ABSTRACT

Title of Document:	COMPETITIVE RESPONSE OF INVASIVE PLANT SPECIES TO NEIGHBOR PRESENCE, IDENTITY, AND PHENOLOGY ACROSS TWO GROWING SEASONS
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Exotic plant species invade many native communities, yet some communities are less susceptible than others. Community properties that determine resistance to or influence on invasive species performance are less studied. The target-neighbor experimental design, originally used for studies of root and shoot competition (Chapter 1), is now commonly used to determine effects of communities of species on a target plant of interest. However, intensity of competition varies between species both within and across seasons, therefore interactions between species are not static. Thus I compared the competitive response of two widespread but relatively little studied

invasive species, early flowering *Hesperis matronalis* and later flowering *Nepeta cataria.*
Both invasives were introduced into native species neighbor communities composed of
either early, late, or a mixture of early and late growth and flowering phenologies and
measured over the course of two years (Chapter 2). Additionally, invasive species
introduction time into native communities was manipulated by either coestablishing the
invasive with the neighbors, or introducing the invasive into established neighbor
communities (Chapter 3).

I predicted that target invasives would experience the most intense competition
(1) when sharing the same growth and flowering phenology as their surrounding native
community, due to temporal niche overlap (2) when introduced into an established native
community versus when coestablished with native neighbors, and also that (3) the overall
effect of neighbor presence and neighbor identity would vary with the age of the
competitors. Finally, I utilized field soil probes and greenhouse plants labeled with ^{15}N
isotopes to determine the relationship between phenology and nitrogen uptake to provide
a mechanistic basis for temporal niche overlap (Chapter 4).

The competitive response of both invasive species' performance was dependent
on plant trait measured, community age, and native neighbor phenology treatment. I
found evidence for a negative effect of temporal niche overlap, but resource pre-emption
by the early phenology neighbors was more prevalent than stronger competition due to a
shared phenology. Despite this, field nitrogen levels were still correlated with species
phenology. This body of work supports the need for more research on the factors
associated with native communities' ability to resist invasion.

Competitive response of invasive plant species to neighbor presence, identity, and
phenology across two growing seasons

By

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Table of Contents

Chapter 1: The importance of analyzing neighbor competitive response in the target-neighbor experimental design

Abstract

The strengths and weaknesses of the different experimental designs used to study plant competition are frequently debated. In the target-neighbor design (a type of additive design where one of the competing species is reduced to a single individual), controls and analyses are used for the target, but not for the neighbors. I conducted a literature review to determine how the target-neighbor design has been typically utilized and analyzed. I found that historically, targets were often smaller than neighbors and introduced after neighbor establishment, thus targets would have little effect on neighbors. However, as co-introduction of targets and neighbors of similar size is now common, the target is more likely to affect the neighbors than in its earlier usage. This can be problematic, since if targets have a significant effect on the neighbors, bias is introduced into the neighbor treatments. As target treatment controls are necessary to determine the absolute effect of neighbors on target growth, I advocate that analysis of the neighbor competitive response serves as a control for the target-neighbor community as a whole.

Introduction

The study of competition among species has led to the formation of many concepts and theories of species coexistence (Darwin 1859, Paine 1974; Harper 1979; Sax 2006). Among plants in particular, there is a long history of empirical experiments designed to examine inter- and intraspecific interactions (Clements 1929; Connell 1983). Knowledge of plant interactions is essential for understanding species' distributions (Pelissier et al. 2010), succession (Tognetti et al. 2010), evolution (Darwin 1859; Pfennig and Pfennig 2010), as well as the spread of invasive species (Holdredge and Bertness 2011; Murrel et al. 2011). A thorough understanding of interspecific interactions is especially important as climate change alters interactions between plant species within a community (Dunnet and Grime 1999; Adler et al. 2009) and often appears to benefit invasive species (Bradley et al. 2010; Willis et al. 2010). Though there has been much research into the mechanisms of plant competition, the role of competition in structuring plant communities is still a source of debate (e.g., Went 1973; Craine 2005).

Multiple types of experimental designs have been utilized to address a myriad of questions involving plant competition. The advantages and drawbacks to these designs are well-documented (Gibson et al. 1999) and frequently debated (Cousens 1991; Gibson et al. 1999; Freckleton et al. 2009). The majority of the experimental designs still in use have not changed substantially since their original introduction, (Gibson et al. 1999), though some, such as the replacement series, have lost favor to designs with fewer confounding factors. The optimal analyses for the data from each of these experiments are also debated, as are the most informative metrics of competition that can be derived

from the data (Freckleton and Watkinson 2000; Freckleton et al. 2009; Weigelt and Jolliffe 2003; Onofri et al. 2010).

Manipulative plant competition experiments, typical of ecological experiments, usually contain some form of control treatment. These control treatments serve as a comparison to the plants in competition, and most often take the form of either a single plant grown without competitors, or as a monoculture of the species of interest. One particular plant competition experimental design, the target-neighbor design, is unique in this aspect in that one of experimental groups, the "neighbors", is not controlled (Gibson et al.1999). The target-neighbor design is a form of additive design where one of the competing species is reduced to a single individual (the target) and the density or identity of the surrounding individuals (the neighbors) is manipulated (Goldberg & Fleetwood 1987). This design is advantageous because it allows for focus on the response of an individual plant, rather than the mean of a population (Gibson et al. 1999). Though density and proportions can be confounded in certain circumstances, this is not an issue when neighbor density is held constant and only neighbor identity is manipulated as a treatment.

Within a single target-neighbor design experimental plot, each plant belongs to one of the two groups: target or neighbors. The outcome of the experiment is then dependent on the interactions between these two groups, and their respective competitive effects and competitive responses (Goldberg and Landa 1991). The competitive effect of an individual is its influence on a competitor, while the competitive response is its reaction to the presence of that competitor (Panetta and Randall 1993). In a system where only two individuals or groups (e.g., target and neighbor) are planted in

competition with one another, the competitive effect of one group is equal to the competitive response of the other group. However, in a target-neighbor experiment with two or more different targets and two or more neighbor treatments, the competitive effects and competitive responses of both the targets and the neighbor communities are separate from one another. The competitive effect of a neighbor treatment is its influence *on* the targets. The competitive response of a neighbor treatment is the neighbors' *reaction to* the targets. Likewise, target competitive effect is the influence of that target on its neighbors, and target competitive response is the target's reaction to the neighbors. Different plant traits may even be associated with the respective competitive effect and competitive response of a species (Wang et al. 2010), indicating the importance of understanding both aspects of competition in an experiment.

One of the earliest appearances of the target-neighbor design was the chapter "Competition in Underplanted Cultures" in the book Plant Competition by Clements et al. (1929). To separate the effects of competition for light and water, *Helianthius annuus* cultures were planted at various densities in a pot with a separate cylinder sunk in the center. Once the neighbors reached a certain size, seed was sown in the center cylinder. The central plant (sometimes multiple plants) served as a phytometer that grew beneath larger conspecifics. Following this precedent, the target-neighbor design was traditionally used for studies focusing on separation of above- and below-ground competition (Mcphee and Aarssen 2001).

Today, however, the target-neighbor design has been used less frequently for separation studies and is now used almost solely in experiments where targets and neighbors are grown without separation, and only neighbor density or identity is

manipulated.   It is due to this shift from its original conception and the recent increase in target-neighbor design usage that I believe the design and its subsequent analysis should be reexamined.

Review of target-neighbor usage

I conducted a literature review of peer-reviewed ecological journals in order to determine how target-neighbor experiments were utilized and how their results were analyzed. I searched ISI Web of Science using the search terms "target neighbor" or "target neighbour" (American and British spellings, respectively) and "plant," with no restriction on year through June 2012. From these results I selected manipulative studies where targets were deliberately planted into a neighbor community consisting of at least two neighbors. I omitted those studies where targets were planted with only one neighbor, as these are more accurately defined as pairwise designs.   Studies were then divided into those where the experiment was entirely manipulative, with all plants deliberately planted in a target-neighbor design, and those where the target was planted into a natural or unstructured (i.e. seeds were randomly sowed) neighbor community. This was done because when targets are transplanted into a natural or unstructured community, neighbor identity, number, and position are a challenge to control across replicates and consistent neighbor measurements would not be feasible in this situation. Additionally, I noted whether the experiment separated above-ground from below-ground competition.

Those papers that were entirely manipulative were then characterized based on four points: (1) whether the neighbor competitive effect or target competitive response was reported (both are measures of neighbor influence on the target), (2) whether more

than one target treatment was utilized, (3) whether neighbor competitive *response* was reported, and (4) whether the targets and neighbors were planted simultaneously, or the targets were planted after neighbor establishment,

I found 64 total studies that utilized the target-neighbor design (Appendix A). I focused my review on the 42 studies that met my criteria of a manipulative target-neighbor design where both the target and neighbors were deliberately planted. All but one of these studies reported a neighbor competitive effect or target competitive response, as is expected with the target-neighbor design. Only 22/42 studies (52.4%) used at least two different target treatments in their experimental designs and could have potentially analyzed a separate neighbor competitive *response*. Of these 22, only one actually reported neighbor competitive response, where they found a significant target x neighbor treatment interaction (Cheplick and Kane 2004). One other article reported neighbor response to an herbicide treatment but not to the target treatments (Thijs et al. 1994). My results show that in the common usage of the target-neighbor design, the influence of the neighbors on the target is known, but an analysis of the influence of the target on the neighbors (the neighbor competitive response) was almost universally missing.

Without an analysis of neighbor competitive response in an experimental design with two more or more separate target treatments, valuable information about the treatments may be missed. This analysis provides greater insight into interactions between targets and neighbors through determination of whether neighbor community characteristics vary across different target treatments. In any experimental design where plants are manipulated as a treatment, variation is inherently present in that independent

variable. If there is a significant effect of the target treatment on neighbor growth, the growth of the neighbors will be dependent on the growth of their respective targets, and the characteristics of the neighbor communities will vary along with the target. Thus, neighbor treatments are no longer uniform across targets and bias is introduced into that treatment. This could lead to "unfair" comparisons, especially if measurements are made only once at the conclusion of the experiment (Gibson et al. 1999). Consequently, the conclusions made from comparisons of different targets grown within the same neighbor treatment may no longer be valid.

Just as target treatment controls are necessary to determine the absolute effect of neighbors on target growth, analysis of the neighbor competitive response serves as a control for the target-neighbor community as a whole. When neighbor competitive response is not reported, the implicit assumption is that neighbor characteristics such as height or biomass are equivalent across all target treatments (Fig. 1A). If this is true, then the targets would have a neutral effect on neighbors, and neighbor characteristics would not vary in response to the targets. This also means that any variation in overall treatment plot characteristics, such as plot biomass, is dependent solely on variation among the different target species. There are several non-mutually exclusive reasons for this result. For instance, if neighbors were established prior to the targets, resource pre-emption by the neighbors could result in only the targets experiencing a reduction in growth. Alternatively, targets may be considerably smaller than neighbors, or targets and neighbors could have different resource requirements, causing the target to have little influence on neighbor growth.

If targets are planted simultaneously with the neighbors, or are of a similar size or larger than the neighbors, it is more likely that there would be a negative effect of a target on the neighbors, and that variation in target size would lead to differences in neighbor competitive response (Fig 1B). Though intensity of competitive effect and response are not necessarily correlated (Wang et al. 2010), plants that have strong competitive effect on neighboring individuals often have a limited competitive response. A target with these characteristics, or a target that is a stronger competitor than the neighbors, would also be likely to influence neighbor characteristics. This pattern may also imply that maximum plot biomass is restricted by limited resource availability. In this case an increase in target biomass would lead to a proportional decrease in neighbor biomass.

Facilitation between target and neighbors can also occur (e.g. through nitrogen fixation or soil moisture retention by one of the partners) resulting in a positive relationship between target and neighbor biomass (Fig. 1C). Brooker et al. (2008) suggest that facilitation is an underappreciated phenomenon that should be measured in more than just extreme environments. Although it is more likely for the neighbors to facilitate the target than vice versa, since there are multiple neighbors surrounding the single target, actual target-neighbor relationships are dependent on relative sizes and planting times of the targets and neighbors.

Target-neighbor design – past and present

Although there has been a clear shift in the usage of the target-neighbor design and the increased probability that targets will influence neighbor characteristics, the results presented from target-neighbor analyses have remained largely unchanged.

Neighbor competitive response is still omitted from the results of target-neighbor studies. The reason behind this omission may be that the effect of targets on the neighbors is simply not expected. The expectation of the target having a neutral effect on the neighbors is not unreasonable, as the target-neighbor design itself minimizes the impact of the target on the neighbors, especially if the target is planted after neighbors have already established (Goldberg and Fleetwood 1987). The target is surrounded by neighbors on all sides, ensuring (if neighbors are sufficiently near or dense) that the target will experience a measurable effect of competition. By design, the neighbors have more physical space to grow outward away from the target and each other, and thus are less likely to experience significant competitive effects from the target or other neighbors. Also, most early usages of the target-neighbor design were in above-ground and below-ground separation studies, which usually relied on planting target seedlings into pre-established communities (Appendix A). Since this arrangement results in the target being substantially smaller than the neighbors, there would likely be little effect of the target on the neighbors (Cook and Ratcliff 1984).

It is for these reasons that in some of the earlier uses of the target-neighbor design neighbor analyses were largely ignored. Clements et al. (1927) reported the effect of the treatments on the phytometers (targets) but not on the neighbors. This was reasonable as the goal of the study was to analyze the response of a target seedling to larger neighbors. Welbank (1961) recognized that focusing only on target responses and not neighbor responses was an incomplete analysis of their competitive interactions. However, Welbank specified that this incomplete approach was justified in order to simplify the experiment, as it was especially practical for studies of crop-weed competition where

crop effects on weeds would not be interesting. Likewise, the smaller target crops would not be as likely to affect the larger weed neighbors (Welbank 1961).

In recent target-neighbor studies however, targets and neighbors are typically co-established, and separation of above- and below- ground competition is uncommon. Prior to 2000, 13/35 target neighbor studies had coestablished the targets and neighbors, compared to 25/29 studies since 2000 (Appendix A). Several authors explicitly stated that targets and neighbors were coestablished in order to eliminate size biases in competition (e.g., Hwang and Lauenroth 2008). These practices increase the probability the targets and neighbors will be similar in size, and thus that targets will have a significant effect on neighbor growth. This shift in practice has made analysis of neighbor competitive response more relevant than in earlier studies when targets were smaller or planted into an established neighbor community.

It is likely that neighbor competitive effects may only be of concern when a difference is obvious to the researcher. Alternatively, neighbor competitive response may be analyzed more frequently, but non-significant results often remain unreported in the literature. Regardless of whether a significant difference in neighbor competitive response is expected, statistical confirmation should not be disregarded, as the omission of neighbor competitive response causes species interactions to be only partially quantified. A partial solution to this problem is to use neighbor biomass as a predictor or covariate during analysis in a per-unit-biomass comparison (e.g. Howard 2001; Weigelt et al. 2002). Though this approach accounts for variation in neighbors across target treatments, this method does not reveal whether the neighbor treatments themselves vary with different targets. This approach is also limited in that it accounts only for

differences in biomass and not other traits that could provide insight into competitive interactions. Height and growth form influence competition for light, and flowering phenology provides insight into resource usage throughout the season (Thimann 1980; Cleland et al. 2006). A formal analysis of neighbor competitive response is thus the most effective way to determine whether significant variation is present among neighbor treatments in response to different targets.

A significant difference in neighbor competitive response is indicated by a significant effect of target treatment on neighbor characteristics, or a significant target x neighbor interaction when measuring neighbor characteristics. If either of these conditions is met, it would require that caution be taken during interpretation of the neighbor competitive effect and target competitive response, as the neighbors could no longer be considered a consistent treatment. In the single study that did report neighbor competitive effect (Cheplick and Kane 2004) the objective was to analyze the growth of targets grown with neighbors of either the same or different maternal families. Unlike most target-neighbor studies, they were *not* comparing effects across neighbor treatments, and it was therefore not necessary to qualify their conclusions as a result of the target-neighbor interaction. If comparisons are being made across neighbor treatments, as is typical, and the difference in neighbor treatments is minor or isolated to a single target-neighbor pairing, a determination should be made as to whether this difference is great enough to call into question the validity of the other experimental results. For example, in Fig. 1B, if the neighbor biomass when planted with Target A were lower, and the neighbor biomass when planted with Target C were higher, would the anticipated change in their respective target biomass means (determined through a correlation between target

11

and neighbor biomass) be great enough to alter the significance of the target biomass results? If not, then the analysis can proceed as planned, with the caveat that the neighbor means are not equal. However, where target influence causes neighbor means to have a large difference, targets should be analyzed separately from one another. Alternatively, overall treatment plot characteristics could be analyzed, instead of separating target and neighbor traits. In addition to providing insight into target-neighbor interactions and guidance on analysis, information on neighbor competitive response can also be used to inform future decisions on planting distance and plant arrangement.

I hope that in future applications of this experimental design, the competitive effects and responses of both targets and neighbors will be considered in order to provide a more complete understanding of plant competition. Though the analyses and controls in any experiment depend on the questions of interest, it is important to take into account the interactions of each of the species or species mixtures. Measurement and analysis of neighbor competitive response is a simple and straightforward technique to ascertain the effects of competition on all species involved in the target-neighbor or similarly constructed experimental designs.

Figure 1: Graphic of target-neighbor experimental design target and neighbor treatment of biomass means for both illustrating potentially different outcomes when grown together. The neighbor treatment (white bars) is shown with the three target treatments (dark bars A,B, and C) in three possible scenarios: A) Neighbors are not differentially affected by targets (no diffence in neighbor competitive response), B) Neighbors are differentially affected in a negative fashion by target biomass (competition), C) Neighbors are differentially affected in a positive way by target biomass (facilitation).

Figure 1.
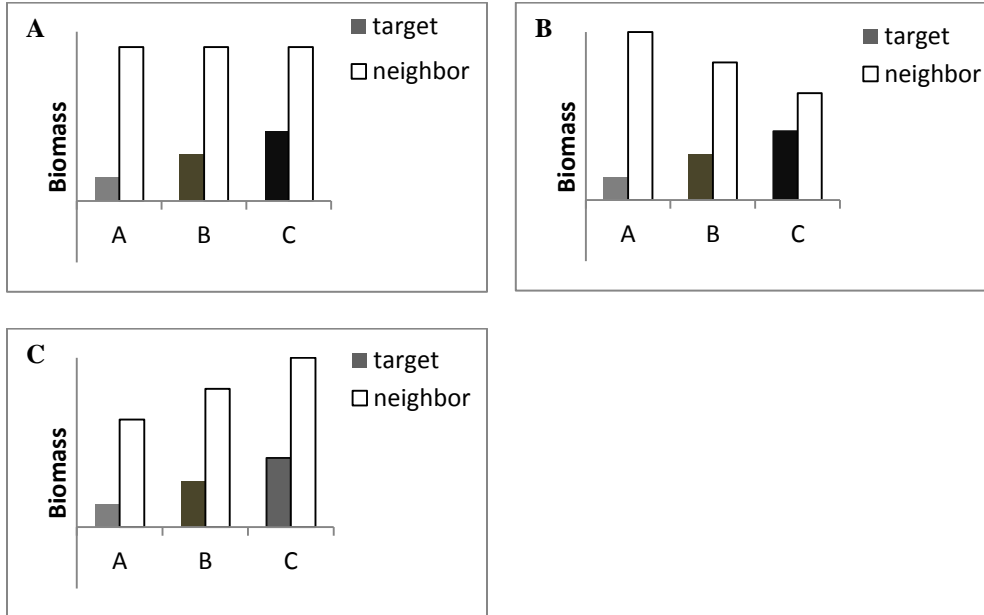
Chapter 2: Shifts in competitive response over time: Neighbor presence, identity, and phenology influence invasive plant performance across two years

Abstract

The direction and intensity of species interactions are variable and dependent on individual plant physical and physiological traits. However, interspecific interactions are not static, and the intensity and direction of interactions among species change over time. In this study, I utilize a target-neighbor design to compare the competitive response of two target invasive species across two years to several different neighbor communities. Specifically I investigated the effects of neighbor presence and absence, identity (intraspecific and interspecific competition), and the role of growth and flowering phenology on both the target invasive and neighbor performance. I co-established perennial invasive targets with perennial native neighbor seedlings and conspecifics in experimental communities in the field and measured phenological traits, morphological characteristics, and biomass over two growing seasons.

The competitive response of both target invasives' vegetative biomass, seed mass, and flowering traits were strongly influenced by the neighbor treatments, though both intraspecific and interspecific responses varied based on invasive target species, plant trait, and year. Neighbor presence had a significant negative effect on target invasive performance in the first year of the experiment, but no effect in year two.   Little evidence of stronger competitive interactions was detected when invasives and neighbors shared similar growth and flowering phenologies. However, early flowering neighbors had a greater effect on the late flowering target invasive *N. cataria*, suggesting resource pre-

16

emption. This study illustrates the importance of multi-year and multi-trait competition studies, as studies at one life stage alone are likely to under- or over-estimate the actual importance of competition in structuring communities.

Introduction

The influence of neighboring individuals on plant growth has long been studied (Clements et al. 1929, Harper 1977). Though most plants require similar resources, such as light, water, and soil nutrients, the direction and intensity of species interactions are dependent on individual species' physical, physiological, and phenological traits. The competitive effects (influence on neighbors) and competitive responses (reaction to neighbors) of different species grown together are dependent on the interplay of those species' respective traits (Goldberg 1987). Furthermore, these interspecific interactions are not static such that various growth stages, the plant traits measured, environmental variables, and introduction time can also influence plant interactions and the assessment of competitive outcomes (Gibson et al. 1999). Field studies along with complementary observational studies are essential to understanding plant species interactions, but by design there is often little or no control over plant arrangement, growth, and number.

Experimental studies in the field where plants are allowed to grow or volunteer with little interference or artificially are most representative of natural systems. Such studies are the basis of many hypotheses and theories on plant interactions, including the R* theory of plant competition (Fargione and Tilman 2002), and the CSR theory describing plants as competitors, stress tolerators, and ruderals (Grime 1977, Craine 2005) among others. In more manipulative studies, where plant arrangement, number,

17

and order is pre-determined and controlled, more precise questions about the role of these factors in plant interactions can be answered (Gibson et al. 1999).

One of the most fundamental approaches to manipulative field designs involves the development of competitive hierarchies to determine patterns of individual species dominance over or subordinance to their neighbors (Keddy and Shipley 1989). Designs involving only two species grown together in competition make control of density and number easier, but these pairwise design studies are among the least natural. Experimental designs where multiple species are simultaneously grown in competition with one another while taking into account factors such as size and density allow for a more realistic examination of competition as plants in the wild are often surrounded by a local neighborhood of various species.

Unlike field studies where plant position is not strictly controlled (e.g. Tilman 2002), in many manipulative competition studies, where plants deliberately planted in a specific pattern either in pots or the field, interactions are examined among species at only a single life-history stage (Mangla et al. 2011). This measurement timeframe is most frequently within the first season of growth (Farrer and Goldberg 2010 Suding and Goldberg 1999; Mangla et al. 2011). This approach can be problematic; however, as competitive results extrapolated from the seedling stage may not be representative of long-term outcomes, especially in perennial species (Mangla et al. 2011, Zhang and Lamb 2012). Plant age is related to but distinct from plant size, as plants within their first year of growth are not only smaller, but exhibit different phenologies and physiological responses than mature individuals (Zhang and Lamb 2012). The intensity and direction of interactions among species can also change over time as plants age (Farrer et al. 2009,

Leger and Espelend 2010, Zhang and Lamb 2012). Manipulative field studies that follow the competitive responses of individual plants as they age across years are necessary to better understand competitive interactions in nature (Zhang and Lamb 2012).

To determine the effects of plant age on competitive effect and competitive response in varying local neighborhood scenarios, I examined the effects of neighbor plant presence/absence, conspecific vs. heterospecific identity, and plant phenology. The presence of a neighbor plant in close proximity is usually predicted to have a negative effect on plant growth, especially if the neighbor exhibits similar traits. However, experimental results reveal positive, non-significant, and negative responses to neighbors, with some responses dependent on the ages of one or both of the neighbors (Farrer et al. 2010). As plants grow and progress through successive reproductive and physiological stages, changes in the competitive effects and competitive responses of species are likely to occur (Mangla et al. 2011; Zhang and Lamb 2012). For example, seedling germination and survival can be facilitated by neighbor presence, though neighbors may have a less positive or even negative effect as either or both plants increase in size (Bertness and Callaway 1994; Farrer and Goldberg 2010).

Niche theory predicts that conspecifics should compete more intensely with one another than heterospecifics, due to more similar resource requirements and competitive effects (Tilman 1982). If one species is able to deplete resources to a lower level than another, the species with the lower R* would be expected to experience less intense competition when grown with heterospecifics with a higher R*. However, experimental results have not shown a consistent pattern in either direction, with species-specific results that can be dependent on abiotic conditions such as water and soil nutrient

availability (Luo et al. 2010; Farrer and Goldberg 2011). These varying results conform more closely with the neutral theory of plant competition, where differences between inter- and intraspecific competition are predicted to be random (Hubbell 2005, Leger and Espeland 2010).

Flowering phenology is less frequently studied in plant competition experiments, yet it is an important trait that is related to the success of many invasive plant species (Wolkovich and Cleland 2011). Plant resource uptake is closely correlated with flowering phenology, with species growing, flowering, and fruiting at different times of the year, thus resource demand within a community fluctuates within a season (Thimann 1980, Chesson et al. 2001). Therefore species differentially impact the community over the course of the season (Schemske et al. 1978, Motten 1986, Osada et al. 2003). Temporal niche segregation has been shown in multiple plant communities (Martinkova et al. 2002, Golluscio et al. 2005), and more diverse communities have been shown to utilize more resources due to seasonal complementarity (Hooper and Vitousek 1998; Stevens and Carson 2001, Fargione and Tilman 2005). Observed phenological shifts due to recent climate change have brought the subject to the forefront (Cleland et al. 2006, Korner and Basler 2010, Miller-Rushing and Primack 2008). In studies where phenology is included as a functional trait in an experiment, it is often confounded with differences in growth form (e.g. grass vs. forb), life history (annual, biennial, or perennial) life history, or photosynthetic pathway (C_3 vs. C_4; e.g. Hooper and Vitousek 1998, Dukes 2002). Only when these confounding factors can be eliminated or controlled for can the absolute effects of phenology be quantified.

In this study, I quantify the performance of two invasive species with disparate

flowering phenologies and their native species neighbors while manipulating neighbor

presence and absence, conspecific and heterospecific neighbor identity, neighbor growth

and flowering phenology, and phenological functional group diversity. I co-established

all seedlings in the field utilizing a target-neighbor design where the invasive species

were targets and the native species were the neighbors. I predicted that 1) the intensity of

target response to neighbor presence/absence would be species-specific, 2) intraspecific

competition would be more intense than interspecific competition, 3) target invasives

would experience the most intense competition when grown with native neighbors of the

same phenological functional group, and 4) that increased neighbor functional group

diversity would reduce average target success. Furthermore, as I measured plant

performance across two years, I predicted that growth, flowering, fruiting, competitive

responses would be dependent on plant age.

Methods

Study Species- Native species (Appendix B, Table 1) were selected based on flowering

phenology data garnered primarily from Strausbaugh and Core's "Flora of West

Virginia" (1977) and "Finding Wildflowers in the Washington-Baltimore Area" by

Fleming, Lobstein, and Tufty (1995). The USDA Plants Database (USDA, NRCS

http://plants.usda.gov) was checked to confirm species' native status was correct, all

native species were perennial, and that the study site was within each of the species'

native range.

The native species were chosen as congeneric pairs and represent two

phenological functional groups: early-season flowering and late-season flowering phenology. Functional groups were designed around flowering phenology since flowering time is known as a good indicator of changes in resource use (Thimann 1980, Cleland et al. 2006) (Appendix B). Plant growth and flowering phenology are tightly correlated, and soil nutrient uptake typically decreases when a plant produces reproductive structures (Thimann 1980).

In order to limit non-phenological variation among species, all target invasives and native neighbors were chosen with respect to average size, growth form, and habitat such that all experimental species were herbaceous perennial forbs of comparable size. Each experimental species also utilized the C_3 photosynthetic pathway. The early flowering functional group native species were *Asclepias viridis, Coreopsis lanceolata, Liatris squarrosa,* and *Helenium flexuosum,* while the late flowering functional group was comprised of *A. tuberosa, C. tripteris, L. pilosa,* and *H. autumnale.* All native species were members of the family Asteraceae, except for the *Asclepias* species in the Asclepiadaceae.

The two invasive species (Appendix B, Table 1) were also chosen based on phenology, with *Hesperis matronalis* (Brassicaceae) the early-flowering invasive and *Nepeta cataria* (Lamiaceae) the late-flowering invasive. *Nepeta cataria* is a perennial, and *H. matronalis* grew as a polycarpic perennial in this study, although it can also grow as a biennial. These invasive species have been investigated little in terms of their effects on ecosystems or invasibility (but see Hwang and Lauenroth 2008). Anecdotally, they are both known to be weedy and aggressive (USDA, NRCS http://plants.usda.gov).

Site- Research plots were located at the Blandy Experimental Farm (78°3'30" W, 39°3'28" N) a University of Virginia research station located in Boyce, Virginia. Blandy is located within the Shenandoah Valley, and consists primarily of old fields and small woodlots. The experimental field site was located in an old field dominated by *Fescue* spp. grasses interspersed with patches of *Onopordum acanthium* (scotch thistle). Site preparation took place in 2007, and included spraying a glyphosate-based herbicide, mowing, and finally burning the site to remove vegetation. Landscaping fabric was placed between all plots to reduce the growth of non-experimental species. Removal of non-experimental species was done by hand as necessary (Appendix B*).*

Planting design- I utilized a target-neighbor experimental design modified from that used by Goldberg and Fleetwood (1987). The target-neighbor design is a type of additive design that focuses on the competitive response of an individual plant, the target (Gibson et al. 1999). In this experiment the invasive species served as the targets and the native species served as the neighbors, with the exception of the intraspecific competition treatments where the invasive was surrounded by its conspecifics. The target invasive was planted in the center of the plot surrounded by an octagonal ring of eight neighbors. Each neighbor was planted 15 cm from the target invasive, a distance selected to allow plants to experience competition, yet limit mortality. Due to this octagonal arrangement, each of the eight neighbors was approximately 11.5 cm from adjacent neighbors. All seedlings were germinated in the University of Maryland greenhouse, and all targets and neighbors were planted in the field in early April 2008. Plants that experienced mortality within three weeks of planting were promptly replaced.

Experimental Treatments- There were five separate neighbor treatments into which the target invasives were planted: (1) with all of the early-flowering functional group native neighbors, (2) with all of the late-flowering functional group native neighbors, (3) among a mixture of early- and late-flowering functional group native neighbors, (4) in its own invasive monoculture, and (5) planted 'solo' without any neighbors. The early native neighbor treatment was comprised of each of the four early native species, while the late native neighbor treatment was comprised of each of the four late native species. The mixture of early and late native species treatment, hereafter 'mixed native neighbors', was comprised of three sub-treatments which represented the three of the six possible combinations of two early and two late species that had the greatest range in phenologies (Appendix B, Table 1). These sub-treatments were treated as a single treatment for analysis. Both monoculture and solo treatments are frequently used as controls in target-neighbor experiments (K. Barry Dissertation, Chapter 1).

In order to de-couple phenological functional group diversity from species diversity and avoid the sampling effect issues outlined by Huston (1997), treatments were designed so that species and generic diversity was constant across the early, late, and mixed native neighbor treatments. This was arranged by planting four native neighbor species in each treatment, so that there was always a species from each of the four genera (*Asclepias*, *Coreopsis*, *Liatris*, and *Helenium*) present, with each species represented by two individuals in order to complete the ring of eight native neighbors. The conspecific neighbors were always planted opposite each other in the plot, with planting *order* (which pairs were adjacent to each other) randomized within each treatment plot. This

phylogenetically controlled design also further limited non-phenological variation amongst the treatments. Thus, the early and late native neighbor treatments each represent a single functional group designated by their phenology, while the mixed natives treatments contains two functional groups, both early and late phenology.

Unexpectedly, both *Liatris* species experienced near 100% mortality. This mortality altered the experimental design by reducing the number of native neighbors in the early, mixed, and late phenology treatments from eight to six. The loss of the two *Liatris* species also caused a difference in the number of neighbors between the native neighbor treatments (six) and the invasive monoculture treatments (eight). However, there was no significant effect of this difference in neighbor number on any neighbor community or target invasive traits (data not shown), so analyses within native neighbor treatments, and between native neighbor treatments and monoculture treatments, were conducted as initially planned. The few *Liatris* individuals that survived were extremely small, and the effect of their presence within the neighbor treatments was deemed to be negligible. Additionally, each mixed neighbor treatment now contained either one early and two late species (Mixed sub-treatments A and B) or two early and one late species (Mixed sub-treatment C), as opposed to containing two early and two late species as originally designed (Appendix B). This caused a late phenology species bias to be present in the mixed treatments and led to a cautious interpretation of mixed native neighbor comparisons.

Measurements- In order to understand all interactions between the targets and the neighbors, plant performance measurements were made on the targets and each of the

neighbors of every treatment. After planting in April 2008, I measured the longest length (from leaf tip to leaf tip) of each seedling in a subset of plots from each treatment to serve as a covariate for all subsequent morphological measurements. Once flowering began (June in 2008, April in 2009), I recorded date of first flowering for each individual plant, and once each week flower production on individual plants was measured until the end of September. On Asteraceae species, counts were on the number of capitula, but these were analyzed along with counts of individual flowers for the other species. From these phenological data peak flowering date (the day when the greatest number of flowers were open) and total flower count (the sum of all weekly counts) were calculated for each species (Appendix B).

Plant height on all plots and above-ground biomass on a sub-set of plots were measured in November 2008, after the majority of plants had begun to senesce. The remaining plants were allowed to naturally senesce that winter and regrow from their roots the following spring, and were measured again and harvested in October 2009 (Appendix B). Biomass in both years was separated into vegetative biomass (stems and leaves) and reproductive biomass (fruits/seeds). All plant material was dried at 60° C in a drying oven for nine days before it was weighed. Among the native species, fruits were weighed, but in the invasive species seeds were removed from their fruits and weighed independently. This approach was necessary due to the difficulty in cleanly separating *N. cataria* fruits from connected leaves and stems. Fruits of *N. cataria* along with any attached stems and leaves were ground down using a series of screens and sieves, so that the hard seeds could be removed from the processed vegetative material. For consistency between the invasives, seeds were also separated from fruits and weighed independently

for *H. matronalis*. Seed mass was highly correlated with fruit mass for both of the invasives and each of the neighbor species, allowing us to use these two separate measures of reproductive effort for the target and neighbor species (data not shown). It was not possible to effectively separate the roots of the targets and neighbors, subsequently roots were not harvested in the field.

There were originally 15 replicates planted for each experimental treatment, with the mixed neighbor treatments containing 45 replicates (due to the three mixed neighbor sub-treatments that were combined). In total, 420 experimental plots composed of over 3100 plants were established, representing 10 experimental treatments for each of the two years. Replication varied slightly from the original design due to neighbor mortality and planting error.

Analyses – In addition to analyzing the target competitive response (the effect of the neighbor treatments on the targets), I also chose to analyze the neighbor competitive response (the effect of the target treatments on the neighbors). I took this approach to determine whether there was any difference in performance of neighbor treatments when planted with the early or late target invasive. This was also done to identify any bias within the neighbor treatments across the two invasive targets as both targets and neighbors can influence one another during growth (See Chapter 1).

The target and neighbor competitive responses were analyzed with a mixed model ANOVA in SAS version 9.2 (SAS Inc., Cary, N.C) using the Mixed procedure. The target invasive competitive response full model consisted of neighbor treatments (five levels - early, late, and mixed neighbors, as well as target monoculture and solo

treatments), target invasive treatment (two levels - early and late) and year (two levels -

2008 and 2009). Blocks (the three fenced enclosures in the field) were treated as a

random factor.

Statistical contrasts were used to determine differences of the response variables

between groups based on my predictions. The effects of phenology and functional group

diversity were tested by contrasting the early and late treatments, and the mean of the

early and late against the mixed treatment, respectively. Differences between

intraspecific and interspecific competition were tested by contrasting the monoculture

treatments against the combined mean of the early, mixed, and late treatments. To

determine whether there was overall an effect of neighbor presence or absence, I

performed a contrast between the solo treatment and the mean of all other treatments.

Four contrasts were conducted, so a Bonferroni corrected p-value of .05/4 = .0125 was

used to limit experiment-wise error (Appendix B). Target survivorship was tested with a

logit-transformed model in Proc GLIMMIX, while Spearman Rank correlations were

used to determine the relationship between target biomass and neighbor biomass.

The native neighbor competitive response was calculated for morphological

traits by taking the sum of the measurements of each of the individual neighbors within

each treatment plot. The date of first flower and peak flower date were analyzed by

taking the mean of each species in a plot, then by taking the average of each species'

flowering time mean to get a plot mean. The native neighbor full model analysis

included neighbor treatment (three levels - early, late, and mixed), target invasive (early

and late), and year (2008 and 2009). Tukey-Kramer multiple means comparison tests

were used to differentiate between mean comparisons.

When necessary, data were transformed to meet ANOVA assumptions. All

means in tables and graphs were appropriately back-transformed. When data

transformations were not sufficient to meet ANOVA assumptions, I utilized the non-

parametric Scheirer-Ray-Hare extension of the Kruskal-Wallis test (Sokal and Rohlf

1994). Non-parametric multiple means comparisons were calculated with a Dunn's test

all-pairwise comparison (Hollander and Wolfe 1999).

Results

Target Invasive

Significant higher-level interactions between year of measurement, neighbor treatment,

and target invasive prevented analysis of most of these main effects. Thus, results are

presented separately for each invasive by year.

Survivorship- Year 1 survivorship was high for both target invasives (86.5% for early

target invasive *H. matronalis*, 96.2% for late target invasive *N. cataria)*. There was no

effect of neighbor presence/absence, neighbor phenology, or functional group diversity

on survivorship of either target invasive. However, early *H. matronalis* targets grown

with conspecific neighbors had significantly lower survival (63.2%) than targets grown

with heterospecifics (89.8%) ($F_{1,108}$=6.45, p<0.0125).

In year 2 of the study (2009), there was a marked decline in average survivorship

of early invasive *H. matronalis* (64.3%), mostly due to over-winter mortality. *Hesperis*

matronalis targets grown without neighbors had lower survivorship (16.67%) than those

grown with neighbors (64.25%) ($F_{1,55}$=6.79, p<0.0118). Growth with conspecific

neighbors also led to lower survivorship (23.07%) than growth with heterospecific

neighbors (77.98%) in *H. matronalis*, indicating facilitation by the native neighbors

($F_{1,55}$=9.08, p<0.0039).

In contrast, year 2 late invasive *N. cataria* survivorship only declined slightly to

88.8% of the original plants, with no effect of neighbor presence/absence or

heterospecific / conspecific identity. As in the first year of the study, year 2 native

neighbor phenology and functional group diversity did not affect survivorship of either

target invasive species.

Target invasive characteristics- In year 1, the early target invasive *H. matronalis* did not

flower or fruit, but in year 2, *H. matronalis* average date of first flower was over 46 days

earlier than late target invasive *N. cataria*, ($F_{1,77}$=317.76, p<0.0001), with a peak

flowering date of over 65 days earlier ($F_{1,87}$=128.53, p<0.0001). On average, the late

invasive *N. cataria* was significantly larger than early invasive *H. matronalis* in terms of

vegetative biomass in both year 1 ($F_{1,78}$=4.38, p=0.0395) and year 2 ($F_{1,83}$=73, p<0.0001)

(Figure 1). The late invasive *N. cataria* also produced significantly more flowers

($F_{1,109}$=146.02, p<0.0001) and significantly greater seed mass ($F_{1,93}$=83.12, p<0.0001)

than early invasive *H. matronalis* in year 2 when both species reproduced (Appendix C,

Table 2).

Effect of neighbor presence / absence- The presence of neighbors reduced the vegetative

biomass of both the early invasive *H. matronalis* ($F_{1,41}$=10.62, p<0.0023) and late

invasive *N. cataria* ($F_{1,37}$=62.21, p<0.0001) in year 1, however neighbors had no effect

on the biomass of either target invasive in year 2 (Figure 1). Late invasive *N. cataria*

showed the more extreme response to neighbor presence, as solo targets produced over

13.5 times as much biomass as those targets surrounded by neighbors. Neighbor

presence also reduced late invasive *N. cataria* flower production ($F_{1,65}=205.55$,

p<.0.0001) and led to a later date of first flower in Year 1 ($F_{1,68}=7.73$, p=0.007)

(Appendix C, Tables 1 and 2), though *N. cataria* peak flowering date was unaffected.

Nepeta cataria seed mass followed the same pattern as vegetative biomass, with seed

mass significantly reduced by neighbor presence ($F_{1,114}=43.62$, p=<0.0001) in year 1. In

year 2, neighbor presence had no effect on any flowering traits or seed mass for either of

the target invasives.

Effect of intra- vs. interspecific neighbors – The target invasive vegetative biomass

response to intraspecific competitors was species- and year-specific. Intraspecific

competition led to a lower vegetative biomass for early invasive *H. matronalis* in year 1

($F_{1,41}=10.67$, p=0.0022) but not in year 2 ($F_{1,28}=0.7$, p=0.4114), and for the late invasive

N. cataria in year 2 ($F_{1,57}=10.59$, p=0.0019), but not in year 1 ($F_{1,37}=0.1$, p=0.7593)

(Figure 1). There was no difference between intra- and interspecific competition on any

flowering variables or seed mass for *N. cataria* in year 1 or *H. matronalis* in year 2

(Appendix C, Tables 1 and 2). *Nepeta cataria* flower counts could not be conducted in

year 2 in monocultures due to the density and position of stems, thus the effects of

intraspecific competition on phenological variables could not be tested.

Effect of native neighbor phenology - There was no effect of native neighbor phenology

31

on early invasive *H. matronalis* or late invasive *N. cataria* vegetative biomass, flower count, date of first flower, or peak flowering date in either year 1 or year 2. However, the seed mass of late target *N. cataria* was significantly less when planted with early native neighbors in year 1 ($F_{1,35}$=19.11, p<0.0001), providing some support for the resource pre-emption hypothesis. However, there was no effect of neighbor phenology on the seed mass of either target in year 2 (Appendix C, Tables 1 and 2).

Effect of phenological functional group diversity – Comparison of the mixed neighbor treatments to the average of the early and late neighbor treatments allowed the effect of functional group diversity to be analyzed. Late invasive *N. cataria* date of first flower was significantly earlier in the mixed neighbor treatments that contained two functional groups than in the average of the two single functional group treatments ($F_{1,68}$=7.78, p=0.0069) in year 1. This indicates that greater functional group diversity led to an earlier initial flowering date in the late invasive. However, there were no significant effects of phenological functional group diversity on any other measured traits of either *N. cataria* or *H. matronalis* in either year (Appendix C, Table 1).

Native neighbors

Native neighbor community characteristics – Survivorship of the native neighbors was equivalent across target treatments in both years, though the number of viable plots (those that were not removed due to excessive neighbor mortality) decreased from 96% in Year 1 to 47% in Year 2 (not including plots that were previously harvested), largely due to

over-winter mortality.

In year 1, when all plants were transplanted into the field, neighbor flowering phenology was later than expected, as the experimental species needed to reach a minimum size or resource storage requirement before initiation of flowering. As expected, date of first flower of the early natives was significantly earlier than the mixed natives and late natives ($F_{2,225}=42.11$, $p<0.0001$; Appendix C, Table 3), but there was no difference in peak flowering date. Flowering phenology was more typical in year 2, as all the surviving natives that were established in year 1 emerged from established roots that spring. Early neighbors and mixed neighbors had an earlier date of first flowering ($F_{2,75}=5.76$, $p=0.0047$) and peak flowering date ($F_{2,75}=6.89$, $p=0.0018$) than late neighbors.

Native Neighbor Competitive Response

To verify that native neighbor treatment performance was similar when planted with both the early and late target invasive treatments, I analyzed native neighbor competitive response to the targets. If there is an unknown effect of target treatment on the neighbors, this could lead to bias in the interpretation of the target results (K. Barry Dissertation, Chapter 1). In year 1, there was no effect of target invasive treatment on any of the measured neighbor traits (Appendix C, Table 3). In Year 2, however, there was a significant negative effect of the late target invasive *N. cataria* on late native neighbor vegetative biomass ($F_{1,75}=6.43$, $p=0.0133$; target x neighbor interaction: $F_{2,75}=6.5$, $p=0.0025$), fruit mass ($F_{1,75}=3.04$, $p=0.0805$), and flower count ($F_{1,19}=16.18$, $p=0.0007$) (Appendix C, Table 3). The late target invasive *N. cataria* also caused a

reduction in fruit mass in the mixed neighbor treatments.

Discussion

The competitive responses of the target invasive species to neighbor presence/absence, conspecific or heterospecific identity, and phenology were highly variable and strongly dependent on neighbor treatment, year, and plant trait measured. The dependence of competition on plant age, trait, and even phenology have been shown before (Farrer et al. 2009, Leger and Espelend 2010, Zhang and Lamb 2012). However, differences in experimental species, habitats, settings, experimental designs, and methods, along with measurements, and species-specific responses among studies make direct comparisons between different studies difficult. The examination of competitive interactions in this study among species in varying contexts using similar metrics of performance demonstrated the high degree to which the results of plant competition can be context-dependent.

Effect of neighbor presence/absence

In year 1, when both target invasives and native neighbors were co-established as seedlings, neighbor presence reduced the vegetative biomass of both the early invasive *H. matronalis* and late invasive *N. cataria*. This result was expected and is consistent with other target-neighbor studies examining the effects of competitor presence on individual target plants, including *H. matronalis* (Hwang and Lauenroth 2008). However, the negative influence of neighbor presence was only temporary, as the presence of neighbors did not affect the growth of either of the target invasives in year 2. This result

could be due to a decrease in the competitive effect of the neighbors or an increased tolerance of competition by the target. However, the native neighbors were on average taller and produced more biomass in their second year of growth, making a decrease in their competitive effect unlikely. After establishment in their first year of growth, both of the invasives' competitive responses to neighbors were minimal, a characteristic that may aid in colonization of established communities. The limited response by the invasive species may be due to a greater allocation of resources to root growth and higher leaf photosynthetic rates (Drenovsky et al. 2008), since they are related to slower initial above-ground plant growth but ultimately lead to establishment in dense plant communities. Some invasives have also been shown to perform better than native species in deep shade (Reinhart et al. 2006). In the early target *H. matronalis*, the change in competitive response may be due in part to the change in growth form from a rosette in year 1 to a bolting stem in year 2, a life history strategy common among biennial and in some perennial species. Energy stored during the rosette stage is reallocated towards stem production in the second or subsequent years of growth. As bolting plants draw resources from stored energy reserves in their roots, their current requirements for light and nutrients are subsequently reduced (Bazzaz 1997), limiting their competitive response to neighbors. Alternatively, allocation of biomass towards below-ground growth may have made above-ground biomass more susceptible to neighbor competition in the first year. This is in contrast to subsequent years, where above-ground biomass, due to the presence of reproductive structures, would be of greater importance.

The lack of any competitive response of late invasive *N. cataria* to neighbors in year 2 and the significant decline in neighbor performance in plots that contained *N.*

35

cataria indicates that established *N. cataria* individuals were strong competitors for resources. *Nepeta cataria* total flower count responded strongly to competitor presence in year 1, but flowering schedule (date of first flower and peak flowering date) was relatively fixed across treatments. Competitor presence caused *N. cataria* targets to begin flowering an average of 10 days later than solo plants, though there was no correlation between target invasive biomass and average flowering date (r=-0.207, p=0.2729). Stress from competition may have caused this delay in flowering, as limiting resources (including soil nutrients, water, and light) are well known to affect flowering phenology in plants (see Harper 1977). Between years, late invasive *N. cataria* demonstrated considerably more variability in flowering time, with year 1 plants in 2008 initiating flowering nearly three weeks later than year 2 plants in 2009, showing that even a late-flowering experimental species experienced a delayed phenology in their first year of growth.

There was a negative correlation between target biomass and neighbor biomass for both target invasives when averaged across all neighbor treatments, but this relationship was driven by the solo treatments for both targets. This is due to the negative logarithmic (L-shaped) relationship between target and neighbor biomass, a well-known effect (Goldberg and Fleetwood 1987), where the greatest decrease in target biomass is at low neighbor biomass values. There is little effect of increasing neighbor biomass after the inflection point (e.g. Howard 2001, Daneshgar and Jose 2009), indicating both a saturation point at low neighbor biomass and a limited response to neighbors above this threshold size.

Initial target invasive size was a poor predictor of future size, with no relationship

between size (height) as a seedling and size (height or biomass) at the end of year 1, and no relationship between year 1 size (height or biomass) and year 2 size (height or biomass). Similarly, overwinter mortality was not associated with plant size for either target invasive (data not shown). Though *N. cataria* vegetative biomass was highly correlated with fruit mass within a season, biomass and height in year 1 were not correlated with fruit mass in year 2. While vegetative biomass is often used as a proxy for reproductive biomass (Gibson 1999), I found vegetative biomass to be a poor predictor of reproductive output in subsequent years. More dynamic estimates including measurements of photosynthesis, respiration, and nutrient resorption are known to provide better estimates of future reproductive output than static estimates such as biomass (Ashman 1993).

I also analyzed neighbor competitive response to determine whether the target invasive treatments had a differential influence on neighbor treatment characteristics. In Year 2 the late native neighbor treatments produced significantly less vegetative biomass, fruit mass, and flowers when grown with the late invasive *N. cataria* compared to when grown with the early invasive *H. matronalis* (Figure 1). By measuring both the competitive response of the targets to their respective neighbors and the competitive response of the neighbors to the different targets, I discovered a differential competitive response in Year 2 that otherwise would not have been detected. The competitive effect of the late invasive on the late neighbors could have potentially led to an unintended bias in my analysis of the target invasive results. However, despite the differential response of the late neighbor to the two invasives, I determined that this result would not adversely affect the planned analyses and interpretation of the target competitive response

(Appendix D).

Intraspecific and interspecific competition

Niche theory predicts that intraspecific competition should be more intense than

interspecific competition due to greater similarity between competing individuals

(Hutchinson 1959). The relative intensity of intraspecific competition is believed to

contribute to species diversity within a community, but relatively few studies test these

predictions among plants in natural communities (Silvertown 2004). Studies that

explicitly compare intra- and inter-specific competition have not shown a consistent

pattern between their relative intensities (Gurevitch et al. 1992; Farrer and Goldberg

2010). The intensity of inter- and intraspecific competition appears to be largely species-

specific, with some species facilitating one another and performing significantly better in

monoculture than with heterospecifics (e.g. Weigelt et al. 2007). However, performance

can also be dependent on abiotic factors such as water availability (Wiegelt et al. 2005,

Luo et al. 2010) and nutrient timing (James and Richards 2007). As the number and

placement of individuals in my experimental plots was strictly controlled, Tilman's R*

hypothesis (Fargione and Tilman 2002) could not be directly tested, as plots were not

allowed to each establish an equilibrium. However, the differences in monoculture

results across years may indicate that the R* for these species populations could fluctuate

with plant age. These species-specific outcomes appear to fit the predictions of the

neutral theory more than niche theory (Leger and Espeland, 2010), since intraspecific

competition was not consistently stronger than interspecific competition with target

species or year. However, these results and the disparate results of others may in part be

caused by artifacts from different experimental designs (Farrer and Goldberg 2010), as studies in more natural old field communities without controlled plant densities show that non-neutral process are dominant in community assembly and invader resistance (Fargione, Brown, and Tilman, 2003, Tilman 2004).

Intraspecific competition reduced the biomass of the early invasive *H. matronalis* rosette in Year 1, but there was no difference between intra- and interspecific competition in Year 2 when *H. matronalis* bolted and flowered. Intraspecific competitive intensity may have been reduced due to the taller and narrower bolting growth form of *H. matronalis* leading to less competition for light. Soil nutrient uptake in year 2 was also likely more limited than in the first year, when plants were storing energy for later reproduction. Despite similar responses in biomass (and other traits) of surviving plants in both treatments in year 2, *H. matronalis* monoculture treatments experienced some of the highest over-winter morality of any of the neighbor treatments. Though I did not directly measure leaf litter, the rapid decomposition of *H. matronalis* leaves (K. Barry, personal observation) suggest that heterospecific litter may have been important in facilitating over-winter survival of *H. matronalis* targets, as leaf litter can facilitate plant survival over winter (Farrer et al. 2010). However, the direction of neighbor effects changed with year and season, as heterospecific neighbors reduced *H. matronalis* growth in Year 1, then facilitated overwintering rosettes, and finally had no effect on bolting individuals in Year 2. Contrasting trends between plant survival and subsequent vegetative and reproductive biomass have been shown before (Callaway et al. 1996, Farrer and Goldberg 2010), as neighbors can simultaneously facilitate seedling establishment by reducing the probability of mortality but later limit the total size and

probability of reproduction of adults.

Effect of phenology on competition

I found little support for effects of neighbor phenology or phenological group diversity on target invasive competitive response, indicating that temporal partitioning of resources was not a major factor in these artificial communities. Analysis of the neighbor treatments did reveal strong competition between the late target *N. cataria* and the late neighbors, though this competition was realized through a reduction in neighbor biomass, not target biomass. This result partially supports the fluctuating resources hypothesis, and illustrates the potential of even a single *N. cataria* individual to affect neighbor community growth. The habit of early invasive *H. matronalis* individuals to emerge rapidly and early and reproduce as a bolting stem in year 2 may have limited the effect of neighbor competitors and provided a temporal escape from both late phenology and early phenology neighbors. This hypothesis could not be directly tested as sequential size measurements were not made across the season. The only target invasive trait that was affected by neighbor phenology was late invasive *N. cataria* seed mass, which was lower when grown with early native neighbors. This indicates that resource pre-emption may have been more important to competitive outcomes than a shared phenology with neighbors. The effects of competition were also detected between late invasive *N. cataria* and the mixed native neighbors, though it was mixed native neighbor fruit mass that was reduced, not an *N. cataria* trait. It was unclear why the fruit mass of the mixed native neighbors, but not the early or late neighbors, would be affected, but it may be due to higher order interactions among the natives and between the natives and target invasive

40

in the mixed native species combinations.

Species that grow, flower, and fruit at times when neighbors are using fewer resources experience less competition for those resources (Shea and Chesson 2002). This provides an advantage in resource pre-emption, which is believed to be one of the most important factors in plant competition (Craine et al. 2005). Several studies have shown examples of how temporal separation of growth is an important aspect in the interactions between alien and native species (Hooper 1998, Fargione et al. 2003, Crawley 2004). Early growing species (such as *Lonicera* spp.) take advantage of limited competition for light and soil nutrients early in the season (McEwan et al. 2009, Wolkovich and Cleland 2011), while also precluding the usage of resources by later-growing species. Even a slightly earlier emergence date can make the difference between becoming one of the few dominant or many subordinate individuals within a population or a community (Verdu and Traveset 2005).

The lack of phenological complementarity amongst the target invasive individuals and their neighbors could have been caused by several factors. Resource competition may not have been closely enough correlated with flowering phenology in these species, or variation within neighbor functional groups may have made phenological effects harder to detect. Although I attempted to control for non-phenological variation as much as possible, some neighbor species were considerably larger than others (both *Helenium* spp. were consistently larger than other natives, while both *Asclepias* spp. were consistently smaller). It is also possible that the phenological treatments were simply not different enough for there to be phenological complementarity. I selected congeneric species pairs that were all perennial forbs to determine whether phenological

complementarity could be demonstrated independent of major differences in growth form or life history. Within these *a priori* constraints, variation in phenology in the selected experimental species was not as great as phenological variation across other species that did not fit the other criteria. Plants that were very similar in growth form, habitat, and other characteristics except for widely different phenologies were a challenge to find. This is in part because many extremely early flowering plant species are biennials or spring ephemerals that do not maintain growth or biomass during the majority of the growing season, largely avoiding above-ground competition with other later flowering species altogether. Complementarity in phenology may be inextricably tied to variation in life history, growth form, and other traits, and thus difficult to test truly independent of those traits. Temporal variability of resources such as rain is well known to drive phenological differences in arid systems, but in temperate systems in particular, more work is needed to separate the influence of plant phenology from other correlated traits such as growth form (e.g., forb vs. grass), life history (perennial vs. biennial), and photosynthetic pathway (C_3 or C_4).

In summary, though neighbor presence/absence and conspecific or heterospecific neighbor identity affected the performance of the target invasives, I found little evidence for complementarity in native neighbor phenology to limit invasive establishment and success in my experimental study system. However, if I had terminated the experiment after a single season, I would have concluded that native neighbors have a strong influence on target invasive growth and neighbor presence and would ultimately limit target invasive species population growth. Instead, target invasive size reduction was only temporary, as mature plants showed no residual effects of suppression at the

seedling stage. Though multi-year competition studies are not uncommon (Tilman 1999, Schmidkte et al. 2010), many manipulative competition experiments are terminated after a single season of growth (Gibson, 1999, Leger and Espelend 2010, Farrer and Goldberg 1999, Mangla et al. 2010). Target-neighbor experiments that last for multiple years are particularly uncommon (but see Farrer and Goldberg, 2010). As competition is typically most intense at the seedling stage or during the first year of growth, results from seedling or young plant competition experiments often are considered to be representative of mature plant competition (Farrer and Goldberg 2010; Mangla et al. 2010). However, as competitive effects on targets and competitive responses of neighbors may change over time, my results show that even second season outcomes cannot be predicted by first year results. The importance of multiyear studies in understanding perennial plant interactions should not be underemphasized. To achieve a greater understanding of the outcomes of competition, experiments should include multiple stages of a plant's life history. Otherwise, competition studies are likely to over-estimate or under-estimate the actual importance of competition in influencing long-term community composition.

Figure 1. Vegetative biomass competitive response means ($\pm$ one SE) for target invasives and native neighbors: Year 1 (a) and Year 2 (b) for early (*Hesperis matronalis*) and late (*Nepeta cataria*) target invasive vegetative biomass by neighbor treatment. Means for (a) and (b) are presented on a $\log_{10}$ scale. Year 1 (c) and Year 2 (d) native neighbor vegetative biomass by target invasive treatment. Comparisons were conducted within each neighbor treatment. Means sharing the same letter are not significantly different at $a=.05$. Note differences in scale of y-axis between targets (a and b) and neighbors (c and d).

Figure 1.
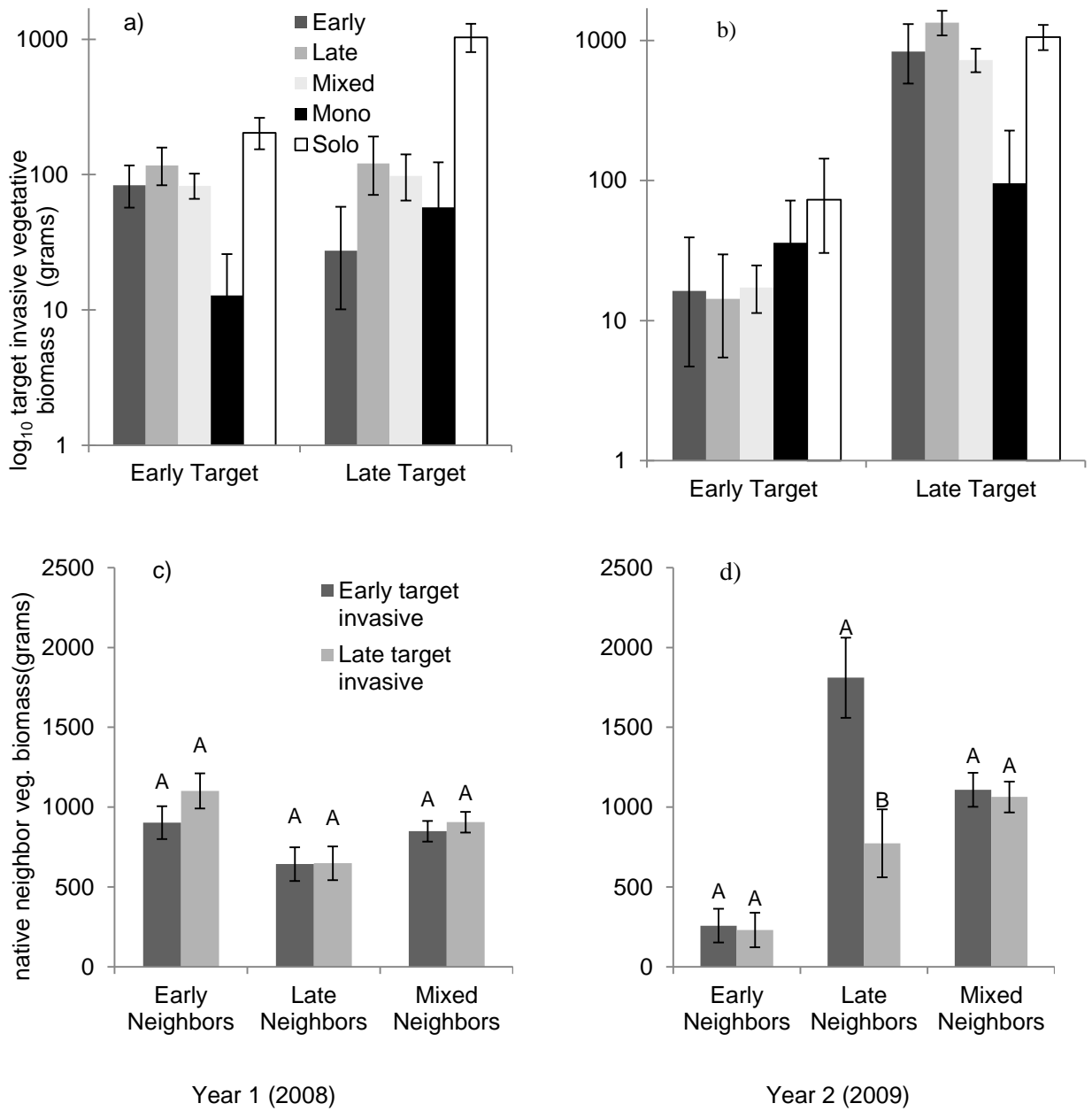
Chapter 3: The role of introduction time and neighbor phenology in the competitive response of two invasive species

Abstract

Differences in introduction time have been shown to play a large role in the subsequent establishment and spread of invasive species. Within a season, factors such as native plant phenology can also affect invasive species success. In this study, I use a target-neighbor design to quantify the effect of introduction time and native neighbor species phenology on the performance of two invasive species, early flowering *Hespersis matronalis* and later flowering *Nepeta cataria*. Introduction time of the invasive species was manipulated by either coestablishing the invasives as seedlings simultaneously with native neighbors, or introducing the invasive seedlings one year after native neighbor communities were established. I predicted that target invasives would experience the most intense competition when (1) introduced into an established native community versus when coestablished concurrently with native neighbors, and (2) when invasive species shared the same growth and flowering phenology as their surrounding native community, due to temporal niche overlap and a lack of phenological complementarity.

Native neighbor species phenology had a greater influence on target invasive performance than introduction time of the invasive. However, the intensity of the two invasive species' competitive responses varied between invasive species and the performance trait examined. Only the early invasive *H. matronalis* experienced greater competition when introduced into established native communities as predicted. Both *H.*

matronalis and the late invasive *N. cataria* exhibited a reduction in biomass when introduced into late phenology native communities, demonstrating an effect of phenological complementarity on the late invasive, but not the early invasive. This result suggests the presence of other unmeasured covarying factors that caused both targets to react in a similar fashion to the native neighbor phenology treatments. Reproductive performance of coestablished *N. cataria* suggests that resource pre-emption may be more important in invasive species establishment than native community phenological complementarity. I advocate for further investigations of the early stages of invasive species establishment to provide additional insights into factors that may prevent their establishment and spread.

Introduction

The existence of a latency period or lag time after exotic plant introduction and before exotic plant invasion has long been recognized (Elton 1958; Richardson et al. 2006). These latency periods vary considerably in their length (Daehler 2009; Kowarik et al. 1995), though for the majority of invasive plant species there is little information on this stage of their invasion (Marsico 2010). Several factors are hypothesized to contribute to this lag period, including the gradual increase in genetic diversity obtained through multiple introductions or hybridization with native species, time needed to adapt to climatic and abiotic conditions of the new range, selection for traits that confer competitive ability, or elimination of costly traits related to defense against predators and pathogens of the native range (Elton 1958; Kowarik et al. 1995; Barney et al. 2008; Meyer et al. 2004). A given invasive species may benefit from one or more of these

adaptations once introduced into the novel range. Because many introduced non-native

species are unnoticed at first due to a small population size and limited range, the small

fraction of introduced plants that ultimately become invasive remain undetected as

'sleeper weeds' until they begin a rapid range expansion (Richardson et al. 2006).

Consequently, the latency period between the introduction and invasive spread of an

exotic plant is a crucial, but challenging time period to study. For those introduced

species that have already become invasive, this latency period has already passed.

Despite the inability to observe the original latency period in a species that has

already become invasive, much can be learned about temporal changes in invasive

interactions with native communities through experimental manipulations. Within a

season numerous factors can affect invasive success including rainfall patterns, abiotic

conditions, and native plant phenology. Across seasons, more mature plant communities

have been shown to resist invasion to a greater degree than newly established or recently

disturbed communities (Sheley, Mangold, and Anderson 2006; McGlone et al. 2011;

Catford et al. 2012). However, the effect of introduction time on invasive species success

warrants further study.

All plant propagules, regardless of species or origin, must overcome the

limitations of space, light, water, and soil nutrients imposed by the existing plant

community. The success of a given propagule is a function of the characteristics of the

propagule, the environment, and the native community (Harper 1977). Introduction into

a community during a period when competition for resources is lower (and thus more

resources are available) would then increase the probability of successful establishment

and growth. The fluctuating resources hypothesis states that an invasive species should

benefit when there is an increase in the availability of unused resources (Davis et al. 2000). Resource availability can occur either through an influx of resources into the system, or a decrease in the usage or uptake of resources from the system. Resource usage within a community is a function of both community phenology and community age, as communities with higher resource complementarity take up resources over a longer time period within a season, and communities of an intermediate age typically take up more resources than very young or very old communities (Sheley, Mangold, and Anderson 2006).

To assess the role of invasive species introduction time on their competitive performance within native species communities, I utilized a target-neighbor design to determine the response of two invasive species to different introduction times into experimental native neighbor communities exhibiting variable phenologies. One target invasive exhibits an early-season growth and flowering phenology, and the other invasive species exhibits a relatively later growth and flowering phenology. Both invasives were planted into native communities consisting of plants with early, late, or a mixture of early and late growth and flowering phenologies. Introduction time was manipulated by coestablishing half of the invasive targets simultaneously with native neighbors, and introduction of the remaining invasive targets one year after native neighbor communities were established. I predicted that target invasives would experience the most intense competition when (1) introduced into an established native community versus when coestablished simultaneously with native neighbors, and (2) when invasive species shared the same growth and flowering phenology as their surrounding native community, due to temporal niche overlap and a lack of phenological complementarity.

50

Methods

Study Species- The native species chosen for the artificial communities in this study were selected based on flowering phenology data garnered primarily from Strausbaugh and Core's "Flora of West Virginia" (1977) and "Finding Wildflowers in the Washington-Baltimore Area" by Fleming, Lobstein, and Tufty (1995). The USDA Plants Database (USDA, NRCS http://plants.usda.gov) was used to confirm that (1) all native species were perennial, (2) native status was correct, and (3) the study site was within each species' native range. My goal was to limit variation among species other than growth and flowering time. In this study flowering time was used as a proxy for resource competition, as soil nutrient uptake typically decreases when a plant produces reproductive structures (Thimann 1980), and flowering time is known as a good indicator of changes in resource use (Thimann 1980; Cleland et al. 2006). Plant species were chosen with respect to maximum size, growth form, and habitat such that all invasive and native neighbor species used in this study are herbaceous perennial forbs of comparable size. Species were chosen as congeneric pairs and represent two phenological functional groups: an early-season or late-season growth and flowering phenology. This phylogenetically controlled design was chosen so that in every treatment plot with native neighbors, there was always a species from each of the four genera (*Asclepias*, *Coreopsis*, *Liatris*, and *Helenium*) present. The native species in the early flowering functional group were *A. viridis, C. lanceolata, L. squarrosa,* and *H. flexuosum,* while the late flowering functional group was comprised of *A. tuberosa, C. tripteris, L. pilosa,* and *H. autumnale.* All native species were in the Asteraceae, except for the *Asclepias* species in

51

the Asclepiadaceae. The two invasive species were also chosen based on phenology, with *Hespersis matronalis* (Brassicaceae) the early-flowering invasive, and *Nepeta cataria* (Lamiaceae) the late-flowering invasive. *Nepeta cataria* is a perennial, and *H. matronalis* grew as an iteroparous perennial in my study, although it can grow also as a biennial (USDA, NRCS http://plants.usda.gov). These two invasive species have been investigated little in terms of their effects on ecosystems or invasibility, though anecdotally, they are known to be weedy and aggressive (Hwang and Lauenroth 2008, USDA, NRCS http://plants.usda.gov).

Site - Research plots were located at the Blandy Experimental Farm (78°3'30" W, 39°3'28" N) a University of Virginia research station located in Boyce, Virginia. Further details on site preparation can be found in K. Barry Dissertation (Chapter 2).

Planting design - I utilized a target-neighbor design modified from that used in Weigelt et al. (2007). This design was chosen because it allowed focus on the competitive response of an individual plant, the target. The target invasive was planted in the center of the plot, surrounded by a ring of eight native species neighbors, resulting in an octagonal planting design. Native neighbors were planted 15 cm from the target invasive, a distance selected to allow plants to experience competition, yet limit mortality. Each of the eight native neighbors was approximately 11.5 cm from adjacent neighbors in the plot. In order to understand all plant species interactions and ascertain that neighbor competitive response was consistent across target invasive treatments, I also measured

the traits of the surrounding neighbor individuals, as both targets and native neighbors can influence one another during growth (K. Barry Dissertation, Chapter 1).

Experimental Treatments - Both of the target invasives (the early phenology *H. matronalis* and late phenology *N. cataria*) were planted individually in each of four neighbor phenology treatments – early native neighbors, late native neighbors, mixed native neighbors, and solo (no neighbors) treatments. The early-flowering and late-flowering native neighbor phenology treatments were comprised of the four early and four late native species, respectively. Mixed native neighbor treatments contained both early and late species. Three out of six possible mixed treatment species combinations were planted but were analyzed as a single treatment due to neighbor mortality (see below).

Target invasive introduction time was manipulated by coestablishing half of the target invasives simultaneously in their native neighbor experimental plots all as seedlings in April 2008. The other native neighbor communities were also planted at the same time, but without a target invasive. These treatment plots overwintered and emerged from roots in the spring of 2009, when target invasive seedlings were introduced into the center of these established native neighbor plots. There were initially 15 replicates for each of the 12 original target-neighbor combinations (two invasives x early, late, three mixed plots and solo targets) within each introduction time treatment. In total, 360 experimental plots composed of over 2760 plants were established. Plots were randomly arranged within each of three fenced enclosures which also served as blocks for statistical analysis.

The species diversity and generic diversity of the neighbors were designed to be constant across each of these treatment plots, with the eight neighbors represented by four native species (two replicates per species) in each treatment plot. The conspecific neighbors were planted opposite each other, with planting *order* (which pairs were adjacent to each other) randomized within each treatment plot. Phenological functional group type (early, late and mixed) and number (early and late treatments each had one functional group, while the mixed treatment was comprised of two functional groups – both early and late) could then be manipulated while maintaining a constant species diversity. This approach de-coupled functional group diversity from species diversity, and avoids the sampling effect issues outlined by Huston (1997).

Unexpectedly, both *Liatris* species experienced near 100% mortality. This mortality altered the experimental design by reducing the number of native neighbors in the early, mixed, and late treatments from eight to six. The loss of the two *Liatris* species also caused a difference in the number of neighbors between the native neighbor treatments (six) and the invasive monoculture treatments (eight). However, there was no significant effect of this difference in neighbor number on any neighbor community or target invasive traits (data not shown), so analyses within native neighbor treatments, and between native neighbor treatments and monoculture treatments, were conducted as initially planned. The few *Liatris* individuals that survived were extremely small, and the effect of their presence within the neighbor treatments was deemed to be negligible. Additionally, each mixed neighbor treatment now contained either one early and two late species (Mixed sub-treatments A and B) or two early and one late species (Mixed sub-treatment C), as opposed to containing two early and two late species as originally

54

designed. This caused a late phenology species bias to be present in the mixed treatments and led to a cautious interpretation of mixed native neighbor comparisons.

Measurements- Once flowering began (June in 2008, April in 2009), date of first flowering was recorded on all individuals within each plot (both target invasive and native neighbor species) and flower production was measured weekly on all individuals until the end of September. From these phenological data I calculated the total flower production (the sum of all weekly counts) for each individual. After height of all species was measured *in-situ* (November 2008 for coestablished plots and November 2009 for introduced plots), the above-ground biomass for each plant in each plot was harvested. All plants were dried at 60° C in a drying oven for nine days before they were weighed. See K. Barry Dissertation (Chapter 2) for additional details on field measurements. Among the native species, fruits were weighed, but the invasive species seeds were removed from their fruits and weighed independently. This approach was necessary due to the difficulty in cleanly separating *N. cataria* fruits from connected leaves and stems. Fruits of *N. cataria* along with any attached stems and leaves were ground down using a series of screens and sieves, so that the hard seeds could be removed from the processed vegetative material. For consistency between the invasives, seeds were also separated from fruits and weighed independently for *H. matronalis*. Seed mass was highly correlated with fruit mass for both of the invasives and each of the neighbor species, allowing us to use these two separate measures of reproductive effort for the target and neighbor species (data not shown). It was not possible to effectively separate the roots of the targets and neighbors, subsequently roots were not harvested in the field.

Analyses –To assess target invasive species competitive response I utilized the natural log response ratio (lnRR), which allowed for a direct comparison of competitive intensity of different treatments through comparison of competitive effects to their respective solo treatment values (James and Richards 2009). The lnRR was calculated using the following equation (with vegetative biomass as an example): $\log_e$ (biomass of target invasive grown with neighbors / biomass of target invasive in solo (no competition) treatments). The natural log response ratio was calculated in the same way for all other target invasive species traits (seed mass, flower production, and date of first flowering). A negative lnRR value indicates competition from native neighbors, while a positive value indicates facilitation by native neighbors. An lnRR that is not significantly different from zero indicates no effect of native neighbors on invasive species performance.

In addition to analyzing the target invasive species competitive response (the effect of the native neighbor phenology treatments on the target invasive species), I also chose to analyze the native neighbor competitive response (the effect of each invasive target species on their native neighbors). I took this approach to determine whether there was any difference in native neighbor phenology treatments when planted with either the early or late target invasive species, and thus identify any bias within the native neighbor treatments across the two invasive targets (K. Barry Dissertation, Chapter 1). All native neighbor phenology treatment plots traits were analyzed at the plot level using original values, as the native neighbor data, lacking a comparable neighbor "solo" treatment, could not be transformed with lnRR. Neighbor phenology treatment plot height,

vegetative biomass, and fruit mass were calculated by taking the sum of the measurements of each of the individual neighbors for each treatment plot. Neighbor date of first flowering was analyzed by taking the mean of each species in a plot, then by taking the average of each species' flowering data mean to get a plot mean.

The target and neighbor competitive responses were analyzed with a mixed model ANOVA in SAS version 9.2 (SAS Inc., Cary, N.C) using the Mixed Procedure. The full target invasive model consisted of three neighbor treatments (early, late, and mixed neighbors), two target invasive treatments (early and late) and two target invasive introduction times (coestablished and introduced). Due to frequent significant interactions between factors, the two target invasive species were analyzed separately, and then, if neighbor x invasive introduction time interactions persisted, the effect of neighbors on each target invasive was further analyzed separately by invasive introduction time.

To determine the intensity of competition across different late invasive *N. cataria* plant traits, the lnRR of vegetative biomass, flower production, and seed mass were also analyzed by introduction time (coestablished and introduced) in a separate mixed model analysis. The full native neighbor model included the same factors as the target invasive full model. Blocks were treated as a random factor for both invasive target and native neighbor analyses. Target survivorship was analyzed using Proc GLIMMIX using a logit link. Relationships between variables were determined using Spearman correlations.

Tukey-Kramer adjusted multiple means comparison tests were used to differentiate between treatment means. When necessary, data were transformed to meet

ANOVA assumptions. All reported means were appropriately back-transformed. When data transformations were not sufficient to meet ANOVA assumptions, I utilized the non-parametric Scheirer-Ray-Hare extension of the Kruskal-Wallis test (Sokal and Rohlf 1994). Non-parametric multiple means comparisons were calculated with a Dunn's test all-pairwise comparison (Hollander and Wolfe 1999).

Results

Target invasive competitive response

Survivorship was high for both target invasives (early invasive *H. matronalis*: 89.21%, late invasive *N. cataria*: 96.89%), with a marginally significant difference in survivorship between the two invasives ($F_{1,97}$=3.45, p<0.0663). However, there was no effect of introduction time or native neighbor phenology treatment on the survivorship of either invasive species (Table 1).

When both invasive species were examined together in the same model I found that neighbor presence (independent of neighbor phenology) reduced the vegetative biomass of the late target invasive *N. cataria* over twice as much as that of the early target invasive *H. matronalis* ($F_{1,111}$=20.21, p<0.0001).

Early invasive *H. matronalis* vegetative biomass was reduced more by introduction into an established native community than by coestablishment with native neighbors ($F_{1,56}$=3.36, p=0.072; Table 2). Additionally, only those *H. matronalis* targets that were introduced into the established community were influenced by native neighbor phenology (Figure 1a), such that mixed native neighbor phenology treatments significantly reduced *H. matronalis* biomass more than early native neighbor phenology

treatments, with no significant difference between early and late phenology neighbor treatments. Early invasive *H. matronalis* plants did not flower within their first year of growth, thus only vegetative biomass data are available for this target invasive.

Late invasive *N. cataria* showed no main effect of either introduction time or native neighbor phenology on vegetative biomass, although there was a significant interaction between the two factors ($F_{2,54}$=5.27, p=0.0081) (Table 2). When late invasive *N. cataria* was introduced into established native neighbor communities, competition was more intense for *N. cataria* when grown with late and mixed phenology neighbors than with early neighbors (Figure 1b). Coestablished *N. cataria* biomass was unaffected by differences in native neighbor phenology.

Late invasive *N. cataria* seed mass and flower production results followed a similar pattern to vegetative biomass, although there were differences in which treatments were significantly different. In coestablished *N. cataria* treatments, both flower production ($F_{2,43}$=4.47, p=0.0172) and seed mass ($F_{2,87}$=7.4, p=0.0011) were significantly reduced only by the early phenology native neighbors. Coestablished late invasive *N. cataria* growing with early native neighbors flowered significantly later than those growing with late neighbors. Among the introduced *N. cataria* the opposite trend was seen, with flower production reduced significantly more by the presence of late and mixed phenology native neighbors ($F_{2,28}$=6.02, p=0.0067) than early or mixed phenology neighbors. Date of first flowering and seed mass of introduced *N. cataria* were unaffected by native neighbor phenology treatments.

Late invasive N. cataria trait response comparison

Late invasive *N. cataria* targets coestablished with native neighbors experienced significantly greater reduction in seed mass than *N. cataria* targets that were introduced into established neighbor communities ($F_{1,114}$=58.15, p<0.0001; Figure 2). No effect of introduction time was detected on the competitive responses of *N. cataria* in terms of vegetative biomass or flower production. Looking at the magnitude of the overall trait responses pooled across introduction time, flower production was the performance trait most reduced by native neighbor presence, followed by seed mass, then vegetative biomass ($F_{2,248}$=12.59, p<0.0001) (Figure 3).

Native Neighbor community competitive response

Native Neighbor community characteristics were dependent on neighbor phenology and varied with target introduction time, but responded similarly to the two invasive species (Table 4). The experimental design dictated planting the "introduced" neighbor plants was at the same time as the "coestablished" plants, thus the introduced native neighbors treatments were one year older than the coestablished neighbor communities at harvest time. Among native neighbors coestablished with the target invasives, the early neighbors had greater vegetative biomass (Figure 1c), seeds mass, and flower production, but were shorter in stature than either the mixed or late neighbor phenology treatments (Table 5).

In the native neighbor phenology treatments grown with introduced target invasives, early neighbors produced significantly less biomass (Figure 1d), fewer flowers, and less fruit mass compared to the mixed and late neighbors (Table 5). However, there was no differential effect of target invasive species on any aspect of native neighbor

performance, indicating that native neighbor treatments were similar to one another, and no neighbor bias occurred between the two target invasive treatments (K. Barry Dissertation, Chapter 1).

Discussion

Invasive introduction time and native neighbor phenology were both determinants of target invasive performance, though competitive responses varied between the early and late phenology target invasives and the plant trait examined. Across all experimental treatments, the presence of native neighbors independent of phenological treatment had a greater effect on late invasive *N. cataria* than on early invasive *H. matronalis*, with the biomass of *N. cataria* reduced twice as much as that of *H. matronalis*. The target invasives were predicted to experience greater competition when grown with neighbors exhibiting the same growth and flowering phenologies. However, only the late invasive *N. cataria* when introduced into established late native neighbor communities fit this prediction. Target invasives were also expected to experience greater competition when introduced into a community, as opposed to when they were coestablished with neighbors. However, this was only true for the early target invasive *H. matronalis*, as *N. cataria* performance was not affected by introduction time into the experimental native communities.

Plant introduction time into a population or community is known to be an important determinant of its subsequent establishment, growth and reproductive success. Small differences in germination date can lead to considerable variation in subsequent growth, biomass and fruit production (Brenton and Facelli 2008; Abraham, Corbis, and

61

D'Antonio 2009). I had predicted that competitive intensity would be greatest for targets introduced into an established community due to the differences in plant size and ability to acquire resources between the seedling target invasives and the established native neighbors. However, I observed no consistent effect of introduction time on target invasive biomass. Only the biomass of the early invasive *H. matronalis* was reduced significantly when introduced into an established native neighbor community, as late invasive *N. cataria* was strongly affected by neighbors independent of introduction time.

Low tolerance for competitor presence is not necessarily related to the strength of competitive effect on neighbors (Goldberg and Werner 1983), and competitive effect and response can also change across life history stages (Lamb and Cahill 2006; Zhang and Lamb 2012). The presence of first year late invasive *N. cataria* plants had no effect on its native neighbors, but invasive individuals just one year older can resist the effects of their native neighbors entirely, and exert a competitive effect strong enough to reduce native neighbor vegetative and reproductive traits (K. Barry Dissertation, Chapter 2). This change in competitive response between *N. cataria* seedlings and adult plants may be due to a shift in competitive response strategies from a persistent, slow growth rate, to an escape strategy of rapidly growing and outcompeting neighbors (Zhang and Lamb 2012).

This target invasive species-specific response in introduction time may stem from differences in growth form and shade tolerance. Early invasive *H. matronalis* plants grew only as a rosette in this experiment with flowering occurring only in their second year of growth (K. Barry Dissertation, Chapter 2). Rosettes are inherently limited in height, as all leaves originate at or near ground level, leading to a relative disadvantage

for the rosette when competing for light with plants that exhibit upright growth forms (Sletvold 2005). Consequently, the greater tolerance that early invasive *H. matronalis* exhibits with neighbors present may be due to a higher tolerance for limited light availability than late invasive *N. cataria*, a factor known to drive differences in competitive response in native and invasive species (Goldberg and Werner 1983, Hager 2004). However, despite a potential greater tolerance of low light compared to late invasive *N. cataria*, light still appears to be a limiting resource for growth in some instances, as neighbor presence has been found to reduce *H. matronalis* biomass even with addition of increased water and soil nutrients (Hwang 2010).

The phenology of the native neighbor communities was shown to be more influential than the invasive targets' own introduction times in determining target invasive performance. When target invasives were coestablished with native neighbors, neighbor phenology did not have a significant effect on target biomass. However, other performance traits, including late invasive *N. cataria* seed mass and flower production, showed a consistently greater inhibition when late invasive *N. cataria* was grown with early neighbors than with mixed or late neighbors, contrary to my prediction. The invasive targets introduced into pre-existing established neighbor communities showed a much stronger response to neighbor phenology than those coestablished with native neighbors, and both early and late introduced target invasives were most inhibited when grown with the late neighbor treatments. These results suggest that in coestablished communities, competition through resource pre-emption may be more important than temporal niche overlap in reducing invasive species reproductive success (Craine et al. 2005).

Phenological complementarity is thought to benefit species that grow either earlier or later than their neighbors such that they have temporal competitive release (Wolkovich and Cleland 2011). I had predicted greater competitive intensity in treatments where there was phenological overlap between the target invasives and native neighbors, that is, when there was no phenological complementarity. However, only the coestablished late invasive *N. cataria* results fit my prediction, as it was less successful in the late neighbor treatments. The early invasive *H. matronalis* responded similarly to neighbor phenology as *N. cataria*, with *H. matronalis* also experiencing reduced growth when grown with the late neighbors. Subsequently, my data provide only partial support for temporal variation in plant phenology as a mechanism leading to unutilized resources available for invasive establishment (Gerlach and Rice 2003; Wolkovich and Cleland 2011), a prediction of the fluctuating resources hypothesis (Davis et al. 2000).

The similarity of the early and late target invasive biomass competitive responses suggests the presence of other factors causing both targets to react in a similar fashion to the neighbor phenology treatments. One factor that appears to be driving the responses is neighbor biomass. The pattern of competitive intensity for the two invasives was closely associated with the presence of a significant correlation between target biomass and neighbor biomass. Across the coestablished invasive treatments there was no effect of neighbor phenology treatment on invasive target vegetative biomass. Similarly, no relationship was found between coestablished target invasive and native neighbor total biomass (vegetative plus reproductive biomass) for either early invasive *H. matronalis* (r=-0.202, p=0.2510, n=56) or late invasive *N. cataria* (r=-0.186, p=0.2933, n=54). Conversely, target invasives introduced into established native communities were more

strongly affected by their native neighbors' phenology, and there was a negative

relationship between the total biomass (vegetative and reproduction) of native neighbors

and total biomass of each invasive species (*H. matronalis:* r=-0.519, p=0.0039, n=56; *N.*

cataria: r=-0.606, p=0.0010, n=54). Thus introduced target invasive biomass was related

to neighbor biomass, but coestablished target biomass was independent of the biomass of

neighbors.

The differential influence of native neighbor biomass on target invasive growth

indicates that phenological complementarity may be partially driven by other traits that

are known to be correlated with phenology. Bolmgren and Cowan (2008) found that

across the Swedish flora, there was a positive relationship between flowering phenology

and plant height, such that later flowering plants were taller than those that flowered early

in the season. Among my introduced native neighbor treatments, the late phenology

neighbor treatments plants were significantly taller and produced significantly more

biomass than the early neighbors (Figure 1c; Table 5). Native neighbor phenology

treatment characteristics differed among coestablished neighbors in their first year of

growth, and while the early neighbors were also significantly shorter, they produced

significantly more biomass than the mixed and late neighbor phenology treatments

(Figure 1c). Thus neighbor biomass data mirrors the responsiveness of the target

invasive treatments to neighbor phenology, such that neighbor treatments with the

strongest effect on invasive targets were those neighbors that produced the greatest

vegetative biomass.

Most notably, it was differences in phenology among the already established

native neighbors, and not the coestablished neighbors, that most determined differences

in target invasive species response. If this relationship is consistent across plant

communities, invasive species newly introduced into established communities should

show a strong negative response to temporal niche overlap from the native community

and exert little influence of their own onto the established native community due to their

relatively small size (Goldberg and Werner 1983). However, it is unknown whether this

advantage would continue among older groups of plants (or mature communities), as the

advantage of a progressively older native perennial plants in an herbaceous community

would likely decrease within a few years with respect to relative size. However, size

hierarchies are common within populations of individual species and plant communities

(Dudash 1991). The decreasing size difference between introduced and established

plants would be due to the smaller relative differences in age between introduced and

established plants after each year, and the limited lifespans of many herbaceous plants

restricts the potential age differences. Except in long-lived species, any age advantages

that natives would have over an introduced invasive would likely be short-term.

Moreover, once invasive plants become established, the influence of natives upon

invasives could be reduced or reversed. However, the inhibitory effect of accumulated

plant material (i.e. grass thatch or leaf litter) (Facelli and Pickett 1991; Evans et al. 2011)

could continue even after the life of an individual plant. Thus longer-term and wider-

scale studies are needed to determine the community-level outcomes of invasive species

introduction time.

The intensity of late invasive *N. cataria* competitive response varied with the

fitness traits measured and whether they were vegetative or reproductive performance

measures. The introduced *N. cataria* experienced less severe competition resulting in

less of a reduction in seed mass than when coestablished with native neighbors (Figure 2). This greater relative allocation to seeds mass when *N. cataria* has limited access to resources due to the presence of established neighbors may be an adaptation to increase current seed output at the expense of uncertain future survival and growth (Harper 1977). Reproductive traits (flower production and seed mass) showed a more extreme response to neighbor presence than vegetative biomass. Analogous results have been found in many species (e.g., Lovett Doust 1989; Tremmel and Bazzaz 1995), as reproductive allocation has been shown to decrease when plants are grown in competition. Noteworthy is the intensity of competition on flower production compared to the lesser effect on seed biomass (and vegetative biomass) (Figure 3). Any community effect of decreasing sexual reproduction in *N. cataria* is uncertain, as even small individuals exhibit prolific seed production. The extreme response of flower production compared to the more modest decline in fruit production also indicates that resources or pollen deposition may be limiting conversion of flowers to fruits in my experimental field plot communities (Knight et al. 2005).

Characteristics of the native community are major determinants of invasive species success in their introduced range. However, both the introduction time of an invasive species relative to the age of the native community, as well as the growth and flowering phenology of each also plays an important role in determining invasive species establishment and performance. The increasing number of introductions of non-native species warrants more research to focus on the earliest stages of introduction, so that invasive lag times and initial interactions with native communities can be better understood.

Acknowledgements

Table 1. Average percent survivorship ±1 SE (in parentheses) for early invasive *H. matronalis* and late invasive *N. cataria* by introduction time and native neighbor phenology treatment.

	Early Invasive	Late Invasive
Coestablished Invasive		
Early Neighbors	0.933 (±.071)	1.000 (±0.000)
Late Neighbors	0.867 (±0.090)	1.000 (±0.000)
Mixed Neighbors	0.893 (±0.046)	0.978 (±0.025)
Introduced Invasive		
Early Neighbors	0.714 (±0.166)	1.000 (±0.00)
Late Neighbors	1.000 (±0.00)	1.000 (±0.00)
Mixed Neighbors	0.944 (±0.060)	0.917 (±0.059)

Table 2. Target invasive natural log response ratio (lnRR) mixed ANOVA results. ETI and LTI represent the early target invasive (*H. matronalis*) and the late target invasive (*N. cataria*) respectively. DFF: date of first flower. The early target invasive did not produce flowers or fruit in any of the treatments. Seed mass data could not be transformed to meet ANOVA requirements, so the variables Neighbor and Introduction Time were analyzed separately with non-parametric tests. Bold typeface indicates a significant p-value, and italic typeface indicates a marginally significant p-value.

	Vegetative Biomass		Seed mass	Flower Production	DFF
	ETI	LTI	LTI	LTI	LTI
Neighbor	$F_{2,56}=1.48$ p=0.2365	$F_{2,54}=0.63$ p=0.5389	$F_{2,113}=5.02$ p=**0.0082**	$F_{2,73}=2.14$ p=0.1244	$F_{2,54}=7.09$ p=**0.0018**
Introduction Time	$F_{1,56}=3.36$ p=*0.0720*	$F_{1,54}=0.78$ p=0.3818	$F_{1,114}=58.15$ p<**0.0001**	$F_{1,73}=0.15$ p=0.7016	$F_{1,54}=0.01$ p=0.9879
Neighbor x Introduction Time	$F_{2,56}=2.16$ p=0.1245	$F_{2,54}=5.27$ p=**0.0081**	N/A	$F_{2,73}=10.36$ p=**0.0001**	$F_{2,54}=5.78$ p=**0.0053**

Table 3. Late target invasive (*N. cataria*) competitive natural log response ratio (lnRR) means ±1 SE (in parentheses) by introduction time and native neighbor phenology treatment. DFF: date of first flower. Replication (number of experimental plots) is noted by n. More negative lnRR values indicate a greater competitive response by the invasive species.

	Seed mass	n	Flower Production	n	DFF	n
Coestablished Invasive						
Early Neighbors	-1.310 (±0.755)	3	-1.390 (±1.358)	3	0.108 (±0.029)	3
Late Neighbors	-2.815 (±0.010)	14	-7.072 (±0.960)	14	0.053 (±0.020)	2
Mixed Neighbors	-2.184 (±0.222)	26	-5.751 (±0.502)	27	0.023 (±0.013)	7
Introduced Invasive						
Early Neighbors	-4.073 (±0.032)	3	-6.548 (±0.821)	3	0.179 (±0.086)	3
Late Neighbors	-3.533 (±0.180)	6	-4.482 (±0.569)	6	-0.113 (±0.105)	14
Mixed Neighbors	-3.592 (±0.112)	17	-3.913 (±0.399)	22	0.116 (±0.056)	27

Table 4. Native neighbor competitive response mixed ANOVA results for vegetative biomass, fruit mass, plant height, and

flower production. Bold typeface indicates a significant p-value, and italic typeface indicates a marginally significant p-value.

	Vegetative Biomass		Fruit mass		Height		Flower Production	
	Co-established	Introduced	Co-established	Introduced	Co-established	Introduced	Co-established	Introduced
Neighbor	$F_{2,63}=8.22$ p=**0.0007**	$F_{2,58}=11.48$ p<**0.0001**	$F_{2,63}=9.57$ p=**0.0002**	$F_{2,58}=4.46$ p=**0.0158**	$F_{2,145}=24.94$ p<**0.0001**	$F_{2,59}=11.18$ p<**0.0001**	$F_{2,149}=15.96$ p<**0.0001**	$F_{2,57}=7.73$ p=**0.0011**
Target Invasive	$F_{1,63}=1.57$ p=0.2146	$F_{1,57}=0.01$ p=0.9217	$F_{1,63}=0.64$ p=0.4263	$F_{1,57}=0.86$ p=0.3578	$F_{1,146}=0.05$ p=0.8227	$F_{1,59}=0.02$ p=0.8993	$F_{1,149}=15.96$ p=0.2241	$F_{1,57}=0.02$ p=0.8766
Neighbor x Invasive	$F_{2,63}=0.56$ p=0.5714	$F_{2,58}=0.21$ p=0.8138	$F_{2,63}=0.80$ p=0.4554	$F_{2,58}=0.33$ p=0.7178	$F_{2,146}=0.15$ p=0.8606	$F_{2,59}=0.41$ p=0.6626	$F_{2,149}=0.62$ p=0.5369	$F_{2,57}=0.82$ p=0.4462

Table 5. Native neighbor competitive response means ±SE (in parentheses) for fruit mass, height, and flower production means ± SE by target introduction time and native neighbor phenology treatment. Replication (number of experimental plots) is noted by n. Early and late invasives species experimental treatment plots are pooled since there was no significant difference between them.

| Native Neighbor	Fruit mass (g)		Height (cm)		Flower Production (n)	
Coestablished targets		n		n		n
Early	208.34 (±24.32)	50	291.40 (±14.92)	44	2489.751 (±292.250)	44
Late	141.87 (±24.67)	58	496.94 (±19.96)	42	2294.161 (±283.610)	42
Mixed	122.84 (±20.20)	171	338.90 (±10.22)	136	1330.871 (±159.087)	136
Introduced targets						
Early	81.87 (±28.01)	7	191.67 (±248.26)	6	758.07 (±280.65)	7
Late	136.59 (±31.12)	21	665.59 (±40.72)	21	1982.29 (±404.17)	21
Mixed	171.84 (±27.07)	53	512.58 (±29.63)	53	1833.76 (±327.27)	53

Figure 1. Vegetative biomass competitive response means $\pm$ SE. Early target invasive (*Hesperis matronalis* (a) and late target invasive (*Nepeta cataria*) (b) natural log response ratio (lnRR) results when either coestablished or introduced into native neighbors phenology treatments. Means for (a) and (b) are presented on a $\log_{10}$ scale. More negative values indicate more intense competition. Native neighbor phenology treatment competitive response to early and late target invasives when invasives are coestablished (c) and introduced (d). Comparisons were conducted within each native neighbor phenology treatment. Means sharing the same letter are not significantly different at $a=.05$. Note differences in scale of y-axis between targets (a and b) and neighbors (c and d).

Figure 2. Late invasive *Nepeta cataria* natural log response ratio (lnRR) competitive responses means $\pm$ 1 SE of vegetative biomass, flower production and seed mass by introduction time. More negative values indicate more intense competition. Means sharing the same letter are not significantly different at $a=.05$. The early invasive *Hesperis matronalis* did not flower or fruit, precluding analysis.
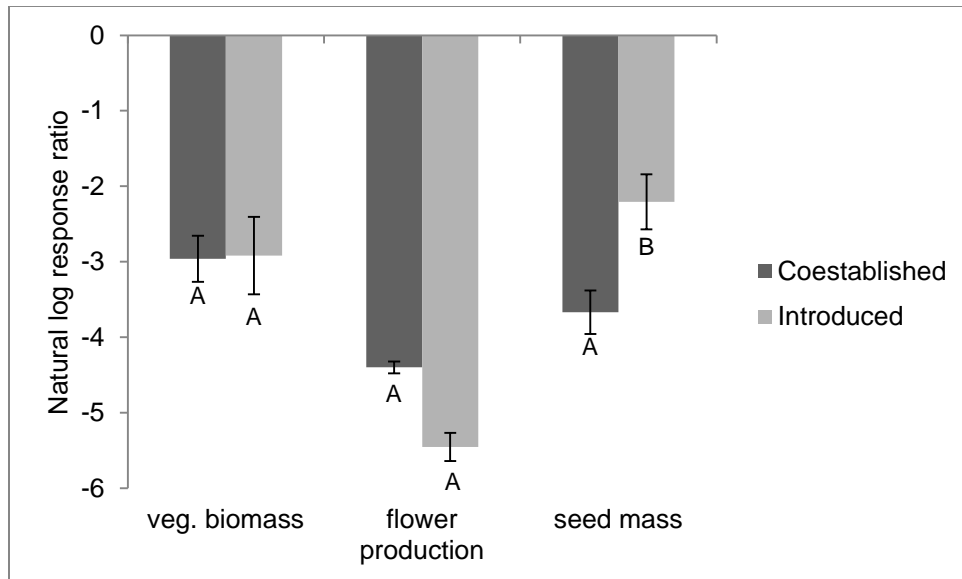
Figure 3. Late invasive *N. cataria* lnRR competitive responses means $\pm$1 SE of vegetative biomass, flower production and seed mass. Results are pooled across introduction time. More negative values indicate more intense competition. Means sharing the same letter are not significantly different at $a=.05$.
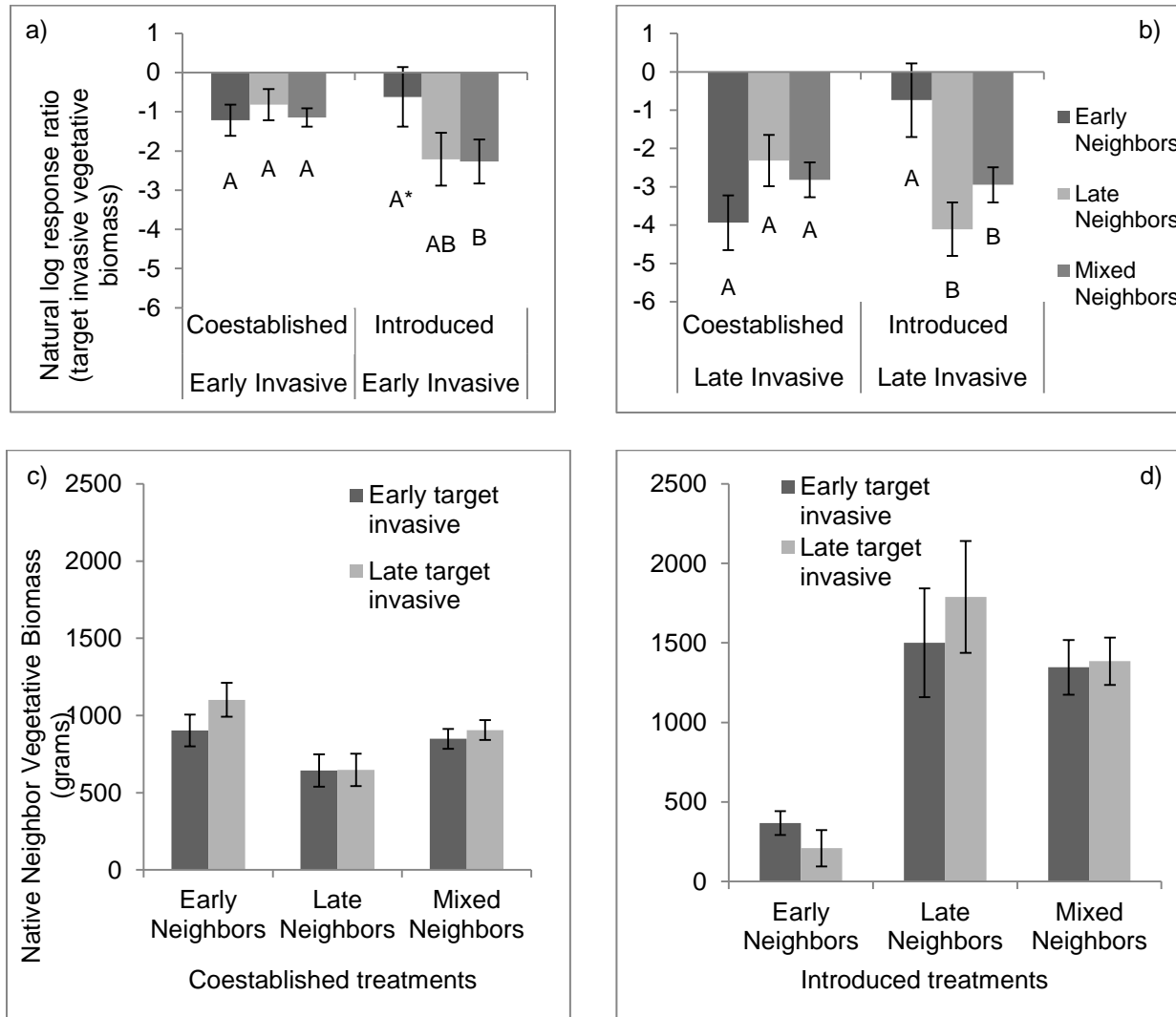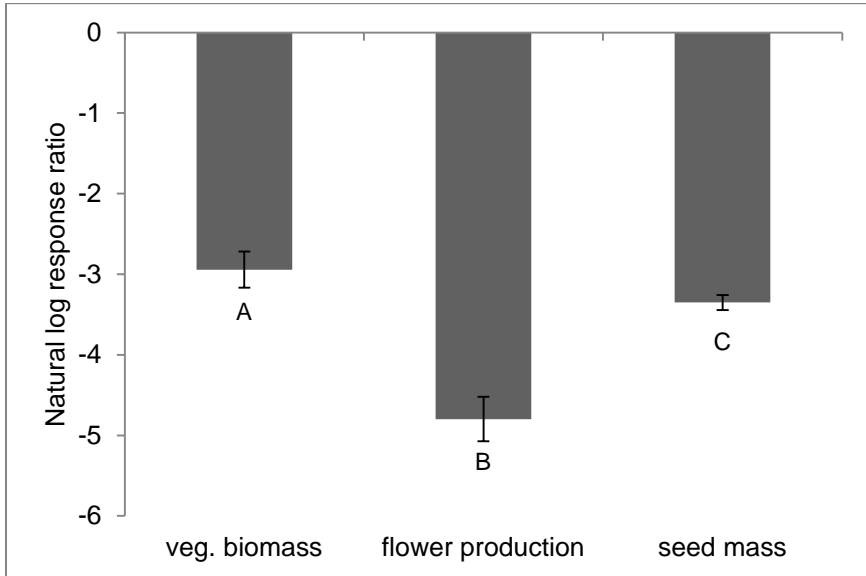
Figure 1.

Figure 2.

Figure 3.

Chapter 4: The role of plant phenology on native and invasive species growth and soil nitrogen uptake.

Abstract

Soil nutrient resources are essential to plant growth. Plant roots compete for access to nitrogen, but not necessarily at the same locations within the soil profile and time periods within a season, or even in the same chemical forms. Through phenological complementarity, plants requiring the same resources may partially avoid competition with one another because of nutrient usage at different times of the season. This complementarity can be especially pronounced between natives and invasive species, where temporal niche segregation allows some invasive species to establish during periods within a season when there is less competition for resources.

My experimental approach was to compare biomass production and nitrogen uptake in both field and greenhouse experiments. I compared early and late flowering native and invasive monocultures, as well as polycultures comprised of early, late and a mixture of early and late flowering native species. I predicted that (1) flowering phenology would be correlated with nitrogen uptake schedule for both native and invasive species, and (2) invasive species would produce more biomass and uptake more nitrogen than native species.

In the field I found some evidence for phenological complementarity, with the greatest growth and biomass accumulation in my mixed flowering native field plots.

Additionally, field nitrogen levels over the course of the season significantly differed between early and late phenology treatments. In the field native species accumulated significantly more biomass than invasive species, contrary to my prediction. However, in the greenhouse invasive species accumulated more biomass than native species. I detected no clear patterns between phenology or between invasive and native species status, and their nitrogen uptake in the greenhouse. More work is required to understand how plant resource dynamics interact to affect community primary productivity and resistance to non-native species invasion.

Introduction

Soil nutrient resources are essential to plant growth, and experimental (Ashton et al. 2010; Brown and Rice 2010) and theoretical studies (Craine 2005a) have done much to elucidate below-ground competition for resources. Plants primarily acquire nutrients through the soil, particularly nitrogen, the most important macronutrient for plant growth (Chapin 1980). Plant roots compete for access to nitrogen, but not necessarily at the same locations within the soil profile and time periods within a season, or even in the same chemical forms (Fargione and Tilman 2005; Ashton et al. 2010). By experimentally increasing plant community diversity, both primary productivity (Hille Ris Lambers et al. 2004) and soil nitrogen usage have been shown to increase (Hooper and Vitousek 1998). This can be due to several factors, including a simple sampling effect of larger plants (Huston 1997), or the presence of plants with nitrogen fixing *Rhizobium* (Fargione et al. 2007) A third cause, niche complementarity, is due to niche differences among species such that there is a greater production of biomass or usage of

resources in a polyculture than in a monoculture (Qin et al. 2003). Increased species trait

complementarity and greater biomass production are thought to limit invasive species

success (Wolkovich and Cleland 2011).

Variation in plant nitrogen uptake as plants grow, flower, and fruit is better

understood among agricultural species, but is less studied in natural systems of native or

invasive species. Nitrogen uptake has been shown to decrease once flower production

starts in poinsettia (Scoggins and Mills 1998), and rice (Sheehy et al. 2004), decrease

during flowering in tomato (Terabyashi et al. 1991), and plateau at flowering in corn

(Dharmakeerthi et al. 2006). As such, flowering time is known as a good indicator of

changes in resource use in plants (Thimann 1980, Cleland et al. 2006).

Through complementarity of growth and flowering phenology, plants requiring

the same resources experience less competition from one another because of nutrient

usage at different times of the season (Qin et al. 2003). Complementarity between

natives and some invasive species can be especially pronounced, where temporal niche

segregation allows some invasives to grow during periods when native community

primary productivity, and thus resource uptake, is low (Wolkovich and Cleland 2011).

To investigate the role of temporal niche overlap in the potential ability of a

native community to resist invasion by a non-native species I compared biomass

production and nitrogen uptake in complementary field and greenhouse studies.

Specifically, I compared both native and invasive species exhibiting both early and late

growth and flowering phenologies. In field plots, invasive species monocultures and

native species monocultures and polycultures were planted to determine the effects of

native or invasive status and phenological functional group diversity on treatment plot

biomass and soil nitrogen level. In the greenhouse, plants were grown singly in pots without competition, fertilized with ^{15}N isotopes, and harvested in a staggered design to determine the relationship between plant growth, phenology and plant nitrogen content. I predicted that growth and flowering phenology would be correlated with nitrogen uptake schedule for both native and invasive species, and that invasive species would produce more biomass and take up more nitrogen than native species.

Methods

Study Species- Experimental species were chosen with respect to plant size, growth form, and habitat such that all native and invasive species used in the study are herbaceous perennial forbs of comparable size. The native species were chosen as congeneric pairs and represent two phenological functional groups: early-season flowering and late-season flowering. This phylogenetically controlled design was chosen so that in every native species polyculture treatment, there was always a species from each of the four genera (*Asclepias*, *Coreopsis*, *Liatris*, and *Helenium*) present. The early flowering functional group native species were *Asclepias viridis, Coreopsis lanceolata, Liatris squarrosa,* and *Helenium flexuosum,* while the late functional group was comprised of *Asclepias tuberosa, Coreopsis tripteris, Liatris pilosa,* and *Helenium autumnale.* The two invasive species were also chosen based on phenology. *Hespersis matronalis* (Brassicaceae) was the early-flowering invasive, and *Nepeta cataria* (Lamiaceae) was the late-flowering invasive. These invasive species have been investigated little in terms of their effects on ecosystems or invasibility (but see Hwang and Lauenroth 2008), though anecdotally they are known to be weedy and aggressive (USDA, NRCS http://plants.usda.gov).

Field Experiment

Site- Research plots were located at the Blandy Experimental Farm (78°3'30" W,

39°3'28" N) a University of Virginia research station located in Boyce, Virginia. Further

details on site preparation can be found in K. Barry Dissertation (Chapter 2).

Experimental Treatments- I utilized a target-neighbor design for all experimental plots

modified from that used in Goldberg and Fleetwood (1987). One central plant, the target,

was planted in the center of the plot, surrounded by a ring of eight neighbors, resulting in

an octagonal planting design. This planting approach was followed because this study is

embedded within a larger field experiment to assess the role of native species phenology

on invasive species establishment (See K. Barry Dissertation, Chapters 2 & 3). This

experimental design was beneficial because it allowed for a consistent planting pattern to

be used for both single species monocultures and multi-species polycultures. It also

provided greater control of plant number and density than if plants were sown or spaced

randomly. Neighbors were planted 15 cm from the targets, a distance selected to allow

plants to experience competition, yet limit mortality. Due to this octagonal arrangement,

each of the eight neighbors was approximately 11.5 cm from adjacent neighbors. The

targets and neighbors were planted in the center of a 0.6 meter x 0.6 meter plot, with plots

spaced one meter apart from one another. Each plot was surrounded by landscaping

fabric to prevent growth of non-experimental species.

Each of the eight native species and the two invasive species were planted as

monocultures using the target-neighbor design planting pattern. In addition, there were

five native species polycultures – one treatment containing early phenology native species, one treatment containing late phenology native species, and three treatments containing combinations of early and late native species (the "mixed" treatments). The early and late treatments were composed of the four early and four late native species, respectively. I chose three out of the six possible combinations of early and late species that had the most disparate phenologies to form the "mixed" species treatments. Across the native polycultures, species diversity and generic diversity of the neighbors was designed to be constant, with the eight neighbors in each treatment plot represented by four species (two replicates per species). The conspecific neighbors were planted opposite each other, with planting *order* (which pairs were adjacent to each other) randomized within each treatment plot. This approach de-coupled functional group diversity from species diversity, and avoids the sampling effect issues outlined by Huston (1997). Phenological functional group number and functional group type could then be manipulated while maintaining a constant species diversity. In each native species polyculture plot a random species was selected from the neighbors of that plot to fill the target position.

Unexpectedly, both *Liatris* species experienced near 100% mortality. The loss of the two *Liatris* species caused a difference in number of species between the remaining monocultures and the polycultures, and also caused the mixed treatments to contain either two early and one late species, or vice versa. Because of these issues, all mixed neighbor treatments were analyzed together as one treatment, hereafter "mixed treatment". However, there was no effect of this difference in plant number per plot on treatment plot biomass (data not shown), so further analyses proceeded as initially planned. Plant

species mortality across experimental treatments was low (data not shown), except for the *H. matronalis* monocultures (K. Barry Dissertation, Chapter 2).

All plants in the experimental plots were planted in Spring 2008, allowed to overwinter, and then grow back in 2009. This approach was taken to give the experimental plots time to establish and demonstrate the phenology of mature plants, as well as avoid phenological artifacts during the first year of growth from seedling transplantation into the field. Above-ground biomass of all plants within each experimental plot was harvested in October 2009, dried in an oven for nine days at $60\,^{\circ}\mathrm{C}$, and then weighed. There were initially eight replicates planted for each of the eight native species monocultures and the native species polycultures (early, late and mixture of early and late species), and 15 replicates for the invasive monocultures. The unequal sampling is due to the larger experimental design (K. Barry Dissertation, Chapters 2 & 3).

Field Soil Probe Experiment – PRS (Plant root simulator)$^{\mathrm{TM}}$ probes from Western Ag Innovations, Inc. were used to detect available soil nitrogen. The probes consist of a resin membrane placed inside a 15 cm long flat plastic peg, which is then hammered into the ground. A pair of these probes (one for attracting anions and one for cations) was inserted into the ground near the center of the plot for four replicates of each treatment (monocultures of invasive and native species, and the three native polycultures (early, late, and mixed phenology). In the mixed phenology treatments, at least one of each subtreatment was selected. After approximately three weeks in the ground, all probes were removed and replaced by a fresh set. The first set of probes was inserted on June 9$^{\mathrm{th}}$, 2009, followed by replacements at June 30$^{\mathrm{th}}$, July 22$^{\mathrm{nd}}$, and August 18$^{\mathrm{th}}$. The last set

of probes (those placed on August 18th) was collected on September 9th. The probes were refrigerated upon removal from the ground and sent back to Western Ag Innovations for analysis for the anion ammonium (NH_4^+) and the cation nitrate (NO_3^-). Use of this technique allowed discernment of nitrogen availability during different stages of plant growth and phenology throughout the season. In order to limit costs, the four replicates per experimental treatment group at each of the four collection times were combined and processed as two replicates, upon which statistical analyses were performed.

Greenhouse Experiment

Experimental treatments – All plant species were germinated in flats in a mist room at the University of Maryland greenhouse, with *Coreopsis tripteris* and *Asclepias viridis* requiring a five month cold/moist stratification beforehand. Stratification was performed by sowing seeds in greenhouse flats and placing them outside to overwinter in cold frames. Chicken wire was used to prevent animal encroachment and disturbance of flats. Twenty replicates of each of the native species (except the two *Liatris spp.*) were sown. All native species and the two invasives were transplanted as seedlings of similar size singly into 6 inch (15.24 cm) diameter pots on July 25th (with an ~14.5-hour non-augmented day length) and divided into five blocks on two greenhouse benches at the University of Maryland research complex. There was little mortality across species, except for *A. viridis*, which limited replication for that species. After two weeks of growth with ambient temperatures (target day time temperature was 24°C throughout experiment) humidity was increased to 60% and day length was augmented with sodium vapor lights to a 16-hour day. Plants were watered frequently (every other day to twice a

day, as needed) in amounts of approximately 100 mL, with more added if soil was still dry. This approach was taken to prevent nitrogen from leaching from the plots. Longest stem length, or height of the highest leaf tips of rosettes, was measured approximately every two weeks. The initial stem length measurement was used as a covariate for the biomass and stem length analyses. Stem length was highly correlated with biomass (data not shown), thus only biomass results are presented here.

Staggered harvests were originally planned for every four weeks so that species could be harvested during different stages of their phenology (vegetative growth only, bud production, flowering, fruiting). The first harvest took place on August 26th. The 2nd harvest was conducted earlier than initially planned on September 10th, because the late invasive *N. cataria* had already begun to flower. However, by four weeks later, none of the other species had even produced buds, and thus the 3rd and 4th harvests were delayed (to December 8th, and December 22nd, respectively) to provide more time for phenological advancement. Unfortunately, even with long day length and warm temperatures that were expected to promote flowering, only three of the eight species: invasive *Nepeta cataria*, and natives *Asclepias tuberosa* and *Helenium flexuosum* ultimately flowered by the termination of the experiment four months after planting. Approximately one quarter of the 20 replicates were collected during each harvest.

Plants were fertilized weekly with a modified Hoagland's solution providing 20 mg of nitrogen per week throughout the experiment. Two weeks prior to the 2nd, 3rd, and 4th harvests, the replicates that were to be harvested were fertilized with a Hoagland's solution containing the stable isotope ^{15}N in place of unlabeled nitrogen, and were not fertilized again so that plants would take up nitrogen only in the form of the ^{15}N during

that time period. Two days before this ^{15}N fertilization, the pots were thoroughly leached

with ~400 mL of water to flush out any unlabeled nitrogen from previous fertilizations.

The first set of replicates was not fertilized with ^{15}N because it was harvested shortly

after planting and nitrogen uptake was expected of all species at this time as plant roots

grow following transplantation.

Aboveground biomass of each replicate was dried in an oven at $60\,°C$ for 9 days

and weighed. Roots could not be reliably separated from the soil to collect a

homogenous sample from each species, thus below-ground biomass was not measured.

Samples of leaves and flowers from each plant replicate were collected at harvest time for

preparation for the subsequent nitrogen analyses.

Greenhouse nitrogen measurements of leaves and flowers   Nitrogen measurements were

made through analysis with a stable isotope mass spectrometer on finely ground leaf and

flower samples. This revealed the percent nitrogen content and ^{15}N isotope concentration

for each leaf and flower sample. Due to the limited changes in growth and flowering

phenology among the plants only a subset of harvests that represented differences in

phenology were analyzed. At the 2nd harvest leaves of all plant species (six natives two

and invasives) were analyzed, as well as the flowers of *N. cataria*. At the 4th harvest only

the leaves and flowers of the three flowering species were analyzed (natives *A. tuberosa*

and *H. flexusosum,* and invasive *N. cataria*).

To reduce the total number and expense of nitrogen analyses, the four replicates

were combined to produce two replicates for each the selected species / harvest

combinations. Though replication was lowered, this increased the precision of the combined replicates while reducing the necessary number of mass spectrometer analyses.

Statistical Analyses –

To assess differences in biomass accumulation and nitrogen uptake among field experimental plots all analyses were conducted at the plot level. For biomass, this was done by taking the sum of the biomasses of each of the individuals in each treatment plot. Data from nitrogen probes were analyzed both with a repeated measures analysis, and also through a single total plot nitrogen metric calculated by summing the amount of both forms of available nitrogen (NH_4^+ and NO_3^-) across all four probe sampling time periods across the season. Although the probes and the calculated total nitrogen metric do not provide information on the absolute amount of nitrogen present in the soil, they are useful for comparing differences in nitrogen detected across the season among experimental treatment plots.

Field experiment biomass and soil probe data full statistical models included all eleven treatments (six native monocultures, two invasive monocultures, and three native polycultures). These treatments were further grouped and analyzed by functional group category (invasive monoculture, native monoculture, the 1-functional-group early and late phenology polycultures, and the 2-functional-group mixed phenology polyculture). Analyses were conducted with a mixed model ANOVA in SAS version 9.2 (SAS Inc., Cary, N.C) using the Mixed Procedure. Regression slopes were compared using a mixed model ANOVA, and Spearman correlations were used to determine the relationship between biomass and soil nitrogen.

In the greenhouse experiment plant growth and nitrogen uptake results were analyzed with a mixed model ANOVA of plant biomass and a mixed model repeated measures analysis of plant height / longest stem length. Plant stem length at time of planting was used as a covariate for both of these analyses, but the covariate did not substantially change the results of either trait, and thus was dropped from the final model. All nitrogen analyses for leaves and flowers (percent nitrogen and 15N isotope ratio) were conducted with a mixed model ANOVA.

In both field and greenhouse analyses, statistical contrasts were used to test differences between early and late phenology treatments (independent of native / invasive status) and native and invasive treatments (independent of phenology), respectively. Means were compared using Tukey-Kramer multiple means comparisons. When necessary, data were transformed to meet ANOVA assumptions. All measured character means in tables and graphs were appropriately back-transformed. When data transformations were not sufficient to meet ANOVA assumptions, I utilized the non-parametric Scheirer-Ray-Hare extension of the Kruskal-Wallis test (Sokal and Rohlf 1994). Non-parametric multiple means comparisons were calculated with a Dunn's test all-pairwise comparison (Hollander and Wolfe 1999).

Results

Field experiment –

When all experimental treatments were analyzed together I detected a significant treatment effect on above-ground biomass ($F_{10,54}=49.16$, $p<0.0001$), with a 34-fold difference in biomass between the largest biomass response (late native *C. tripteris*) and

smallest biomass response (early invasive *H. matronalis*) (Figure 1). Late phenology

treatments as a whole (late invasive and the three late native species monocultures, as

well as the late- species native polycultures) produced an average of ~7 times more

biomass than early phenology treatments (the early invasive and the three early native

species monocultures, as well as the early species native polycultures) ($F_{1,54}$=218.12,

<0.0001). Functional group category (invasive monoculture, native monoculture, the 1-

functional-group early and late polycultures, and the 2-functional-group mixed-

phenology polyculture) significantly influenced experimental plot biomass ($F_{3,63}$=2.98,

p=0.0381), as the native species mixed phenology treatment produced significantly more

biomass than the invasive monocultures (Figure 2). Finally, a contrast was conducted to

determine if native species groups pooled together (monocultures, 1-functional and 2-

functional) and invasive species monocultures pooled together accumulated biomass

differentially, but no significant difference was detected.

When all field soil probe treatments were analyzed in one model I found that total

nitrogen level (both forms of nitrogen, NH_4^+ and NO_3^-, summed across all four sampling

periods) varied significantly across treatments ($F_{10,19}$=6.66, p=0.0002), with a pattern

similar to, but not as variable as the biomass results (Figure 3). When all early phenology

treatments and all late phenology treatments were respectively pooled, I found that total

nitrogen level was significantly lower in late phenology plots than in the early phenology

plots, mirroring the biomass production of those treatments ($F_{1,19}$=9.44, p=0.0063).

When I examined the overall main effect of functional group category on soil nitrogen,

an a-priori contrast showed that native species treatments reduced soil nitrogen more than

the invasive species treatments ($F_{1,26}$=4.81, p=0.0374).

Across all field treatments (all native and invasive monocultures and all polycultures) I detected a relationship between treatment plot biomass and soil nitrogen availability, such that less nitrogen was detected in plots with greater biomass (r=-0.403, p=0.0274) (Figure 4). Subsequently, when plot biomass was used as a covariate for the nitrogen analyses, there was no longer an effect of phenology (early vs. late) on nitrogen level ($F_{1,28}$=1.36, p=0.258), though native species treatments still reduced nitrogen to a greater degree than invasive species treatments ($F_{1,25}$=5.72, p=0.0246), opposite my prediction.

Over the course of the field season, soil nitrogen increased in the early phenology treatments during the fourth sampling period (Fig 5a), but soil nitrogen generally continuously decreased for all late phenology season treatments (Figure 5b). A comparison of regression slopes indicated that the pooled early-treatment and pooled late-treatment regression slopes were significantly different from one another ($F_{1,218}$=5.11, p=0.0248). The growing difference in nitrogen levels (NH_4 and NO_3 combined) throughout the season is especially evident between the two invasive species, as during the first two sampling periods there was no significant difference in nitrogen levels, but a significant difference between nitrogen levels was detected in the last two sampling periods (Figure 6).

Greenhouse Experiment –

When I examined all harvest data collectively I found that biomass of all native and invasive plant species predictably increased across time ($F_{3,134}$=80.53, p<0.0001) and varied with species ($F_{7,134}$=82.01, p<0.0001) (Figure 7). I also detected a significant

species x harvest time interaction ($F_{21,134}=14.12$, p<0.0001) indicating that the plant species accumulate biomass at different rates. On average, the late phenology species produced significantly more biomass than early phenology species ($F_{1,134}=29.78$, p<0.0001). Additionally, the invasive species produced more biomass than the native species ($F_{1,134}=126.68$, p<0.0001) as predicted. Both of these results were largely driven by the late invasive *N. cataria*, which attained the greatest above ground biomass of all the experimental species in the greenhouse (Figure 7).

At the 2nd harvest there was a marginally significant effect of species on leaf percent nitrogen content ($F_{1,8}=2.95$, p=0.0765), and the invasive species had a higher percent nitrogen than native species ($F_{1,8}=5.11$, p=0.0537) (Table 1). However, no difference was detected in percent nitrogen content overall between early and late phenology species. Furthermore, I detected no differences in leaf ^{15}N isotope concentration, indicating that by the second harvest nitrogen uptake rates were similar across all of the study species independent of whether they were native or non-native (Table 1).

At the 4th harvest only the three flowering species (natives *A. tuberosa* and *H. flexusosum,* and invasive *N. cataria*) were analyzed for nitrogen content. No differences were detected in percent nitrogen or in ^{15}N isotope concentration between the leaves and flowers of the flowering species (Table 1).

When comparing plant species across the 2nd and 4th harvest dates of the three flowering species, both early native *H. flexuosum* ($F_{1,3}=13.8$, p=0.0339), and late invasive *N.cataria* ($F_{1,3}=4.28$, p=0.0839), showed a decrease in leaf percent nitrogen over time. However, no differences were detected among the three species in ^{15}N isotope

concentration between the two harvest dates, indicating that nitrogen uptake was similar across species across time. Since there were no differences between the floral and leaf tissue samples collected, only leaf analyses are shown.

Discussion

In the field experiment the vegetative biomass accumulated across the season was highly variable for the polycultures and the monocultures and of native and invasive species. Greater biomass production is a characteristic often associated with invasive plant species (Gerlach and Rice 2003; Van Kleunen et al. 2009; but see Schamp and Aarsen 2009), and though I found differences between biomass of invasives and natives when grown singly in the greenhouse, this pattern was not found in the plant monocultures in the field. Late phenology treatment field plots (including monocultures and polycultures) produced considerably more biomass than early phenology treatments, a relationship also found in K. Barry Dissertation (Chapter 3). Additionally, when field treatments were grouped according to their functional group categories, the invasive monocultures accumulated significantly less biomass than the mixed phenology native species treatment plots, possibly indicative of phenological complementarity. Mixed phenology treatments did not produce more biomass than the average of native monocultures, however, meaning that there was no overyielding within plots.

Phenological complementarity allows for greater biomass production due to shifts in temporal resource usage, and plays an important role in the maintenance of species diversity and greater primary productivity within some plant communities (Hooper 1998; Qin et al. 2003; Fargione and Tilman 2005; Wolkolwich and Cleland 2012). Temporal

93

niche segregation has been shown in different plant communities both between grasses and forbs (Martinkova et al. 2002), and woody and herbaceous plants (Golluscio et al. 2005). More diverse communities (those with a greater number and richness of species) have also been shown to use more resources due to seasonal complementarity (Hooper and Vitousek 1998; Fargione and Tilman 2005). However, fewer studies have used functional groups based on plant growth and flowering phenology to examine differential resource utilization without confounding factors of life history or growth form (Hooper and Vitousek 1998, Dukes 2002) as conducted in this study.

Total soil nitrogen levels in my field experiment were similar to, but not as variable across treatments as trends in biomass production. Although plot biomass was inversely correlated with soil nitrogen level, the amount of variation in the biomass-nitrogen relationship (Figure 4) indicates the association between above-ground biomass and below-ground nitrogen uptake is loose. Controlling for plot biomass caused the difference between early and late phenology plots to disappear, again suggesting a link between plant phenology and plant biomass. Across the Swedish flora species that flowered later in the season were taller than plants that flower earlier due to a longer growing season (Bolmgren and Cowan 2008), but relationships between flowering phenology and biomass can also vary with community age (K. Barry Dissertation, Chapter 2; Zhang and Lamb 2012).

The true nitrogen uptake dynamics in the field plots may vary from my results, as the soil nitrogen probes were utilized at a constant depth and in consistent locations within the experimental plots. It was possible that greater soil nitrogen uptake occurred outside of the area sampled by the soil probes, as variation in phenology can also be

94

correlated to variation in rooting depth (Fargione and Tilman, 2005). Sampling at multiple depths may be needed to better elucidate nitrogen uptake dynamics.

The role of nitrogen uptake dynamics is not well understood among many invasive species. In the field, soil nitrogen remained lower in late phenology plots than in early phenology plots, indicating that towards the end of the season, the late phenology treatments were taking up more nitrogen than the early phenology treatments that had finished producing fruit and had begun to senesce. The differences in soil nitrogen between the early invasive and late invasive exemplify this result (Figure 6) and indicate that timing of nitrogen uptake can be as important as the quantity of nitrogen uptake. In this way, plants with similar soil nutrient requirements compete less intensely when they are utilizing nitrogen at different times during the season (Fargione and Tilman, 2005).

This differential uptake in soil nitrogen over time would be a logical mechanism of phenological complementarity, as flowering time is known to be correlated with resource uptake in many plants (Cleland et al. 2006). Phenological complementarity is found more frequently in ecosystems where resources are discontinuously available, or in grasslands containing C_3 and C_4 species. These findings of correlations between plant phenology and nitrogen uptake in a temperate research system of plants with the same photosynthetic pathway and growth form are informative because they show that complementarity can be present even where resources are more plentiful and plants are otherwise similar on habit. Phenological complementarity is not limited to soil nutrients, as there can be both spatial and temporal complementarity for water, light, or physical space (Wolkovich and Cleland 2011). For instance, though I found phenological complementarity in biomass production, I did not detect significant complementarity of

95

nitrogen uptake. This may have been due to probe placement and my limited sampling design. Complementarity among the mixed plots could also have been due to temporal differences in competition for other resources other than nitrogen.

One of the proposed mechanisms of invasive species success is greater nitrogen use efficiency than native species (Drenovsky et al. 2008). Higher nitrogen use efficiency (or, alternatively, reduced nitrogen requirement), benefits a species by allowing it to survive in areas where nitrogen is limiting, either due to lack of nitrogen in the soil or inability to access nitrogen or high nitrogen uptake by competitors. Plants with the lowest nitrogen requirements should have the greatest competitive advantage, as once nitrogen levels are sufficiently low, those plants with lowest nitrogen requirements would be most successful (Tilman 1985; Craine 2005b). In the field, I found that the invasive treatments used significantly less nitrogen than the native treatments, indicating that the invasives are able to produce as much biomass as native monocultures while using less nitrogen. The invasives would then have an advantage over the native species when nitrogen was limiting in an environment.

The goal of the greenhouse experiment was to provide greater insight into the relationship between nitrogen uptake and growth and phenology of the individual invasive and native species when grown without competitors. Not surprisingly, biomass of species generally increased over time, though biomass accumulation was not consistent for all species (Figure 7). When grouped by phenology, late phenology species produced more biomass than early phenology species, the same pattern that was seen in the field experiment. Unexpectedly, the late invasive *N. cataria* was the first to flower of any of the species, and flowered continuously until the end of the experiment.

96

The rapid growth and large size of *N. cataria* drove the relationship of higher biomass of invasive over native species. These characteristics of *N. cataria* along with its early flowering and higher percentage of leaf nitrogen than the native species potentially indicates that *N. cataria* exhibits plasticity such that it is able to take advantage of favorable conditions and flower more quickly after germination than it did when growing under more natural conditions in the field. Though plasticity may benefit some invasive species (Knop and Resusser 2012), it may not be a major contributor of invasive success in non-native plants as a whole (Palacio-López and Gianoli 2011), and requires further investigation.

Percent nitrogen content of leaves and flowers and leaf ^{15}N isotope concentration differed little among species and sampling periods (data not shown). These limited differences may be due to the slow growth, small sample sizes, and lack of phenological progression in this greenhouse experiment. If all native and invasive species had flowered as planned, the results would likely have been more conclusive. Nonetheless, the gross difference between plant performance and phenology between greenhouse and field experiments suggests caution should be taken when extrapolating greenhouse results to performance of plants in the field, as countless comparative studies have done (e.g. Diamond 1986).

Overall, I found strong evidence in the field for a relationship between plant phenology and nitrogen uptake throughout the season. I also detected evidence for complementarity between native species of different phenologies. Competition between neighbors of similar phenology has been shown to reduce competitor biomass and other fitness traits (K. Barry Dissertation, Chapter 2, Hooper 1998, Anten and Hirose 1999).

However, more work is required to determine how plant resource dynamics interact to affect community primary productivity and resistance to non-native species invasion. Furthermore, data on invasive plant nutrient uptake and usage should be systematically collected to provide more insight into how to help predict or mitigate their spread.

Table 1. Greenhouse leaf nitrogen means $\pm$ 1 SE (in parentheses) of early and late phenology plant species. Only the three flowering species were sampled for nitrogen at the 4th harvest. NA indicates non-flowering plants where sampling was not conducted because they were senescing.

	H. matronalis	*N. cataria*	*A. viridis*	*C. lanceolata*	*H. flexuosum*	*A. tuberosa*	*C. tripteris*	*H. autumnale*
Functional group	early invasive	late invasive	early	early	early	late	late	late
Harvest 2								
percent N	3.961 ($\pm$0.029)	3.093 ($\pm$0.087)	2.952 ($\pm$0.087)	2.992 ($\pm$0.279)	3.222 ($\pm$0.051)	3.361 ($\pm$0.013)	3.269 ($\pm$0.022)	2.832 ($\pm$0.472)
N15 isotope	168.156 ($\pm$167.293)	164.182 ($\pm$57.594)	128.344 ($\pm$30.838)	241.246 ($\pm$137.508)	217.999 ($\pm$90.926)	303.385 ($\pm$89.053)	185.794 ($\pm$184.286)	239.166 ($\pm$9.073)
Harvest 4								
percent N	NA	2.394 ($\pm$0.327)	NA	NA	2.849 ($\pm$0.073)	3.311 ($\pm$0.163)	NA	NA
15N isotope	NA	106.944 ($\pm$41.316)	NA	NA	198.003 ($\pm$111.193)	202.899 ($\pm$77.599)	NA	NA

Figure 1. Field experiment plots of aboveground biomass (Means $\pm$ 1 SE) by treatment. Displayed from left to right: two invasive species monocultures, early phenology native monocultures, late phenology native monocultures, and three native polycultures.

Figure 2. Field experiment plots of aboveground biomass (means $\pm$ 1 SE) by functional group category. Means sharing the same letter are not significantly different.

Figure 3. Field experiment plots total nitrogen metric (means and $\pm$ 1 SE) by treatment. Displayed from left to right: two invasive species monocultures, early phenology native monocultures, late phenology native monocultures, and three native polycultures.

Figure 4. Field treatments correlation between biomass and total nitrogen metric (NH_4 and NO_3 across all field treatments.

Figure 5. Field soil nitrogen levels (means $\pm$ 1 SE) across sampling periods of one growing season for all a) early phenology and b) late phenology species.

Figure 6. Field nitrogen levels (means $\pm$ 1 SE) of early invasive *H. matronalis* and late invasive *N. cataria* throughout one season. Means during the last two sampling periods were significantly different between the early and late invasive species.

Figure 7. Greenhouse biomass (means $\pm$ 1 SE) for plant species at each of the four harvest times. Early native *Asclepias viridis* means are present but extremely small.

1
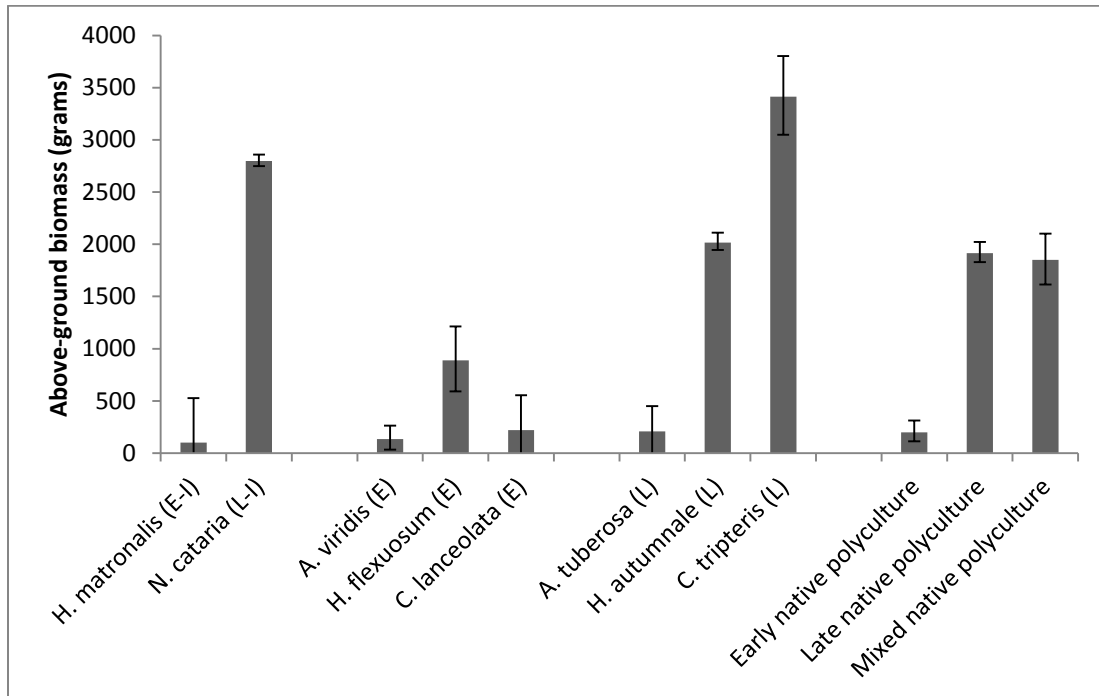
Figure 1.

Figure 2.

Figure 3.

Figure 4.

Total nitrogen metric level vs. Treatment biomass (grams)

104

Figure 5.

b)

(Chart: Soil nitrogen level vs Soil probe placement date)

Legend:
- N. cataria (L-I)
- C. tripteris(L)
- H. autumnale(L)
- A. tuberosa(L)
- Late polyculture

Y-axis: Soil nitrogen level (0.0 to 500.0)
X-axis: Soil probe placement date (T1:6/30/09, T2:7/22/09, T3:8/18/09, T4:9/10/09)
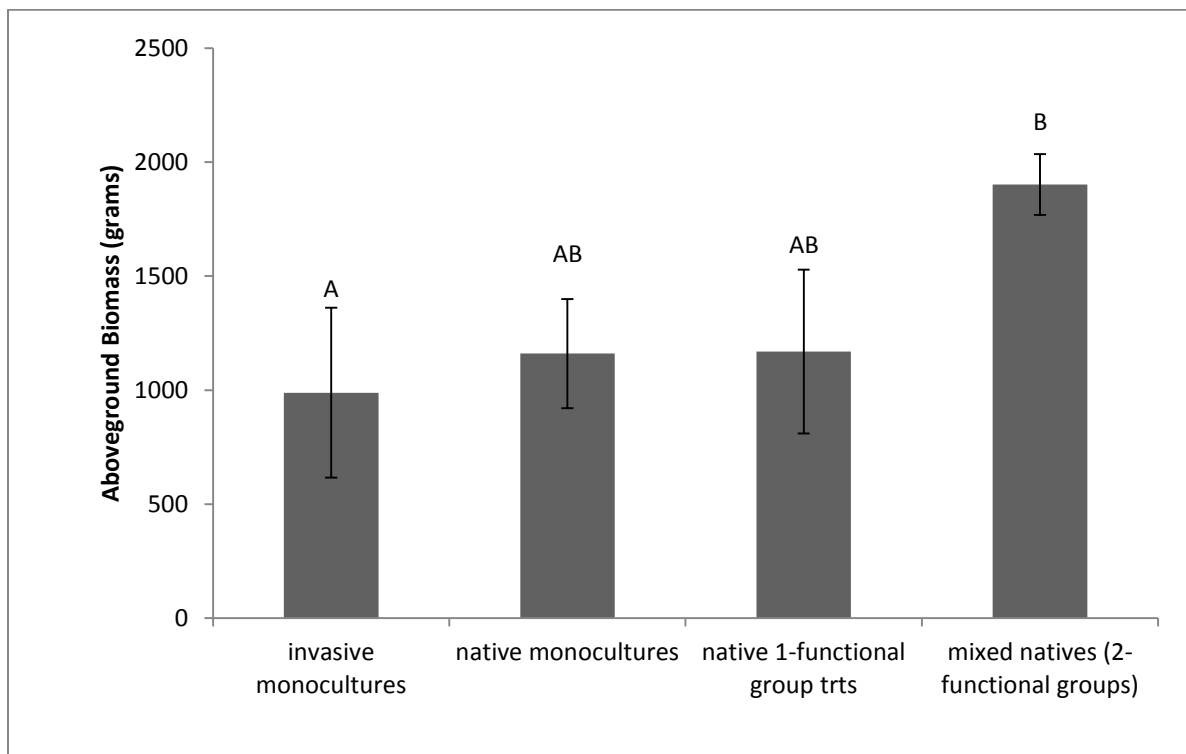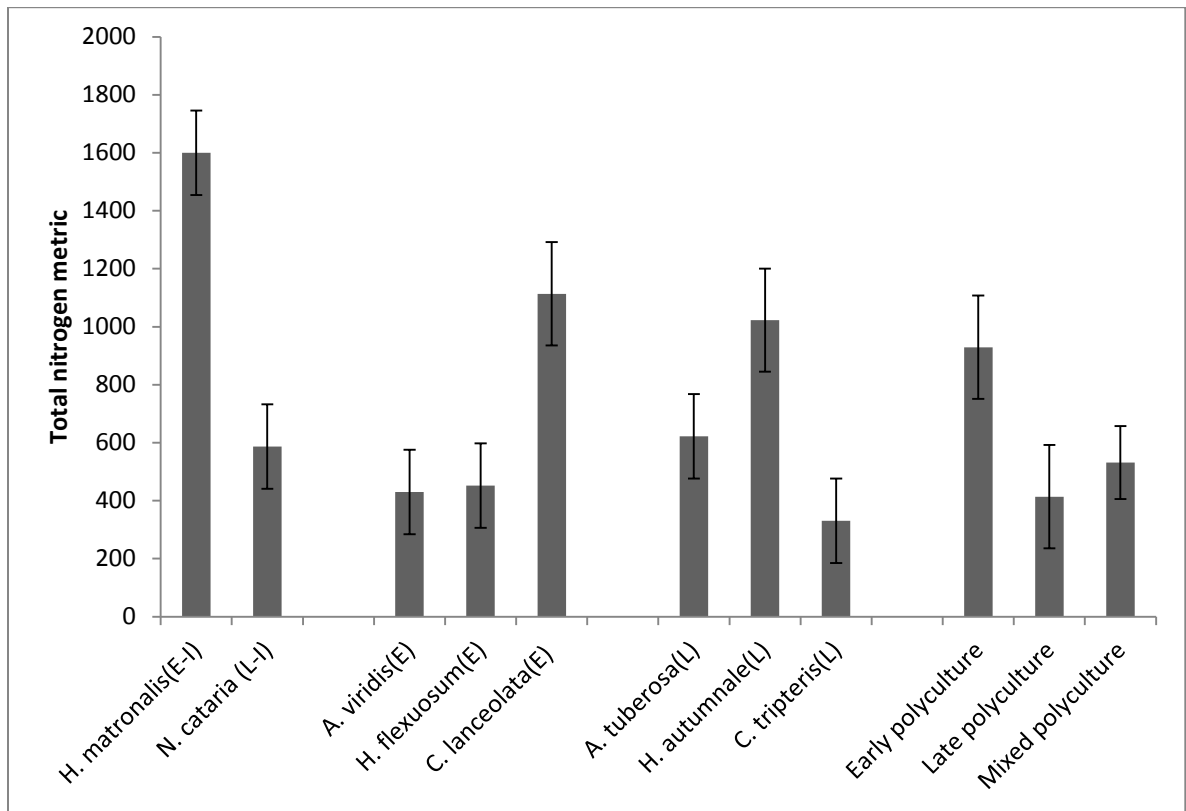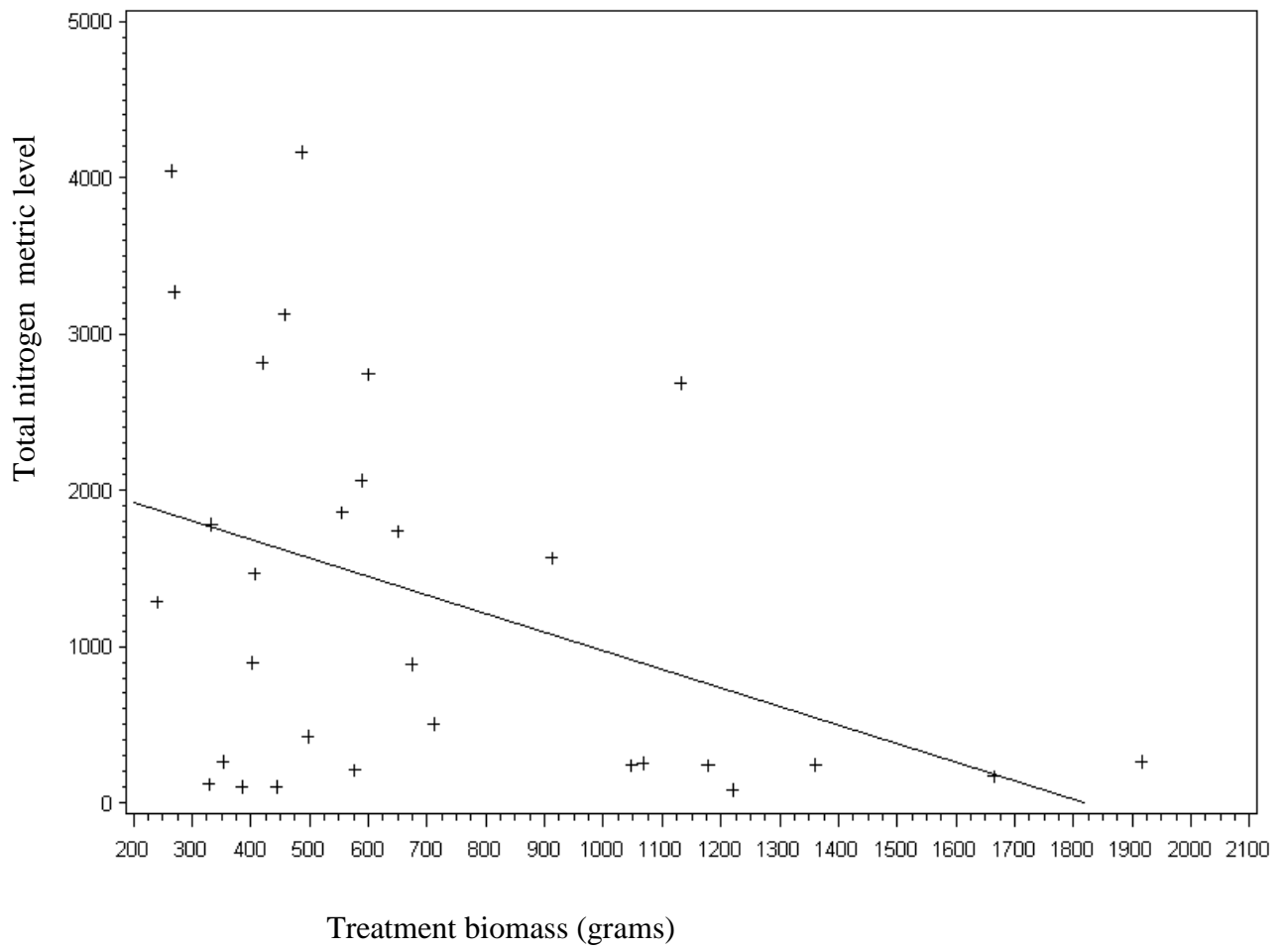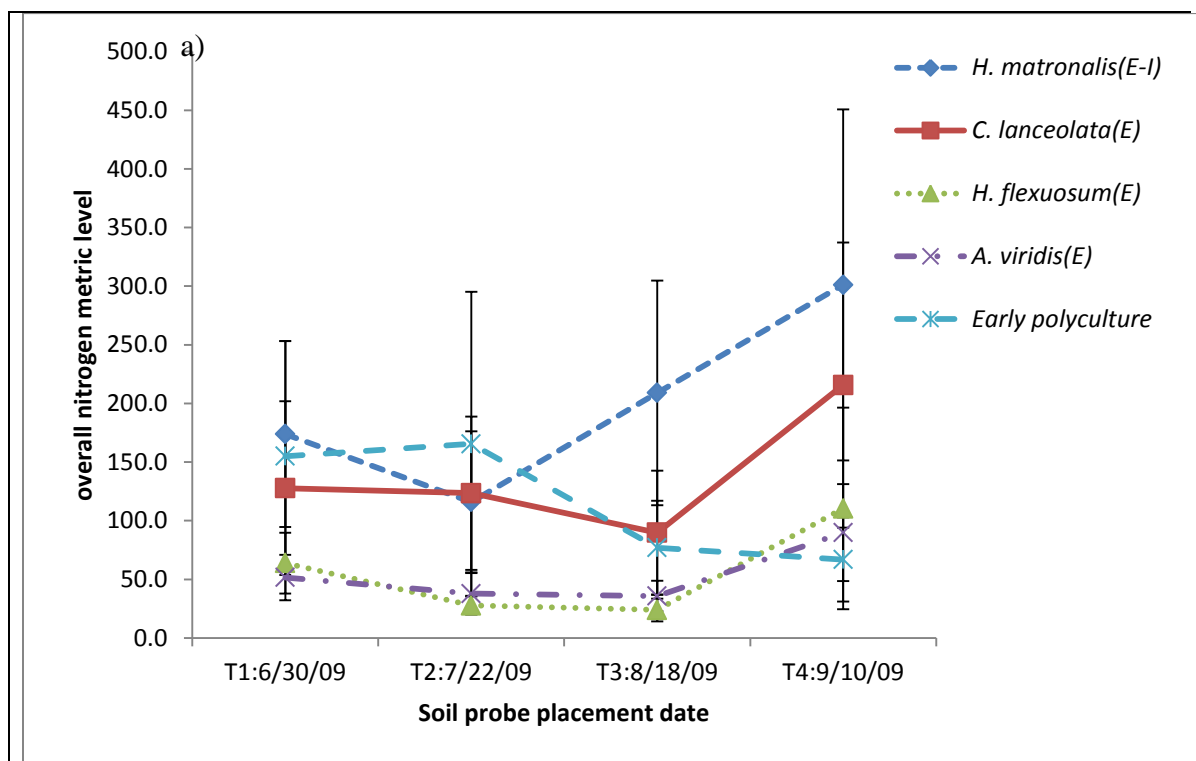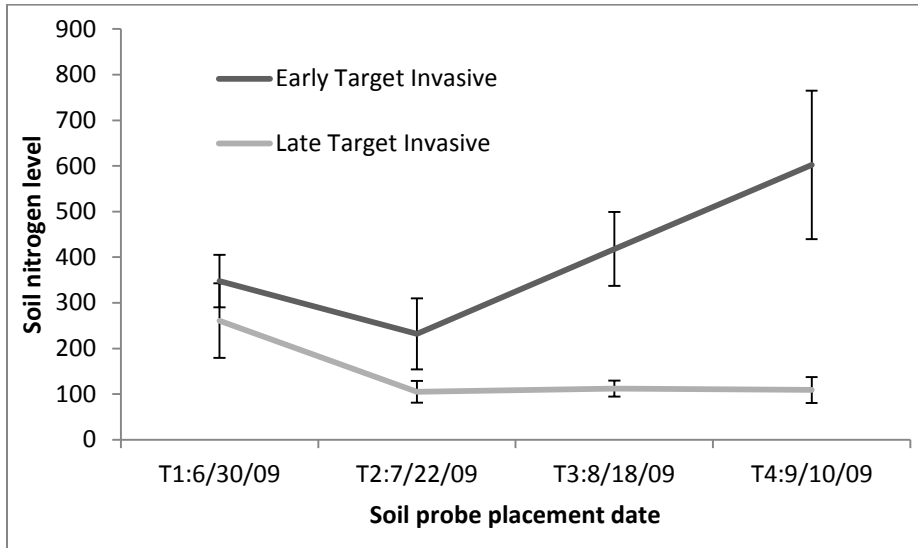
Figure 6.

Figure 7
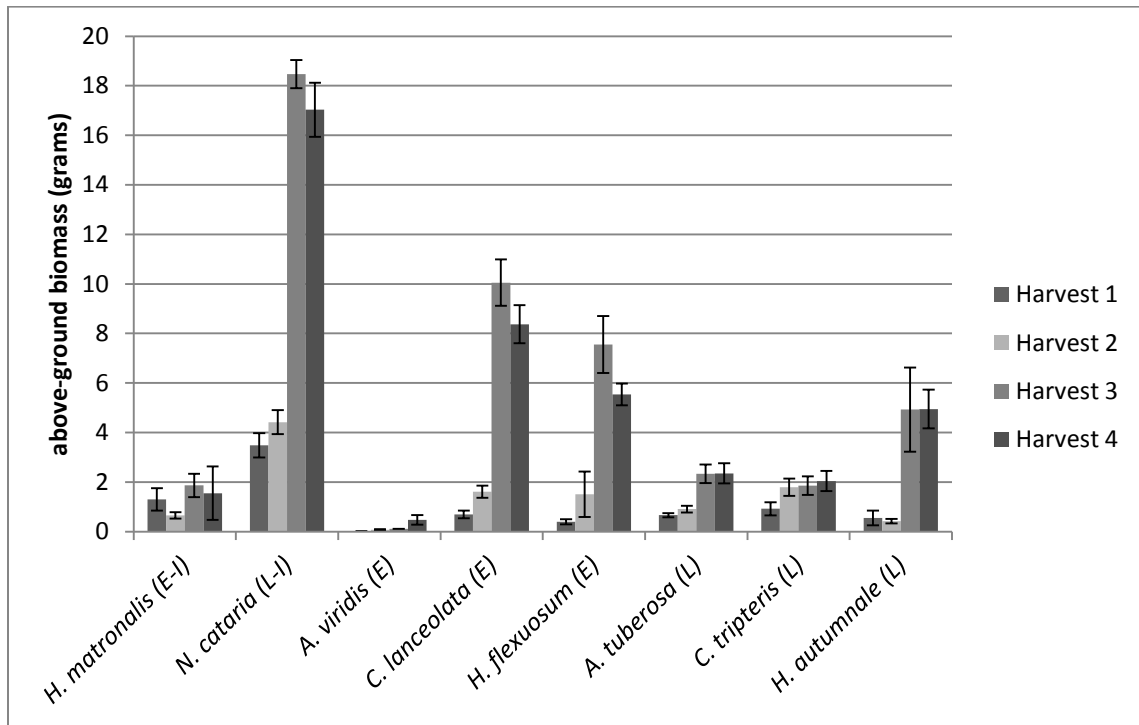
Survey results of studies utilizing the target-neighbor design

Articles found during literature search of the target-neighbor experimental design listed in chronological order. Column headings and entries are as follows:

Comm: Was neighbor community planted as part of the experiment or was the target planted into a natural or unstructured community? (planted/natural)

Type: Did experiment involve separation of above-ground and below-ground competition or not? (a-b/no)

Order: Were neighbors established before the target, or were targets and neighbors planted together? (neighbors/same)

NCE: Was neighbor competitive effect reported or target competitive response (both are measures of neighbor influence on the target) reported? (yes/no)

T#: Was there more than one target treatment per neighbor treatment? (yes/no)

NCR: Was neighbor competitive response to targets reported (yes/no)

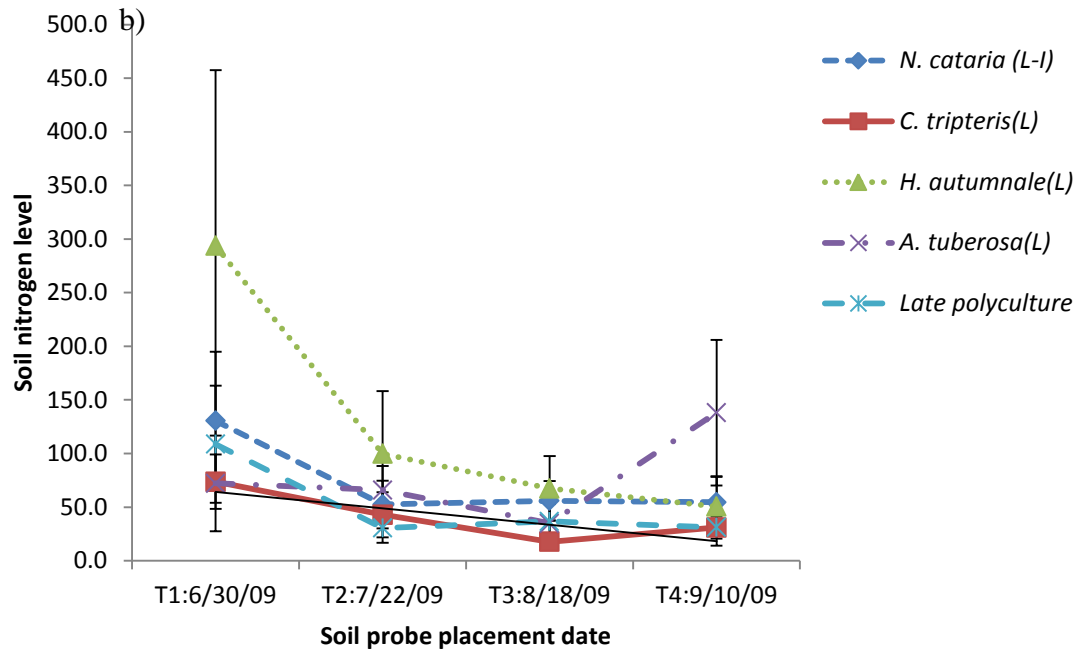| Author (year)	Comm.	Type	Order	NCE	T#	NCR
Clements (1927)	planted	a-b	neighbors	yes	no	no
Welbank (1961)	planted	a-b	neighbors	yes	no	
Cook and Ratcliff (1984)	natural	a-b	neighbors			
Cook and Ratcliff (1985)	natural	a-b	neighbors			
Goldberg and Fleetwood (1987)	planted	no	neighbors	yes	yes	no
Reichenberger and Pyke	natural	a-b	neighbors			

(1990)

Goldberg and Landa (1991)	planted	no	together	yes	yes	no
Denslow et al. (1991)	natural	a-b	neighbors			
Gill and Marks (1991)	natural	a-b	neighbors			
Wilson and Tilman (1991)	natural	a-b	neighbors			
Marvel et al. (1992)	planted	a-b	together	yes	no	
Perera et al. (1992)	planted	a-b	together	yes	no	
Putz and Canham (1992)	natural	a-b	neighbors			
Seager et al. (1992)	natural	a-b	neighbors			
Hartnett et al. (1993)	planted	no	together	yes	yes	no
Panetta and Randall (1993)	planted	no	together	yes	yes	no
Tremmel and Bazzaz (1993)	planted	no	together	yes	yes	no
Wilson (1993a)	natural	a-b	neighbors			
Wilson (1993b)	natural	a-b	neighbors			
Wilson and Tilman (1993)	natural	a-b	neighbors			
Lindquist et al. (1994)	planted	no	together	yes	no	
Thijs et al. (1994)	planted	no	together	yes	no	
Dillenberg et al. (1995)	planted	a-b	together	yes	no	
DiTomasso and Watson (1995)	planted	no	together	yes	no	
Tremmel and Bazzaz (1995)	planted	no	together	yes	yes	no
Belcher et al. (1995)	natural	a-b	neighbors			

Gerry and Wilson (1995)	natural	a-b	neighbors			
Wilson and Tilman (1995)	natural	a-b	neighbors			
Gibson and Skeel (1996)	planted	no	together	yes	no	
Moora and Zobel (1996)	planted	no	together	yes	no	
Twolan-Strutt and Keddy (1996)	natural	a-b	neighbors			
van Auken and Bush (1997)	natural	a-b	neighbors			
Vila (1997)	natural	a-b	neighbors			
Peltzer et al. (1998)	natural	a-b	neighbors			
Leishman (1999)	planted	no	neighbors	yes	yes	no
Cahill (2003)	natural	a-b	neighbors			
Cahill and Casper (2000)	natural	a-b	neighbors			
Humhprey and Pyke (2001)	planted	no	together	yes	no	
Howard (2001)	natural	no	neighbors			
Ronsheim and Anderson (2001)	planted	no	together	no	no	
Cahill (2002)	natural	a-b	neighbors			
Keddy et al.(2002)	planted	no	neighbors	yes	no	
Weigelt et al. (2002)	planted	no	together	yes	yes	no
Franks (2003)	planted	no	together	yes	yes	no
Cheplick and Kane (2004)	planted	no	together	yes	yes	yes
Osunkoya et al. (2005)	planted	no	together	yes	yes	no

Weigelt et al. (2005)	planted	a-b	together	yes	yes	no
Harbur and Owen (2006)	planted	no	together	yes	no	
Jankju-Borzelabad and Griffiths (2006)	planted	no	neighbors	yes	no	
Ramseier and Weiner (2006)	planted	no	together	yes	no	
James and Richards (2007)	planted	no	together	yes	yes	no
Rajaniemi (2007)	planted	no	together	yes	no	
Weigelt et al. (2007)	planted	no	together	yes	yes	no
Hwang and Lauenroth (2008)	planted	no	together	yes	yes	no
Pollnac et al. (2008)	planted	no	together	yes	yes	no
Schmidt et al. (2008)	planted	no	together	yes	yes	no
Daneshgar and Jose (2009)	planted	no	neighbors	yes	no	
Collins et al. (2010)	planted	no	together	yes	yes	no
Jiang et al. (2010)	planted	no	together	yes	no	
Luo et al. (2010)	planted	no	together	yes	yes	no
Song et al. (2010)	planted	no	together	yes	yes	no
West et al. (2010)	planted	no	together	yes	no	
Farrer and Goldberg (2011)	planted	no	together	yes	yes	no
Nagashima and Hikosaka (2011)	planted	a-b	together	yes	yes	no
Dyer et al. (2012)	planted	no	together	yes	no	no
Zhang and Lamb (2012)	planted	no	together	yes	yes	no

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Appendix B

Supplemental methods:

Flowering time as a proxy for resource competition - Nitrogen uptake has been shown to decrease once flower production starts in poinsettia (Scoggins and Mills 1998), and rice (Sheehy et al. 2004), decrease during flowering in tomato (Terabyashi et al. 1991), and plateau at flowering in corn (Dharmakeerthi et al. 2006).

Experimental Site Design - Landscaping fabric was secured around all plots to suppress weed growth, and a 7-ft deer fence and 3-ft chicken-wire fence were erected to prevent large and small mammal herbivory, respectively. The experimental plots were randomly arranged within three fenced enclosures which served as blocks for the statistical analysis.

Experimental Treatments - Within our experimental parameters, there were six possible combinations of early and late species to form the "Mixed native neighbor" treatment. Choosing only one of these possible combinations would limit the scope of inference for the treatment, as the results would be only species-specific, so I selected three combinations (Mixed A, Mixed B, and Mixed C) that had the most extreme growth and flowering phenologies and originally planned to assess these three specific species combinations separately.

Phenological Measurements - I counted all open flowers that had undergone anthesis and had not begun to wilt. Though individual flowers were not marked across censuses, preliminary observations indicated that individual flowers were not open for greater than a week, most likely leading to an underestimate of actual flower production (K. Barry pers. obs.).

Morphological Measurements - In October 2008 and 2009, morphological measurements included height (longest stem length if stems grew at an angle), stem diameter, and canopy length and width were made *in-situ* on each of the replicates. Due to different growth forms among plants and strong correlations with biomass, these measurements were not used further in the analyses presented here.

Statistical Contrasts - The coefficients of these four contrasts would have been orthogonal with one another, except for uneven replication across treatments. To compensate for the lack of orthogonality, a Bonferonni corrected p-value was used to determine significance (Sokal and Rohlf 1994).

*Covariate analysis* - The longest length of seedlings in Spring 2008 was used as a covariate for all 2008 data, but it was not significant in any target invasive or native neighbor models. Similarly, none of the correlations between the covariate and any of the fall 2008 measurements were significant. Subsequently, this covariate was removed from the model and was not used in the final analyses.

Table 1. Experimental species, target / neighbor status, phenological functional group, and native neighbor treatment combinations. "E" denotes a plant in the early-flowering functional group, while "L" denotes a plant in the late-flowering functional group. The presence of each native neighbor species in their respective treatments is indicated by an "x". Target invasive solo treatments and target invasive monocultures not shown.

Species	Target/ Neighbor Status	Family	Pheno-logical Func. Group	Native Neighbor Treatment Combinations				
				Early	Late	Mix. A	Mix. B	Mix. C
Asclepias viridis	Native Neighbor	Asclepiadaceae	E	x		x		x
Asclepias tuberosa	Native Neighbor	Asclepiadaceae	L		x		x	
Coreopsis lanceolata	Native Neighbor	Asteraceae	E	x			x	x
Coreopsis tripteris	Native Neighbor	Asteraceae	L		x	x		
Liatris squarrosa	Native Neighbor	Asteraceae	E	x		x	x	
Liatris pilosa	Native Neighbor	Asteraceae	L		x			x
Helenium flexuosum	Native Neighbor	Asteraceae	E	x				
Helenium autumnale	Native Neighbor	Asteraceae	L		x	x	x	x
Hesperis matronalis	Target Invasive	Brassicaceae	E					
Nepeta cataria	Target Invasive	Lamiaceae	L					

Figure 1. Graphic of experimental treatments. Native species indicated by solid lines, invasive species by dotted lines. Phenological functional groups represented by "E" (early) and "L" (late). Both early and late target invasives were planted within plots containing (from left to right) early neighbors, late neighbors, early and late neighbors (mixed species plots), their invasive conspecifics (monocultures), and no competition (solo treatments).
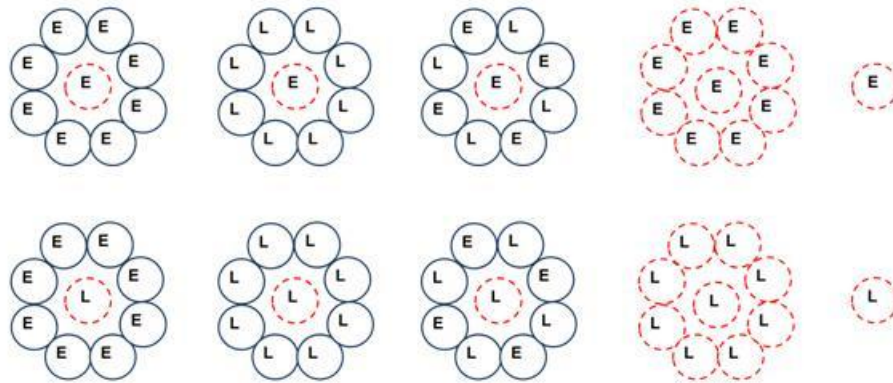
Appendix C

Supplemental Results:

Native Neighbor Characteristics - In Year 1, late native neighbors produced significantly less biomass than both the early native neighbors and mixed native neighbors (($F_{2,63}=8.22$, p=0.0007). In Year 1, plant height of the late neighbors > mixed neighbors > early neighbors ($F_{2,212}=44.83$, p<0.0001). In Year 2, biomass trends were partially reversed with late neighbors producing more biomass than the early neighbors ($F_{2,75}=11.2$, p<0.0001); there was also a significant target x neighbor interaction ($F_{2,75}=6.5$, p=0.0025). Neighbor height in Year 2 followed the same pattern as in Year 1 ($F_{2,74}=17.2$,p<0.0001), (Supplement, Table 2). Fruit mass was more conserved across treatments, as in Year 1 early native neighbors produced significantly more fruit mass than the late or mixed native neighbor treatments ($F_{2,63}=9.57$, p=0.0002), while in Year 2 there was no effect of experimental treatment on neighbor fruit mass.

Relationship between target and neighbor biomass –In Year 1, there was a significant negative correlation between early invasive *H. matronalis* total (vegetative + reproductive) biomass and neighbor biomass (r=-0.332, p=0.035), though this relationship disappeared when solo targets were removed from the analysis (r=0.035, p=0.7892). A similar pattern was seen for late invasive *N. cataria* total biomass and neighbor biomass in Year 1 (with solo treatment: r=-0.715, p<0.0001; without solo treatment: r=0.2048, p =0.2257), and in Year 2 (with solo treatment r=-0.221, p=0.0848:

without solo treatment: r=-0.237, p=0.1121). In Year 2 no significant relationship was

detected between *H. matronalis* total biomass and neighbor biomass (r=-0.227,

p=0.2032)

1

2 **Table 1.** Target invasive competitive response contrast results by year and target invasive. ETI: early target invasive, LTI: late

3 target invasive, DFF: date of first flower. The early target invasive (*Hesperis matronalis*) did not flower or fruit in 2008,

4 designated by "NA". Late target invasive (*Nepeta cataria*) monoculture flowers could not be measured in Year 2. Bold

5 typeface indicates a significant Bonferonni-adjusted p-value (<0.0125).

Contrast	Vegetative biomass		Seed mass		Flower count		Date of first flowering		Peak flower date	
Year 1	ETI	LTI	ETI	LTI	ETI	LTI	ETI	LTI	ETI	LTI
Solo vs neighbor	$F_{1,41}=10.62$ p<0.0023	$F_{1,37}=62.21$ p<0.0001	NA	$F_{1,114}=43.62$ p=<0.0001	NA	$F_{1,65}=205.55$ p<0.0001	NA	$F_{1,68}=7.73$ p=0.007	NA	$F_{1,71}=0.27$ p=0.6028
Intra vs inter	$F_{1,41}=10.70$ p=0.0022	$F_{1,37}=0.10$ p=0.7593	NA	$F_{1,93}=0.29$ p=0.594	NA	$F_{1,65}=0.18$ p=0.6711	NA	$F_{1,68}=1.75$ p=0.1897	NA	$F_{1,51}=0.31$ p=0.5784
Early vs late	$F_{1,41}=0.49$ p=0.4862	$F_{1,37}=3.87$ p=0.0565	NA	$F_{1,35}=19.11$ p=0.0001	NA	$F_{1,65}=2.33$ p=0.1319	NA	$F_{1,68}=2.68$ p=0.106	NA	$F_{1,17}=0.00$ p=0.992
1 f-group vs 2	$F_{1,41}=0.33$ p=0.5717	$F_{1,37}=1.01$ p=0.3215	NA	$F_{1,88}=4.06$ p=0.047	NA	$F_{1,65}=4.46$ p=0.0386	NA	$F_{1,68}=7.78$ p=0.0069	NA	$F_{1,46}=0.19$ p=0.6648
	ETI	LTI	ETI	LTI	ETI	LTI	ETI	LTI	ETI	LTI
Year 2										
Solo vs neighbor	$F_{1,28}=1.73$ p=0.1991	$F_{1,55}=3.87$ p=0.0541	$F_{1,31}=0.08$ p=0.782	$F_{1,57}=0.69$ p=<0.4106	$F_{1,38}=0.0,$ p=.9566	$F_{1,57}=0.3,$ p=0.5833	$F_{1,23}=0.11$ p=0.7484	$F_{1,57}=2.21$ p=0.143	$F_{1,23}=0.12$ p=0.7302	$F_{1,55}=0.49$ p=0.4855
Intra vs inter	$F_{1,28}=0.70$ p=0.4114	$F_{1,57}=10.6$ p=0.0019	$F_{1,29}=0.48$ p=0.4955	$F_{1,57}=3.94$ p=0.0519	$F_{1,33}=0.08$ p=.7771	NA	$F_{1,23}=3.09$ p=0.0919	NA	$F_{1,23}=2.19$ p=0.1525	NA
Early vs late	$F_{1,28}=0.01$ p=0.9218	$F_{1,56}=0.95$ p=0.335	$F_{1,6}=0.37$ p=0.5677	$F_{1,57}=2.34$ p=0.1319	$F_{1,6}=1.20,$ p=.3153	$F_{1,57}=0.11$ p=0.7447	$F_{1,23}=0.67$ p=0.4231	$F_{1,57}=0.36$ p=0.5503	$F_{1,23}=0.00$ p=0.9959	$F_{1,56}=0.44$ p=0.5093
1 f-group vs 2	$F_{1,28}=0.02$ p=0.8777	$F_{1,57}=1.62$ p=0.2089	$F_{1,26}=0.79$ p=0.3829	$F_{1,57}=0.16$ p=0.6917	$F_{1,29}=0.20,$ p=.6566	$F_{1,57}=1.22$ p=0.2741	$F_{1,23}=0.64$ p=0.4333	$F_{1,57}=0.37$ p=0.5545	$F_{1,23}=0.00$ p=0.9753	$F_{1,57}=0.34$ p=0.6253

Table 2. Target invasive competitive response means ±1 SE by year and treatment. ETI: Early target invasive, LTI: late target invasive, DFF: date of first flower. The early target invasive did not flower or fruit in Year 1, designated by "NA". Flower count was not measured on the late target invasive monoculture treatment in Year 2. Replication (number of experimental plots) noted by n.

	Seed mass				Flower Count				DFF				Peak Flower Date			
Year 1																
TRT	ETI		LTI	n	ETI		LTI	n	ETI		LTI	n	ETI		LTI	N
Early	NA		0.043 ±.042	17	NA		30.992 ±58.856	6	NA		229.416 ±0	6	NA		236.17 ±8.74	6
Late	NA		1.684 ±.753	20	NA		220.064 ±151.876	13	NA		218.218 ±0	13	NA		236.62 ±5.94	13
Mixed	NA		1.652 ±.472	53	NA		360.564 ±171.821	29	NA		213.201 ±0	29	NA		243.43 ±4.04	29
Mono	NA		0.066 ±.060	5	NA		101.012 ±132.185	5	NA		213.201 ±5.499	5	NA		240.60 ±9.57	5
Solo	NA		60.637 ±8.893	20	NA		8893.674 ±1581.826	20	NA		208.514 ±0	20	NA		244.26 ±4.91	20
Year 2	ETI		LTI		ETI		LTI		ETI		LTI		ETI		LTI	
Early	0.800 ±.799	3	39.213 ±36.253	3	0.799667 ±.800	3	13087 ±4489.05	3	140.028	1	183.67 ±3.056	3	146.0067 ±9.76	3	212.54 ±5.01	3
Late	4.048 ±3.454	5	124.037 ±29.848	14	13.3186 ±8.894	5	14375 ±2078.03	14	131.3064 ±5.108	3	181.64 ±1.415	14	145.9428 ±5.63	5	208.95 ±2.48	14
Mixed	5.365 ±2.210	20	89.231 ±18.577	26	25.47767 ±10.106	23	9796.83 ±1443.83	27	130.1889 ±2.220	15	183.85 ±1.019	27	146.1561 ±2.52	23	212.30 ±1.92	27
Mono	3.062 ±1.603	3	7.794 ±16.162	3	15.79548 ±9.136	4	NA		145.865 ±6.069	4	NA		155.8361 ±5.57	4	NA	
Solo	0.573 ±.109	2	77.340 ±22.046	16	12.86137 ±7.983	5	13272 ±.002	17	138.675 ±4.657	5	180.47 ±1.284	17	146.4558 ±4.40	5	209.13 ±2.29	17

Table 3. Native neighbor community competitive response means ±1 SE by neighbor treatment and year. Means for individual target treatments (ETI: early target invasive, LTI: late target invasive) are only noted below when there was a significant effect of target invasive or target x neighbor interaction in the analysis. DFF: date of first flower. "NA" notes where flower count analyses were not conducted in 2008 because floral census ended before all plants finished flowering. Replication (number of experimental plots) noted by n.

Native Neighbor	Fruit mass ETI	n	Fruit mass LTI	n	Height ETI	n	Height LTI	n	Flower Count ETI	n	Flower Count LTI	n	DFF	n	Peak Flower Date	n
2008																
Early	208.34 ±24.32	50			291.40 ±14.92	44			NA				211.29 ±2.08	44	236.24 ±2.45	44
Late	141.87 ±24.67	58			496.94 ±19.96	42			NA				232.80 ±2.10	43	233.78 ±2.47	43
Mixed	122.84 ±20.20	171			338.90 ±10.22	136			NA				231.40 ±1.39	141	236.28 ±1.35	141
2009	ETI		LTI		ETI		LTI		ETI		LTI					
Early	179.15 ±49.28	4	54.14 ±31.77	3	209.25 ±46.00	3	110.25 ±46.00	3	27.52 ±5.92	4	18.52 ±6.23	3	182.57 ±5.02	7	200.00 ±4.60	7
Late	164.93 ±30.42	7	70.07 ±14.07	14	728.86 ±59.51	7	514.74 ±42.08	14	50.46 ±4.33	7	29.12 ±3.06	14	204.13 ±7.02	21	218.22 ±5.98	21
Mixed	166.03 ±23.08	24	106.91 ±16.93	29	418.83 ±39.28	24	418.21 35.73	29	41.22 ±2.53	24	38.20 ±2.34	29	192.45 ±5.88	53	204.12 ±4.89	53

Appendix D

Supplemental Discussion:

Interpreting target response with potential bias in neighbor competitive response- In our experiment, if the late neighbor treatment had been uniform across target invasive treatments (with either a decrease in late neighbor biomass when grown with the early target, or an increase when grown with the late target), a subsequent increase in early target biomass or decrease in late target biomass when grown with the late neighbor treatments would be expected. However, the predicted relative direction of change for the affected target means would cause the treatment means to move closer together, and not further apart. This pattern, and the variance among the treatment means, suggest that the adverse effect of the late neighbor treatment inequality on the response of the late target and the significance of the contrasts is minimal, resulting in a robust result.

Appendix E

Table 1: Target invasive competitive response untransformed means $\pm$ 1 SE. ETI and LTI represent the early target invasive (*H. matronalis*) and the late target invasive (*N. cataria*) respectively. DFF: date of first flower. The early target invasive did not produce flowers or fruit in any of the treatments.

	Vegetative biomass		seed mass	flower production	DFF
Coestablished	EI	LI	LI	LI	LI
early	117.046 ($\pm$41.095)	44.082 ($\pm$22.964)	0.043 ($\pm$0.043)	113.000 ($\pm$104.039)	235.167 ($\pm$9.952)
late	149.62 ($\pm$44.769)	158.916 ($\pm$67.687)	1.684 ($\pm$0.754)	485.847 ($\pm$200.764)	222.308 ($\pm$5.442)
mixed	105.679 ($\pm$20.78)	156.824 ($\pm$38.827)	1.622 ($\pm$0.465)	741.207 ($\pm$255.339)	215.242 ($\pm$2.263)
solo	225.506 ($\pm$49.11)	1030.05 ($\pm$128.411)	60.638 ($\pm$8.894)	10103.45 ($\pm$1169.99)	210.1 ($\pm$1.505)
Introduced	EI	LI	LI	LI	LI
early	11.953 ($\pm$4.398)	59.383 ($\pm$12.867)	7.218 ($\pm$5.959)	815.000 ($\pm$518.64)	228.334 ($\pm$17.948)
late	3.599 ($\pm$1.342)	4.309 ($\pm$1.632)	0.019 ($\pm$0.01)	0.834 ($\pm$0.655)	174.5 ($\pm$41.5)
mixed	5.704 ($\pm$1.516)	23.067 ($\pm$9.124)	1.948 ($\pm$0.715)	123.091 ($\pm$58.22)	213.715 ($\pm$6.789)
solo	21.715 ($\pm$8.762)	126.145 ($\pm$27.519)	17 ($\pm$3.984)	1729.100 ($\pm$409.205)	189.7 ($\pm$0.7)

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