasis in stressful and resource-limited environments. If having phenotypically plastic traits conveys a competitive advantage during the secondary invasion stage (Dietz and Edwards, 2006), such traits may be responsible for the expansion of many invasive species.

Phalaris arundinacea L. (Poaceae), reed canarygrass (Fig. 1), is a circumboreal perennial wetland grass whose range within the United States currently spans all states except those in the

Abbreviations: AGB, aboveground biomass; BGB, belowground biomass; C, carbon; N, nitrogen; C/N ratio, carbon to nitrogen ratio; S/R ratio, shoot to root ratio; PI, phenotypic plasticity index.

* Corresponding author. Current address: Department of Plant Biology, Michigan State University, East Lansing 48824, USA. Tel.: +1 517 353 5202; fax: +1 517 353 1926.

E-mail address: marti993@msu.edu (J.P. Martina).



Fig. 1. A tussock of flowering reed canary grass, *Phalaris arundinacea*.

Photograph by Jason Martina.

southeast (USDA NRCS, 2010). It can produce thick crowns of aboveground biomass, a large seed set and is capable of vigorous growth (Lavergne and Molofsky, 2007). Originally thought to have been introduced to the United States around 1850 (Merigliano and Lesica, 1998), more recent research suggests invasive genotypes are a mixture of agronomic cultivars derived from European and native genetic material (Galatowitsch et al., 1999; Lavergne and Molofsky, 2004, 2007). Additionally, repeated introductions into the United States have likely contributed to its invasion success (Lockwood et al., 2005; Lavergne and Molofsky, 2007). Though the negative impacts of *P. arundinacea* invasion have been long assumed, a recent study demonstrated negative effects on native plants and several animal groups (Spyreas et al., 2010).

P. arundinacea is highly aggressive; plants were able to form monotypic stands in just two years when exposed to experimental treatments of prolonged flooding, sedimentation and nutrients (Kercher et al., 2007). In natural populations, its abundance has been correlated with soil-N availability (Martina and von Ende, 2008; Matthews et al., 2009). Many abiotic and biotic factors have been studied with respect to *P. arundinacea* growth, development and biomass allocation. The response of *P. arundinacea* to light (Maurer and Zedler, 2002; Perry and Galatowitsch, 2004),

hydrology (Magee and Kentula, 2005; Kercher et al., 2007), nutrient enrichment (Green and Galatowitsch, 2002; Perry et al., 2004; Gusewell, 2005; Herr-Turoff and Zedler, 2007), sedimentation (Mahaney et al., 2004), genotype (Morrison and Molofsky, 1998) and competition (Budelsky and Galatowitsch, 2000; Perry and Galatowitsch, 2004) has been evaluated. Briefly, *P. arundinacea* responds to nutrient enrichment by increasing total biomass, usually to a much greater degree than natives and other invasives (Green and Galatowitsch, 2002). Low irradiance considerably reduces total biomass production (Perry and Galatowitsch, 2004). *P. arundinacea* grows well in saturated soils, but under flooded conditions growth is reduced (Rice and Pinkerton, 1993; Maurer and Zedler, 2002). The growth form switches from sward to tussock under constant flooding (Herr-Turoff and Zedler, 2007). We consider *P. arundinacea* a model invasive species because of its wide geographic and habitat range, aggressive nature and the expanding body of studies of its ecology. Others have argued similarly: Lavergne and Molofsky (2004) identify it as an ideal system in which to investigate what traits determine successful establishment after a plant colonizes a new region. Kercher et al. (2007) used *P. arundinacea* to explore the complexity of factors that underlie rapid invasions, and found a 3-step invasion and degradation

process was involved: an initial decline of native species exposed to prolonged flooding and sedimentation, with subsequent accelerated *P. arundinacea* population growth, followed by more native species decline.

The purpose of this study was to experimentally study the effects of different levels of light, soil-N and moisture on the growth of *P. arundinacea*. While studies have examined the effects of light, soil-N and moisture singly or in pairs, we know of none that has examined the effect of these factors simultaneously for *P. arundinacea*. Examining combinations of all three factors allows complex interactions to be detected, which can lead to a better understanding of the environmental conditions that influence growth characteristics of common invasive plant species. For example, Lautenschlager (1999) found significant interactions between light, moisture and nutrients on the biomass production of red raspberry (*Rubus idaeus*), a common woodland invader; and these factors partially explained its dominance post-disturbance. Sultan (2001) studied the effects of light, moisture and nutrients on the phenotypic plasticity of reproductive traits of several species of *Polygonum* to determine if the species' plasticity matched their ecological breadth of distribution and found complex high-order species by environment interaction effects. Light, moisture and nutrients are important factors to include in studies of phenotypic plasticity because most plants have to deal with gradients of these resources in natural and altered environments, where *P. arundinacea* can inhibit establishment of native species (Lindig-Cisneros and Zedler, 2002; Perry and Galatowitsch, 2003).

Few studies have examined how the internal dynamics of carbon (C) and nitrogen (N), or leaf chlorophyll content (Meekins and McCarthy, 2000) and leaf soluble protein content, respond to contrasting abiotic conditions in an invasive plant species. If *P. arundinacea* shows plasticity in these physiological traits, as well as the morphological traits usually associated with plant performance (biomass production, shoot/root ratio, etc.), then the former may inform the mechanism for its wide range of abiotic tolerance by allowing for better resource acquisition in variable environments, as Molina-Montenegro et al. (2012) concluded with *Taxraxacum officinale*. In turn a better understanding of plasticity in physiological traits can help elucidate complex resource acquisition strategies that can be important when seeking to predict invasion success with species-specific invasion models (Eppinga et al., 2011).

Models of individual growth and resource allocation in invasive plant species may allow us to better understand invasive plant strategies in natural settings. Lemaire and Millard (1999) developed a conceptual ecophysiological plant growth model to predict biomass and nutrient allocation patterns in plants growing alone and in competition with other plants. The model allows plants to be plastic and predicts that with a decrease in the amount of carbon assimilated (low light levels), there is an automatic increase in the allocation of C to shoot rather than root growth to increase the plant's competitive ability for light. Conversely, if there is a decrease in nitrogen uptake, but C assimilation is high (high light levels), then C is allocated to belowground biomass for root growth. This allocation of C to root growth increases the uptake of nitrogen and other limiting nutrients. Due to the requirement of N for the production of photosynthetic machinery (e.g., chlorophyll and Rubisco), high soil-N conditions can lead to an increase in chlorophyll and Rubisco production to increase photosynthesis (assimilation of C). Implicit in Lemaire and Millard's (1999) model are changes to the plant's shoot and root C/N ratios associated with different abiotic environments, with high light conditions increasing C/N ratios (due to increased C assimilation and storage) and high soil-N conditions decreasing C/N ratios (due to increased N uptake). For a review of the physiological and molecular aspects of how nutrient limitation alters biomass allocation, see Hermans et al. (2006).

In general, we predict (1) *P. arundinacea* will exhibit consistently wide ranges in individual response variables to contrasting abiotic conditions (plasticity), which will be detected by statistically significant three-way interactions (Cook-Patton and Agrawal, 2011) and large phenotypic plasticity index values. Additionally, we predict (2) that allocation patterns will be consistent with those predicted by Lemaire and Millard's (1999) model of plant growth, namely, chlorophyll and soluble protein content should be greatest in the high soil-N conditions, because of the N requirement for chlorophyll and protein production, and shoot and root C/N ratios should decrease with increased N availability and increase with increased light availability as discussed above. We predict (3) that the biomass production of *P. arundinacea* will be the greatest in high-light, high soil-N, saturated soil conditions and light will have the most dramatic effect on biomass production, as other studies have found (Maurer and Zedler, 2002; Perry and Galatowitsch, 2004). Tiller production (i.e., clonal growth) should also be greatest in these "ideal" conditions because maximizing spatial dominance is usually the strategy of clonal plants (Thompson, 1991; Pysek, 1997; Pottier and Evette, 2011), and the clonal growth of *P. arundinacea* has been shown to respond to gradients of light and nutrients (Maurer and Zedler, 2002). Finally, consistent with predictions from Lemaire and Millard's model (prediction 2), we predict (4) plants in high-light, high soil-N, saturated soil conditions will also have high chlorophyll and protein content because plants growing in these conditions should have the required N for high chlorophyll and protein production. The high-light and high soil-N conditions should also result in intermediate shoot and root C/N ratios (compared to other treatment conditions) because high C acquisition (photosynthesis) should be evened out by high N uptake.

2. Materials and methods

2.1. Experimental treatment structure

The growth response and resource allocation of *P. arundinacea* to contrasting levels of light, soil-N and moisture were evaluated using a $2 \times 2 \times 3$ factorial treatment structure in an experiment conducted in the Northern Illinois University (NIU) greenhouse from 22-March to 16-May-2005. Two levels of light were crossed with two levels of soil-N and three levels of moisture, for a total of 12 treatment combinations. The two light levels were high-light (no shade cloth) or low-light (50% shade cloth). Shade cloth was stretched around a PVC frame ($106 \times 80 \times 85$ cm) for the low-light treatment level. Light levels were measured with a Licor Quantum model LI-185A light meter at noon at the beginning of each week to monitor the light level at each plot. Natural light was used to avoid an uneven light gradient that had been observed in a pilot experiment with the same design, but under artificial light. The irradiance for the high-light treatment level was approximately $1100 \text{ micromoles m}^{-2} \text{ s}^{-1}$ and $400 \text{ micromoles m}^{-2} \text{ s}^{-1}$ for the low-light.

The two soil-N levels were low soil-N (no nitrate addition) and high soil-N ($40 \text{ g m}^{-2} \text{ yr}^{-1}$). Nitrate was added as 50 ml aliquots containing calcium nitrate tetrahydrate dissolved in distilled water once a week for four weeks (18-April to 9-May-2005), for a total soil-N per pot equivalent to $40 \text{ g m}^{-2} \text{ yr}^{-1}$. Calcium nitrate tetrahydrate has been used in past studies with *P. arundinacea* (Katterer and Andren, 1999; Katterer et al., 1998; Green and Galatowitsch, 2001, 2002), mainly because it is highly soluble and calcium tends not to change the ionic balance as much as other cations. The N addition of $40 \text{ g m}^{-2} \text{ yr}^{-1}$ is slightly less than the high N addition used in Katterer et al. (1998) ($45 \text{ g N m}^{-2} \text{ yr}^{-1}$) and Green and Galatowitsch (2002) ($48 \text{ g N m}^{-2} \text{ yr}^{-1}$) when testing the effects of fertilization on *P. arundinacea*, and is comparable to N inputs seen around regions

of intense agriculture (Davis et al., 1981; Neely and Baker, 1989). The three moisture levels were dry (watered every other day), wet (watered daily) or saturated (kept in trays of standing water), similar to soil moisture treatments implemented by Wetzel and van der Valk (1998). Soil moisture was measured with a HydroSense™ Moisture Content Measurement System (Decagon Devices, Inc.) to monitor the moisture gradient created by the watering regime. A gradient of ~25% soil moisture for the dry, ~35% soil moisture for the wet and ~45% soil moisture for the saturated treatment was maintained throughout the experiment and is similar to the range of 27–47% soil moisture used in a field experiment testing soil moisture effects on *P. arundinacea* (Morrison and Molofsky, 1998). It should be noted that our dry treatment is named in relation to the other treatment levels, and although plants in the dry treatment received less water than the other levels, they did not show any obvious morphologic signs of drought stress.

The plants were grown in 17 cm diameter pots (17 cm tall). The pots were arranged as a split-plot factorial 2–23 design (Kirk, 1995), with light as the whole plot factor and soil-N and moisture as crossed subplot factors. The experiment was conducted on five greenhouse benches; total replication equaled five ($n=60$ pots). Each bench contained a high-light and a low-light plot, which were randomly assigned to a side. Within each light level (plot), each of the six nitrate \times moisture (subplot) treatment combinations were randomly assigned to one of the six positions.

On 22-March-2005 seed stock harvested from the Boyle Grassland (property of NIU) were sown into flats of Pro-mix watered with tap water. Seeds were allowed to germinate and grow for three weeks before 60 seedlings of relatively equal size were separated and transplanted to the pots described above. Plants were grown in these pots until the end of the experiment. The soil used was a mixture of 50% sand and 50% loam, which was recommended by Graustein (1995) because of *P. arundinacea*'s rooting behavior of aggregating around large particles in the Pro-mix substrate. This aggregation made it difficult to fully wash soil from roots when harvesting plants (Graustein, 1995).

2.2. Response variables

The response variables measured for each plant at the end of the experiment were: aboveground biomass (AGB), belowground biomass (BGB), shoot/root (S/R) ratio, total number of tillers, AGB tiller⁻¹, shoot C/N ratio, root C/N ratio, total leaf chlorophyll and leaf soluble protein content. Aboveground plant material was harvested on 16-May-2005 and belowground material on 17-May-2005. The total number of tillers was counted for each pot. Two leaf tissue samples were taken from each plant, one for the chlorophyll and protein measurement (~0.25 g, following Wintermans and De Mots, 1965 and Jones et al., 1989, respectively) and the other for the shoot C/N ratio measurement (~20 mg, partially following Saarinen, 1998). Weights for individual leaf samples were recorded as wet weight. Later, their dry weights were estimated by interpolation from a wet weight vs. dry weight regression plot and added to the AGB total for each plant. Both tissue samples were taken from the same leaf blade, which was selected from the middle of the tallest stem. This was done to ensure the leaf blade was neither relatively old nor young, because this could bias physiological measurements. After tissue samples were collected, the remaining AGB was clipped at the soil surface and dried at 60 °C for 72 h. The pots containing soil and root material were kept overnight at 5 °C to inhibit growth. On 17-May-2005 the roots and rhizomes were washed free of soil and dried at 60 °C for 72 h. Before roots were dried, root tissue samples (~30 mg) were taken and immediately placed in a –70 °C freezer for later C/N analysis.

The tissue samples collected for chlorophyll and protein measurements were immediately placed in cryotubes and frozen in

liquid nitrogen. After the samples were fully frozen, they were placed in a –70 °C freezer. Total chlorophyll was analyzed spectrophotometrically following Wintermans and De Mots (1965). Leaf soluble protein content was analyzed colorimetrically by the Coomassie blue protein assay method (Bradford, 1976). The tissue samples collected for the shoot and root C/N ratio measurements were lyophilized, crushed and analyzed for their C and N content with a Carlo Erba NA-1500 Elemental Analyzer (Verardo et al., 1990).

2.3. Statistical analysis

The experiment was conducted as a three-factor split-plot factorial design structure using five greenhouse benches. The benches were treated as blocks, with one high-light and one low-light “whole plot” assigned randomly to each block. Within each whole plot, there were six pots, assigned randomly to one of the six treatment combinations of moisture \times soil-N. Light, moisture and soil-N were considered fixed factors, and block a random factor: light had a randomized complete block design structure, while moisture and soil-N had a completely randomized design structure (Milliken and Johnson, 2009). Three separate multivariate analysis of variance (MANOVA) analyses were performed for three sets of pairs of response variables: (1) AGB and BGB, (2) shoot C/N ratio and root C/N ratio and (3) leaf chlorophyll content and leaf soluble protein content. The multivariate analysis was used to increase the probability of detecting correlated responses in individual replicates to treatment effects that may be missed in univariate analyses and to reduce the risk of type I error from multiple univariate tests (Scheiner, 2001; Cook-Patton and Agrawal, 2011). In addition, because of the integrated nature of plant phenotypes, multivariate analyses may be useful in detecting suites of correlated traits that may respond differently to variation in environmental conditions (Edwards et al., 2011). The Rao *F* approximation to Wilk's λ was used as the test statistic for each MANOVA. Separate ANOVAs were then run to assess which factors significantly influenced individual response variables.

AGB, BGB, S/R ratio and root and shoot C/N ratio were log transformed and chlorophyll and leaf soluble protein content square root transformed, to correct for heteroscedasticity and non-normality (Zar, 1999). Statistical analysis was performed using GenStat12.1 (VSN International, 2010).

We estimated phenotypic plasticity at the population level for particular traits by averaging across individuals in different environmental treatments (Vitasse et al., 2010). This is more similar to phenotypic plasticity *sensu lato*, than *sensu stricto* (investigating plasticity along known genetic lines) as discussed by Valladares et al. (2006). Phenotypic plasticity *sensu lato* is more focused on the responses of species or populations to variable environments (Callaway et al., 2003; Valladares et al., 2005, 2006). A recent study of the morphologic plasticity in canopy structure of *P. arundinacea* during invasion described plasticity at a population level, similar to our design (Herr-Turoff and Zedler, 2007). It should be noted that the responses we see in this study are at the population level and not across genetically identical lines. We calculated the phenotypic plasticity index (PI: ranging from 0 to 1) for each response variable as the difference between the maximum mean value and the minimum mean value divided by the maximum mean value (Valladares et al., 2000a) across levels of a treatment. PI was calculated independently for each treatment factor (light, soil-N, and moisture), as well as among all three-way treatment combinations (Valladares et al., 2000b). Using trait PIs allows variables with divergent ranges in variation and/or that are expressed in different units to be compared. A phenotypic plasticity index based on maximum

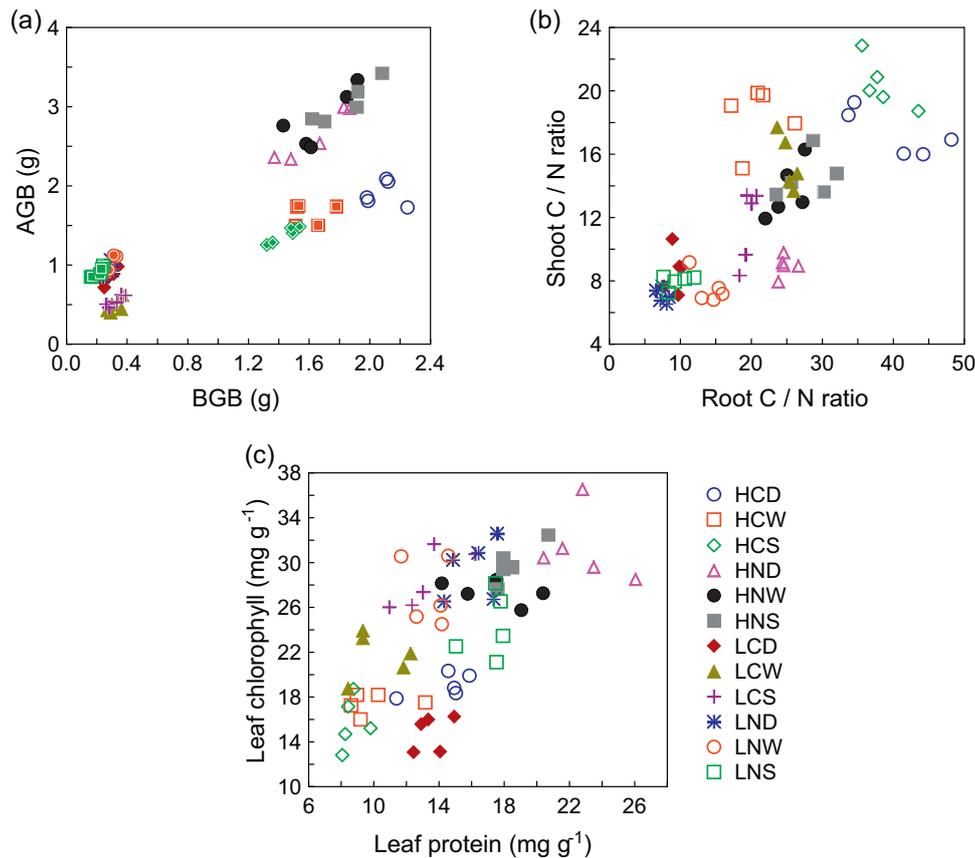


Fig. 2. Bivariate scatterplots of (a) aboveground (AGB) and belowground (BGB) biomass, (b) shoot and root carbon (C)/nitrogen (N) ratios and (c) leaf chlorophyll and leaf soluble protein of *Phalaris arundinacea* grown in high- and low-light conditions at two soil-N levels and three moisture levels. Summary of statistical analysis by MANOVA are presented in the Results section. Treatment combinations in legend: H=high-light, L=low-light, C=low soil-N, N=high soil-N, D=dry, W=wet, S=saturated.

and minimum means is robust, simple and widely used (Valladares et al., 2006).

3. Results

3.1. Multivariate responses

Because the three-way interactions were significant for each of the bivariate responses, we will focus on these as the most informative aspect of the multivariate analysis.

3.1.1. Aboveground and belowground biomass

Four clusters occurred in two separate groups in the bivariate plot of AGB and BGB (Fig. 2, three-way interaction: Rao $F_{4,30} = 18.20$, $P < 0.001$). Plants in the low-light plots had lower AGB and BGB compared to those in the high-light plots. Within each group, AGB was greater in the high soil-N pots, with the exception of the low-light, low soil-N, dry plants, which clustered with the high soil-N. There were opposing trends in the responses of AGB and BGB to the moisture levels in the high soil-N compared to the low soil-N pots: AGB and BGB increased with increasing moisture level in the high soil-N pots, but decreased with increasing moisture level in low soil-N pots. This was consistent with follow-up MANOVA analysis of the high-light treatment level, in which there was a significant moisture \times soil-N interaction (Rao $F_{4,14} = 6.39$, $P = 0.004$), although the slopes of the best-fit linear curves for the two soil-N levels were not statistically different ($t_{26} = 1.40$, $P = 0.174$).

3.1.2. Shoot C/N and root C/N ratios

Although an overall positive trend was present in the bivariate plot of shoot versus root C/N ratios, there were clusters associated with particular treatment combinations (Fig. 2, three-way interaction; Rao $F_{4,30} = 15.30$, $P < 0.001$). Plants in the low-light, high soil-N treatment combinations, as well as the low-light, low soil-N, dry plants, had low shoot and root C/N ratios. Plants grown in the high-light, low soil-N, dry and saturated moisture conditions had the highest shoot and root C/N ratios. However, the high-light, low soil-N, wet plants had lower shoot C/N ratios than the other high-light, low soil-N plants, which placed them at the periphery of the cluster described below. The third cluster of plants, located at lower C/N ratios, was from the high-light, high soil-N, wet and dry, as well as the low-light, low soil-N, wet treatment combinations. Finally, plants grown in the high-light, high soil-N, dry treatment combination, along with the other two low-light, low soil-N, saturated replicates, had lower shoot C/N ratios than those in the previously described cluster.

3.1.3. Leaf chlorophyll and soluble protein content

There was a positive trend in the bivariate plot for leaf chlorophyll content and leaf soluble protein content (Fig. 2). Generally, those plants with the highest amounts of chlorophyll and protein were in the high-light, high soil-N pots. The low-light, high soil-N plants had intermediate protein levels and intermediate to high levels of chlorophyll, but the percentages of chlorophyll and protein varied among the three moisture treatment levels (three-way interaction, Rao $F_{4,30} = 10.10$, $P < 0.001$). Chlorophyll levels generally increased with increasing moisture in the low-light, low soil-N

Table 1
F-values, significance levels and df for three-factor split-plot ANOVA analysis for the effect of light, soil-N and moisture on aboveground biomass (AGB), belowground biomass (BGB), AGB/tiller, total tillers, shoot/root (S/R) ratio, shoot C/N ratio, root C/N ratio, total leaf chlorophyll and leaf soluble protein content.

| Source | df | AGB | BGB | AGB per Tiller | Total Tillers | S/R Ratio | Shoot C/N | Root C/N | Chlorophyll | Protein |
|-----------|----|------------|------------|----------------|---------------|------------|-----------|-----------|-------------|-----------|
| Light | 1 | 3174.07*** | 6427.93*** | 210.19*** | 243.75*** | 907.73*** | 148.22*** | 1671.42 | 4.53 | 10.67* |
| Soil-N | 1 | 506.64*** | 2.199 | 62.49*** | 66.33*** | 1451.65*** | 154.90*** | 107.00*** | 262.26*** | 127.47*** |
| Moisture | 2 | 7.66** | 5.00* | 3.73† | 0.81 | 22.16*** | 72.90*** | 30.32*** | 1.37 | 27.93*** |
| L × N | 1 | 51.22*** | 4.85 | 15.81** | 1.59 | 72.68*** | 0.08 | 17.90** | 35.60*** | 24.51*** |
| L × M | 2 | 2.18 | 5.08* | 1.53 | 7.27** | 49.51*** | 8.65** | 121.64*** | 14.02*** | 13.33*** |
| N × M | 2 | 17.69*** | 8.08** | 3.84† | 1.17 | 35.68*** | 2.57 | 9.37** | 18.74*** | 2.55 |
| L × N × M | 2 | 5.31† | 25.71*** | 4.02† | 3.30 | 39.23*** | 20.42** | 27.44*** | 32.83*** | 0.87 |

* $p < 0.05$.
** $p < 0.01$.
*** $p \leq 0.001$.

pots, although protein levels in the low-light, low soil-N, wet treatment combination were more variable. The plants in the wetter treatment levels of the high-light, low soil-N treatment combination had the lowest chlorophyll and protein levels, but protein content tended to increase as moisture decreased.

3.2. Univariate responses

3.2.1. Aboveground biomass (AGB)

The moisture and soil-N treatments did not affect AGB in the low-light plants, whereas AGB increased with moisture level in the high soil-N, high-light condition and decreased with moisture level in the low soil-N, high-light condition (Fig. 3, three-way interaction, Table 1). *P. arundinacea* greatly increased AGB production in high light compared to low light (significant main effect, Table 1).

3.2.2. Belowground biomass (BGB)

The pattern for BGB production was similar to AGB production. Moisture and soil-N treatments did not affect BGB in the low-light condition, whereas BGB increased with moisture level in the high soil-N, high-light condition and decreased with increased moisture in the low soil-N, high light condition (three-way interaction, Fig. 3, Table 1). BGB was significantly greater in highlight compared to lowlight (significant main effect, Table 1).

3.2.3. Shoot/root (S/R) ratio

The moisture and soil-N treatments did not affect S/R ratio in the high-light conditions; however, the S/R ratio increased with moisture level in the low-light, high soil-N treatment

combination (Fig. 4, three-way interaction, Table 1). In the low-light, low soil-N treatment combination, the S/R ratio was greatest in the dry moisture level and least in the wet and saturated moisture levels. The S/R ratio was the greatest overall in the low-light, high soil-N combination.

3.2.4. Total tillers and AGB tiller⁻¹

Soil-N addition significantly increased the total number of tillers. Total tiller production was greater at the intermediate moisture level under high-light conditions, but least at that moisture level in the low-light plants (moisture x light interaction, Fig. 5, Table 1). AGB tiller⁻¹ decreased with increasing moisture in the low soil-N pots. That trend was absent at the higher soil-N level, but AGB tiller⁻¹ was significantly greater across both light levels (three-way interaction, Fig. 6, Table 1).

3.2.5. Shoot C/N ratio

In the low-light, high soil-N condition, moisture did not significantly affect shoot C/N ratio, whereas in the high-light condition shoot C/N ratio increased with soil moisture in the high and low soil-N conditions (Fig. 7, three-way interaction, Table 1). Shoot C/N ratio was greatest in the low soil-N condition and least in the high soil-N condition (significant main effect, Table 1). Because of the strong treatment effects on shoot C/N ratio, a nonlinear function was fitted to the shoot C/N vs. shoot %N plot and the shoot C/N ratio vs. shoot %C plot, to assess the strength of the relationships. There was a strong negative curvilinear relationship between shoot C/N ratio and shoot %N ($P < 0.001$, $R^2 = 0.96$), but no relationship between shoot C/N ratio and shoot %C ($P = 0.11$, $R^2 = 0.04$), implying

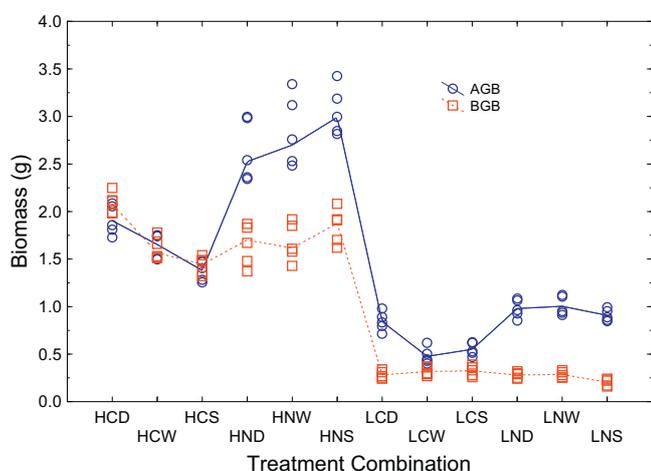


Fig. 3. Dot plots and loess fits of aboveground (AGB) and belowground biomass (BGB) of *Phalaris arundinacea* grown in high-light and low-light conditions at two soil-N levels and three moisture levels. Results of statistical analysis by ANOVA are in Table 1. Coding of treatment combinations as in Fig. 2.

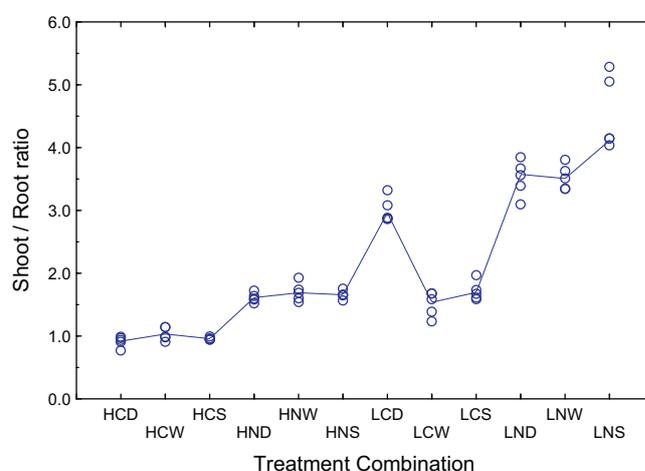


Fig. 4. Dot plot and loess fit of shoot/root ratio of *Phalaris arundinacea* grown in high-light and low-light conditions at two soil-N levels and three moisture levels. Results of statistical analysis by ANOVA are in Table 1. Coding of treatment combinations as in Fig. 2.

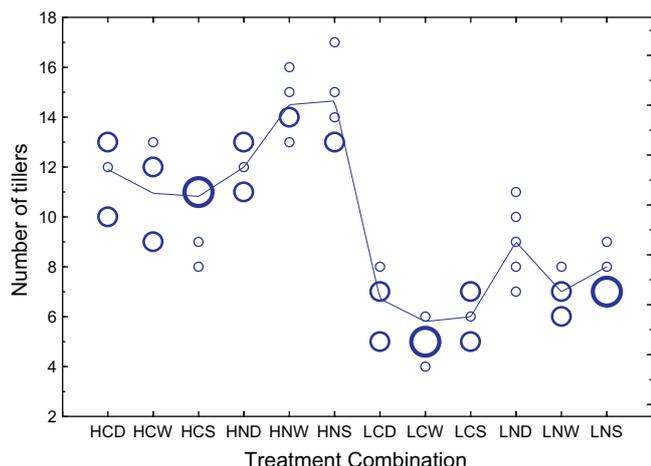


Fig. 5. Bubble dot plot and loess fit of number of tillers per pot of *Phalaris arundinacea* grown in high-light and low-light conditions at two soil-N levels and three moisture levels. Size of dot proportional to number of equivalent values. Results of statistical analysis by ANOVA are in Table 1. Coding of treatment combinations as in Fig. 2.

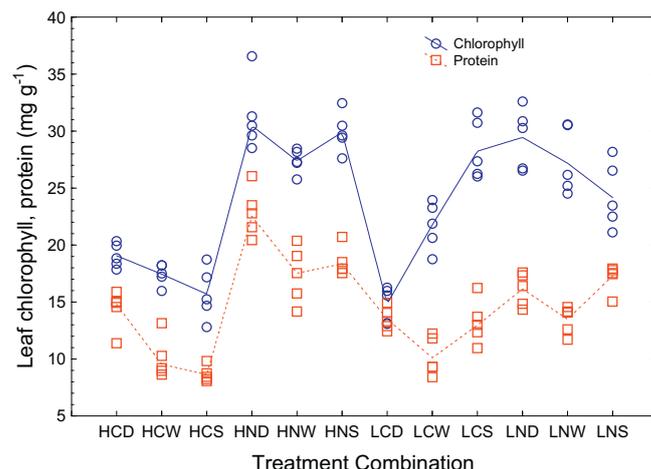


Fig. 8. Dot plot and loess fit of leaf chlorophyll and leaf soluble protein content of *Phalaris arundinacea* grown in high-light and low-light conditions at two soil-N levels and three moisture levels. Results of statistical analysis by ANOVA are in Table 1. Coding of treatment combinations as in Fig. 2.

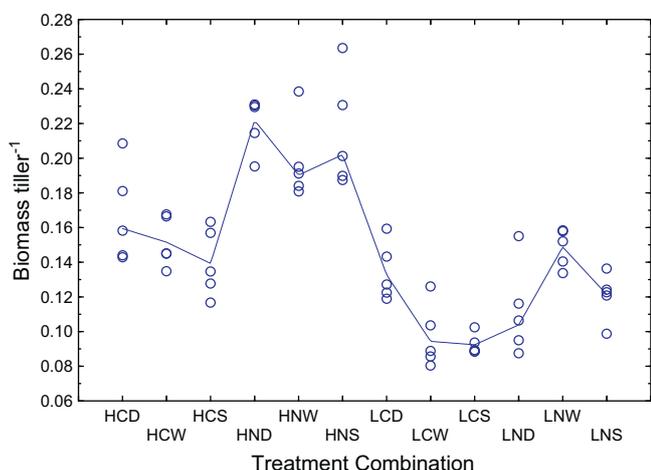


Fig. 6. Dot plot and loess fit of biomass per tiller of *Phalaris arundinacea* grown in high-light and low-light conditions at two soil-N levels and three moisture levels. Results of statistical analysis by ANOVA are in Table 1. Coding of treatment combinations as in Fig. 2.

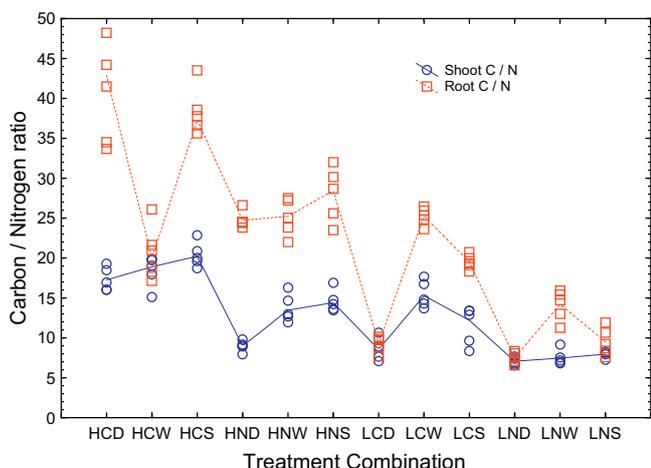


Fig. 7. Dot plots and loess fits of plant tissue carbon/nitrogen ratios in shoots and roots of *Phalaris arundinacea* grown in high-light and low-light conditions at two soil-N levels and three moisture levels. Results of statistical analysis by ANOVA are in Table 1. Coding of treatment combinations as in Fig. 2.

that any treatment effect on shoot C/N ratio was due to changes in shoot %N.

3.2.6. Root C/N ratio

In the low-light treatment level, root C/N ratio showed similar responses in both soil-N conditions: root C/N ratio was least in the dry moisture level and greatest in the wet and saturated moisture levels. In the high-light, low soil-N treatment combination, root C/N ratio was the least in the wet and greatest in the dry and saturated treatment levels and relatively constant throughout the moisture regime in the high-light, high soil-N conditions (significant 3-way interaction, Fig. 7, Table 1). Root C/N ratio was also the least in the high soil-N compared to the low soil-N condition (significant soil-N main effect, Table 1). A nonlinear function was fitted to the root C/N vs. root %N plot and a linear function to the root C/N ratio vs. root %C plot to assess the strength of the relationships. There was a strong negative curvilinear relationship between root C/N ratio and root %N ($P < 0.001$, $R^2 = 0.99$) and a weaker, but still significant, positive linear relationship between root C/N ratio and root %C ($P < 0.001$, $R^2 = 0.63$), implying that treatment effects on root C/N ratio were due to both changes in root %C and %N.

3.2.7. Leaf chlorophyll content

Chlorophyll content was mostly unaffected by moisture in the high-light conditions (compared to low-light), but increased with increasing moisture level in the low-light, low soil-N treatment combination, and decreased with increasing moisture in the low-light, high soil-N conditions (three-way interaction, Fig. 8, Table 1). Chlorophyll content was the greatest in the high soil-N treatments (significant soil-N main effect, Table 2).

3.2.8. Leaf soluble protein

There were two significant two-way interactions for leaf soluble protein content: (1) in the low soil-N condition, plants grown at the low-light treatment level produced more soluble protein than those grown in the high-light treatment level. For the high soil-N condition, the opposite occurred (Fig. 8, Table 1). (2) Leaf soluble protein content in the low-light treatment level increased from the wet to the saturated soil moisture treatment levels, but in the high-light condition, leaf soluble protein content was not significantly different in the wet and saturated moisture levels. Also, leaf soluble protein content was greater in the dry condition relative to the wet and saturated conditions in both light conditions (Fig. 8, Table 1).

Table 2
Mean phenotypic plasticity index (PI) and percent change (%) of *Phalaris arundinacea* to light, soil-N and moisture. PI was calculated by the difference between the maximum mean and the minimum mean divided by the maximum mean of each trait. Percent change represents the percent difference across treatment levels. Total plasticity is the plasticity index calculated among all 12 treatment combinations, which represents the greatest possible plasticity expressed for each trait.

| Plant Trait | Light | | Soil-N | | Moisture | | Total Plasticity | |
|----------------|-------|--------|--------|-------|----------|-------|------------------|--------|
| | PI | % | PI | % | PI | % | PI | % |
| AGB | 0.65 | 182.80 | 0.40 | 68.02 | 0.08 | 8.17 | 0.84 | 536.60 |
| BGB | 0.84 | 507.79 | 0.02 | 1.92 | 0.11 | 12.70 | 0.90 | 924.51 |
| Total Biomass | 0.73 | 268.01 | 0.26 | 35.12 | 0.09 | 9.95 | 0.84 | 514.24 |
| Shoot/Root | 0.56 | 126.02 | 0.45 | 81.02 | 0.14 | 16.37 | 0.80 | 395.51 |
| Tillers | 0.44 | 79.90 | 0.22 | 28.40 | 0.05 | 4.84 | 0.65 | 188.00 |
| Biomass/Tiller | 0.35 | 54.85 | 0.23 | 29.60 | 0.10 | 11.62 | 0.58 | 138.05 |
| Root C/N | 0.52 | 108.71 | 0.29 | 41.02 | 0.14 | 16.45 | 0.81 | 437.63 |
| Shoot C/N | 0.38 | 60.53 | 0.35 | 53.26 | 0.24 | 30.96 | 0.65 | 188.63 |
| Chlorophyll | 0.04 | 3.74 | 0.31 | 44.92 | 0.05 | 4.78 | 0.53 | 111.20 |
| Protein | 0.09 | 9.74 | 0.34 | 50.50 | 0.24 | 30.94 | 0.62 | 164.14 |
| Mean | 0.46 | 140.21 | 0.29 | 43.38 | 0.12 | 14.68 | 0.72 | 359.85 |

Leaf soluble protein content was the greatest in the high soil-N condition compared to the low soil-N condition (significant main effect, Table 1).

3.3. Mean phenotypic plasticity index

The mean PI was greatest for the light treatment (0.46), and the maximum PI values, which were related to biomass, also were associated with this main effect (Table 2). The mean PI for soil-N was considerably lower (0.29), but was >50% greater than for the moisture main effect. This pattern of decreasing plasticity (light > soil-N > moisture) was consistent for all plant traits, except for chlorophyll and protein, in which the greatest plasticity was associated with soil-N and the least with light. Total plasticity values, which were based on individual treatment combinations and represented the greatest possible plasticity for each plant trait, were larger than any of the PI values for the main effects (Table 2).

4. Discussion

We made four main predictions for the growth and resource allocation of *P. arundinacea* to contrasting abiotic conditions, which briefly, were as follows: (1) *P. arundinacea* will exhibit consistently wide ranges in mean values of individual response variables to contrasting abiotic conditions; (2) allocation patterns will be consistent with those predicted by Lemaire and Millard's (1999) model of plant growth; (3) biomass and tiller production of *P. arundinacea* will be the greatest in the high-light, high soil-N, saturated soil condition, and light will have the most dramatic effect on biomass production; (4) plants in the high-light, high soil-N, saturated soil condition also will have high chlorophyll and protein content, and intermediate shoot and root C/N ratios, relative to the other treatment combinations.

Variation in light, soil-N and moisture substantially affected the growth response and resource allocation of *P. arundinacea* in this study. The presence of a significant three-way interaction for seven of the nine response variables in this factorial experiment supported prediction 1 and showed that tests of these resources individually will not accurately describe how *P. arundinacea* will respond in the field where gradients of all three occur. For example, AGB increased in high-light, high soil-N conditions as moisture increased, but in high-light, low soil-N conditions, AGB decreased with increasing moisture level. That moisture can have contrasting effects on AGB depending on soil-N levels at high light levels has not been shown experimentally previously. This suggests in disturbed sites, where there are large areas of bare ground (high light levels) due to sedimentation, *P. arundinacea* will respond positively to high soil moisture as long as N is available, otherwise increasing

soil moisture will decrease its growth. This could explain why *P. arundinacea* is a major invader in prairie pothole wetlands where there are both high nutrients (due to agricultural fertilizers) and disturbed, bare ground (flooding by the removal of drainage tiles) (Seabloom and van der Valk, 2003).

The greatest biomass and tiller production occurred in the high-light, high soil-N, saturated treatment combination, which corresponded to relatively high chlorophyll and protein production and intermediate shoot and root C/N ratios, confirming predictions 3 and 4. These results demonstrate that greater biomass and tiller production can be associated with changes in internal resource allocation. Additionally, because leaf chlorophyll and protein production, as well as shoot and root C/N ratios, followed predicted patterns, it is likely that *P. arundinacea* is adjusting these physiological traits to take advantage of ideal growing conditions, i.e., increased N uptake followed by high production of plant molecules requiring N in high soil-N conditions and increased C assimilation in high light conditions (Lemaire and Millard, 1999).

In further support of prediction 3, low light levels had an overwhelmingly negative effect on both above- and belowground biomass production. It is clear from our results and others' (Kercher and Zedler, 2004; Perry and Galatowitsch, 2004) that *P. arundinacea* needs high light levels to successfully grow and invade. In the low-light, high soil-N treatments, S/R ratio increased with increasing moisture level, which indicated that more biomass was being allocated to the shoots when nitrate and moisture were abundant in the soil. Also, the S/R ratio in the low-light treatment was greater than in the high-light treatments, presumably because more biomass was being allocated above ground for increased C assimilation (photosynthesis) in the low-light conditions (Lemaire and Millard, 1999; Maurer and Zedler, 2002), which has been seen in other herbaceous species (Saarinen, 1998).

Increased tiller production in suitable environments is an important aspect of the growth strategy of vegetatively reproducing plants because clonal growth (tiller production) enables a seedling to successively colonize surrounding areas once established (Hutchings and de Kroon, 1994). This could partially explain the pattern in vegetative reproduction we observed for *P. arundinacea* of increased tiller production with increasing moisture levels in the high-light, high soil-N condition, but decreased with increasing moisture level in the high-light, low soil-N condition. One possible reason for this response is clonal growth can greatly increase the ability of *P. arundinacea* to compete for nearby resources (Klimes et al., 1997; Herr-Turoff and Zedler, 2007) and since this pattern seems correlated with AGB and BGB production, it is likely a result of ideal conditions promoting the best growth strategy. Tiller production still occurred in the low-light conditions, but the tillers were not as robust as those in the high-light conditions, as demonstrated by the lower AGB tiller⁻¹ in the former (see

Fig. 6). The general increase in AGB tiller⁻¹ in high soil-N conditions in all light and moisture treatment levels (except in low-light conditions) was to be expected, as *P. arundinacea* should have used the additional soil-N to produce taller, more robust stems that can compete for light, as predicted by Lemaire and Millard (1999). Herr-Turoff and Zedler (2007) also observed taller, heavier *P. arundinacea* shoots at high N additions along with greater lateral spread in flooded conditions. Overall, as we found, *P. arundinacea*, did best at high soil-N, fully saturated conditions (highest biomass production, greater competitor) (Herr-Turoff and Zedler, 2007).

The general trend of shoot and root C/N ratios was that plants grown in low soil-N, high-light conditions had both high shoot and root C/N ratios compared to plants grown in high soil-N, low-light conditions (Fig. 2). This was expected (prediction 2), because with decreased N uptake (low soil-N conditions) and increased C assimilation (high light levels), plants should have had a high C/N ratio for both roots and shoots. Conversely, with increased N uptake (high soil-N conditions) and decreased C assimilation (low light levels), plants should have had a low C/N ratio for both roots and shoots. These results support Lemaire and Millard's (1999) model of plant growth and allocation, which states that internal C/N ratios can be altered in expected ways by the abiotic environment. Overall, root C/N ratio was greater in the high-light conditions than in the low-light conditions, which supports the prediction that in high-light environments more C was being assimilated in the shoots and allocated to the roots for maintenance (respiration), energy storage, growth or root exudation (Lemaire and Millard, 1999). The root C/N ratio also was lower in the high soil-N treatments levels than in the low soil-N treatment levels; this confirmed that *P. arundinacea* was absorbing N from the soil, thus lowering the root C/N ratio (Saarinen, 1998).

Plants grown in high soil-N conditions had high levels of both chlorophyll and soluble protein, while plants grown in low soil-N conditions had low levels of both response variables, which further supported prediction 2. One obvious explanation for this trend is that N was required for both chlorophyll and protein production (Field and Mooney, 1986); thus, plants grown in high soil-N conditions presumably had more N available for chlorophyll and protein production (e.g. Rubisco) than plants grown in the low soil-N conditions. This illustrates that when resources are abundant, *P. arundinacea* can use those resources to up-regulate physiological processes for increased photosynthesis and growth.

P. arundinacea had the greatest plasticity in response to the main effect of light compared to soil-N and moisture (Table 2). This is not surprising given the sensitivity *P. arundinacea* has shown to gradients of light (Maurer and Zedler, 2002; Perry and Galatowitsch, 2004) and suggests that *P. arundinacea* can adjust both morphological and physiological traits according to the light environment. Although chlorophyll and soluble protein content showed little plasticity in response to light, they were plastic to variation in soil-N, implying that *P. arundinacea* can adjust metabolic construction based on resource supply. Conversely, in a similar study investigating the effects of light, nutrients and density on *Alliaria petiolata* (garlic mustard), Meekins and McCarthy (2000) found *A. petiolata* responded to shade treatments by increasing chlorophyll production, but soil nutrient addition had no effect. For most plant traits, *P. arundinacea* responded the least to variation in moisture, which could be due to a lack of plasticity potential, or, alternatively, could be due to an interaction with moisture and another factor. The lack of plasticity in response to variation in soil moisture alone is consistent with the results of Morrison and Molofsky (1998) who found the biomass production of *P. arundinacea* did not respond to a soil moisture treatment that was almost identical to ours (27–47% compared to 25–45% soil moisture). Our results also support those of Kercher and Zedler (2004) who found *P. arundinacea* a

superior competitor across a wetland hydrologic gradient under high nutrient conditions.

Interactions between light, soil-N and moisture likely accounts for PI values for Total Plasticity being greater than PI values for the main effects. The high Total Plasticity mean PI value (0.72, Table 2) indicates the high level of plasticity *P. arundinacea* can express across gradients of light, soil-N and moisture, and further demonstrates the importance of considering these factors simultaneously both in the field and in models of *P. arundinacea* growth and dominance. Furthermore, even though it is potentially problematic to evaluate PI values among species (Valladares et al., 2006), *P. arundinacea*'s PI values in this experiment are high compared to published values in similar abiotic conditions for both invasive and noninvasive species (Valladares et al., 2000a,b; Balaguer et al., 2001; Peperkorn et al., 2005).

Alliaria petiolata, a biennial herb currently invading many North American temperate deciduous forests, responded similarly to nutrient addition and light availability as *P. arundinacea*. *Alliaria petiolata* increased total biomass in high-light and high-nutrient conditions, as well as increased shoot/root ratio in the low-light conditions (Meekins and McCarthy, 2000). Leaf chlorophyll content was the greatest in the two low-light treatment levels, compared to the high-light treatment level. Overall, light had the greatest effect on growth and reproduction (Meekins and McCarthy, 2000). These results suggest that *A. petiolata* and *P. arundinacea* are responding to variation in light and soil-N similarly, and this response could allow for invasion of natural ecosystems. *Lythrum salicaria* (purple loosestrife), an aggressive wetland plant, showed great phenotypic plasticity to a gradient of soil moisture. Shoot mass was the greatest in the high-water condition and decreased as soil moisture decreased (Mal and Lovett-Doust, 2005).

Not all invasives respond positively to increased nutrient availability. In a study of *Isatis tinctoria* (dyer's woad), an invasive biennial herb of the western United States, high soil-N availability failed to alter any growth or physiological response variable (Monaco et al., 2005). The authors predicted this lack of a response to soil-N availability because *I. tinctoria* is a stress-tolerator adapted for low nutrient sites. Instead, variation in light and soil moisture explained all the differences in the growth and physiological variables measured. Surprisingly, variation in light availability did not alter the shoot/root ratio of *I. tinctoria* (Monaco et al., 2005). These results suggest that the conditions for invasion can be species- or context-specific, and management techniques targeting only one or two abiotic factors might not be adequate for control of all invasives in a habitat. *Isatis tinctoria* not responding to available soil-N and *A. petiolata* responding differently to shade treatments (increased chlorophyll production in low light conditions, see above) is likely related to the ecosystems they invade (disturbed, low nutrient rocky slopes and forest understories, respectively) and could help explain the highly plastic responses seen in *P. arundinacea* in this study. Wetlands are highly variable ecosystems where resources, hydrology and disturbances are dynamic (Zedler and Kercher, 2004). One way to cope with abiotic variability is to be phenotypically plastic (Richards et al., 2006), which might suggest that the most aggressive wetland invaders are those species with the greatest plasticity. Future studies should investigate the plasticity of the most successful wetland invaders in a common experimental design to determine if their plasticity is equal to or greater than that of *P. arundinacea*. Furthermore, future studies should investigate if the phenotypic plasticity of *P. arundinacea* and other common wetland invaders varies among populations as was seen for *Microstegium vimineum*, an invader of eastern North American forests (Droste et al., 2010). Differences in plasticity among populations could either be due to differences in the original genetic makeup of the founding populations, or from natural selection acting to increase

plasticity. In either case, as suggested by Droste et al. (2010), management practices of reducing gene flow between populations could reduce the evolution of greater plasticity, and, therefore, potentially decrease the invasion potential of the target species.

5. Conclusion

More detailed data on variation in traits of plant species can help improve the parameterization of species-specific variables in plant models of growth and dominance, which in turn can lead to more reliable predictions (Cannas et al., 2003; Savage et al., 2007). Recently, Eppinga et al. (2011) modified a classical plant model of interspecific competition to investigate the feedback effect of litter dynamics and evolutionary change in *P. arundinacea* on its ability to invade a native sedge species. Analyses of the model produced a number of intriguing predictions, one of which was that the tissue C/N ratio of *P. arundinacea* played an important role in the outcome of competition by altering both *P. arundinacea*'s nitrogen/light consumption ratio/vector (evolutionary change) and litter quality. Moreover, the synergy of having both positive litter feedbacks and increases in C/N ratios operating could cause critical transitions to alternative stable states in which *P. arundinacea* was dominant, notably, with only a ~5% change in the C/N ratio. However, in their analyses tissue C/N ratio (shoot/root ratio as well) was varied independently of resource conditions. Our results show that both tissue chemistry and shoot/root ratio can vary depending on the availability of each of the three plant resources. It would be useful to investigate whether having parameters of plant growth vary with resource levels in invasion models such as Eppinga et al. (2011) significantly affects the robustness of their predictions.

Acknowledgements

We thank Lesley Rigg and Gabriel Holbrook for comments on early drafts of the manuscript. We also thank the Holbrook and Loubere labs for assistance with physiological parameters determination. The NIU greenhouse staff assisted us with material preparation and plant harvesting. Financial support was provided by the Department of Biological Sciences at Northern Illinois University and a University Fellowship.

References

- Balaguer, L., Martinez-Ferri, E., Valladares, F., Perez-Corona, M.E., Baquedano, F.J., Castillo, F.J., Manrique, E., 2001. Population divergence in the plasticity of the response of *Quercus coccifera* to the light environment. *Functional Ecology* 15, 124–135.
- Bradford, M.M., 1976. A rapid and sensitive method for quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72, 248–254.
- Budelsky, R.A., Galatowitsch, S.M., 2000. Effects of water regime and competition on the establishment of a native sedge in restored wetlands. *Journal of Applied Ecology* 37, 971–985.
- Callaway, R.M., Pennings, S.C., Richards, C.L., 2003. Phenotypic plasticity and interactions among plants. *Ecology* 84, 1115–1128.
- Cannas, S.A., Marco, D.E., Paez, S.A., 2003. Modelling biological invasions: species traits, species interactions, and habitat heterogeneity. *Mathematical Biosciences* 183, 93–110.
- Cook-Patton, S.C., Agrawal, A.A., 2011. Relatedness predicts phenotypic plasticity in plants better than weediness. *Evolutionary Ecology Research* 13, 527–542.
- Davidson, A.M., Jennions, M., Nicotra, A.B., 2011. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecology Letters* 14, 419–431.
- Davis, C.B., Baker, J.L., van der Valk, A.G., Beer, C.E., 1981. Prairie pothole marshes as traps for nitrogen and phosphorus in agricultural runoff. In: Richardson, B. (Ed.), *Proceedings of the Midwest Conference on Wetland Values and Management*. Freshwater Society, Navarre, MN, pp. 153–163.
- Davis, M.A., Grime, J.P., Thompson, K., 2000. Fluctuating resources in plant communities: a general theory of invasibility. *Journal of Ecology* 88, 538–534.
- Dietz, H., Edwards, P.J., 2006. Recognition that causal processes change during plant invasion helps explain conflicts in evidence. *Ecology* 87, 1359–1367.
- Droste, T., Flory, S.L., Clay, K., 2010. Variation for phenotypic plasticity among populations of an invasive exotic grass. *Plant Ecology* 207, 297–306.
- Edwards, K.R., Bastlova, D., Edwards-Jonasova, M., Kvet, J., 2011. A comparison of univariate and multivariate methods for analyzing clinal variation in an invasive species. *Hydrobiologia* 674, 119–131.
- Eppinga, M.B., Kaproth, M.A., Collins, A.R., Molofsky, J., 2011. Litter feedbacks, evolutionary change and exotic plant invasion. *Journal of Ecology* 99, 503–514.
- Field, C., Mooney, H.A., 1986. The photosynthesis-nitrogen relationship in wild plants. In: Givnish, T.J. (Ed.), *On the Economy of Plant Form and Function*. Cambridge University Press, Cambridge, pp. 25–55.
- Galatowitsch, S.M., Anderson, N.O., Ascher, P.D., 1999. Invasiveness in wetland plants in temperate North America. *Wetlands* 19, 733–755.
- Graustein, J.S., 1995. Competitive effect of an invasive grass, *Phalaris arundinacea* L., on the growth of a rhizomatous sedge, *Carex stricta* L. Masters Thesis, Northern Illinois University, DeKalb.
- Green, E.K., Galatowitsch, S.M., 2001. Differences in wetland plant community establishment with additions of nitrate-N and invasive species (*Phalaris arundinacea* L. and *Typha xglauca* Godr.). *Canadian Journal of Botany* 79, 170–178.
- Green, E., Galatowitsch, S.M., 2002. Effects of *Phalaris arundinacea* and nitrate-N addition on establishment of wetland plant communities. *Journal of Applied Ecology* 39, 134–144.
- Gusewell, S., 2005. Responses of wetland graminoids to the relative supply of nitrogen and phosphorus. *Plant Ecology* 176, 35–55.
- Hermans, C., Hammond, J.P., White, P.J., Verbruggen, N., 2006. How do plants respond to nutrient shortage by biomass allocation. *Trends in Plant Science* 11, 610–617.
- Herr-Turoff, A., Zedler, J.B., 2007. Does morphological plasticity of the *Phalaris arundinacea* canopy increase invasiveness? *Plant Ecology* 193, 265–277.
- Hutchings, M.J., de Kroon, H., 1994. Foraging in plants: the role of morphological plasticity in resource acquisition. *Advances in Ecological Research*, vol. 25. Academic Press, London, pp. 159–238.
- Jones, C.G., Hare, J.D., Compton, S.J., 1989. Measuring plant protein with the Bradford Assay. *Journal of Chemical Ecology* 15, 979–992.
- Katterer, T., Andren, O., 1999. Growth dynamics of reed canarygrass (*Phalaris arundinacea* L.) and its allocation of biomass and nitrogen below ground in a field receiving daily irrigation and fertilization. *Nutrient Cycling in Agroecosystems* 54, 21–29.
- Katterer, T., Andren, O., Pettersson, R., 1998. Growth and nitrogen dynamics of reed canarygrass (*Phalaris arundinacea* L.) subjected to daily fertilization and irrigation in the field. *Field Crops Research* 55, 153–164.
- Kercher, S.M., Zedler, J.B., 2004. Flood tolerance in wetland angiosperms: a comparison of invasive and noninvasive species. *Aquatic Botany* 80, 89–102.
- Kercher, S.M., Herr-Turoff, A., Zedler, J.B., 2007. Understanding invasion as a process: the case of *Phalaris arundinacea* in wet prairies. *Biological Invasions* 9, 657–665.
- Kirk, R.E., 1995. *Experimental Design: Procedures for the Behavioral Sciences*. Brooks/Cole Publishing, Pacific Grove.
- Klimes, L., Klimesova, J., Hendricks, R., van Groenendael, J., 1997. Clonal plant architecture: a comparative analysis of form and function. In: de Kroon, H., van Groenendael, J. (Eds.), *The Ecology and Evolution of Clonal Plants*. Backhuys Publishers, Leiden, pp. 1–29.
- Lautenschlager, R.A., 1999. Environmental resources interactions affect red raspberry growth and its competition with white spruce. *Canadian Journal of Forest Research* 29, 906–916.
- Lavergne, S., Molofsky, J., 2004. Reed canary grass (*Phalaris arundinacea*) as a biological model in the study of plant invasions. *Critical Reviews in Plant Sciences* 23, 415–429.
- Lavergne, S., Molofsky, J., 2007. Increased genetic variation and evolutionary potential drive the success of an invasive grass. *Proceedings of the National Academy of Sciences of the USA* 104, 3883–3888.
- Lemaire, G., Millard, P., 1999. An ecophysiological approach to modeling resource fluxes in competing plants. *Journal of Experimental Botany* 50, 15–28.
- Lindig-Cisneros, R., Zedler, J.B., 2002. *Phalaris arundinacea* seedling establishment: effects of canopy complexity in fen, mesocosm and restoration experiments. *Canadian Journal of Botany* 80, 617–624.
- Lockwood, J.L., Cassey, P., Blackburn, T., 2005. The role of propagule pressure in explaining species invasions. *Trends in Ecology and Evolution* 20, 223–228.
- Magee, T.K., Kentula, M.E., 2005. Responses of wetland plant species to hydrologic conditions. *Wetlands Ecology and Management* 13, 163–181.
- Mahaney, W.M., Wardrop, D.H., Brooks, R.P., 2004. Impacts of sedimentation and nitrogen enrichment on wetland plant community development. *Plant Ecology* 175, 227–243.
- Mal, T.K., Lovett-Doust, J., 2005. Phenotypic plasticity in vegetative and reproductive traits in an invasive weed, *Lythrum salicaria* (Lythraceae), in response to soil moisture. *American Journal of Botany* 92, 819–825.
- Martina, J.P., von Ende, C.N., 2008. Correlation of soil nutrient characteristics and reed canarygrass (*Phalaris arundinacea*: Poaceae) abundance in northern Illinois (USA). *The American Midland Naturalist* 160, 430–437.
- Matthews, J.W., Peralta, A.L., Soni, A., Baldwin, P., Kent, A.D., Endress, A.G., 2009. Local and landscape correlates on non-native species invasions in restored wetlands. *Ecography* 32, 1031–1039.
- Maurer, D.A., Zedler, J.B., 2002. Differential invasion of a wetland grass explained by tests of nutrients and light availability on establishment and clonal growth. *Oecologia* 131, 279–288.
- Meekins, J.F., McCarthy, B.C., 2000. Responses of the biennial forest herb *Alliaria petiolata* to variation in population density, nutrient addition and light availability. *Journal of Ecology* 88, 447–463.
- Merigliano, M.F., Lesica, P., 1998. The native status of reed canary grass (*Phalaris arundinacea*, L.) in the inland Northwest, USA. *Natural Areas Journal* 18, 223–230.

- Milliken, G.A., Johnson, D.E., 2009. Analysis of Messy Data Volume 1: Designed Experiments. Chapman and Hall/CRC, Boca Raton, FL.
- Moles, A.T., Gruber, M.A.M., Bonser, S.P., 2008. A new framework for predicting invasive plant species. *Journal of Ecology* 96, 13–17.
- Molina-Montenegro, M.A., Penuelas, J., Munne-Bosch, S., Sardans, J., 2012. Higher plasticity in ecophysiological traits enhances the performance and invasion success of *Taraxacum officinale* (dandelion) in alpine environments. *Biological Invasions* 14, 21–33.
- Monaco, T.A., Johnson, D.A., Creech, J.E., 2005. Morphological and physiological responses of the invasive weed *Isatis tinctoria* to contrasting light, soil-nitrogen and water. *Weed Research* 45, 460–466.
- Morrison, S.L., Molofsky, J., 1998. Effects of genotypes, soil moisture, and competition on the growth of an invasive grass, *Phalaris arundinacea* (reed canary grass). *Canadian Journal of Botany* 76, 1939–1946.
- Neely, R.K., Baker, J.L., 1989. Nitrogen and phosphorus dynamics and the fate of agricultural runoff in Northern Prairie Wetlands. In: van der Valk, A.G. (Ed.), *Northern Prairie Wetlands*. Iowa State University Press, Ames, IA, pp. 92–131.
- Peperkorn, R., Werner, C., Beyschlag, W., 2005. Phenotypic plasticity of an invasive acacia versus two native Mediterranean species. *Functional Plant Biology* 32, 933–944.
- Perry, L.G., Galatowitsch, S.M., 2003. A test of two annual cover crops for controlling *Phalaris arundinacea* invasion in restored sedge meadow wetlands. *Restoration Ecology* 11, 297–307.
- Perry, L.G., Galatowitsch, S.M., 2004. The influence of light availability on competition between *Phalaris arundinacea* and a native wetland sedge. *Plant Ecology* 170, 73–81.
- Perry, L.G., Galatowitsch, S.M., Rosen, C.L., 2004. Competitive control of invasive vegetation: a native wetland sedge suppresses *Phalaris arundinacea* in carbon-enriched soil. *Journal of Applied Ecology* 41, 151–162.
- Pimentel, D., Zuniga, R., Morrison, D., 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecological Economics* 52, 273–288.
- Pimentel, D., Lach, L., Zuniga, R., Morrison, D., 2000. Environmental and economic costs of nonindigenous species in the United States. *BioScience* 50, 53–65.
- Pottier, J., Evette, A., 2011. Spatial pattern and process at the plant neighborhood scale: insights from communities dominated by the clonal grass *Elymus repens* (L.) Gould. *Journal of Vegetation Science* 22, 973–983.
- Pysek, P., 1997. Clonality and plant invasions: can a trait make a difference? In: de Kroon, H., van Groenendael, J. (Eds.), *The Ecology and Evolution of Clonal Plants*. Backhuys, Leiden, pp. 405–427.
- Rice, J.S., Pinkerton, B.W., 1993. Reed canarygrass survival under cyclic inundation. *Journal of Soil and Water Conservation* 48, 132–135.
- Richards, C.L., Bossdorf, O., Muth, N.Z., Gurevitch, J., Pigliucci, M., 2006. Jack of all trades, master of some? On the role of phenotypic plasticity in plant invasions. *Ecology Letters* 9, 981–993.
- Saarinen, T., 1998. Internal C:N balance and biomass partitioning of *Carex rostrata* grown at three levels of nitrogen supply. *Canadian Journal of Botany* 76, 762–768.
- Savage, V.M., Webb, C.T., Norberg, J., 2007. A general multi-trait framework for studying the effects of biodiversity on ecosystem functioning. *Journal of Theoretical Biology* 247, 213–229.
- Scheiner, S.M., 1993. Genetics and evolution of phenotypic plasticity in plants. *Annual Review of Ecology, Evolution, and Systematics* 17, 667–693.
- Scheiner, S.M., 2001. MANOVA multiple response variables and multispecies interactions. In: Scheiner, S.M., Gurevitch, J. (Eds.), *Design and Analysis of Ecological Experiments*, 2nd ed. Oxford University Press, Oxford, pp. 99–115.
- Schlaepfer, D.R., Glatli, M., Fischer, M., van Kleunen, M., 2010. A multi-species experiment in their native range indicates pre-adaptation of invasive alien plant species. *New Phytologist* 185, 1087–1099.
- Seabloom, E.W., van der Valk, A.G., 2003. Plant diversity, composition, and invasion of restored and natural prairie pothole wetlands: implications for restoration. *Wetlands* 23, 1–12.
- Sher, A.A., Hyatt, L.A., 1999. The disturbed resource-flux invasion matrix: a new framework for patterns of plant invasion. *Biological Invasions* 1, 107–114.
- Spyreas, G., Wilm, B.M., Plocher, A.E., Ketzner, D.M., Matthews, J.W., Ellis, J.L., Heske, E.J., 2010. Biological consequences of invasion by reed canary grass (*Phalaris arundinacea*). *Biological Invasions* 12, 1253–1267.
- Sultan, S.E., 2001. Phenotypic plasticity for fitness components in *Polygonum* species of contrasting ecological breadth. *Ecology* 82, 328–343.
- Thompson, J.D., 1991. The biology of an invasive plant. *Bioscience* 41, 393–401.
- U.S. Department of Agriculture, Natural Resources Conservation Service 2010. PLANTS Database, [Online]. Available: <http://plants.usda.gov/>, accessed 2 February 2012.
- Valladares, F., Martinez-Ferri, E., Balaguer, L., Perez-Corona, E., Manrique, E., 2000a. Low leaf-level response to light and nutrients in Mediterranean evergreen oaks: a conservative resource-use strategy. *New Phytologist* 148, 79–91.
- Valladares, F., Wright, S.J., Lasso, E., Kitajima, K., Pearcy, R.W., 2000b. Plastic phenotypic response to light of 16 congeneric shrubs from a Panamanian rainforest. *Ecology* 81, 1925–1936.
- Valladares, F., Dobarro, I., Sanchez-Gomez, D., Pearcy, R.W., 2005. Photoinhibition and drought in Mediterranean woody saplings: scaling effects and interactions in sun and shade phenotypes. *Journal of Experimental Botany* 56, 483–494.
- Valladares, F., Sanchez-Gomez, D., Zavala, M.A., 2006. Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. *Journal of Ecology* 94, 1103–1116.
- van Kleunen, M., Weber, E., Fischer, M., 2010. A meta-analysis of trait differences between invasive and non-invasive species. *Ecology Letters* 13, 235–245.
- van Kleunen, M., Dawson, W., Dostal, P., 2011. Research on invasive-plant traits tells us a lot. *Trends in Ecology and Evolution* 26, 317.
- Verardo, D.J., Froelich, P.N., McIntyre, A., 1990. Determination of organic carbon and nitrogen in marine sediments using the Carlo Erba NA-1500 Analyzer. *Deep Sea Research* 37, 157–165.
- Vitasse, Y., Bresson, C.C., Kremer, A., Michalet, R., Delzon, S., 2010. Quantifying phenological plasticity to temperature in two temperate tree species. *Functional Ecology* 24, 1211–1218.
- Wetzel, P.R., van der Valk, A.G., 1998. Effects of nutrient and soil moisture on competition between *Carex stricta*, *Phalaris arundinacea*, and *Typha latifolia*. *Plant Ecology* 138, 179–190.
- Wintermans, J.F.G.M., De Mott, A., 1965. Spectrophotometric characteristics of chlorophyll and their pheophytins in ethanol. *Biochimica et Biophysica Acta* 109, 448–453.
- Zar, J., 1999. *Biostatistical Analysis*, 4th ed. Prentice Hall, New Jersey.
- Zedler, J.B., Kercher, S., 2004. Causes and consequences of invasive plants in wetlands: opportunities, opportunists, and outcomes. *Critical Reviews in Plant Science* 23, 431–452.