Biol Invasions (2013) 15:1819–1832
DOI 10.1007/s10530-013-0411-5

ORIGINAL PAPER

Leaf litter variation influences invasion dynamics in the invasive wetland grass *Phalaris arundinacea*

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Received: 5 August 2011 / Accepted: 3 January 2013 / Published online: 23 January 2013

Abstract High litter mass is hypothesized to produce an invader-directed invasion by changing ecosystem properties such as nutrient cycling rates and light availability. An invasive plant species that stimulates litter accumulation may induce a positive feedback when it benefits from high litter conditions. *Phalaris arundinacea* is an invasive wetland grass that may induce positive litter feedback, as it produces abundant litter that varies in quality due to a wide range of foliar C:N content. In this study we investigated the range of growth responses within native and invasive genotypes of *Phalaris* that varied in initial foliar C:N levels (high C:N content was present in the invasive genotypes) when grown under varying litter mass. Overwintering with high litter reduced establishing tiller survivorship and the presence of litter delayed tiller emergence by 2 weeks. Overall, genotypes exhibited high trait plasticity in response to litter. Our results indicate that high litter mass can stimulate *Phalaris* growth, specifically for the genotypes with high initial C:N foliar tissue. Additionally, genotypes with initially high C:N ratios exhibited plastic responses consistent with a Master-of-some strategy indicating that their performance under high litter may depend upon the nutrient conditions under which they are grown. This study provides evidence for conditions that may lead to a positive feedback in *Phalaris*' introduced range. Future studies should investigate how changing litter quantity alters nutrient cycling and competitor growth.

Keywords C:N ratio · Ecosystem engineer · Intraspecific variation · Invasion · Leaf litter · *Phalaris* · Positive feedbacks · Reed canary grass · Wetland

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Introduction

One of the key challenges in invasive plant ecology is to predict which introduced species are most likely to become invasive and have large impacts on the ecosystems invaded (Hulme et al. 2011; Simberloff et al. 2011; Thompson and Davis 2011; van Kleunen et al. 2011). Among the invaders that will have the largest impacts are those that directly modify ecosystems (i.e. ecosystem engineers), including cascading effects on the native community (Crooks 2002; Cuddington and Hastings 2004). If an invader-directed

change leads to habitat conditions that promote its own growth and/or suppresses the native community, the invasive plant species has produced a positive feedback that may be difficult to reverse (Brooks et al. 2004; Ehrenfeld 2010). If the invasive species' environmental alterations are extensive, the native species may no longer be able to compete or survive within the modified ecosystem (Mack and D'Antonio 1998; Ehrenfeld 2003), reducing biodiversity (Zedler and Kercher 2004).

Simple changes in litter dynamics by ecosystem engineers can result in direct effects on both the physical and biological systems of an ecosystem (Treseder and Vitousek 2001; Ehrenfeld 2003). Litter can play a central role for invasive macrophyte establishment and dominance (Holdredge and Bertness 2011) via competition suppression (Minchinton et al. 2006) and promotion of its own growth (Farrer and Goldberg 2009). The establishment of a new plant species can cause shifts in litter production (quantity), and indirectly affect the strength of plant–plant interactions (Ehrenfeld 2003; Farrer and Goldberg 2009). For example, a new species with high litter production can limit the quantity and quality of light, as well as reduce the soil-seed contact for establishing species growth (Facelli and Pickett 1991). Additional litter mass may also be a source of nutrients if freed through decomposition and the timing of when the nutrients become available may impact which species is successful (Zedler 2009).

A new species' litter can also vary by composition (quality) compared to the native ecosystem, and ecosystem engineers have been shown to shift nutrient cycling within an ecosystem due to alterations of litter chemistry and physical properties (see Wedin and Tilman 1990; Van Vuuren et al. 1992; Ehrenfeld et al. 2005; Zedler 2009). Litter with low carbon to nitrogen ratios (C:N) has higher decomposition rates; senesced biomass with high available N is consumed rapidly by detritivores resulting in thin litter cover but high available nutrients for plant uptake (Hobbie 1992; Allison and Vitousek 2004). This positive feedback has been demonstrated in invaded ecosystems (Cameron and Spencer 1989; Ehrenfeld et al. 2001), where high N litter composition modified the microbe community. In contrast, poor quality litter with high C:N results in slow nutrient cycling due to a linear decrease in N mineralization (Hefting et al. 2005) and decomposition rates (Hobbie 1992; Liao et al. 2008), causing high litter mass and possibly suppression of native competitors (Zedler and Kercher 2004; Hovstad and Ohlson 2008; Farrer and Goldberg 2009).

The invasive wetland grass, *Phalaris arundinacea* (*Phalaris* hereafter), can form monocultures and has considerable litter production in its introduced range (Lesica 1997). However, despite its dominance, field studies have shown that invasive *Phalaris* individuals are poor competitors relative to native co-occurring species (Morrison and Molofsky 1998; Morrison 2002). *Phalaris* performance varies by genotype however, as demonstrated by Lavergne and Molofsky (2007) in a greenhouse common garden study where invasive *Phalaris* genotypes emerged earlier, produced more tillers and had greater final biomass compared to native *Phalaris* genotypes. Furthermore, analysis of the same genotypes in greenhouse conditions show the invasive genotypes have a wider range of foliar C:N ratios, which are 12 % higher on average compared to the native *Phalaris* genotypes due to lower N content (Fig. 1; Molofsky et al. unpub. data, also see Eppinga et al. 2011). These higher foliar C:N ratios could be responsible for altering ecosystem properties and led Eppinga et al. (2011) to hypothesize that evolutionary shifts leading to higher C:N litter could induce a positive litter feedback and a critical ecosystem transition from a native-dominated

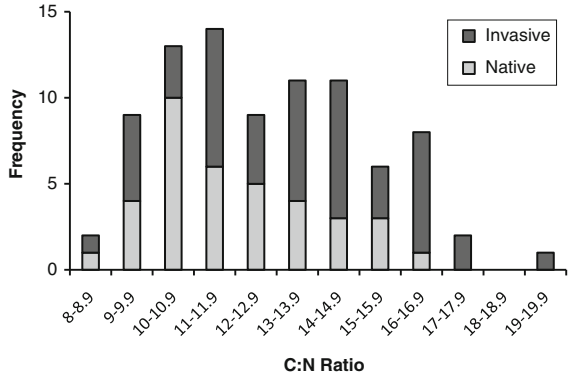

Fig. 1 Introduced *Phalaris arundinacea* genotypes exhibited a wider and higher range of C:N values compared to native (European) genotypes ($F_{1,\ 84} = 7.37$, $p = 0.008$). The analysis was conducted on newly formed foliar tissue from the *Phalaris genotype collection* at the University of Vermont (86 total genotypes: 49 invasive and 37 native) representing the European and North American origin centers and margins (Lavergne and Molofsky 2007). All plants were grown under uniform greenhouse conditions

low-litter wetland toward an invader dominated high-litter ecosystem state (assuming N is limiting to decomposition). Occurrence of such a litter feedback under field conditions, however, would require that high C:N genotypes perform better under high litter conditions. Field evidence is still lacking that this requirement can be fulfilled.

In this study we assessed the range of response of *Phalaris* genotypes to litter application. More specifically, using a common garden study in the field, we examined the plasticity in trait response of *Phalaris* to varying levels of litter mass deposition. We hypothesized that invasive genotypes show a broader range of survival and growth responses to litter than native ones, with the genotypes known to exhibit high leaf tissue C:N ratios performing better than the genotypes known to exhibit low leaf tissue C:N ratios.

Methods

Study species

Reed canary grass, *Phalaris arundinacea* (L.), is a circumboreal, cool season C_3 wetland grass. *Phalaris* spreads via seeds and asexually through dense rhizomes and tillers, which can fragment and produce new individuals. Individual culms can grow to 2 m in height. Starting in 1850, Eurasian *Phalaris* was routinely introduced to the US for wet pasture forage, soil erosion control and wastewater treatment (Apfelbaum and Sams 1987; Lavergne and Molofsky 2004). In the introduced range, *Phalaris* forms large monotypic stands, displacing native plant communities (Barnes 1999) and altering water and sediment movement (Lavergne and Molofsky 2006).

Experimental design

Phalaris genotype collection

Genotypes used in the following experiments were selected from the *Phalaris genotype collection* maintained at the University of Vermont. The genotypes had been previously identified via allozyme analysis (Lavergne and Molofsky 2007) and included 49 invasive and 37 native specimens. The genotype collection had representatives from the species native range center (Czech Republic, 49°00′N, 14°46′E, n = 23) and margin (France, 43°37′N, 3°52′E, n = 14), as well as the invasive range center (Vermont, 44°28′N, 73°9′W, n = 23) and margin (North Carolina, 35°19′N, 83°38′W, n = 26).

Common garden experiment

The aim of the *Common garden experiment* was to determine how the quantity of litter affects survivorship and growth of *Phalaris* genotypes that differ in their foliar C:N content (when grown under the same conditions). Specifically, we selected 16 genotypes that exhibited the extreme ranges of foliar C:N (Fig. 1; Molofsky et al. unpub. data, see Eppinga et al. 2011): four native origin genotypes with low C:N from France and the Czech Republic (C:N values between 8.9 and 10.1), as well as twelve invasive (introduced origin) genotypes from North Carolina and Vermont—four with low C:N (8.8–9.5) and eight with high C:N (16.4–19.3). C and N content measurements were determined from 25 mg ground and dried (60 °C for 2 days) new foliar tissue samples using a dynamic flash combustion technique (Flash EA 1112 NC Soil Analyzer, CE Elantech, Lakewood, NJ).

We vegetatively propagated clones of each genotype prior to planting in the field (Collins et al. 2010). Replicates were generated via tiller cuttings from existing genotype collection, applying Hormodin® 1 (OHP, Mainland, PA) rooting hormone to each node, and covering tillers with saturated Pro-Mix® BX (Premier, Rivière-du-Loup, QC) soil for 6–8 weeks under spring greenhouse conditions (22–26 °C day/16–20 °C night with 12 h days). Prior to planting, new plants were standardized to 2 green leaves, 10 cm of stem, 5 cm of fine root and 2 cm of rhizome (with 1 developing bud). Using eight replicates per treatment in a full-factorial design, we planted 384 tillers (16 genotypes × 3 litter treatments × 8 replicates) on June 16, 2009 in a wetland (UVM Biological Research Complex, Burlington, VT, 44°27′N, 73°11′W). Each tiller was randomly planted in a 25 cm by 25 cm (1/16 m^2) plot from which all other vegetation was removed. Each plot was separated by 1.5 m to ensure there was no interaction between neighboring plots. Tillers were watered for 2 weeks as needed and weeded monthly. After 2 weeks, any dead tillers were replaced.

Field measurements and litter application

At the end of the growing season (September 23–25, 2009), survivorship, maximum stem height, and leaf number of the tallest tiller was recorded for each replicate. Litter was applied to the replicates at three levels: 0, 52.7, and 106.0 g per 1/16 m^2 using dried *Phalaris* field litter. The 0 g per 1/16 m^2 served as the control. The applied litter levels matched surveyed monotypic *Phalaris* stands levels (± 2 S.E. about the mean) neighboring the wetland site. Specifically, we harvested the aboveground biomass (living and dead) of ten replicate 1/16 m^2 quadrates from two different stands on September 16, 2009 (five from each). This and additional harvested litter was homogenized, dried at 60 °C for 2 days prior to field application. A 1/4 m^2 plastic netting with 1 cm mesh was staked down upon every replicate to maintain litter treatment levels (before the start of the growing season, the center 15 cm of the netting was cut out and removed to avoid blocking developing tillers but still keeping the litter in place). A large netting size was used to permit a wide range of detritivores access (Killham and Wainwright 1981; Armbrecht et al. 2004).

Over the 2010 growing season, we determined the emergence date (when the first tissue developed) and monthly measurements (tiller number, maximum stem height and number of leaves upon that stem) through September 15, 2010. At the end of the growing season (October 4–6, 2010), we harvested above and below ground biomass for surviving individuals. The root biomass was washed, then all biomass was dried (60 °C for 2 days) prior to weighing.

Statistical analysis

To determine the effect of litter treatments (X) on replicate survivorship (Y) at the beginning and end of growing seasons, we used a log-likehood ratio test. Differences in replicate emergence date (Y) between litter treatments (X) could not be analyzed using parametric methods—no transformation compensated for the non-normally distributed data. Therefore, we turned to non-parametric analyses treating the litter treatment as an ordinal variable, using Kendall's robust line-fit method (Sokal and Rohlf 1995) with MATLAB v.7.4.0.287.

Differences in growth rate (Y; height, number of leaves or tillers) over the 2010 growing season between litter treatments (X_1), origin (X_2), C:N (X_3; nested within X_2), or their interaction, were determined through repeated-measures ANCOVA using Greenhouse-Geisser Epsilon to correct sphericity and final 2009 tiller counts as the covariate to remove replicate variation prior to litter application using JMP v.9.0.0.

Differences in final growth measurements (Y) between treatments (see X_i above) were assessed using ANCOVA, with final 2009 tiller count used as the covariate to remove replicate variation prior to litter application. A Box-Cox transformation was used when needed to meet normality assumptions (tested with a Shapiro–Wilk *W* test and noted in Table 1) using JMP v.9.0.0. When appropriate, the back-transformed means and ±1 S.E. are presented.

Litter Response Contrasts (LRC)

LRC by treatment

To better compare final replicate responses to litter, a Litter Response Contrast (LRC; Eq. 1) was utilized in two population *t* tests between the applied litter treatments (X_1; low or high), parsed by C:N content (X_2; low or high) and origin (X_3; native or invasive). Mean LRC values and standard errors were generated by treatment through the construction of bootstrap replicates sampling litter treatment–control pairs (with replacement) from the original data (Efron and Tibshirani 1993; n = 50,000 bootstrap replicates per treatment). We used the random permutation function as implemented in MATLAB v.7.4.0.287 to construct these bootstrap replicates. The Litter Response Contrast reads in equation form:

$$\mathrm{LRC} = \frac{[Yi_{\mathrm{Litter}} - (\beta i * (Zi - \bar{Z}i)] - [Yi_{\mathrm{No}} - (\beta i * (Zi - \bar{Z}i)]}{[Yi_{\mathrm{Litter}} - (\beta i * (Zi - \bar{Z}i)] + [Yi_{\mathrm{No}} - (\beta i * (Zi - \bar{Z}i)]} \quad (1)$$

This LRC is a dimensionless index for the effect of litter on treatment replicates (differences in replicate responses between litter treatments, Yi_{Litter}, and no litter controls, Yi_{No}). Values range from −1 to 1, with positive values indicating a positive effect. Previous work has shown this index has strong statistical and mathematical properties and it has been used to compare net effects on plant performance (Armas et al. 2004;

Table 1 Effect of litter and design treatments on *Phalaris* growth responses

Variable	Error *df*	Litter (*df* = 2)	Origin (*df* = 1)	C:N[origin] (*df* = 2)	Litter × C:N[origin] (*df* = 3)
Tiller count	–	–	–	–	–
Leaf count	199	0.31[a]	0.79[a]η	1.72η	–
Maximum height	199	3.62**	2.86[a]*	2.43[a]	3.77[a]η**
Root biomass	202	0.19	1.39	4.80**	0.13
Shoot biomass	197	1.43	1.59	3.46*	0.46
Root:shoot ratio	195	1.70	0.48	0.07[a]	2.56
Total biomass	195	1.09	1.59	4.16**	0.11
Mass per tiller	195	3.86**	0.03	9.92**	0.01

F statistic values shown from ANCOVAs with significance indicated by asterisks (**$p < 0.05$, *$p < 0.1$). Previous year's final tiller counts were used as the covariate unless noted by η indicating final heights were used as the stronger correlate. Responses were Box Cox transformed when needed to achieve a normal distribution (with few exceptions: [a]No transformation needed; – denotes when no transformation achieved normality). Assumptions could not be met to perform parametric analyzes on tiller count. See Fig. 3 for norms of reaction of the litter × C:N[origin] treatments

Carvalho et al. 2010; Eppinga et al. 2010). The final replicate traits from September 2010 [Yi; number of tillers (Y_1), number of leaves (Y_2), maximum stem height (Y_3), root mass (Y_4), shoot mass (Y_5), root:shoot ratio (Y_6), total biomass (Y_7), and mass per tiller (Y_8)] were modified by the difference of a covariate (Zi; September 2009 tiller count or height) and its mean ($\bar{Z}i$) to remove replicate variation prior to litter application if a correlation was found, specifically using the covariate slope (β) with the strongest pairwise correlation for each Yi. Significant differences from no litter controls were determined when data frequency occurred above/below the control (i.e. 95 % confidence interval).

LRC by genotype

To further investigate how individual genotypes responded to litter, a LRC was again utilized to test between litter treatments, Yi_{Litter}, and no litter controls, Yi_{No}, drawing only from one genotype at a time opposed to pooling all replicates of the same treatment. The statistical approach itself, however, is the same as described in the *LRC by treatment* section.

We tallied the significant responses by treatment group to summarize the large number of *LRC by genotype* comparisons (16 genotypes × 2 litter treatments vs controls × 8 traits = 256 responses—16 responses due to low replication). Specifically, significant responses to litter were categorized as a positive or negative (where all positive responses of the eight final traits were associated with positive LRC values with the exception of the root:shoot ratio, where a negative value would typically be interpreted as a positive sign of fitness (Perry et al. 2004)). Using a contingency analysis, we tested for significant differences between the number of significant growth traits (+ or −) in each treatment group [litter treatments (X_1; low or high), C:N content (X_2; low or high) and origin (X_3; native or invasive)] in JMP v.9.0.0. Comparisons of origin were only done within the low C:N genotypes because high C:N genotypes did not occur in the native populations and we eliminated the high C:N genotypes to keep the number of replicates consistent between the native and invasive genotypes and remove the confounding factor of differences in C:N ratios.

Results

Common garden experiment

Survivorship, emergence and growth rates

The survival rate of *Phalaris* that overwintered with high litter (49 %) was lower than low litter (63 %) and no litter treatments (68 %; $\chi^2_{df=2,N=378} = 9.91$, $p = 0.007$). Plants with high litter continued to have lower survivorship through the end of the experiment (Fig. 2; $\chi^2_{df=2,N=378} = 10.59, p = 0.005$). Survivorship

did not vary by origin or C:N content. The presence of any litter also delayed springtime emergence—production of any aboveground tissue trailed controls by 14.2 ± 2.3 days (Kendall's tau = 0.42, $T_{N=22,582}$ = 9.56, $p < 0.0001$).

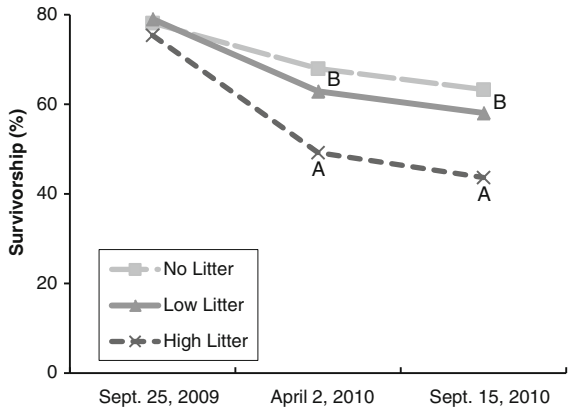

Fig. 2 Survivorship of *Phalaris* replicates was lower in high litter treatments after litter was applied in 2009. *Different letters* signify significant differences between treatments (Tukey–Kramer HSD, $\alpha > 0.05$)

Over the growing season, repeated-measures ANCOVA show that stems grew at a faster rate in high litter treatments ($F_{4.65, 456} = 3.29, p = 0.008$). In addition, the invasive genotypes grew faster than the natives ($F_{2.33, 456} = 5.25$, $p = 0.004$). The litter amount did not affect the rate of production for the number of leaves ($F_{4.60, 450} = 1.48, p = 0.198$) or the number of tillers ($F_{4.36, 433} = 1.42$, $p = 0.223$), and there were no significant interactions between the growth rates during the growing season and C:N ratio.

Final growth measurements

After a full growing season with litter, parametric analyses show that *Phalaris* leaf number and tiller number did not vary among litter treatments (see Table 1 for *F* values); however high litter replicates exhibited a wide range of responses (Fig. 3). Under high litter, plants produced 29 % (back-transformed ±1 S.E. = +20, −18) taller tillers compared to low litter treatments (Table 1), but did not differ from no litter controls. Additionally, plants in the high litter

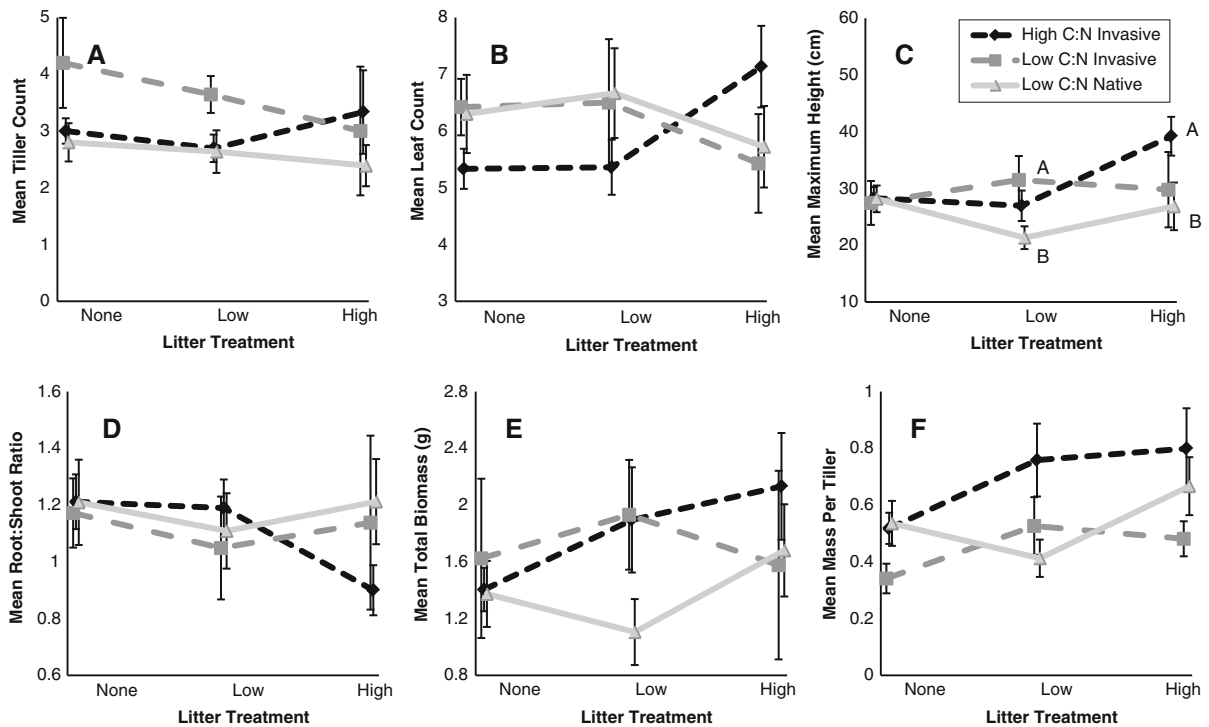

Fig. 3 Norms of reaction in final measured traits, separated by litter treatment, foliar C:N content and origin. **A** tiller count, **B** leaf count, **C** maximum stem height, **D** root:shoot ratio, **E** total biomass, and **F** mass per tiller (±1 S.E.). *Different letters* in Figure **C** signify significant differences between treatments (Tukey–Kramer HSD, $\alpha > 0.05$)

treatments had 19 % (+9, −8) greater mass per tiller than no litter controls (Table 1).

There were no significant differences in traits among origin types (Table 1), however, when parsing origin types by C:N traits, differences emerged between root biomass, total biomass and per tiller biomass (Table 1). Invasive plants with high C:N ratios had a 28 % (+15, −13) higher per tiller mass compared to their low C:N tissue counterparts, and a trend towards higher shoot biomass. Although there were limited significant differences when comparing the interaction of C:N[origin] and litter treatments, there was a consistent positive pattern of the invasive high C:N plants responding to increasing litter (Fig. 3). The interaction did show significant effects on maximum stem heights (Table 1; Fig. 3C), with invasive high C:N tillers under high litter and invasive low C:N tillers under low litter outperforming native genotypes on average by 56 % (+35, −28) and 26 % (+35, −28), respectively.

Litter Response Contrasts

LRC by treatment

Upon pooling individual values for group LRC trait comparisons, we discovered that low C:N content genotypes grown in high litter produced fewer tillers compared to controls (Fig. 4A; LRC estimates; tiller count; $p = 0.040$), while the effect was marginal in low litter ($p = 0.090$). The native genotypes (all low C:N) also had shorter stem heights than controls when grown in low litter (Fig. 4C; LRC estimates; height $p = 0.028$). In contrast, genotypes with high C:N content had increased growth traits compared to controls in the presence of low litter (Fig. 4F; LRC estimates; mass per tiller $p = 0.041$) and high litter (Fig. 4B–F; LRC estimates; leaf count $p = 0.022$, height $p = 0.007$, root:shoot $p = 0.014$, total biomass $p = 0.041$, mass per tiller $p = 0.026$, shoot biomass—not shown $p = 0.029$).

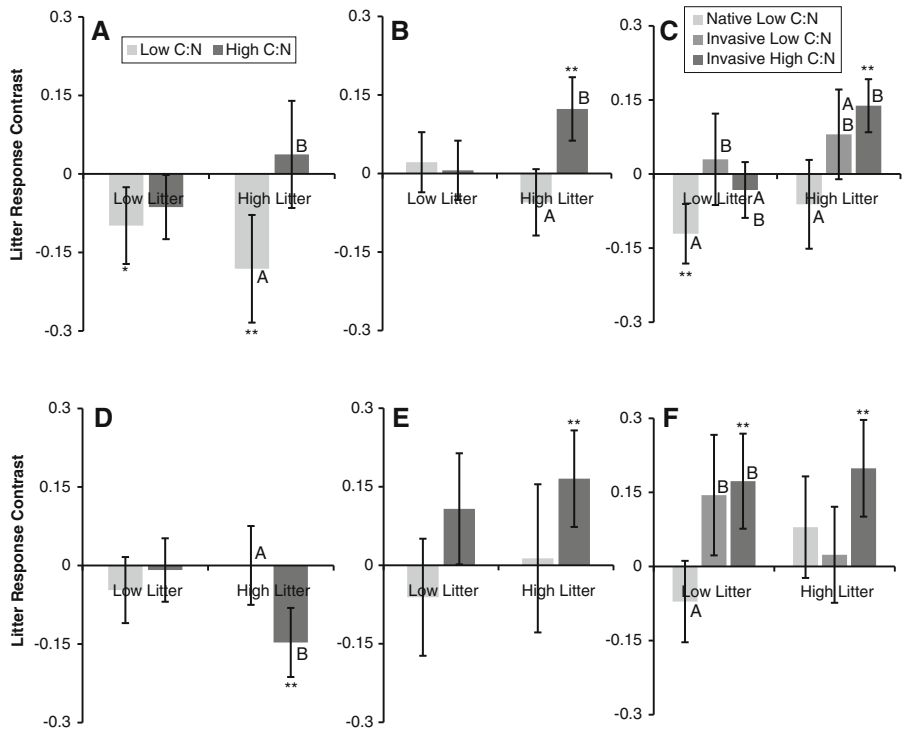

Fig. 4 Mean pairwise differences (LRC; Eq. 1) between litter treatments and no litter controls comparing values of: **A** tiller count, **B** leaf count, **C** maximum stem height, **D** root:shoot ratio, **E** total biomass, and **F** mass per tiller (±1 S.E.), separated by C:N group. Low C:N genotypes were split between origin (native or introduced) when their responses differed. Columns noted with *different letters* indicate significant differences between treatment contrasts. *Asterisks* indicate litter effects that are significantly different from controls (*$p < 0.1$; **$p < 0.05$)

High C:N genotypes had greater number of leaves compared to low C:N genotypes when grown in high litter (Fig. 4B; LRC estimates; $p = 0.023$) with additional growth traits trending towards higher growth levels in the high C:N genotypes (Fig. 4A, D; LRC estimates; tiller count $p = 0.069$; root:shoot $p = 0.074$; height—not shown $p = 0.063$). The effects of litter were further explained when low C:N genotypes were separated by origin. The mass per tiller of native genotypes was marginally reduced when grown under low litter compared to the low C:N invasive genotypes (Fig. 4F; LRC estimates; $p = 0.078$) but significantly reduced compared to high C:N invasive genotypes (Fig. 4F; LRC estimates; $p = 0.030$). Similarly, there was a trend towards low C:N native genotypes being shorter than low C:N invasive genotypes under low litter (Fig. 4C; $p = 0.092$) and were shorter compared to high C:N invasive genotypes under high litter (Fig. 4C; $p = 0.033$).

LRC by genotype

Although we did find consistent differences for the LRC by treatment, examining responses by individual genotypes show a wide range of variation in responses to litter. No one genotype shows positive responses (or negative responses) consistently across the range of final measured traits (see "Appendix"). Additionally, we summarized the 240 genotype responses to find differences in the frequency of significant LRC (Table 2). Under high litter, 11 % of high C:N genotype contrasts were positive, compared to only 4 % in the low C:N genotypes (Fischer's Exact Test; $p = 0.023$). In contrast, low C:N genotypes in high litter exhibited 17 % negative responses compared to only 3 % in the high C:N genotypes (Fischer's Exact Test; $p = 0.023$). In low litter, groups with the same low C:N content switched in response based on origin—native genotypes yielded more negative responses (22 %) compared to invasive genotypes (3 %) (Fischer's Exact Test; $p = 0.010$). All other genotype-level frequency comparisons from the contingency analysis did not yield significant differences.

Discussion

For *Phalaris* establishment, the presence of high litter suppresses overwintering survival (Fig. 2) and any litter delays emergence rates by 2 weeks. These results were unforeseen in previous modeling work by Eppinga et al. (2011) but are in line with the findings of Lenssen et al. (2000), which show the negative effects of litter (light/physical obstruction) on emergence and growth of *Phragmites australis*. Along with light, litter also buffers temperature (Facelli and Pickett 1991; Ehrenfeld et al. 2005). We estimate that the plants with litter were initially buffered from 4 % (121–130 GGD) of the 2010 VT growing season using Growing Degree Days (GDD; Adams and Galatowitsch 2006) as a

Table 2 Frequency of genotypes with significant responses compared to controls after one full year of litter application

Initial Foliar Tissue	Origin	Low Litter			High Litter		
		# of Responses	% Significant		# of Responses	% Significant	
Low C:N	Native	32	+3%	-22%	32	+6%	-13 %
	Invasive	32	+19%	-3%	16	+0%	-25%
	Combined	64	+22%	-13%	48	+4%	-17%
High C:N	Invasive	64	+8%	-5%	64	+11%	-3%
	Total	128	+9%	-9%	112	+8%	-9%

240 individual genotype LRC indices were tallied to assess significant responses against no litter controls (when 95 % of a CI occurred above/below the control; see "Methods" and "Appendix"). Positive growth responses of the eight final traits were associated with positive LRC values. The two grey boxes indicate contrasts where treatment trait responses had significant switches in direction (Fischer's Exact Test). Due to low surviving replicates, two genotypes (16 responses) were omitted from the high litter, low C:N, invasive treatment group

parameterization of heat accumulation over the growing season (base temperature = 0 °C, temperature values obtained from Burlington, VT NCDC). This negative litter effect seems especially inhibiting for *Phalaris*, as it gains its competitive advantage through rapid early growth, which allows it first access to newly freed nutrients and to establish a dominant position for light (Morrison and Molofsky 1998; Wetzel and Valk 1998). However, when competitors are growing in litter, one must consider that each plant has to overcome initial litter suppression and future studies should examine whether *Phalaris* is better adapted to emerge through litter than its competitors.

Although litter delayed emergence, plants in high litter still outperformed other treatments despite the shorter growing season—growing faster and ending with taller tillers and greater mass per tiller (Table 1). When the final growth traits were contrasted by treatment group, the high C:N invasive plants outperformed the native genotypes, especially when grown in high litter (Fig. 3), producing lower R:S ratios while keeping root masses constant (Fig. 4; Table 1), similar to *Phalaris* growth in high nutrient conditions (Wetzel and Valk 1998). Low C:N invasive genotypes only outperformed native genotypes in height and mass per tiller when growing under low litter (Fig. 4). The LRCs indicate that the high C:N plants may utilize available nutrients at a higher rate for the traits measured (Table 2; Fig. 4).

Invasive litter has been shown to improve growing conditions by increasing soil nutrient quality (Minchinton et al. 2006; Farrer and Goldberg 2009); and studies using invasive wetland plants such as *Typha* × *glauca* (Farrer and Goldberg 2009) and *Phragmites australis* (Holdredge and Bertness 2011) also showed that an invasive plant species will have positive growth in high levels of its own litter (Farrer and Goldberg 2009). Additionally, due to the stimulation of growth with high litter, the high C:N plants in our study maintain high litter production despite the lower survivorship from overwintering with high litter (if plants were considered a population).

Any variation in decomposition rate and N cycling could play a significant role in litter feedbacks, as decomposers cycle detritus more rapidly and could stimulate growth if N is limiting (Zedler 2009). Few studies have documented how decomposition rates vary by litter *quantity* in similar environments however. One may hypothesize that high litter mass may provide a more favorable environment for decomposers (e.g. high moisture, ample supply of food) allowing for increased rates of decomposition (Niklasch and Joergensen 2001). It is also unknown how recalcitrant litter will affect the ecosystem or litter decomposition in following years, and should be considered in future studies, as treatments with litter had recalcitrant litter at the end of the experiment that may act as a physical barrier to competitor growth (Facelli and Pickett 1991; Minchinton et al. 2006; Zedler 2009) and may increase the degree of nutrient retention in the detritosphere (Hefting et al. 2005).

Our study looked at the establishment of new tillers that were independent of the main stand over a short time-frame; however, in tillering perennials, spreading shoots receive energetic support via rhizomes (which may provide higher survivorship when under stress; Hartnett and Bazzaz 1983). Additionally, Collins et al. (2010) demonstrated the presence of like genotype neighbors promoted positive frequency-dependent interactions in *Phalaris*, which resulted in increased stem height and aboveground biomass production. Because of this, one might hypothesize that established plants growing in high litter could show stronger contrasts that support the positive litter feedback mechanism.

Many genotypes show strong shifts in response to litter levels changing—sometimes reversing the direction of phenotypic traits with added litter ("Appendix"). Although no one genotype had a positive response to litter in all measured traits, our results indicate that increased litter mass can stimulate *Phalaris* growth, specifically for the genotypes with high initial C:N foliar tissue (Fig. 4; Table 2). The greater aboveground biomass per amount of belowground biomass produced in the invasive high C:N genotypes may allow for greater litter production. While this study was not designed to investigate the presence of a positive litter feedback driving the invasion of *Phalaris* in North American wetlands, it does suggest that a number of necessary requirements for such a litter feedback are fulfilled in the field. More specifically, our study shows that there is phenotypic variation in biomass production and that C:N variation can lead to differential growth responses to litter deposition. Future studies should investigate whether a positive litter feedback mechanism is acting to promote *Phalaris* dominance in the introduced range

and how nutrients are released in systems with increased litter production.

Although we did not test the C:N of litter produced by our field-grown plantings, [N] has been shown to decline in foliar tissue consistently over growing seasons for two perennial grass species (Dohleman et al. 2012) and likely changes over the season in our genotypes. Another study (Eppinga and Molofsky 2012) using the same genotypes (grown under low nutrient greenhouse conditions) also noted the final C:N ratios can switch with environmental conditions. These studies indicate that the high C:N genotypes are phenotypically plastic and can perform better in an environment with high litter under conditions in which nutrients are plentiful, but the effect may only occur under specific nutrient conditions (like our field site; Collins et al. 2010). Thus, the high C:N genotypes appear to follow a Master-of-some strategy (sensu Richards et al. 2006).

To explain species performance, it is not necessarily the origin of the genotypes that matters, but rather the traits that individual genotype expresses (Levine et al. 2003; Cohen et al. 2011). Thus, if high C:N genotypes have faster stem growth rates under high litter, produce more total biomass under high litter and high C:N litter decomposes at a slower rate (Hobbie 1992; Liao et al. 2008), then we have the preconditions for high C:N genotypes to promote their own growth and potentially set up a positive feedback that may, under certain conditions, promote invasiveness.

A shift in a trait after a species is introduced to a new region, such as a change in its C:N ratio, may be required before an introduced plant species comes to dominate a community and may be one explanation for why certain species experience a lag phase before they become aggressive (Crooks and Soule 1999; Sakai et al. 2001). Identifying the causes of invasiveness is important for remediation and management (Jordan et al. 2008). The possibility of an invader-directed feedback mechanism points to the need to manage litter to control invasive wetland plants (Lavergne and Molofsky 2006; Zedler 2009; Holdredge and Bertness 2011; e.g. by burning to allow N volatilization; Sharrow and Wright 1977) as part of an ecosystem reengineering for natives (Suding et al. 2004; Yelenik and Levine 2010), as well as a need to limit the chance for individuals with novel traits to arise through immigration or through subsequent hybridization (Lavergne and Molofsky 2007; Simberloff 2011).

Acknowledgments Sincerest thanks to our colleagues at the Odum Conference 2009, Rensselaerville, NY, our reviewers, as well as K. Alley, D. Barrington, R. Collins, A. DeSenna, M. Harlacher, D. Ross, L. Schmitt, E. Sorel, S. Strella and H. Tobi, for their contributions throughout the project. This research was supported by a USDA Hatch and USDA-NRI 2006-03645 Grant awarded to J. Molofsky.

Appendix

See Fig. 5.

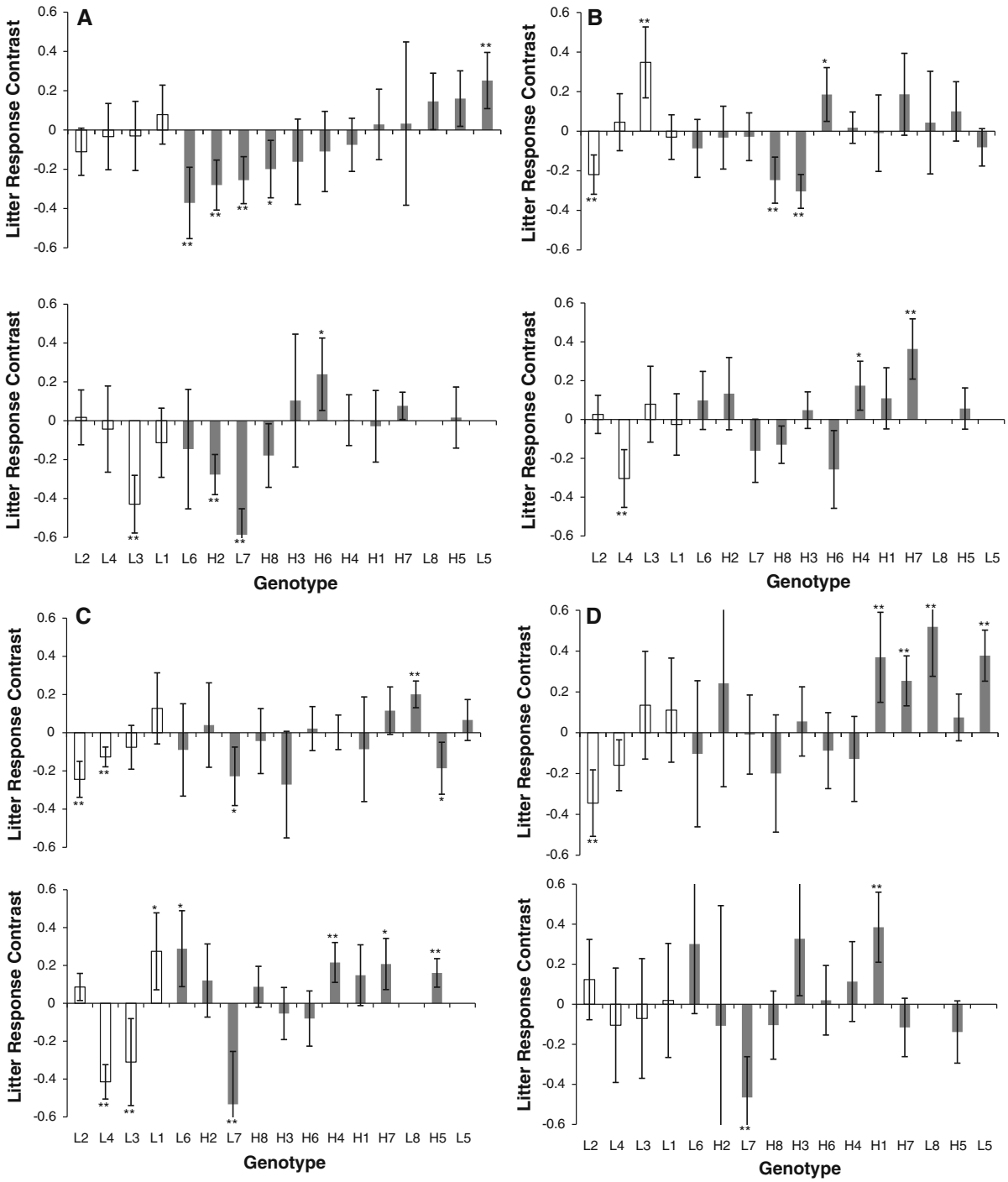

Fig. 5 Genotype mean differences (LRC; Eq. 1) between low litter-no litter treatments (*top figure*) or high litter-no litter treatments (*bottom figure*) for final measured traits: **A** tiller count, **B** leaf count, **C** maximum stem height, **D** root biomass, **E** shoot biomass, **F** root:shoot ratio, **G** total biomass, and **H** mass per tiller (±1 S.E). Genotypes are ranked uniformly (by the Figure **A** low litter-no litter tiller count LRC values). Native genotypes are on the *left side* (*hollow bars* numbered L1-4 for low C:N) while invasive genotypes are in *solid bars* numbered L5-8 (for low C:N) and H1-8 (for high C:N). *Asterisks* indicate litter effects that are significantly different from controls (*$p < 0.1$; **$p < 0.05$). Note Figure **E** has a larger *y*-axis range than the other figures. Due to low numbers of surviving replicates, two genotypes (#L5 and L8) were omitted in high litter figures

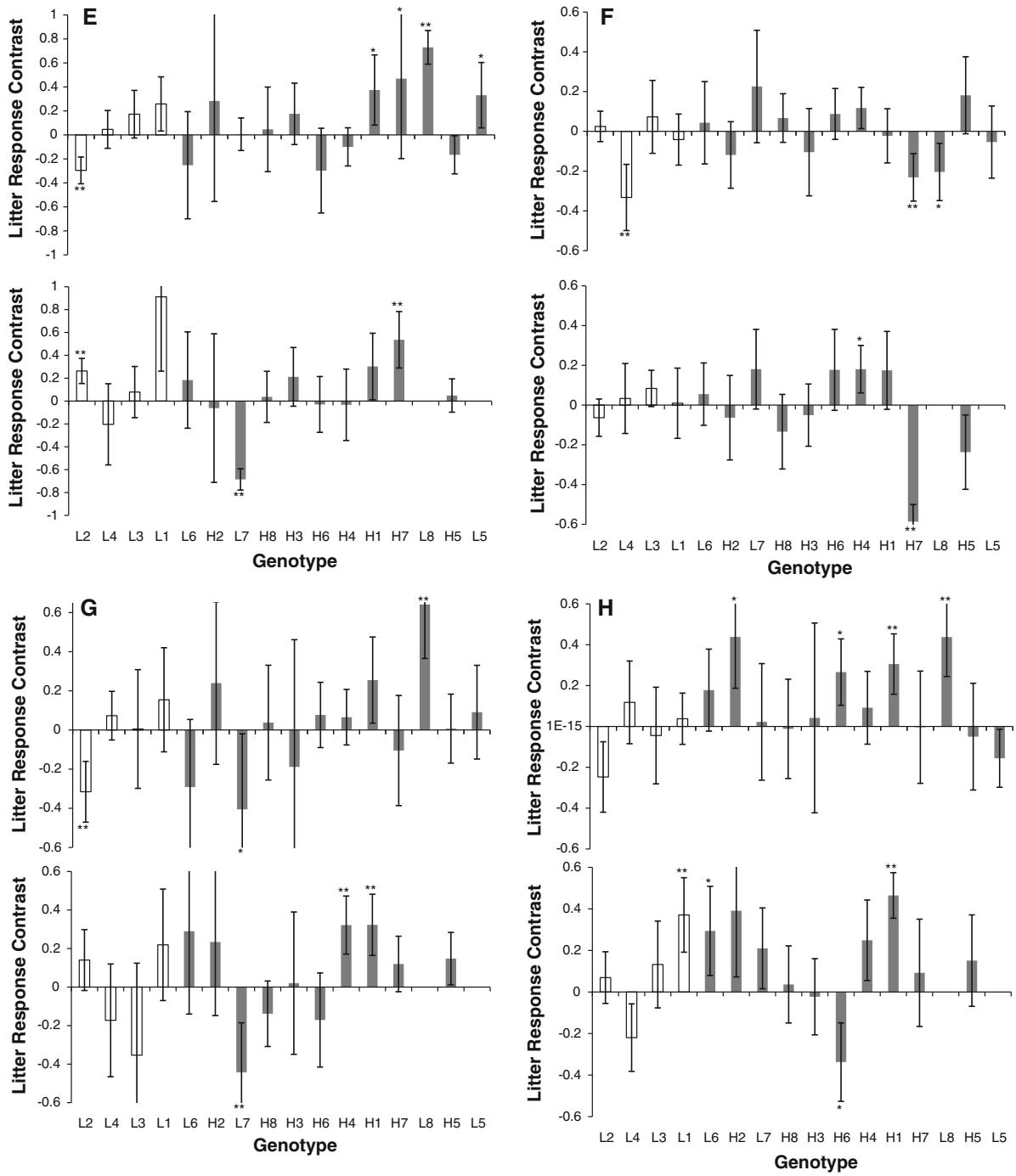

Fig. 5 continued

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