POPULATION ECOLOGY

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The invasive shrub, *Lonicera maackii*, reduces growth and fecundity of perennial forest herbs

Abstract Effects of invasive plant species on native plant species are frequently assumed or inferred from comparisons, but rarely quantified experimentally. Such quantification is important to assessing risks and impacts of invasives. We quantified the effects of *Lonicera maackii*, an exotic shrub invasive in many eastern North American forests, on survival, growth, and reproduction of three perennial herbs: *Allium burdickii*, *Thalictrum thalictroides*, and *Viola pubescens*. We predicted that the spring ephemeral, *A. burdickii*, would be most impacted, due to early leaf expansion of *L. maackii*. Field experiments were carried out in two deciduous forest stands, one (Gregg's Woodlot, GW) disturbed and the other (Western Woods, WW) relatively undisturbed. In each stand, individual herbs were transplanted into a blocked design of 60 plots where *L. maackii* was present, absent, or removed, and monitored for 5 growing seasons. *Lonicera maackii* did not affect survival of transplants, but reduced growth and final size of individuals of all three species. For two of the species, *A. burdickii* and *V. pubescens*, *L. maackii* reduced the proportion of live plants flowering in both stands, and reduced the seed or fruit number per flowering individual in GW. For *T. thalictroides* the proportion flowering was not affected, but seed number per flowering plant was reduced by *L. maackii* in both stands. For all three species, cumulative seed production over the course of the study was reduced by *L. maackii*. Overall, effects on the spring ephemeral, *A. burdickii*, were similar to effects on the other herbs. Because mortality of these established individuals was not affected, short-term studies might conclude forest herbs are unaffected by invasive shrubs.

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However, the growth and reproduction impacts documented here suggest that populations are impacted in the long-term.

Introduction

Biological invasion by non-native species is considered one of the major threats to the global environment (Vitousek et al. 1996; Mack et al. 2000), and invasive plants in particular (Cronk and Fuller 1995) are considered to have major impacts on plant populations, communities, and ecosystems. In the United States, exotic plant species cost approximately $34 billion in damage and control each year (Pimentel et al.44">2000).

However, assessing the impact of an invasive plant species requires quantifying the per-capita effects of individual plants (Parker et al. 1999), and this has rarely been done (D'Antonio and Kark 2002). Effects of invasive plants on native species are often inferred from comparative studies; relatively few experiments (Witkowski 1991; Midgley et al. 1992; Dillenburg et al. 1993; Huenneke and Thomson 1995; McCarthy 1997; Meekins and McCarthy 1999; Gould and Gorchov 2000; Gorchov and Trisel 2003) have been done to quantify impacts. Experiments involving multiple species are particularly desirable as they will enable us to assess whether the impacts of an invasive species on different native species are similar, as would be predicted from neutral models (Hubbell 2001; Davis 2003), or whether native species with niches similar to the invasive are more strongly affected.

One of the many species of exotic shrubs invading forests in the eastern United States is *Lonicera maackii* (Rupr.) Herder, Amur Honeysuckle. This robust, multi-stemmed deciduous shrub is native to northeast Asia and was introduced to the USA in 1898. Subsequently it was the subject of cultivar improvement and promoted for landscaping and wildlife benefits by the USDA (Luken

and Thieret 1995). *Lonicera maackii* has currently escaped cultivation in 30 eastern and central states (Trisel and Gorchov 1994; USDA, NRCS 2001).

The ability of *L. maackii* to dominate both disturbed areas and forest understories has been attributed to plastic branch architecture and biomass allocation patterns (Luken 1988; Luken et al. 1995, 1997). Early expansion (March) and late senescence (November) of leaves (Trisel 1997) give *L. maackii* a photosynthetic advantage over native deciduous shrubs and trees. This extended leaf display decreases the amount of light reaching the forest floor, and in turn may reduce photosynthetic rates and growth of smaller shrubs, herbs, saplings and seedlings. Gould and Gorchov (2000) hypothesized that *L. maackii* most negatively affects species most dependent on irradiance before canopy leaf-out, a special case of the niche overlap hypothesis. Support for this hypothesis comes from experiments on three forest annuals; *L. maackii* reduced survival to reproductive age only for the two earlier species, although it reduced fecundity of survivors for all three (Gould and Gorchov 2000).

Among forest stands in southwest Ohio forests, abundance of *L. maackii* is negatively correlated with density and species richness of tree seedlings and with herb cover (Hutchinson and Vankat 1997). Within stands, areas with *L. maackii* have lower density and species richness of native shrubs (Medley 1997) and tree seedlings (Collier et al. 2002), and lower cover and species richness of herbs (Collier et al. 2002). These patterns suggest a negative effect of *L. maackii*, but could alternatively be due to greater recruitment of *L. maackii* in sites that are depauperate for some reason, such as past disturbance. However, removal of *L. maackii* has been shown to increase survival of tree seedlings (Gorchov and Trisel 2003), in addition to the above cited effects on forest annuals. Perennial herbs might be less responsive to invasive shrubs because of their tolerance to low resource conditions, limited seedling establishment, or slow vegetative reproduction (McCarthy 1997).

In order to test whether perennial herbs are suppressed by invasive shrubs in deciduous forests, we investigated the response of three herb species to removal of *L. maackii* over a 5-year period. Specifically, we quantified how *L. maackii* affects the survival, growth, and fecundity of individuals of these three species. Furthermore, to investigate whether impact was related to dependence on irradiance before canopy leaf-out, we examined whether the response of the herbs was related to leaf phenology, specifically whether the spring ephemeral was more negatively affected than the two full-season perennials.

Materials and methods

Study sites

Field experiments were carried out in two deciduous forest stands near Oxford, Ohio (39°30′N, 84°45′W). Western Woods (WW) is a 40 ha relatively undisturbed stand dominated by *Quercus rubra*, *Fraxinus* spp., *Acer saccharum*, and *Fagus grandifolia* with a tree basal area (BA) of 24.9 m^2/ha (Gould and Gorchov 2000). *Lonicera maackii* was the most common shrub, with a density of 0.3 shrubs/m^2 (Gould and Gorchov19">2000). Gregg's Woodlot (GW) is a 7 ha stand dominated by *Carya ovata*, *Fraxinus* spp., *C. laciniosa*, and *Q. rubra*. Compared to WW, BA was less (21.4 m^2/ha) and *L. maackii* density was greater (0.7 shrubs/m^2; Gould and Gorchov 2000), perhaps due to greater anthropogenic disturbance. Selective cutting, livestock grazing, and burning were practiced in the first half of the twentieth century, but the forest has remained untouched since 1970 (T. Gregg, personal communication).

Study species

We studied three perennial herbs differing in leaf phenology. *Allium burdickii* (Hanes) A.G. Jones (Liliaceae), is a spring ephemeral, found in rich, upland woods in the upper Midwest and Plains states (Jones 1979). This taxon is considered *A. tricoccum* Solander var. *burdickii* Hanes by McNeal and Jacobsen (2002). It produces two to three ephemeral leaves that senesce before the scape and umbellate inflorescence develop in early June. Genetic individuals (genets) can reproduce vegetatively via bulb division to form genetically identical daughter ramets.

Thalictrum thalictroides (L.) Eames and Boivin (Ranunculaceae), also called *Anemonella thalictroides* (L.) Spach, is a full-season perennial widely distributed across the eastern United States in dry or moist woods (Gleason and Cronquist 1991). Because of its early leaf expansion (March), Lubbers and Christensen (1986) considered *T. thalictroides* a spring ephemeral, but we considered it a full-season perennial because it senesces its leaves from July to September, as much as 3 months after *A. burdickii* and other spring ephemerals. Flowering occurs in April and May, and the single-seeded achenes mature beginning in late May. *T. thalictroides* produces only basal leaves the first growing season.

Viola pubescens Aiton var. *pubescens*. (Violaceae) is a full-season, widespread perennial found in woods and meadows (Gleason and Cronquist 1991, Ballard 1994). Leaf expansion begins in late March or early April and the leaves last through summer (unpublished data). Like most *Viola* species, *V. pubescens* produces both chasmogamous (CH) flowers and cleistogamous (CL) flowers. CH flowers are capable of both outcrossing and selfing and are larger and showier than CL flowers, which can only self (Mattila and Salonen 1995). In our population CH flowers opened in late April while CL flowers developed from late May until the end of the growing season. CH fruits mature in May and early June, whereas CL fruits mature from June to late summer. The seeds develop within capsules and have elaiosomes; they are likely ant-dispersed given evidence that ants disperse the seeds of *V. pensylvanica* (Culver and Beattie 1978), a species now included in *V. pubescens* (Ballard 1994).

Experimental design

We used a blocked design for field experiments at each site. At WW we used 20 blocks established by Gould and Gorchov (2000) in November 1994; each block contained one plot where *L. maackii* was present, one where *L. maackii* was absent, and one where *L. maackii* was removed (*n* =60 plots). Within each block, plots were placed 2–10 m apart to minimize differences in slope, drainage, and canopy cover. The *L. maackii* -absent plot was placed where there was no evidence of live or dead *L. maackii*, and each of the two other plots was placed where there were at least two *L. maackii* individuals >1 m tall within a 1.5 m radius. One of the latter plots was randomly assigned to the removal treatment; *L. maackii* shrubs were removed by excavating the meristematic burl. Overhanging branches and resprouting stems of *L. maackii* were trimmed annually in the removal plots.

At GW we used 30 blocks, each with a *L. maackii* -present plot and a *L. maackii* -removed plot 2–10 m away, originally selected by

Gould and Gorchov (2000) from a pool of 60 present and 60 removal plots established at the site in 1992. Each plot was initially centered on a large *L. maackii* shrub and randomly assigned to treatment. Removal of *L. maackii* began in 1992 when stems of the central *L. maackii* were cut to 25 cm in height (Gorchov and Trisel 2002). Continued removal of resprouting stems from 1992 to 1995 led to the death of most of these central shrubs. After 1995 sprouts from the few survivors were trimmed annually, and all other *L. maackii* within 2 m were removed. No *L. maackii* -absent plots were included due to the high density of *L. maackii* at GW.

Individuals of each herb species were collected from local populations. The underground parts were separated from the stems and leaves, rinsed, air dried, weighed, and numbered. For each herb species, four individuals were randomly assigned for planting into each of the 60 treatment plots at both sites (n =240 individuals/species/site). Bottomless poultry-wire planting cages (83 cm×60 cm×60 cm), which protected the herbs (but not *L. maackii*) from mammalian herbivores, were placed to the north of the center of each treatment plot under the shade of the central *L. maackii* individual. Placement of individuals within the cage was determined randomly using a 4×4 Latin Squares method. Individuals were transplanted within 1–3 days of collection; *A. burdickii* in June 1995, *T. thalictroides* in May 1996, and *V. pubescens* in September 1997. There was no significant treatment difference in the initial weight of transplants for any species at either site (one-way ANOVA; all GW df =1; all WW df =2; all P >0.05).

The herbs were monitored weekly for survival, growth, and fecundity from April to September each year beginning the spring following transplanting. Other plants emerging within the planting cages were removed by hand (generally only aboveground parts) at each census to prevent aboveground competition from masking a treatment effect. Growth was determined by annual changes in size, measured as leaflet number for *T. thalictroides*, total leaf number (basal leaves + stem leaves, which correlated strongly with number of stems) for *V. pubescens,* and total leaf width, which was shown to correlate strongly with ramet biomass in the closely related *A. tricoccum* (Nault and Gagnon 1993), for *A. burdickii*.

Flower production for *T. thalictroides* and *V. pubescens* was recorded as number of flowers. The small, numerous flowers of *A. burdickii* could not be counted non-destructively, so only the presence or absence of a scape (inflorescence) was noted.

Fruits of *A. burdickii* were collected once seed maturation began in the population. Fruits were counted in the laboratory and opened to obtain seeds. Seeds were dried to determine mean seed weight per individual.

Thalictrum thalictroides flower and seed (achene) counts were made in the field. Seeds were not collected for weighing because achenes from a single flower do not mature and dehisce synchronously and it was difficult to determine when they were mature.

Fruits of *V. pubescens* were counted in the field, but seed counts and weights were difficult to obtain because seeds are released following capsule dehiscence. To optimize seed collection, Coleman lantern mantles were tied around the peduncles of fruits as they matured. Bagging depended on fruit type and length. Few CL fruits were bagged because they often have short peduncles (<10 mm). Rather than bagging, CL fruits were monitored closely, and the peduncles were pinched off when the capsules matured. At most, 50% of the fruits at a site were collected due to difficulties with bagging and collecting fruits before they dehisced. Seeds of *V. pubescens* were not collected in 1998 because few plants were reproductive their first year. In 1999 and 2000, seeds were counted and weighed from all collected fruits. The number of seeds per fruit was only analyzed for fruits with complete seed counts. Only mature, full seeds were counted and weighed. In 1999, the seeds were oven-dried before weighing, but in 2000 seeds were only air-dried because a subset was needed for another experiment.

As a measure of cumulative growth, we obtained biomass of each surviving individual. Individuals were harvested, separated into below- and above-ground parts, dried at 55°–65°C, and weighed. Vegetative *A. burdickii* individuals were harvested 15–30 May 2001; flowering individuals were harvested 1 week later. For *A. burdickii*

bulb mass but not root mass was included in total biomass because roots were incompletely harvested. *T. thalictroides* was harvested from 14 to 22 June 2001. Before drying, the number of tubers was counted for each individual. *V. pubescens* was harvested from GW 26–29 April and from WW 10–17 May 2002.

Statistical analysis

Treatment effects on survivorship were analyzed using the LIFETEST procedure in SAS version 8.01 (Fox 1993; Allison 1995). Because mortality of the herbs during their first year was likely due to transplant shock rather than treatment, the initial number of individuals alive was set as the number alive in the spring after transplanting.

Analysis of variance (ANOVA) was used to detect treatment effects on growth and reproduction (except proportion flowering) separately for each species and year using the GLM (general linear model) procedure in SAS. The same analysis was used for harvest biomass of each species, and for average weight per tuber and number of tubers per *T. thalictroides* plant. The blocked experimental design required a mixed model approach in which treatment effects were fixed and block and interaction effects were random. We used the TEST option in the RANDOM statement to calculate expected mean squares, the combinations of these that were appropriate for the F tests, and the appropriate degrees of freedom (SAS 1989). Most variables were log-transformed to improve normality and ensure homogeneous variance. Post hoc comparisons of means among present, absent, and removal treatments at WW were done using Bonferroni multiple pairwise comparisons.

In any given year, analyses of growth included all live plants. For *A. burdickii*, daughter ramets produced vegetatively were not considered separate individuals. As in many studies involving plant reproduction, analyses of fecundity variables were more complicated due to hierarchical variables that are sequential in their appearance (Mitchell 1993). Thus, only plants flowering in a given year were analyzed for numbers of flowers, fruits, and seeds.

To detect treatment differences in the number of live individuals flowering each year we constructed 2×2 contingency tables for GW and 3×2 tables for WW, and used likelihood ratio chi-square (G^2) tests (SAS PROC FREQ). When the treatment effect was significant at WW, we constructed separate 2×2 tables for each pair of treatments and carried out separate G^2 tests on each. G^2 tests were also used to determine whether treatment effected vegetative reproduction in *A. burdickii*, by comparing the number of harvested plants that had 1 versus >1 bulbs (ramets).

For *V. pubescens*, we tested for treatment effects on CH and CL flowers and fruits separately, but only CL variables were normally distributed. To determine the effect of treatment on CH flower and fruit production, we used non-parametric generalized linear models (PROC GENMOD, SAS version 8.01; see McKenzie and Halpern 1999; Berg and Redbo-Torstensson 1999; and Guisan and Theurillat 2000 for use in ecology). Specifically, we fitted a Poisson distribution and used a non-linear logarithmic function to link the response and predictor variables. To correct for overdispersion, the d-scale option in PROC GENMOD was used to change the scale parameter from 1 to the square root of the deviance divided by the degrees of freedom. The models included treatment and block predictor variables as in the ANOVA models used for the other demographic variables. Pairwise comparisons for WW were built into the models using contrast statements.

Before testing treatment effects on the number of seeds per *V. pubescens* fruit we tested whether seed number differed between CH and CL fruits. There were no significant differences at GW in 1999 (ANOVA; F =0.54) and 2000 (F =0.07), or at WW in 1999 (F =0.44), but CH fruits produced more seeds per fruit than CL fruits at WW in 2000 (F =4.73; P <.05) necessitating separate analyses. In 2000 only, average seed weight of *V. pubescens* was analyzed separately for CH and CL fruits, because seed collection was complete enough to determine that CH seeds were 1.4 times heavier than CL seeds at both sites (two-way ANOVA; F =6.23 P <.05 at

GW; $F = 12.62$; $P < 0.001$ at WW). In analyses of both seed number and weight, there were not enough blocks with values from all treatments, so the block effect was removed from the ANOVA model.

Cumulative fecundity per individual over the study was quantified as the total number of seeds for *A. burdickii* and *T. thalictroides* and total number of fruits (CH, CL, and total) for *V. pubescens*. Cumulative fruit and seed counts could not be analyzed by standard parametric tests because of large proportions of zeros, a form of overdispersion. For this reason we used a zero inflated Poisson (ZIP) model, which attributes some fraction $(1-\pi)$ of the observed zeros to a "zero-inflation" component, and the remainder (π) to a Poisson distribution [Po(x,μ)] (Böhning et al. 1997; Ridout et al. 1998). Thus, for each treatment our model [f(x; π, μ)=$(1-\pi)$ (Po(x,0)+π Po(x, μ)] fit two parameters: a zero inflation probability $(1-\pi)$ and a Poisson mean (μ). Block effects were not included in the models. We tested whether π and μ differed between treatments using SAS code (PROC NLMIXED) written by M. Hughes. For WW, all pairwise comparisons were tested, with Bonferroni significance adjustment. A generalized coefficient of determination (R^2) was calculated by comparing the log-likelihood of the fitted model [L(β)] with that of the null model (with only an intercept, and no treatment effect) [L(0)], following the formula of Nagelkerke

(1991): $R^2 = \{1 - [L(0)/L(\beta)]^{2/n}\}/\max(R^2)$, where n = sample size and $\max(R^2) = 1 - L(0)^{2/n}$.

Results

Allium burdickii

Survival of *A. burdickii* did not differ significantly among treatments at either study site (Fig. 1a, b).

Among survivors at GW, individuals in the removal treatment were significantly larger than in the present treatment in all study years except 1996 (Fig. 2a, Table 1). By 2000, the sum of leaf widths on individuals in the removal treatment was over 1.5 times that of individuals in the present treatment. Final harvest biomass was significantly greater for individuals in the removal treatments (Fig. 3a, Table 1). In part this was due to greater vegetative reproduction; 17% of survivors in the removal treatment

Fig. 1 The proportion of individuals alive each year out of the number alive the first spring following transplantation for each treatment for *Allium burdickii* at **a** GW and **b** WW, *Thalictrum thalictroides* at **c** GW and **d** WW, and *Viola pubescens* at **e** GW and **f** WW. Sample sizes for each treatment and statistics for survival analysis (SAS PROC LIFETEST) are provided on each graph; for GW $df = 1$, for WW $df = 2$
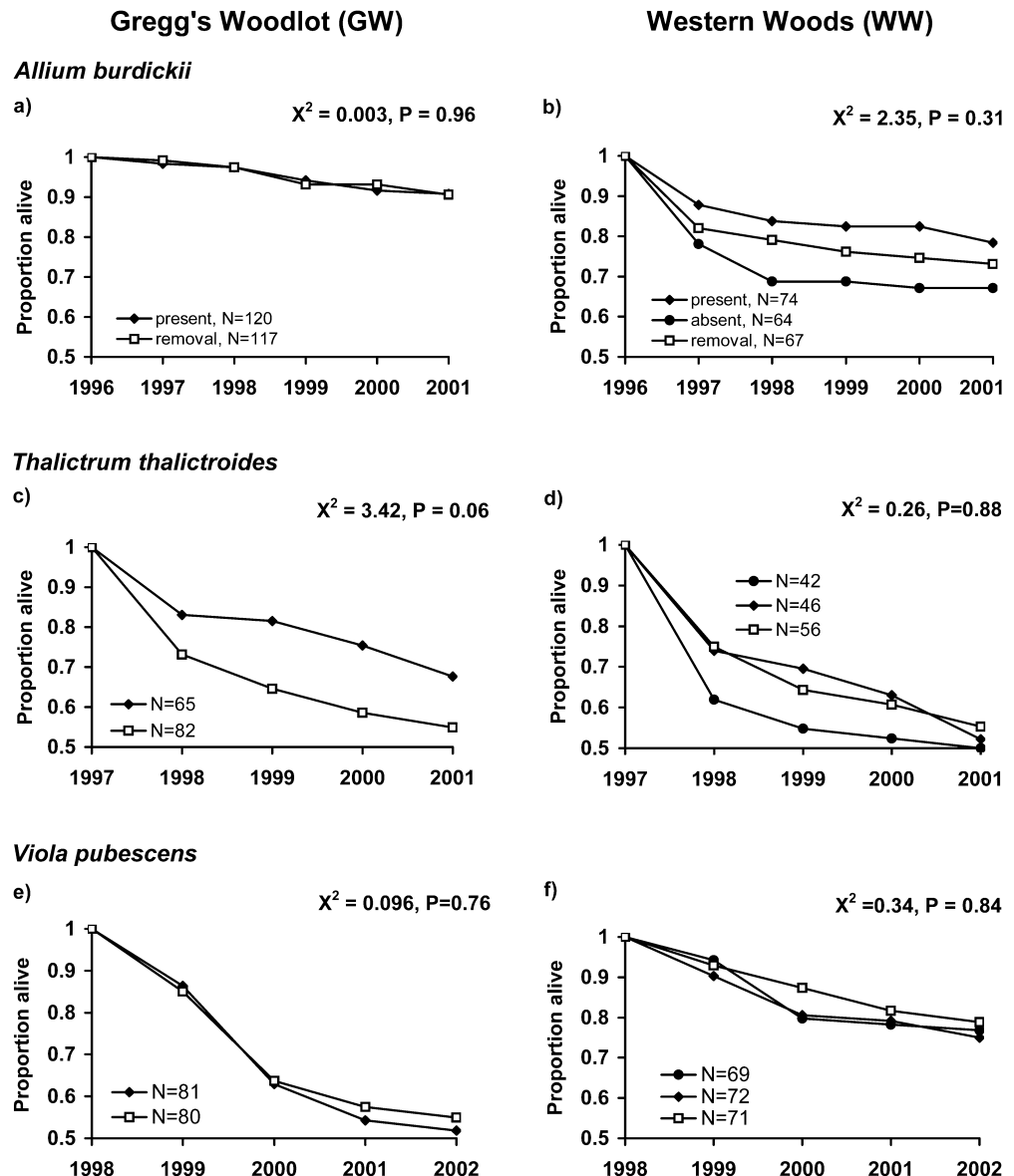
were comprised of 2 or more ramets (bulbs), compared to 5% of those in the present treatment (G^2 =9.09, df =1, P =0.0026). At WW individuals in the present treatment were generally smallest, but a significant treatment difference was detected only in 2000 (Fig. 2b, Table 1). That year, leaves were 1.4 times wider on individuals in the removal treatment than in the present treatment, but neither of these treatments differed significantly from the absent treatment. Final biomass was significantly greater for individuals in the removal and absent treatments than for those in the present treatment at WW (Fig. 3b, Table 1). There was no significant difference among treatments in the proportion of survivors comprised of >1 ramet (G^2 =3.08, df =2).

At GW, surviving individuals in the removal treatment were more likely to flower than those in the present treatment in 1997 (G^2 =9.63, P =0.0019), 1999 (G^2 =11.29, P =0.0008), and 2000 (G^2 =6.57, P =0.0100, all df =1), but there was no treatment effect in 1996 (G^2 =0.01) or 1998 (G^2 =0.20) (Fig. 4a). At WW, fewer individuals flowered in 1999 where *L. maackii* was present than where it was absent (G^2 =4.83, df =1, P =0.0280) or removed (G^2 =5.20, df =1, P =0.0226) (Fig. 4b). Treatments did not differ in proportion flowering in other years (1996: G^2=0.19; 1997: G^2=0.57; 1998: G^2=2.12; 2000: G^2=1.26; all df =2).

Fruit production per flowering individual was significantly higher where *L. maackii* was removed for every year except the first (1996) at GW, but there was no treatment effect on fruit production at WW (Table 1). Flowering individuals at GW matured more seeds in the removal treatment in each of the last four study years (Fig. 5a, Table 1). At WW there was a treatment effect on seed number only in 1998, but that year there were no significant pairwise contrasts (Fig. 5b, Table 1). The

Fig. 2 Mean (+SD) size of surviving individuals in each treatment for each year at GW (**a**, **c**, **e**) and WW (**b**, **d**, **f**). The measure of size for *Allium burdickii* (**a**, **b**) is the sum of leaf widths, for *Thalictrum thalictroides* (**c**, **d**) it is the number of leaflets, and for *Viola pubescens* (**e**, **f**) it is the number of leaves. For each year×site, different letters indicate significantly different (*P* <0.05) treatment means based on analysis of variance and, for WW, Bonferroni multiple comparison test (Tables 1, 2, 3)

Fig. 3 Mean (+SD) dry biomass at harvest for individuals of *Allium burdickii, Thalictrum thalictroides*, and *Viola pubescens* in each treatment at GW (**a, c, e**) and WW (**b, d, f**). Different letters indicate significantly different (*P* <0.05) treatment means based on analysis of variance and, for WW, Bonferroni multiple comparison test (Tables 1, 2, 3)
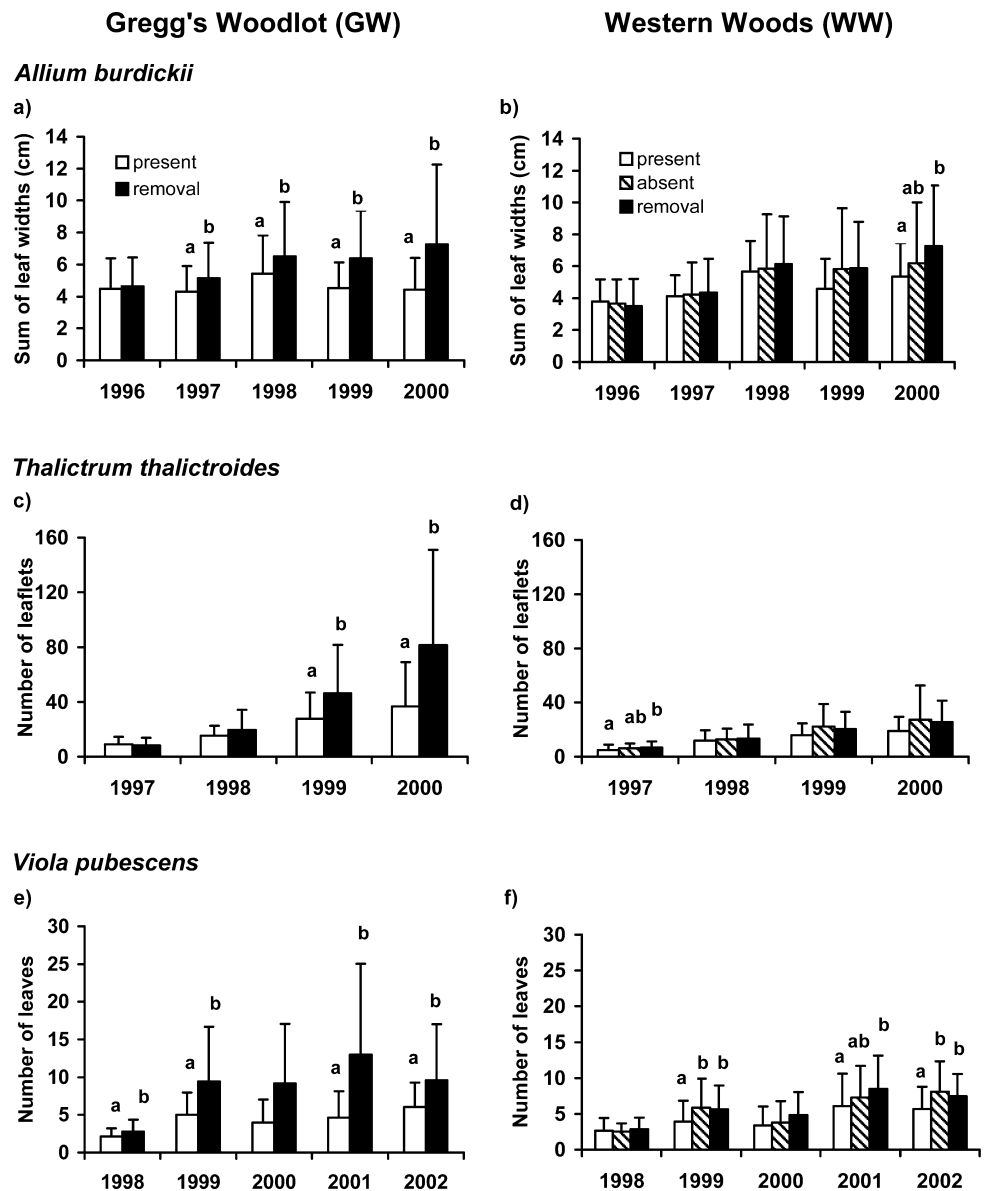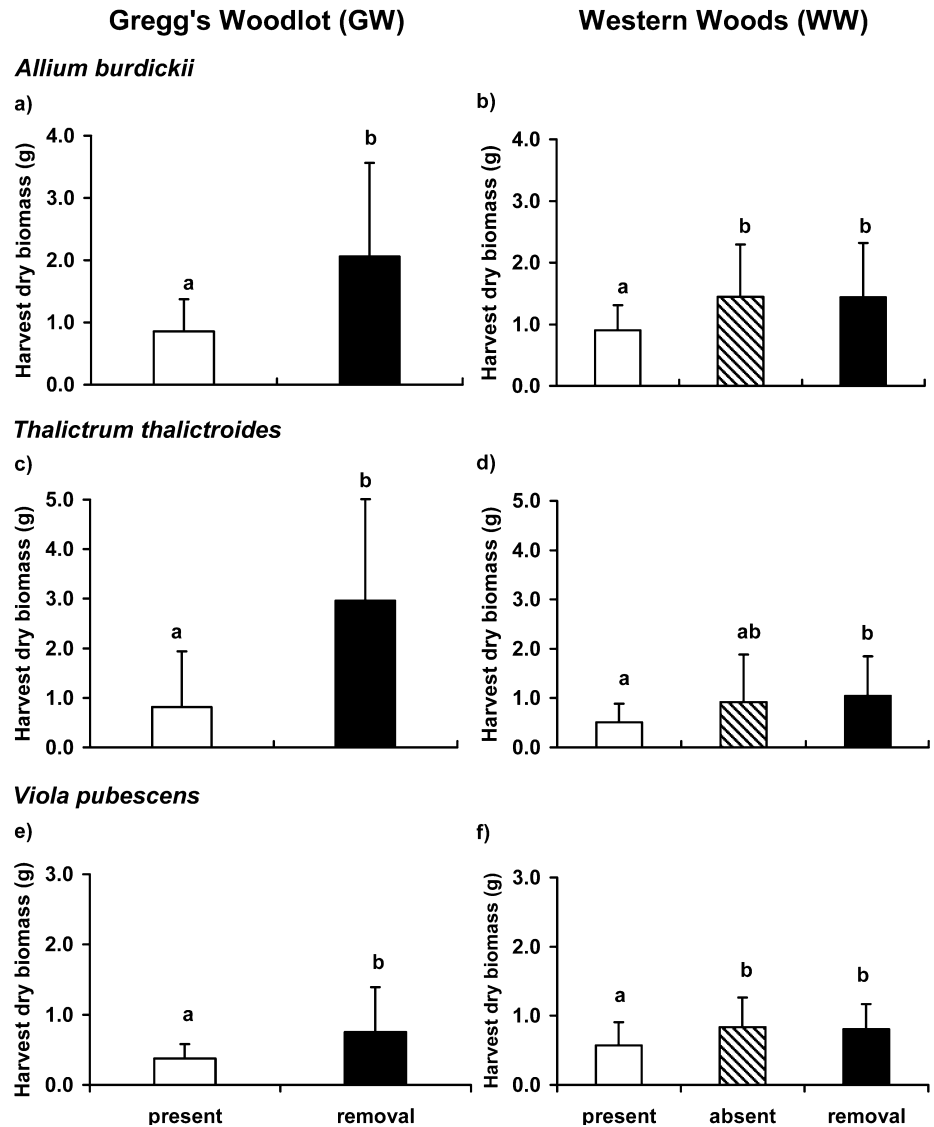

Gregg's Woodlot (GW)   Western Woods (WW)

average weight of seeds was significantly higher for individuals in the removal treatment in 1996, 1999 and 2000 at GW, but at WW there were no significant differences among treatments in any year (Table 1).

Over the 5-year experiment, individuals in the removal treatment produced 1.6 times as many seeds as individuals in the present treatment at GW (Fig. 6a); the distributions of seed counts in each treatment differed little from a Poisson distribution, and the Poisson mean was significantly higher in the removal treatment (Table 5). At WW cumulative seed production was 1.4× greater in the removal treatment than in the present treatment, with the absent treatment intermediate (Fig. 6b). These three treatments had significantly different Poisson means, but did not differ in zero-inflation probabilities (Table 5).

Thalictrum thalictroides

Survival of *T. thalictroides* did not differ among treatments at either site, although there was a trend (*P* =0.0644) at

GW for higher survival where *L. maackii* was present (Fig. 1c, d).

At GW, survivors in the removal treatment were larger than those in the present treatment in the last two years for which complete leaflet counts were obtained, averaging nearly twice as many leaflets in 1999, and more than 2× in 2000 (Fig. 2c, Table 2). At harvest, individuals in the removal treatment had significantly (over 3×) greater biomass (Fig. 3c, Table 2), due both to significantly more tubers and significantly greater average tuber weight (Table 2). However, at WW, treatment only affected size in 1997, when individuals in the removal treatment had significantly more leaflets than those in the present treatment (Fig. 2d, Table 2). At harvest the overall treatment effect was not significant (Fig. 3d, Table 2), but the Bonferroni test indicated that removal treatment plants had significantly greater biomass than those in the present treatment. Average tuber weight was higher in the removal treatment than in absent or present treatments, but the average number of tubers was not significantly affected (Table 2).

Table 1 Two-factor mixed GLM analysis of variance results for size and reproduction variables measured for *Allium burdickii* individuals grown in different treatments and blocks. At GW there were 30 blocks and the treatments were *Lonicera maackii* present and removal; at WW there were 20 blocks, and a third treatment, *L. maackii* absent, was included. Italicized variables were log-transformed

| Site	Year	Dependent variable	n	Treatment df	Treatment F	Block df	Block F	Treatment×Block df	Treatment×Block F
GW	1996	*Total leaf width*	236	1, 29.1	0.30	29, 29	0.74	29, 176	1.64*
		Fruits	139	1, 35.8	3.91§	29, 25.6	2.59**	27, 81	0.47
		Seeds	139	1, 33.1	3.32	29, 26	2.20*	27, 81	0.68
		Mean seed weight	131	1, 74	12.24***	29, 74	1.63*	26, 74	0.65
	1997	*Total leaf width*	233	1, 29.5	6.02*	29, 29	1.26	29, 173	1.50§
		Fruits	156	1, 34.2	22.95***	29, 26.6	1.30	26, 99	0.94
		Seeds	156	1, 32.7	13.87***	29, 26.5	1.26	26, 99	1.15
		Mean seed weight	151	1, 94	0.21	29, 94	1.91*	26, 94	1.00
	1998	*Total leaf width*	231	1, 29.5	5.33*	29, 29	2.95**	29, 171	1.85**
		Fruits	194	1, 32.1	10.30**	28, 28	1.61	28, 136	1.10
		Seeds	194	1, 30.6	4.76*	28, 28	1.31	28, 136	1.71*
		Mean seed weight	167	1, 31.6	0.55	28, 25.8	3.59***	26, 111	1.22
	1999	*Total leaf width*	220	1, 30.7	31.87***	29, 29	1.96*	29, 160	1.53§
		Fruits	103	1, 22.2	6.62*	26, 19.4	1.35	20, 55	3.00***
		Seeds	103	1, 22.7	10.42**	26, 19.2	1.58	20, 55	2.44**
		Mean seed weight	98	1, 52.0	9.14**	26, 52.0	0.63	18, 52	1.03
	2000	*Total leaf width*	198	1, 32.7	26.10***	29, 29	2.83**	29, 138	1.31
		Fruits	161	1, 40.4	28.37***	29, 26.9	2.94**	26, 104	0.62
		Seeds	161	1, 37.8	24.83***	29, 26.7	2.57**	26, 104	0.74
		Mean seed weight	154	1, 31.3	8.20**	29, 25.6	2.10*	25, 98	1.18
		Harvest biomass	184	1, 29.0	45.43***	29, 26.0	2.35*	26, 127	2.26**
WW	1996	Total leaf width	206	2, 41.2	0.67	19, 38.7	1.32	37, 147	0.96
		Fruits	32	2, 8.1	0.57	13, 6.7	0.39	8, 8	0.86
		Seeds	32	2, 8.1	0.77	13, 7.2	0.43	8, 8	1.40
		Mean seed weight	32	2, 8.0	0.05	13, 7.9	0.52	8, 8	8.34**
	1997	Total leaf width	167	2, 38.3	0.37	19, 37.2	1.55	32, 113	1.37
		Fruits	86	2, 27.8	1.14	18, 26.5	1.48	24, 41	1.6
		Seeds	86	2, 27.9	1.76	18, 26.6	1.51	24, 41	1.58
		Mean seed weight	83	2, 38	0.73	18, 38	1.09	24, 38	0.73
	1998	*Total leaf width*	159	2, 33.9	0.02	19, 32.67	2.14*	29, 108	1.86*
		Fruits	106	2, 24.2	3.01	18, 23.2	0.62	22, 63	2.17**
		Seeds	105	2, 24.2	3.54*	18, 23.1	1.1	22, 62	2.09*
		Mean seed weight	101	2, 59	2.45	18, 59	2.07*	21, 59	1.18
	1999	*Total leaf width*	156	2, 31.3	2.63	19, 30.7	2.13*	28, 106	2.24**
		Fruits	55	2, 16.7	0.79	15, 13.0	2.13	15, 22	1.59
		Seeds	55	2, 16.3	0.83	15, 13.5	2.58*	15, 22	2.06
		Mean seed weight	55	2, 17.2	1.17	15, 12.4	1.17	15, 22	1.22
	2000	*Total leaf width*	148	2, 30.9	5.40**	19, 29.9	3.16**	27, 99	1.79*
		Fruits	114	2, 27.2	2.00	19, 28.8	0.85	24, 68	1.57
		Seeds	116	2, 26.8	1.16	19, 27.4	2.32*	25, 69	3.32***
		Mean seed weight	115	2, 69.0	2.38	19, 69.0	0.87	24, 69	1.51
		Harvest biomass	137	2, 30.3	6.28**	18, 28.7	1.77	26, 90	1.67*

* *P*=<0.05
** *P*=<0.01
*** *P*=<0.001
§ *P*=<0.06

Treatment did not affect the likelihood of flowering at GW (1997: G^2=0.04; 1998: G^2=0.03; 1999: G^2=0.06; 2000: G^2=0.10; 2001: G^2=0.00; all *df* =1) (Fig. 4c). At WW individuals in the removal treatment were more likely to flower in 2000 than those in the present (G^2 =4.50, *df* =1, *P* =0.0340) or absent treatments (G^2 =6.01, *df* =1, *P* =0.0142) (Fig. 4d). However, there were no treatment effects on proportion flowering in other years (1997:

G^2=1.51; 1998: G^2=0.87; 1999: G^2=0.12; 2001: G^2=3.08; all *df* =2).

At GW reproductive individuals in the removal treatment averaged significantly more flowers in 1999, 2000, and 2001 (Table 2), and more seeds in 1999 and 2000 (no data for 2001) than those in the present treatment (Fig. 5c, Table 2). Treatment effects on reproduction at WW were confined to the last 2 years of the study. In 2000 both flower and seed production were significantly higher in the

Fig. 4 The proportion of live
individuals that flowered in each
treatment for each year for *Alli-
um burdickii* at **a** GW and **b**
WW, *Thalictrum thalictroides* at
c GW and **d** WW, and *Viola
pubescens* at **e** GW and **f** WW.
Different letters indicate signif-
icant differences ($P < 0.05$)
among treatments based on
likelihood ratio chi-square tests
(see text for details)
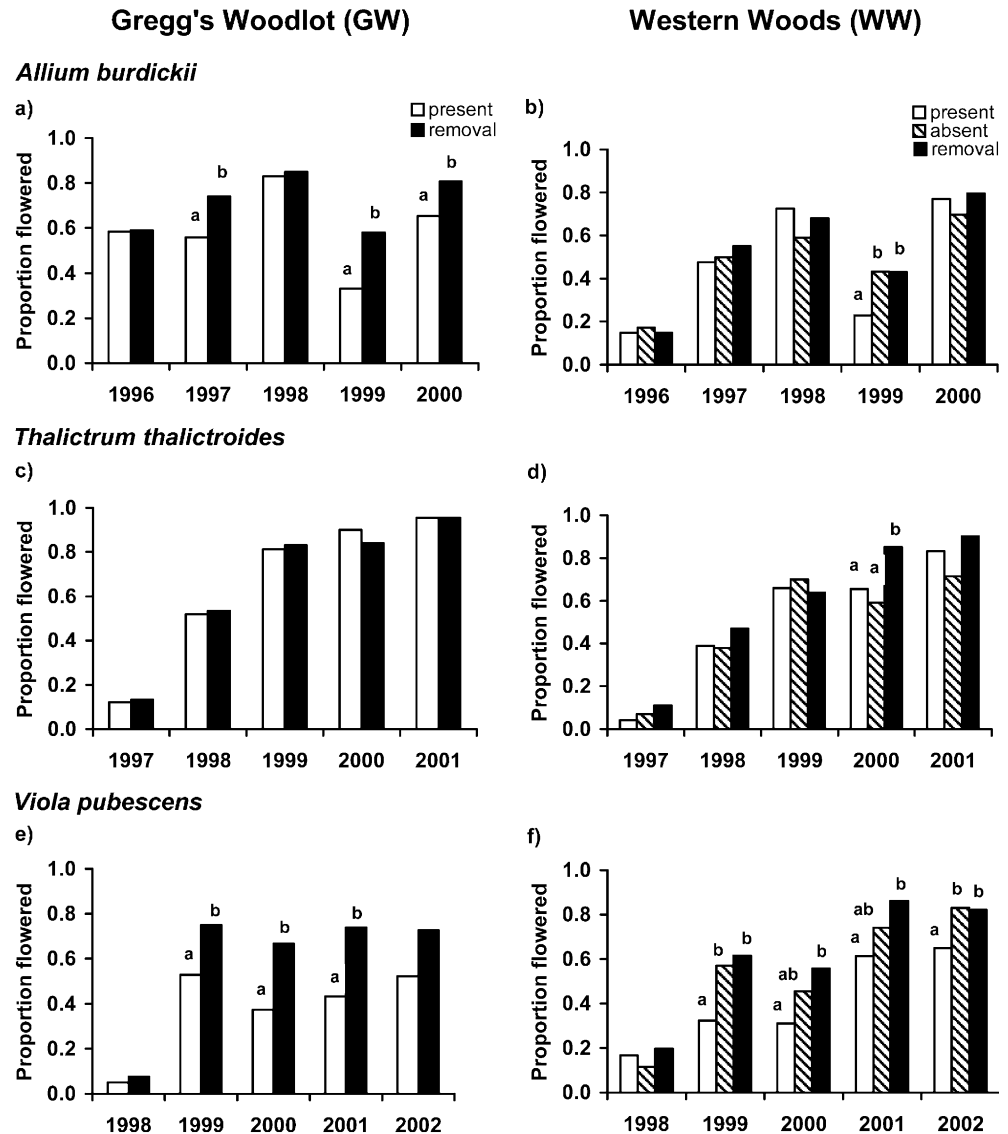
absent treatment than in the present and removal
treatments (Fig. 5d, Table 2). In 2001 there was a
marginal ($P = 0.0628$) effect of treatment on flower
number; the only pairwise difference was greater flower
number in removed versus present treatment individuals.

Cumulative seed production over the 4-year period
differed significantly among treatments at both sites. At
GW individuals in the removal treatment averaged twice
the seed production as those in the present treatment
(Fig. 6c), with treatments differing significantly in Poisson
mean but not in zero-inflation probability (Table 5). At
WW plants in the absent and removal treatments averaged
1.6× as many seeds as those in the present treatment
(Fig. 6d). All pair-wise comparisons of treatment Poisson
means were significantly different, but treatments did not
differ in zero-inflation probability (Table 5).

Viola pubescens

Survival of *V. pubescens* was not significantly affected by
treatment at either site (Fig. 1e, f).

At GW surviving individuals in the removal treatment
were larger than those in the present treatment, averaging
significantly more leaves in every year but 2000 (Fig. 2e,
Table 3), when there was a significant treatment×block
interaction. At harvest, individuals in the removal treat-
ment averaged more than twice the biomass as those in the
present treatment (Fig. 3e, Table 3). At WW individuals in
the removal and absent treatments averaged significantly
more leaves than those in the present treatment in 1999
and 2002, and those in the removal treatment averaged
significantly more than those in the present treatment in
2001 (Fig. 2f, Table 3). Harvest biomass in the removal
and absent treatments was significantly greater than that in
the present treatment (Fig. 3f, Table 3).

Treatment did not affect the proportion of survivors at
GW that flowered in the first year of study (1998:

Fig. 5 Mean (+SD) reproductive output per flowering individual in each treatment for each year at GW (**a**, **c**, **e**) and WW (**b**, **d**, **f**). Reproductive output was quantified as number of seeds for *Allium burdickii* (**a**, **b**) and *Thalictrum thalictroides* (**c**, **d**) and number of fruits for *Viola pubescens* (**e**, **f**). Different letters indicate significantly different (*P*<0.05) treatment means based on analysis of variance and, for WW, Bonferroni multiple comparison test (Tables 1, 2, 3). The *asterisk* indicates an overall treatment effect, but no significant pairwise differences

Gregg's Woodlot (GW)

Allium burdickii

Thalictrum thalictroides

Viola pubescens

Western Woods (WW)
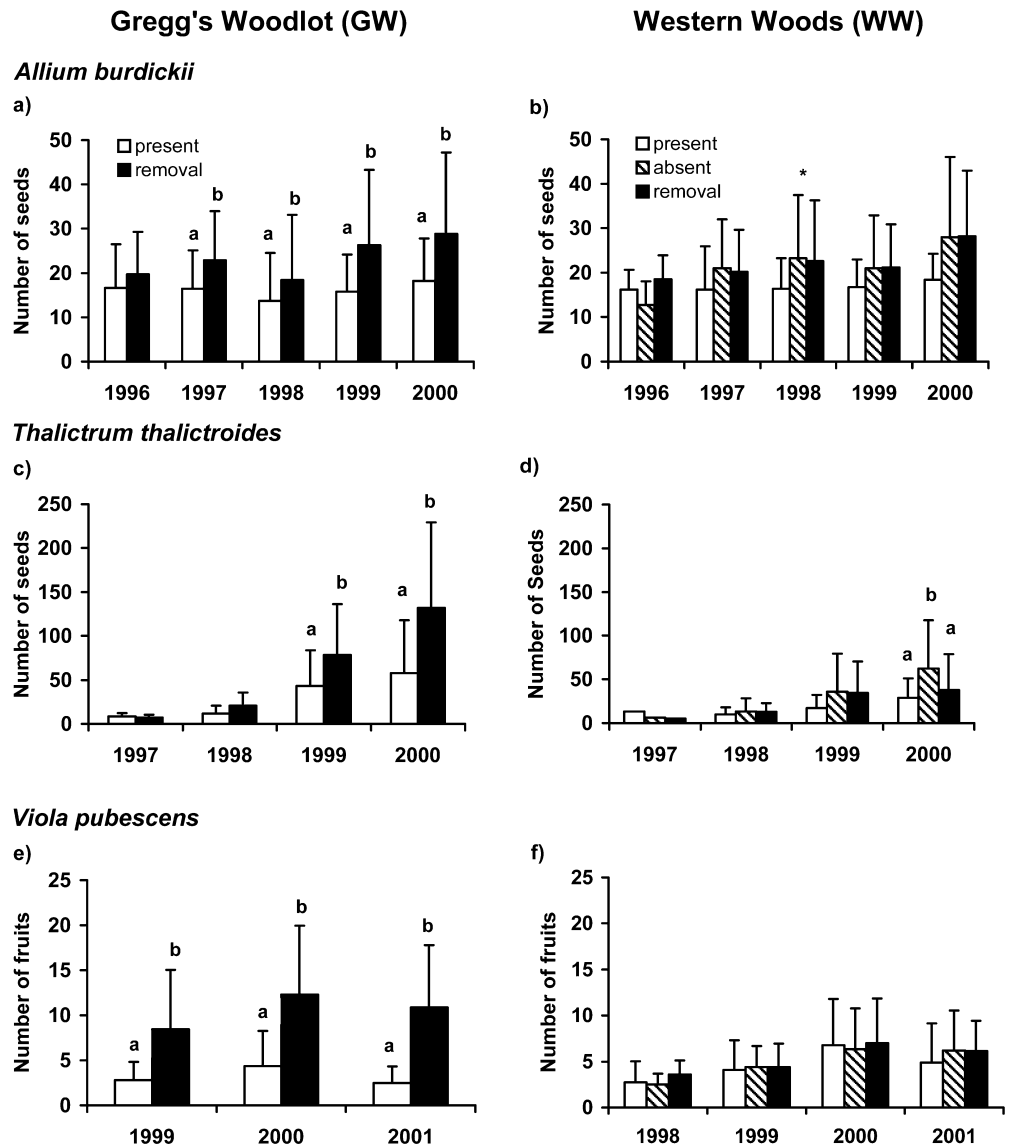
G^2=0.46), but flowering was significantly higher in the removal treatment in 1999 (G^2 =7.42, *P* =0.0065), 2000 (G^2 =8.44, *P* =0.0037), and 2001 (G^2 =8.93, *P* =0.0028), and marginally higher in 2002 (G^2 =3.84, *P* =0.0501, all *df* =1) (Fig. 4e). At WW, treatment did not affect flowering in 1998 (G^2 =1.99, *df* =2), but did in the 4 subsequent years (Fig. 4f). In 1999 flowering was lower in the present treatment than in the removal (G^2 =18.94, *P* <0.0001) and absent treatments (G^2 =8.06, *P* =0.0045); the same pattern occurred in 2002 (present vs removal: G^2=4.30, *P* =0.0381; present vs absent: G^2=4.67, *P* =0.0307). In 2000 and 2001 significantly more plants flowered in the removal treatment than in the present treatment (2000: G^2=7.00, *P* =0.0082; 2001: G^2=9.45, *P* =0.0021).

The numbers of cleistogamous (CL) flowers and fruits exceeded the numbers of chasmogamous (CH) flowers and fruits each year at both sites (Miller 2001, unpublished data). At GW, the numbers of CH flowers and fruits, CL flowers and fruits, as well as the numbers of total flowers and fruits per reproductive individual, were all signifi-

cantly greater for individuals in the removal treatment in 1999, 2000, and 2001 (Fig. 5e, Tables 3, 4). In 2002, only CH flower counts were complete at the time of harvest; these did not differ significantly between treatments (Table 4). At WW there were no significant treatment effects on any of these flower or fruit variables, except the number of CH fruits was marginally (*P* =0.055) affected in 1999, when individuals in the absent treatment averaged twice as many CH fruits as those in the present treatment, with the removal treatment intermediate (Fig. 5f, Tables 3, 4).

At GW the number of seeds per fruit was significantly higher in the removal treatment in 1999 (*F* =8.65, *df* =1, 105, *P* =0.004), but not in 2000 (*F* =0.23, *df* =1, 141). Average seed weight did not differ between treatments either year (1999: *F* =0.03, *df* =1, 44; 2000 CH fruits: F=0.09, *df* =1, 15; 2000 CL fruits: *F* =0.00, *df* =1, 36). At WW there was no treatment effect on seeds/fruit in 1999 (*F* =0.42, *df* =2, 95) or 2000 (CH fruits: F=0.40, *df* =2, 11; CL fruits: *F* =0.92, *df* =2, 270). Treatments did not differ

Fig. 6 Mean (+SD) cumulative reproductive output of an individual in each treatment at GW (**a**, **c**, **e**) and WW (**b**, **d**, **f**) over the course of the experiment for *Allium burdickii* (1996–2000), *Thalictrum thalictroides* (1997–2000), and *Viola pubescens* (1998–2001). For *V. pubescens*, chasmogamous (*CH*) and cleistogamous (*CL*) fruits are distinguished, but standard deviations are for total fruits. Significance tests for treatment effects are reported in Table 5. Significance of treatment effects on *V. pubescens* are not indicated on this graph, because separate tests were carried out on CH and CL fruits (see Table 5)
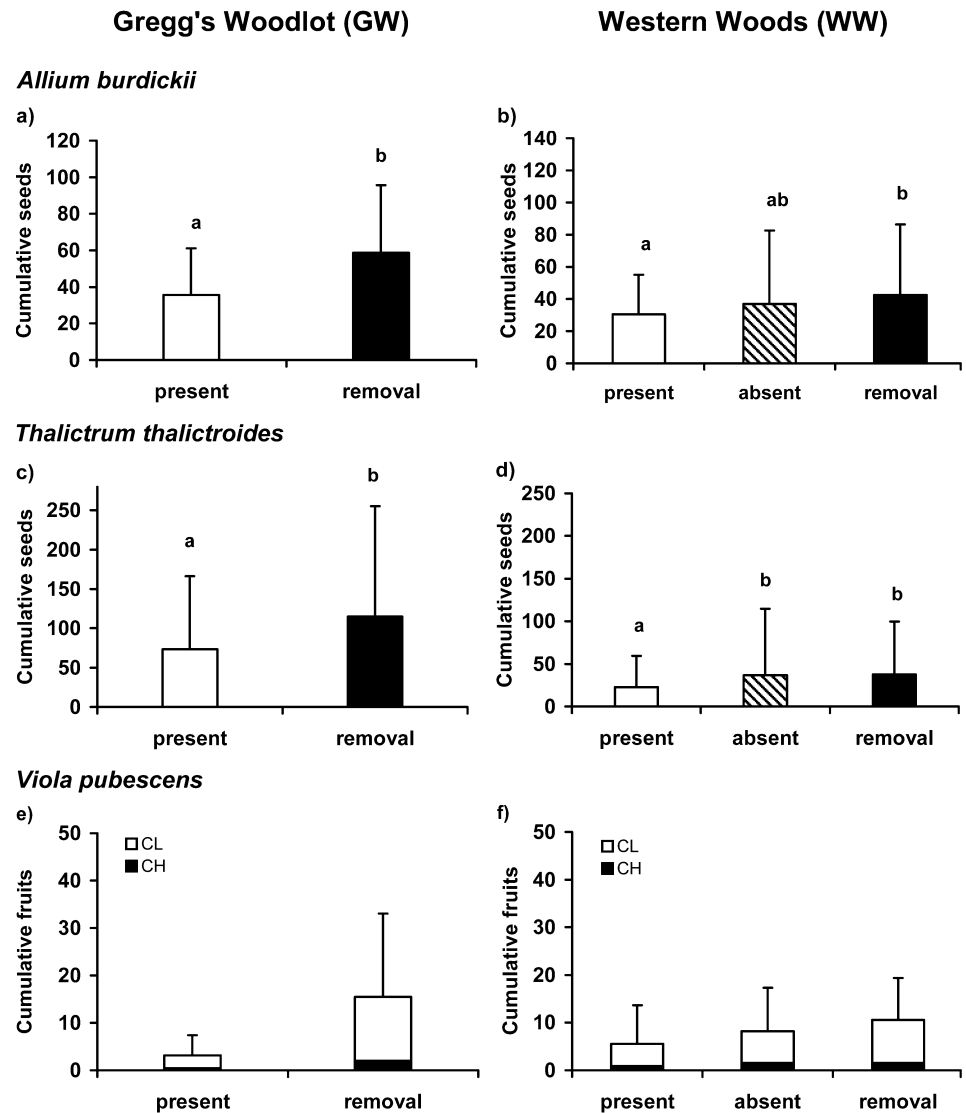
in average seed weight in 1999 ($F = 1.35$, $df = 2$, 45), or for seeds in CH fruits in 2000 ($F = 3.05$, $df = 2$, 9). However, there was a marginally significant treatment effect on average seed weight of CL fruits in 2000 ($F = 3.09$, $df = 2$, 59, $P = 0.0531$); CL fruits in the absent and removal treatments had seeds that averaged 1.8 mg while those in the present treatment averaged 1.4 mg.

Treatments affected cumulative CH and CL fruit production similarly. At GW, individuals growing where *L. maackii* was removed averaged more than 4× as many CH fruits and more than 5× as many CL fruits as those growing where *L. maackii* was present (Fig. 6e). The removal treatment had a significantly lower zero-inflation probability for CL fruits, and significantly higher Poisson means for both CH and CL fruits (Table 5). At WW, individuals in the removal treatment averaged nearly twice as many CH and CL fruits as those in the present treatment, with the absent treatment intermediate (Fig. 6f). Compared to the present treatment, the removal treatment had significantly lower zero-inflation probabilities for both CH and CL fruit counts (Table 5). Furthermore, the

Poisson mean for CL fruits was significantly higher in the removal treatment than in the present or absent treatments (Table 5).

Discussion

We found that the invasive shrub, *Lonicera maackii*, significantly reduced the growth and reproduction, but not survival, of all three species of perennial herbs. Effects appear to be cumulative, as treatments frequently did not differ in the early years of the study, and became stronger over time.

Survivorship

The lack of a negative effect of *Lonicera maackii* on the survival of any of these three understory species (Fig. 1) suggests that perennial herbs are somewhat less sensitive to competition from this invasive shrub than are some

Table 2 Two-factor mixed GLM analysis of variance results for size and reproduction variables measured for *Thalictrum thalictroides* individuals grown in different treatments and blocks (see Table 1 for details). All dependent variables were log-transformed, as indicated by italics. Seed counts at WW in 1997 were not analyzed due to low sample sizes. Seed counts in 2001 were not analyzed because plants were harvested before all seeds were mature

Site	Year	Dependent variable	n	Treatment		Block		Treatment×Block	
				df	F	df	F	df	F
GW	1997	*Leaflets*	147	1, 38.3	0.14	28, 22.5	3.05**	24, 93	0.46
		Seeds	19	1, 2.0	0.93	12, 1.7	0.27	2, 3	2.07
	1998	*Leaflets*	113	1, 29.3	1.72	27, 21.0	1.53	20, 64	0.67
		Flowers	60	1, 11.9	3.80	23, 12.0	1.38	9, 26	0.78
		Seeds	60	1, 10.1	0.12	23, 10.1	0.75	9, 26	2.04
	1999	*Leaflets*	105	1, 21.9	6.67*	26, 17.5	1.01	18, 59	1.25
		Flowers	87	1, 19.1	12.13**	26, 16.8	0.99	17, 42	2.44**
		Seeds	87	1, 18.9	10.85**	26, 16.8	1.26	17, 42	2.76**
	2000	*Leaflets*	98	1, 25.8	24.70***	26, 16.1	1.89	17, 53	0.66
		Flowers	87	1, 18.9	20.71***	25, 14.1	0.70	15, 45	1.26
		Seeds	87	1, 18.7	10.24**	25, 14.1	0.89	15, 45	1.32
	2001	*Flowers*	85	1, 14.9	15.71**	26, 11.7	0.66	13, 44	2.03*
		Harvest biomass	89	1, 15.4	18.71***	27, 13.6	0.55	14, 46	2.84**
		Tubers	86	1, 15.6	8.11*	27, 13.1	0.77	14, 43	2.07*
		Mass per tuber	86	1, 43.0	12.56***	27, 43	0.86	14, 43	1.04
WW	1997	*Leaflets*	144	2, 35.0	3.36*	19, 33.5	1.50	28, 94	0.97
	1998	*Leaflets*	102	2, 31.6	0.03	17, 21.3	2.05§	23, 59	0.68
		Flowers	44	2, 13.9	1.68	16, 8.8	1.06	13, 12	0.97
		Seeds	44	2, 14.2	1.97	16, 7.8	1.02	13, 12	0.78
	1999	*Leaflets*	91	2, 28.2	1.02	17, 20.5	1.90	22, 49	1.31
		Flowers	60	2, 19.4	2.24	17, 16.6	1.05	16, 24	0.94
		Seeds	60	2, 18.1	1.93	17, 16.4	0.93	16, 24	1.53
	2000	*Leaflets*	84	2, 28.5	1.77	17, 18.7	2.13§	21, 43	1.05
		Flowers	61	2, 19.9	5.01*	17, 9.1	1.20	14, 27	0.62
		Seeds	61	2, 20.2	3.87*	17, 8.9	1.39	14, 27	0.59
	2001	*Flowers*	63	2, 18.5	3.23§	17, 16.0	1.30	16, 27	1.28
		Harvest biomass	74	2, 22.9	2.20	17, 18.7	2.56*	19, 35	1.43
		Tubers	73	2, 21.8	0.84	17, 18.7	1.52	19, 34	1.77
		Mass per tuber	73	2, 34.0	7.91**	17, 34.0	1.67	19, 34	1.06

* $P = <0.05$
** $P = <0.01$
*** $P = <0.001$
§ $P = <0.07$

forest annuals (Gould and Gorchov 2000) and tree seedlings (Gorchov and Trisel 2003). This might be attributable to the stored reserves in underground perennating organs. If the herbs had been exposed to herbivory by mammals, a loss of leaf tissue would have exacerbated the negative impacts of *L. maackii*, resulting in higher mortality. Thus our exclusion of mammals may have prevented the manifestation of a treatment effect on survival.

Growth

As predicted, *L. maackii* negatively affected growth of all three herb species in at least one, if not several, years over the course of the experiment. Effects were generally stronger, and manifest sooner, at the more disturbed site (GW). Significant treatment effects usually emerged after one or two years, then persisted and expanded in subsequent years.

L. maackii treatment affected growth of *A. burdickii* and *V. pubescens* more consistently than growth of *T. thalictroides* (Fig. 2). This is not entirely consistent with our hypothesis that *L. maackii* has a stronger effect on growth of spring ephemerals than full-season perennials. The similarity of responses of the first two species is surprising given that *A. burdickii* is probably less tolerant of shade, based on Rothstein and Zak's (2001) finding that the closely related and phenologically similar *A. tricoccum* has a higher light compensation point than *V. pubescens* and the semi-evergreen *Tiarella cordifolia*. Growth of *T. thalictroides* differed more between stands than among treatments (Fig. 2c, d). The very rapid growth of this herb at GW, combined with high mortality at both sites (Fig. 1c, d), suggests its life history strategy differs from those of the other two herbs; it readily takes advantage of high resource levels, such as those at GW (particularly where *L. maackii* was removed), but has reduced ability to persist and grow under the low resource conditions in the more mature WW.

The lack of a significant treatment effect on total leaf production of *V. pubescens* at both sites in 2000 was unexpected, given the presence of a significant treatment effect the previous year (Fig. 2e, f). At GW, individuals were about the same size in 2000 as they were in 1999, but *L. maackii* reduced the size of individuals in the present

Table 3 Two-factor mixed GLM analysis of variance results for size and reproduction variables measured for *Viola pubescens* individuals grown in different treatments and blocks (see Table 1 for details). All variables were log-transformed as indicated by italics. Flower and fruit counts at GW in 1998 were not analyzed due to small sample size

Site	Year	Dependent variable	n	Treatment		Block		Treatment×Block	
				df	F	df	F	df	F
GW	1998	*Leaves*	161	1,33.7	7.97**	29, 28.3	1.04	28, 102	1.53
	1999	*Leaves*	138	1, 32	15.49***	29, 26.4	2.09*	26, 81	1.43
		CL flowers	90	1, 24.8	25.99***	27, 21.8	1.18	21, 40	1.28
		Total flowers	90	1, 25.7	33.70***	27, 22.0	1.41	21,40	1.02
		CL fruits	90	1, 25.9	32.75***	27, 22.0	1.42	21, 40	0.99
		Total fruits	90	1, 27.0	40.88***	27, 22.2	1.73	21, 40	0.82
	2000	*Leaves*	100	1, 18.7	1.11	26, 16.3	1.11	16, 56	2.41**
		CL flowers	53	1, 8.4	10.20*	19, 7.7	0.60	8, 24	3.57**
		Total flowers	53	1, 8.5	11.42**	19, 7.6	0.85	8, 24	2.65*
		CL fruits	53	1, 8.4	12.19**	19, 7.7	0.70	8, 24	3.44**
		Total fruits	53	1, 8.6	13.83**	19, 7.7	1.00	8, 24	2.31§
	2001	*Leaves*	90	1, 24.4	25.01§	27, 14.2	3.11*	15, 46	0.51
		CL flowers	53	1, 9.6	17.90**	20, 8.9	1.38	9, 22	2.32§
		Total flowers	53	1, 9.9	25.71***	20, 8.9	2.02	9, 22	1.54
		CL fruits	53	1, 10.3	31.78***	20, 8.9	1.48	9, 22	1.12
		Total fruits	53	1, 10.3	29.52***	20, 8.9	2.02	9, 22	1.05
	2002	*Leaves*	86	1, 20.0	13.69**	27, 12.1	2.20	14, 43	0.76
		Harvest biomass	86	1, 19.6	12.48**	27, 12.2	1.48	14, 43	0.82
WW	1998	*Leaves*	212	2, 39.5	0.11	19, 38.6	1.75	38, 152	1.58*
		CL flowers	34	2, 9.1	1.47	16, 8.4	0.82	9, 6	2.40
		Total flowers	34	2, 9.1	1.39	16, 8.5	0.74	9, 6	3.26
		CL fruits	34	2, 9.1	1.34	16, 8.3	0.83	9, 6	2.09
		Total fruits	34	2, 9.1	1.38	16, 8.4	0.66	9, 6	2.45
	1999	*Leaves*	194	2, 41.9	9.32***	19, 39.6	3.05**	38, 134	1.16
		CL flowers	98	2, 33.9	1.16	19, 31.5	2.56**	29, 47	1.74*
		Total flowers	98	2, 35.3	2.00	19, 32.2	2.69**	29, 47	1.37
		CL fruits	98	2, 36.1	1.61	19, 32.6	2.22*	29, 47	1.21
		Total fruits	98	2, 37.5	2.30	19, 33.2	2.25*	29, 47	1.02
	2000	*Leaves*	175	2, 39.3	2.37	19, 38.1	2.18*	37, 116	2.37***
		CL flowers	77	2, 28.7	0.88	17, 21.0	1.84	21, 36	0.86
		Total flowers	77	2, 29.7	0.94	17, 21.1	1.87	21, 36	0.76
		CL fruits	77	2, 26.2	0.08	17, 23.3	0.98	21, 36	1.16
		Total fruits	77	2, 30.2	0.90	17, 21.1	1.78	21, 36	0.72
	2001	*Leaves*	169	2, 39.2	2.93&	19, 36.9	1.76	36, 111	2.02**
		CL flowers	125	2, 38.3	1.71	19, 34.0	1.54	32, 71	1.75*
		Total flowers	125	2, 37.7	1.65	19, 33.8	1.64	32, 71	1.95**
		CL fruits	125	2, 37.0	1.72	19, 33.6	1.45	32, 71	2.19**
		Total fruits	125	2, 35.9	1.60	19, 33.2	1.53	32, 71	2.82***
	2002	*Leaves*	163	2, 39.5	4.28*	19, 36.2	1.08	35, 106	1.56*
		Harvest biomass	160	2, 38.6	4.93*	19, 36.0	1.92*	35, 103	1.91**

* $P = <0.05$

** $P = <0.01$

*** $P = <0.001$

§ $P = <0.06$

& $P = 0.065$ but removal treatment significantly greater than present treatment in Bonferroni tests.

treatment only in some blocks (treatment×block interaction). A significant interaction was also detected that year at WW, but in addition, plants were smaller in 2000 compared to 1999. Despite their reduced size, individuals produced more flowers and fruits, which suggests that in 2000, resources were allocated to reproduction rather than growth.

Fecundity

As with growth, reproduction was affected by *L. maackii* earlier and more strongly at GW, and effects on *T. thalictroides* were less consistent than effects on the other two species (Figs. 4, 5). *L. maackii* reduced both proportion flowering and fruits/seeds per flowering individual in *V. pubescens* and *A. burdickii*, but only seeds per flowering individual in *T. thalictroides*. It is not clear why the response of the first two species was similar, given that they have contrasting leaf and reproductive

Table 4 Analysis of Deviance tables from generalized linear models on chasmogamous (CH) flower and fruit numbers per flowering *V. pubescens* individual. These variables could not be analyzed by ANOVA due to non-normal distributions. *P* values of significant chi-square tests are in **bold**. Samples sizes were too low in 1998 for statistical analyses

Site	Year	Dependent variable	Source	Deviance	*df*	Chi-Square	Pr>Chisq
GW	1999	CH Flowers	Intercept	51.07			
			Treatment	43.78	1	23.63	**<.0001**
			Block	11.11	23	105.85	**<.0001**
		CH Fruits	Intercept	135.60			
			Treatment	113.04	1	22.32	**<0.0001**
			Block	60.66	27	51.81	**0.0028**
	2000	CH Flowers	Intercept	76.06			
			Treatment	67.97	1	7.61	**0.0058**
			Block	34.01	19	31.96	**0.0316**
		CH Fruits	Intercept	67.51			
			Treatment	62.26	1	4.74	**0.0294**
			Block	35.35	19	24.36	0.1827
	2001	CH Flowers	Intercept	118.67			
			Treatment	99.73	1	12.43	**0.0004**
			Block	47.24	20	34.44	**0.0233**
		CH Fruits	Intercept	92.93			
			Treatment	82.09	1	9.23	**0.0024**
			Block	36.42	20	38.87	**0.0069**
	2002	CH Flowers	Intercept	78.38			
			Treatment	74.82	1	2.55	0.1103
			Block	39.05	24	25.65	0.3713
WW	1999	CH Flowers	Intercept	35.98			
			Treatment	35.39	2	1.13	0.5680
			Block	24.66	19	20.45	0.3678
		CH Fruits	Intercept	136.69			
			Treatment	129.22	2	5.80	0.0550
			Block	99.11	19	23.40	0.2204
	2000	CH Flowers	Intercept	3.52			
			Treatment	3.47	2	1.59	0.4520
			Block	0.48	13	105.40	**0.0001**
		CH Fruits	Intercept	9.42			
			Treatment	9.34	2	0.24	0.8885
			Block	5.99	13	9.52	0.7329
	2001	CH Flowers	Intercept	174.48			
			Treatment	174.01	2	0.36	0.8367
			Block	137.76	19	27.11	0.1022
		CH Fruits	Intercept	169.87			
			Treatment	169.21	2	0.50	0.7802
			Block	136.28	19	24.89	0.1643
	2002	CH Flowers	Intercept	83.40			
			Treatment	82.38	2	1.91	0.3851
			Block	55.45	19	50.02	**0.0001**

phenologies; *A. burdickii* produces a single inflorescence as leaves are senescing whereas *V. pubescens* continues to produce CL flowers and fruits throughout the growing season.

Phenology provides a possible explanation for why seed mass in *A. burdickii* was more consistently reduced by *L. maackii* than seed mass of *V. pubescens* (Tables 1, 3); for *A. burdickii* seed mass is the only variable that can be adjusted in response to resource levels late in the season, whereas in *V. pubescens* the response may be manifest in reduced growth, CL flower production, or seeds per flower.

Because *V. pubescens* produces CH and CL flowers on the same individual, each individual is subject to the costs and benefits associated with producing both flower types. Because CL flowers are less costly (Schemske 1978; Waller 1979) their production is thought to be more resistant to environmental variability (Schemske 1978); thus one might predict less sensitive to competition from an invasive. However, we found that both CH and CL

Table 5 Model-generated estimates of zero inflation probability and Poisson mean of cumulative fruit and seed counts for each *L. maackii* treatment for three perennial herb species. Significance tests and coefficient of determination calculation are described in Materials and methods

Site	Species	Response variable	*n*	Treatment	Mean	Zero-inflation probability		Poisson mean		Coefficient of deter-mination
						Parameter estimate	F^a	Parameter estimate	F^a	
GW	*Allium*	Seeds	234	Present	35.68	0.04	1.19	37.26	576.75***	0.92
				Removal	58.64	0.02		59.67		
	Thalictrum	Seeds	147	Present	73.63	0.25	3.38	97.67	1357.84***	1.00
				Removal	114.59	0.39		187.92		
	Viola	CH fruits	161	Present	0.46	0.51	1.48	0.94	19.40***	0.26
				Removal	2.01	0.36		3.14		
	Viola	CL fruits	161	Present	2.63	0.41	5.47*	4.48	321.39***	0.93
				Removal	13.50	0.24		17.7		
WW	*Allium*	Seeds	205	Present	30.50	0.19	1.50	37.62a	138.91***	0.76
				Absent	36.88	0.31		53.64b		
				Removal	42.51	0.28		59.33c		
	Thalictrum	Seeds	145	Present	22.65	0.48	1.30	43.42a	123.92***	0.83
				Absent	36.86	0.55		81.47c		
				Removal	37.67	0.39		61.34b		
	Viola	CH fruits	212	Present	0.89	0.5b	3.05*	1.77	1.92	0.07
				Absent	1.55	0.39ab		2.52		
				Removal	1.59	0.22a		2.05		
	Viola	CL fruits	212	Present	4.64	0.43b	5.32**	8.14a	10.69***	0.14
				Absent	6.68	0.26ab		9.03a		
				Removal	8.93	0.18a		10.93b		

[a]*F* tests for GW have *df*=1, for WW *df*=2. For WW, treatments with different letters were significantly different based on pairwise comparisons with Bonferroni adjustment

* *P*<0.05

** *P*<0.01

*** *P*<0.001

reproduction were reduced by *L. maackii* (Table 4), similar to Matilla and Salonen's (1995) finding that both CL and CH flower production in *V. mirabilis* were reduced in low light. Each year, and in each treatment, CL flower and fruit production exceeded CH reproduction, similar to patterns reported for *V. sororia* (Solbrig et al. 1980) and *Oxalis acetosella* (Berg and Redbo-Torstensson 1998). The predictable seasonal shift from CH to CL flower production we observed in *V. pubescens* suggests the transition is controlled by daylength rather than resources, as it is in other *Viola* spp. (Evans 1956; Mayers and Lord 1983).

L. maackii suppressed the long-term reproductive success of all three perennial herb species at both sites (Fig. 6). For *A. burdickii* and *T. thalictroides*, our ZIP analysis finding that treatments did not differ in zero-inflation probability but did differ in Poisson mean (Table 5) indicates that, at least over a 5-year period, *L. maackii* does not reduce the likelihood of reproduction, but does reduce fecundity of reproductive individuals.

In contrast, for cumulative production of CL fruits in *V. pubescens*, both parameters were significantly affected by treatment, indicating that *L. maackii* both reduced the likelihood of reproducing *and* reduced CL fruit production of reproductive plants. Although treatment effects on CH fruit production were similar in magnitude to those on CL fruit production, only the zero-inflation probability was significantly affected at WW, and only the Poisson mean at GW. We believe the lack of statistical significance in both parameters is more likely due to the consistently lower levels of CH fruiting, rather than qualitative differences in how *L. maackii* affects CH versus CL reproduction.

Consequences for populations

For all three understory perennial herbs, *L. maackii* reduced growth and fecundity, but not adult survivorship. For herbaceous perennials in general, population growth rates are most sensitive to rates of individual growth (progression) and stasis (survival within same stage class) and less sensitive to recruitment of seeds and seedlings, clonal growth, and retrogression, based on elasticity values of transition matrices (Silvertown et al.49">1993). Therefore, the reductions in individual growth caused by *L.*

maackii that we documented for all three herb species are likely to significantly reduce population growth rates, and may be more important than the reductions in fecundity or the lack of effects on survival. We can make more specific inferences for *A. burdickii* because stage transition matrices for *A. tricoccum*, which has a very similar life history, have been parameterized (Nault and Gagnon 1993). Their elasticity analysis indicates that growth and vegetative reproduction of larger ramets had the greatest effect on population growth rate. Since our study of *A. burdickii* incorporated vegetative reproduction within growth, the reductions in growth of established ramets caused by *L. maackii* is likely to significantly reduce population growth rate.

Mechanisms

This experiment did not test the mechanism(s) by which *L. maackii* reduces growth and reproduction of native herbs, but it does provide some insights. If allelopathy were important, demographic rates of the herbs in the *L. maackii* absent treatment at WW would be higher than in the removal treatment, where allelopathic toxins could have accumulated in the soil before the shrubs were removed. However, we rarely detected higher demographic rates in the absent treatment (only *T. thalictroides* flowers and seeds per flowering plant in 2000 and cumulative seed production), suggesting allelopathy was unimportant. In fact, demographic rates in the absent treatment were frequently intermediate between those in the present and removal treatments, and in some cases (% of *T. thalictroides* fruiting in 2000, cumulative seed production of *Allium*, and cumulative CL fruit production of *Viola*) were significantly lower than in the removal treatment. We hypothesize that lower rates in the absent versus the removal treatment are attributable to greater competition with established herbs in and near the plots, as the removal plots probably initially had less herb biomass due to suppression by *L. maackii*.

Competition is the most likely mechanism by which *L. maackii* alters herb demography. Even if the community is not resource limited upon introduction (Davis et al. 2000), this shrub probably competes with native herbs for resources shortly after it colonizes. Light is frequently the limiting resource for understory herbs. The shady conditions caused by a dense *L. maackii* understory begin as early as March and continue through November, significantly reducing light for herbs that depend on the period before canopy leaf-out for much or most of their annual carbon gain. Consistent with this light competition hypothesis, herbs in the removal treatment consistently had higher demographic rates at GW, the site with lower tree basal area and a more open canopy.

In this more disturbed stand (GW) *L. maackii* had a greater impact on these herbs, consistent with the hypothesis that disturbance favors invasives by altering the selection regime that would otherwise favor locally-adapted native species (Byers 2002), for example by changing the seasonal pattern of light availability. We do not think the greater impact at GW was due to this site's higher density of *L. maackii* or larger plot size, because impacts at both sites were presumably due primarily to the large shrub in the plot center.

Microsite factors also influenced the effect of *L. maackii* on these herbs. Significant block effects were prevalent in the analyses, which suggest blocks differed in environmental factors such as light penetration or soil moisture that affected the herbs. The significant treatment×block interactions for some of the demographic variables may also have been due to differences in light or soil moisture. In wetter or more shaded blocks, the herbs would have benefited from *L. maackii* removal due to increased sun exposure, whereas in gaps or drier blocks, herbs might have been water-stressed by increased light. Although there was some year-to-year variation in treatment effects, suggesting some interaction with weather; in no year did *L. maackii* have a significant positive effect on any demographic parameter of any of the study species.

Conclusions

It is possible that the negative effects of *L. maackii* on the growth and reproduction of these herbs is no stronger than that which would be caused by a comparable biomass of native shrubs. However, native shrubs are very sparse at both study sites and in other stands in this region; e.g. in an old-growth stand at nearby Hueston Woods State Park, the dominant shrub, *Lindera benzoin*, has a density of only 0.0066 stems >1 cm dbh per m^2 (Foré et al. 1997), two orders of magnitude less than *L. maackii* at GW and WW. Thus, regardless of whether *L. maackii* has a greater per-capita or per-gram competitive effect than native shrubs, its high density means that its invasion subjects perennial herbs to a level of shrub competition not experienced in undisturbed forests.

The fact that impacts on the spring ephemeral, *A. tricoccum*, were similar to impacts on the two full-season herbs, fails to support our hypothesis that impacts correlate with a critical overlap in phenology. However, full season herbs such as *V. pubescens* fix most of their carbon before canopy closure (Rothstein and Zak 2001), and thus might be as sensitive to *L. maackii* shading as spring ephemerals. Based on our original hypothesis, we predict that summer herbs, those that grow primarily after canopy leaf-out, are less impacted by early leafing invasive shrubs, than any of the herbs included in this study.

Except for Gould and Gorchov (2000), this is one of the first studies to show experimentally the direct effects of an invasive plant on native herbs, and to assess the cumulative effects over five years. Our findings that effects of *L. maackii* on herb growth and reproduction were generally not detectable until the second or later year of the study highlight the need for multiple-year studies to assess the effects of invasive species on native species.

Populations of native perennials are clearly at risk from *L. maackii* and other invasive plants that expand early and

shade the forest floor. This risk could be overlooked by short-term studies of herb cover or density if, as shown in this study, the invader does not increase mortality of adult herbs. However, reductions in growth and reproduction of individual herbs, such as those caused by *L. maackii*, will likely reduce native herb population sizes over time. Even if native plants respond slowly, precautions should be made for combating invasive plants before negative impacts are realized.

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