Two co-occurring invasive woody shrubs alter soil properties and promote subdominant invasive species

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Summary

1. Though co-occurrence of invasive plant species is common, few studies have compared the community and ecosystem impacts of invaders when they occur alone and when they co-occur. Prioritization of invasive species management efforts requires sufficient knowledge of impacts – both among individual invasive species and among different sets of co-occurring invaders – to target resources towards management of sites expected to undergo the largest change.

2. Here, we observed differences in above- and below-ground impacts of two invasive woody shrubs, *Lonicera maackii* and *Ligustrum sinense*, among plots containing both shrubs (mixed), each species singly or lacking both species (control).

3. We found additive and non-additive effects of these co-occurring invasives on plant communities and soil processes. Mixed plots contained two times more subdominant invasive plant species than *L. maackii* or *L. sinense* plots. Compared to control plots, mixed plots had three times the potential activity of β-glucosidase, a carbon-degrading extracellular soil enzyme. *L. maackii* plots and mixed plots had less acidic soils, while *L. sinense* plots had higher soil moisture than control plot soils. Differences in soil properties among plots explained plant- and ground-dwelling arthropod community composition as well as the potential microbial function in soils.

4. *Synthesis and applications.* Our study highlights the importance of explicitly studying the impacts of co-occurring invasive plant species singly and together. Though *Lonicera maackii* and *Ligustrum sinense* have similar effects on ecosystem structure and function when growing alone, our data show that two functionally similar invaders can have non-additive impacts on ecosystems. These results suggest that sites with both species should be prioritized for invasive plant management over sites containing only one of these species. Furthermore, this study provides a valuable template for future studies exploring how and when invasion by co-occurring species alters above- and below-ground function in ecosystems with different traits.

Key-words: aboveground, belowground, biological invasion, community effects, dominant invader, invasion impact, *Ligustrum sinense*, *Lonicera maackii*, strong invader

Introduction

An ecosystem's level of invasion is the actual or relative number of non-native invasive species coexisting within a habitat (Chytrý *et al.* 2008a). Habitats vary greatly in their level of invasion (Chytrý *et al.* 2008b; Catford *et al.* 2012) owing to habitat characteristics such as distance from propagule sources or the competitive ability of the species within the habitat. Though some habitats have high levels of invasion, a disproportionate amount of research has focused on understanding the effects of single

rather than multiple invaders (Kuebbing, Nuñez & Simberloff 2013). The impacts of co-occurring invaders are likely to vary by species, ecosystem and the specific properties measured. Similarly, the impacts of co-occurring invaders are likely to differ from those of single-species invasions. For example, for eight non-native grassland species, the change in relative above-ground biomass when the species were grown in monoculture and mixture plots varied through time and by species (Isbell & Wilsey 2011). In the first year of growth, the four non-native grasses underyielded, the two forbs overyielded, and the two legumes showed no change when grown with other non-natives. Through time, the species that consistently

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underyielded were likely to be lost from mixture plots. Here, the differential response of non-native species when found singly versus together provides important management information, suggesting that prioritization could initially focus on species that overyield in mixture.

Habitats that exhibit high levels of invasion will contain multiple interacting non-native and native species. While the impacts of co-occurring invasives are not well documented, there is ample evidence that single invaders have notable impacts, particularly on native plant communities (Vilà *et al.* 2011; Simberloff *et al.* 2013). Invasive plants can competitively exclude native species owing to increased above-ground biomass production (Vilà *et al.* 2011), increased shading caused by extended leaf phenology (Fridley 2012) or alteration of ecosystem structure (Simberloff 2011). When invasive plants decrease space, light and resource availability in a community, the abundance and fitness of native populations and the diversity of entire communities decline (Vilà *et al.* 2011).

Modification of plant communities by invaders can link to changes in below-ground communities and *vice versa* (Pyšek & Richardson 2010; Vilà *et al.* 2011; Simberloff *et al.* 2013), because plant and soil communities are connected by nutrient flows between above- and below-ground systems (Wardle *et al.* 2004). Invasive plants can affect soil processes directly by changing soil nutrient cycles or by altering the quality and quantity of nutrient inputs (Ehrenfeld 2010) or indirectly by changing microbial communities' structure or function (Kourtev, Ehrenfeld & Haggblom 2003). For example, *Alliaria petiolata* (M. Bieb.) Cavara and Grande secretes secondary allelopathic compounds that decrease the abundance and diversity of arbuscular mycorrhizal fungi, which reduces the growth of native plants that depend on these beneficial soil organisms (Stinson *et al.* 2006). This feedback potentially explains *A. petiolata's* competitive dominance in its non-native range (Lankau & Strauss 2011). In instances such as this, the below-ground effect of an invasive plant manifests in above-ground native plant communities. Again, though there is much evidence of invaders altering above- or below-ground communities singly, we have much less documentation of how co-occurrence of invaders might change these impacts (but see Ehrenfeld & Scott 2001).

Here, we use an observational study to ask how the occurrence of one or two invasive woody shrub species changes the above- and below-ground impacts in deciduous forests of the southeastern USA. Linking above- and below-ground effects of invasion will help to inform management decisions by directing management towards above-ground biomass removal or soil remediation efforts (Kardol & Wardle 2010). Likewise, a better understanding of the impacts of invasives on resident communities and ecosystems is the first step in any invasive management plan (Buckley 2008), and ranking impacts of single invasive species is useful for prioritization of where to expend limited resources (Parker *et al.* 1999). When habitats

contain multiple invaders, it becomes necessary to understand how the impacts differ when non-native species co-occur. As level of invasion increases, the combined impacts of co-occurring invaders can decrease, stay the same, increase linearly (additively) or increase nonlinearly (non-additively). Interactions among the invaders may lead to a variety of responses, such as when positive interactions among invaders lead to a non-additive increase in impacts (i.e. invasional meltdown, Simberloff & Von Holle 1999). Though true invasional meltdown examples are uncommon (Simberloff 2006), cases of non-additive impacts should rank highest in prioritization of management efforts because of the acceleration of impacts when the invaders co-occur.

Two ubiquitous and invasive woody shrubs in southeastern forests are Chinese privet *Ligustrum sinense* Lour. and Amur bush honeysuckle *Lonicera maackii* (Rupr.) Herder. The rapid invasion of *L. maackii* across the southeastern USA at the end of the 20th century (Luken & Thieret 1996) included encroachment into forests previously invaded by *L. sinense*. Although most studies focus on the impacts of each shrub singly, forests now contain a mosaic of areas where each shrub is found alone and where they co-occur. How these species interact in the non-native range is unknown, but they are functionally similar species (i.e. understorey woody shrubs) that have similar impacts when they occur singly.

The presence of each shrub correlates with decreased native plant diversity (Hutchinson & Vankat 1997; Collier, Vankat & Hughes 2002), reduced plant growth (Miller & Gorchov 2004; Greene & Blossey 2011) and decreased insect abundance (Goodell, McKinney & Lin 2010; Ulyshen, Horn & Hanula 2010). Additionally, their presence altered soil nutrient availability and decomposition rates (Mitchell, Lockaby & Brantley 2011; Poulette & Arthur 2012; Trammell *et al.* 2012). Previous work on interactions among co-occurring invasive plants suggests that functionally dissimilar species, such as those that can or cannot fix nitrogen, might be more likely to have non-additive impacts (Vitousek & Walker 1989). This hypothesis implies that non-additive effects of functionally similar species might be uncommon and thus best ignored by managers. In fact, previous findings in areas invaded by one or the other of our focal species indicate that co-invasion by both species will be additive or neutral relative to one-shrub areas because they are both woody shrubs. However, these assumptions have never been tested. We hypothesize that even seemingly functionally similar invasive plants can interact and have non-additive effects.

Materials and methods

We surveyed naturally occurring stands of *Lonicera maackii* and *Ligustrum sinense* in three forests near Knoxville, Tennessee, USA: Ijams Nature Center (35°57′19·29″N, 83°51′56·3″W), which is a 70-ha nature park managed for pedestrian-only recreation; IC King (35°53′58·88″N, 83°56′41·65″W), which is a 49-ha

recreation site managed for mountain biking and hiking; and Forks of the River Wildlife Management Area (35°57′13·04″N, 83°51′3″W), which is a 134-ha natural area managed for biking, hiking and hunting. Hunting restrictions prevented our access to this site during plant and arthropod sampling times; thus, we present only soil data from this site. All sites are hardwood forests, dominated by ash *Fraxinus* spp., oak *Quercus* spp., maple *Acer* spp. and beech *Fagus grandifolia*, and there are no current forest management plans.

Ligustrum sinense is a semi-deciduous shrub whose native range covers much of China, Vietnam and Laos (Nesom 2009). First introduced to the USA in 1852 (Dirr 2009), *L. sinense* occurs in 18 states (EDDMapS 2012). *Lonicera maackii* is a deciduous shrub native to central and north-eastern China, Korea and Japan. The first North American record of *L. maackii* was in Ottawa, Canada, in 1896 (Luken & Thieret 1996). *Lonicera maackii* occurs in 29 states (EDDMapS 2012).

Within each site, we located three blocks that contained four circular 6-m diameter (*c.* 113 m^2) plots. Each plot was defined by one of the following vegetation types: *L. sinense* only, *L. maackii* only, *L. maackii* and *L. sinense* co-occurring, or *L. maackii* and *L. sinense* (control plot) for a total of 36 plots (3 sites × 3 blocks × 4 treatments). The 'invaded' treatments had >75% foliar cover of the respective vegetation type. To determine percentage canopy openness and leaf area index (LAI), we analysed hemispherical photographs taken in the plot centre with a fisheye lens mounted on a 1-m tripod with Gap Light Analyzer software (Frazer, Canham & Lertzman 1999). All photographs were taken on cloudless days in late July between 06·30 and 08·30 h.

We sampled plant communities at Ijams and IC King in late May of 2010 when herbaceous cover was highest. Within each plot, we counted all plants in four haphazardly placed, non-overlapping 0·5-m^2 quadrats, noting the identity, abundance (number of stems) and percentage cover of all species present.

We sampled ground-dwelling arthropods with dug-in pitfall traps at Ijams and IC King. We set three traps per plot, the first placed at the plot's centre (directly under the centre plant) and the subsequent traps *c.* 1 m apart in a line. Each trap consisted of a 266-mL plastic cup filled with a soap and water mixture (*c.* 1 drop liquid soap per litre of water to relax surface tension) with a styrofoam plate secured above to prevent rain from overflowing the traps. We trapped arthropods for 48 h on 15–17 September 2010 and identified all individuals to morphospecies.

We measured the potential activity of three extracellular enzymes, soil pH and gravimetric water content. We analysed enzymes important in soil carbon (β-glycosidase), nitrogen (β-N-acetylglucosaminidase [nagase]) and phosphorus (phosphatase) cycling (Sinsabaugh & Follstad Shah 2012). On 13 October 2010, we sieved (2-mm mesh size) and homogenized four 10-cm mineral soil cores (5·08 cm diameter) collected within 1 m of each plot centre. Soil pH was calculated from 10-g slurries of field-moist soil and 20 mL of deionized water with a pH conductivity meter (Denver Instruments Model 220, New York, USA). Soil moisture content was calculated as the percentage difference in weight after 20 g of field-moist samples was placed in a drying oven at 105 °C *c.* 48 h. Enzyme activity was assayed by mixing 1 g of field-moist soil with 125 mL of a 50 mmol L^{-1} sodium acetate buffer and stirring the mixture in a stir plate for 2 min. We measured enzyme activity on 96-well plates that were divided into

blank controls, reference standards and negative controls, replicating each eight times (see Saiya-Cork, Sinsabaugh & Zak 2002). We incubated β-glycosidase plates for 2 h and nagase and phosphatase plates for 0·5 h and then measured fluorometric activity using a Modulus fluorometer (Turner Biosystems, California) at an excitation of 365 nm and an emission of 450 nm. After corrections, we report potential enzyme activity as nmol h^{-1} g^{-1}.

We used mixed-effect nested ANOVAs to test for the effects of invasive plants (*L. maackii*, *L. sinense*, mixture or control) and habitat variation (block and site) on soil pH, soil moisture, canopy openness, LAI, potential soil enzyme activity (β-glycosidase, nagase and phosphatase), as well as plant and arthropod abundance, richness and diversity (Shannon's diversity index). The main effect of invasive plant cover type was modelled as a fixed factor nested within block and site, which were modelled as random factors. We calculated F-ratios and estimated variance components according to Quinn & Keough (2002). Transformations were necessary to meet the normality assumption of ANOVA; β-glycosidase activity, arthropod abundance, arthropod diversity and plant abundance were log-transformed, and nagase activity and LAI were square-root-transformed.

We used unconstrained (PERMANOVA and non-metric multidimensional scaling) and constrained (redundancy analysis) multivariate analyses to describe differences in plant and arthropod community composition and potential soil microbial function. Unconstrained multivariate techniques are useful for examining broad patterns, whereas constrained ordinations allow tests for relationships between explanatory and response variables (Borcard, Gillet & Legendre 2011). For all multivariate analyses, we created presence/absence matrices with plots as rows and species as columns. *Lonicera maackii* and *L. sinense* individuals were excluded from the plant matrix because we wanted to test the influence of their presence on the remainder of the resident community. We combined our enzyme activity measurements into a single matrix to assess total microbial function. Potential activity of extracellular enzymes can be used as an indicator of the nutrient demand of microbial organisms and thus as a proxy for potential microbial function in soils (Sinsabaugh & Follstad Shah 2012).

To detect patterns in community composition among vegetation types and blocks, we used two-way permutational multivariate analysis of variance, which is a more robust test than traditional multivariate analysis of variance (PERMANOVA; Anderson 2001). For each PERMANOVA, we calculated Bray–Curtis similarity matrices on the log-transformed community matrix. We treated 'blocks' as random factors and 'invasive plant vegetation type' as a fixed factor, and we calculated pseudo-F and P-values from 9999 permutations of the original data with type III sum of squares. Owing to software limitations, we were unable to include the nested 'site' factor in this analysis.

We used unconstrained redundancy analysis (RDA) to test for the environmental variables correlated with changes in community composition (Legendre & Gallagher 2001). We created Hellinger-transformed plot-by-species community matrices using plant cover, arthropod abundance and potential soil enzyme activity data. The full environmental matrix included the variables percentage canopy openness, LAI, soil moisture, soil pH and density of *L. maackii* or *L. sinense* stems (see Table S1, Supporting information). To select the most significant variables for each RDA, we used the ordistep function (R vegan package,

v. 2.15.1), and the most significant environmental variables were then included in the final model, excluding collinear variables [i.e. variables with a variance inflation factor (VIF) >10, Legendre & Gallagher 2001]. We tested for significance of the full RDA model with the anova.cca function (R vegan package, v. 2.15.1), which is an ANOVA-like test using 10 000 step permutations. All analyses were performed in R software program (R Core Team 2013).

Results

We found an additive effect of co-occurring invasive shrubs on subdominant invasive plant richness. Subdominant invasive species richness was twice as high in mixed plots (4·00 ± 0·73 SE) compared to *L. maackii* or *L. sinense* plots (2·00 ± 0. 37 SE and 2·00 ± 0·40 SE, respectively; Table 1 and Fig. 1a). Other invasive plants sampled included *Ailanthus altissima* (Mill.) Swingle, *Albizia julibrissin* Durazz., *Celastrus orbiculatus* Thunb., *Dioscorea oppositifolia* L., *Glechoma hederacea* L., *Hedera helix* L., *Lonicera japonica* Thunb. and *Vinca minor* L.

The presence or absence of *L. maackii* and *L. sinense* was associated with different soil properties. Mixed plots had triple the potential β-glycosidase extracellular enzyme activity (103 ± 34 nmol h^{-1} g^{-1}; mean ± SE) compared to control plots (35 ± 11 nmol h^{-1} g^{-1}), a marginally significant difference ($\alpha < 0.1$; Fig. 2a). *L. sinense* plots had 30% higher gravimetric water content (0·31 ± 0·02 SE) than control plots (0·22 ± 0·02 SE; Fig. 2e). Soils in control plots were more acidic (5·81 ± 0·30 SE) than those in *L. maackii* plots (6·34 ± 0·2 SE) and mixed plots (6·33 ± 0·27 SE; Fig. 2d). We did not find significant variation in nagase or phosphatase potential activity among plots that differed in the presence or absence of *L. maackii* or *L. sinense* (Fig. 2b,c).

We found that blocks and sites also explained variation in many forest features, including plant community composition (total cover, total richness and native richness) ground-dwelling arthropod abundance, soil properties (nagase or phosphatase potential activity) and canopy properties (canopy openness and LAI; Table 1).

Overall community composition was less similar among blocks than among plots that varied in the presence or absence of *L. maackii* or *L. sinense*. Plant community composition varied significantly by block (Fig. 3a; PERMANOVA pseudo-$F_{5,15} = 4.09$, $P = 0.0001$), but not by vegetation type (Fig. 3a; PERMANOVA pseudo-$F_{3,15} = 1.37$, $P = 0.10$; see Appendix S1, Supporting information). Ground-dwelling arthropod community composition varied significantly by block (Fig. 3d; PERMANOVA pseudo-$F_{5,15} = 2.81$, $P = 0.0001$), but not vegetation type (Fig 3e; PERMANOVA pseudo-$F_{3,15} = 1.30$, $P = 0.08$; see Appendix S2, Supporting information). Finally, potential soil microbial function varied significantly by block (Fig. 3g; PERMANOVA pseudo-$F_{8,24} = 4.44$, $P = 0.001$), but not by vegetation type (Fig. 3h; PERMANOVA pseudo-$F_{3,24} = 2.02$, $P = 0.13$; Table S2, Supporting information).

We used constrained multivariate analysis to define which plot properties were the most important in describing differences in above- and below-ground community composition and function. Soil pH and LAI were selected as the most important environmental predictors of plant community composition (Table 2 and Fig. 3c). The two RDA axes explained *c*. 19% of the variation (axis 1, 11·3%, $P < 0.001$; axis 2, 7·4% $P = 0.019$). Soil pH, LAI and *L. sinense* density were the best predictors for arthropod community composition, and these variables explained 19% of the variation in community composition (Table 2, Fig. 3f; axis 1, 7·1%, $P = 0.002$; axis 2, 6·7% $P = 0.018$; axis 3, 5·5%, $P = 0.270$). Together, soil moisture, soil pH and *L. maackii* density explained 35% of the variation in potential soil microbial function (Table 2, Fig. 3i; axis 1, 19·8%, $P = 0.009$; axis 2, 15·3% $P = 0.028$; axis 3, <0·001%, $P = 0.990$).

Discussion

We found that when two functionally similar invasive plants co-occur, their combined community and ecosystem impacts were not always equal to the sum of the impacts of each individual invader. This suggests that scientists and managers cannot use studies on single invasive species to infer impacts when invaders co-occur. We show that the co-occurring invasive woody shrubs, *Lonicera maackii* and *Ligustrum sinense*, had neutral, additive and non-additive effects on various attributes of resident communities and ecosystems when they grew separately and together. Importantly, both shrubs either singly or in mixture were associated with differences in soil properties, indicating these shrubs can have more subtle impacts on forest soils and that even with the removal of the species from invaded forests, soil legacies might persist (Ehrenfeld 2010; Simberloff *et al.* 2013). Likewise, plots with both shrubs contained more subdominant invasive plant species than other plots, indicating that removal of these two dominant invaders might lead to re-invasion of the area by subdominant invaders at the site (Hulme & Bremner 2006; Cox & Allen 2008; Pearson, Ortega & Columbus 2009). Whether this is a common occurrence in other ecosystems or for other co-occurring invasive species is currently unknown and an area for further work. A comprehensive understanding of invader impacts across habitats that may vary in level of invasion would provide an informed foundation for developing hypotheses regarding the impacts of non-native communities as well as better predictive tools for the types of invasive plant combinations that are most likely to have the greatest impacts.

Increasing non-additive impacts should be a concern for invasive species managers who would like to restore native ecosystem function. In our study, mixed plots had three times higher potential activity of β-glycosidase (Fig. 2a), an enzyme that breaks down cellulose,

Table 1. Plots dominated by two invasive shrubs, *Lonicera maackii* and *Ligustrum sinense*, were associated with variation in the number of subdominant invasives present, potential activity of carbon-degrading soil enzyme β-glycosidase, soil pH and soil moisture. Nested ANOVA accounted for strong variation among plots containing each invader singly, plots with both species or control plots lacking either shrub. Plots were blocked within sites [see Table S3 (Supporting information) for full ANOVA tables]. Percentage (%) values represent estimates of variance components for each model error term, and bold *P*-values are significant at $P < 0.1$

| Dependent variable	Invasive shrub [block (site)]			Block (site)			Site		
	%	*F*	*P*	%	*F*	*P*	%	*F*	*P*
Plant community									
Total cover	0·0	$0.27_{3,15}$	0·85	22·3	$7.39_{4,3}$	**0·07**	0·5	$1.90_{1,4}$	0·24
Total richness	4·3	$2.10_{3,15}$	0·14	0·0	$0.94_{4,3}$	0·54	2·8	$5.32_{1,4}$	**0·08**
Native richness	0·8	$1.19_{3,15}$	0·35	0·0	$0.75_{4,3}$	0·62	2·8	$10.39_{1,4}$	**0·03**
Non-native richness	9·6	$4.11_{3,15}$	**0·03**	16·3	$1.32_{4,3}$	0·43	0·0	$0.003_{1,4}$	0·96
Diversity	4·6	$2.18_{3,15}$	0·13	1·7	$1.05_{4,3}$	0·50	0·2	$1.27_{1,4}$	0·32
Arthropod community									
Abundance	1·1	$1.61_{3,15}$	0·23	55·3	$5.73_{4,3}$	**0·09**	0·0	$0.26_{1,4}$	0·63
Richness	0·6	$1.16_{3,15}$	0·36	12·4	$1.74_{4,3}$	0·34	0·0	$0.07_{1,4}$	0·80
Diversity	2·5	$1.62_{3,15}$	0·23	0·0	$0.52_{4,3}$	0·73	0·0	$0.57_{1,4}$	0·49
Soil properties									
β-glucosidase	6·8	$3.05_{3,18}$	**0·06**	0·4	$1.01_{4,3}$	0·52	0·2	$1.25_{2,4}$	0·38
Nagase	0·0	$0.44_{3,18}$	0·72	40·3	$11.6_{4,3}$	**0·04**	0·0	$0.41_{2,4}$	0·69
Phosphatase	0·2	$1.11_{3,24}$	0·37	50·4	$8.37_{6,3}$	**0·05**	0·0	$0.45_{2,6}$	0·66
pH	3·5	$3.28_{3,24}$	**0·04**	46·5	$3.29_{6,3}$	0·18	0·4	$1.26_{2,6}$	0·35
Gravimetric water content	6·0	$3.06_{3,24}$	**0·05**	0·0	$0.24_{6,3}$	0·93	0·0	$0.33_{2,6}$	0·73
Plot properties									
Canopy openness	0·0	$0.56_{3,24}$	0·65	25·0	$5.77_{6,3}$	**0·09**	0·0	$0.59_{2,6}$	0·58
Leaf area index	0·2	$1.13_{3,24}$	0·36	41·9	6.12	**0·08**	0·0	$0.79_{2,6}$	0·49
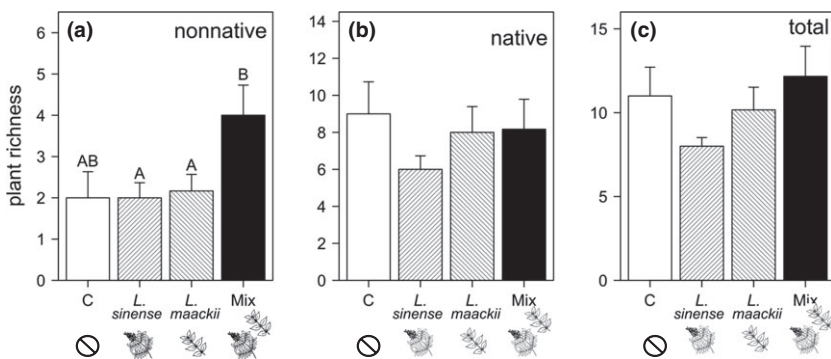
Fig. 1. Subdominant invasive plant richness (a) in plots containing two invasive shrubs (Mix) was double that of plots containing each shrub, *Ligustrum sinense* or *Lonicera maackii*, or control plots (C) where neither shrub was present. Plant richness of native species (b) and total richness (c) did not vary among plots.

compared to control plot soils. High carbon-degrading enzyme activity suggests high decomposition rates, which could be caused by high-quality invasive plant leaf litter stimulating microbial activity (Sinsabaugh & Follstad Shah 2012). Invaded sites often have higher leaf decomposition rates (Ehrenfeld 2010), and leaf litter from *L. maackii* and *L. sinense* is higher in quality (i.e. lower leaf C : N) and decomposes faster than native woody species' litter (Blair & Stowasser 2009; Mitchell, Lockaby & Brantley 2011; Arthur *et al.* 2012). Though we did not study decomposition, our results suggest that adding leaf litter from these two invasive plants is different than adding litter from one of them alone, resulting in a non-additive impact of co-occurring invasive plant litter. An invader-induced change in nutrient cycling may drive invader dominance and decrease native plant abundance and diversity (Kourtev, Ehrenfeld & Haggblom 2003).

Though we did not find a difference in the native plant community between control and invaded plots (Table 1, Fig. 2b), we did see an increase in subdominant invasive plant richness in mixed plots.

Sites with more than one invasive species are susceptible to reinvasion if management includes only the removal of dominant invaders and not subdominant invaders (Hulme & Bremner 2006). Thus, the presence of subdominant invasive plants is a management concern. The additive effect of *L. maackii* and *L. sinense* on invasive plant richness (Table 1, Fig. 1a) suggests that the likelihood of re-invasion is higher in areas with both invaders because there are twice as many invasive species present to take their place. Contrary to our findings, richness of invasive and native species was equally low in riparian plots that contained the herbaceous vine *Vinca major* L., the grass *Arundo donax* L. or both invasive species relative to plots
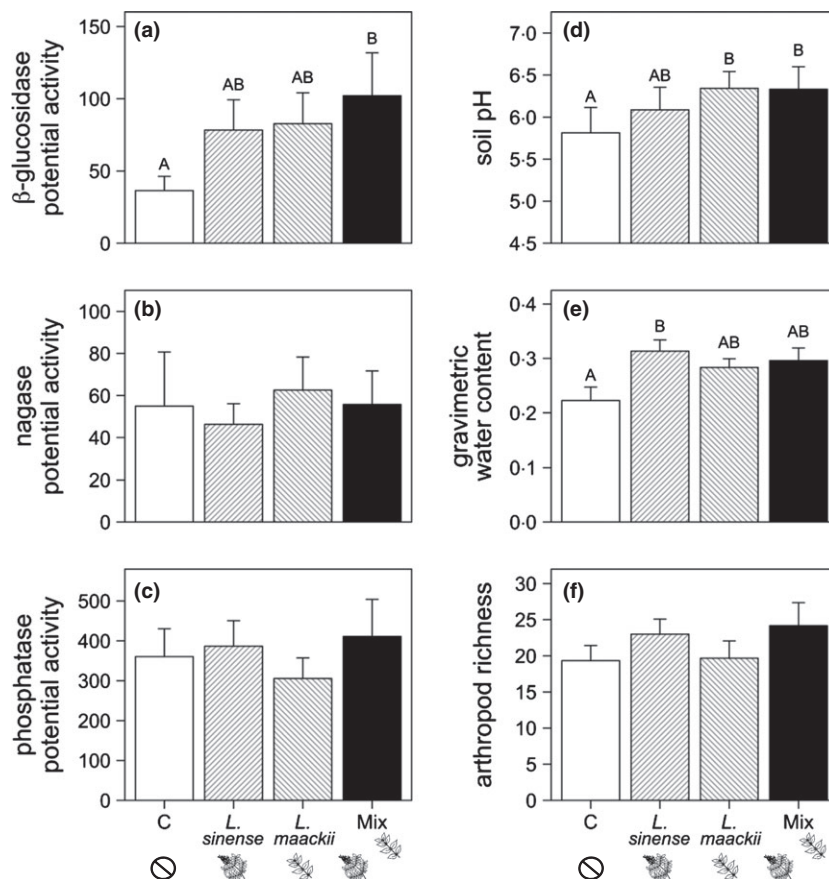
Fig. 2. Potential activity of the soil enzyme β-glucosidase (a), soil pH (d) and soil moisture (e) varied in plots that differed in the presence of zero (control plots: C), one (*Ligustrum sinense* or *Lonicera maackii* plots) or two (mixture plots: Mix) invasive woody shrub species. Potential activity of the soil enzymes nagase (b) and phosphatase (c) and arthropod richness (f) did not vary among plot types. Bars represent mean and standard error.

where both invaders were absent (Cushman & Gaffney 2010). While studies across ecosystems are few, these two contrasting results indicate that additive and non-additive effects of invasive species might depend on the invaded ecosystem, the species involved and functional differences between the co-occurring invaders.

The presence of each invasive shrub was related to differences in soil properties. Soil moisture differences between *L. sinense* and control plots suggest that *L. sinense* performance is higher in moist microsites, which is in accord with observations that the species is particularly invasive in riparian areas (Merriam 2003; Miller 2010). Soils in control plots were more acidic than soils in *L. maackii* plots and mixture plots (Fig. 2d). Other forest invaders, including *L. maackii*, affect the pH of soils when they invade (Ehrenfeld & Scott 2001; Schradin & Cipollini 2012), which suggests that *L. maackii* might be changing soil properties rather than selecting less acidic sites.

It is important to note that we consistently found a strong signal of variation among blocks and sites in canopy and soil properties, plant community structure, and plant and arthropod community composition (Table 1, Fig. 3a,d,g). Small-scale habitat heterogeneity such as this is common in forested ecosystems (Chávez & Macdonald 2010; Douda *et al.* 2012), especially younger secondary forests such as the ones we sampled (Moora *et al.* 2007). We found that small-scale (block) environmental heterogeneity affected the community structure and spatial

distribution of plants, as in other forest studies (Chávez & Macdonald 2010; Douda *et al.* 2012). The variation in enzyme activity among blocks could be due to the variation in soil pH, the most important driver of enzyme activity globally (Sinsabaugh & Follstad Shah 2012). Interestingly, we did not find an effect of block or site on arthropod diversity, although habitat heterogeneity is an important predictor of arthropod diversity (Tews *et al.* 2004; Báldi 2008), particularly microhabitat heterogeneity in forested systems (Ziesche & Roth 2008). We may have failed to detect variation in arthropod community owing to our single-sampling period or because our plots did not vary in percentage shrub cover or vertical structure complexity, which have the largest effect on forest arthropod diversity (González-Megías, Gómez & Sánchez-Piñero 2007; Janssen, Fortin & Hébert 2009). Finally, the presence of subdominant invasive species in mixed plots may contribute to the variation we found in other plot properties (Peltzer *et al.* 2009), although our observational design precludes our disentangling these effects.

Environmental variation in abiotic site properties such as soil nutrients and soil moisture can moderate the impact of invasive species (Pyšek *et al.* 2012), as we found in our redundancy analysis. The impacts of invaders on soil nutrient pools can be dampened or magnified by variation in soil nutrient availability or soil texture (Scharfy *et al.* 2009. Soil pH and LAI varied among blocks and were selected as significant variables in redundancy
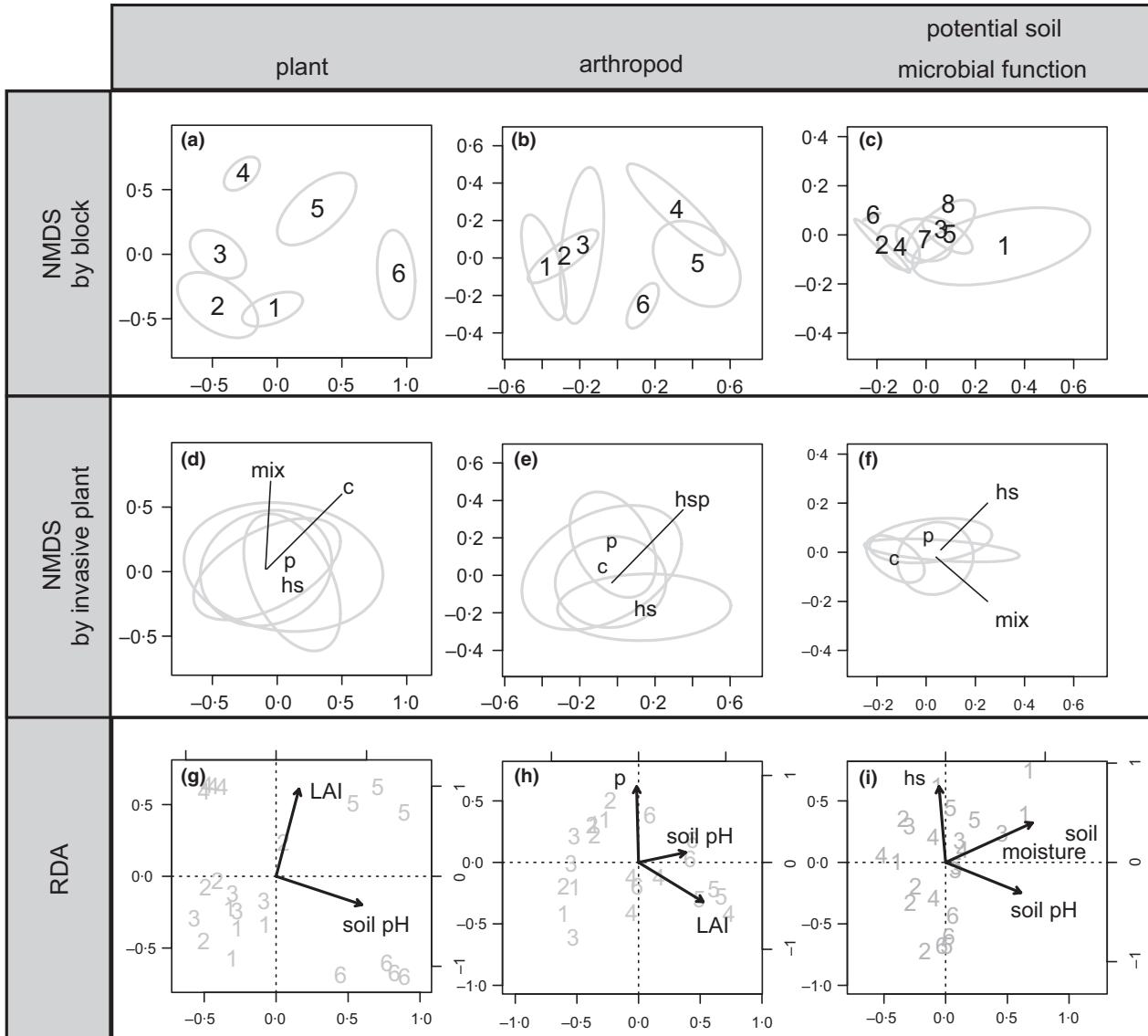
Fig. 3. Understorey plant- and ground-dwelling arthropod communities, as well as potential soil microbial function, showed more variation among blocked plots (a, b, c) than among plots that differed in the presence of zero (control plots, c), one (*Ligustrum sinense*, p or *Lonicera maackii*, hs) or two (mixture plots: m) invasive woody shrub species (d, e, f). Unconstrained non-metric multidimensional scaled plots based on Bray–Curtis dissimilarity matrices for each taxon show centroids and 95% confidence limits based on standard deviation of individual points. All ordination stress values were <0·2. Constrained redundancy analysis (g, h, i) depicts the most significant variables driving community composition for each taxon. Variables included in models included leaf area index (LAI), soil moisture, soil pH and invasive plant average density (*L. sinense*, p; *L. maackii*, hs) across blocked plots (grey numbers).

Table 2. Soil pH, soil moisture, leaf area index (LAI) and number of invasive plant stems (*Lonicera maackii* or *Ligustrum sinense*) affect potential soil function and composition of understorey plant- and ground-dwelling arthropod communities. All unconstrained redundancy analyses were conducted on Hellinger-transformed abundance matrices

Model	Variables	Constrained variance	Adjusted R^2	F_{perm}
Plant community	Soil pH, LAI	0·19	0·11	2·42***
Arthropod community	*Ligustrum* Stems, soil pH, LAI	0·19	0·09	1·77***
Potential soil function	*Lonicera* stems, soil pH, soil moisture	0·35	0·25	3·43*

P-values are based on 9999 permutations of the data, and asterisks (*) indicate significance levels: ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

analysis, explaining *c.* 19% of the variation in plant and arthropod community composition (Table 2, Fig. 3c,f); this result is similar to other findings (Barbier, Gosselin & Balandier 2008; Chávez & Macdonald 2010; Douda *et al.* 2012). However, our redundancy analysis detected direct and indirect influences of invasive shrubs on plant and arthropod communities as well, despite strong microhabitat variation. The density of *L. sinense* stems helped explain compositional differences in arthropod communities (Table 2, Fig. 3f). Removal of dense monotypic stands of *L. sinense* can increase beetle diversity compared to untreated or uninvaded sites, indicating *L. sinense* stem density is important for ground-dwelling arthropod fauna (Ulyshen, Horn & Hanula 2010). Soil pH was an important predictor in all of our redundancy analyses (Table 2). Higher soil pH was associated with *L. maackii*, indicating that the potential effect of the shrub on soil properties could indirectly affect forest community composition.

Interestingly, we found that *L. maackii* and *L. sinense* had greater effects on below-ground soil properties than on above-ground plant or arthropod communities. Effects of invaders on soils can feed back to above-ground communities, particularly invaders that might change ecosystem carbon cycling. The invasive grass *Microstegium vimineum* (Trin.) A. Camus increases carbon fixation and shunts more carbon into below-ground communities, affecting above- and below-ground food webs (Bradford *et al.* 2012). We found that when the invasive shrubs co-occur, there was a threefold increase in carbon-degrading enzyme activity. This below-ground change could have long-term consequences for above-ground communities.

Though we found no difference among plant communities in invaded plots, previous studies show that *L. maackii* and *L. sinense* are associated with decreased native plant abundance or diversity (Hutchinson & Vankat 1997; Collier, Vankat & Hughes 2002; Hartman & McCarthy 2008; Hanula, Horn & Taylor 2009; Greene & Blossey 2011). Thus, impacts of invasive plants can vary in their frequency and reliability across ecosystems (Hulme *et al.* 2013). Other studies typically compared forested sites across a larger spatial scale, such as forested stands with and without *L. sinense* (Hanula, Horn & Taylor 2009) or sites that varied in level of invasion (Hutchinson & Vankat 1997; Hartman & McCarthy 2008; Greene & Blossey 2011; but see Collier, Vankat & Hughes 2002). Our comparison of plots at a smaller spatial scale suggests that environmental variation may be more important at this scale for determining plant community composition.

Currently, few studies test how the influence of co-occurring invaders differs from that of single invaders (but see Cushman & Gaffney 2010), making comprehensive recommendations for management of co-occurring invaders difficult. As the level of invasion increases within habitats, managers are faced with two scenarios. First, they must choose specific sites to manage, which may vary in level of invasion. Second, managers must choose which invaders to manage within any given habitat. We suggest that managers of sites with *L. maackii* or *L. sinense* initially prioritize sites containing both species because they cause non-additive impacts, but that these same sites should be monitored for potential re-invasion by subdominant invaders. As we expand our knowledge of how impacts of co-occurring invaders differ from those of single invaders across different ecosystems and invasive species combinations, we will build a larger body of research that will enable us to develop better hypotheses for predicting the impacts of co-occurring invasive plants.

Acknowledgements

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. List of environmental variables used in unconstrained redundancy analysis.

Table S2. Full ANOVA tables from PERMANOVA models.

Table S3. Full ANOVA tables for nested ANOVA models.

Appendix S1. List of plant species identified.

Appendix S2. List of arthropod species identified.