Effects of mechanical management of Rhamnus cathartica on fungal communities

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Abstract

Fungi provide valuable services to ecosystems; however little is known about the effects exotic invasive plants have on fungal communities. The objective of this study was to examine the effects of mechanical treatments of *Rhamnus cathartica* (commonly known as buckthorn) on fungal communities. The study was completed at two invaded sites in southeastern Wisconsin during June and July of 2006. Three blocks were studied per site, blocks consisted of: two mechanical management (cut back and cut plus girdle) and an unaltered control. Fungal communities were studied by analysis of sporocarp diversity, hyphal extractions and root arbuscular mycorrhizal abundance. Results indicated in all cases treated plots varied from control therefore fungal communities were affected by the mechanical management of *Rhamnus cathartica*. In the case of *Rhamnus cathartica*, its symbiosis with arbuscular mycorrhizae may increase ability to invade. Fungal community changes induced by exotic invasives may also affect ecosystem function.

Keywords: arbuscular mycorrhizae; fungal community; hyphae; *Rhamnus cathartica*; sporocarp

Introduction

Ecosystem functions are important to all species that inhabit them. Exotic invasive plants are being researched because they are a threat to these ecosystem functions (Ehrenfeld 2003). In order to understand a plant's full potential for invasiveness, its relationship with all aspects of an ecosystem need to be studied. This paper will first review the importance of fungi to ecosystem function. Then it will explain what invasive plants are, focusing in-depth at the invasive *Rhamnus cathartica* (buckthorn). The purpose of this study was to determine if a correlation exists between *R. cathartica* and the diversity and abundance of fungal communities.

Fungi

Fungi are abundant in most ecosystems and affect both biotic and abiotic components (Dighton 2003). Ecosystem services provided by fungi include biomass

regulation, food sources, decomposition, nutrient mineralization and nutrient accessibility (Dighton 2003). These services make them valuable to the ecosystems they inhabit.

Though many are familiar with mushrooms, the mushroom fruiting body (sporocarp) is only a small portion of the fungus. Further, not all fungi produce mushrooms. The larger fungal component consists of long threads of hyphae ranging between 5 to 10µm in diameter; these hyphae can be miles long (Campbell, Reece and Simon 2004, Dighton 2003). The hyphae absorb nutrients from their surroundings and help to stabilize the soil. Since there are many species that are difficult to distinguish, the fungi are usually classified into three major groups based on how they get their food: parasitic, saprophytic and mycorrhizal (Arora 1986).

Although parasitic fungi only make up 30% of the fungi species known, they are important as population regulators (Campbell, Reece and Simon 2004). A well known example of population disturbance by a fungal parasite was the potato blight of Ireland (Austin Bourke 1964). Not only did the fungal infection drastically reduce the population of the potato crop but also reduced the Irish people's population, who were dependant on the crop. Some studies suggest that even microbial parasites can effect population of top predators (Wardle and Yeates 1993). There are three kinds of parasites: castrators (affect reproduction of host), killers (kills off host) and debilitators (those that cause lesions and chronic infections) (Dighton 2003).

Saprotrophic fungi are commonly referred to as the decomposers. They decompose plant and animal matter. The decomposition process is important in the recycling on nutrients back into the soil for plant intake (Dighton 2003). Saprotrophic

fungi release extracellular enzymes that degrade organic molecules by mineralization.

Degraded nutrients, now as inorganic molecules, can then be utilized for plant uptake.

Mycorrhizal fungi have a mutualistic relationship with plants. These plants receive nutrients from mycorrhizal fungi while the fungi receive carbon from the plant (Dighton 2003). The fungi also are a secondary root system for the plant that helps the plant obtain nitrogen, phosphorus and magnesium. The mycorrhizal mutualistic relationship is a factor for plants that includes the enhancement of competitive ability (Carey, Marler and Callaway 2004). M. Bundrett (1991) suggested that these mycorrhizal relationships are so prominent that 95% of vascular plants have them. Three of the most common categories of mycorrhizae include ericoid mycorrhizae (EM), ecotomycorrhizae (ECM) and arbuscular mycorrhizae (AM).

Ericoid mycorrhizae (EM) are associated with members of the ericales (Dighton 2003). The ericale group's members are sclerophyllous evergreens living in nitrogen and phosphorus poor habitats (Read and Perez-Moreno 2003). This makes the EM relationship essential in obtaining nitrogen and phosphorus for these plants. The EM penetrates the root cortical cells with hyphal coils. Nutrient exchange takes place at the surface of these coils.

Ectomycorrhizae (ECM) generally has their associations with coniferous and deciduous trees (Dighton 2003). The ECM fungi form between root cortical cells. This forms a Hartig net which exists outside the endodermis of the host root (root tips). These structures appear to be root extensions but can be visually identified by their swollen "Y" branching pattern. ECM also has the capacity to act as decomposers (Zhu and Ehrenfeld 1996).

Arbuscular mycorrhizae are mainly the zygomycete species. They are mostly associated with herbaceous plants, grasses and a few trees. Arbuscular mycorrhizals penetrate within the host plant's root cortical cells and develop an arbuscule within the cell (Dighton 2003). The surface area of the arbuscule is where the majority of the nutrient exchange takes place. Hyphae of the fungi spread out of the cell and into the soil to bring the nutrients in (Dighton 2003). A study done by Lund University in Sweden found that plants with AM relationships obtained 500 to 600 times the amount of phosphorus than the same species without the AM relationship (Bolan 1991). This study is an example of how important these symbioses can be for nutrient uptake in plants.

Studies, like Carey, Marler and Callaway (2004) have begun to look at mycorrhizae and their relation to invasive plants. Their study found that arbuscular mycorrhizals transferred carbon from the native *Festuca idahoensis* to the exotic invasive plant *Centaurea maculosa*. The study suggests exotic plants that form mycorrhizal relationships may develop as invasives by surpassing native competitiveness. In other words, increased nutrient availability due to mycorrhizal fungi could increase the invasibility of the exotic plant (Rothstein, Vitousek and Simmons 2004).

Invasive plants

An exotic invasive plant can best be described as a plant that alters habitats by replacing a diversity of native plants with a non-indigenous single species (Culliney 2005). These invader plants flourish due to the lack of predation, competitors and disease (Smith and Smith 2006). Studies of these invasive plants have been increasing and have included examining ecological and economic impacts. Research on invasive species

typically begins with an examination of the individual invasive species characteristics followed by experiments that examine how that species directly impacts an ecosystem. In combination, these studies determine the appropriate level of concern and often can suggest management options.

Most non-indigenous plants were originally introduced by human activities accidentally or deliberately, depending on species (Cronk and Fuller 2001). Many of the invasive plants in the Midwest were brought here as ornamentals while others were brought accidentally like *Potamogeton crispus* commonly known as curly-leaf pondweed (Czarapata 2005). Once introduced, the seedlings can be distributed by animal excrement, machinery, or carried by wind and water.

Establishment of invasive plants creates a number of ecological problems. Some ecological problems caused from invasive plants include loss of biodiversity due to degradation of food sources, species extinction, soil composition changes and changes in hydrologic cycles (Czarapata 2005). *Euphorbia esula* (leafy spurge) is one such invasive that replaces native grasses and changes grazing land because cattle and horses will not eat it.

Invasive plants inflict economic costs by causing damages and losses. In fact, recent research done by David Pimentel et al., (2000) suggests the economic costs of invasive plants in the U.S. cost 26.4 billion dollars a year based on estimated losses (23.4 billion) and cost of management and eradication (3 billion). Though complete eradication is desirable, it is often not possible due to the plant (Culliney 2005).

Managers, then often aim to control rather than eliminate the species, using one of three approaches: biological, chemical and mechanical control.

Biological controls are usually animals, anthropods and fungal agents. The agents released are usually exotic predators, parasites or pathogens of the invasive plant. This control method can be less expensive than chemical and mechanical methods and is usually less damaging to the environment (Culliney 2005). Once the biological controls establish themselves, they can spread naturally over large areas and need little human labor for monitoring. Problems with using biological controls include the possibility of the control attacking non-target plants, their own potential to become invasive and difficulty in initial establishment.

Chemical control methods are often effective treatments but should be handled cautiously. They can include such herbicides as Glyphosate (commonly known as Roundup) and Triclopyr (Brush-B-Gon). Some common applications methods include wide-spray application, cut-stump treatment (placing herbicide directly on a cut plant), basal bark (applying treatment around base of the tree, or injection of herbicide into the trunk of the tree (Czarapata 2005). Chemical treatments are often effective but have risks of damaging non-target plants and ground contamination.

Mechanical methods are often the most practical for individuals but are labor intensive. These control methods include hand-pulling, girdling, cutting and burning (Culliney 2005). Controlled burning can be effective because many invasives can not tolerate fires. However, controlled burns often require permits and professional application and may not be feasible in densely wooded areas. Girdling and cutting can require minimal tools from pocket knives to hand saws. Treatments are meant to shock the plant to death and/or to expose the plant to parasites (Czarapata 2005).

Management of invasive plants maybe easier and cheaper if factors that help naturalize the invasive plants are known, so that specific traits can be targeted. Studies, like those done by Kathleen Knight and Peter Reich (2005), have begun to help determine relations of specific factors. For example patch versus landscape scales can show an opposite relationship between invasive plants and diversity, so treatment my have to be approached differently at these two scales (Knight and Reich 2005). There are many possible factors aiding the invasibility of plant, including early leaf emergence, late senescence, fungal relationships and generalist behaviors. The ability to leaf out early and/or senescence late allows the exotic plant to obtain sunlight and soil nutrients sooner and/or longer than the native plants (Czarapata 2005). Fungal relationships also can aid in the invasiveness of a plant. For example carbon parasitism by arbuscular mycorrhizae has been shown to aid the *Centaurea maculosa* in out-competing neighboring plants for carbon (Carey et al. 2004). Many invaders are also generalists, having the ability to tolerate different habitats and soil extremes (Stewart and Graves 2006). One such invader is the *R. cathartica*.

Rhamnus cathartica

The *Rhamnus cathartica* (buckthorn) is a common invader of the Midwestern forests and has become invasive throughout Canada and the Northeastern U.S. (Frappier et al., 2004). These European shrubs were brought here as ornamental hedge bushes in the mid 1800s. Prized for its dense foliage, they grow between 3-9 meters tall and quickly form dense thickets (Knight and Reich 2005).

R. cathartica has a few known advantages that aid in its invasiveness. One advantage was suggested by a study done by Robin Harrington et al. (1989), in which they found that R. cathartica leafs out early and senesces late, obtaining 27% to 35% more annual carbon gain than the native plants. It's early leafing and dense foliage allows it to shade out the native plants. Seeds are spread either by bird or are dropped below parent plants. Even though buckthorn berries are attractive to birds, the berries act as a laxative to the bird, resulting in energy loss for the bird and the spread of the seed in excrement (Czarapata 2005). There a no other known animals that eat R. cathartica.

R. cathartica does have a symbiotic relationship with AM fungi (Knight 2005). Studies on other invasive plants (like spotted knapweed) have found exotic plants to be aided in invasibility by carbon parasitism through AM soil fungi (Carey et al., 2004), though little is known about if this relationship affects the invasibility of R. cathartica. We know that R. cathartica makes an impact on the animal and plant systems (Czarapata 2005) but what about the fungal community? Understanding how R. cathartica affects the fungal community will improve our ability to predict changes of ecosystem functions when this plant invades.

Objectives

R. cathartica is an invasive shrub that affects both animal and plant systems, but little is known about its effects on the fungal community other than it has a symbiotic relationship to AM soil fungi. To examine this relationship, I first will manage the *R. cathartica* in disturbed deciduous forests and then test for differences in the fungal community. I expect that the management of *R. cathartica* will result in changes of the

abundance and diversity within the subterranean fungal community. If management is effective in a die back of *R. cathartica*, then I expect there will be a decline in arbuscular mycorrhizal abundance.

Methods and Materials

Sites

The study took place at two sites in Kenosha County Wisconsin; Pringle Nature Center of Bristol (N42° 31', W -88° 0') and Hawthorn Hollow of Kenosha (N42° 38', W -87° 52'). These locations are now nature preserves that were previously agricultural land. The areas of study at the sites were preserved in the 1970's at Pringle and 1915 at Hawthorn Hollow. Sample areas at the sites were chosen because they have been successfully invaded by the *R. cathartica* and have had little alteration by humans since the time of preservation. The nearest routine human disturbance is trail maintenance (mowing, wood chip dispersing and tree trimming) roughly between 5 and 20 meters from sample location.

To investigate fungal response to the management of *R. cathartica* three areas (40m by 10m) were chosen and marked in the corners with flags. Each of these blocks was then measured and flagged to contain 3 (10m by 10m) plots, with a 5m buffer zone (see fig. 1). Each block contained a control plot, a cut plot and cut plus girdle plot. Plots were assigned a treatment by a random dice roll.

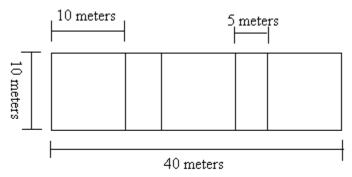


Figure 1. Block method. Block is 40m by 10m. Plots are 10m by 10m with a 5m buffer zone.

Management

Mechanical treatments of *R. cathartica* were chosen for a couple of reasons. First, mechanical treatments above ground reduced the amount of soil disturbance. Secondly, mechanical tools are easily accessible and low in cost. Chemical treatments were not chosen because of concern that the chemicals may also affect fungal communities (which would disrupt the results of this study). Biological controls could not be used because to date there is no such control for *R. cathartica* (Czarapata 2005).

The two mechanical treatments used in this experiment are cutting and girdling.

The cut treatment was simply trimming the shrubs as low possible using a hedge pruner.

Girdling was chosen because the method stops the flow of sugars hence decreasing the life of the plant. However, the cut method was used along with the girdle because the trees had already begun to fruit. I wanted to remove the canopy before the seeds could be dispersed. The control plots were unaltered.

Sampling

Sampling started one week after treatments were completed. It included weekly soil sampling and sporocarp identification with abundance counts. Sampling was done once a week starting June 20th, 2006 and ending July 13th, 2006. Soil samples were collected from 2 random locations within the plot by using a 4 inch Lillian Vernon garden shovel. Specimens from the two locations were pooled into a single ziplock bag and were then frozen until further testing.

Sporocarps were also sought out once weekly. Two field guides, Mushrooms

Demystified (Arora 1986) and National Audubon Society Field Guide to North American

Mushrooms (Lincoff 1981), were used for identification of sporocarps. Abundance was

counted and documented. If in-field identification was not possible, a specimen was

placed on an index card and then wrapped in wax paper to be identified at the school lab.

Laboratory analysis

Soil samples were examined using hyphal extractions and root arbuscular mycorrhizal counts. To extract hyphae, roughly half of each soil sample was air dried, the rest was frozen. For each dried sample 10g of soil was mixed with a sodium metaphosphate solution. After solutions were mixed, they were passed through a 425um brass sieve. A syringe then was used to collect 10ml of the sieved solution. Afterwards the syringe was attached to a filter apparatus containing a cellulosic filter and a polycarbonate filter. The solution was then passed through the filter apparatus. The polycarbonate filters were removed and slide mounted. At 400x magnification under a compound microscope, I counted all the hyphae in one viewing screen and identified whether they were non-mycorrhizal or mycorrhizal. The hyphae could be identified by

visual characteristics like branching angle and presence of septae (for an example see figure 2).

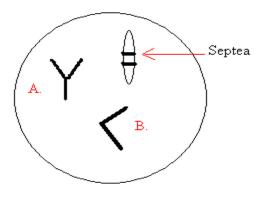


Figure 2. Characteristics of mycorrhizal and non-mycorrhizal hyphae extracted from soil. Mycorrhizal hyphae (A.) may have a sixty degree "Y" branching. Non-mycorrhizal hyphae (B.) may have a 90 degree branch and/or septae.

Arbuscular mycorrhizal fungi were counted by staining roots infected by arbuscular mycorrhizal fungi (Koske and Gemma 1989) and permanently mounting them on slides. The PVLG mounting medium was modified from Koske and Gemma (1989) to use 5ml lactic acid instead of the suggested 50ml in order to increase hardening. For each soil sample, five roots (2cm length, 2mm diameter) were hand selected, washed, bleached and then stained with a trypan blue solution. These stained roots were then slide mounted and viewed under a compound microscope at 400x magnification (see figure 3). Arbuscular mycorrhizals were counted, documented and averaged per soil sample.

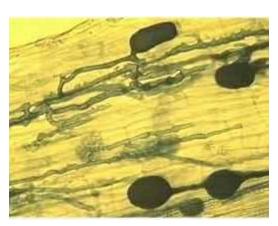


Figure 3. Arbuscular mycorrhizals of clover root. Picture taken from Deacon (2006).

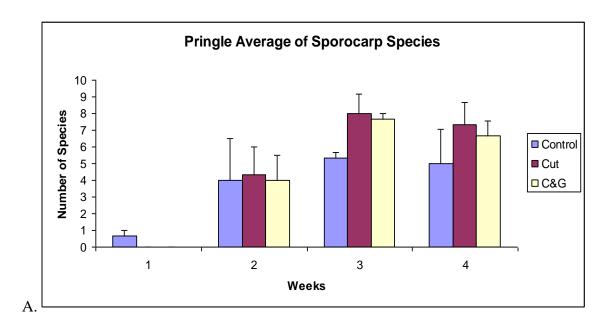
Statistical analysis

Results from the sporocarp identification and soil sample tests were visually and statistically analyzed to determine whether treatments differed from the control.

Comparisons, for which there were clear trends, were analyzed using t-tests to determine if differences were significant.

Results

No significant differences between treatments in the diversity of sporocarps were found (*P*>0.09 at Pringle and *P*>0.26 at Hawthorn, Figure 5). However, trends of species diversity, at both sites, increased over the four week period, by weeks 3 and 4 the treated plots had more species diversity than the control. The only dominant species Pringle Nature Center had in common with Hawthorn Hollow was the *Mycorrhaphium adustum* commonly called Kidney Shaped Tooth. Sporocarp identification gave a total over the 4 weeks; 47 known species of fungi, 9 identified to the genus, 2 unknown species of LBMs (little brown mushrooms) and 6 identified slime molds (for a total list see appendix 1.). At Hawthorn Hollow the dominant species included: *Marasmius rotula*, *Mycorrhaphium adustum*, *Plicaturopsis crispa*, *Trametes versicolor*, *Trichaptum biformis*. Other dominant species at Pringle included *Arcyria denudata*, *Crepidotus mollis*, *Omphanlina ericetorum* and *Schizophllum commune*. Dominant species accounted for 75 % total fungal abundance.



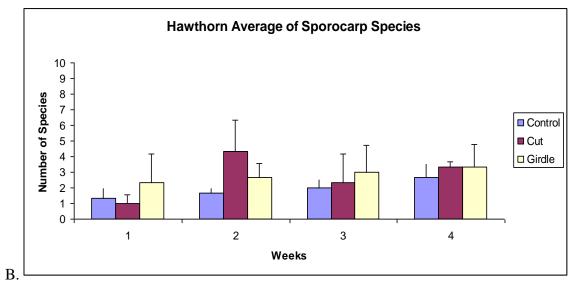
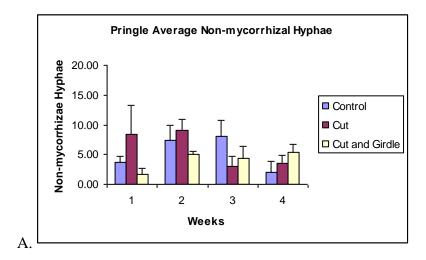
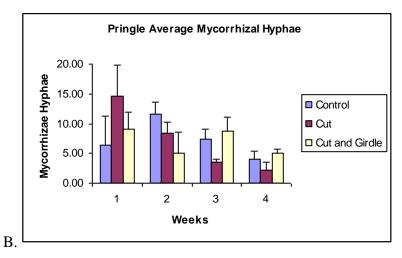


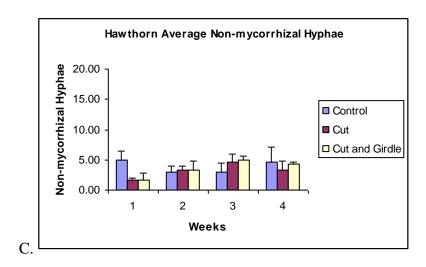
Figure 5. Mean and standard error of sporocarp species found at Pringle (A) and Hawthorn (B).

Though hyphae extraction results were not significantly different (P>0.62 at Pringle and P>0.13 at Hawthorn) among treatments, there were clear trends (figure 6). The non-mycorrhizal hyphae averages at Pringle had only small variances. Pringle's mycorrhizal hyphae averages overall declined. The cut plot had a continual decline while the control and the cut plus girdle plot varied week to week. It is noted that plot standard

error in week 1 was high. Hawthorn's non-mycorrhizae hyphal count was similar to Pringle's in that it hardly changed over the four weeks and the standard error results were the most consistent of the hyphal extractions. Hawthorn's cut plot also decreased in mycorrhizal fungi as well as its control and cut plus girdle plots.







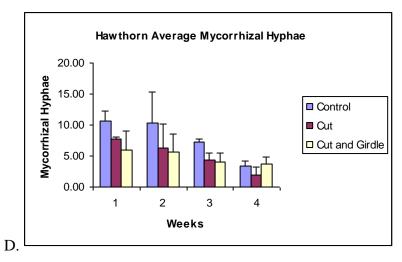
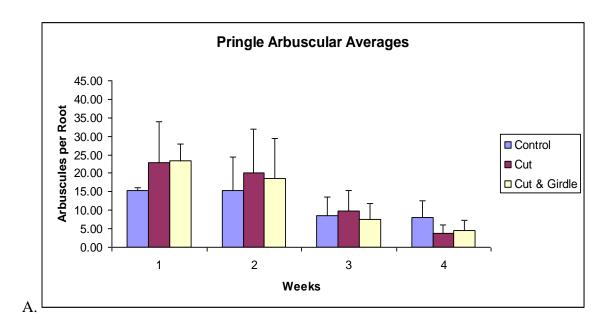


Figure 6. Hyphal mean and standard error. Non-mycorrhizal hyphae Pringle (A) and Hawthorn (C). Mycorrhizal hyphae at Pringle (B) and Hawthorn (D).

There are trends in the arbuscular averages at both sites although there were no significant differences (P>0.67 at Pringle and P>0.37 at Hawthorn, figure 7). Arbuscular mycorrhizal averages at Pringle decreased over time. The treated plots began with more arbuscular mycorrhizae averages but by week 4 both were less than control. Hawthorn's results were not the same as Pringles. The control and the cut plus girdle plot stayed

almost the same. The cut plot decreased in weeks 2 and 3, then returned in week 4 similar to the first week.



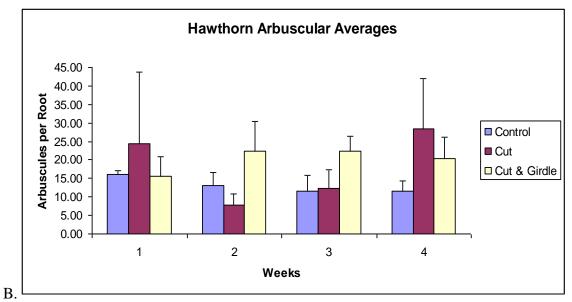


Figure 7. Arbuscular mean and standard error found per root at Pringle (A) and Hawthorn (B).

Discussion

This study examined fungal community response to the mechanical management treatments (cut back and cut plus girdle) of *R. cathartica*. Fungal response in all points of analysis (sporocarp diversity, soil hyphal and root arbuscular mycorrhizal abundance) did have an important general pattern even though t-test analysis suggests results are not statistically different. The important trend in all cases was that the treatment results varied from the control. This trend suggests that the hypothesis was supported; the mechanical treatments of *R. cathartica* affect fungal communities.

Specific data analysis was applied to sporocarp diversity, hyphal extractions and root arbuscular mycorrhizal abundance. The prediction of treatments affecting fungal communities was supported in sporocarp diversity analysis. Although there was an overall increase in sporocarp diversity, treated plots had greater diversity trends than the control plots. The hypothesis was also supported by hyphal analysis which found patterns of decline in mycorrhizal hyphae. However variances in the arbuscular mycorrhizae abundances (decline at Pringle but not at Hawthorn) did not completely support the hypothesis. Site differences may have been due to site differences such as trample and seasonal growth.

Differences between site results may have been due to a few circumstances, like plot trample, seasonal growth and climate. The first plots to be managed were at Hawthorn. Shrubs at this site were older and larger, so removal may have caused a significant amount of trample. Sampling was also done in the peak of the growing season in which there was a few days of precipitation, this may have had influences on results. Other studies suggest the management of buckthorn should take place during the winter

season to avoid such variables as trample and soil disturbance (Frappier 2004). This would be useful for future studies since buckthorn can be easily identified and seasonal native plants will have already died. Multiple year census studies will also be useful in determining climate affects and seasonal difference in the fungal community.

A continuation of this specific study will occur this summer. Sporocarp diversity and abundance as well as hyphal extractions and arbuscular abundance will continue to be analyzed. The continuation will assist in the analysis of result patterns and determine if results are seasonal or treatment related. The study will also include the measure of regrowth of *R. cathartica* and the effectiveness of mechanical treatments. Other future studies should include the examination of specifically *R. cathartica* roots pre and post treatment focusing on arbuscular relations. Arbuscular mycorrhizal symbiosis with the shrub may provide insight to an aiding invasive factor.

Overall this study has shown that *R. cathartica* does impact the fungal community. This is important because changes in fungal communities can disrupt ecosystem services like biomass regulation, decomposition and nutrient accessibility. Knowledge of fungal relations with invasive plants may provide predictions of how ecosystems will change once invaded and may also determine specific factors aiding invasibility.

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Appendix 1. Species of sporocarps identified by scientific name.

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Arcyria denudate	Inocybe pyriodora	Schizophllum commune
Ascocoryne sarcoides	LBM (little brown mush.)	Polyporus elegans
Auricularia auricular	Lycogala epindendrum	Polyporus mori
Boletus bi-color	Marasmius spp.	Sparassis crispa
Calocera viscosa	Marasmius rotula	Stereum spp.
Calvatia cyathiformis	Mycena spp.	Stereum ostrea
Cantharellus spp.	Mycena acicula	Stereum striatum
Certiomyxa fruticuosa	Mycena subcaerula	Tectella patellaris
Clavicorona pyxidata	Mycorrhaphium adustum	Trametes versicolor
Climacocystis borealis	Nidularia pulvinata	Tremella mesenterica
Coprinus atramentarius	Omphalina ericetorum	Trichaptum biformis
Cortinarius spp.	Panaeolus sphinctrinus	Tyromyces chioneus
Crepidotus herbarum	Paxillus involutus	Underwodia columnaris
Crepidotus mollis	Peniophora rufa	
Dacrymyces palmatus	Perenniporia subacida	
Exidida glandulosa	Peziza vesciculosa	
Galiella rufa	Pleurotus ostreatus	
Glocophyllum spp.	Plicaturopsis crispa	
Gymnopus subnudus	Polyporus arcularius	
Hericium spp.	Polyporus badius	
Helvella macropus	Polyporus elegans	
Heterobasidion annosum	Polyporus mori	
Hygrophorus spp.	Pouzarella nodospora	
Hypomyces chrysospermus	Russula compacta	
Inocybe spp.	Russula fragilis	
Inocybe lacera	Sarcoscypha occidentalis	