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# Site Characteristics as Predictors of *Lonicera maackii* in Second-growth Forests of Central Kentucky, USA

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**ABSTRACT:** Non-native invasive plants can negatively affect the abundance and survival of native plant species and alter ecosystem function. Amur honeysuckle (*Lonicera maackii*) is an invasive shrub that is an increasingly onerous problem for forest managers in eastern North America. We examined site-specific characteristics related to the presence and abundance of Amur honeysuckle, and other non-native invasive plants, in 15 second growth forests in central Kentucky. Individual remnants were characterized by: (1) frequency and cover of non-native invasive and native shrubs, (2) overstory tree basal area, (3) forest floor litter depth, (4) species composition of leaf litter, and (5) soil characteristics. Of the measured variables, the only statistically significant relationships we discovered were between Amur honeysuckle and characteristics of the forest floor. We found significant negative relationships between Amur honeysuckle presence and both forest floor litter depth ( $P = 0.01$ ) and the percent of oak (*Quercus* spp.) litter on the forest floor ( $P = 0.004$ ). The abundance (percent cover) of Amur honeysuckle was significantly and negatively related to forest floor litter depth ( $P = 0.03$ ). These findings suggest that forest floor mass creates a barrier to invasion by exotic plants and that forests dominated by oak species may be more resistant to invasion by Amur honeysuckle. While this study did not identify a causal relationship between litter depth and oak litter and the presence of Amur honeysuckle, our findings do suggest that older forests, and those dominated by oak, may be more resistant to the invasion of Amur honeysuckle.

*Index terms:* forest floor, invasive species, leaf litter, *Lonicera maackii*, oak

## INTRODUCTION

The invasion of forest ecosystems by non-native invasive plants (NNIP) is of global concern due to their negative effects on biodiversity and ecosystem function (e.g., Vitousek 1990; Hobbs and Humphries 1995; Mack et al. 2000). In hardwood forests of the eastern United States, land managers face considerable challenges in controlling the expansion of NNIP, particularly those that are spreading into sites with intact forest canopies (Webster et al. 2006). There is a growing body of literature examining multiple facets of forest invasion, addressing the impacts of invaders on the success of native species (Hutchinson and Vankat 1997; Mack et al. 2000; Meiners et al. 2001; Gorchoy and Trisel 2003; Stinson et al. 2006; Hartman and McCarthy 2007), characteristics of invading plants (Bazzaz 1986; Luken 1988), and mechanisms of establishment within ecological systems (Ellsworth et al. 2004).

The extent of invasion is often inconsistent among locations within a region, and an important task for invasion ecology is to understand the drivers of this variability (Brothers and Spingarn 1992; Hutchinson and Vankat 1998; Bartuszevige et al. 2006; Chabrierie et al. 2007). Within forests, site characteristics contribute to regulation of invasion by non-native species (Brothers and Spingarn 1992; Huebner and Tobin 2006; Weber and Gibson 2007). Abiotic

factors that may regulate invasion include landscape structural features, such as the proportion of the landscape in agricultural use, the amount of edge present in the landscape, and land-use history (Lundgren et al. 2004). Site features such as soil pH have also been related to invasive species success (Xiong and Nilsson 1999; Johnson et al. 2006). Though more controversial, some research asserts that biotic features of the recipient community may also limit invasion. For instance, there is some evidence that high species diversity may create resistance to NNIP (Maron and Marler 2008), although that proposition has also been challenged (Von Holle 2005).

One of the most widespread and problematic NNIP in the eastern United States is the invasive shrub Amur honeysuckle (*Lonicera maackii* (Rupr.) Herder) (Luken and Thieret 1996; Barger et al. 2008; Center for Invasive Species and Ecosystem Health 2008). A suite of traits has been identified that may contribute to this species' invasion success. For instance, its seeds are widely bird-dispersed, do not require a period of dormancy, and are able to germinate year-round (Luken and Goessling 1995; Luken and Thieret 1996; Hidayati et al. 2000). Once established, Amur honeysuckle is both morphologically and physiologically plastic in response to changing environmental conditions (Luken et al. 1995). This species has a longer growing season than that of native plant

species (McEwan et al. 2009a) and may also benefit from allelopathic compounds it exudes, both in suppression of potential plant competitors (Dorning and Cipollini 2006; McEwan et al. 2010) and in resistance to insect herbivores (Cipollini et al. 2008; McEwan et al. 2009b). Amur honeysuckle is known to cause reduced recruitment and diversity of tree seedlings (Hartman and McCarthy 2004), have negative impacts on annual and perennial forest herbs (Gould and Gorchoff 2000; Miller and Gorchoff 2004), and initiate a general loss in native plant species richness and abundance (Hutchinson and Vankat 1997; Collier et al. 2002).

Prior research has examined patterns of Amur honeysuckle distribution in individual forest stands (Deering and Vankat 1999; Johnson et al. 2006), as well as broad, regional-scale, colonization patterns (Luken and Thieret 1996; Hutchinson and Vankat 1998). For example, Hutchinson and Vankat (1998) found that, in an agricultural matrix, Amur honeysuckle distribution was positively correlated with forest cover and forest connectivity, but was negatively correlated with large areas of agricultural lands that may act as a barrier to spread. Bartuszevige et al. (2006) found that distance to the nearest town was the only significant factor in determining the presence of Amur honeysuckle in woodlots; percent cover (abundance) of honeysuckle was correlated with the amount of edge in the landscape.

Biotic attributes of the forest may also play a role in regulating Amur honeysuckle. For instance, although this species can become established in second growth forests, it is found in less abundance and with lower density than in other locations (Luken and Goessling 1995; Hutchinson and Vankat 1997; Hartman and McCarthy 2007). Further, tree basal area and increasing diversity of woody species have been found to be negatively related to Amur honeysuckle density (Bartuszevige et al. 2006). Bartuszevige et al. (2007) found that Amur honeysuckle individuals were associated with areas of low leaf mass and that establishment was low in treatments with deep leaf litter. During field reconnaissance in preparation for site

selection for this study, observational evidence that fewer Amur honeysuckle plants were located in forests dominated by oaks (*Quercus* spp.) suggested that oaks may play a role in conferring resistance to Amur honeysuckle invasion (H.N. Wilson, pers. observation).

The objective of this study was to determine site-specific characteristics of second-growth forests that are associated with the establishment and proliferation of invasive species, with particular focus on Amur honeysuckle. We hypothesized ( $H_1$ ) that soil physicochemical characteristics (e.g., soil pH; Johnson et al. 2006) would influence the presence and abundance of NNIP at the 15 study sites. Evidence that woodlots with higher species diversity may exhibit lower densities of Amur honeysuckle (Bartuszevige et al. 2006) led to the hypothesis ( $H_2$ ) that the presence of native shrub species, such as coralberry (*Symphoricarpos orbiculatus* Moench), spicebush (*Lindera benzoin* (L.) Blume), mapleleaf viburnum (*Viburnum acerifolium* L.), and pawpaw (*Asimina triloba* (L.) Dunal), inhibits establishment of Amur honeysuckle. Mature forest interiors have been found to be relatively resistant to invasion by NNIP (Brothers and Spingarn 1992); therefore, it was hypothesized that ( $H_3$ ) forest characteristics such as basal area and the species composition of the vegetation would affect the establishment of Amur honeysuckle in the study sites. Finally, previous research demonstrating that forest floor characteristics are important for limiting Amur honeysuckle (Bartuszevige et al. 2007), coupled with field observations, led to the hypothesis ( $H_4$ ) that honeysuckle presence and abundance would be reduced in the presence of deep litter layers with greater proportions of oak litter.

## METHODS

### Study Area

This study was conducted in the Inner Bluegrass (IBG) physiographic region of central Kentucky. The region is characterized by a patchwork of forest remnants

dissected by urban and rural development and agricultural land use. Forests of the IBG are generally dominated by sugar maple (*Acer saccharum* Marsh.), common hackberry (*Celtis occidentalis* L.), black walnut (*Juglans nigra* L.), chinkapin oak (*Quercus muehlenbergii* Engelm.), shumard oak (*Quercus shumardii* Buckley), red oak (*Quercus rubra* L.), white oak (*Quercus alba* L.), hickory (*Carya* Nutt. spp.), ash (*Fraxinus* L. spp.), black cherry (*Prunus serotina* Ehrh.), elm (*Ulmus* spp.), black locust (*Robinia pseudoacacia* L.), honey locust (*Gleditsia triacanthos* L.), and Ohio buckeye (*Aesculus glabra* Willd.) (Braun 1950; Wharton and Barbour 1991). Soils of the region developed from Middle Ordovician Lexington Limestone and tend to be highly fertile (Wharton and Barbour 1991). The IBG is characterized by mild karst topography and gently rolling hills surrounding approximately 160 km of limestone outcroppings and deep gorges along the Kentucky River.

### Site Selection

Fifteen second-growth forest remnants were selected from a suite of potential public and private sites. Basic selection criteria were: (1) a minimum forest age of approximately 50 years, (2) an absence of previous removal of invasive species, and (3) a minimum area of 10 ha. A total of 26 potential forest remnants were visited to determine whether the remnant met selection criteria, and 15 final sites were selected (Table 1). Through conversations with landowners, cross-referencing areas with historical aerial photographs when available, and collection of tree cores (where permitted), it was determined that maximum tree age ranged from 56 to 95 years, with an average stand age of 70 across the sites (Table 1). Size of the study forest remnants ranged from 10 ha to 330 ha and the remnants were under varying ownerships, both private and public. Aerial photographs were used in conjunction with topographic maps provided by the Kentucky Geological Survey (KYGEONET) and National Land Cover Data to reference the 15 selected forest remnants using a geographical information system (GIS) (ESRI ArcView 9.1).

**Table 1. Descriptive characteristics of 15 forested study remnants in the Inner Bluegrass Region of central Kentucky. Calculations were made only using trees  $\geq 10$  cm dbh. Frequency (FRQ: %) and cover (COV: %) of *Lonicera maackii* are as measured along study transects in each site.**

Study Site	Size (ha)	Age (yrs)	BA (m <sup>2</sup> /ha)	DEN (stems/ha)	<i>Lonicera maackii</i>	
					FRQ	COV
Archer	72	68	29.1	407.6	0	0
Bright	10	-	32.1	601.9	100	63.75
Crossen	110	78	34.6	670.8	100	3.27
Crutcher	65	60	26.9	404.3	100	8.98
Dumaine	10	-	41.5	841.7	100	33.99
Grider	10	56	35.8	471.4	100	37.23
Hicks	20	63	35.8	613.4	100	49.38
Jim Beam	47	-	31.1	692.2	100	53.64
LAC <sup>a</sup>	121	66	37.1	637.1	100	25.58
LHC <sup>b</sup>	99	-	28.6	510.4	100	28.61
Polly's Bend	101	62	23.9	339.4	50	12.19
Raven Run	297	-	33.6	546.5	71	21.62
Sally Brown	256	73	30.4	436.2	80	0.69
Sowers	162	77	30.2	547.8	0	0
Tom Dorman	331	95	41.9	524.7	50	0.03

<sup>a</sup>Life Adventure Center of the Bluegrass

<sup>b</sup>Lower Howard's Creek

## Sampling

Random coordinates were selected in each forest site using Hawth's Analysis Tools (Beyer 2004) within ArcView (ESRI ArcView 9.1). In the field, these coordinates were located using a Global Positioning System unit, and marked the starting point of 100 m transects used for data collection. A minimum of three transects were established in each site; for larger sites, transects were established at a density of 1 transect per 35 ha. To avoid vegetation composition changes associated with elevation gradients, each transect followed the topographic contour. The direction of the transect along the contour was determined at random, or when necessary, in the sole direction that would accommodate the full length of the transect. Points were discarded if they located a transect in a landscape position that would encounter obstacles to sampling, such as steep cliffs, drainages that changed the aspect of the line, forest edge, floodplains or streamside areas, or patches of young forest that did not appear to meet the minimum age requirement of 50 years.

Along each transect, the following characteristics were measured: (1) frequency and cover of non-native invasive and co-occurring native understory shrubs; (2) basal area and species of trees  $\geq 10$  cm diameter at breast height (dbh; 1.37 m) and understory trees (2.5 to 10 cm dbh); (3) litter depth; (4) species composition of the leaf litter (oak vs. other species); (5) soil pH; and (6) soil carbon (C) and nitrogen (N) concentrations. Stands were sampled from mid-May to early August.

Vegetation sampling was accomplished using a combination of line-intercept sampling (Bonham 1989) and the point-center quarter method (PCQM; Cottom et al. 1953). Non-native and co-occurring native understory shrubs were recorded along the entire 100-m length of each transect using line-intercept sampling (Bonham 1989) wherein measurements were made of the distance along which any portion of target plants touched the transect. Forest stand composition was measured using the PCQM method with sample points established at 20-m intervals along each

transect, for a total of six sample points per transect. At each sample point, dbh and distance to the nearest tree 2.5 to 10 cm and  $\geq 10$  cm dbh in each of four quadrants was measured. For each transect, species composition, stem density, basal area (BA), relative stem density (RD), relative basal area (RBA), and relative frequency (RF) were calculated for canopy ( $\geq 10$  cm dbh) and understory (2.5 to 10 cm dbh) stems.

Soil and leaf litter samples were collected every 20 m along each transect at the same sample points used for PCQM data collection. To sample the leaf litter (Oi layer), a wooden template (28 cm x 28 cm) was placed 2 m up-slope from each PCQM point. After measuring the depth (cm) of the leaf litter at the inside center point of the template, the leaf litter was cut along the inside frame of the template with a serrated knife. Leaf litter was removed and bagged for later sample processing. Litter samples were sorted into oak and non-oak species, oven-dried at 60 °C, and weighed. Mean litter mass, litter depth, and oak as a

percent of total litter mass were estimated for each transect.

Following the removal of the leaf litter, the top 5 cm of soil, including the Oe and Oa if present, was collected using a 2-cm diameter soil core. Soil samples were refrigerated at 4 °C until processed. Samples were passed through a 2-mm mesh sieve; soil pH was measured using a 5-gram subsample of mineral soil mixed in a 1:2 soil to water slurry (Hendershot et al. 1993). The remaining soil samples were composited by transect, oven-dried at 60 °C, and analyzed for total C and N concentration using a LECO CN 2000 analyzer (LECO Corp., St Joseph, MI).

### Data Analysis

While multiple NNIP were encountered and initially analyzed for dominance in the 15 forest remnants examined for this study, only Amur honeysuckle occurred frequently enough and with enough cover to be included as a response variable in analysis. Relative cover of Amur honeysuckle was arcsine-square root transformed prior to analysis to attain normal distribution of the response variable according to the Shapiro-Wilk test for normality.

To address the effects of site-specific characteristics on the abundance (% cover) of Amur honeysuckle in forest remnants, statistical analyses were conducted using a linear mixed model (PROC MIXED) in SAS 9.2 (SAS Institute 2008), using the transects as replicates. The effects of site-specific characteristics on the presence/absence of Amur honeysuckle in forest remnants were addressed using a generalized linear mixed model (PROC GLIMMIX) in SAS 9.2 (SAS Institute 2008), also using transects as the unit of replication. The GLIMMIX procedure fits a generalization of a logistic regression model for the binary response of presence/absence while allowing for normally distributed random effects (i.e., site-specific effects) in the model (Schabenberger 2005). Model fit was evaluated using the generalized Chi-square statistic divided by the model degrees of freedom, a metric that is expected to be close to the value of 1 if the data distribution closely fits the model. Percent cover of the three most

frequent native shrub species, coralberry, spicebush, and pawpaw, were analyzed to address their effects on Amur honeysuckle presence and abundance. The influence of tree species dominance was analyzed by measuring absolute stem density, absolute basal area of all trees sampled, total basal area of tree species, and relative basal area of dominant tree species. BA measurements were grouped into canopy trees (dbh  $\geq$  10 cm) and understory trees (dbh 2.5 to 10 cm). Dominant tree species were defined by relative importance values. To examine the relationship between forest floor characteristics and Amur honeysuckle (presence and abundance), other variables in analysis included forest floor depth, leaf litter mass, and % of oak litter present in the leaf litter in statistical analysis. Soil characteristics included: (1) % soil C, (2) % soil N, (3) C:N, and (4) soil pH. For statistical analysis, mean pH for each transect was represented as  $H^+$  concentration.

Before including variables in statistical models, they were first analyzed using the SAS CORR Procedure (SAS Institute 2008) to determine multicollinearity using Pearson's correlation coefficient. Variables with absolute correlations with any other covariates of less than 0.4 ( $|r| < 0.4$ ), where  $r$  indicates the strength of pair-wise linear relationship, were retained. The 13 variables retained for analysis were: (1) % cover of spicebush, (2) % cover of coralberry, (3) % cover of pawpaw, (4) total BA of trees  $\geq$  10 cm dbh, (5) RBA of ash trees  $\geq$  10 cm dbh, (6) RBA of hickory  $\geq$  10 cm dbh, (7) RBA of oak  $<$  10 cm dbh, (8) RBA of ash  $<$  10 cm dbh, (9) RBA of common hackberry  $<$  10 cm dbh, (10) depth of the forest floor leaf litter, (11) % oak litter in the forest floor, (12)  $H^+$  concentration, and (13) soil % C. Percent oak present in the Oi layer was correlated with oak RBA ( $r = 0.6$ ,  $P < 0.0001$ ). Percent soil C and % total N were strongly correlated ( $r = 0.98$ ,  $P < 0.0001$ ), and only % soil C was retained for analysis so that the relationship between soil chemical characteristics and Amur honeysuckle could be examined. Percent soil C was chosen for inclusion over % N because % N had a more significant correlation with total BA (tree species  $\geq$  10 cm dbh ( $r = -0.26$ ,  $P = 0.04$ )) than did % soil C ( $r = 0.23$ ,

$P = 0.07$ ). C:N ratio was correlated with the % of oak litter in the forest floor ( $r = 0.53$ ,  $P = 0.0001$ ) and, therefore, was not included in the regression analysis. Total litter mass was correlated with litter depth ( $r = 0.64$ ,  $P < 0.0001$ ); litter depth was retained in the model.

To determine the site-specific characteristics that best explained the binary response of Amur honeysuckle presence or absence on individual transects, a generalized linear mixed model for binary response with backwards elimination of variables was used (PROC GLIMMIX). For the 13 remnants where Amur honeysuckle was present on at least one transect, determination of significant site-specific variables explaining the abundance of Amur honeysuckle was determined with linear mixed model analysis using backwards elimination of variables (PROC MIXED). Site was maintained as a random effect for both models. Variables were removed individually until only those variables with  $P \leq 0.05$  remained in the model.

Non-metric multidimensional scaling (NMDS) ordination using PC-ORD software (Kruskal 1964; Mather 1976) was used to relate variation in the presence of invasive species in forest remnants and environmental variables. NMDS is a method suited to the ordination of non-normal or discontinuous data (McCune and Mefford 1999). The ordination of NNIP and environmental attributes included a vegetation matrix with 64 transects (rows) and 7 variables (columns) and an environmental matrix with 64 transects and 8 variables. Variables for the vegetation matrix were: (1) Amur honeysuckle, (2) wintercreeper (*Euonymus fortunei* (Turcz.) Hand.-Maz.), (3) multiflora rose (*Rosa multiflora*), (4) European privet (*Ligustrum vulgare* L.), (5) Japanese honeysuckle (*Lonicera japonica* Thunb), (6) garlic mustard (*Alliaria petiolata* (M. Bieb.) Cavara & Grande), and (7) burning bush (*Euonymus alatus* (Thunb.) Siebold). Variables for the environmental matrix were: (1) litter depth, (2) % oak leaf litter, (3) % soil organic matter, (4) % C, (5) % N, (6) ratio of C:N, (7) BA of  $\geq$  10 cm dbh oak, and (8) BA of  $<$  10 cm dbh oak. Variables used for NMDS analysis included all environmental data as well

as oak BA because we were specifically interested in examining the relationship between oak and NNIP. These eight variables were chosen prior to statistical analysis. The preliminary ordination was run using the Sorensen (Bray-Curtis) distance measure. Starting configurations were randomly selected by PC-ORD. The parameters for the preliminary NMDS ordination involved a 6-dimensional array stepped down to a 2-dimensional solution. Instability criterion was 0.00001, with 400 iterations, 40 runs of real data and 50 runs of Monte Carlo randomized data (McCune and Grace 2002).

The final parameters for the ordination of NNIP and environmental attributes used the starting configuration from the preliminary run, and had a 2-dimensional solution. Instability criterion was 0.00001, with 400 iterations, 1 real run with no randomizations, and no step-down in dimensionality. Stability of the solution was assessed by PC-ORD using a plot of stress vs. iteration. The proportion of variance represented by each axis was based on the coefficient of variation ( $r^2$ ) between the distance in the ordination space and distance in the original space, as generated by PC-ORD.

## RESULTS

Basal area (trees  $\geq 10$  cm dbh) ranged from 23.9 – 41.9 ( $m^2/ha$ ), with total stem densities ranging from 339.4 – 841.7 stems/ha (Table 1). Forest remnants varied in tree species composition with oak-maple, oak-maple-hickory, and maple-ash communities most common. Sugar maple was a dominant species on most sites (10 of 15) and oak was a dominant species on 7 of 15 sites. Dominant oak species included chinkapin oak (5 sites), white oak (2 sites), and northern red oak (1 site).

Amur honeysuckle was found in 13 of 15 sites. On those 13 sites, frequency varied between 50% – 100% and cover varied between 0.03% – 64% (Table 1). The frequency and cover of other NNIP encountered were substantially less than that of Amur honeysuckle. Multiflora rose occurred on 10 of 15 sites, and had the highest frequency among the other NNIP, ranging from 0 to 83%, but with only 0

to 3% cover. European privet, Japanese honeysuckle and wintercreeper also had lower frequency (ranging from 0 to 67%) and cover (ranging from 0 to 5.2%) than Amur honeysuckle.

Based on multivariate tests, none of the measures of forest characteristics were significant factors in Amur honeysuckle presence or abundance. The shrub layer of the forest remnants in this study was often sparse with a small number of native shrubs and small trees encountered in sampling. We identified no significant relationships between the cover of the most important native shrubs (4.1% total cover), coralberry (3.5%) and pawpaw (1.8%), and either the presence or abundance of Amur honeysuckle.

Despite a reasonable range in each of the soil chemical characteristics, we found no statistically significant relationships between these characteristics and Amur honeysuckle presence or abundance. Across the 15 forest remnants, soil pH ranged from 4.1 to 6.9 (average of 5.6), percent C ranged from 3% to 14% (mean of 5.5%), percent N ranged from 0.3% to 1.2% (mean 0.45%), and C:N ranged from 10.9 to 15.5 (mean 12.2).

There was a general pattern wherein sites with the highest litter depth had the least or no Amur honeysuckle (Figure 1). Generalized linear mixed models (PROC GLIMMIX) showed that forest floor litter depth (0 cm to 4.65 cm;  $P = 0.01$ ) and the percent of oak litter on the forest floor (0.2% to 66%;  $P = 0.004$ ) were the only statistically significant predictors of Amur honeysuckle presence. Oak species found in the litter, in order of abundance, were chinkapin oak, white oak, northern red oak, black oak (*Q. velutina* Lam.), and shumard oak. A linear mixed model analysis (PROC MIXED) revealed that the only significant predictor of Amur honeysuckle abundance in second growth forests was leaf litter depth ( $P = 0.03$ ).

To examine the response of Amur honeysuckle presence to litter depth and % oak in the leaf litter, the two variables were modeled together, using a generalized linear mixed model (PROC GLIMMIX). The

model showed no significant interaction between litter depth and % oak litter ( $P = 0.2$ ). Estimated probabilities of presence over varying % oak in litter were calculated and plotted for three fixed litter depths (Figure 2). Leaf litter depths of 0.5 cm, 2.2 cm, and 3.0 cm were chosen because they were representative of the low, moderate, and thick forest floor depths found across the study sites. The probability of finding Amur honeysuckle was calculated as:

Probability of honeysuckle presence =

$$\frac{\exp(b_0 + (b_1 * \% \text{ oak litter}) + (b_2 * \text{litter depth}))}{1 + \exp(b_0 + (b_1 * \% \text{ oak litter}) + (b_2 * \text{litter depth}))}$$

where  $b_0 = 7.75$ ,  $b_1 = -0.1104$ , and  $b_2 = -1.2099$  (Figure 2). The model predicts Amur honeysuckle probability presence at nearly 100% where oak litter is less than 10% of total litter mass. Where modeled litter depth is 0.5 cm, Amur honeysuckle presence remains close to 100% until oak litter is roughly 40% of litter mass. At modeled litter depth of 2.2 cm, the likelihood of finding Amur honeysuckle drops to 67% when oak litter content is 40% of litter mass. With modeled litter depth of 3.0 cm, the likelihood of finding Amur honeysuckle is reduced to 44% when oak litter content is 40% of litter mass.

Non-metric multidimensional scaling arrayed NNIP along a gradient in ordination space relative to measured environmental variables (NMDS; Figure 3). The NMDS final solution included two axes (Monte Carlo test:  $P = 0.02$ ), which explained a large portion of variation in the data set (Axis 1  $r^2 = 0.718$ , Axis 2  $r^2 = 0.189$ ). Final stress (7.2) and final instability (0.00001) of the ordination strongly suggested a reliable final configuration (McCune and Grace 2002). Of the eight environmental variables measured, those that had an  $r^2 > 0.2$  were C:N ratio, litter depth, % oak in leaf litter, and oak RBA ( $\geq 10$  dbh oak; Figure 3). Burning bush, Amur honeysuckle, wintercreeper, and European privet were found in the mid-right portion of the diagram, associated with remnants that had shallow leaf litter depths and less oak. Multiflora rose and Japanese honeysuckle were found more centrally along axis one, suggesting a wider tolerance of conditions; however, the location of Japanese honeysuckle near

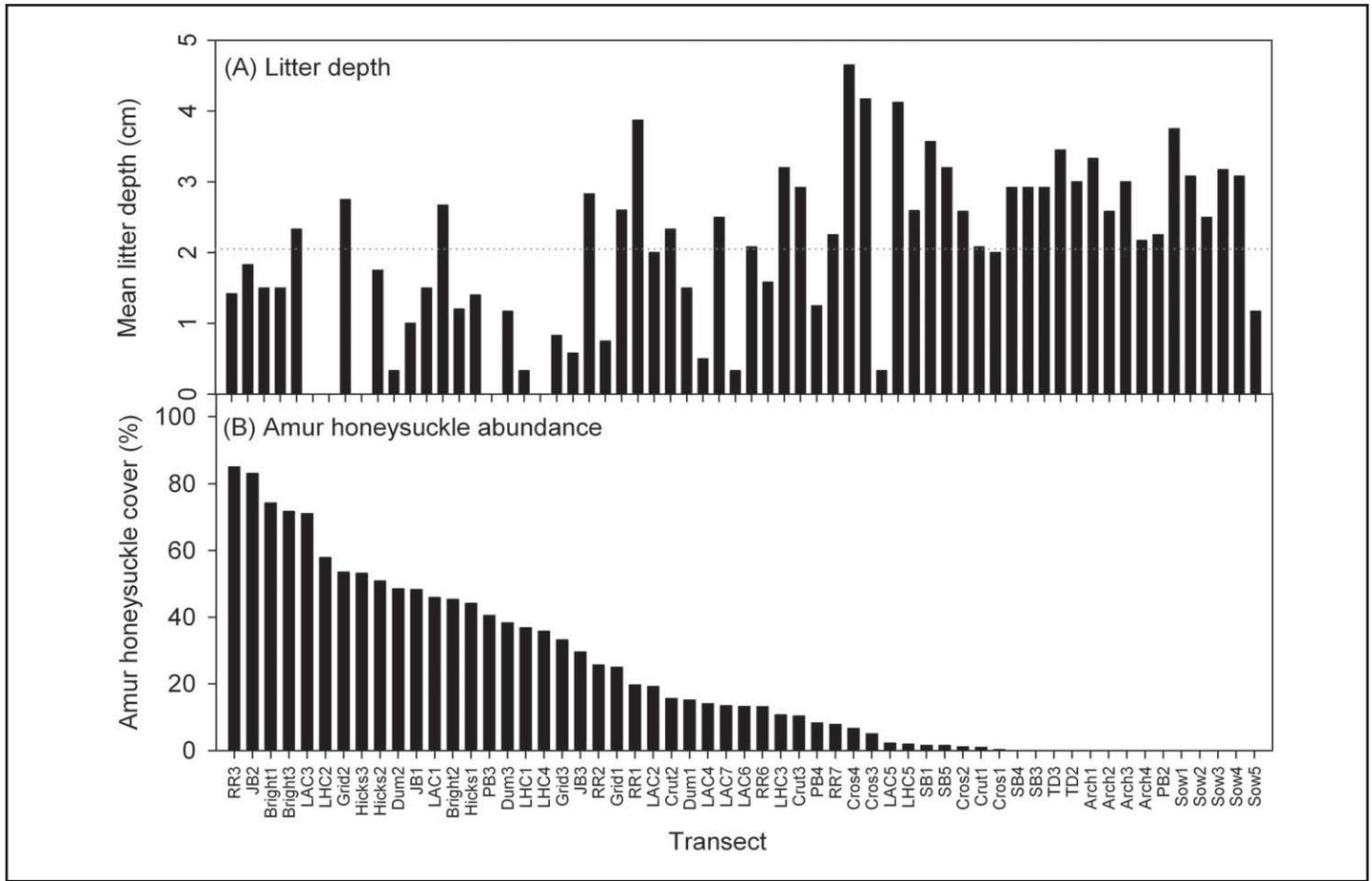


Figure 1. Mean litter depth (A) and percent Amur honeysuckle abundance (B) by transect, shown to represent declining percent cover along the x-axis.

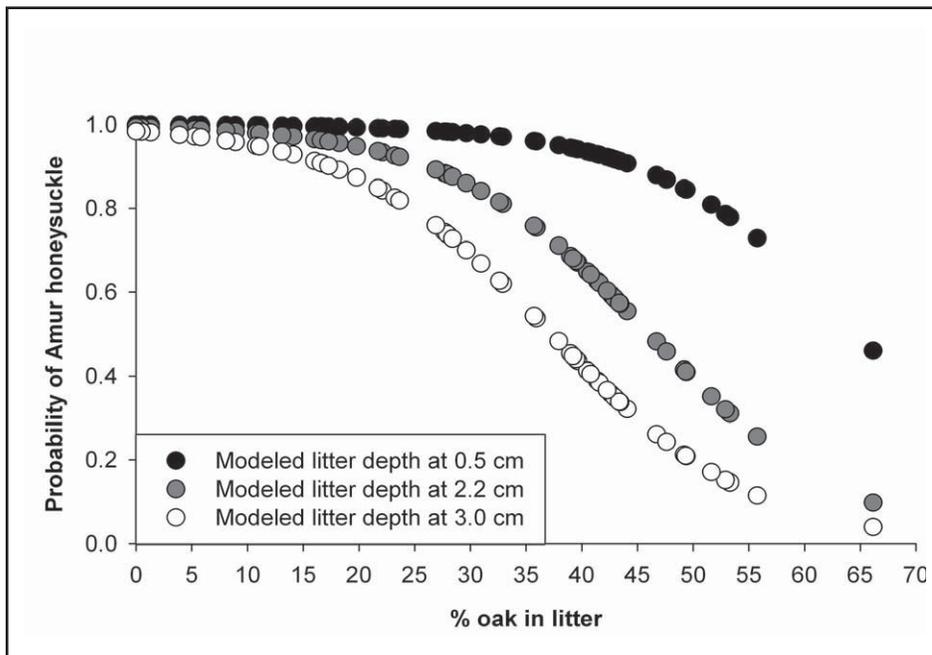
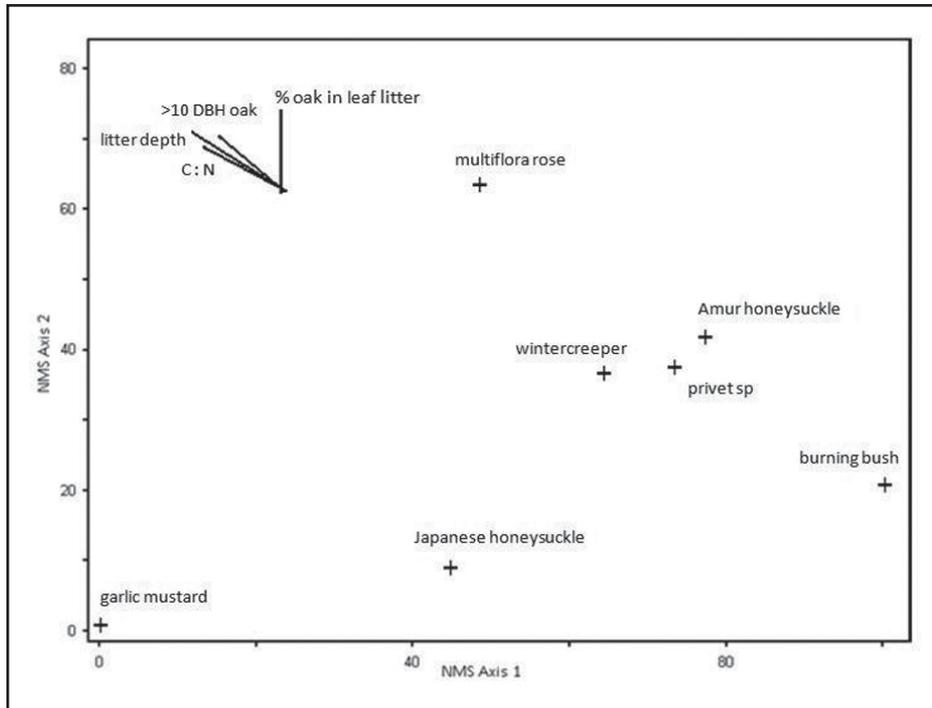


Figure 2. The probability of finding Amur honeysuckle in a forest as determined by the percent of oak found in the forest floor (up to 66 %) as measured on individual transects, at modeled litter depths of 0.5 cm, 2.2 cm, and 3.0 cm, to capture the range of variability.

the bottom of the diagram indicates a negative correlation with % oak litter on the forest floor. Garlic mustard was located in the lower-left portion of the diagram suggesting greater tolerance of leaf litter depth in a forest (Figure 3). Overall, litter depth appeared to be the most influential environmental attribute, with most of the NNIP species arrayed directly opposite of the litter depth vector.

## DISCUSSION

Invasion within individual forests is known to be regulated by site-specific characteristics including both biotic and abiotic factors, as well as limitations of propagule dispersal, which was not addressed in this study. For example, invasion success has been shown to be influenced by soil characteristics such as pH (Xiong and Nilsson 1999; Johnson et al. 2006). We hypothesized ( $H_1$ ) that soil features would be a significant predictor of NNIP; however,



**Figure 3.** Non-metric multidimensional scaling of environmental attributes and invasive plant species (Axis 1  $R^2 = 0.718$ , Axis 2  $R^2 = 0.189$ , final stress 7.2) demonstrated the strongest relationships between the vegetation data and the ratio of C:N, litter depth,  $\geq 10$  cm dbh oak, and % oak in leaf litter (expressed by vectors).

in our study, soil characteristics were not significantly correlated with the presence or abundance of NNIP. This suggests soil characteristics are not useful for predicting invasion success in this study system. The intentional selection of relatively uniform second-growth forest habitats during our site selection process may have negated the potential influence of soil, as these sites may have been more similar to one another in soil characteristics than in other studies implemented over larger regions. Although research has found NNIP are often associated with high-nutrient soils (Kourtev et al. 1998; Ehrenfeld et al. 2001; Johnson et al. 2006), it is difficult to experimentally distinguish between the following three possibilities: (1) NNIP are more successful in sites with high-levels of nutrients, (2) NNIP cause these site conditions through alterations of ecosystem processes, or (3) a historical land-use practice (agriculture) simultaneously caused high-nutrient levels and facilitated invasion of NNIP. Further work is needed to address these issues in this system and others.

Our hypothesis ( $H_2$ ) that Amur honeysuckle would be negatively associated with native

shrub species was not supported. We found no relationship between the abundance of native shrub species and either presence or abundance of Amur honeysuckle, perhaps due to the overall paucity of native shrubs. The most abundant native shrub, spicebush, was found on  $< 5\%$  of the total transect distance sampled, while Amur honeysuckle was present on ca. 20% of this same transect distance. It is unclear whether a denser native shrub layer would be competitive with Amur honeysuckle and limit its abundance. These data do suggest that native forest understories in the study region are relatively free of native shrubs, which may provide an opportunity for further Amur honeysuckle expansion.

Mature forests have been shown to be more resistant to invasion than recently disturbed or early successional forests (e.g., Huebner and Tobin 2006). For instance, Brothers and Spingarn (1992) demonstrated that high light environments in edge habitats were strongly associated with invasive species presence, while deep shade in forest interiors was (apparently) an impediment to invasion. Johnson et al. (2006) demonstrated that historical land use was an important

predictor of invasive species presence in modern stands. We partially eliminated the role of disturbance by specifically focusing only on established second-growth forests, and within that context hypothesized ( $H_3$ ) that forest characteristics, such as forest basal area and the species composition of the forest vegetation, would affect the establishment of NNIP. This hypothesis was not supported, as these characteristics were not significant in explaining either the presence or abundance of Amur honeysuckle. This finding is in opposition to other studies that found woodlots with relatively high basal area had lower Amur honeysuckle invasion, such as Hutchinson and Vankat (1997; basal area range: 10.8 – 46.4  $m^2 ha^{-1}$ ) and Bartuszevige et al. (2006; basal area range: 10.3 – 35.7  $m^2 ha^{-1}$ ). While direct measures of light availability would provide a more direct measure of overstory impact than basal area, our data suggest that second-growth forests are vulnerable to Amur honeysuckle invasion and that aspects of the overstory vegetation, including basal area, are not good predictors of invasion success.

Bartuszevige et al. (2007) demonstrated experimentally that increasing leaf litter depth constrains Amur honeysuckle seedling establishment. Hence, we hypothesized ( $H_4$ ) that NNIP success would be related to the depth of leaf litter on the forest floor, and further hypothesized that the proportion of oak in the litter would also be a factor. This hypothesis was supported by the negative relationship between Amur honeysuckle presence and leaf litter depth and the proportion of oak in the litter. These findings suggest that interactions at the forest floor may be relevant to the distribution and abundance of certain NNIP. While the correlations between litter depth and percent of oak litter with Amur honeysuckle presence were negative, it was beyond the scope of this observational study to determine if there is a mechanistic process associated with litter depth and Amur honeysuckle, or if forest floor depth is a surrogate for other uninvestigated variables. There are several forest floor-related hypotheses to consider for future research. Deep leaf litter may: (1) inhibit germination by acting as a physical barrier between the seed and soil therefore limiting access to nutrients

and water; (2) prevent emerging seedlings from acquiring adequate light for success; or (3) cause an alteration of soil chemistry making the microsite inhospitable for germination (Facelli and Pickett 1991). Greenhouse studies have provided evidence that germination of NNIP (Ellsworth et al. 2004), including *Lonicera* spp. (Hidayati et al. 2000), are not negatively influenced by being placed under leaf litter. These studies did not address the potential inhibiting effect of litter depth on successful germination and seedling development of Amur honeysuckle in natural environments. Under natural forest conditions, seeds are generally scattered on top of the litter layer and must move through the litter to contact the soil to ensure germination and establishment success. The time on the surface of the soil, and the need to move through the layers, may increase predation, desiccation of seeds, or desiccation of emerging radicals and initial seedlings. Further, the interactions of natural forest conditions cannot be replicated in a greenhouse setting and it may be those interactions that work together to limit germination.

While this study suggests that leaf litter acts to inhibit the success of Amur honeysuckle establishment, other studies have found that the decomposition of NNIP litter creates a positive feedback, encouraging further NNIP succession (Ehrenfeld et al. 2001; Allison and Vitousek 2004; Ashton et al. 2005). It is now well-established that Amur honeysuckle litter decomposes and releases nitrogen more rapidly than the litter of native species in the same forests (Arthur et al. 2012; Poulette and Arthur 2012; Trammell et al. 2012), and may, thereby, contribute to a reduced litter layer in systems in which it has invaded while simultaneously altering soil carbon and nitrogen pools. As oak leaves decompose, releasing tannins and other secondary compounds into the soil, the microenvironment may become inhospitable to Amur honeysuckle seeds or seedlings. While the interactions among NNIP, litter decomposition, and decomposer organisms were not addressed in this study, these may be important for explaining the negative effects of oak litter on the presence of Amur honeysuckle. Further research is needed to explore the role, and mechanisms, of the forest floor

as a predictor of NNIP success.

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