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Regional Patterns of Floristic Diversity and Composition in Forests Invaded by Garlic Mustard (*Alliaria petiolata*)

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Abstract - The impacts of invasive species on native plant communities are often studied on small spatial scales but may vary across regionally heterogeneous landscapes. Comparisons of vegetation across several similar sites with and without an invasive species present can be logistically challenging but highly informative to both scientists and land managers. We examined regional geographic variation in the diversity and composition of 8 replicate northeastern forest-understory plant communities invaded by the non-native species *Alliaria petiolata* (Garlic Mustard). Despite variation in underlying soil conditions and horizon development, several native species and their associated functional groups were either negatively or positively associated with Garlic Mustard invasion at the regional scale, and soil moisture and pH were higher in invaded plots across all sites. Most tree species were less common at invaded sites, but high tree-seedling abundances at some sites led to regionally higher seedling abundance in the presence of Garlic Mustard. Our study highlights the importance of species-specific responses, as well as site-specific soil conditions, for better understanding potential impacts of invasion.

Introduction

It is well established that invasion by non-native plants can impact diversity and composition of native plant communities (Callaway and Ridenour 2004, Callaway et al. 2004, Elton 1958, Klironomos 2002, Simberloff and Von Holle 1999), and that a wide range of environmental disturbances can facilitate invasions of non-native plants in general (Dukes and Mooney 1999). In northeastern North American deciduous forests, research has documented correlations between plant invasions and landscape features such as fragmentation and past land-use (Motzkin et al. 1996, 1999). However, effects of individual invasive plants on the local flora are generally documented for just 1 or a few adjacent forest stands, and management of individual species is generally done on an ad-hoc, local basis (e.g., Kueffer et al. 2013). A fundamental knowledge gap remains in understanding interactions between forest flora and invasion by individual species of concern (Murphy and Romanuk 2014). Understanding whether and how specific invasive plants co-vary with floristic and environmental patterns at a regional scale can improve our understanding of the invasion process and bolster effective management (Kueffer et al. 2013).

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The invasive plant *Alliaria petiolata* (M.Bieb.) Cavara & Grande (Garlic Mustard; Brassicaceae) is widely known to invade intact forest-understory plant communities. A number of mechanisms including escape from natural enemies, phenotypic plasticity, and early phenology have been postulated for its success in North American forest habitats (Rodgers et al. 2008a). Several studies have found that it suppresses native plant growth in temperate deciduous forests of North America via phytochemical disruption of mutualisms with mycorrhizal fungi (Burke 2008, Cantor et al. 2011, Castellano and Gorchov 2012, Hale et al. 2016, Koch et al. 2011, Stinson et al. 2006, Wolfe et al. 2008). Garlic Mustard may also alter the native flora through direct competition and/or asynchronous capture of light, water, and nutrients (Myers and Anderson 2003, Whigham 2004), and through effects on nutrient cycling (Rodgers et al. 2008b), suppression of germination (Prati and Bossdorf 2004), and interactions with insect and mammalian herbivores (Dávalos et al. 2015a, 2015b; Kalisz et al. 2014). However, many local studies showing a direct displacement or inhibition of native plants in the field (Nuzzo 1999, Rodgers et al. 2008a, Stinson et al. 2007, Waller et al. 2016) are confounded by others showing little difference in the community composition of invaded and non-invaded sites (Davis et al. 2014, Nuzzo et al. 2009, Rodgers et al. 2008a, Rooney and Rogers 2011), a decrease in impact over time (Lankau et al. 2009), and co-occurrence with other invasions, soil and canopy disturbance, and herbivory—all of which also impact forest flora (Dávalos et al. 2015a 2015b; Eschtruth and Battles 2009; Kalisz et al. 2014; Knight et al. 2009; Nuzzo, et al. 2009). Studies that consider plant community assemblages of invaded forests in the context of geographic variation, particularly in soil variation at a regional scale, are missing from a vast literature on local ecology of Garlic Mustard. Land managers currently working to control Garlic Mustard at a local-stand scale would also benefit from a better understanding of plant community associations with Garlic Mustard invasion and their geographical variation.

We conducted comparative community-level analyses in 8 northern hardwood forests to test whether forest patches invaded by Garlic Mustard are floristically distinct from nearby non-invaded patches in diversity, density, and species composition. Based on prior studies, we predicted that Garlic Mustard presence would be associated with reduced plant diversity and that plant community composition would differ between invaded and non-invaded areas. We also predicted that Garlic Mustard invasion would be associated with an increase in other non-native plants and plants associated with disturbance, and with a decline in species dependent on mycorrhizae. We were interested in whether the floristic composition and diversity of invaded/non-invaded forest habitats is consistent across similar forests within a broader regional landscape, and, if not, which environmental and abiotic variables best predict differences in plant community composition.

Field-site Description

We surveyed and compared the forest understory vegetation in 8 distinct northern hardwood ecosystems of the northeastern US, where Garlic Mustard is a known management concern. Study forests were dominated by *Acer saccharum* (Sugar Maple), *Fraxinus americana* (White Ash), *Quercus rubra* L. (Red Oak), and *Pinus strobus* L. (White Pine), the presence of which suggest secondary regrowth following agricultural abandonment (Hall et al. 2002, Thompson et al. 2013). The study sites were located in an area from eastern and central Massachusetts to the Berkshire and Taconic Mountains, spanning 251 km and elevations of 40 m to 404 m (Table 1). In addition to choosing a similar canopy composition for each site, we controlled for site history by (a) verifying with landowners/stakeholders that Garlic Mustard has been present for at least 2 decades, (b) excluding sites with clear evidence of past agricultural use (i.e., confirming that plots were situated on soils with a shallow and disorganized Ap horizon indicative of use as unimproved pasture and/or woodlot; Motzkin et al. 1996), and (c) mapping spatial coordinates of the sites to available historical forest-cover maps (Motzkin and Foster 2009) and aerial photography (Army Map Service aerial photography courtesy of the US Geological Survey) to verify approximate forest age (Motzkin and Foster 2009).

Table 1. Physical descriptions of the 8 study sites, listed from southwest to northeast. Lat. = latitutde, long. = longitude, moist. = moisture, and elev. = elevation.

Site	Land stewardship	Lat. (°N), long. (°W)	Soil order, texture	Mean soil moist. (%)	Elev. (m)	Slope, aspect	Dominant canopy species
West Point (WP)	Department of Defense	41.3793, 74.0192	Inceptisol, Clay loam	35.6	343.2	20.5% 116.5°	Sugar Maple –Red Oak
Black Rock (BR)	Black Rock Forest Consortium	41.4207, 74.0104	Inceptisol, Sand clay loam	33.3	212.7	24.8%, 321.5°	Sugar Maple –Red Oak –Green Ash
Pittsfield State Forest (PF)	Massachusetts State	42.4868, 73.2998	Spodosol, Silt clay loam	35.2	360.5	4.6%, 102.2°	Sugar Maple –Black Cherry –Beech
Questing Forest (QF)	Trustees of Reservations	42.1211, 73.2542	Spodosol, Sand clay loam	34.3	404.4	18.7%, 297.0°	Sugar Maple –White Ash
McLennan Forest (MC)	Trustees of Reservations	42.2215, 73.1732	Spodosol, Clay loam	22.8	340.7	28.5%, 218.9°	Sugar Maple
River Road (RR)	Private	42.5365, 72.5691	Inceptisol, Clay loam	19.1	40	12.2%, 109.4°	Sugar Maple –White Ash –Silver Maple
Harvard Forest (HF)	Harvard University	42.5294, 72.1904	Inceptisol, Clay loam	26.9	315.7	17.2%, 253.2°	Sugar Maple –White Ash –Black Cherry
Drumlin Farm (DF)	Mass Audubon	42.4094, 71.3272	Entisol, Clay loam	24.2	74.3	13.3%, 80.1°	White Pine –Sugar Maple

Methods

Understory-community sampling

At each site, we established six 2 m × 2 m plots within patches of forest where Garlic Mustard has had a known presence for at least 2 decades. Garlic Mustard mean densities were 65 ± 12.6 SE plants per m^2 across all sites, including first- and second-year plants. We located the plots at random points along a transect spanning the length of the invaded patch with a minimum interval of 5 m. We chose invaded sites with a minimum density of 20 adult Garlic Mustard plants per m^2, a cut-off value used in prior work (Stinson et al. 2007). Given that the phytochemical effect of Garlic Mustard is known to decline at distances of ≥20 m from a patch (Wolfe et al. 2008), we selected non-invaded plots within 20–200 m of invaded plots along transects of a corresponding length and with a similar slope and aspect. In July 2013, May 2014, and August 2014, we identified to species and counted all vascular plants <1 m in height in each plot, following the species nomenclature in *Flora Novae Angliae* (Haines 2011). We also obtained estimates of Garlic Mustard seedling and adult densities in each plot. Although non-native taxa were sometimes present in both types of plots, we did not select our plots with regard to the presence of other non-native species, but rather considered their presence as a response variable to be generated from our floristic survey.

Environmental data

At each site we measured slope, aspect, percent slope, and canopy closure as well as soil texture (% sand, silt, and clay), moisture, and pH during the peak growing season for Garlic Mustard (June). We used a magnetic compass to determine aspect, a clinometer to measure slope, and a spherical densiometer to calculate canopy closure. We averaged 4 canopy-closure measurements at each plot (Strickler 1959). We employed a Theta Probe ML3 soil moisture sensor (Delta T Devices, LTD., Cambridege, UK) to quantify bulk soil moisture at 3 random points per plot and averaged those values to generate a seasonal estimate and then quantified moisture of the organic soil layer using the gravimetric water-content method (Jarrell et al. 1999). We determined the texture of mineral soil using the hydrometer method (Elliott et al. 1999) and measured pH of soil suspensions using a digital pH meter (Robertson et al. 1999).

Based on known linkages between soil geological history and vegetation (DeGasperis and Motzkin 2007, Motzkin et al. 1996, Schimel and Chadwick 2013, Soil Survey Staff 2017), we also mapped the spatial coordinates of our sites on local soil-surveys to identify soil-order classification. Soils at our sites included entisols with little to no horizon development in the relatively urban eastern-most site, inceptisols with moderate horizon development and weathering dominating the central portion of the study area in central Massachusetts and the Taconic Mountains, and spodosols with an accumulation of humus and several horizons dominating the western Berkshire Mountains (Soil Survey Staff 2017).

Analysis

Multivariate analyses. We utilized PC-ORD v. 5.10 (MjM Software, Gleneden Beach, OR) to conduct non-metric multidimensional scaling analysis (NMDS) of the full compositional data-set using averaged species densities for each of the 48 plots and Bray-Curtis dissimilarities. To reduce the effect of rare species, we removed species with an occurrence of ≤2 individuals (McCune and Grace 2002), resulting in a count of 61 species for analysis out of the 112 total observed. We found no major difference in species composition across sample dates, and thus created a single (averaged) compositional vector of community composition from our 3 census dates for each site. We considered Garlic Mustard invasion status as part of the sampling design and thus did not include Garlic Mustard density or occurrence as dependent variables. To reduce variation in species densities and to analyze plant communities in terms of relative abundance, we applied general relativization by species prior to analysis (McCune and Grace 2002). We employed the autopilot mode in PC-ORD for the NMDS analysis (medium thoroughness, 100 runs with real data, 0.000001 stability criterion, 10 iterations to evaluate stability, 500 maximum iterations). We visually inspected plot locations on a 2-dimensional plot of NMDS scores for grouping by site and invasion status, and by environmental variables (soil-texture category, soil order; Table 1) to determine if plant-community composition was ordered by these variables. We assigned each of the 61 species in the NMDS ordination a soil-order association based on occurrence within, or closest distance to, convex hulls around plot groupings by soil order. We calculated the proportions of plants within various disturbance and mycorrhizal categories, as described below, within each soil order.

To test for the relative effects of Garlic Mustard presence and soil order on plant community composition, we used multi-response permutation procedures (MRPP) on a Bray-Curtis dissimilarity matrix weighted by groups, which was performed in PC-ORD.

Univariate analyses. We used generalized linear mixed models (GLMMs) to test for effects of Garlic Mustard invasion, soil order, and their interaction on (1) density and species diversity (total species richness, Shannon diversity, and Pielou's evenness) of the total plant community, (2) density and diversity of plant functional groups, (3) proportional density and species richness of plants according to their associations with mycorrhizal fungi, and (4) soil moisture. We also used GLMMs to test for soil-order effects on Garlic Mustard density, and for the effects of Garlic Mustard densities on mycorrhizal plant density in invaded plots only.

The plant functional groups were forbs, tree seedlings, shrubs (including woody vines and ground cover), ferns and fern allies, graminoids (including sedges and rushes), and non-natives (all non-native plants). We assigned species' tolerance to disturbance according to descriptions in Haines (2011); those identified as occurring in disturbed/anthropogenic habitats were designated "disturbance-tolerant", and those without this identification were designated "disturbance-intolerant" (see Supplementary Table 1, available online at http://www.eaglehill.us/NENAonline/suppl-files/n25-3-N1636-Haines-s1, and, for BioOne subscribers, at https://dx.doi.

org/10.1656/N1636.s1). We categorized species as mycorrhizal or non-mycorrhizal (hereafter, mycorrhizal status), and further classified mycorrhizal plants by mycorrhizal type (endomycorrhizal or ectomycorrhizal), which we determined from published accounts (see Supplementary Analysis Methods and Supplementary Table 2 for additional information, available online at http://www.eaglehill.us/NENAonline/suppl-files/n25-3-N1636-Haines-s1 and, for BioOne subscribers, at https://dx.doi.org/10.1656/N1636.s1). Only species for which we could find published information on mycorrhizal associations were included in these analyses.

We analyzed proportions of plant density and species richness. Density and diversity were much higher for mycorrhizal than non-mycorrhizal plants, and proportions varied widely among mycorrhizal types; thus, all analyses were performed on each mycorrhizal category separately. In all GLMMs, we set site as the random effect. We conducted these analyses in the GLIMMIX procedure in SAS/STAT® software, Version 14.1 of the SAS System for Windows (© 2002–2012 by SAS Institute Inc., Cary, NC).

To test for species-specific differences by Garlic Mustard invasion status, we analyzed species densities using a zero-inflated Poisson (ZIP) model and the GENMOD procedure in SAS/STAT software. We deemed P-values ≤0.05 significant for all analyses and adjusted P-values for multiple comparisons using Bonferroni corrections in the MRPP and Tukey's HSD in the GLMMs.

Results

Community composition

The 3-dimensional NMDS solution had a stress of 19.7, with 15.9%, 14.7%, and 17.3% of variation explained by axes 1, 2, and 3, respectively. Plant communities in non-invaded and invaded plots were separated in the ordination but varied by soil order in degree of separation (Fig. 1A). Plots on entisol soils were distinct from plots on all other soil orders, and communities on inceptisol and spodosol soils overlapped only slightly (Fig. 1A). Of the included environmental variables, soil pH had the strongest correlation with community composition in the ordination; pH was positively associated with axis 2 and was higher for inceptisols and spodosols compared to entisols (Pearson's $r^2 = 0.21$ for axis 2). Entisols, inceptisols, and spodosols were associated with progressively decreasing proportions of disturbance-tolerant and non-native plant species (Fig. 1B, C; also see Supplementary Table 3 and Supplementary Fig. 1, available online at http://www.eaglehill.us/NENAonline/suppl-files/n25-3-N1636-Haines-s1 and, for BioOne subscribers, at https://dx.doi.org/10.1656/N1636.s1), but the proportion of mycorrhizal species did not vary with soil order (0.90, 0.94, and 0.93 mycorrhizal for entisol, inceptisol, and spodosol, respectively). Plant community composition, as determined by MRPP, varied significantly among soil orders, but not with Garlic Mustard invasion status (see Supplementary Table 4, available online at http://www.eaglehill.us/NENAonline/suppl-files/n25-3-N1636-Haines-s1 and, for BioOne subscribers, at https://dx.doi.org/10.1656/N1636.s1).

Based on these findings, we included soil order as a factor in the remainder of the analyses, but we excluded data from the single entisol site, due to a lack of replication. However, we provide supplementary figures that include the entisol-site data for qualitative comparison (see Supplementary Figs. 2–6, available online at http://www.eaglehill.us/NENAonline/suppl-files/n25-3-N1636-Haines-s1 and, for BioOne subscribers, at https://dx.doi.org/10.1656/N1636.s1).

Total plant density and diversity, and soil characteristics

There were no significant effects of Garlic Mustard presence on total plant community density or species diversity (See Supplementary Table 2, available online at http://www.eaglehill.us/NENAonline/suppl-files/n25-3-N1636-Haines-s1), and soil order did not significantly affect Garlic Mustard density ($F_{1,35}$ = 1.66, P = 0.206). However, bulk soil moisture ($F_{1,34}$ = 5.36, P = 0.0267) and organic soil pH ($F_{1,34}$ = 17.37, P = 0.0002) were higher on average in invaded than non-invaded plots (Fig. 2A, B). Garlic Mustard density had a positive correlation with organic soil moisture in invaded plots ($F_{1,13}$ = 4.92, P = 0.045; Fig. 2 C), but it was not correlated with organic soil pH ($F_{1,13}$ = 0.82, P = 0.381)

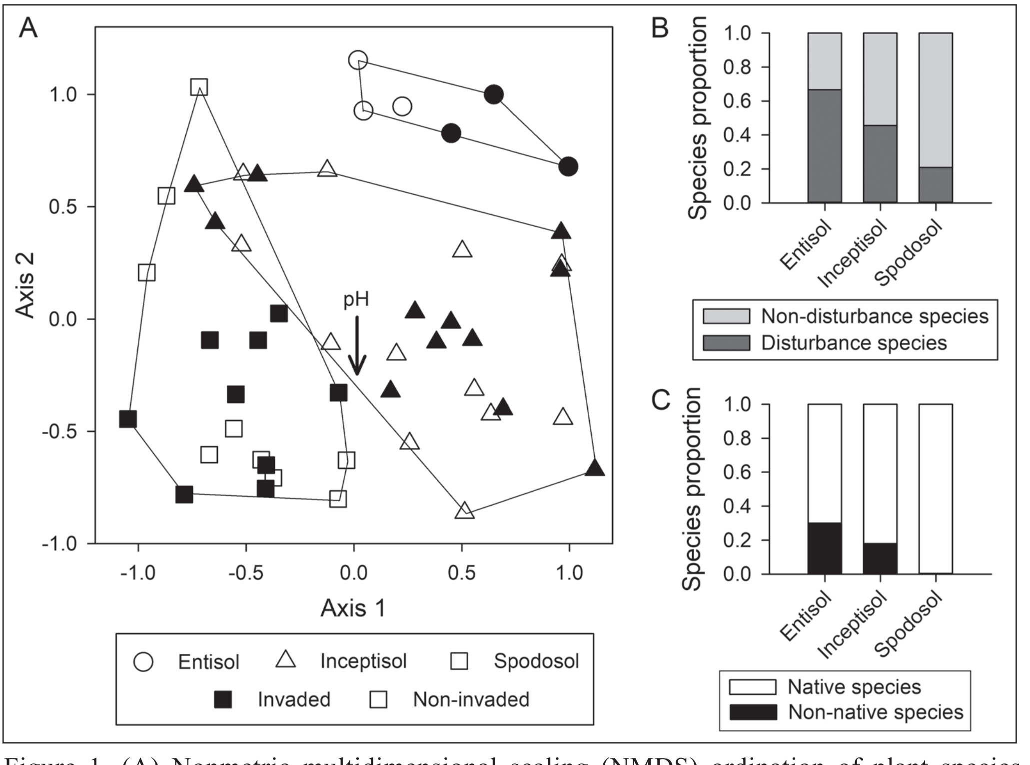

Figure 1. (A) Nonmetric multidimensional scaling (NMDS) ordination of plant species densities, separated by soil order; (B) proportions of species by disturbance tolerance; and (C) nativity associated with each soil order in the NMDS. In (A), convex hulls are shown for each soil order, and the arrow represents soil pH correlation with axis 2 (r^2 = 0.21; all other environmental variables had r^2 < 0.2 for both axes). Garlic Mustard was not included in these analyses. Different symbols in (A) represent plots in the following soils and invasion treatments: circles = entisol, triangles = inceptisol, squares = spodosol, open symbols = non-invaded, and closed symbols = invaded.

Density and diversity of functional groups

Proportional densities and proportional species richness of functional groups varied by Garlic Mustard invasion status and soil order (Fig. 3). For all sites together, ferns, non-natives, and shrubs tended to have higher total proportional densities in non-invaded plots, but tree seedlings showed the opposite pattern, with higher proportional densities in invaded plots at all sites and overall. Total proportional density of forbs was also higher in non-invaded plots, apparently driven by a relationship with spodosol sites. Regarding species richness, ferns and trees trended toward higher richness in non-invaded than invaded plots, but forb richness was lower in non-invaded than in invaded plots. There was higher Shannon diversity ($F_{1,33}$ = 8.42, P = 0.007), Pielou's evenness ($F_{1,31}$ = 14.2, P = 0.001), and forb richness ($F_{1,33}$ = 6.05, P = 0.019), in invaded than non-invaded plots, but forb density did not vary significantly by invasion status ($F_{1,33}$ = 0.03, P = 0.853) (Fig. 4). No other functional group was significantly affected by Garlic Mustard invasion (See Supplementary Table 2, available online at http://www.eaglehill.us/NENAonline/suppl-files/nX25-3-N1636-Haines-s1 and, for BioOne subscribers, at https://dx.doi.org/10.1656/N1636.s1).

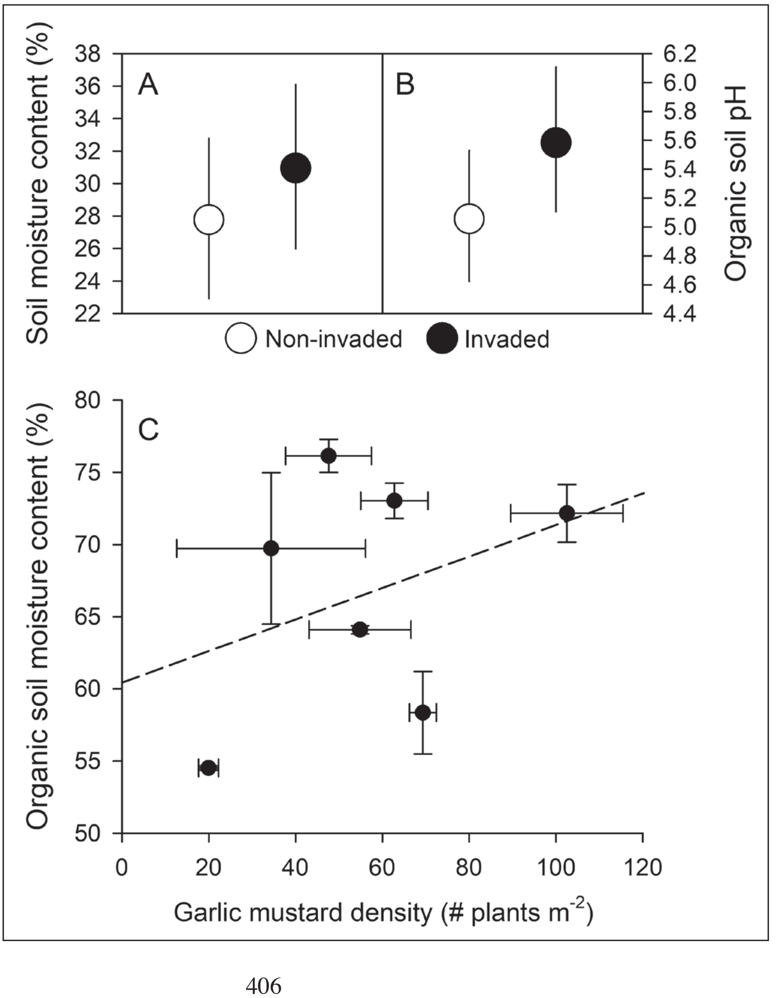

Figure 2. Effects of Garlic Mustard presence on (A) soil moisture and (B) organic soil pH, and (C) Garlic Mustard density effects on organic soil moisture. Soil moisture and organic soil pH were significantly higher in invaded than non-invaded plots (P = 0.0267 and 0.0002, respectively). The dashed line in (C) represents the positive, significant effect of Garlic Mustard density on organic soil moisture (P = 0.045; analysis based on a GLMM, which has no r^2 equivalent such as that supplied by linear regression analysis).

Among the 40 most abundant species in our study, some native species were clearly affiliated with Garlic Mustard presence or absence, while others were not (Table 2). The forbs *Maianthemum canadense* (Canada Mayflower) and *Erythronium americanum* (Trout Lily) were significantly less abundant in invaded than non-invaded plots. Seedlings of the native trees *Acer rubrum* L. (Red Maple) and White Ash were also negatively associated with Garlic Mustard. Native species that were positively associated with Garlic Mustard included the forbs *Tiarella cordifolia* (Foamflower) and *Impatiens capensis* (Jewelweed), and Sugar Maple and *Prunus serotina* (Black Cherry) seedlings.

Density and diversity of disturbance-tolerant and mycorrhizal dependent species

There was a disturbance × soil × Garlic Mustard invasion effect on the density of species with regard to disturbance tolerance (Table 3); invaded inceptisols and

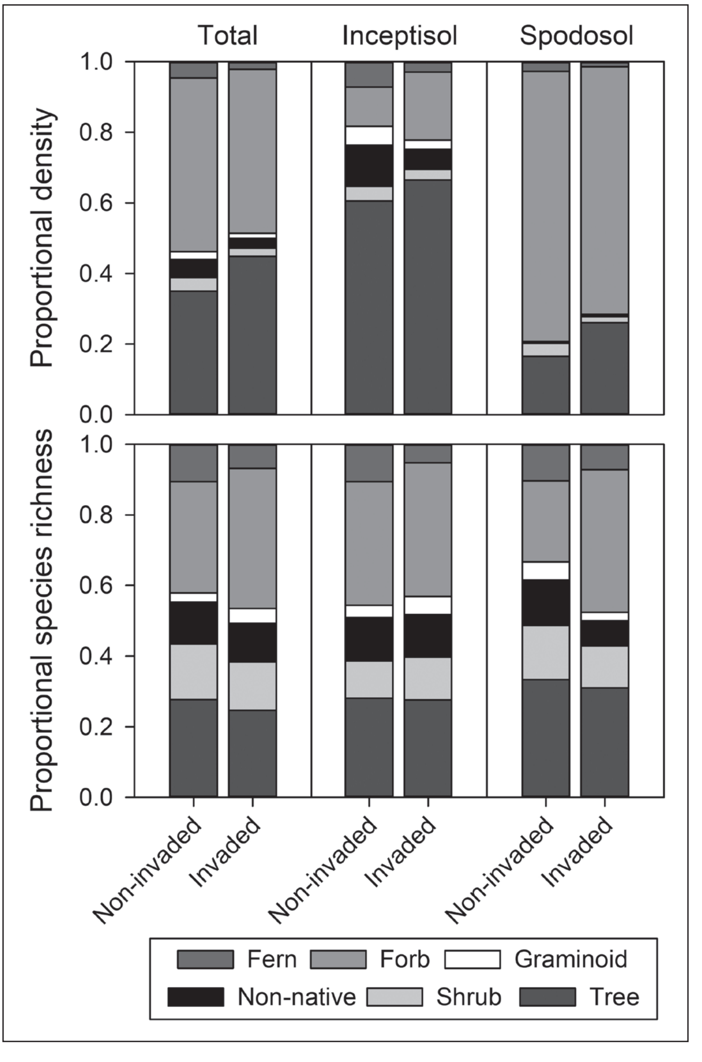

Figure 3. Proportions of functional-group density and species richness in non-invaded and invaded plots for all data combined (Total) and by soil order. Plant functional groups occur in descending alphabetic order (ferns, forbs, graminoids, non-natives, shrubs, trees) in each stacked bar. Garlic Mustard was not included in these analyses.

Table 2. The 40 species with highest density in the study by invasion association. Species negatively associated with Garlic Mustard have higher densities in non-invaded (N-inv.) than in invaded (Inv.) plots, positively associated species have the opposite association, and species that do not differ in densities by invasion status are neutral. Species in each category are sorted from highest to lowest maximum density. *P*-values and density estimates are from zero-inflated Poisson tests of density differences between invaded and non-invaded plots; *P*-values with an asterisk (*) are significant using the Bonferroni correction for multiple comparisons. Additional columns indicate number of sites and soil orders (I = inceptisol, S = spodosol) each species occurred in, and whether or not the species is considered disturbance tolerant. [Table continued on following page.]

	Functional	Density			No. of	Soils		Disturbance
Species	group	N-Inv.	Inv.	*P*	sites	I	S	tolerant?
Negative Garlic Mustard association								
Maianthemum canadense Desf. (Canda Mayflower)	Forb	20.400	2.300	<0.001*	4	X	X	Yes
Erythronium americanum Ker-Gawl. (Trout Lily)	Forb	19.500	12.900	<0.001*	3	X	X	No
Osmundastrum cinnamomea (L.) C. Presl (Cinnamon Fern)	Fern	3.810	1.720	0.002*	1	X		No
Lysimachia borealis (Raf.) U. Manns & A. Anderb. (Starflower)	Forb	2.960	0.608	0.003	2		X	No
Microstegium vimineum (Trin.) A. Camus (Japanese Stiltgrass)	Non-native	2.290	1.020	0.048	1	X		Yes
Fraxinus americana L. (White Ash)	Tree	2.120	0.600	<0.001*	7	X	X	No
Euonymus alatus (Thunb.) Sieb. (Burning-Bush)	Non-native	1.830	0.370	<0.001*	6	X	X	Yes
Rubus hispidus L. (Bristly Blackberry)	Shrub	1.330	0.083	0.030	2		X	Yes
Acer rubrum L. (Red Maple)	Tree	1.180	0.097	0.004*	3	X	X	No
Carex appalachica J. Webber & P.W. Ball (Appalachian Sedge)	Graminoid	1.120	0.471	0.016	5	X	X	No
Tussilago farfara L. (Coltsfoot)	Non-native	0.936	0.028	0.009	3	X	X	Yes
Dryopteris sp. (Wood Fern)	Fern	0.875	0.279	0.012	4	X	X	No
Parthenocissus quinquefolia (L.) Planch. (Virginia-Creeper)	Shrub	0.726	0.341	0.041	5	X	X	Yes
Positive Garlic Mustard association								
Tiarella cordifolia L. (Foam-Flower)	Forb	16.900	59.900	<0.001*	1		X	No
Acer saccharum Marsh. (Sugar Maple)	Tree	7.120	9.530	<0.001*	7	X	X	No
Impatiens capensis Meerb. (Jewelweed)	Forb	0.028	4.210	<0.001*	5	X	X	Yes
Persicaria sp. (smartweed)	Forb	0.028	2.750	<0.001*	1	X		No
Prunus serotina Herh. (Black Cherry)	Tree	0.689	1.670	<0.001*	6	X	X	Yes
Rubus sp. (blackberry)	Shrub	0.009	1.380	0.002	3	X	X	Yes
Circaea canadensis (L.) Hill (Broad-Leaved Enchanter's-Nightshade)	Forb	0.341	1.170	0.036	3	X	X	No
Sanguinaria canadensis L. (Blood-Root)	Forb	0.028	0.750	0.014	1	X	X	No
Symphyotrichum sp. (American-Aster)	Forb	0.028	0.608	0.030	2	X		No

Table 2, continued.

Species	Functional group	Density		*P*	No. of sites	Soils		Disturbance tolerant?
		N-Inv.	Inv.			I	S	
Neutral Garlic Mustard association								
Mitchella repens L. (Partridge-Berry)	Shrub	7.250	0.000	1.000	1		X	No
Arisaema triphyllum (L.) Schott (Jack-In-The-Pulpit)	Forb	3.030	2.590	0.156	6	X	X	No
Onoclea sensibilis L. (Sensitive Fern)	Fern	1.690	2.330	0.406	3	X	X	No
Carex pensylvanica Lam. (Pennsylvania Sedge)	Graminoid	1.580	2.120	0.352	3	X	X	No
Carpinus caroliniana Walt. (American Hornbeam)	Tree	1.370	1.640	0.633	1		X	Yes
Viburnum acerifolium L. (Maple-Leaved Viburnum)	Shrub	1.240	0.000	1.000	1	X	X	No
Eurybia divaricata (L.) Nesom (White Wood-Aster)	Forb	0.583	1.210	0.091	3	X		No
Fagus grandifolia Ehrh. (American Beech)	Tree	1.070	0.000	1.000	1		X	No
Viola pubescens Ait. (Yellow Forest Violet)	Forb	0.000	1.070	1.000	1		X	No
Dryopteris carthusiana (Vill.) H.P. Fuchs (Spinulose Wood Fern)	Fern	0.891	0.466	0.226	3	X	X	No
Polygonatum biflorum (Walt.) Ell. (King Solomon's-Seal)	Forb	0.409	0.740	0.185	4	X	X	Yes
Celastrus orbiculatus Thunb. (Asian Bittersweet)	Non-native	0.734	0.516	0.349	4	X	X	Yes
Lactuca canadensis L. (Tall Lettuce)	Forb	0.000	0.705	1.000	1		X	Yes
Trillium sp. (wakerobin)	Forb	0.517	0.687	0.433	4	X	X	No
Vitis riparia Michx. (River Grape)	Shrub	0.608	0.083	0.081	2	X	X	Yes
Geum fragarioides (Michx.) Smedmark (Appalachian Barren-Strawberry)	Forb	0.000	0.608	1.000	1		X	Yes
Toxicodendron radicans (L.) Kuntze (Poison-Ivy)	Shrub	0.534	0.216	0.073	4	X		Yes
Maianthemum racemosum (L.) Link (Feathery False Solomon's-Seal)	Forb	0.479	0.440	0.864	4	X	X	No

non-invaded spodosols had lower densities than species that are not disturbance tolerant compared to disturbance-tolerant species in either soil order (Fig. 5). However, there were no correlations between Garlic Mustard densities and disturbance-tolerant plant-density ($F_{1,13}$ = 1.75, P = 0.209) or species richness ($F_{1,13}$ = 0.89, P = 0.363), or non-disturbance tolerant plant density ($F_{1,13}$ = 1.15, P = 0.303) or species richness ($F_{1,13}$ = 0.04, P = 0.849). Total mycorrhizal plant proportional density and species richness did not vary significantly with Garlic Mustard invasion status or soil order (Table 3), nor were there correlations between Garlic Mustard densities and mycorrhizal plant density ($F_{1,13}$ = 0.52, P = 0.484) or species richness ($F_{1,13}$ = 0.52, P = 0.484).

Discussion

Few studies have examined regional variation in associations between Garlic Mustard invasion and forest understory plant communities, with prior research largely restricted to individual or a few adjacent local sites (e.g., Dávalos et al. 2015a, 2015b; Rodgers et al. 2008a; Stinson et al. 2007). Here, we demonstrate few direct effects of invasion on overall plant community patterns but some species-

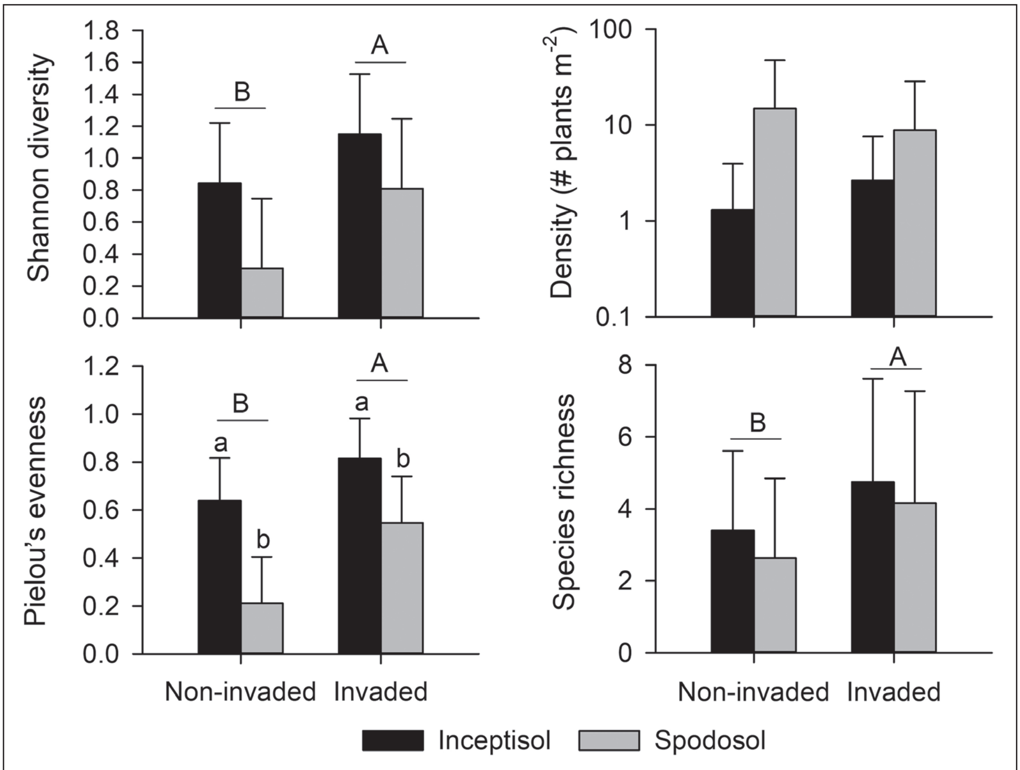

Figure 4. Effects of Garlic Mustard invasion and soil order on diversity, density, evenness, and richness of forbs. Different letters represent significantly different (P < 0.05) findings between invaded and non-invaded plots, and among invasion/soil order combinations; upper-case letters represent invasion effects, and lower-case letters represent soil order effects. Garlic Mustard was not included in these analyses.

Table 3. Generalized linear mixed models output for effects of invasion on density and species richness of disturbance-tolerant and mycorrhizal plants (not including Garlic Mustard) with respect to soil order and disturbance.

Characteristic	Effect	Density	Species richness
Disturbance tolerance			
	Invasion	$F_{1,71} = 2.06$, $P = 0.155$	$F_{1,71} = 5.33$, $P = 0.024$
	Disturbance	$F_{1,71} = 77.30$, $P < 0.001$	$F_{1,71} = 30.50$, $P < 0.001$
	Soil	$F_{1,5} = 0.04$, $P = 0.855$	$F_{1,5} = 6.48$, $P = 0.052$
	Invasion × Disturbance	$F_{1,71} = 1.48$, $P = 0.227$	$F_{1,71} = 1.77$, $P = 0.187$
	Invasion × Soil	$F_{1,71} = 2.45$, $P = 0.122$	$F_{1,71} = 0.00$, $P = 0.962$
	Disturbance × Soil	$F_{1,71} = 1.68$, $P = 0.199$	$F_{1,71} = 12.40$, $P = 0.001$
	Invasion × Disturbance × Soil	$F_{1,71} = 5.27$, P = 0.025	$F_{1,71} = 1.72$, $P = 0.193$
Mycorrhizal			
	Invasion	$F_{1,33} = 0.10$, $P = 0.753$	$F_{1,32} = 1.74$, $P = 0.197$
	Soil	$F_{1,5} = 1.17$, $P = 0.330$	$F_{1,32} = 3.05$, $P = 0.090$
	Invasion × Soil	$F_{1,33} = 1.03$, $P = 0.316$	$F_{1,32} = 0.15$, $P = 0.703$

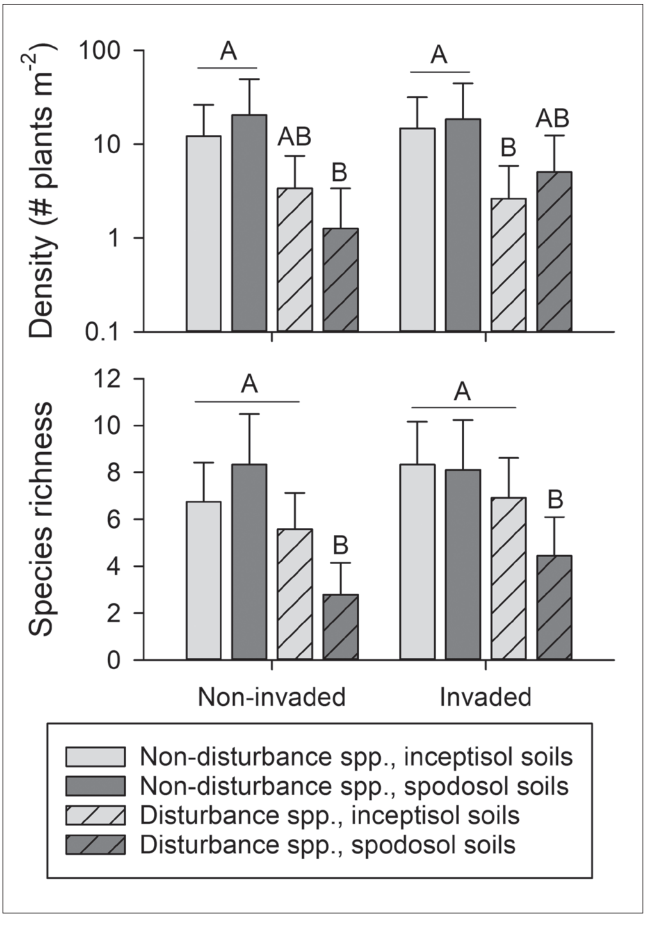

Figure 5. Effects of Garlic Mustard presence and soil order on plant density and species richness of disturbance-tolerant and non-tolerant plants. Different letters represent significantly different ($P < 0.05$) invasion/disturbance combinations, as determined by Tukey's HSD tests. Garlic Mustard was not included in these analyses.

specific trends in negative or positive associations with Garlic Mustard across several temperate deciduous forests of the northeastern US. We also show that site-to-site variation in soil order exists regarding interactions between Garlic Mustard and native vegetation, and that local variation in soil moisture and pH may also affect densities of both Garlic Mustard and other species in the community.

Species-specific responses to Garlic Mustard

Our work provides a regional context to prior studies showing reduced abundances of spring ephemerals, forbs, and some tree seedlings with Garlic Mustard invasion at the local level (Nuzzo 1999, Rodgers et al. 2008a, Stinson et al. 2007, Waller et al. 2016). Of the individual taxa that were scarce under Garlic Mustard invasion, Canada Mayflower, Trout Lily, and *Lysimachia borealis* (Starflower), are known to be mycorrhizal (Brundrett and Kendrick 1990), as are *Osmundastrum cinnamomea* (Cinnamon Fern) (Berch and Kendrick 1982) and seedlings of Red maple and White Ash. The affiliation of the tree-seedling functional group with Garlic Mustard invasion appears to be driven largely by high abundances of Sugar Maple, and in spite of lower species-level abundances of White Ash and Red Maple within invaded patches in our study. The Sugar Maple seedlings were largely in the 0–2-y age group, and occurred in mesic sites with canopy Sugar Maples; thus, the co-occurrence of Sugar Maple seedlings may be an incidental consequence of local canopy structure or timing of seedling emergence, and of Garlic Mustard and Sugar Maple's shared affinity for mesic sites (Rodgers et al. 2008a). All 3 tree-seedling species mentioned above are mycorrhizal obligates known to decline in growth in the presence of Garlic Mustard (Stinson et al. 2006), so it is unclear whether mycorrhizal suppression by Garlic Mustard is a major driver of all tree seedling abundances at our sites. Other mechanisms may interact with Garlic Mustard invasion to influence densities of other taxa, including higher soil moisture and pH in the invaded patches in our study. Elsewhere, soil moisture and pH have both been important at the local scale (Anderson and Kelley 1995, Meekins and McCarthy 2001), as have other factors not measured here, such as direct competition for nutrients (Poon and Maherali 2015), interactions with deer, which prefer native species to Garlic Mustard (Kalisz et al. 2014) and other exotic taxa (Boyce 2015).

Regionally consistent effects of Garlic Mustard invasion on native plant assemblages

Despite variation across soil orders and a general lack of Garlic Mustard effects on many of the measured community variables, 2 lines of evidence suggest that forest patches invaded by Garlic Mustard have different patterns of functional-group assemblages compared to uninvaded patches across the region. First, despite higher soil moisture at sites with Garlic Mustard, proportional densities and total densities of key fern-species that tend to prefer moist sites were lower at invaded sites. Lower fern densities could be due to competition or potential mycorrhizal suppression by Garlic Mustard (West et al. 2009), although allelopathy and/or high monospecific densities common among ferns themselves may have suppressive effects on Garlic Mustard itself (Stewart 1975). Second, and somewhat surprising, was a regionally consistent dip in non-native functional-group densities at sites with Garlic Mustard, which counters established theories of "invasional meltdown" and invasion "hotspots" which predict co-invasions in areas with recent disturbance and/or high resource availability (Stohlgren et al. 1999) and create feedbacks between disturbance and degradation of the native flora (Simberloff and Von Holle 1999). One explanation for reduced exotics in invaded plots in our study is our intentional sampling of intact forested sites, as opposed to forest edges or fragments where exotics are generally more common (Goldblum and Beatty 1999, Yates et al. 2004). Overall, our community-level data suggest that it is possible to categorize these functional groups as affiliates or non-affiliates with Garlic Mustard invasion at the regional scale. However, regional variability in environmental and vegetation patterns may counter predictions arising from smaller-scale experiments showing local effects of Garlic Mustard on native plants, or from broader geographic studies suggesting co-occurrences of exotics during the invasion process.

Management implications

Taken together, our results highlight the need to consider species-specific responses, landscape-level heterogeneity in soil conditions, and general plant community associations when evaluating ecological impacts and management priorities related to invasive plants.

For certain individual species, a regional pattern of negative association with Garlic Mustard invasion suggests the need for coordinated management. These taxa include the mycorrhizal fungi-dependent herbaceous plants Trout Lily and Canada Mayflower, as well as White Ash and Red Maple seedlings. However, our data generally suggest against the practice of managing for high diversity (Roberts and Gilliam 1995), especially if abundances of native forest understory species are a priority, given higher overall diversity in sites with Garlic Mustard. While Garlic Mustard did not tend to occur with other non-natives at a regional scale, the 3-way interaction between disturbance tolerance, invasion status, and soil order indicates frequent co-occurrences and thus the need for co-management of disturbance-tolerant species with Garlic Mustard invasion, particularly on inceptisols. Finally, given Garlic Mustard's affiliation with higher soil moisture, mesic sites may pose a greater management problem than drier sites. Ours is one of the first to capture landscape-level variation in vegetation patterns pertaining to Garlic Mustard invasion, and additional studies at this spatial scale could be highly informative for understanding broader geographic patterns of impact and management issues for Garlic Mustard.

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