Research article

Eco-evolutionary litter feedback as a driver of exotic plant invasion

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Introduction

Successfully established exotic plants may actively modify the habitats in their new range, which induces cascading effects on ecosystem functioning (Jones et al., 1994; Brookes, 2002; Cuddington and Hastings, 2004; Cuddington et al., 2007). In this context, much attention has been paid to invasive plants affecting nutrient cycling, thereby changing the partitioning of carbon and nutrients between soil, litter and living biomass pools (Vitousek et al., 1987; Witkowski, 1991; Ehrenfeld, 2003). Many examples highlight the potential for invasive species to accelerate nutrient cycling, but invasive species may decelerate nutrient cycling as well (Liao et al., 2008; Ehrenfeld, 2010). Both acceleration and deceleration of nutrient cycles may induce a positive feedback in the invasion process if the net effect of the ecosystem change is beneficial to the invader itself (Hobbs, 1992; Allison and Vitousek, 2004; Eppinga et al., 2011).

Another line of research suggests that post-introduction evolution of exotic plants may contribute to their success (Müller-Schärer et al., 2004; Bossdorf et al., 2005; Strayer et al., 2006). Post-introduction evolution may be driven by altered selection pressures in the new range. Possible factors altering selection pressure include different environmental conditions (Sakai et al., 2001; Leger and Rice, 2007), release from natural enemies (Blossey and Nötzold, 1995; Blair and Wolfe, 2004), and lack of co-evolution with newly encountered competitors (Bais et al., 2003; Callaway and Ridenour, 2004). Post-introduction evolution may be more likely when there have been multiple introduction events of a species into the new range, resulting in admixture between subpopulations that are isolated from each other in the native range.
ity, dead, critically, result, availability reflected could feedback rates the invasive range better in their invasive range (Hierro et al., 2005). Importantly, the mechanisms described above may induce these evolutionary changes over very short timescales (Whitney and Gabler, 2008).

Until now, the role of post-introduction evolution has received little attention in empirical studies of invasive plants and nutrient cycling. Positive eco-evolutionary feedback would occur when a newly evolved genotype (from here referred to as ‘invasive genotype’) affects nutrient cycling in a way that creates more favorable conditions for this novel genotype than genotypes originating from the native range (from here referred to as ‘native genotypes’) or other plant species. Previous studies of eco-evolutionary feedback between organisms and resource dynamics have been mainly theoretical modeling exercises (Laland et al., 1999; Odling-Smee et al., 2003; Kylafis and Loreau, 2008). A notable exception, however, is a number of studies showing that there is a genetic basis for variation in tannin concentrations in the leaves of Populus spp. (Schweitzer et al., 2004), which may affect nutrient cycling in its native habitat (Schweitzer et al., 2005; Pregitzer et al., 2010; Smith et al., 2012). In this system, there may be positive eco-evolutionary feedback because the genotypes decelerating nutrient cycling show a tendency toward increased production of fine roots (Fischer et al., 2006). Theoretical modeling suggests that similar eco-evolutionary feedback could occur between invasive exotic plants and nutrient cycling (Eppinga et al., 2011), but empirical evidence for the latter is even more scarce.

A key trait, affecting nutrient cycling is the C:N ratio of the dead plant material becoming part of the litter or detritus pool (Hobbie, 1992; Hooper and Vitousek, 1998; Ehrenfeld, 2003). Although leaf tissue C:N ratio is strongly determined by nutrient availability (e.g. Hobbie, 1992), genotypic variation in uptake and allocation strategies can create differences in C:N ratio between genotypes at a given soil nutrient level. A previous model study suggested that post-introduction evolution toward higher C:N ratios could create a positive eco-evolutionary feedback in the invasion process (Eppinga et al., 2011). More specifically, genotypes with a higher leaf tissue C:N ratio could affect nutrient cycling by stimulating the accumulation of recalcitrant litter (e.g. Thormann et al., 1999), which may induce a positive eco-evolutionary feedback if genotypes with high C:N ratios also have higher growth rates under that litter. Although the main driver of trait variation seems to have been genetic recombination of previously isolated subpopulations (i.e., admixture; Lavergne and Molofsky, 2007), model analysis revealed that eco-evolutionary feedback could be a secondary feature exacerbating the invasion process (Eppinga et al., 2011). This model exercise yielded one prediction of how eco-evolutionary feedback would be reflected in field data, and two predictions of how eco-evolutionary feedback could be tested for experimentally (Eppinga et al., 2011). More specifically, occurrence of this eco-evolutionary feedback would be reflected by (see Appendix A for details): (1) field data showing higher litter:biomass ratios in the invasive range; (2) higher C:N genotypes benefiting more from experimental litter additions than low C:N genotypes; (3) this beneficial effect on high C:N genotypes inducing a critical transition toward an invader-dominated state when a critical amount of litter is added to a native species-dominated community that is growing under low nutrient conditions.

The aim of this study was to empirically test these three model predictions, thereby examining to what extent exotic plant invasion could be driven by eco-evolutionary feedback between leaf tissue C:N ratio and litter accumulation. For this test we used the wetland grass Phalaris arundinacea (Phalaris from here), which is invasive in North America. Previous studies revealed that Phalaris is a weak competitor for nutrients but a strong competitor for light. Because decomposition of litter releases nutrients but reduces light availability, the presence of litter may increase the competitiveness of Phalaris (Perry and Galatowitsch, 2004; Perry et al., 2004; Eppinga et al., 2011). Also, novel genotypes occurring in the invasive range have higher C:N ratios than native genotypes when grown under the same (high) nutrient and light conditions (Lavergne and Molofsky, 2007; Eppinga et al., 2011).

First, we compared field observations of litter:biomass ratios in Phalaris-dominated plots in native and invasive ranges. Based on previous model predictions, we hypothesized that litter:biomass ratios are higher in the invasive range (Fig. 1A and B). Second, we measured the response to litter of low C:N and high C:N genotypes when grown in isolation. A requirement for eco-evolutionary feedback would be that the high C:N genotypes respond more positively to litter (Fig. 1C and D). Third, we introduced low and high C:N Phalaris genotypes into native species-dominated mesocosms with low nutrient conditions, which were then exposed to varying litter levels (Fig. 1E and F). For these conditions, previous model predictions suggest that occurrence of an eco-evolutionary feedback would be reflected by similar growth of low and high C:N genotypes under low levels of litter, but better growth of high C:N genotypes under high levels of litter (Eppinga et al., 2011; Fig. 1E and F).

Material and methods

Study species

Phalaris arundinacea (reed canary grass, Poaceae, Phalaris from here) is a cool season (C₄) grass that is one of the most aggressive invaders of North American wetlands (Galatowitsch et al., 1999; Lavergne and Molofsky, 2004; Zedler and Kercher, 2004). Evidence is accumulating that the success of this species is at least partly due to post-introduction evolution, which has been stimulated through multiple introductions of disparate European populations (Lavergne and Molofsky, 2007). Multiple introductions have lead to novel genotypes of smaller genome size, forming invasive populations with high genetic variability and phenotypic plasticity (Lavergne and Molofsky, 2007; Lavergne et al., 2010). Previous experiments show that invasive genotypes have on average a higher growth rate and a higher leaf tissue C:N ratio than native genotypes when grown under the same nutrient and light conditions (as further explained in the description of the experimental design of the mesocosm experiment).

Field survey

Study areas

We studied four sites in Phalaris’ native range (South Bohemia, Czech Republic), and four areas in its invasive range (Vermont, USA). The four sites in the native range were all in the vicinity of the town of Třeboň: 1: Halámkyn 48°51’N, 14°54’E; 2: Krabonoš 48°48’N, 14°55’E; 3: Nová Hlína 49°02’N, 14°48’E; 4: Mokré Louky 49°01’N, 14°46’E). The climate in this region is sub-oceanic,
Fig. 1. Explanation of how the three hypotheses for the current study were derived from a previous theoretical model study (Eppinga et al., 2011, see Appendix A for details). (a) In field sites where Phalaris is present, eco-evolutionary feedback between Phalaris’ C:N ratio and litter accumulation may induce higher litter:biomass ratios. (b) Occurrence of this feedback would be reflected by higher litter:biomass ratios in invasive ranges than in the native range. Note that the colored dots in (a) correspond to the same-colored bars in (b). (c) High C:N genotypes respond more positively to litter. (d) The more positive response to litter of high C:N genotypes results in stronger positive litter feedback on high C:N genotypes. (e) Under low nutrient conditions, there may be a threshold in litter:biomass ratio above which high C:N Phalaris genotypes, but not low C:N genotypes, could induce a critical transition toward a Phalaris-dominated state. (f) This model prediction would be reflected by high C:N genotypes doing much better in high litter treatments as compared to all other treatments.

characterized by an average temperature of 7.4 °C, with monthly average temperatures ranging between −2.2 °C and 17.7 °C, and an average annual precipitation rate of 627 mm (Návratilová and Hájek, 2005; Káplová et al., 2011). The field sites can be characterized as floodplain sedge grasslands with organic but silty soils (Káplová et al., 2011). In all sites Phalaris was the dominant species, but co-occurring species were Carex acuta, Alopecurus pratensis and Deschampsia cespitosa (Káplová et al., 2011).

The four field sites in the invasive range were all in the vicinity of the town of Burlington (1: Botanical Research Complex of the University of Vermont 44°27’N, 73°11’W, 2: Shelburne Bay 44°24’N, 73°14’W 3: Ethan Allen Homestead 44°30’N, 73°14’W,
4: Chimney Corner 44° 34′N, 73° 09′W). The climate in this region is humid-continental, characterized by an average temperature of 7.3 °C, with monthly average temperatures ranging between −7.8 °C and 21.4 °C, and an average annual precipitation rate of 877 mm (Sisson and Gyakum, 2004). The field sites can be characterized as floodplain sedge grasslands. In all sites Phalaris was the dominant species, but co-occurring species were Carex stricta, Solidago canadensis and Phragmites australis.

Field sampling and measurements

Field sampling took place on 12–13 September 2010 in Vermont and 22–23 September 2010 in the Czech Republic. At each field site, we selected an area of approximately 1 ha, in which we established eight 50 × 50 cm plots within patches that were dominated by Phalaris. Plots were separated by at least 2 m. In each plot, we harvested aboveground biomass. Because harvests were undertaken near the end of the growing season, harvested aboveground biomass could be used as an estimate for the annual aboveground biomass production (Wassen et al., 2005; Eppinga et al., 2008). After the living aboveground biomass was removed, we harvested the litter that was present on the ground. To determine the dominance of Phalaris in the selected plots, both aboveground biomass and litter from other species were kept separately from Phalaris material. Other plant species only comprised a very small portion of aboveground biomass and litter in all plots (<10% in all plots in both ranges), and was not significantly different between native and invasive ranges (Mann–Whitney U test; U = 492, p = 0.78). This explains why results for litter/biomass ratios in native and invasive ranges did not depend on whether only Phalaris was considered or whether the pooled biomass and litter of all species was considered.

Statistical analysis of field survey

All variables were tested for normality using a Shapiro–Wilks W test. Because none of the variables was normally distributed, and sufficient normality could not be achieved by transformation, we turned to non-parametric analyses instead. More specifically, we tested for differences in litter density, biomass density and litter/biomass ratio between native and invasive ranges (4 × 8 = 32 plots per range) using Mann–Whitney U tests.

Mesocosm experiment

Experimental setup

To test our second hypothesis, low and high C:N genotypes of Phalaris were grown in mesocosms under four different levels of litter (no, low, medium, high; determination of specific litter amounts is explained below). To test our third hypothesis, we also performed the same litter treatments for the Phalaris genotypes that were introduced in mesocosms that were dominated (cover >90% in all mesocosms) by the native species Carex stricta. For each C:N strategy, six different genotypes were selected. Using a full-factorial design, this lead to a total of 2 (low/high C:N strategy) × 4 (litter levels) × 2 (isolation/competition) × 6 (genotypes per C:N strategy) = 96 mesocosms. All mesocosms and litter used in this experiment were taken from one field site in the invasive range that was also used in the field survey described above (Biological Research Complex of the University of Vermont).

We selected six low C:N genotypes and six high C:N genotypes from a collection of native and invasive genotypes maintained in the greenhouse at the University of Vermont (Lavergne and Molofsky, 2007). A total of 210 different genotypes has been collected from two populations in the native habitat (in France and in the Czech Republic) and from two populations in the invasive habitat (Vermont and North Carolina). For further details see Lavergne and Molofsky (2007). Leaf tissue C:N ratios for a subset of these genotypes has been measured in a previous experiment, in which 46 native and 56 invasive genotypes were grown under the same nutrient and light conditions (Molofsky et al. unpublished data; see also Eppinga et al., 2011). Observed C:N ratios and root:shoot ratios in this previous experiment suggest that nutrient availability was relatively high (see Appendix B for details). For the low C:N genotypes, we selected three native genotypes and three invasive genotypes, to ensure that potential differences in response to litter were due to differences in C:N strategy rather than other traits that may have been subject to evolutionary development (Blossey and Nötzold, 1995; Sakai et al., 2001; Bais et al., 2003; Lavergne and Molofsky, 2007). High C:N genotypes were all selected from the invasive range, because only invasive genotypes exhibited high C:N ratios in the previous experiment (Molofsky et al. unpublished data; see also Eppinga et al., 2011). In this previous experiment there was a substantial difference in C:N ratio between the low and high C:N genotypes (two sample t-test, t90 = 8.13, p < 0.001): the average C:N ratio (±1 s.e.) of the six low C:N genotypes was 10.3 (±0.3)g Cg N−1, whereas the average C:N ratio of the high C:N genotypes was 16.4 (±0.8)g Cg N−1. In another previous experiment (Lavergne and Molofsky, 2007), the same set of high C:N genotypes attained higher biomass after 11 weeks than the same set of low C:N genotypes (t92 = 2.83, p = 0.007): biomass of the high C:N genotypes was 33 (±1.0)g, whereas that of the low C:N genotypes was 29 (±1.3)g. This variation in biomass was due to variation in belowground biomass (One-way ANOVA, F1,42 = 6.60, p = 0.014), rather than aboveground biomass (One-way ANOVA, F1,42 = 0.007, p = 0.934). As the eco-evolutionary feedback encompasses production of calcitrant aboveground litter, these results suggest that a difference in C:N ratio is the key factor driving more positive responses of high C:N genotypes to litter (see Appendix A). For other traits we found no significant differences in leaf number (Mann–Whitney U test, U = 197, p = 0.28), tiller number (Mann–Whitney U test, U = 177, p = 0.13) and attained height (One-way ANOVA, F1,42 = 0.011, p = 0.917) between the high C:N and low C:N genotypes used in this study.

Each genotype was grown under all four litter treatments, with and without competition. Hence, eight identical individuals of each genotype were needed for the experiment. These eight identical individuals of each genotype were obtained by vegetative propagation of clones. For each genotype, we applied growth hormone (Hormodin® 1, OHP, Mainland, PA) to each node of a set of tiller cuttings. These tiller cuttings were subsequently grown for 6–8 weeks under spring greenhouse conditions (22–26 °C day/16–20 °C night with 12 h days) and watered when needed. Prior to planting, all individuals were standardized to 1 green leaf, 8 cm of stem, 5 cm of fine root and 2 cm of rhizome with 1 developing bud (following Collins et al., 2010).

Two Carex stricta-dominated patches were selected in the field, from which mesocosm cores of 15 cm diameter and 13 cm depth were taken. From the same two areas, soil from the upper 15 cm was taken to fill pots for the treatments in which Phalaris was grown in isolation. The standardized Phalaris individuals (see above) were subsequently planted in the mesocosm environment, using pots with a height of 15.9 cm and a volume of 3.6 L. The plants were grown for one week to overcome transplantation shock (following Collins et al., 2010), after which litter treatments were applied.

Levels of litter application were based upon an initial field survey (at the same site where mesocosms were harvested) of litter densities at the beginning of the growing season (April 2010). During this part of the growing season, litter density is relatively high, because little of last year’s biomass has decomposed during the previous winter. This thick litter layer is characteristic of the conditions under which new shoots have to establish. In eight 25 cm × 25 cm plots, we measured an average litter density of 920 g m−2, which
was then assigned to be the litter level of the medium litter treatment. The low litter treatment was set at two standard deviations below the observed mean litter density (320 g m$^{-2}$), whereas the high litter density treatment was set at two standard deviations above the mean observed density (1520 g m$^{-2}$). Litter used in the experiment was harvested in October 2009, subsequently dried and then stored until the start of the experiment in June 2010.

After litter application, the plants in the mesocosms were grown for 10 weeks (June–September 2010) under common garden conditions at the University of Vermont. To simulate wetland conditions, pots were subject to hypoxic conditions (following Perry et al., 2004). We used plastic tubs with a volume of 32 L, which could store six pots. Water levels were maintained at the same level as the soil surface in the pots (Perry et al., 2004). Once a week, tubs were flushed, meaning that all water was removed and replaced by nutrient-poor tap water. Usually, nutrients are added after flushing to maintain levels of nutrient availability (e.g. Kumar et al., 1995). In this experiment, however, we did not add nutrients after flushing, meaning that a fraction of the available dissolved nutrients has been washed out with each flushing event. Hence, nutrient availability inevitably decreased over the course of the experiment. The weekly flushing of tubs was also used to randomize all the pots over the different tubs in the experiment.

Due to the weekly flushing procedure, the current experiment was carried out under relatively low nutrient availability (see Appendix B for details). Under these conditions, nutrient availability is determined by the nutrient replenishment rate rather than the actual size of the dissolved nutrient pool (Binkley and Hart, 1989). As a result, average nutrient uptake by plants may be orders of magnitude larger than the average available nutrient pool as determined through a timeseries of measurements in soil or pore water (Bridgham et al., 2001). Under such circumstances, the most reliable indicators of nutrient availability are provided by the chemical composition of plant tissue and a plant’s allocation pattern (Wassen et al., 1995; Güsewell and Koerselman, 2002; Perry et al., 2004). Previous studies of Phalaris considered nitrogen as the most important limiting nutrient (Green and Galatowitsch, 2002; Lavergne and Molofsky, 2004; Perry et al., 2004), which we also focused on in this study. Thus, indicators that could be used for nitrogen availability in the current experiment were the nitrogen content of leaf tissue and Phalaris’ root:shoot ratio. As explained in detail in Appendix B, both these indicators suggest that nitrogen availability in the current experiment was at a level at which litter addition may induce a critical transition in ecosystem states for high C:N genotypes, but not for low C:N genotypes (Fig. 1E and F).

After ten weeks, aboveground biomass and belowground biomass were harvested for Phalaris and native vegetation separately. Distinct color differences between the roots of Phalaris and the roots of native vegetation enabled isolation of the majority of Phalaris root biomass, although a small fraction of Phalaris’ fine roots in the competition treatments may have been missed in the separation process. After separation, roots were washed. Both aboveground and belowground biomasses were dried at 70°C for one week (Eppinga et al., 2008) after which dry weight was measured. A subsample of 5–10 mg from each aboveground biomass sample was analyzed for C and N content, using a dynamic flash combustion technique.

**Statistical analysis of mesocosm experiment**

We calculated a net litter effect for each treatment using:

$$L_{\text{eff}} = \frac{(X_{\text{litter}} - X_{\text{No}})}{X_{\text{litter}} + X_{\text{No}}}$$

where $L_{\text{eff}}$ is a dimensionless property for litter effect, $X_{\text{litter}}$ is the biomass reached in the litter treatment and $X_{\text{No}}$ is the biomass reached in the no litter treatment. In equation (1), overbars indicate averages. The value of $L_{\text{eff}}$ can range between $-1$ and $1$, with positive values indicating positive effects. This index is a suitable indicator for net effects on plant performance and has strong mathematical and statistical properties (Armas et al., 2004).

In the context of plant-soil feedbacks, this index has previously been applied to quantify effects of soil biota on plant performance (Carvalho et al., 2010) and effects of plant composition on nutrient availability (Eppinga et al., 2010).

Differences in litter effects between low and high C:N genotypes can be tested with a t-test that uses the $L_{\text{eff}}$ values and their standard deviations (following Eppinga et al., 2010). Because we did not consider replicated pairs in our study, and because the relatively small sample sizes for genotype × litter treatments ($n = 6$) hamper the possibilities to achieve normal distributions even after transformation, we used a bootstrap technique (Efron and Tibshirani, 1993) to estimate the $L_{\text{eff}}$ and its standard deviation. We generated 100,000 bootstrap replicates to estimate the mean of $L_{\text{eff}}$ and its standard error, using a random permutation function as implemented in Matlab (v. 7.7.0, Mathworks 2008; Eppinga et al., 2010).

Apart from determining differences between low C:N and high C:N genotypes, $L_{\text{eff}}$ values can also be used to test whether a litter effect is significantly positive or negative. This was determined by the fraction of the bootstrap samples that had an index value of 0 or the opposite sign (Fox, 2008; Carvalho et al., 2010). For example, if a mean $L_{\text{eff}}$ value was calculated to be +0.25, and only two percent of all bootstrap replicates had an index value that was negative or zero, the conclusion was that the effect was significantly positive, with a p-value of 0.02. This procedure can be written in equation form as:

$$p_{\text{LEff}} = 0 = \frac{1}{n} \sum_{i=1}^{n} H\left(\left| L_{\text{eff},i} \right| + \left| L_{\text{eff}} \right| - \left| L_{\text{eff},i} + L_{\text{eff}} \right| \right).$$

In which $H()$ depicts the Heaviside function, $L_{\text{eff}}$, the index value of the $i$th bootstrap replicate, $L_{\text{eff}}$ the mean index value of all bootstrap replicates and $n$ the total number of bootstrap replicates.

Our hypothesis for the competition treatments was that all treatments would converge to either an ecosystem state in which Phalaris was outcompeted or an ecosystem state in which Phalaris became dominant. Thus, rather than expecting an increasing trend in Phalaris biomass with litter application, we expected two distinct states (Fig. 1E), with only the high C:N genotypes under high litter application levels achieving high biomass (Fig. 1F). This test of the possibility of a critical transition is known as divergence of states due to different initial settings (Scheffer et al., 2003; Schröder et al., 2005). In our mesocosm experiment, the differences in initial settings were due to differences in litter application, which were hypothesized to affect high C:N genotypes but not low C:N genotypes (Fig. 1E). It is important to note that in a previous experiment, growth differences between low and high C:N genotypes did become apparent within the same timespan (10–11 weeks, Lavergne and Molofsky, 2007) that was used in the current experiment. The more dominant Phalaris becomes over native species, the closer it approaches the biomass levels reached when grown in isolation (i.e. without any competition). Therefore, the Phalaris response variable that was used in the analysis was percent of total biomass reached in isolation for the same litter treatment (in line with Perry and Galatowitsch, 2004). The hypothesis was tested by means of a One-Way ANOVA between the eight groups (2 C:N strategies and four litter levels) and a post hoc Tukey test. Normality
(tested with a Shapiro–Wilk W test) of the *Phalaris* response variable was achieved through log-transformation.

**Results**

**Field survey results**

Field observations showed that litter density in *Phalaris*-dominated patches was higher in invasive than in native ranges (Fig. 2A, Mann–Whitney U test; \( U = 281, p = 0.002 \)). This result could not be explained by increased productivity of *Phalaris* in the invasive range. On the contrary, aboveground biomass density was lower in the invasive range (Fig. 2B, Mann–Whitney U test; \( U = 333, p = 0.016 \)). From these results, it follows that the observed litter:biomass ratio was higher in the invasive range than in the native range (Fig. 2C, Mann–Whitney U test; \( U = 199, p < 0.001 \)).

It should be noted, however, that large variation in aboveground biomass and litter densities were observed within both invasive and native ranges (Fig. 2). We therefore performed an additional test to examine whether this result depended on one or two outlier sites. More specifically, we removed the field site with the highest litter:biomass ratio (Shelburne Bay, invasive range) and the field site with the lowest litter biomass ratio (Nová Hlína, native range) from the dataset, and tested again for differences in litter:biomass ratio between ranges. This reduced dataset still showed that the litter:biomass ratio was higher in the invasive range than in the native range (Mann–Whitney U test; \( U = 165, p = 0.011 \)), showing that the result was not explained by the presence of outlier sites in the dataset.

**Mesocosm experiment results**

**Litter effects on low C:N and high C:N genotypes**

Contrary to previous experimental results under high nutrient availability, the high C:N genotypes did not show a higher biomass production than low C:N genotypes in the absence of litter (Fig. 3A). However, these high C:N genotypes did experience a net positive effect of litter (Fig. 3B–D). For aboveground biomass of high C:N genotypes, positive litter effects were observed in all treatments (Fig. 3C, bootstrap estimates; low litter: \( p = 0.039 \), medium litter: \( p = 0.005 \), high litter: \( p = 0.022 \)). For total biomass and belowground biomass of high C:N genotypes, positive litter effects were observed in the low and medium litter treatments (Fig. 3B–D, total biomass; low litter: \( p = 0.006 \), medium litter: \( p = 0.013 \), belowground biomass; low litter: \( p = 0.01 \), medium litter: \( p = 0.026 \)). Although the total and belowground biomass response of high C:N genotypes to litter also tended to be positive under high litter application (Fig. 3B and D), a significantly positive litter effect was not observed (Fig. 3B and D, total biomass: \( p = 0.06 \); belowground biomass: \( p = 0.11 \)). The low C:N genotypes responded quite differently to litter than the high C:N genotypes in that positive litter effects were not observed for any of the biomass response variables in any of the litter treatments (Fig. 3B–D). These results fulfill the prerequisite for eco-evolutionary feedback, in that high C:N genotypes (which may stimulate litter accumulation) benefited from high litter conditions whereas the low C:N genotypes did not (Fig. 3B–D).

**Litter effects on competition between *Phalaris* and the native plant community**

When grown in competition with native species, however, both low C:N and high C:N genotypes reached about 30% of the biomass that was reached when grown in isolation with the same amount of litter (Fig. 4). Hence, there were no significant differences between low C:N and high C:N genotypes for any of the litter treatments (One-way ANOVA; \( F_{3,39} = 0.74, p = 0.64 \)). Thus, although the high C:N genotypes responded positively to litter when growing in isolation, none of the litter treatments were strong enough to improve their performance when growing in competition with native species. Therefore, these data provided no support for the second hypothesis.

![Fig. 2. Biogeographical comparison of litter and biomass pools in *Phalaris*-dominated patches at four sites in *Phalaris*’ native range (South Bohemia, Czech Republic) and four sites in *Phalaris*’ invasive range (Vermont, USA). Bars indicate mean values, error bars indicate ±1 s.e. Asterisks indicate litter effects that are significantly different from zero: *p < 0.05*, **p < 0.01**, ***p < 0.001. (a) Litter:biomass ratios were higher in the native range. (b) Litter densities were higher in the invasive range. (c) Aboveground biomass densities were higher in the native range.](image-url)
One of the assumptions that were made in the previous model study was that high C:N genotypes would conserve their C:N trait under low nutrient conditions (Eppinga et al., 2011). Analysis of C:N ratios in the current experiment, however, contradicted this assumption. In general, C:N ratios where higher in the current experiment (see Appendix B for details) than in a previous experiment (Molofsky et al. unpublished data; see also Eppinga et al., 2011), indicating lower nutrient availability in the current experiment. The low C:N genotypes had an average C:N ratio of 10 gC gN⁻¹ in the previous experiment, but an average of 34 (±5.8) gC gN⁻¹ in the isolation treatments of the current experiment. Although the high C:N genotypes had a higher C:N ratio in the current experiment as well, the increase was not as large; the high C:N genotypes had an average C:N ratio of 16 gC gN⁻¹ in the previous experiment, but an average of 30 (±4.4) gC gN⁻¹ in the current experiment. Thus, genotypes that reached high leaf tissue C:N ratios in the previous experiment actually had lower C:N ratios in the isolation treatments of the current experiment than the previously identified ‘low C:N’ genotypes (two sample t-test, t₄₀ = 2.46, p = 0.018). These observations suggest that the two genotype groups differ in their plasticity in leaf tissue C:N ratio in response to changes in nutrient availability. (However, to avoid changing the initial terminology, we will keep referring to the two genotype groups as ‘low C:N’ and ‘high C:N’ genotypes in the ‘Discussion’ section).

**Discussion**

**Field survey: higher litter biomass ratios in the invasive range**

The field survey results corroborated previous model predictions of litter:biomass ratios being higher in *Phalaris*’ invasive range, which would be expected in case of eco-evolutionary feedback occurring in this invasive range (Figs. 1 and 2; Eppinga et al., 2011).
Previous theoretical studies have highlighted the potential importance of eco-evolutionary feedbacks between (invasive) plants and nutrient cycling (Odingle-Smee et al., 2003; Kylafis and Loreau, 2008; Post and Palkovacs, 2009), but empirical tests of these findings are sparse. Most empirical studies on invasive plants and nutrient cycling have reported acceleration of decomposition rates and a trend toward lower C:N ratios in biomass (Liao et al., 2008; Ehrenfeld, 2010). Invasion of Phalaris, however, may be aided by deceleration of nutrient cycling and subsequent litter accumulation (Zedler, 2009; Eppinga et al., 2011). Litter manipulation experiments with other invasive macrophytes also suggest that litter feedbacks may enhance the success of these species (Farrer and Goldberg, 2009; Vaccaro et al., 2009). To our knowledge, however, litter accumulation by these other invaders has not yet been compared to accumulated litter levels in their native range.

The main aim of the field survey was to empirically test previously derived model predictions (Fig. 1A and B; Eppinga et al., 2011). It should be noted that causal relationships cannot be inferred from this kind of field observations. Therefore the aim of such an empirical test is to try falsifying a hypothesis or provisionally accepting a hypothesis and making it subject to further empirical testing (Eppinga et al., 2008, 2010). In this study, biogeographical differences in litter:biomass ratio between native and invasive ranges could be caused by many other factors that influence litter density, such as climate (Aerts, 1997) and flooding frequency (Zedler, 2009). Thus, corroboration of field observations with model predictions does not prove that the model is correct. However, if we would not have found any difference in litter:biomass ratios between ranges or even a reversed pattern (highest litter:biomass ratios in the native range), this would have made a strong case against the hypothesis of evolutionary development creating positive litter feedbacks and its importance for Phalaris invasion in North American wetlands. Because we did not falsify our hypothesis in this manner, the field observations encourage further research on this topic.

**Mesocosm experiment: positive litter feedback, but no threshold dynamics**

Our results suggest that the interaction between Phalaris and litter density may indeed result in a positive feedback, but only for high C:N genotypes that evolved in the invasive range. Hence, positive litter feedback may occur in the invasive range due to evolutionary changes, providing a possible mechanism explaining why Phalaris may be more successful in the invasive range, although not necessarily by reaching higher biomass densities (Figs. 2 and 3).

The results of the mesocosm experiment also suggest, however, that this positive litter feedback can only develop in nutrient-rich environments. More specifically, comparing these results with previous experiments suggests that the selected high C:N genotypes in this study only exhibit this advantageous trait under high nutrient availability (Figs. 3 and 4; Lavergne and Molofsky, 2007; Molofsky et al. unpublished data, see also Eppinga et al., 2011). In other words, the expression of a higher C:N ratio in invasive genotypes seems environmentally driven. This challenges one of the assumptions of the previous model study (Eppinga et al., 2011) in which a higher C:N ratio of invasive Phalaris genotypes was considered to be maintained in various environments (ranging from low to high nutrient conditions). A possible explanation for the experimental results reported here is that invasive genotypes have adapted to high nutrient and high litter conditions, rather than being superior genotypes under all environmental conditions. This notion is in compliance with a recent study (Jakubowski et al., 2011) showing that selective breeding has led to agronomic Phalaris genotypes that perform well in a particular environment (nutrient-rich uplands), but not under all environmental conditions. Further evidence for the occurrence of eco-evolutionary feedback could therefore be provided by studying fitness changes of Phalaris due to litter application under higher nutrient availability. This will not only require the study of interactions between Phalaris and other species, but also looking at intraspecific competition between genotypes.

Our results suggest that the invasive high C:N genotypes in our experiment may not fit within a jack-of-all-trades scenario (as previously modeled), but it is more likely that they fit within a Master-of-some scenario, in that they have higher fitness than native genotypes only in specific environments (Richards et al., 2006). Therefore, it is questionable whether flexible traits such as the leaf tissue C:N ratio can be used to predict invasion success of particular genotypes of a species over a wide range of environmental conditions. Therefore, it is recommended that future assessments focus on trait variation within invasive populations, and how this variation influences fitness over a range of environmental conditions (Suding et al., 2008).

In our mesocosm experiment, we examined the fate of perturbations of a stable (native species-dominated) state. This perturbation comprised not only introduction of Phalaris but also varying amounts of litter. According to theory, introduction of high C:N genotypes and high amounts of litter would be a strong enough perturbation to induce a transition toward another (Phalaris-dominated) stable state. Although stability and a final outcome of competition cannot be expected in such a short-term experiment (Gibson et al., 1999; Schröder et al., 2005), one would expect divergent trajectories of Phalaris growth if the perturbation were strong enough to switch toward another stable states’ basin of attraction (Scheller et al., 2003). It should also be noted that our experimental setup did not evaluate all possible effects of litter on native species. Importantly, previous studies have shown that litter may suppress germination of seeds and seedling emergence (Goldberg and Werner, 1983; Suding and Goldberg, 1999; but see Farrer and Goldberg, 2011). Because such negative effects were not considered in the current study, litter effects on native vegetation (and thereby indirectly on Phalaris), may have been quantified in a somewhat conservative way. In addition, there may be time lags between litter accumulation and species responses that exceed the timescale considered in our mesocosm experiment (Molofsky et al., 2000; Farrer et al., 2010). Nevertheless, the driving mechanisms inducing the critical transition in the tested model, competition for soil resources and light and differential growth responses of low C:N and high C:N Phalaris genotypes, do occur on a timescale that was captured within the experiment (Perry and Galatowitsch, 2004; Perry et al., 2004; Lavergne and Molofsky, 2007).

In this paper, we focused on predictions from the previous model related to the occurrence of eco-evolutionary litter feedback in the invasive range (Eppinga et al., 2011). This model also made predictions that were outside of this scope, because they were not related to evolutionary change. Instead, these other predictions focused on the mechanisms through which litter affects resource competition. Empirical testing of these resource competition-related predictions will be the subject of further research. Litter decreases light availability and increases nutrient retention, and model predictions suggest that there is synergy between these mechanisms (Eppinga et al., 2011). A two-year field experiment is currently running in which the response to various litter treatments of Phalaris and native species is measured. These treatments involve a mulched litter treatment (providing nutrients, but not reducing light) and an artificial litter treatment (not providing nutrients, but reducing light) to test for synergistic effects. These results may provide further insights into the environmental conditions (i.e. resource supply conditions) under which evolutionary
changes in Phalaris’ response to litter (Fig. 3) most strongly affect the outcome of competition between Phalaris and native species.

Perspectives

Recent discussions in invasion ecology have highlighted the need to identify key traits that enable exotic plants to actively modify their new ranges (Hulme et al., 2011; Thompson and Davis, 2011a,b; Van Kleunen et al., 2011). Our results suggest that if such key traits result from eco-evolutionary feedback mechanisms, their presence may go unnoticed when: (1) the invasive population is considered as a single population and only its mean trait values are examined, thereby neglecting potential differences between native and novel (invasive) genotypes; (2) only a narrow range of environmental conditions is considered, because eco-evolutionary feedback may only occur within a particular range of environmental conditions. This latter notion suggests that eco-evolutionary feedback may select for specialist genotypes, rather than superior genotypes. Hence, post-introduction evolution of invasive species into multiple novel environments may boost trait variation at the genotype level. This trait variation may thus not only be due to a high level of phenotypic plasticity of certain genotypes (as observed for Phalaris by Lavergne and Molofsky, 2007; Lavergne et al., 2010), but evolutionary development of specialist genotypes may add to the observed phenotypic variation as well.

Many examples have been reported of invasive species inducing positive feedbacks by actively modifying the new range (Crooks, 2002; Cuddington et al., 2007). Our results suggest that such feedbacks may not only arise from predetermined selection pressures in new ranges, but could also be induced by evolutionary development of the invasive species within these ranges. Although some studies have highlighted mechanisms that may reduce the success of invading exotic plants over time (e.g. Niijer et al., 2007), eco-evolutionary feedback may make an invader more competitive over time, at least within a certain range of environmental conditions. Identification of these eco-evolutionary feedbacks in an early stage of the invasion process could be assisted by examining the performance of invasive species at the genotype level.

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Appendix A. Derivation of model predictions and hypotheses

In this Appendix, we provide the theoretical underpinning for the conceptual graphs shown in Fig. 1 of the main text. The hypotheses for the current study were derived from the model framework first proposed by Eppinga et al. (2011), who considered competition for light and soil nitrogen between Phalaris and a native species. This model was parameterized based on previous pot experiments, which revealed that Phalaris outcompeted other species at high levels of soil nitrogen (Perry et al., 2004).

The first hypothesis was tested by means of a field survey. The field survey focused on field sites in both native and invasive habitats where Phalaris was dominant. In the previous model, these conditions are met at high nutrient availability (a soil nitrogen availability in the absence of plants of S=15 mg kg−1 or higher). The graph shown in Fig. 1A of the main text is a conceptual representation of a model simulation showing how the litter:biomass ratio changes with increasing Phalaris’ carbon:nitrogen (C:N) ratio when parameterized for these high nutrient conditions (Fig. A1).

The second hypothesis of the study was related to the response of low C:N and high C:N Phalaris genotypes when grown in isolation, and being exposed to varying amounts of litter. To generate a model prediction for these conditions, the previously developed model framework (Eppinga et al., 2011) needed to be modified. More specifically, the following modifications were made to the model so that it could be applied to the isolation treatments of the mesocosm experiment in this study: (1) rather than considering competition between Phalaris and native species, we only consider performance of different Phalaris genotypes when grown in monoculture; (2) we assume that Phalaris’ growth was limited by soil nitrogen (N); (3) a fixed amount of litter was applied, and the chemical characteristics of the litter do not depend on the genotype considered. These modifications yield the following equations:

\[
\frac{dP}{dt} = \frac{gN}{kP + N} P - mP \tag{A1}
\]

\[
\frac{dN}{dt} = a(S - N) - \frac{qP}{\rho_{\text{Root}}} \frac{gN}{kP + N} P + \frac{q^2_{\text{Litter}}}{\rho_{\text{Root}} \rho_{\text{Litter}}} dD_{\text{Applied}} \tag{A2}
\]

In which \( P \) is Phalaris density (g m−2), \( N \) is nitrogen availability in the soil (mg kg−1), and \( D_{\text{Applied}} \) the amount of litter applied in each treatment (g m−2), \( g \) is Phalaris’ relative growth rate (day−1), \( kP \) is the nitrogen availability at which Phalaris reaches half its maximum growth rate (mg kg−1), \( m \) is the mortality rate (day−1), \( a \) is the turnover rate of nutrient supply (day−1), \( S \) is nitrogen availability in the absence of plant uptake (mg kg−1), \( qP \) is the genotype-specific nitrogen content of plant tissue (g m−2), \( q^2_{\text{Litter}} \) the nitrogen content of the applied litter (g m−2), \( \rho_{\text{Root}} \) is the rooting depth of Phalaris, \( \rho_{\text{Litter}} \) is the nitrogen content of the applied litter (g m−2), note that the same litter was applied to all...
genotypes in the experiment), $d$ is the decomposition rate of the litter (day$^{-1}$), $Q_{\text{litter}}$ is the nitrogen content of Phalaris litter (g N g$^{-1}$) at which it decomposes at rate $d$, and $\alpha$ is the nutrient-litter feedback coefficient (dimensionless). Eqs. (A1) and (A2) can be solved to equilibrium, yielding:

$$\dot{N} = \frac{mk_p}{g - m}$$

(A3)

$$\dot{p} = \frac{(g - m)\alpha d D_{\text{Applied}} q_{\text{litter}}^2 - a \rho b_{\text{Root}} Q_{\text{litter}} (m(k_f + S) - gS)}{(g - m) m q_p Q_{\text{litter}}}$$

(A4)

In which the hats indicate equilibrium. Eq. (A4) shows that the density that Phalaris can reach depends on the amount of litter applied ($D_{\text{Applied}}$). Therefore, the biomass response to litter application can be calculated as follows:

$$\frac{d\dot{p}}{dD_{\text{Applied}}} = \frac{\alpha d q_{\text{litter}}^2}{m q_p Q_{\text{litter}}}$$

(A5)

A crucial observation from Eq. (A5) is that the genotype-specific leaf tissue nitrogen content $q_p$, occurs in the denominator. This means that the higher the genotype's C:N ratio (i.e. the smaller $q_p$), the more it benefits from litter application. Note that this is a general observation, independent of particular parameter settings. Eq. (A4), however, shows that the biomass of high C:N genotypes (i.e. genotypes having smaller values of $q_p$) will also be higher in the absence of litter. Because high C:N genotypes are predicted to do better under both no litter and with litter treatments, it is not obvious what the resulting litter effect $L_{\text{EFF}}$ will be (which considers the relative performance under litter compared to the no litter treatment). Another complication is that the above equations are based on long-term equilibrium values, but a 10 week mesocosm experiment is considered here. We can address both issues by constructing an equation of predicted biomass after $t = 70$ days as a function of the amount of litter applied for both low C:N and high C:N genotypes:

$$P_{\text{LCN}} (t = 70) = P_{\text{NO,LCN}} + \frac{\alpha d q_{\text{litter}}^2 D_{\text{Applied}}}{m q_p Q_{\text{litter}}}$$

(A6)

$$P_{\text{HCN}} (t = 70) = P_{\text{NO,HCN}} + \frac{\alpha d q_{\text{litter}}^2 D_{\text{Applied}}}{m q_p Q_{\text{litter}}}$$

(A7)

In Eqs. (A6) and (A7), $P_{\text{NO,LCN}}$ and $P_{\text{NO,HCN}}$ were parameterized based on the performance of the selected genotypes in a previous pot experiment of similar duration ($P_{\text{NO,LCN}} = 29g$, $P_{\text{NO,HCN}} = 33g$; Lavergne and Molofsky, 2007). The second term in Eqs. (A6) and (A7) overestimates the effect of litter because of the equilibrium approximation, and because a constant nutrient release over time is assumed in this model framework, whereas in the experiment release rates will go down when litter reduces in quantity and quality over time. This point was addressed by including a damping factor, $\tau$. We parameterized $\tau$ in such a way that high litter application could increase Phalaris' performance by 50%–100%, which seems a reasonable maximum limit given the duration of the mesocosm experiment. For all other parameter values, we followed Eppinga et al. (2011). From Eqs. (A4) and (A5) it follows that high C:N genotypes respond more positively to litter (Fig. A2A), and that this is also reflected in litter effects that are more strongly positive for high C:N genotypes for the three treatment levels used in this study (320, 920 and 1520 g m$^{-2}$). The results shown in Fig. A2 form the quantitative justification of the conceptual Fig. 1C and D presented in the main text.

Finally, the third hypothesis was related to the occurrence of a critical transition toward dominance of high C:N Phalaris genotypes when litter is added to a native-dominated community under low nutrient conditions. The conceptual Fig. 1E and F are based on simulations performed by Eppinga et al. (2011), who used a nitrogen availability in soil in the absence of plants of $S = 9$ mg kg$^{-1}$.

### Appendix B. Indicators for nutrient availability

In this Appendix, we consider several indicators of nutrient availability for the mesocosm experiment described in the main text. In general, the leaf tissue C:N ratio of plants increases with decreasing nitrogen availability (Hobbie, 1992; Wassen et al., 1995; Gusewell, 2004). Previous experiments under high nutrient availability (Lavergne and Molofsky, 2007; Molofsky et al. unpublished data, see also Eppinga et al., 2011) lead to an average Phalaris' leaf tissue C:N ratio of 12.8 (±0.2) gC gN$^{-1}$ (n = 102, Molofsky et al. unpublished data). A few observations of Phalaris' leaf tissue C:N ratio in the wetland where the mesocosms for the current study were taken showed similar values (n = 48, average C:N ratio 13.0 (±1.8) gC gN$^{-1}$ (Kaproot, Eppinga and Molofsky, unpublished data). In the current experiment, however, leaf tissue C:N ratios of both low C:N and high C:N genotypes when grown in isolation were much higher (n = 48, average C:N ratio 31.6 (±0.8) gC gN$^{-1}$), suggesting lower nitrogen availability than in previous experiments and in field conditions under which Phalaris can invade. Looking more closely at the C:N ratio per litter treatment, the mean C:N
ratio of high C:N genotypes declined with increasing amount of litter applied (Fig. B1). A similar pattern was found for low C:N genotypes, although the high litter treatment formed an exception to this trend (Fig. B1). A possible explanation for these decreasing C:N ratios could be that there was an increase in nutrient availability due to litter decomposition. It should be noted, however, that these trends were not statistically significant (non-parametric regression: Low C:N: $T_{24} = -0.14$, $p > 0.1$; High C:N: $T_{24} = -0.14$, $p > 0.1$), possibly due to the large variation between pots and the degree of replication when parsing out the data to individual C:N strategy x litter treatments. When aggregating the data at a higher level, clearer trends were observed as reported in the main text. On the other hand, it should be noted that the increasing biomass of high C:N genotypes (see main text) implies that total nutrient uptake of high C:N genotypes did increase with increasing litter application.

In addition, a previous nitrogen fertilization experiment showed that *Phalaris*’ root:shoot ratio decreases with increasing nitrogen availability (Perry et al., 2004). More specifically, Perry et al. (2004) found that under low nitrogen conditions (achieved by amending active carbon to the soil and no or little nitrogen application during the experiment), the root:shoot ratio of *Phalaris* when grown in isolation was approximately ranging between 1.1 and 1.5 (Perry et al., 2004). When grown in competition with *Carex hystericina* under these nutrient conditions, *Phalaris* was outcompeted (Perry et al., 2004). Under high nitrogen availability, the root:shoot ratio of *Phalaris* when grown in isolation reduced to an approximate range between 0.1 and 0.6 (Perry et al., 2004). When grown in competition with *C. hystericina* under these nutrient conditions, *Phalaris* suppressed *C. hystericina* (Perry et al., 2004). In previous experiments, the low and high C:N genotypes used in this study had root:shoot ratios of 0.63 (±0.01) ($n = 90$, Lavergne and Molofsky, 2007) when grown in isolation, which is similar to ratios observed in the high nitrogen availability treatments of Perry et al. (2004). In the current experiment, however, we observed an average root:shoot ratio of 1.6 (±0.07) ($n = 48$) when *Phalaris* genotypes were grown in isolation, corresponding to the low nitrogen availability treatments of Perry et al. (2004). Because Perry et al. (2004) observed that *Phalaris* was outcompeted when growing with native species and attaining these root:shoot ratios, these indicators suggest that the experimental conditions of our current experiment were appropriate to test our second hypothesis about the effect of litter addition on the growth of low and high C:N *Phalaris* genotypes in native-dominated mesocosms (see Fig. 1E and F in the main text).

**Fig. B1.** Measured C:N ratios in the leaf tissue of low and high C:N *Phalaris* genotypes when growing in isolation. Although mean C:N ratios declined with increasing amount of litter applied (the only exception being the high litter treatment of low C:N genotypes), there were no trends in C:N ratios that were statistically significant. In the graph, all treatments were considered as groups in a One-Way ANOVA followed by a post hoc Tukey test, same letters indicating no significant differences ($p > 0.05$).

**References**


