INFLUENCE OF LOW DENSITY GARLIC MUSTARD PRESENCE AND HARDWOOD LEAF LITTER COMPOSITION ON LITTER DWELLING ARTHROPOD DIVERSITY

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ABSTRACT.   Garlic mustard (*Alliaria petiolata* (M. Bieb.) Cavara & Grande) is a non-native plant that commonly invades hardwood forest understory plant communities. Such invasions have the potential of restructuring forest communities and influencing community function. Litter dwelling arthropods were collected from areas with and without garlic mustard, and were identified to family. Forest characteristics, including canopy cover, forest basal area, litter depth, and soil moisture, were also measured. Plot locations with and without garlic mustard did not differ in the forest characteristics. However, arthropod richness was significantly reduced in areas with garlic mustard compared to areas without. Arthropod richness and diversity were positively related to leaf litter species diversity. In nonmetric multidimensional scaling ordination, mature garlic mustard density influenced a few arthropod taxonomic groups. However, it is likely that forest characteristics that facilitate the intensity of garlic mustard colonization (i.e., canopy cover, moisture) may be part of that influence. Additionally, leaf litter species richness provided a strong relationship with the majority of taxonomic groups. While garlic mustard presence may have a minor influence on the litter dwelling arthropod community, leaf litter richness and diversity play a major role in defining the arthropod community diversity and individual taxonomic group abundances. Management to control garlic mustard in forests may have little impact on leaf litter dwelling arthropods, especially if the litter layer remains intact.

Keywords: *Alliaria petiolata*, diversity, garlic mustard, litter dwelling arthropods, Tullgren-Berlese trap

INTRODUCTION

Addition of non-native plants to forest understory communities not only alters the composition of the community, but also alters community function (Gordon 1998; Maskell et al. 2006). Environmental characteristics (e.g., pH, fertility, light, moisture) are often different between common habitats with and without non-native plant species (Maskell et al. 2006). Much of the time, alterations to a community structure or function are subtle without fully reconstructing the native plant community (Mandryk & Wein 2006). In addition to the plant community, changes to arthropod communities by adding an exotic species may be variable, but may provide further insight into the importance of such plant additions (Marshall & Buckley 2009; Simao et al. 2010).

Garlic mustard (*Alliaria petiolata* (M. Bieb.) Cavara & Grande [Brassicaceae]) is a Eurasian plant species introduced to North America prior to 1868 (Nuzzo 1993). As a monocarpic, obligate biennial, garlic mustard seeds germinate in early spring and plants subsequently overwinter as leaf rosettes (Cavers et al. 1979). An erect stem is produced during the following spring, flowering in late spring, and seeds are dispersed during mid- to late-summer (Cavers et al. 1979; Anderson et al. 1996). Garlic mustard success in forest understories likely stems from its ability to grow under a wide range of light conditions and to further acclimate to the current condition in which it is growing (Cavers et al. 1979, Anderson & Dhillion 1991). Additionally, self-pollination ensures a single individual within a forest can easily begin the establishment of a population (Anderson et al. 1996). Self-pollination serves non-native forest invaders by allowing them to colonize rapidly as a result of micro-site disturbances, as seen in other forest understory species (Oswalt & Oswalt 2007; Marshall & Buckley 2008).

Major management concerns regarding garlic mustard invasion center on the restructuring of understory plant communities. Numerous studies

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have investigated the environmental and plant community changes that occur following garlic mustard invasion and management activities to remove the species (e.g., McCarthy 1997; Hochstedler et al. 2007; Stinson et al. 2007; Rodgers et al. 2008). One possible mechanism for altering plant communities is the potential ability for garlic mustard to out-compete some neighboring species (Meekins & McCarthy 1999). A second possible mechanism may be related to the reduction in arbuscular and ecto-mycorrhizal fungi, which would influence competitive abilities (Roberts & Anderson 2001; Stinson et al. 2006; Wolfe et al. 2008). However, Lankau (2011) found recovery and potential resistance of soil microbial communities in response to garlic mustard invasion. Overall management of garlic mustard may be as simple as reformed and proper deer management, demonstrated as a complex interaction of deer overabundance facilitating garlic mustard success (Kalisz et al. 2014).

Arthropod communities respond to nonnative plant colonization with alteration to trophic and physical structure (Marshall & Buckley 2009; deHart & Strand 2012). deHart & Strand (2012) found shifts in predator feeding behavior, likely due to shifts in prey sources due to garlic mustard invasion. However, Dávalos & Blossey (2004) found no change in predatory ground beetle richness or abundance with colonization by garlic mustard. While certain sites may exhibit decreases in arthropod abundances due to garlic mustard invasion, other sites exhibit no relationship (Dávalos & Blossey 2004). In contrast, springtail abundance does correlate positively with garlic mustard invasion (Alerding & Hunter 2013).

Leaf litter absence or disturbance typically has a direct impact on arthropod communities in forests (Sayer 2005). With decreased litterfall, abundances in arthropods also decrease (David et al. 1991). This is likely related to leaf litter providing buffers against temperature and moisture changes, as well as food sources (both the litter itself and prey) (David et al. 1991). Additionally, the physical structure influences predator-prey interactions, increasing numbers of already abundant predators and increasing numbers of most prey (Bultman & Uetz 1984). Abundances of soil and litter arthropods are greater in older leaf litter comprised of several tree species compared to single species litter or younger leaf litter of several species (Kaneko & Salamanca 1999; Hansen 2000).
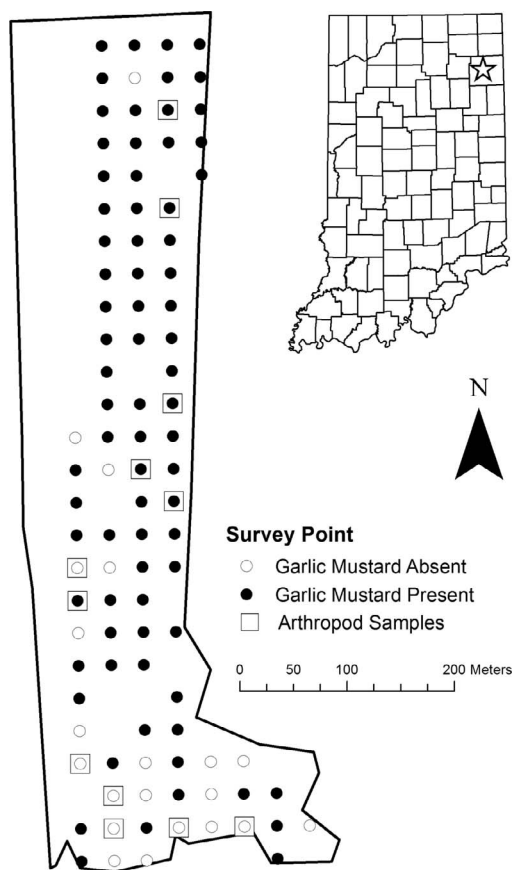

Figure 1.—Forest location (star) in Indiana and survey plot locations within forest.

We were interested in investigating arthropod community responses to established low density garlic mustard populations, as well as other forest characteristics. The objectives of this study were to quantify differences in leaf litter arthropod community abundance and diversity in areas with and without garlic mustard, and to test relationships between arthropod community diversity and leaf litter composition.

METHODS

A systematic grid of points (30 m spacing) was used to locate areas within a mature, second growth hardwood forest (41° 7' 20" N, 85° 8' 14" W; 13.7 ha; Fig. 1) in Fort Wayne, Allen County, Indiana, with and without garlic mustard. The overstory of this forest is dominated by *Acer saccharum*, *Tilia americana*, *Ulmus americana*, and *U. rubra*, with *Prunus serotina*, *Quercus rubra*, and *Q. velutina* being common

(Arvola et al. 2014). Across all plant strata, this forest is less diverse than others in the region and the understory is less dense overall (Arvola et al. 2014). The forest occurs in the Auburn Morainal Complex physiographic division and dominated by Blount-Morley silt loam soils (Franzmeier et al. 2004). At each grid point, all garlic mustard individuals within a 5 m radius circular plot were counted as mature (flowering/producing seed) or immature (leaf rosette) during July 2012. At each point, forest characteristics of percent canopy cover was measured with a concave spherical densiometer using standard techniques (Lemmon 1956); forest basal area was measured with a 10-factor prism using standard techniques (Avery & Burkhart 2002); litter depth was measured to the nearest 0.5 cm; and percent volumetric soil moisture was measured with a 12 cm Field Scout TDR probe (Spectrum Technologies, Plainfield, IL). The relationship between leaf litter moisture and soil moisture is variable and highly dependent on litter age (Nelson 2001).

Six points each with and without garlic mustard were randomly selected from the grid survey. Leaf litter was collected (down to mineral soil surface) within 1 m^2 quadrats centered at the selected grid points during September 2012. Arthropods were sorted from the litter samples using Tullgren-Berlese funnel traps (Southwood & Henderson 2000) with a 25 watt incandescent bulb as the light and heat source, stored in 70 percent ethanol at 0 °C until identified, and identified to the finest taxonomic level (typically family) using Triplehorn & Johnson (2005). Taxonomic nomenclature followed ITIS (2015). After arthropods were sorted from litter (approximately 72 hours), all intact leaves were identified to species using Barnes & Wagner (2004) and Jackson (2004), and counted. Arthropod family richness (count) and diversity (Shannon index) were calculated for each plot, as well as leaf species richness and diversity.

Arthropod and leaf richness and diversity, as well as forest measures, were compared between areas with and without garlic mustard using a Student's t-test. Relationships between leaf richness and diversity, and arthropod richness and diversity were identified using simple linear regression. Nonmetric multidimensional scaling (NMDS) ordination was used to compare arthropod taxonomic group abundance dissimilarities and relate those to environmental factors (R^2 cuttoff = 0.2). Statistical analysis was conducted using base package of R (version 3.1.1, The R Foundation for Statistical Computing) and vegan package for ordination (metaMDS function), vector correlations (envfit function), and species accumulation curves with rarefaction method (specaccum function) (version 2.2-1, Oksanen et al. 2015).

RESULTS

A total of 98 grid points were surveyed, with 77 of those having garlic mustard present (Fig. 1). The six randomly selected points with garlic mustard had a mean of 45.6 (SD ± 30.8) mature and 10.2 (SD ± 5.7) immature individuals, which did not differ from the other grid points with garlic mustard (t = 0.21, df = 75, p = 0.838; t = 0.07, df = 75, p = 0.944; respectively). Additionally, forest characteristics of canopy cover, basal area, litter depth, and soil moisture, did not differ between plots with garlic mustard selected for arthropod sampling and those not selected (t = −0.43, df = 75, p = 0.668; t = −0.30, df = 75, p = 0.766; t = −0.94, df = 75, p = 0.352; t = −0.58, df = 75, p = 0.561; respectively). Similarly, these four environmental variables did not differ between plots without garlic mustard selected for arthropod sampling and those not selected (t = −0.74, df = 19, p = 0.470; t = −0.437, df = 19, p = 0.667; t = 1.22, df = 21, p = 0.236; t = 0.217, df = 19, p = 0.831; respectively). Species accumulation curves for both arthropods and leaf litter both exhibited negative exponential functions (Fig. 2).

Further analysis includes only plots selected for arthropod sampling. Forest structure (canopy cover and basal area) and soil moisture did not significantly differ between plots with and without garlic mustard (Table 1). However, litter depth was significantly greater in plots with garlic mustard present (Table 1). Of the diversity measures, only arthropod richness was significantly different between plots with and without garlic mustard (Table 1). Additionally, arthropod abundance (count of individuals) was not significantly different between plots with and without garlic mustard (t$_{(2),10}$ = 1.17, p = 0.269; Table 2). Similarly, leaf abundance was not significantly different between plots with and without garlic mustard (t$_{(2),10}$ = 0.59, p = 0.569; Table 3). We pooled the plots for simple linear regression analysis. Leaf species richness had no significant influence on arthropod family richness or diversity (Fig. 3 A, B). However,
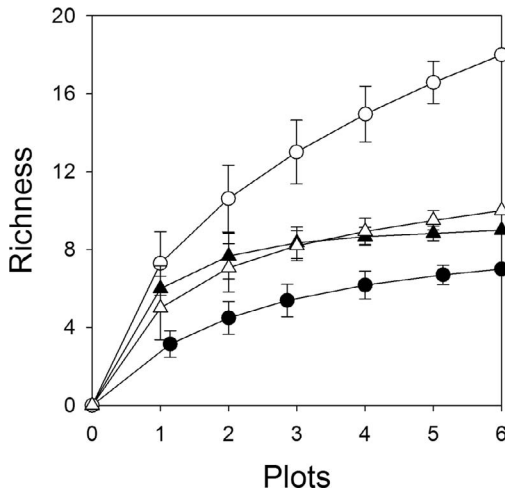
Figure 2.—Species accumulation curves for arthropods and leaf litter sampling (with standard deviation). Circles represent arthropods; triangles represent leaf litter. Open symbols indicate garlic mustard absence; closed symbols indicate garlic mustard presence.

arthropod richness and diversity were positively related with leaf diversity (Fig. 3 C, D). Arthropod abundance was not significantly related to leaf litter richness or diversity.

NMDS ordination of arthropod taxonomic group abundances resulted in a stress of 0.105 with three dimensions using an alternative Gower dissimilarity, which includes weights to exclude double zeros (i.e., joint absences between taxonomic groups; Anderson et al. 2006). Additionally, joint vectors displayed over the NMDS plot provide a visual representation of the influence environmental factors have on arthropod taxonomic groups (Fig. 4). Presences of vectors represent significant correlations, while direction and length of the vectors represent direction of influence and intensity of relationships, respectively. Armadillidiidae (woodlice) and Chrysomelidae (leaf beetles)

were positively influenced by mature garlic mustard densities, while Araneidae (spiders) and Passandridae (flat bark beetles) were negatively influenced. However, leaf litter species richness had a strong influence on most of the arthropod taxonomic groups, positive for some and negative for others (Fig. 4). Using tree species as the environmental factor for joint vectors, none of the tree species were correlated with the arthropod taxa and failed to meet the cutoff to be included in the figure.

DISCUSSION

Garlic mustard is a common understory invader of hardwood forests and has been the focus of extensive management (Stinson et al. 2007). Our study investigated the relationships between garlic mustard presence and leaf litter dwelling arthropods. While garlic mustard presence did result in limited decreased arthropod richness, leaf litter diversity had a clear, strong influence on the richness and diversity of arthropods. Additionally, leaf litter richness had a direct impact on the abundance of most of the arthropod taxonomic groups. Our interpretation of these results is that garlic mustard had a minor role in determining the litter dwelling arthropod community, while the litter layer "community" structure (i.e., diversity, richness) had a major role in defining the arthropod community. Likely the role of garlic mustard in this forest is less important because of the density in our sample locations (55.8 total individuals per plot = 0.7 individuals per m^2). Nuzzo (1999) demonstrated that garlic mustard becomes a fixture in forest communities and varies in density and cover annually. However, when disturbances occur in forests, garlic mustard quickly increases in number (Nuzzo 1999). Our study site is likely in the sustaining population stage of garlic mustard occurrence and the low density nature of this plant in the forest understory may

Table 1.—Comparisons of mean canopy cover, basal area, litter depth, soil moisture, arthropod richness and diversity, and leaf richness and diversity between plots with and without garlic mustard (standard error). Asterisk (*) indicates significant one-tailed t-test.

Garlic mustard	Canopy cover (%)	Basal area (m²/ha)	Litter depth (cm)	Soil moisture (%)	Arthropod		Leaf litter	
					Richness	Diversity	Richness	Diversity
Present	88.5 (1.9)	29.1 (2.5)	4.2 (0.4)	27.3 (6.6)	2.3 (0.4)	0.71 (0.17)	6.0 (0.3)	0.47 (0.15)
Absent	89.4 (1.3)	31.8 (3.2)	2.0 (0.8)	27.2 (6.4)	5.0 (1.1)	1.06 (0.28)	5.0 (0.7)	0.92 (0.23)
$t_{(2),10}$	0.38	0.67	−2.38	−0.02	2.27	1.06	−1.29	1.64
p-value	0.712	0.521	0.019*	0.987	0.046*	0.312	0.226	0.135

Table 2.—Arthropod taxa total abundances with frequency in parentheses (number of plots) in areas with garlic mustard present and absent.

Class	Order	Family	Present	Absent
Arachnida	Araneae	Araneidae	3 (2)	4 (2)
	Oribatida		0	4 (2)
	Pseudoscropiones		0	9 (5)
Chilopoda			0	2 (2)
Diplopoda			0	1
Insecta	Coleoptera	Apionidae	0	2 (1)
		Chrysomelidae	4 (4)	7 (4)
		Curculionidae	0	1
		Nitidulidae	0	2 (1)
		Passandridae	2 (1)	0
		Staphylinidae	1	1
	Hemiptera	Aphididae	1	0
		Aradidae	0	1
		Lygaeidae	0	1
		Miridae	3 (1)	0
		Nabidae	0	1
	Hymenoptera	Formicidae	0	348 (2)
		Ichneumonidae	0	1
	Psocodoea	Trogiidae	0	5 (1)
Malacostraca	Isopoda	Armadillidiidae	7 (4)	19 (3)
		Total	21 (6)	410 (6)

have minimal influence on the overall arthropod community.

Our plots randomly selected for arthropod sampling were not different in forest structure compared to the population of plots with and without garlic mustard. The only differing forest characteristic was litter depth (greater in garlic mustard plots). This result is similar to Bartusze-vige et al. (2007), who found garlic mustard seedling survival was greater in undisturbed litter and also had a clear inverse relationship

Table 3.—Leaf litter taxa total abundances with frequency in parentheses (number of plots) in areas with garlic mustard present and absent.

Family	Species	Present	Absent
Betulaceae	*Ostrya virginiana*	15 (3)	35 (5)
Fagaceae	*Fagus grandifolia*	24 (5)	45 (4)
	Quercus alba	21 (3)	8 (4)
	Quercus palustris	76 (6)	112 (6)
	Quercus velutina	18 (5)	16 (3)
Lauraceae	*Sassafras albidum*	1	0
Magnoliaceae	*Liriodendron tulipifera*	0	5 (1)
Rosaceae	*Prunus serotina*	0	2 (1)
Salicaceae	*Populus deltoides*	9 (3)	2 (2)
Sapindaceae	*Acer rubrum*	8 (4)	11 (3)
	Acer saccharum	19 (6)	1
	Total	191 (6)	237 (6)

with reductions in litter depth. Variability in forest structure (i.e., our measures of canopy cover, basal area, litter depth) could influence arthropod abundances and diversity (Jeffries et al. 2006). However, with those forest structure characteristics remaining relatively similar between treatments, we interpret changes in arthropod diversity, richness, and taxonomic group abundances to be influenced more so by the leaf litter layer composition and less by the presence and absence of garlic mustard.

There was a significant difference in arthropod richness between areas with and without garlic mustard, with garlic mustard presence reducing richness. However, since this difference did not extend to arthropod diversity, we argue that garlic mustard is then a minor influence on the arthropod community. Because richness and Shannon's diversity index are positively correlated (Stirling & Wilsey 2001), we would expect to see the influence of garlic mustard in both arthropod richness and diversity if it were a strong or major influence. We did find this strong or major influence on arthropod richness and diversity with leaf litter diversity. Increases in leaf litter diversity significantly increased arthropod community richness and diversity. While not compared statistically, it would be difficult to argue the leaf litter richness and
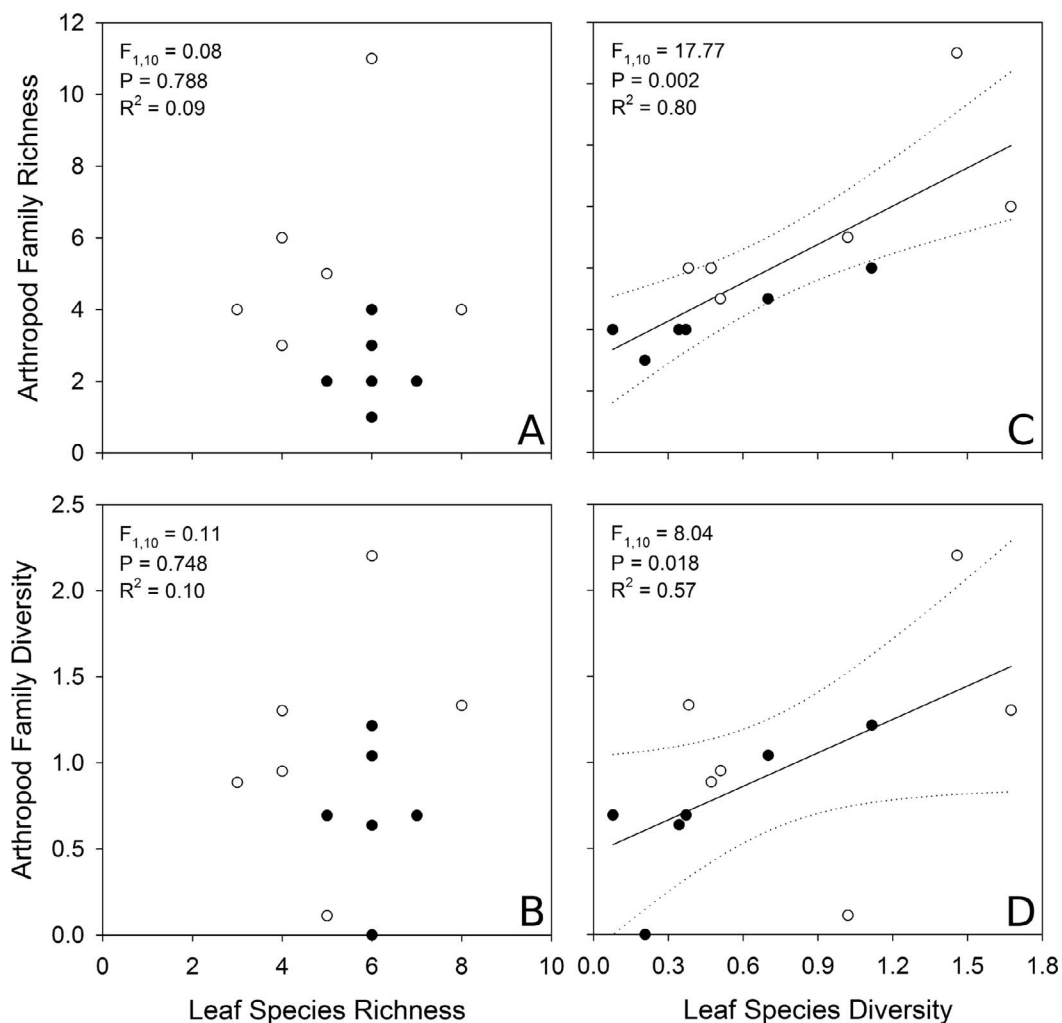
Figure 3.—Simple linear regression relationships between arthropod richness (A, C) and diversity (B, D) and leaf litter richness (A, B) and diversity (C, D) for pooled plots with (closed circles) and without (open circles) garlic mustard. Dotted lines indicate 95% confidence intervals.

diversity (Table 1) differ from overstory richness and diversity, as the mean overstory richness and diversity for this forest has been reported as 4.7 and 0.99, respectively (Arvola et al. 2014). With this similarity in the litter and overstory, garlic mustard likely had no influence on the overstory trees producing litter. In terms of composition, leaf litter in plots with garlic mustard were 37% similar to the overstory composition, while plots without garlic mustard were 48% similar, using data presented in Arvola et al. (2014). Many of the overstory trees reported by Arvola et al. (2014) that differed from our leaf litter were single individuals and not widespread

dominating species. While garlic mustard green rosettes may accelerate leaf litter decomposition (Rodgers et al. 2008), the density of garlic mustard in our study forest is likely too low to dramatically change decomposition rates.

Most arthropod groups were not heavily influenced by garlic mustard density, aligning with the vector origin in the NMDS plot. The strong positive influence by garlic mustard density on Armadillidiidae, potentially classified as alkaliphiles (van Straalen & Verhoef 1997), is likely related to an increase in soil and litter pH in garlic mustard colonized areas (Rodgers et al. 2008). Conversely, Araneidae and Passandridae may
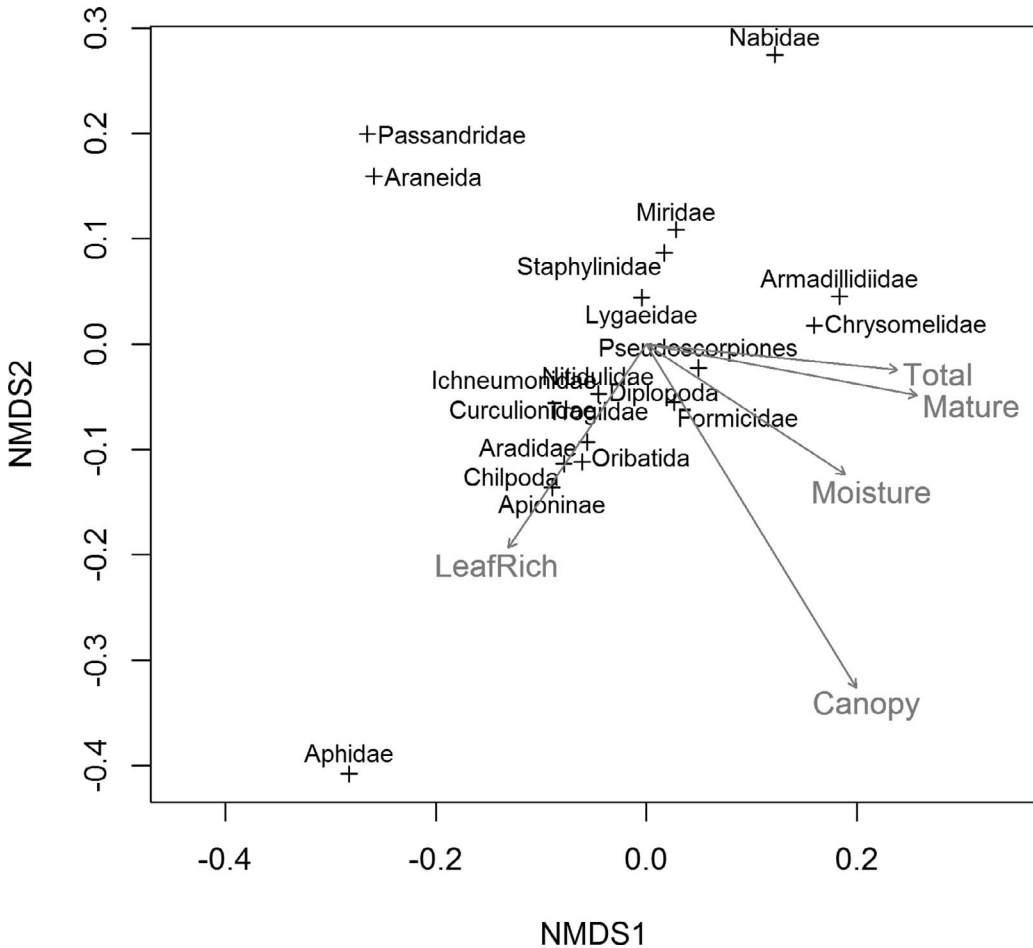
Figure 4.—Nonmetric multidimensional scaling (NMDS) ordination of arthropod taxonomic groups captured. Joint vector direction represents positive influence on groups and length represents intensity of influence. Vectors include percent canopy cover (Canopy), leaf litter species richness (LeafRich), percent volumetric soil moisture (Moisture), count of mature garlic mustard plants (Mature), and count of mature and immature garlic mustard plants (Total).

have been more influenced by canopy cover, soil moisture, or other spatial characteristics not measured in this study (e.g., course woody debris), as evidenced by the alignment and length of the vectors (Sanderson et al. 1995). Leaf litter species richness seems to have had the strongest influence on the most taxonomic groups. While leaf litter species diversity provided significant linear regression equations for arthropod diversity, litter richness influenced individual groups more than litter diversity. The alignment of taxonomic groups with the vector direction visually represents this relationship. Groups such as Aphididae (positive) and Nabidae (negative) had the most dramatic relationships with leaf litter richness.

Because leaf litter encompasses a broad range of habitat characteristics (e.g., pH, temperature, moisture, nutrients, shelter), leaf litter species richness would influence many of those and result in arthropod community organization (Bultman & Uetz 1984; Burghouts et al. 1992). It should be noted that NMDS uses the dissimilarity calculations for the entire arthropod community sampled and correlations of forest characteristics in relation to the community. This leads to minor interpretation issues of the two presentations of data (Fig. 4, Table 2). However, for the entire community, leaf litter layer richness influences the taxonomic groups strongly, as evidenced by the direction and length of the vector.

Garlic mustard presence, albeit at low density, in a hardwood forest had minimal influence on litter dwelling arthropods. While arthropod richness was significantly reduced in areas with garlic mustard, abundance and diversity of captured arthropods were not different. Additionally, other forest characteristics, such as canopy cover and soil moisture, that facilitate garlic mustard colonization intensity, may have had more influence on arthropods. There can be positive and negative influence by canopy cover and soil moisture depending on the arthropod family (Greenberg & Forrest 2003). Finally, leaf litter species diversity and leaf litter species richness may have the most important roles in determining arthropod diversity and individual taxonomic group abundances, respectively. While management of garlic mustard may be important for other communities, the leaf litter dwelling arthropods may not be affected. Further research on density dependent impacts is necessary in order to define an acceptable density for management decisions. While low density garlic mustard may have limited impacts, those low density populations may have the greatest seed production per individual facilitating rapid population responses to disturbance (Nuzzo 1999; Pardini et al. 2009). Since litter dwelling arthropods may not be impacted in any great way by garlic mustard, recovery time of such communities following management and restoration may be minimal.

ACKNOWLEDGEMENT

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