Effects of Purple Loosestrife (Lythraceae) on Wetland Biodiversity and the Impact of the Biological Control Insects, *Galerucella calmariensis* L.

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SYNOPSIS

Lythrum salicaria (Purple Loosestrife) is an exotic perennial from Eurasia that has inhabited wetlands of North America since the early 1800s. As a prolific invasive species, *L. salicaria* has the ability to spread aggressively, grow rapidly, and disrupt wetland communities within which it becomes established. As a result, *L. salicaria* may potentially reduce biodiversity and habitat quality of the wetlands within which it resides. There are no natural predators of *L. salicaria* endemic to North America. In recent decades, several insect species have been successfully introduced to biologically control the spread of *L. salicaria.* Pierce Cedar Creek Institute has introduced *Galerucella calmariensis*, a species of leaf eating beetle, as a method of biological control to help manage the spread of *L. salicaria* within the wetland communities on their property. This study will evaluate *L. salicaria*'s impact in the wetlands at Pierce Cedar Creek Institute by 1) mapping the extent of current *L. salicaria* infestation across Institute property using GPS data points and GIS mapping, 2) examining the wetland characteristics associated with invasions of *L. salicaria* using ordination techniques to analyze species data and measured environmental variables for three wetland types: an uninvaded wetland, an invaded and managed wetland, and a heavily invaded unmanaged wetland, 3) calculating several measure of species diversity, including a Floristic Quality Assessment for each of the three wetland types, and, 4) measuring the effectiveness of the introduced biological control agent, *Galerucella*, using standard monitoring protocols. Our study will provide baseline data required for effective

monitoring of the wetland communities at Pierce Cedar Creek Institute and will help guide the implementation of future management strategies for controlling the invasive species *L. salicaria*.

INTRODUCTION

Non-native, invasive plant species are of management concern because of the negative impact they have on ecosystem processes and the associated economic consequences of invasion (Mack et al. 2000; Pimentel et al. 2000). Invasive species can alter the basic ecological properties of a community (i.e. dominant species, patterns of nutrient cycling, plant productivity), they often out-compete native plant species, and they degrade habitats for wildlife (Mack et al. 2000; Lavoie 2010).

Purple loosestrife (*Lythrum salicaria* L., Lythraceae) is thought to be one of the most invasive plant species in the world (Lavoie 2010). The International Union for the Conservation of Nature considers purple loosestrife one of the 100 worst invasive species (Invasive Species Specialist Group 2013), the *Prioritized List of Invasive Alien Plants of Natural Habitats in Canada* ranks purple loosestrife second on its list (Catling and Mitrow 2005), and *L. salicaria* has been listed as a noxious weed in 33 of the United States (USDA 2015).

Lythrum salicaria is of Eurasian decent and was likely introduced to North America in the early 1800s through the dumping of ships ballast and the import of raw wool or sheep containing seed (Thompson et al. 1987). Additionally, *L. salicaria* seed was sold in American and Canadian nurseries as early as 1829 (Mack 1991). Since then *L. salicaria* has dispersed widely among wetland habitats altering their basic structure (Thompson et al. 1987). The plant appears to be spreading at a rate of 100,000 ha per year and the annual costs associated with the control of purple loosestrife and the economic losses and damages from purple loosestrife invasion are about 45 million US dollars (Pimentel et al. 2001; Pimentel 2009). Because of its rapid range expansion *L. salicaria* has been identified as a serious threat to wetland habitats. It is reported to out-compete other wetland flora, degrade habitats for wetland fauna (Thompson et al. 1987; Blossey et al. 2001; Brown et al. 2006), and alter nutrient cycling (Emery and Perry 1995, 1996; Templer et al. 1998). However, several studies have found little evidence that *L. salicaria* negatively impacts the biological diversity of wetlands (Anderson 1995; Hager and McCoy 1998; Treberg and Husband 1999; Hager and Vinebrooke 2004). Studies by Rachich and Reader (1999), Farnsworth and Ellis (2001) and Hager and Vinebrooke (2004) indicate that *L. salicaria* invasions are dependent on disturbance, which suggests that wetland invasion by *L. salicaria* may actually be an indicator of anthropogenic disturbance. The question over the extent of ecological harm that *L. salicaria* actually causes remains unresolved (Lavoie 2010).

Plant functional group studies have showed evidence that altering the functional diversity within a community can impact ecosystem processes (Tilman et al. 1997; Hooper and Vitousek 1998; Reich et al. 2004). This suggests that the introduction of an invasive species such as *L. salicaria*, which would likely be a novel functional group within the wetland community, may alter ecosystem processes within the wetland (Mahaney et al. 2006). Boutin and Keddy (1993) identified the ability to acquire nutrients, ability to compete, tolerance to stress, and ability to disperse as distinctive functional traits for wetland plant species. Using these traits, they identified three functional groups: interstitial, matrix, and ruderal species. For example, *Carex lacustris* Willd., is an interstitial species which has a low compact growth form, shallow root system, relatively low seed production, and a shoot system that invests more energy in light capture than the root system invests in nutrient capture (Mahaney et al. 2006). Mahaney et al. (2006) identified *L. salicaria* as a ruderal species well-known for its ability to prolifically produce seeds. A mature plant can produce around 2.7 million seeds per year which creates large seed banks in areas where *L. salicaria* is found. However seed dispersal may be limited (Thompson et al. 1987; Yakimowski et al. 2005). Other functional traits of *L. salicaria* include tall erect stems that grow to about 2.7 m, a persistent perennial tap root with a shallow spreading root system, and the ability to rapidly regenerate following cutting. Lastly, Mahaney et al. (2006) identify *Typha angustifolia* L. (narrow-leaved cattail), *Typha latifolia* L. (broad-leaved cattail), and *Phalaris arundinaceae* L. (reed canary grass) as matrix species whose functional traits include very tall shoots, deep well-developed roots and rhizomes, and a strong competitive ability for soil nutrients. The differences between how these functional groups use and allocate resources may impact wetland ecosystem processes resulting in environmental gradients associated with percent soil moisture, the amount of available soil nitrogen and phosphorus, soil water depth, and disturbance history. In turn, altered ecosystem processes and properties may be correlated with species diversity within wetland habitats (Hager and Vinebrooke 2004; Mahaney et al. 2006).

Ecologists frequently use ecological indices to measure community structure, evaluate changes in species composition, and draw inferences about habitat quality. The indices most frequently used are Shannon's Diversity Index (H'), Simpson's Diversity Index (D), and Shannon-Wiener Evenness (E). Calculated values for Shannon's Diversity Index begin at zero (0) with values more distant from 0 representing higher diversity. Calculated Shannon-Weiner Evenness values range from 0 – 1 where 1 indicates complete evenness of species distribution. Simpson's Diversity Index is generally considered to be the most meaningful and robust of the diversity measures (Margurran 2004). Calculated values for Simpson's Index range from 0 to 1, with values closer to 0 representing higher diversity. In addition to these diversity indices, Floristic Quality Assessments (FQA) are used to identify natural areas, facilitate comparisons between sites, and assess the floristic quality of natural, restored, and created habitats (Swink and Wilhelm 1994; Mortellaro et al. 2012).

The basis of a Floristic Quality Assessment (FQA) is the concept of species conservatism or “nativeness”. The concept is based on the idea that the observer has a certain level of confidence as to where a plant species is likely to occur; ranging from highly disturbed, man-made habitat to highly intact remnant native habitat (Swink and Wilhelm 1994; Mortellaro et al. 2012). FQA uses the aggregate conservatism of all species found on a site as a measure of the site’s ecological integrity. The method requires the *a priori* assignment of a “coefficient of conservatism” (C value) to each native species; C values range from 0 to 10 (Swink and Wilhelm 1994). A 0 C value is assigned to ruderal species; those plant that that are obligate to ruderal or disturbed areas such as cracks in sidewalks, road sides, excavated lands, parking lot edges, industrial sites, and agricultural fields. A C value of 10 is assigned to those species that are obligate to high quality natural areas. These are native species whose presence provide the highest level of confidence that the area in which they are found are the most intact (pre-European settlement) remnant natural habitats. Typically those species display specific adaptations to a narrow range of the environment conditions. C values ranging from 1 – 3 indicate the species has varying affinity to ruderal areas, values from 4 – 6 indicate varying affinity to natural areas, and values from 7 – 9 indicate varying affinity to high quality natural areas. For example, a C value of 5 would be applied to a species that is obligate to remnant natural habitats but for which the quality of the natural area is low (Mortellaro et al. 2012). C values should only be applied at a regional or state level because the behavior of plant populations can differ throughout the species range (Deboer et al. 2011).

FQA metrics derived for a given area include species richness, Mean C, Mean Floristic Quality Index (FQI) and mean wetness (Swink and Wilhelm 1994). Mean C is the average of the C values applied to each species across the sample site. The FQI is an indicator of native vegetative quality for an area. It adds a weighted measure of species richness by multiplying the Mean C by the square root of the total number of native species (Swink and Wilhelm 1994). FQI is designed to reduce subjectivity, and produce an objective standard by which the quality of plant communities can be evaluated in a repeatable fashion (Mortellaro et al. 2012). In general, higher Mean C and Mean FQI values indicate greater floristic integrity and a lower level of disturbance to a site. However the Mean C and Mean FQI values are affected by the timing, sampling effort, and accuracy of the species inventory (Deboer et al. 2011).

Detecting differences in species composition within various wetlands and whether an invasive species has specific abiotic preferences or preferred species associations (i.e. native, weedy, or other nonnative species) requires testing at the species level. The determination of species assemblages along environmental gradients is useful for land management as it can allow one to predict the conditions that may promote invasion (Hager and Vinebrooke 2004; Mahaney et al. 2006). Ordination, or gradient analysis, is a useful tool for examining the associations between invasive and native plants and their environments because it combines information about species abundances with environmental information into one analysis. Ordination can be performed using either indirect gradient analysis or direct gradient analysis methods. Indirect gradient analysis first examines species associations and then the environmental factors important in establishing those associations are inferred (McCune and Grace 2002; Palmer 2012). In other words, the species themselves will indicate what environmental variables are important to determining the structure of the community. With direct gradient analysis the ordination axes are derived from environmental data and then the species data is correlated to the ordination axes (McCune and Grace 2002; Palmer 2012). Therefore, direct gradient analysis is most appropriately used when the goal is to learn how species are distributed along specific environmental gradients (e.g. a moisture gradient or disturbance history gradient) (McCune and Grace 2002). Direct gradient analysis methods found that environmental variables such as topography, altitude, soil moisture, soil fertility, grazing regime, and disturbance history were important in distinguishing sites that were invaded by nonnative plants from those that were uninvaded (Kitayama and Mueller-Dombois 1995; Rose et al. 1998; Hager and Vinebrooke 2004).

As with any invasive species, there are many variables to consider when describing the total impact a species has on the ecosystem. Because invasive species, such as *Lythrum salicaria*, often threaten native biodiversity, and because it is unclear whether *L. salicaria* is directly impacting wetland biodiversity or whether it opportunistically moves in following anthropogenic disturbance, it is considered best practice to remove the invasive species in a cost effective and ecologically friendly manner (Lavoie 2010). Controlled burns, flooding, herbicides, and mechanical methods have all been used with limited success in an effort to control *L. salicaria* growth and spread (Blossey 2002). Burns, herbicides, and flooding lack precision and damage native species, and mechanical methods, while effective, are time consuming and costly. Biological control methods are the most cost effective way to reduce *L. salicaria* population size, and the least labor intensive (Wilson et al. 2009). Two insect species, *Galerucella calmariensis* L. and *Galerucella pusilla* Duftschmidt [Coleoptera: Chrysomelidae] are natural enemies of *L. salicaria* and have proven to be effective at biologically controlling its spread (Blossey 1995a, b; Blossey and Schat, 1997; Palmer 2007). Blossey (2002) and Wilson et al. (2009) detail the life cycle of *G. calmariensis* and *G. pusilla*. Both species are leaf-feeding beetles that defoliate *L. salicaria* and attack its terminal bud. Adult beetles overwinter in leaf litter and emerge early in spring just as the host plant is emerging. During the first few weeks of spring, the overwintered, mobile, adults disperse in search of *L. salicaria* populations in which to reproduce. Adult beetles will feed on young plant tissue and create round holes, a characteristic "spothole" defoliation pattern which provides evidence of beetle presence. Females lay two to 10 eggs on the underside of leaves and on the stems of their host plant during May and June. The first instar larvae are concealed within the leaf or flower buds where they feed; later instar larvae are not concealed and feed openly on all aboveground plant parts where their presence can be easily observed. The larvae feed from the leaf underside where they strip all the photosynthetic tissue from the leaf, leaving only the thin upper epidermis intact. This creates a characteristic "window-pane" defoliation pattern which provides additional evidence of larval presence. The mature larvae pupate in the leaf litter beneath the host plant and the new generation beetles then disperse in search of new *L. salicaria* patches.

Galerucella spp. beetles and larvae have been shown to reduce plant height, reduce the size of the inflorescence and reduce seed set (Blossey 1995a, b; Blossey and Schat, 1997; Palmer 2007). Blossey (1995a, b) and Blossey and Schat (1997) found that at high larval densities (>2 to 3 larvae/cm shoot) entire populations of *L. salicaria* could be defoliated, and that at lower densities, plants retain leaf tissue but show reduced shoot growth, reduced root growth, and fail to produce seed. Although reduced reproduction is beneficial, the greatest benefit to native species appears to be the additional light made available by the reduction in *L. salicaria* height and foliage (Palmer 2007; Wilson et al. 2009). The purpose of using biological control methods is to reduce the spread of invasive species and to allow time for native species to regain a foothold.

Roughly 43 percent of the Pierce Cedar Creek Institute property is designated as wetland and *Lythrum salicaria* is a species of concern within those wetlands (Howell 2015). In an effort to control the spread of *L. salicaria, Galerucella calmariensis* beetles have twice been released on Institute property. This study was performed in order to provide baseline data useful for monitoring the wetland communities at Pierce Cedar Creek Institute and to provide information on the effectiveness of the Institutes current *L. salicaria* management strategies. Our specific goals were: 1) to examine *L. salicaria*'s impact in the wetlands at Pierce Cedar Creek Institute by mapping its current distribution and evaluating species diversity using FQA and several diversity indices, 2) to examine the wetland characteristics associated with invasions of *L. salicaria* using ordination techniques to analyze species data and measured environmental variables for an uninvaded wetland, an invaded and managed wetland, and an invaded and unmanaged wetland and, 3) to measure the effectiveness of the *Galerucella* beetles introduced by the Institute as a biological control agent.

METHODS

Study Areas

Our study was based at Pierce Cedar Creek Institute in Barry County, Michigan, USA (T2N R8W S½ sec 19). The Institute property is approximately 267 ha of which roughly 43% is designated as wetland (Howell 2015). These wetlands are managed in an effort to control invasive species such as *Lythrum salicaria*. An additional study area was located south of the institute at Justin's Marsh in Kalamazoo County, Michigan USA (T3S R11W SW¼ sec10). The 35 ha Justin's Marsh is located along Sugarloaf Lake and is considered to be one of the most diverse botanical areas in Kalamazoo County with over 400 plant species documented from the property (SWMLC). The property is owned by the Southwest Michigan Land Conservancy but is not managed for *L. salicaria*.

In an effort to determine the effect of *Lythrum salicaria* on community structure and the impact of the introduced biological control beetle, *Galerucella calmariensis*, we compared three wetland types that differed in *L. salicaria* infestation and land management practices. One wetland type was selected for its heavy infestation of *L. salicaria* and lack of land management efforts, a second type was selected because *L. salicaria* was present but managed by periodic burns, plant removal, and biological control methods, and the third wetland type was selected for its absence of *L. salicaria*.

Six sites were selected for this study representing the three distinct wetland types: *Lythrum salicaria* uninvaded, *L. salicaria* invaded and managed, and *L. salicaria* invaded and unmanaged; hereafter referred to as uninvaded, managed, and invaded. Sites were selected by visual determination based on the presence or absence of *L. salicaria* and its known management status. Sites 1 and 2 were located on the Institute's property and were uninvaded by *L. salicaria* . Sites 3 and 4 were also located on the Institute's property but were invaded by *L. salicaria*. Site 3 was also the site of a *G. calmariensis* beetle release in 2010 (see Fig. 1 for site locations on Institute property). Sites 5 and 6, located at Justin's Marsh, were heavily invaded by *L. salicaria*. While the property is protected by the Southwest Michigan Land Conservancy no efforts have been made to control the spread of *L. salicaria* (pers.comm).

Mapping of Lythrum salicaria

Beginning in May, and continuing throughout the summer of 2015, Institute property was visually surveyed for populations and individuals of *Lythrum salicaria*. Our surveys consisted of walking transects through the prairie fens at the Institute where hydrology and Institute records indicated the likely presence of *L. salicaria*. Once the prairie fens were surveyed we waited until the plant bloomed to facilitate locating it in more obscure or unexpected areas, such as the prairies, old fields and swamp woods located at the Institute. The perimeter of areas that were densely populated by *L. salicaria* were marked using a Global Positioning System (GPS) (Mobile Mapper, Thales Inc., France). In less dense areas individual plants were GPS marked. These populations and individuals were then added to the Institute's ArcMap 10.2 GIS system (Economic and Social Research Institute, Redlands, CA) to create a record and map of *L. salicaria*'s current distribution on Institute property.

Vegetation Sampling

Within the three wetland types, vegetation was sampled using a stratified random sampling design modeled after Hager and Vinebrooke (2004). The design consisted of eight 1.0 m^2 (1 x 1 m) sampling quadrats arranged in a grid of two quadrats by four quadrats. All quadrats were spaced 2 meters apart. For each site the first quadrat was randomly placed in the vegetation at least 2 meters interior to the wetland edge. The long edge of the sampling grid ran parallel with the edge of the wetland. Within each sampling quadrat, species were identified and compiled into a species inventory list with nomenclature following Reznicek and Voss (2012) (Table 1). Vegetation that could not be confidently identified in the field was sampled and taken back to the laboratory for identification. Sampling was completed between 1 July and 6 August 2015, when the growth of most plant species was complete and before their senescence.

From each quadrat we also recorded several environmental parameters including water depth, soil nutrient and pH parameters, shoot biomass for *L. salicaria* and shoot biomass for all non-*Lythrum* species. In addition we recorded stem density, and several inflorescence parameters (see sections below) for *L. salicaria*.

Determination of Shoot Biomass

In an effort to determine whether *Lythrum salicaria* affects community shoot biomass we compared shoot biomass between our three wetland types; uninvaded, invaded and managed, and invaded and unmanaged. Shoot biomass was determined for each quadrat in mid-August following Mahaney et al. (2006). Shoot biomass, including the current year's dead biomass (snow compresses the previous year's biomass), was clipped at ground level in each 1.0 m^2 quadrat. *Lythrum salicaria* was separated from all non-*Lythrum* species. *Lythrum salicaria* and all non-*Lythrum* species were oven dried separately at 80°C and weighed to the nearest 0.01 g.

Soil Analysis

Soil cores (15 cm deep × 8 cm diameter) were collected from the center of 4 of the 1.0 m^2 quadrats at each study site. Large roots were removed by hand. The samples were individually air dried, mixed to ensure uniformity, and sent to the Michigan State University Soil and Plant Nutrient Laboratory for analysis. Samples were analyzed for extractable calcium (Ca), magnesium (Mg), potassium (K), phosphorus (P), Nitrate (NO_3^-), and pH.

Biodiversity and Floristic Quality Assessment

The percent cover for each species within each 1.0 m^2 quadrat at all study sites was visually estimated. For consistency the same member of the research team made all estimates.

Percent cover for each plant species was used to calculate species richness (S), Shannon's Diversity Index (H'), Shannon-Weiner Evenness (E) and Simpsons Diversity Index (D) (Shannon and Weaver 1949; Simpson 1949) for each wetland type: uninvaded, invaded and managed, and invaded and unmanaged. All calculations were performed using PC-ORD for Windows 6.0 (McCune and Mefford 2011). One-way Analysis of Variance (ANOVA) was performed to determine significant differences in diversity parameters between the three wetland types. All ANOVAs were carried out via Analysis of Variance (ANOVA) Calculator One-Way ANOVA from Summary Data (Soper 2015).

A Floristic Quality Assessment (FQA) was performed for all quadrats at each of the six study sites to assess their natural significance. Analyzing FQA, mean C, and FQI averages for each quadrat can provide ecosystem information that is not measured by other diversity indices (Deboer et al. 2011). Percent cover for each species was used to obtain the Floristic Quality metrics, mean C value, Floristic Quality Index (FQI), mean wetness, relative frequency, and the total and percentage of native and non-native species in each sampled quadrat. The mean C, FQI, and mean wetness were calculated with and without non-native species including *L. salicaria*. All metrics were calculated for each quadrat independently and then averaged following Wilhelm and Masters (1999). Quadrat averages for mean C and FQI (especially for total species, native plus non-native) are believed to be a strong indicator of overall site quality (Taft et al. 2006; McIndoe et al. 2008). Analysis of the floristic data was performed using the *Floristic Quality Assessment Computer Program* version 1.0 (Wilhelm and Masters 1999). The metric for mean wetness were obtained from the Michigan FQA database and was included in our analysis because it indicates whether or not hydrophytic vegetation is frequent in the sampled quadrat.

Comparison of Uninvaded, Invaded and Managed, and Invaded and Unmanaged Study Sites

We used ordination techniques to examine species composition and environmental conditions between uninvaded, invaded and managed, and invaded and unmanaged wetlands. The species data set included percent cover for all species, including *L. salicaria.* The environmental variables included species richness, shoot biomass (for all species except *L. salicaria*), Shannon-Wiener index of diversity, Simpson's index of diversity, water depth, each measured soil nutrient, soil pH, and an additional numerical variable indicating wetland type. Species found in fewer than three sampled quadrats were excluded from the analysis to prevent distortion of the variance (McCune and Grace 2002).

Correspondence Analysis (CA) was performed for the species percent cover data because the technique allows for the simultaneous ordering of the sample units and the species based on the patterns of redundant co-occurrence in the species data set (McCune and Grace 2002; Palmer 2012). Conical correspondence analysis (CCA) was then used to examine the relationship between species percent cover and our measured environmental variables because it determines the linear combinations of environmental variables that best explain the patterns of variation in the species data (McCune and Grace 2002; Palmer 2012). Environmental variables were not transformed prior to analysis because CCA is not dependent on parametric distribution assumptions (McCune and Grace 2002; Palmer 2012). Species found in fewer than 3 quadrats were excluded from the analysis to prevent distortion of the variance (McCune and Grace 2002). All ordinations were performed using PC-ORD Multivariate Analysis of Ecological Data Version 6.0 (McCune and Mefford 2011).

Assessment of Gallerucella Beetles and their Effect on Lythrum salicaria Reproduction

Standardized monitoring protocols established by Blossey and Skinner (2000) were used to assess the abundance of *Gallerucella calmariensis* beetles in sites 3 and 4 at Pierce Cedar Creek Institute and measure their impact on the reproductive success of *Lythrum salicaria*. G*allerucella calmariensis* beetles were not present in the uninvaded sites 1 and 2 or the heavily invaded but unmanaged sites 5 and 6. Assessment of insects and plants occurred twice over the course of the research season. The first assessment occurred on June 1, 2015 as late-May/early June is when over-wintered adults emerge and begin feeding on *L. salicaria*. Within each 1.0 m^2 quadrat, beetle abundance was estimated using standardized counts in fixed time intervals. Counts were made by both researchers observing the sample quadrat from different sides. Counts of 1 minute were assigned to each life stage that could be observed: 1 minute for adults, 1 minute for larvae, and 1 minute for eggs. Beetle abundance was recorded using Blossey and Schat's (1997) seven abundance categories: Category 1 is 0 insects, Category 2 is 1 – 9 insects, Category 3 is 10 – 49 insects, Category 4 is 50 – 99 insects, Category 5 is 100 – 499 insects, Category 6 is 500 – 1000 insects, and Category 7 is >1000 insects. In addition to beetle abundance, feeding damage was also assessed in each 1.0 m^2 quadrat by recording the percent leaf area removed by beetle predation and the total number of *L. salicaria* stems >20 cm in height.

A second beetle assessment was performed in late July to determine the effect that *Galerucella calmariensis* had on the reproductive success of *Lythrum salicaria*. For this assessment we calculated *L. salicaria* percent cover, recorded the total number of *L. salicaria* stems >20 cm from all quadrats, and recorded the average height of the five tallest *L. salicaria* plants. For each of the five tallest stems we also recorded the average number of inflorescence spikes per plant, the average length of the terminal inflorescence, and the average number of flower buds per 5 cm from the center piece of the terminal inflorescence spike.

RESULTS

Mapping of Lythrum salicaria

GPS mapping showed that relatively dense populations of *Lythrum salicaria* cover approximately 209,814 m^2 (20.98 ha) of Institute property. In addition to these dense populations, an additional 404 individual plants were located (Fig. 1). *Lythrum salicaria* was found in 5 ecosystems at the Institute; 1) prairie fen, 2) upland prairie, 3) old field, 4) coniferous swamp woods, and 5) deciduous swamp woods. The largest areas and highest density populations of *L. salicaria* were located in the prairie fen habitats found on both sides of Cedar Creek and surrounding Brewster Lake (Fig. 1). Prairie habitat and deciduous swamp woods had less area covered by *L. salicaria*, and only a few individual plants were found in the coniferous swamp woods and the old field habitats.

Biodiversity and Floristic Quality Assessment

We found 27 plant species among the 48 sampled 1.0 m^2 quadrats from our three wetland types. Species richness (S), Shannon's Index (H'), and Simpson's Index (D), as well as Evenness (E) are reported for each of the three wetland types (Table 2). All measures of diversity were compared between the three wetland types using one-way ANOVA (Soper 2015). ANOVA indicated that there was significant difference between all wetland types (p = 0.000) when they were compared as a group. However, when pairs of wetland types were compared, the *Lythrum salicaria* uninvaded and *L. salicaria* invaded and managed sites were not significantly different for any of the measures of diversity (Table 2). In addition, the invaded and unmanaged site was not significantly different from either the uninvaded or invaded and managed sites with regard to Evenness (E). However, the invaded site was significantly different ($p = 0.000$) from both the uninvaded and invaded and managed sites for Species Richness, Shannon's and Simpson's diversity indices (Table 2).

Of the 27 plant species identified from our six study sites only 2 species (7.4%) were non-native (Table 1). In addition, 24 of the species (88.9%) are typically found in wetlands more than 67% of the time (OBL and FACW) and of these 18 species (66.7%) are obligate wetland species meaning that they are found in wetland habitats 99% of the time. *Typha latifolia*, the broad-leaf cattail, was the only plant species common to all wetland sites (Table 1).

The Floristic Quality Assessment (FQA) of our six study sites indicated that approximately 44% of our sampled plant species fell into the ruderal categories (C value = 0 – 4), whereas 56% were ranked as obligates to natural areas (C value = 5 – 10). Approximately 11% were either ranked as 0 or defaulted to 0 because they are non-native species, 37% were ranked with various affinities to ruderal areas, 33% were ranked as obligate to natural areas but the area was of low quality, 19% were ranked with various affinities to high-quality areas, and none of our sampled species were ranked as obligates to high-quality natural areas. The C value for each species is given in Table 1.

The mean C values for all native species across our 6 study sites ranged from 3.2 – 4 indicating ruderal or disturbed habitat (Figure 2). The greater the mean C and FQI values the higher the floristic quality of an area. FQI represents a weighted estimate of species richness which allows for a more meaningful comparison of different areas that may have the same mean C value. Our FQI values across our 6 study sites ranged from 7.2 – 12.3 (Figure 3).

Comparison of Uninvaded, Invaded and Managed, and Invaded and Unmanaged Study Sites

Wetland types contained a range of plant biomass for both *Lythrum salicaria* and all non-*Lythrum* species combined (Table 3). ANOVA found significant difference between all possible pairs for wetland types for both *Lythrum* biomass and non-*Lythrum* biomass at $\alpha = 0.05$ ($p = 0.01$ or lower).

Correspondence Analysis (CA) resulted in a three-dimensional solution representing the strongest compositional gradients (Fig. 4a). Axis 1 accounted for 28.2% of the variation in the species dataset, axis 2 accounted for 22.3% of the variation, and axis 3 10.18%. *Lysimachia terrestris* (L.) Britton, Sterns and Poggenb. *Sagittaria latifolia* Willd. and *L. salicaria* were positively correlated with axis 1 (r = 1.16, 0.93, 0.96 respectively) and axis 2 (r = 0.95, 0.81, and 0.27 respectively) (Fig. 4a).

Conical Correspondence Analysis (CCA) also resulted in a three-dimensional solution with axis 1, 2 and 3 accounting for 27.3%, 21.2% and 6.1% of the variation in the dataset, respectively (Fig 4 B, C). The significant environmental variables with r values greater than positive or negative 0.5 on axis 1 were phosphorus (r = 0.610), potassium (r = - 0.758), calcium (r = - 0.815), magnesium (r = - 0.879), water depth (r = 0.707), non-*Lythrum* biomass (r = - 0.630), Shannon's Diversity (r = - 0.615), Simpson's Diversity (r = - 0.651), and species richness (r = - 0.604). Additionally, wetland type (Invaded [r = 0.708] and Uninvaded [r = - 0.584]) were strong predictors of species density along axis 1 (Table 4). Soil pH and nitrogen were strong predictors of species density along axis 2 (Fig. 4B; Table 4). The managed wetland type was correlated with axis 3 (r = 0.652) (Fig. 4C; Table 4). Our uninvaded site 1 (quadrats UN1A – H) and managed site 3 (quadrats MG3A-H) showed strong associations in ordination space as they shared 7 species in common. Likewise, uninvaded site 2 (quadrats UN2A – H) and managed site

4 (quadrats MG4A – H) showed strong associations as they shared 6 species in common (Fig. 4B; Table 1). Invaded and uninvaded sites were strongly separated along the first axis and less so along the second axis. All of the invaded and unmanaged wetlands (quadrats HI5A – H and HI6A – H) grouped tightly together in ordination space and were positively correlated with axis 1 and to some degree axis 2. The uninvaded sites were all negatively correlated with axis 1 and to some degree axis 2 while the managed sites were negatively correlated with axis 1 and positively correlated with axis 3 (Fig. 4B, C).

Assessment of Gallerucella Beetles and their Effect on Purple Loosestrife Reproduction

The *Gallerucella calmariensis* beetle data represents baseline data. All monitoring data is presented in the seven abundance categories established by Blossey and Schat (1997) in their standardized protocol for monitoring the effectiveness of the beetle in controlling the spread of *Lythrum salicaria*. Our spring monitoring of sites 3 and 4 resulted in category 3 (10 – 49) to category 5 (100 – 499) egg counts with the exception of quadrat MG4D which had a category 1 egg count (0 eggs) (Fig. 5A and B). This resulted from a lack of *Lythrum salicaria* in that quadrat. There were many fewer adult beetles with most quadrats having abundance categories of either 1 (0 insects) or 2 (1 – 9 insects). In only 3 of the 16 quadrats at the two managed sites were adults observed as category 3 (10 – 49 insects). Larvae followed a similar trend as the adult beetles (Fig.5A and B). The actual number of insects counted at each life stage was greater at site 3 than site 4 (Table 5). Site 3 was the approximate location of a past beetle release. The percent leaf area removed from *L. salicaria* by *G. calmariensis* ranged from 5 – 30% in quadrats found to contain *L. salicaria* (Table 6). No beetles were observed at the invaded and unmanaged sites 5 and 6 located at Justin's Marsh.

Late summer monitoring of *Lythrum salicaria* reproductive success took place at sites 3 and 4 on Institute property on July 29, 2015 and at sites 5 and 6 at Justin's Marsh on July 31, 2015. ANOVA indicated that the total number of stems >20 cm produced at Justin's Marsh was significantly greater than the number of stems produced at the managed sites on Institute property (p = 0.000) (Table 6). Similarly the total number of inflorescences produced at the unmanaged sites was significantly greater than the number produce at the managed sites (p = 0.000) (Table 6). In addition to the total number of stems and total number of inflorescences, we also averaged the number of flower buds for the 5 tallest plants. In the managed sites 3 and 4 only 7 of 16 quadrats had flowering plants and the average number of buds in these quadrats ranged from 7 – 16. In comparison, all 16 quadrats at the unmanaged sites 5 and 6 had flowering stems and the average number of buds in these quadrats ranged from 11.2 – 37.2. The total number of buds at each site is given in Table 6.

DISCUSSION

Lythrum salicaria is classified as an obligate wetland hydrophyte, indicating that it almost always occurs in wetland habitats (USDA 2015). Therefore, we were surprised to find that there were a considerable number of *Lythrum salicaria* individuals in the upland prairies at the Institute and that the plant was also located, although in smaller numbers, in the old fields. This may be due to small areas of hydric soils occurring within these habitats. Because we surveyed the plants in these habitats in late July, when they were blooming, areas of hydric soil in upland habitats would not have been readily observed. A comparison between our *L. salicaria* distribution map and the soils map in the Institute's GIS system could confirm this.

Pierce Cedar Creek Institute has employed several land management strategies to control the spread of *Lythrum salicaria*. These include periodic controlled burns, mechanical removal, and biological control through the introduction *Galerucella calmariensis* beetles. As expected, species richness and diversity were higher in sites that were uninvaded by *Lythrum salicaria* than in sites that were heavily invaded. We were pleased to discover that species richness and diversity were higher, although not significantly so, in the managed areas of Institute property indicating that the Institute's management practices are effectively maintaining diversity at approximately the same level as in areas that have not been invaded by *L. salicaria*. In addition, correspondence analysis grouped uninvaded and managed sites together due to similarities in species composition and their similar responses to environmental variables. The heavily invaded and unmanaged sites formed a group that was removed from the uninvaded and managed sites. The heavily invaded sites where associated with increased water depth. This suggests that the land managers at Pierce Cedar Creek Institute should regularly monitor those areas of Institute property that have greater water depth as *L. salicaria* may be more likely to spread into those areas.

Our Floristic Quality Assessment of four sites on Institute property (sites 1, 2, 3, and 4) indicated that a relatively large number (37%) of the sampled wetland species had affinities to ruderal habitats. The remaining species had affinities to natural areas but the mean C values for those areas ranged from 3 – 4 indicating that the natural areas were of low quality. This may be a consequence of the relatively recent history of disturbance on the property. It will be interesting to see if this changes over time in response to the Institutes land management practices.

Lastly, our data clearly indicate that *Galerucella calmariensis* is effectively controlling the growth and reproduction of *Lythrum salicaria* on Institute property as the number of stems, inflorescences and flower buds are far fewer than in those sites that were unmanaged. We were also able to find evidence of beetle activity across institute property indicating that the beetles have spread from the sites of their initial release. Based on the apparent effectiveness of the beetle, we recommend that the Institute release additional beetles in various wetlands across the property.

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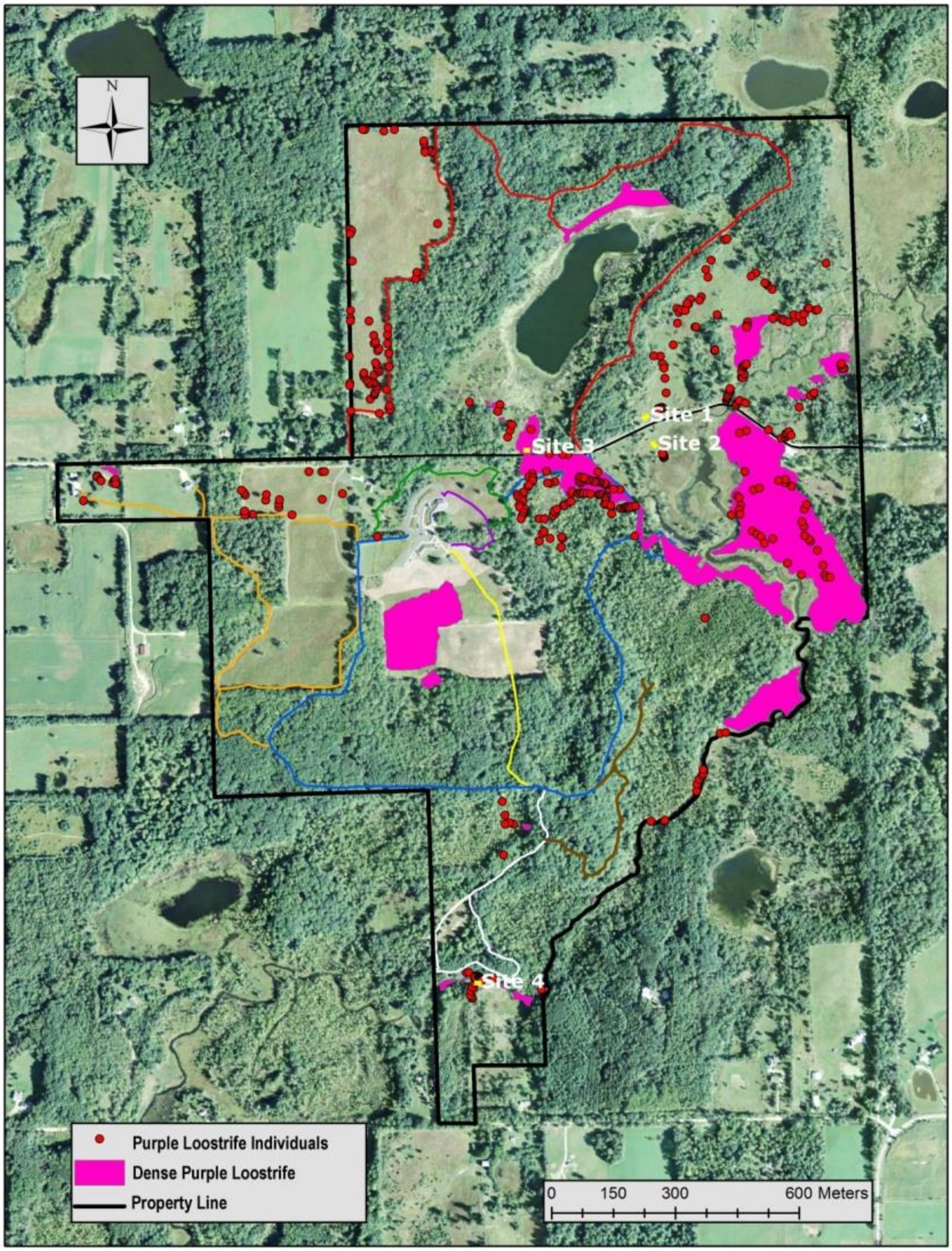

Figure 1. Map of Pierce Cedar Creek Institute property showing locations of study sites. Sites 1 and 2 were uninvaded by *Lythrum salicaria* and sites 3 and 4 were invaded by *L. salicaria* but managed (see text for management practices). Also indicated are the locations of dense stands and individuals of *L. salicaria.*

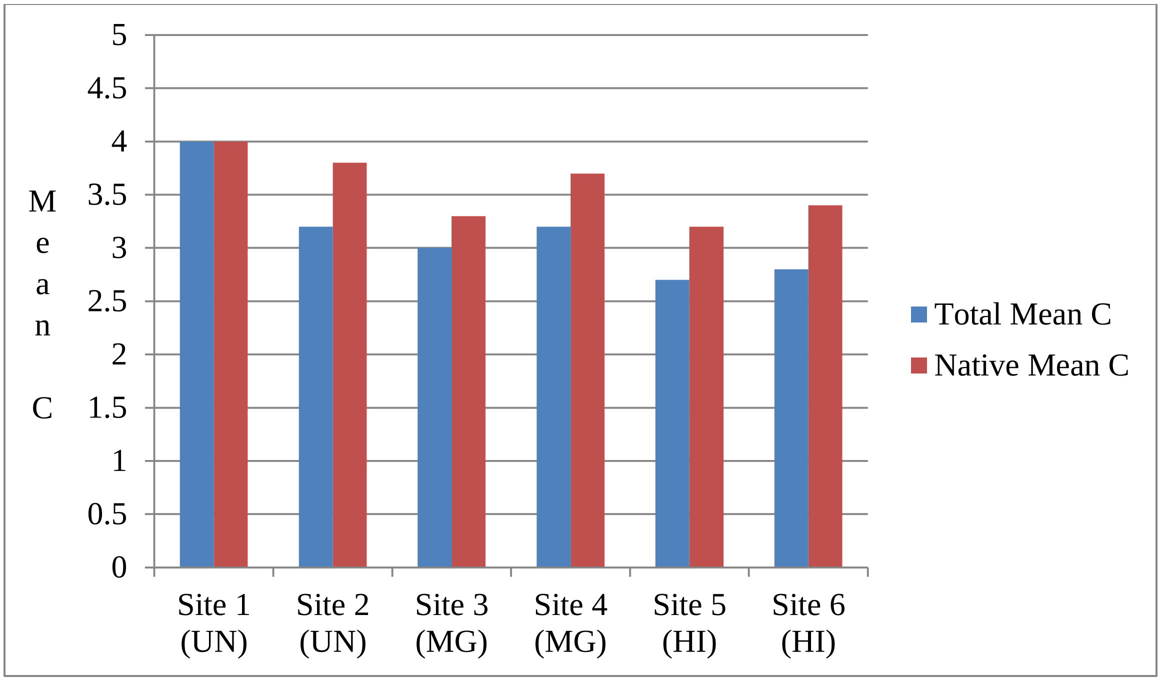

Figure 2. Total and Native Mean C for all sites. UN = uninvaded, MG = invaded and managed, HI = invaded and unmanaged. Sites 1 – 4 were located at Pierce Cedar Creek Institute; sites 5 and 6 were located at Justin's Marsh.

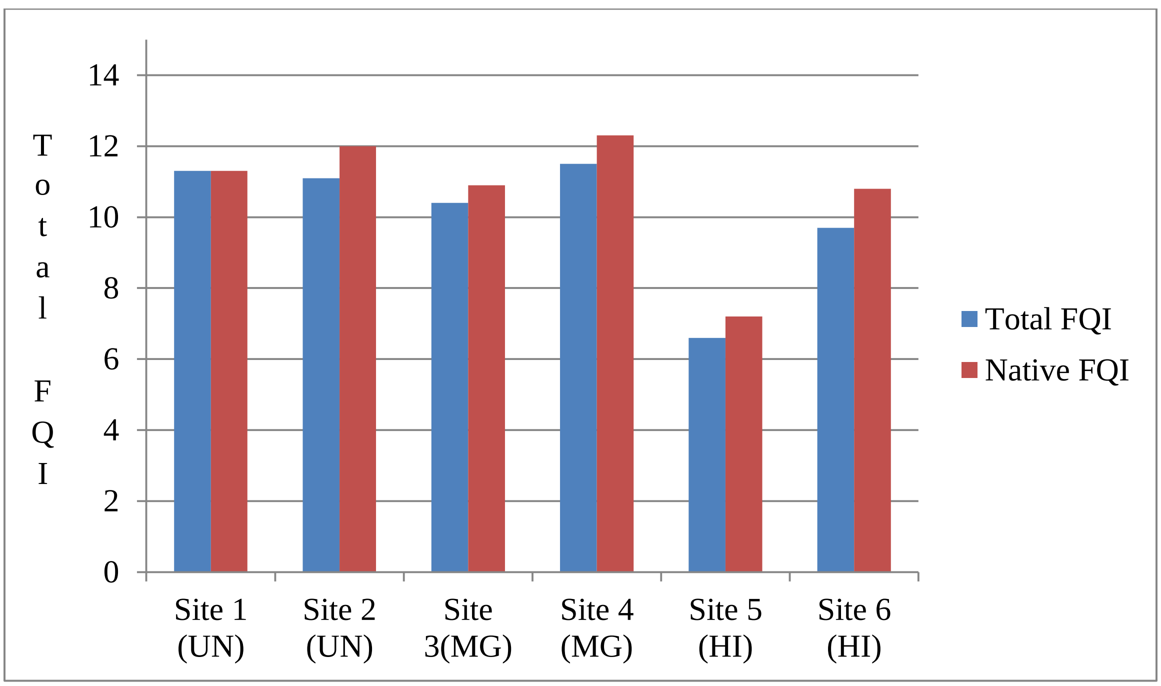

Figure 3. Total and Native FQI for all sites. UN = uninvaded, MG = invaded and managed, HI = invaded and unmanaged. Sites 1 – 4 were located at Pierce Cedar Creek Institute; sites 5 and 6 were located at Justin's Marsh.

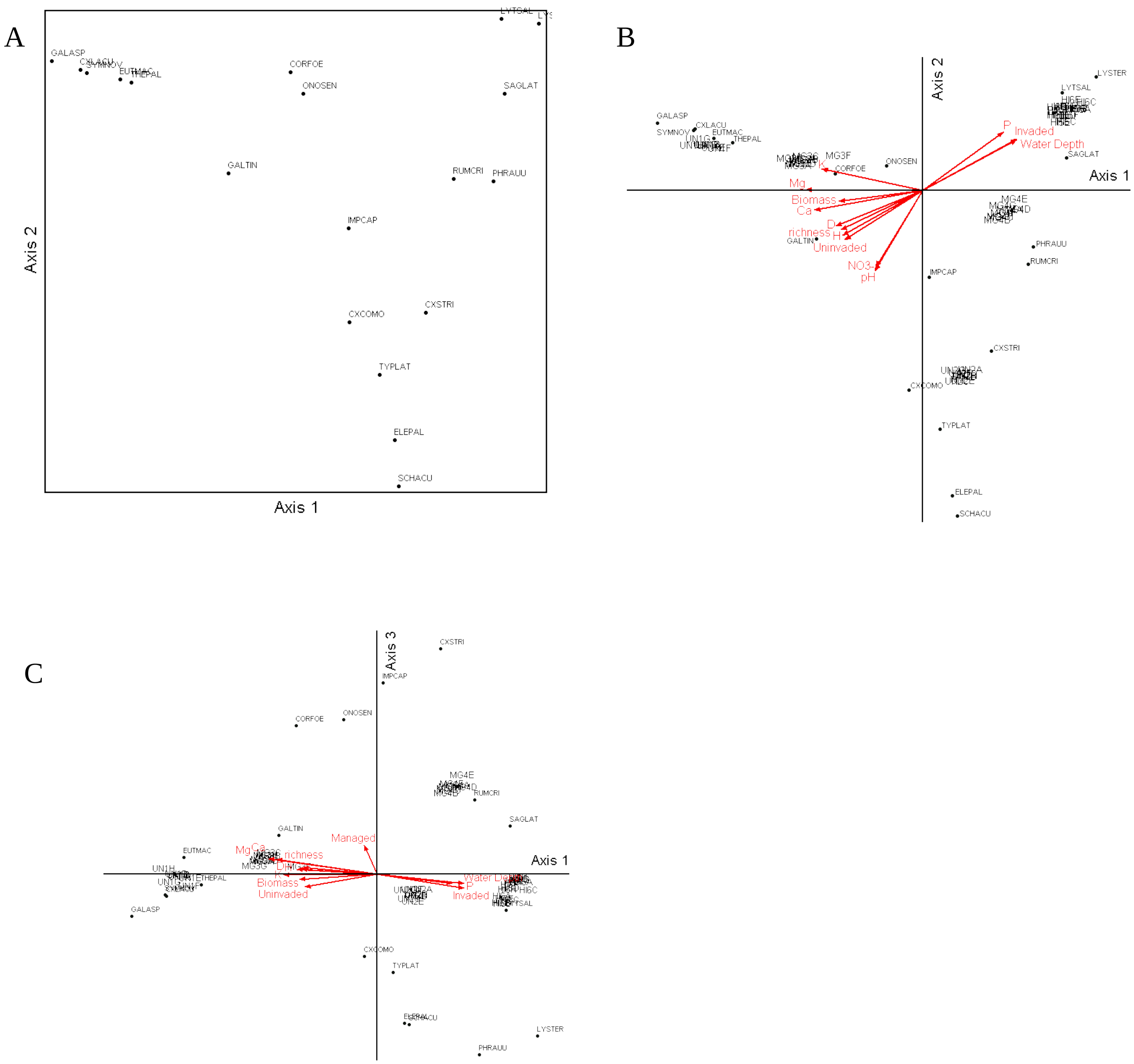

Figure 4. Scatterplot based on (A) Correspondence Analysis of species percent cover, and ordination biplots (B) and (C) based on canonical correspondence analysis of species densities with respect to environmental variables and patch invasion status. In (A), axes 1 and 2 accounted for 28.2% and 22.3% of the variation in the data respectively. In (B), axes 1 and 2 accounted for 27.3% and 21.2% of the variation in the data, respectively and in (C), axes 1 and 3 accounted for 27.3% and 6.1% of the variation, respectively.

A

B

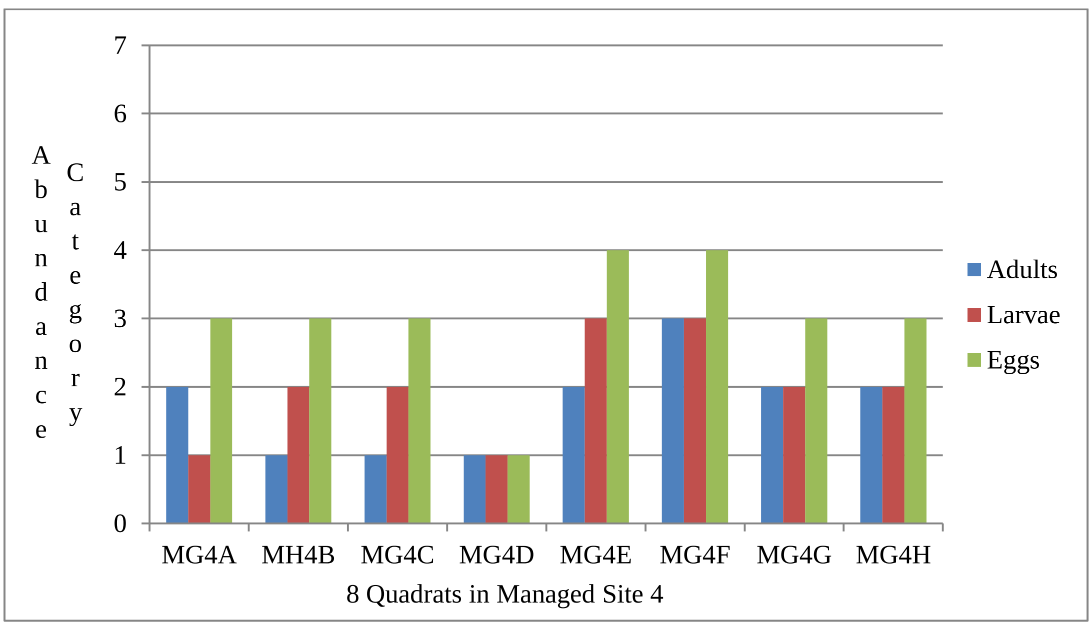

Figure 5. Spring abundance counts for managed site 3 (A) and managed site 4 (B). All counts were made on June 1, 2015. Abundance categories: 1 = 0 insects, 2 = 1 – 9 insects, 3 = 10 – 49 insects, 4 = 50 – 99 insects, 5= 100 – 499 insects, 6 = 500 – 1000 insects, and 7 >1000 insects.

Table 1. Species list indicating the family of each species. Code is our species abbreviation used in ordination . An X indicates the species was present in at least one of the quadrats at a study site. Physiognomy (habit/growth form) abbreviations are as follows: AD = Adventive (non-native) taxa, NT = Native taxa, A = annual, P = perennial, W = woody. C-value is the Coefficient of Conservatism used in determining the floristic quality of a site; * = non-native taxa default to 0 value in FQI calculations. Wet = Michigan wetland indicator status and W = the species wetness coefficient (Reznicek et al. 2014).

Species	**Code**	**Site 1**	**Site 2**	**Site 3**	**Site 4**	**Site5**	**Site 6**	**Physiognomy**	**C-value**	**Wet / W**
Bidens connata	BIDCON					X		NT – A – Forb	5	FACW / -3
Cornus foemina	CORFOE			X	X			NT – Shrub	1	FAC / 0
Carex comosa	CXCOMO		X	X				NT – P – Sedge	5	OBL / -5
Carex lacustris	CXLACU	X		X				NT – P – Sedge	6	OBL / -5
Carex stricta	CXSTRI		X		X			NT – P – Sedge	4	OBL / -5
Eleocharis palustris	ELEPAL		X	X				NT – P – Sedge	5	OBL / -5
Eutrochium maculatum	EUTMAC	X		X	X			NT – P – Forb	4	OBL / -5
Galium asprellum	GALASP	X						NT – P – Vine	5	OBL / -5
Galium tinctorium	GALTIN	X	X	X	X			NT – P – Forb	5	OBL / -5
Geum rivale	GEURIV				X			NT – P – Forb	7	OBL / -5
Impatiens capensis	IMPCAP		X	X	X			NT – A – Forb	2	FACW / -3
Lysimachia terrestris	LYSTER					X	X	NT – P – Forb	6	OBL / -5
Lysimachia thyrsiflora	LYSTHY						X	NT – P – Forb	6	OBL / -5
Lythrum salicaria	LYTSAL			X	X	X	X	AD – P – Forb	*	OBL / -5
Onoclea sensibilis	ONOSEN			X	X		X	NT – Fern	2	FACW / -3
Phalaris arundinacea	PHRAUU		X			X	X	NT – P – Grass	0	FACW / -3
Rosa palustris	ROSPAL				X		X	NT – Shrub	5	OBL / -5
Rumex crispus	RUMCRI		X		X		X	AD – P – Forb	*	FAC / 0
Sagittaria latifolia	SAGLAT		X		X	X	X	NT – P – Forb	4	OBL / -5
Schoenoplectus acutus	SCHACU		X					NT – P – Sedge	5	OBL / -5
Scutellaria lateriflora	SCULAT						X	NT – P – Forb	5	OBL / -5
Sparganium eurycarpum	SPAEUR		X					NT – P – Forb	5	OBL / -5
Symplocarpus foetidus	SYMFOE	X			X			NT – P – Forb	6	OBL / -5
Symphyotrichum novae-angliae	SYMNOV	X		X				NT – P – Forb	3	FACW / -3
Thelypteris palustris	THEPAL	X	X	X			X	NT – Fern	2	FACW / -3
Typha latifolia	TYPLAT	X	X	X	X	X	X	NT – P – Forb	1	OBL / -5
Vitis riparia	VITRIP						X	NT – W – Vine	3	FAC / 0

Table 2. Mean (±SD) and significance for species richness, species evenness, Shannon's index of diversity, and Simpson's index of diversity for 16 quadrats in each of three wetland types: *Lythrum salicaria* uninvaded, invaded and managed, invaded and unmanaged. n = number of species. Analyzed together the three wetland types differed significantly for each of the measures of diversity ($\alpha = 0.05$; $p = 0.01$ or lower). When all possible pairs of strata were analyzed those within a column with the same superscript did not differ significantly at $\alpha = 0.05$.

Wetland Type	n	Species Richness	Evenness (E)	Shannon's Index (H')	Simpson's Index (D)
Uninvaded	17	5.9 (±1.09)[a]	0.868 (±0.071)[a,b]	1.535 (±0.216)[a]	0.732 (±0.076)[a]
Managed	18	6.8 (±1.82)[a]	0.872 (±0.072)[a,c]	1.644 (±0.336)[a]	0.750 (±0.097)[a]
Invaded	13	2.5 (±1.17)	0.667 (±0.319)[b,c]	0.605 (±0.330)	0.367 (±0.193)

Table 3. Biomass in three wetland types (n = 16 quadrats each), *Lythrum salicaria* uninvaded, *L. salicaria* invaded and managed, and *L. salicaria* invaded and unmanaged. (mean (±SD), range) The three wetland types differed significantly for *Lythrum* and non-*Lythrum* biomass ($\alpha = 0.05$; $p = 0.01$ or lower).

Wetland Type	*Lythrum* biomass (g/m^2)	Non-*Lythrum* biomass (g/m^2)
Uninvaded	0.0	354.44 (±158.87), 101-725
Managed	35.63 (±39.38), 0-163	190.81 (±154.25), 88-755
Invaded	293.56 (±139.51), 131-658	84.50 (±90.0), 1-363

Table 4. Correlations of environmental variable with axes calculated in conical correspondence analysis.

Variable	Axis 1	Axis 2	Axis 3
Uninvaded	-0.584	-0.524	-0.293
Managed	-0.103	0.005	0.652
Invaded	0.708	0.539	-0.336
pH	-0.352	-0.810	-0.022
P	0.610	0.619	-0.216
K	-0.758	0.232	-0.024
Ca	-0.815	-0.204	0.331
Mg	-0.879	0.007	0.350
NO3-	-0.359	-0.850	-0.198
Water Depth	0.707	0.544	-0.222
Biomass	-0.630	-0.112	-0.129
H	-0.615	-0.417	0.106
D	-0.651	-0.378	0.106
Richness	-0.604	-0.474	0.146

Table 5. Total number of individuals counted in each life stage (adult, larvae, egg) during the spring abundance count for managed sites 3 and 4. All counts were made on June 1, 2015.

Wetland Type	Eggs	Larvae	Adults
Uninvaded	0	0	0
Managed	797	316	62
Invaded	0	0	0

Table 6. Effects of *Gallerucella calmariensis* feeding on *Lythrum salicaria* growth and reproduction. Observations of % leaf area removed by larvae feeding were made on June 1, 2015 and observations of reproductive success were made on July 29th and 31st. The number of inflorescences and number of buds are totals of the averages of the 5 tallest stems per quadrat.

Wetland Type	Site	% leaf area removed	Number of Stems >20 cm	Number of Inflorescences	Number of Buds
Uninvaded	UN1	0	0	0	0
	UN2	0	0	0	0
Managed	MG3	10	99	49	59
	MG4	10	126	48	22.6
Invaded	HI5	0	359	427	187.6
	HI6	0	328	547	179.5