## Native Vegetation Reestablishment following herbicide treatment of Japanese knotweed and common reed grass

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## **Project Overview**

The Adirondack Park Invasive Plant Program (APIPP) actively manages invasive plant populations in the Adirondacks, including Japanese knotweed (*Reynoutria japonica*) and common reed grass (*Phragmites australis*). The Native Vegetation Reestablishment Project was designed to assess whether sites managed with herbicides to control these two invasive species resulted in non-invasive plant community structures that resemble plant communities in their natural, uninvaded states. APIPP contracted with Adirondack Research to conduct field work in summer 2021 and to write this report. The project results, described in more detail below, indicate that the chemical treatment of invasive species is successful in enabling sites to passively restore to a natural, uninvaded state.

## **Research Summary**

Species richness (number of species present) and density (as measured by total percent ground cover of non-invasive plants, percent bare ground, or average density of the top five, most-common, non-invasive species) of roadside herbicide treatment sites of 35 Japanese knotweed and 36 common reed grass infestations in the Adirondack Park were assessed to determine if successful passive restoration is occurring after the conclusion of treatment.

These infestations had been chemically treated by APIPP's early detection and rapid response (EDRR) teams between the years of 2010 and 2020 to remove the target invasive species. Invasive plant treatment areas ranged in maximum assessment size from about 1 square foot (roughly a couple of plant stems) to roughly 4,828 square feet (0.11 acres) with an average maximum size of 813 square feet or 0.02 acres. Data were collected at 71 locations with both treatment sites and reference sites.

There were over 300 different non-invasive species identified across all sites where data were collected, none of which was present at all sites. Data analysis showed the following.

- Species richness was not significantly different between treatment and reference sites, although it was marginally affected by time since last management for Japanese knotweed.
- Percent ground cover of non-invasive plants did not differ significantly between treatment and reference sites, nor was it affected by time since final chemical treatment (referred to as NPO or number of years with no invasive plants observed).
- Percent bare ground did not differ significantly between treatment and reference sites, nor was it affected by NPO. However, there are some suggestive differences between the first two NPO categories (0 and 1-2 years NPO) in the percent bare ground as well as the percent ground cover of non-invasive plants in common reed grass sites.
- Lastly, the average density of the top five most common non-invasive species across Japanese knotweed or common reed grass sites did not differ significantly between treatment and reference sites, nor was it affected by NPO.

In summary, sites are successfully passively restoring during and after treatment.

Even sites that have not yet reached NPO status (zero years NPO) and may still have the target invasive plant present are statistically similar to nearby reference sites that, to APIPP's knowledge, have never had the target invasive plant present nor have they ever been treated chemically to remove this plant. The one possible exception to this is that the percent of bare ground did have a suggestive difference between the 0-year and 1-2-year NPO categories with treatment sites in the 0-year NPO category having a higher percent of bare ground present. This is not a surprise as sites in this category may have still been under active management throughout the duration of this study and the use of herbicide in the recent past may result in a higher percent bare ground.

The results of this study, in combination with APIPP's records, suggest that the size and density of chemically treated sites decreases overtime, allowing native species to passively restore the infested area even while treatments are still taking place on the subsequently reduced population of target species. Therefore, sites quickly reach a condition that is statistically similar to nearby reference sites shortly after treatment is complete. The results of this study indicate that the chemical treatment of invasive species is successful in enabling sites to passively restore to a natural, uninvaded state.

In its tenure APIPP has successfully eradicated (NPO for 3 or more years), 98 (29% of historically treated) Japanese knotweed and 322 (41% of historically treated) common reed grass sites. Through APIPP's efforts these site have been successfully restored to native cover.

## Introduction

APIPP has been actively treating and monitoring invasive plant populations in the Adirondacks, including Japanese knotweed (*Reynoutria japonica*) and common reed grass (*Phragmites australis*) for over a decade. Following years of successful control of these invasive species at multiple locations, APIPP saw the need to assess whether sites managed by eliminating invasive species with the use of herbicides resulted in non-invasive plant community structures that resemble plant communities in their natural, uninvaded states. APIPP contracted with Adirondack Research in summer 2021 to complete the field work and analysis described below.

Each year, APIPP contracts with an early detection and rapid response (EDRR) team to visit, revisit, assess and perform treatments (both chemical and manual control) of priority terrestrial invasive species infestations within the Adirondack Partnership for Regional Invasive Species Management (PRISM). The New York State Department of Environmental Conservation has established eight PRISMs throughout the state with the goal of combating both terrestrial and aquatic invasive species. APIPP serves as the Adirondack PRISM and as such is responsible for planning regional invasive species management.

Invasive species in New York State are categorized into tiers according to a standard state-wide system. Within these tiers, species rank from Tiers 1 (Early Detection) through 4 (Suppression) with Tier 1 species being those that have not yet reached the PRISM and Tier 4 species being those that are so well established that they cannot be eradicated from the PRISM. A Tier 5 also exists for species that require more research and mapping. The EDRR team focuses its time on surveying and treating species in Tiers 2 through 4, which include both Japanese knotweed and common reed grass as focus species. Both species are considered Tier 4 and are widespread and

established throughout the PRISM. Therefore, APIPP's management of these species is focused on suppression to protect high-priority resources. Because APIPP is managing these two species for suppression, its management and reporting metrics and analyses are currently focused primarily on showing its ability to suppress and prevent known populations of these species from spreading.

As part of APIPP's ongoing data collection from its EDRR team, APIPP has successfully measured reduced post-treatment regrowth of invasives to demonstrate the efficacy of current invasive species management methodology. To show success of these treatments on native ecosystems, however, APIPP saw the need to evaluate re-establishment success of non-invasive plants at historic treatment sites. This is important because, while control efficacy shows how well APIPP's EDRR team can control the growth and spread of an invasive species, it provides less information about how these management efforts influence a community's native species richness and density (as measured by total percent ground cover of non-invasive plants, percent bare ground, or average density of the top five most common non-invasive species) as compared to nearby natural communities that have not been infested and subsequently treated.

The objective of this study was to assess native, non-native, and invasive plant richness and density at the original treatment sites in comparison to plant richness and density found in the surrounding similar habitat (reference sites). In total, 71 sites, split almost evenly between Japanese knotweed and common reed grass, were surveyed in summer 2021.

The approach to assessing the effects of invasive species management on plant re-establishment took into consideration several factors that were predicted, *a priori*, would affect non-invasive plant re-colonization and re-growth. These included original assessed infestation size, number of years of chemical treatment, pre-treatment weed density, and the species of invasive plant treated (Japanese knotweed or common reed grass). While it is suspected that the above factors play a role in the re-establishment of native plants, the goal of this study was more concerned with how sites differed based on time since final chemical treatment (referred to as years of NPO).

Note that two sites may have both had initial chemical treatments in the same year, but one may have been managed annually for the majority of that time and the other may have been NPO for the majority of that time. As a result, these two sites would have had different lengths of time to recover after the conclusion of chemical treatments. Due to these *a priori* assumptions, the study was designed to test for the effects of NPO (time since final treatment) on key attributes of plant re-establishment (see below). Sites were randomly selected and were evenly distributed with respect to the variables mentioned above, which were known to add variation to the explanatory variables.

There are three key questions that were examined:

- 1. Is there a significant difference in **species richness (number of species)** between treatment and reference sites, or over time as categorized by time, in years of NPO?
- 2. Is there a significant difference in the **percent ground cover of all non-invasive plants** or **percent bare ground** between treatment and reference sites, or over time as categorized by time, in years of NPO?

3. Is there a significant difference in the **average density of the five most common noninvasive species** found in sites for each of the two managed invasive plant species between treatment and reference sites, or over time as categorized by time, in years of NPO?

## Methods

## **Experimental Design**

The experimental design consisted of 35 Japanese knotweed and 36 common reed grass sites for a total of 71 sites. Each site was comprised of the historic treatment site survey area, as well as a reference site of similar habitat and nearby location that was not previously infested with the treated invasive plant species. Initial site selection was based on a set of guiding parameters including having been treated with herbicide to remove either Japanese knotweed or common reed grass within the past 11 years.

## **Site Selection**

Sites were randomly selected from a pool of historic Japanese knotweed and common reed grass sites managed by APIPP between the years 2010 and 2020. A distribution analysis was performed to determine the size classes of the treatment sites. Sites were selected so that each categorical group (independent variable), which consisted of a range of years since final chemical treatment took place and where no invasive plants were observed at those sites (NPO), had an even distribution of other variables that were suspected would cause variation in Adirondack Research's analysis. These included original assessed infestation size, number of years of chemical treatment, and pre-treatment weed density. Available data on past treatments was used to determine the best NPO category ranges for each invasive species independently. After determining the best NPO ranges, sites were selected randomly while purposefully keeping the distribution of other attributes even within each of the NPO classes.

Treatment site locations were selected and then marked with a PVC pipe labeled with the site number and inserted into the ground with 3-4 inches protruding above the soil. PVC pipes were placed roughly at the center of the original assessment polygon.

Once a treatment site was located in the field, a reference site was located. Reference sites were selected after locating the treatment site survey area, but before performing vegetation surveys. Reference sites were used for paired comparisons with treatment sites (Tukey's Honest Significant Difference, Tukey's HSD) following any significant or suggestive analysis of variance (ANOVA) results. (An ANOVA is used to determine if there are differences between the means of three or more groups, but it cannot determine where those differences are. A Tukey's HSD is a post-hoc test that can determine which group's mean is different and, therefore, where the difference occurs in the data.) These untreated reference sites were as ecologically similar as possible to treated sites but had not, to Adirondack Research's knowledge, been colonized by either of these invasive species. The reference sites had not received chemical or mechanical invasive species treatments and represent the best examples of similar pre-infestation natural habitats to use as pairwise comparisons to each treatment site.

Since invasive plants can have a large influence on the compositional integrity as well as geomorphological characteristics (Gordon 1998), a nearby area was considered to be similar enough to serve as a reference site if it was in the same geographic area, had similar slope and aspect, similar drainage, and had an overstory consistent with the treatment site.

#### Reference sites were selected based on the following criteria:

- 1. Similar general geographic area as treatment site (ideally adjacent to)
- 2. Similar drainage
- 3. Similar overstory
- 4. Similar slope and aspect
- 5. Similar distance from roads or other manmade or natural objects

## **Field Methods**

The monitoring protocol was designed with three main goals. The first goal was to create a list of all species occurring at each site, identified to species where possible. The second goal was to record a measure of density across all species identified at each site. The third goal was to make comparisons of species occurrence and density at sites where Japanese knotweed and common reed grass were managed to sites that have not been treated or ever had these target species present.

Species occurrence and density were recorded at each of 35 historic Japanese knotweed and 36 common reed grass treatment sites as well as at an equal number of paired reference sites in summer 2021. To keep sampling of each associated treatment and reference site to within one hour combined, grasses and some other difficult to identify species were identified only to the Genera taxon level. Survey time for each site was kept within an hour to make the entire study cost effective and manageable. Based on a similar study APIPP commissioned in 2015(available upon request), it is believed that this was an appropriate amount of time to cover each site with enough attention to detail to get usable and consistent results. Furthermore, plant observations were made at the walking and crouching level without the use of transects or grids.

The goal from a plant identification and abundance point of view was to record as many plant species as possible within about 30 minutes per site. Because time-constrained searches could result in a directional bias toward reduced diversity in larger, more species-rich sites, each treatment and corresponding reference sites was searched for between 10-40 minutes, depending on the size of the site, to observe most apparent species across all sites (Blossey, Jongejons, personal correspondence).

To account for the different effort required to survey larger sites, in site sizes over 0.05 acres, surveys were limited to 2500 square feet. This allowed for consistency in survey time across sites of varying size. For these larger sites, surveys were performed within the constrained 2500 square foot area and that area was centered 2/3 between the center and the outer boundary of the originally assessed infestation (closer to the outer edge than the center).

Three main methods of quantifying plant distribution were employed. This included the Braun-Blanquet scores for each identified species within each site, as well as an estimate of total plant cover and total bare ground as percentage estimates. The Braun-Blanquet scores are categorical estimates of plant cover that are an efficient alternative to more time-consuming stem counts. Using the Braun-Blanquet scale, species with 1-5% cover are assigned to category 1, 5-25% cover to category 2, 25-50% cover to category 3, 50-75% cover to category 4, and 75-95% cover to category 5, and greater than 95 to category 6.

While in the field, data was collected data as numerical values rounded to the nearest 1% for values under 5%, to the nearest 5% for values under 20% and to the nearest 10% for values above 20%. These values were collected so that they could be translated to Braun Blanquet scores, but for purposes of performing ANOVAs, the rounded numerical percentage values were used rather than translations to Braun-Blanquet scores.



FIGURE 1. BRAUN BLANQUET SCALE SHOWING CATEGORICAL VALUES RELATED TO PERCENT COVER ESTIMATES FOR PLANT GROUND COVER.

#### The protocol consisted of the steps outlined below.

- 1. Find treatment site and visualize initial survey boundary.
- 2. Record date, time, general location, and site reference number.
- 3. Place survey marker (PVC pipe) at center of site.
- 4. Select reference site and confirm that both treatment and reference site meet selection criteria.
- 5. Record location at the survey marker for each treatment and reference site.
- 6. Record size of area being surveyed (entire site for sites <2500 square feet, up to 2,500 square feet for sites larger than 2,500 square feet).
- 7. Take photo of site (eye level).
- 8. Take photo of ground cover (65-degree downward angle over representative vegetation of site) to estimate total vegetation cover at site.
- 9. Walk within site boundary and record all plants. For each plant, record Braun-Blanquet scale (1-6) for abundance.
- 10. Estimate total ground cover.
- 11. Estimate total bare ground.
- 12. Repeat steps 3-11 for reference site.
- 13. Save data and proceed to next site.

## **Data Collection**

All field data were collected using a combination of the Survey123 for ArcGIS on iPad tablets along with a paper record of plant species. Paper records were entered into a database after returning from the field at the end of each day. Paper was chosen for recording plant species because of the ease and relative speed of data entry as well as the ability to easily amend records (e.g. when abundance values change upon further discovery). A Bluetooth connected auxiliary GPS receiver (Bad Elf GNSS Surveyor, West Hartford, CT) was used for the tablets as a way to increase location precision and accuracy and to maintain similar accuracy across tablet and smartphone devices.

## **Data Analyses**

Plant lists were compiled for each of the 71 treatment sites as well as for 71 associated reference sites. Each species occurrence was recorded along with that species distribution across each site as estimated using the Braun-Blanquet Scale (Braun-Blanquet, 1932), but recorded in the field as percentage cover rounded to the nearest 5% and 10% as described above for use in ANOVA. Species counts were totaled for each site to assess the number of species between treated sites and reference sites.

Data were examined with the intent of answering the question; Are non-invasive plants growing back after invasive species treatment so as to create ecosystems that resemble nearby plant communities in their uninvaded states?

There were over 300 unique species identified across all of the sites where data were collected, and none of these species was present at every site. Due to the substantial diversity and inconsistency in species composition among sites, multivariate analyses such as Principal Components Analysis were not feasible. The nature of the data, however, did lend itself well to an ANOVA, followed by pairwise comparisons when warranted by statistically significant differences.

These data were analyzed in a similar manner to previous work done in 2015. ANOVAs were developed for four separate responses:

- 1. Species Richness
- 2. Percent Ground Cover
- 3. Percent Bare Ground
- 4. Average Density of Five Most Prevalent Non-Invasive Species at Japanese Knotweed and Common Reed grass Sites

For each of these, the Normality assumption was met by using the untransformed response, or a square root or log transformation.

The ANOVAs examined for each of these four responses were:

- 1. Difference between Treatment and Reference sites
- 2. Difference between NPO categories
- 3. Interactions between Treatment (Treatment and Reference) and NPO categories

If and when these ANOVA's yielded significant differences or suggestive evidence of a difference (usually a P-value under 0.10), a pairwise comparison (Tukey's HSD) was performed.

All data management, exploration, analyses, and visualizations were done using R (Version 4.1.1; R Core Team 2021) and R Studio (Version 1.4.1717; RStudio Team 2021). In addition, the R packages used in the analysis and reporting of these data (in alphabetical order) are gridExtra (Auguie, 2017), lubridate (Grolemund and Wickham, 2011), magrittr (Bache and Wickham, 2020), and tidyverse (Wickham et al., 2019).

## **Box and Whisker Plots**

Box and whisker plots are used throughout this report as they are a convenient way to display the variation in a dataset. In a box and whisker plot, data is displayed in two parts, the box and the whisker, and these two parts help display five values. Figure 1 (below) describes how to interpret a box and whisker plot.



FIGURE 2. DESCRIBES HOW TO INTERPRET A BOX AND WHISKER PLOT.

## Japanese Knotweed Results

## **Japanese Knotweed - Species Richness**

Examining the number of different species per site, as a measure of species richness, reveals that there is no significant difference between the treatment and control sites (P-value = 0.98). There is, however, suggestive evidence of a difference between the NPO categories (P-value = 0.07). Further examination of NPO category differences using pairwise comparisons indicated that there is a significant difference between the 2-3 years and 4-6 years NPO categories (P-value = 0.04, Tukey's HSD). However, there is no evidence of an interaction between treatment and NPO category (P-value = 0.95). Therefore, although NPO categories 2-3 years and 4-6 years are different from one another, this difference is the same for both the treatment and the reference sites and is likely a manifestation of site-specific variables rather than an effect of treatment.



FIGURE 3. BOX AND WHISKER PLOT DISPLAYING SPECIES RICHNESS (Y AXIS) OF JAPANESE KNOTWEED SITES OVER A SPAN OF NPO CATEGORIES (X AXIS). ORANGE BOXES REPRESENT REFERENCE SITES WHILE BLUE BOXES REPRESENT TREATMENT SITES. IN GENERAL TREATMENT SITES HAD GREATER VARIABILITY IN THE MEDIAN NUMBER OF NON-INVASIVE PLANTS, BUT THIS DIFFERENCE WAS NEVER SIGNIFICANT.

TABLE 1. ANALYSIS OF VARIANCE RESULTS EXAMINING SPECIES RICHNESS IN JAPANESE KNOTWEED SITES. THERE IS NO SIGNIFICANT DIFFERENCE BETWEEN THE TREATMENT AND CONTROL SITES (P-VALUE = 0.98). THERE IS SUGGESTIVE EVIDENCE OF A DIFFERENCE BETWEEN THE NPO CATEGORIES (P-VALUE = 0.07). HOWEVER, THERE IS NO EVIDENCE OF AN INTERACTION BETWEEN TREATMENT AND NPO CATEGORY (P-VALUE = 0.95).

| Source                               | Df | Sums of<br>Squares | Mean Square | F value | P value |
|--------------------------------------|----|--------------------|-------------|---------|---------|
| Treatment                            | 1  | 0.06               | 0.06        | 0.00    | 0.98    |
| NPO Category                         | 3  | 871.49             | 290.50      | 2.45    | 0.07    |
| Interaction<br>(Trmt. x NPO<br>Cat.) | 3  | 39.51              | 13.17       | 0.11    | 0.95    |
| Residual                             | 62 | 7,354.71           | 118.62      |         |         |

## Japanese Knotweed - Percent Ground Cover of Non-Invasive Plants

Examining the percent ground cover reveals there is no significant difference between the treatment and control sites (P-value = 0.25). There is also no significant difference between the NPO categories (P-value = 0.18). Furthermore, there is no evidence of an interaction between treatment and NPO category (P-value = 0.76). These results indicate that percent ground cover does not differ between treatment and reference sites and there is no interaction of time.



FIGURE 4. BOX AND WHISKER PLOT DISPLAYING TOTAL PERCENT COVER (Y AXIS) OF JAPANESE KNOTWEED SITES OVER A SPAN OF NPO CATEGORIES (X AXIS). ORANGE BOXES REPRESENT REFERENCE SITES WHILE BLUE BOXES REPRESENT TREATMENT SITES. IN GENERAL, MEDIAN PERCENT COVER DECREASED INSIGNIFICANTLY OVER TIME BUT THIS DECREASE WAS SIMILAR BETWEEN TREATMENT AND REFERENCE SITES.

TABLE 2. ANALYSIS OF VARIANCE RESULTS EXAMINING TOTAL PERCENT COVER OF NON-INVASIVE SPECIES IN JAPANESE KNOTWEED SITES. THERE IS NO SIGNIFICANT DIFFERENCE BETWEEN THE TREATMENT AND CONTROL SITES (P-VALUE = 0.25) OR BETWEEN THE NPO CATEGORIES (P-VALUE = 0.18). Additionally, THERE IS NO EVIDENCE OF AN INTERACTION BETWEEN TREATMENT AND NPO CATEGORY (P-VALUE = 0.76).

| Source                               | Df | Sums of<br>Squares | Mean Square | F value | P value |
|--------------------------------------|----|--------------------|-------------|---------|---------|
| Treatment                            | 1  | 321.43             | 321.43      | 1.32    | 0.25    |
| NPO Category                         | 3  | 1,221.43           | 407.14      | 1.68    | 0.18    |
| Interaction<br>(Trmt. x NPO<br>Cat.) | 3  | 287.46             | 95.82       | 0.39    | 0.76    |
| Residual                             | 62 | 15,063.97          | 242.97      |         |         |

## Japanese Knotweed - Percent Bare Ground

Examining percent bare ground reveals there is no significant difference between the treatment and control sites (P-value = 0.10). There is also no significant difference between the NPO categories (P-value = 0.49). Furthermore, there is no evidence of an interaction between treatment and NPO category (P-value = 0.95). These results indicate that percent bare ground does not differ between treatment and reference sites and there is no interaction of time.



FIGURE 5. BOX AND WHISKER PLOT DISPLAYING PERCENT BARE GROUND (Y AXIS) OF JAPANESE KNOTWEED SITES OVER A SPAN OF NPO CATEGORIES (X AXIS). ORANGE BOXES REPRESENT REFERENCE SITES WHILE BLUE BOXES REPRESENT TREATMENT SITES. IN GENERAL, PERCENT BARE GROUND INCREASED INSIGNIFICANTLY OVER TIME BUT THIS INCREASE WAS SIMILAR BETWEEN TREATMENT AND REFERENCE SITES.

TABLE 3. ANALYSIS OF VARIANCE RESULTS EXAMINING PERCENT BARE GROUND IN JAPANESE KNOTWEED SITES. THERE IS NO SIGNIFICANT DIFFERENCE BETWEEN THE TREATMENT AND CONTROL SITES (P-VALUE = 0.10) or between the NPO categories (P-value = 0.49). Additionally, there is no evidence of an interaction between treatment and NPO category (P-value = 0.95).

| Source                               | Df | Sums of<br>Squares | Mean Square | F value | P value |
|--------------------------------------|----|--------------------|-------------|---------|---------|
| Treatment                            | 1  | 755.71             | 755.71      | 2.78    | 0.10    |
| NPO Category                         | 3  | 668.02             | 222.67      | 0.82    | 0.49    |
| Interaction<br>(Trmt. x NPO<br>Cat.) | 3  | 98.65              | 32.88       | 0.12    | 0.95    |
| Residual                             | 62 | 16,841.90          | 271.64      |         |         |

## Japanese Knotweed – Percent Cover of the Five Most Prevalent Plant Species

The five most prevalent non-invasive species at sites with Japanese knotweed were rough goldenrod (*Solidago rugosa*), Canada goldenrod (*Solidago canadensis*), cow vetch (*Vicia cracca*), queen Anne's lace (*Daucus carota*), and white ash (*Fraxinus americana*). When looking at the average percent cover of the five most prevalent plant species found across all Japanese knotweed sites, there is no significant difference between the treatment and control sites (P-value = 0.19). There is also no significant difference between the NPO categories (P-value = 0.41). Furthermore, there is no evidence of an interaction between treatment and NPO category (P-value = 0.35). Although this p-value is insignificant, the box plots indicate that there are differences between treatment groups among the different NPO categories. In other words, from the figures below, it looks as if average percent cover decreases as the years NPO increases.

Further examination of interactions using pairwise comparisons indicated that these apparent differences are not significant (P-value = 0.49, Tukey's HSD).



FIGURE 6. BOX AND WHISKER PLOT DISPLAYING AVERAGE PERCENT COVER OF THE FIVE MOST COMMON SPECIES (Y AXIS) AT JAPANESE KNOTWEED SITES OVER A SPAN OF NPO CATEGORIES (X AXIS). ORANGE BOXES REPRESENT REFERENCE SITES WHILE BLUE BOXES REPRESENT TREATMENT SITES. IN GENERAL, THE PERCENT COVER OF THESE SPECIES APPEARS TO BE INSIGNIFICANTLY HIGHER IN THE REFERENCE SITES IN SITES WITH ZERO YEARS NPO AND THAT DIFFERENCE BECOMES SMALLER OVER TIME. Table 4. Analysis of variance results examining percent cover of the five most common species in Japanese knotweed sites. There is no significant difference between the treatment and control sites (P-value = 0.19) or between the NPO categories (P-value = 0.41). Additionally, there is no evidence of an interaction between treatment and NPO category (P-value = 0.35).

| Source                               | Df | Sums of<br>Squares | Mean Square | F value | P value |
|--------------------------------------|----|--------------------|-------------|---------|---------|
| Treatment                            | 1  | 102.20             | 102.20      | 1.77    | 0.19    |
| NPO Category                         | 3  | 170.97             | 56.99       | 0.99    | 0.41    |
| Interaction<br>(Trmt. x NPO<br>Cat.) | 3  | 191.77             | 63.92       | 1.10    | 0.35    |
| Residual                             | 62 | 3,587.17           | 57.86       |         |         |

#### Analysis of Variance

## **Common Reed Grass Results**

## **Common Reed Grass - Species Richness**

Examining the number of different species per site, as a measure of species richness, reveals that there is no significant difference between the treatment and control sites (P-value = 0.71). There is also no significant difference between the NPO categories (P-value = 0.90). Furthermore, there is no evidence of an interaction between treatment and NPO category (P-value = 0.97). These results indicate that species richness does not differ between treatment and reference sites and there is no interaction of time.



FIGURE 7. BOX AND WHISKER PLOT DISPLAYING SPECIES RICHNESS (Y AXIS) OF COMMON REED GRASS SITES OVER A SPAN OF NPO CATEGORIES (X AXIS). ORANGE BOXES REPRESENT REFERENCE SITES WHILE BLUE BOXES REPRESENT TREATMENT SITES. IN GENERAL, TREATMENT SITES HAVE FEWER SPECIES PRESENT, BUT THIS DIFFERENCE WAS NEVER SIGNIFICANT.

TABLE 5. ANALYSIS OF VARIANCE RESULTS EXAMINING SPECIES RICHNESS IN COMMON REED GRASS SITES. THERE IS NO SIGNIFICANT DIFFERENCE BETWEEN THE TREATMENT AND CONTROL SITES (P-VALUE = 0.71) OR BETWEEN THE NPO CATEGORIES (P-VALUE = 0.97). Additionally, there is no evidence of an interaction between treatment and NPO category (P-value = 0.97).

| Source                               | Df | Sums of<br>Squares | Mean Square | F value | P value |
|--------------------------------------|----|--------------------|-------------|---------|---------|
| Treatment                            | 1  | 11.20              | 11.20       | 0.14    | 0.71    |
| NPO Category                         | 3  | 47.68              | 15.89       | 0.19    | 0.90    |
| Interaction<br>(Trmt. x NPO<br>Cat.) | 3  | 19.02              | 6.34        | 0.08    | 0.97    |
| Residual                             | 62 | 5,094.67           | 82.17       |         |         |

## Common Reed Grass – Percent Ground Cover of Non-Invasive Plants

Examining the percent ground cover reveals that there is no significant difference between the treatment and control sites (P-value = 0.68). There is also no significant difference between the NPO categories (P-value = 0.23). There is, however, suggestive evidence of an interaction between treatment and NPO category (P-value = 0.07). This means that the difference between treatment groups differs among NPO categories. Based on the box plots, there is an apparent difference between treatment groups in the 0 years and 1-2 years NPO categories but not in the other categories. Further examination of interactions using pairwise comparisons indicated that these differences are not significant.



FIGURE 8. BOX AND WHISKER PLOT DISPLAYING TOTAL PERCENT COVER OF (Y AXIS) OF COMMON REED GRASS SITES OVER A SPAN OF NPO CATEGORIES (X AXIS). ORANGE BOXES REPRESENT REFERENCE SITES WHILE BLUE BOXES REPRESENT TREATMENT SITES. IN GENERAL, PERCENT COVER OF NON-INVASIVE SPECIES WAS GREATER IN REFERENCE SITES BUT THIS DIFFERENCE WAS NEVER SIGNIFICANT.

TABLE 6. ANALYSIS OF VARIANCE RESULTS EXAMINING TOTAL PERCENT COVER OF NON-INVASIVE SPECIES IN COMMON REED GRASS SITES. THERE IS NO SIGNIFICANT DIFFERENCE BETWEEN THE TREATMENT AND CONTROL SITES (P-VALUE = 0.68) OR BETWEEN THE NPO CATEGORIES (P-VALUE = 0.23). HOWEVER, THERE IS SUGGESTIVE EVIDENCE OF AN INTERACTION BETWEEN TREATMENT AND NPO CATEGORY (P-VALUE = 0.07).

| Source                               | Df | Sums of<br>Squares | Mean Square | F value | P value |
|--------------------------------------|----|--------------------|-------------|---------|---------|
| Treatment                            | 1  | 43.21              | 43.21       | 0.17    | 0.68    |
| NPO Category                         | 3  | 1,100.95           | 366.98      | 1.47    | 0.23    |
| Interaction<br>(Trmt. x NPO<br>Cat.) | 3  | 1,824.84           | 608.28      | 2.44    | 0.07    |
| Residual                             | 62 | 15,452.78          | 249.24      |         |         |

## **Common Reed Grass - Percent Bare Ground**

Examining the percent bare ground reveals that there is no significant difference between the treatment and control sites (P-value = 0.51). There is also no significant difference between the NPO categories (P-value = 0.10). There is, however, suggestive evidence of an interaction between treatment and NPO category (P-value = 0.08). This means that the difference between treatment groups differs among NPO categories. Based on the box plots, the treatment percent bare ground is higher than the reference percent bare ground in the 0 years NPO category, but not in the other categories.

Further examination of interactions using pairwise comparisons indicated that there is suggestive evidence of a difference between the treated sites in the 0 years and 1-2 years NPO categories (P-value = 0.07, Tukey's HSD).



FIGURE 9. BOX AND WHISKER PLOTS DISPLAYING PERCENT BARE GROUND (Y AXIS) OF COMMON REED GRASS SITES OVER A SPAN OF NPO CATEGORIES (X AXIS). ORANGE BOXES REPRESENT REFERENCE SITES WHILE BLUE BOXES REPRESENT TREATMENT SITES. IN GENERAL, PERCENT BARE GROUND IS INSIGNIFICANTLY HIGHER IN TREATMENT SITES.

TABLE 7. ANALYSIS OF VARIANCE RESULTS EXAMINING PERCENT BARE GROUND IN COMMON REED GRASS SITES. THERE IS NO SIGNIFICANT DIFFERENCE BETWEEN THE TREATMENT AND CONTROL SITES (P-VALUE = 0.51) or between the NPO categories (P-value = 0.10). However, there is suggestive evidence of an interaction between treatment and NPO category (P-value = 0.08).

| Source                               | Df | Sums of<br>Squares | Mean Square | F value | P value |
|--------------------------------------|----|--------------------|-------------|---------|---------|
| Treatment                            | 1  | 142.86             | 142.86      | 0.43    | 0.51    |
| NPO Category                         | 3  | 2,138.25           | 712.75      | 2.17    | 0.10    |
| Interaction<br>(Trmt. x NPO<br>Cat.) | 3  | 2,301.59           | 767.20      | 2.33    | 0.08    |
| Residual                             | 62 | 20,394.44          | 328.94      |         |         |

#### Analysis of Variance

## **Common Reed Grass – Percent Cover of the Five Most Prevalent Plant Species**

The five most prevalent non-invasive species at sites with common reed grass were flat-topped goldenrod (*Euthamia graminifolia*), tall white aster (*Doellingeria umbellate*), rough goldenrod (*Solidago rugosa*), meadowsweet (*Filipendula ulmaria*), and sensitive fern (*Onoclea sensibilis*). Examining the average percent cover of the five most prevalent plant species found across all common reed grass sites reveals there is no significant difference between the treatment and control sites (P-value = 0.14).

There is also no significant difference between the NPO categories (P-value = 0.34). Furthermore, there is no evidence of an interaction between treatment and NPO category (P-value > 0.99). These results indicate that percent cover of the five most prevalent plant species does not differ between treatment and reference sites and there is no interaction of time.



Analysis of Variance

FIGURE 10. BOX AND WHISKER PLOT DISPLAYING AVERAGE PERCENT COVER OF THE FIVE MOST COMMON SPECIES (Y AXIS) OF COMMON REED GRASS SITES OVER A SPAN OF NPO CATEGORIES (X AXIS). ORANGE BOXES REPRESENT REFERENCE SITES WHILE BLUE BOXES REPRESENT TREATMENT SITES. IN GENERAL, THE PERCENT COVER OF THESE SPECIES APPEARS TO INCREASE INSIGNIFICANTLY OVER TIME.

TABLE 8. ANALYSIS OF VARIANCE RESULTS EXAMINING AVERAGE PERCENT COVER OF THE FIVE MOST COMMON SPECIES IN COMMON REED GRASS SITES. THERE IS NO SIGNIFICANT DIFFERENCE BETWEEN THE TREATMENT AND CONTROL SITES (P-VALUE = 0.14) OR BETWEEN THE NPO CATEGORIES (P-VALUE = 0.34). Additionally, there is no evidence of an interaction between treatment and NPO CATEGORY (P-VALUE = 1.00).

| Source                               | Df | Sums of<br>Squares | Mean Square | F value | P value |
|--------------------------------------|----|--------------------|-------------|---------|---------|
| Treatment                            | 1  | 134.18             | 134.18      | 2.22    | 0.14    |
| NPO Category                         | 3  | 207.26             | 69.09       | 1.14    | 0.34    |
| Interaction<br>(Trmt. x NPO<br>Cat.) | 3  | 1.28               | 0.43        | 0.01    | 1.00    |
| Residual                             | 62 | 3,755.36           | 60.57       |         |         |

## Discussion

The results above explain the significance of differences between variables tested in the study. For example, results describe whether there are significant differences in densities of plants between sites treated to manage invasive species populations as compared to nearby reference sites. Variables including NPO category and the interactions between treatment and NPO category in plant species richness, overall plant density, percent bare ground, or the density of the top five most commonly occurring plant species per reestablishment site type (common reed grass- or Japanese knotweed-managed sites) are also explored.

The results above explain whether variables were significantly different from one another as a result of management practices or time since no invasive plants have been observed (NPO). The overarching question we hoped to answer from this analysis was whether or not sites managed by eliminating invasive species with the use of herbicide chemicals resulted in non-invasive plant community structures that resemble plant communities in their natural, uninvaded states.

#### **Species richness**

Species richness was not significantly different between sites treated chemically to manage invasive species as compared to nearby reference sites. This was true for both species treated: common reed grass and Japanese knotweed. With Japanese knotweed, a significant difference was observed when performing a pairwise comparison across NPO categories, specifically, between the 2-3 years and 4-6 years NPO categories (P-value = 0.04, Tukey's HSD). There were no differences between treatment and reference sites, and likewise no significant interactions. This means that sites (both treatment and reference sites) were different between these NPO categories. There is likely a site-specific difference that is confounding with other factors that influenced the number of species present at the sites as a difference was not seen between treatment and reference plots.

## Percent ground cover of all non-invasive plants

There was no significant difference in the percent ground cover of non-invasive plants between treatment and reference sites. This infers that that plant density was not impacted by herbicide treatment, as percent ground cover of non-invasive plants at treatment sites resembles that at reference sites, regardless of the length of time each site existed with no invasive plants observed. There was a near significant interaction between NPO category and treatment for common reed grass (P-value=0.07), however a further pairwise investigation was insignificant. Overall, percent ground cover was not significantly different between treatment and reference sites, indicating that percent ground cover is approaching a similar state to reference sites as treatment sites near NPO status.

## Percent bare ground

There was no significant difference in percent bare ground between treatment and reference sites, and likewise, there was no significant difference across NPO categories. There was, however, suggestive evidence of an interaction between treatment and NPO category (P-value = 0.08) for common reed grass. Further pairwise testing did not support this, however it is interesting, and it suggests that sites treated for common reed grass become more similar to reference sites after one year of being labeled NPO, but before that they may be more barren. More data may help support this statement.

# Average density of the five most common plant species found in Japanese knotweed sites

When looking at the top five most common non-invasive species found in sites managed for Japanese knotweed, there was not a significant difference between treatment and reference sites. This indicates non-invasive species are returning to Japanese knotweed treatment sites in a way that resembles the plant community structure of reference sites.

## Average density of the five most common plant species found in common reed grass sites

When looking at density of the top five most common non-invasive species found in sites managed for common reed grass, there was no significant difference between the treatment sites and the reference sites. This indicates the five most common non-invasive species are returning to common reed grass treatment sites in a way that resembles the plant community structure of reference sites.

## **Overall conclusion**

Overall, the results are similar to those of the 2015 study with only small differences. This supports the key finding that sites treated chemically to manage either common reed grass or Japanese knotweed result in a similar community of non-invasive plant species as compared to nearby reference sites. This is true even when the invasive plants have received at least one year of treatment and the target invasive plant is still present. It simply looks as if sites are able to revert to a natural state relatively quickly—at least when looking at the variables measured in this study. Sites treated in this study ranged in maximum size from roughly 1 square foot to roughly 4,828 square feet, with an overall average maximum size of 813 square feet or 0.02 acres. Therefore, sites were generally small, allowing for native plants to easily colonize as the target invasive species was selectively targeted for chemical treatment.

The second important thing to mention is that there is a lot of variation in the data presented in this study. While there are no significant differences between treatment and reference sites with regard to plant richness or overall percent coverage, it is possible that significance would be shown with a much larger sample size. However, considering the limited sample size of this study as well as the variation present, the results of this study indicate that, in general, there was not a difference between sites treated to manage invasive plants as compared to those of nearby reference sites. This supports the results obtained in 2015 with a similar sample size that showed similar findings.

#### What to measure in the future

Not much is seen by looking at the number of species. However, slightly better results are seen when looking at differences between treatment and reference sites when examining percent bare ground. Considering the high variation, it may make sense to continually monitor percent bare ground at treatment sites to see if and how that variable changes over time as treatment occurs. An overall percent cover of non-invasive species collected prior to initial treatment may also yield information. This could be a yearly measurement, or it could be an every two- or three-year measurement, revisiting the same site multiple times.

#### Possible issues with how this analysis was performed

The response variables of plant species richness and plant density (percent bare ground, percent ground coverage, and density of the top five most common species) were investigated in response to the independent variable of NPO, or when a site was deemed free from invasive species. This is a good independent variable to use to answer the question of "How long after invasive plants are eradicated from a system does it take for that system to revert back to a natural state?" One potential confounding issue that results from using NPO as an independent variable is that NPO could be affected by site-specific characteristics like soil type or other physical attributes that are not controlled for in an experimental design. An alternative variable, time since initial treatment, was also considered as an independent variable, however that too could be confounded by site characteristics, including, but not limited to, ease of access or visibility from a road (sites managed for longer were also chosen first by APIPP for several reasons). It would also largely be confounded by the length of treatment time, as sites that had the same time since initial treatment may have undergone treatment for different lengths of time and thus may have been subject to differing levels of stress that could impact regeneration. In addition, sites in the NPO 0-year category had the target plant present and treated in 2020. Several of these sites still had the target plants present at the time of this survey. These issues were discussed in length with Adirondack Research's colleagues and a statistician performing the analysis for Adirondack Research. It was concluded was that this analysis was done in an acceptable way to analyze this study, and there are plusses and minuses to either approach.

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