# FINAL REPORT

Restoration of Soil Microbial Function Following Degradation on Department of Defense Lands: Mediating Biological Invasions in a Global Change Context

SERDP Project RC-2326

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This project was designed to better understand the roles of soil biota in (1) mediating native species-NIS interactions and (2) facilitating the recovery of degraded ecosystems. The associated Research Needs were listed as follows: 1. To quantify the functional diversity of soil biota and						
the role of key taxa in maintaining desired functional ecosystem attributes; 2. To identify processes by which desired soil properties and						
associated soil biotic communities are degraded by NIS invasion and test potential mechanisms to restore them; 3. To examine the role of						
disturbance in determining the functional attributes of the soil community; 4. To test how the soil community and nutrient cycling may be						
adversely impacted by regional and global change stressors; 5. To elucidate the role of soil in mediating interactions between native species and NIS; 6. To provide innovative approaches to the study of soil ecology and their implications for management.						
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## List of Acronyms

ANOVA	Analysis of variance
С	Carbon
GM	Garlic mustard
Ν	Nitrogen
NIS	Non-native invasive species
NMDS	Non-metric multidimensional scaling
PI	Principal investigator

## Keywords

Garlic mustard (*Alliaria petiolata*); non-native invasive species; soil microbiota; habitat restoration; global change; nutrient cycling; ecosystem function

## Acknowledgements

We are grateful to the following stakeholders who are participating in this research by granting permission to install plots and carry out the research: West Point Military Academy, Army Corps of Engineers (Indian Hollow), The Trustees of Reservations, Pittsfield State Forest, Harvard Forest, Black Rock Forest Consortium, Mass Audubon Society-Drumlin Farm, and an anonymous private landowner.

# 1. Objective

## **1.1 SERDP Statement and Research Needs**

This project was designed to address the Statement of Need for FY 2013, RCSON-13-02: To better understand the roles of soil biota in (1) mediating native species-NIS interactions and (2) facilitating the recovery of degraded ecosystems. The associated Research Needs were listed as follows: 1. To quantify the functional diversity of soil biota and the role of key taxa in maintaining desired functional ecosystem attributes; 2. To identify processes by which desired soil properties and associated soil biotic communities are degraded by NIS invasion and test potential mechanisms to restore them; 3. To examine the role of disturbance in determining the functional attributes of the soil community; 4. To test how the soil community and nutrient cycling may be adversely impacted by regional and global change stressors; 5. To elucidate the role of soil in mediating interactions between native species and NIS; 6. To provide innovative approaches to the study of soil ecology and their implications for management.

### **1.2 Project objectives**

The general goals of this project were to examine the interactive effects of biological invasion and abiotic global change factors on the functional diversity of soil fungi of Northeastern forest habitats. We focused on management implications for forests disrupted by the invasive plant species *Alliaria petiolata* (garlic mustard). Our specific objectives address RCSON-13-02 Research Needs 1-6, as follows:

*Objective 1: Quantify the functional diversity of soil fungi in forested landscapes affected by NIS invasion.* We used genomic, transcriptomic, and bioinformatics approaches to quantify soil functional diversity in forests invaded by garlic mustard. We focused on the functional role of soil fungi for decomposition of organic compounds, and subsequent implications for native plant performance. This work aims to establish baselines for soil ecosystem recovery, and to determine the extent to which garlic mustard impacts are consistent across landscape gradients of climate and N deposition in the northeastern United States.

*Objective 2: Quantify impacts of garlic mustard on soil biota structure and function, and to test whether removal methods are effective at restoring soil microbial communities.* We imposed experimental eradications of garlic mustard to test potential mechanisms of soil biota recovery, using four datasets as indicators: (a) diversity, composition and function of the soil fungal community; (b) soil carbon and nitrogen cycling, (c) native plant – soil biota feedbacks, and (d) lag times for a-c to recover to reference levels of soil biota structure/function in proximate uninvaded sites.

*Objective 3a: To simulate garlic mustard invasions at an existing, long-term global change experiment to test how multiple stressors affect the soil fungal community and carbon and nitrogen cycling.* We conducted a controlled garlic mustard invasion in a long-term global change experiment at the Harvard Forest to determine how garlic mustard invasion interacts with warming temperatures and anthropogenic N fertilization to alter the structure and function of soil fungal communities and associated C & N cycling dynamics.

*Objective 3b: Determine how global change factors mediate future invasion success.* We can also infer potential effects of invasion and thus future invasion success in a global change context (soil warming and N fertilization).

*Objective 4: To test how soil fungi community recovery mediates long-term garlic mustard invasion impacts on native plant communities.* The multi-year datasets we compiled allow us to examine our recovery indicator data in relation to structural and functional changes in the native plant community. By collecting these data over a multi-year period after garlic mustard eradication (4 years), we were able to identify potential lag times for effective recovery of native vegetation and soil fungal communities, and how this pertains to changes in nutrient cycling dynamics.

*Objective 5: Identify and communicate which garlic mustard eradication methods are most logistically feasible and effective for restoring specific desired ecosystem attributes.* We are in the final stages of interpreting our results in the context of different desired restoration outcomes. For instance, recommendations for biodiversity conservation may differ from those that target maximum decomposition rates or other desired ecosystem function. Based on our data, we will share recommendations with our stakeholders in a workshop setting and create a handout that can guide restoration efforts according to site-specific ecological goals.

### 1.3 Hypotheses to be tested

Within the context of our five study objectives, we test the following hypotheses:

- H1: The abundance and function of soil fungal communities will change in response to garlic mustard invasion, leading to (a) reduced performance and changes in biodiversity of native woody plants, and (b) local suppression of decomposition.
- H2: Global change factors (soil warming and N addition) will exacerbate the effects of biotic invasion by (a) accelerating quantitative declines in mycorrhizae and saprotrophic fungi, (b) altering fungal community structure, and (c) reducing nutrient cycling through decomposition suppression.
- H3: Systematic restoration (e.g., recovery of native plant diversity, soil fungal biological diversity, C and N cycling to pre-invasion levels) will be both <u>more likely and more rapid</u> where stressful global change factors (N deposition and/or warming) are low, than where these abiotic stresses are high.
- H4: Garlic mustard populations may perform better with higher soil nitrogen deposition but decline under warmer conditions, particularly if soil warming is associated with surface drought, leading to plant-soil feedbacks where (a) mycorrhizae and saprotrophic fungal communities are highly suppressed under invasion x high nitrogen conditions due to garlic mustard success and (b) less suppressed under invasion x high temperature conditions due to garlic mustard mortality.

## 1.4 Project components described in this Report

In this Final Report, we discuss progress on our two major experiments, our test eradication (Experiment 1) and our controlled invasion (Experiment 2), with an emphasis on our results informing Objectives 1-5.

# 2. Technical approach

#### 2.1 Summary of project components

Our project has two main components:

1) *Experimental eradications* of garlic mustard at multiple sites distributed across regional environmental gradients (climate and N deposition) in the Northeastern US, taking advantage of DoD lands with active invasions. 2) *A controlled invasion* of garlic mustard within an existing Long-termerm Ecological Research (LTER) site experiment to test how chronic N fertilization and soil warming interact with phytochemical disruption of the soil biota and the plant community. Following one season of experimental invasion, we will eradicate garlic mustard and simulate restoration efforts by transplanting 240 seedlings of red maple and red oak, two dominant native tree species, from locally collected seed stock. This will allow us to assess the impacts of our treatments on native plant-fungal interactions.

The test eradications provide critical high-resolution data for developing NIS control strategies that can be adapted to individual sites, or more broadly across the landscape scale. The controlled invasion complements these regional observations by demonstrating the interactive impacts of invasion, nitrogen deposition, and warming under controlled experimental conditions, and allowing us to disentangle the effects of each factor on plant and soil community response

Methods for the experimental design and measurements are summarized in **Table 1**, below. Rationale for our approach is given in **Box 1**, at right. By integrating metagenomics and other molecular tools with soil biogeochemical analyses and observations of native vegetation, we generated data

#### Box 1 - Methods Rationale

Exp 1 – Test Eradications: *Conducting experimental eradications of garlic mustard at distinct locations across New England will provide an unprecedented opportunity to compare the likelihood and lag times for post-invasion soil restoration under a wide array of geographically variable conditions.* This component of the research takes advantage of DoD installations distributed across regional climate and N gradients in the Northeast, greatly strengthens fundamental research on soil biota with unusually well-replicated applications of cutting-edge molecular techniques, and provides unique, high-resolution data for developing NIS control strategies that can be adapted to individual sites or more broadly across different geographic locations.

Exp 2 – Controlled Invasions: *Experimentally introducing* garlic mustard to an ongoing global change experiment will create the first research platform we know of to simultaneously gauge impacts of biotic and abiotic stress on soil biota and function. This work complements our regional observations by providing controlled experimental conditions under which the interactive impacts of invasion, nitrogen deposition, and warming can be statistically decoupled and examined. Leveraging existing infrastructure at the Harvard Forest allows us to estimate long-term impacts and provide the resulting analysis in a costeffective manner. on the following *soil biota recovery indicators*:

- (a) *Abundance, diversity, composition, and function (gene expression) of the active fungal community;*
- (b) Ecosystem function measured as soil carbon & nitrogen pools and fluxes;
- (c) *Plant-microbial feedbacks including mycorrhizal colonization and growth responses of the plant community.*
- (d) Recovery rates following eradication treatments for recovery indices a-c.

In both experiments, we view the diversity, composition and gene expression of the soil fungi (observations related to recovery indicator a) as the *mechanisms* by which we expect to see effects of our treatments on ecosystem level processes, such as nutrient cycling and plant-fungi feedbacks (recovery indicators b-c).

	Experiment 1 – Test Eradications	Experiment 2 – Controlled Invasions
Treatments	Establish nine sites along temperature and N deposition gradients in New England	Establish 4 global change treatments: Non-treatment control, heated (+5°C), N addition (5 g N m <sup>-2</sup> y <sup>-1</sup> ), heated+N
	Implement four treatments per site: Un-invaded (control), invaded, invaded/pulled, invaded/sprayed Re-invasion was pre-empted by repeating eradication treatments annually as needed.	Establish 2 invasion trts: 100 GM plants m <sup>-2</sup> vs non-invaded control plots nested within each global change plot in a split-plot design. Eradication in second year to preclude re-invasion of experiments/surrounding areas.
Design	Observations of <i>recovery indicators</i> a-d (see below) were made as follows: For gene expression (a): Soil samples will be composited at the patch level: 9 sites x 3 patches per site x 4 treatments per patch = 108 samples. Within site replication = 3 samples/treatment. For vegetation and soil nutrients (b- c): 9 sites x 3 patches x 4 invasion treatments x 3 subplots per trt = 324	Observations of <i>recovery indicators</i> a-d (see below) were made at the plot level, as follows: 4 global change treatments × 2 invasion treatments × 6 replicates = 48 plots

Table 1:	Summary	of ex	perimental	design	and methods
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	samples. Within site replication = 9		
	samples/trt.		
Analysis	General linear model with eradication treatments specified as the main effects and recovery indicators a-d as the dependent variables. Multiple regression approaches to test correlations between geographic environmental variation and recovery indicators.	General linear model (e.g., ANOVA) with soil warming, N addition, and invasion treatments specified as the main effects and recovery indicators a-d as dependent variables. Multiple regression to test causal pathways from invasion, warming, and N addition to measured changes in recovery indicators.	
Recovery Indicator Data	<ul> <li><i>community</i> (baseline sampling in year evaluate treatment effects) with high the (diversity and community composition pipelines previously optimized in the I phospholipid (PLFA) analysis, with a extraction procedure (White <i>et al.</i>, 197)</li> <li>(b) <i>Soil carbon &amp; nitrogen pools and flue</i> respiration collars and a LI-8100A Au Lincoln, NE), soil net nitrogen minera (Binkley and Matson, 1983), total soil soil organic matter chemistry using py 2009).</li> <li>(c) <i>Plant-microbial feedbacks</i> by examining experimentally transplanted native tree period we excavated and examine individe for <i>mycorrhizal colonization</i> with micro McGonigle <i>et al.</i>, 1990; Wolfe <i>et al.</i>, 2000 (Diepen <i>et al.</i>, 2010).</li> </ul>	very rates for recovery indicators a-c using time series analyses of four	

*Further comments:* The expanded methodology for both Experiments, particularly for advanced technical methods for molecular analysis and bioinformatics, is detailed in the initial project proposal (Stinson and Frey #RC2326).

## 3. Results and Discussion

#### 3.1 Experiment 1: Test eradications

*3.1.1 Progress:* We visited and sought permission to work at over fifteen candidate sites for this study and ultimately identified eight study sites in locations with active garlic mustard invasions, including the West Point DoD installation. These sites were strategically distributed across a climatic/N deposition gradient in New England and provide a unique opportunity to observe landscape-level variation in the soil ecological responses to invasion and eradication. We experimentally eradicated garlic mustard successfully from 2013-2018 and report below our findings related to above and below-ground responses to invasion, now published in three peer reviewed journals (Haines et al., 2018, Anthony et al. 2017, and Stinson et al., 2018).

**Table 2:** Location, coordinates and characteristic forest types for eight replicates of the garlic mustard eradication experiment established in 2013.

Site	Northing	Westing	Forest type
Drumlin Farm	42°24'33.79" N	71°19'37.23" W	Central Hardwoods-White pine
Harvard Forest	42°31'45.82" N	72°11'25.49" W	Transition Hardwoods
River Road, Deerfield	42°32'11.25" N	72°34'08.67" W	Transition hardwoods
Pittsfield State Forest	42°29'12.47" N	73°17'59.33" W	Northern Hardwoods
McLennan Forest	42°13'17.44" N	73°10'23.49" W	Northern Hardwoods
Questing Forest	42°07'15.82" N	73°15'14.97" W	Northern Hardwoods
Black Rock	41°25'16.54" N	74°00'34.44" W	Central Hardwoods
West Point	41°22'45.45" N	74°01'09.16" W	Central Hardwoods

## 3.1.2 Results & Discussion:

Vegetation: Tree canopy cover at the study sites is dominated by A. saccharum, A. rubrum, Quercus rubra, Pinus strobus, and Fraxinus Americana, with an understory of seedlings of the same species plus shrubs such as Vaccinium species and Viburnum acerifolium, and low densities of common forest understory plants such as *Mainthemum canadense*, Aster divaricatus, and *Aralia nudicaulis*. Vegetation surveys at these plots have revealed that individual plant species reliant on mycorrhizae, such as trout lily (*Erythronium americanum*) and seedlings of red maple (Acer rubrum) are regionally less abundant in the presence of garlic mustard and that there are mixed effects on overall species composition and diversity (Haines et al., 2018). Below we show a combination of published and unpublished results on vegetation at our eradication sites. Plant communities were more similar to each other with respect to geographic location than presence of garlic mustard, but only when communities were analyzed at the plant species level (nonmetric multidimensional scaling; Figure 1A, below). Garlic mustard presence, however, was more influential in community structure with respect to plant functional groups (Figure 1B, below) and by geography (Urbanowicz et al., 2018) Plant species diversity tends to be higher in plots occupied by garlic mustard compared to areas without, and that this pattern appears to be driven by increased presence of invasive woody species and herbaceous plants in invaded areas. For example, there was higher Shannon diversity in the spring ( $F_{1,32} = 5.97$ , P = 0.0203) and summer ( $F_{1,32} = 5.45$ , P = 0.0259) of 2014, and Shannon evenness in the spring of 2014 ( $F_{1,32} =$ 



**Figure 1:** Overall plant community composition was driven by site proximity rather than invasion status (A). However, plant communities in non-invaded areas were distinct from those in invaded areas when analyzed by functional groups (B). Data from summer 2013.

5.65, P = 0.0236), in areas with garlic mustard than areas without. There were similar, non-significant trends for all diversity indices, including species richness, for most sampling periods (data not shown). Shannon diversity also varied significantly among sites in the spring  $(F_{7,32} = 9.69, P < 0.0001)$  and summer ( $F_{7,32} = 5.36$ , P = 0.0004) of 2014. Patterns of species diversity at individual sites with respect to garlic mustard presence (data not shown) are largely a reflection of diversity and density of invasive woody species and herbaceous species (Figure 2, right).

It is unknown why invasive woody plants are more dense and diverse in



areas with garlic mustard, but there are at least two mechanisms that may be responsible. Soil edaphic characteristics, such as nutrient enrichment resulting from anthropogenic nitrogen deposition, may result in conditions that are more amenable to plant invasion at some sites compared to others (Davis et al. 2000; Urbanowicz et al. 2018).

Alternatively, one or more of the invasive plant species could have changed soil biotic or abiotic conditions in ways that make the sites more prone to invasion by other invasives. Garlic mustard, for example, can disrupt the mutualisms that form between mycorrhizal fungi and plants (Stinson et al. 2006), and this disruption may reduce competition from native plants, providing a competitive advantage to invasive plant species.

Fungal communities: A major finding<br/>from this study is that invasion by<br/>garlic mustard has a homogenizing<br/>effect on soil fungal community<br/>composition (Figure 3, Anthony et al.,<br/>2017). Changes in the soil microbial community<br/>with garlic mustard invasion mirrored those of<br/>the plant community and, in many cases, showed<br/>stronger invasion effects. Soils invaded with<br/>garlic mustard, for example, were much less<br/>variable in microbial community composition<br/>across all study sites than non-invaded soils,<br/>which is suggestive of a garlic mustard<br/>homogenizing effect (Figure 3).diversit<br/>site and<br/>different<br/>to other<br/>to other<br/>to other

These changes are largely due to shifts in microbial community composition in several classes of fungi, including a reduction in relative abundance of Agaricomycetes with invasion, but an increase in multiple other groups, including Dothideomycetes and Sordariomycetes (Figure 3). These changes, similar to the plant community, represent broad increases in diversity of soil fungi in the presence of garlic mustard compared to when absent. There were also significant differences in fungal diversity across sites, and these changes were present in both the organic horizon (i.e., shallow soil) and in the mineral horizon (deeper soils). These changes in microbial community composition were correlated with functional responses in soil edaphic conditions, including a lower C:N ratio (Figure 4A), and an increase in soil pH (Figure 4B), with garlic mustard invasion.

**Figure 2:** Invasive shrub density (A) and forb Shannon diversity (B) show similar patterns with each other regarding site and invasion status. Bars with different letters have different estimates; no letters indicate no difference compared to other estimates.



**Figure 3.** The dominance of fungal communities is reversed with invasion. Garlic mustard is associated with lower relative abundances of symbiotic (mycorrhizal) fungi and higher relative abundances of decomposer (saprotrophic) fungi (Anthony et al., 2017). Bars represent the mean relative abundance across six forests. Significantly different relative abundances in association with invasion are indicated using an asterisk.



**Figure 4**: The relationship between fungal richness and soil properties. The two strongest predictors of fungal richness are the (A) C:N ratio and (B) soil pH.



*Earthworms:* Another discovery from our project is that garlic mustard invasion interacts with non-native earthworms to affect native plants. Long term experimental eradication of garlic mustard reduces earthworm biomass, challenging the theory from correlative studies that earthworms facilitate plant invasions (Stinson et al., 2018; Figure 5). A decline in earthworms with the removal of garlic mustard suggests instead that earthworms respond to plant invasion.

Figure 5. Eradication of garlic mustard also reduces biomass of non-native earthworms in forest soils, challenging correlative studies suggesting that plant invasions follow earthworm disturbance, and instead demonstrating facilitation of earthworms by garlic mustard (Stinson et al., 2018).

*Additional manuscripts:* We are developing additional manuscripts that will assess the effectiveness and impact of our long-term garlic mustard removal treatments on plants and soils at our study sites. This research is anticipated to result in the publication of one manuscript focusing on plant community effects (Coates-Connor et al., in prep), and one manuscript on soil biotic community effects, of garlic mustard presence and eradication (Anthony et al., in prep) at no additional cost to the project.

#### 3.2 Experiment 2: Controlled invasion

*3.2.1 Progress to date*: We implemented the experimental invasion in spring of 2015 using both seedling and second-year garlic mustard plants (**Figure 6**). Prior to fruit maturation, all garlic mustard plants were harvested to prevent accidental spread of garlic mustard seed in the Harvard Forest. By experimentally adding garlic mustard the exisiting global change experiment at the Harvard Forest, we found that invasion mitigates positive effects of soil warming on symbioses between arbuscular mycorrhizal fungi (AMF) and red maple seedlings. This suggests that

invasion will continue to suppress red maple regeneration, even under predicted warming trends that might otherwise benefit this species (Figure 7; Wheeler et al., 2016; Wheeler et al., 2017). One additional publication is anticipated at no ccost to the project, testing for interactive effects of warming, nitrogen deposition, and invasion on the underlying fungal microbial communities in the soil.



**Figure 6:** Schematic showing garlic mustard invasion subplot design within the existing Long-Term Ecological Research plots, with both seedling and second-year rosette stages present after experimental transplant.



Figure 7. Effects of invasion by garlic mustard on the mycorrhizal colonization of red maple seedling roots in plots undergoing nitrogen addition, soil warming and soil warming + nitrogen addition, and control treatments at the Harvard Forest (Wheeler et al., 2017).

Seedling

Second-

year rosette

## 4. Final Conclusions

*Objective 1: Quantifying the functional diversity of soil fungi in forested landscapes affected by garlic mustard.* The results presented above indicate a clear pattern of garlic mustard impacts on both soil microbes and vegetation that is consistently observed across several geographically distributed sites experiencing garlic mustard invasions. We find that forest patches invaded by garlic mustard consistently have floristic and soil communities that are distinct from nearby patches of forest not affected by the invasion (Anthony et al., 2017; Haines et al., 2018; Stinson et al., 2018). Invasion is associated with taxonomic and functional shifts in the microbial community that are correlated with biogeochemical processes. In particular, C:N ratios and microbial diversity increase with invasion, suggesting that decomposer taxa are less efficient and/or abundant at sites invaded by garlic mustard.

In addition, significant effects of site on both floristic and microbial community diversity demonstrate the importance of local variation and suggest the need for more site-specific evaluation of long-term management plans. Specifically, sites that share a similar native tree canopy structure and composition are more similar in their response to invasion than sites with dissimilar canopy, and invasive shrubs often accompany garlic mustard invasions. Our analyses of plant community composition indicate that, for restoring ecosystem attributes such as plant functional groups, there will likely be a need to remove invasive woody plant species as well as garlic mustard.

*Objective 2: Quantify impacts of garlic mustard on soil biota structure and function, and to test whether removal methods are effective at restoring soil microbial communities.* We have demonstrated clear effects of garlic mustard on soil biota including fungi and earthworms (Anthony et al., 2017; Stinson et al., 2018). We have also documented and expect to publish additional work detailing the effects of eradication on (a) diversity, composition and function of the soil fungal community; (b) soil carbon and nitrogen cycling, (c) native plant – soil biota feedbacks, and (d) lag times for a-c to recover to reference levels of soil biota structure/function in proximate un-invaded sites. To our knowledge, this is the first study to document impacts of garlic mustard on soil microbes with such high molecular resolution and at a broad landscape scale.

Objective 3a: Simulate garlic mustard invasions at an existing, long-term global change experiment to test how multiple stressors affect the soil fungal community and carbon and nitrogen cycling. Our controlled garlic mustard invasion in a long-term global change experiment has shown that garlic mustard invasion interacts with warming temperatures and anthropogenic N fertilization to alter the mycorrhizal colonization and growth of red maple seedlings, a key canopy species in the forests at our study sites (Wheeler et al., 2016; 2017). Two upcoming publications will detail how these treatments also alter the structure and function of soil fungal communities, fungal gene abundance, and associated C & N cycling dynamics (Anthony et al., in prep; Moore et al., in prep).

*Objective 3b: Determine how global change factors mediate future invasion success.* We determined that warming has the potential to interact with garlic mustard invasion to affect tree seedling performance and thus the impact and success of further invasions, such that tree seedlings are suppressed in response to the combined effects of warming and invasion (Wheeler et al., 2017). We further showed that specific landscape drivers affect forest invasion by garlic mustard using survey data obtained during our site selection process (Urbanowicz et al., 2018). We also had hoped to take advantage of our experimental invasion to also test how garlic mustard itself is affected by global change factors at no additional cost to the budget, but prioritized the original tasks outlined in our proposal and did not complete this endeavor.

*Objective 4: To test how soil fungi community recovery mediates long-term garlic mustard invasion impacts on native plant communities.* The multi-year datasets we compiled indicate significant lag times for effective recovery of native vegetation and soil fungal communities, and species that are negatively correlated with invasion (Haines et al., 2018; Anthony et al., in prep; Moore et al., in prep).

*Objective 5: Identify and communicate which garlic mustard eradication methods are most logistically feasible and effective for restoring specific desired ecosystem attributes.* We have developed an informational pamphlet based on our data from this project. We have initiated a stakeholder workshop scheduled for January 16, 2019. At this event we will share recommendations with our stakeholders in a workshop setting and distribute the new handout to help guide restoration efforts and site-specific ecological goals.

### **Literature Cited** \* indicates publication resulting from this project.

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