# IMPACTS, GENETIC DIVERSITY, AND LAND MANAGEMENT STRATEGIES OF POST-FIRE *PAULOWNIA TOMENTOSA* INVASIONS IN THE LINVILLE GORGE WILDERNESS AREA, BURKE COUNTY, NC

A Thesis
By
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# Abstract

## IMPACTS, GENETIC DIVERSITY, AND LAND MANAGEMENT STRATEGIES OF POST-FIRE *PAULOWNIA TOMENTOSA* INVASIONS IN THE LINVILLE GORGE WILDERNESS AREA, BURKE COUNTY, NC

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*Paulownia tomentosa* (princess tree) is an exotic-invasive tree species in North America with widespread naturalized populations. Native to East Asia, *P. tomentosa* was introduced to North America in the 1840s as an ornamental tree and timber species and remains widely planted due to its showy flowers and rapid growth. Few studies have quantified its impact on native plant communities, characterized its genetic diversity within and across populations, or compared the effectiveness of different management strategies. As exotic-invasive plants expand their ranges, effective land management will rely on an understanding of impacts, dispersal strategies, and control methods associated with plant invasion.

Multiple large-scale *Paulownia tomentosa* invasions have been promoted in the Linville Gorge Wilderness Area (LGWA), Burke County, NC following a series of wildfires since 2000. Chapter 1 evaluates the effects of varied post-fire *P. tomentosa* invasions on native plant populations and the genetic diversity within and among distinct *P. tomentosa* populations separated by a geographic barrier in the LGWA. Plant communities significantly shifted between invaded and non-invaded plots in all invaded areas with decreased diversity indices in invaded plots of multiple invasions. Genetic diversity was extremely low within and among *P.*

*tomentosa* populations indicating that dispersal is high within the LGWA and that one highly competitive genotype promotes the tree's invasive success. Chapter 2 compares the efficacy and non-target effects of manual and chemical treatment of *P. tomentosa* invasions in the LGWA. Each treatment method successfully reduced *P. tomentosa* populations with ~ 1% cover remaining one year following treatment. I observed no significant differences between plant community composition in manually and chemically treated plots, although diversity indices consistently trended negatively in chemically treated plots. This information concerning plant community impacts, invasion genetics, and control strategies provides a new framework of the role *P. tomentosa* plays in invaded areas and may help inform and guide effective land management.

# Dedication

This project is dedicated to Kayah Gaydish (1979-2015) whose infectious enthusiasm and tireless hard-work will leave a lasting impact on the Linville Gorge Wilderness.  Her superb knowledge and dedication to management in the gorge made this project possible.  Kayah serves as a steadfast model for anyone interested in conservation and wild places.

# Acknowledgements

I would like to thank my advisor Dr. Mike Madritch for lending his expertise, advice, and encouragement to explore ideas that allowed me to grow as a biologist over these two years. My other committee members Dr. Howie Neufeld and Dr. Gary Walker also provided valuable input during the project's planning and review phases. I also owe an enormous amount of gratitude to my excellent field crew- Gretchen Bailey, James Filloramo, and Laura Kraus. While not knowing exactly what they had signed up for, these folks remained good-natured and hard-working even when bushwhacking through briers in search of a supposedly great field site. This project would never have lifted off the ground without you. Kate Lis and Kira Perzell Mandell were invaluable for their extensive lab work. Drew Jenkins was also extremely helpful in guiding me through plant identification and lending an expert eye when I got stuck. Lastly, my fellow graduate students were key in helping me plan, implement, and interpret many facets of my project. In particular, Angela Langevin and Richie Hum were frequently willing to talk things out.

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# Foreword

Chapter 1 of this thesis will be submitted to *Biological Invasions*, an international peer-reviewed journal published by Springer International Publishing.  Chapter 2 will be submitted to *Natural Areas Journal*, an international peer-reviewed journal published by the Natural Areas Association.  Both chapters have been formatted in the style guide for *Biological Invasions*.

# CHAPTER 1: COMMUNITY EFFECTS AND GENETIC DIVERSITY OF POST-FIRE *PAULOWNIA TOMENTOSA* INVASIONS IN THE LINVILLE GORGE WILDERNESS AREA, BURKE COUNTY, NC

**Abstract.** Many naturalized populations of the invasive tree *Paulownia tomentosa* exist in North America, yet little research has quantified its effect on native communities. A series of recent wildfires in the Linville Gorge Wilderness Area (LGWA) promoted multiple large-scale *P. tomentosa* invasions in this ecologically important area. To measure community shifts caused by these *P. tomentosa* invasions across fire regimes, I sampled vegetation in paired invaded and non-invaded plots in mature and immature invasions within two burn areas of the LGWA. Community composition shifted in response to *P. tomentosa* invasion across all invasion stages and burn areas. Species richness and Shannon diversity values decreased in invaded plots in the 2000/2007 and 2000/2013 burn areas. Overall community structure differed in invaded plots within immature invasions ($p = 0.004$). Some individual life form groups also comprised higher or lower aggregated percent covers based on invasion in each burn/invasion treatment. The distribution of *P. tomentosa* age classes in both burn areas indicates that fire strongly promotes invasion but is not necessary for subsequent recruitment. Additionally, preliminary genetic analyses among distinct *P. tomentosa* populations revealed very low genetic diversity suggesting that one introduction took place in the LGWA and possibly a highly competitive genotype may aid the tree's invasive success. This information regarding community shift and strong post-fire recruitment by *P. tomentosa* may inform management decisions by prioritizing *P. tomentosa* control, particularly before and after wildfire or prescribed burns.

## Introduction

Invasive plant species are key drivers of large-scale environmental change (Vilà et al. 2011; Pyšek et al. 2012). Invasive plants influence ecosystem services and functions by outcompeting native species, altering ecosystem properties, impacting plant diversity, and altering native disturbance regimes (Ehrenfeld 2010; Vilà et al. 2011; Pyšek et al. 2012). By altering ecosystem functions and services, invasive species can also cause significant economic impacts on society (Pimentel et al. 2005; Pejchar and Mooney 2009). Stemming from these myriad impacts, research characterizing and quantifying ecological impacts of invasive plants has progressively emerged (Pyšek et al. 2012). However, quantitative assessments comparing invaded and uninvaded communities are relatively scarce when considering the thousands of invasive plants that exist globally (Hulme et al. 2013; but see Vilà et al. 2011). Furthermore, research quantifying community shifts has focused disproportionately on a small number of high-profile invasive plants (e.g., *Bromus tectorum*, *Fallopia japonica*, *Phragmites australis*) with roughly one third of all publications focusing on just nine species (Hulme et al. 2013).

Recent research has demonstrated that native plant community shifts in response to invasion vary in scope and magnitude by species and life form (Hedja et al. 2009; Vilà et al. 2011; Fried et al. 2014). In a field study investigating community impact of eight invasive plants, Fried et al. (2014) found both increases and decreases in species richness and diversity within invaded plots. Variable impacts of invasive species on native biodiversity are widespread in the literature (Stohlgren et al. 2002; Hejda and Pyšek 2006; Meffin et al. 2010; Vilà et al. 2011). Stochastic responses coupled with a focus on a limited number of invasive plants often leads to poorly-supported, speculative predictions of community effects after plant invasion. This speculation may lead to poorly-prioritized invasive species management and ill-informed general hypotheses about plant invasion impacts.

The impact of invasive plants on native plant communities can vary heavily with time since invasion (Strayer et al. 2006). However, most research has focused on single-aged invasions with no consideration of invader age that may provide limited understanding of the invader's effect on the native community (Strayer et al. 2006). For example, tree species occupy different niches based on size and age. These changes in niches over time could lead to differing community impacts as an invasive tree grows from a seedling to canopy level tree and affect ecosystem traits such as canopy cover and nutrient cycling (Gulezian and Nyberg 2011; Vilà et al. 2011; Staska et al. 2014). Plant invasion can also alter the successional trajectory of some areas (Sullivan et al. 2007; Kuebbing et al. 2014). These temporally dependent impacts may not be understood if research focuses only on single-aged invasions. Thus, studying multiple populations of an invader with varying times since invasion may help elucidate its effect over time.

Fire often acts as a large-scale disturbance that promotes recruitment of some invasive plants (Keeley 2006; Kuppinger et al. 2010; Pyšek et al. 2012). Once established, these invaders may also alter the fire regime of an area and promote large-scale community shifts through increased fire frequency and intensity (Keeley 2006; Pyšek et al. 2012). The relationship of fire and invasive plants potentially complicates management efforts as prescribed fire is increasingly used as a management tool (Brose et al. 2013). Conditions created by prescribed fire may promote plant invasion and thwart management goals since post-fire invasion can drastically alter plant community structure and composition (Keeley 2006). Post-fire invasions may deter recruitment of fire promoted/dependent plants that the management plan intended to encourage (Keeley 2006). The response of some invasive plants to fire (Kuppinger et al. 2010; Pyšek et al.

2012) suggests additional research in varied post-fire invasions is necessary to better inform fire-related management efforts.

Population genetics has emerged as a powerful tool for tracking introduction histories, dispersal abilities, and genetic diversity of invasive plants (Estoup and Guillamaud 2010; Yu et al. 2014). Genetic diversity is an important factor contributing to invasive ability of plants with two distinct diversity levels often promoting successful invasion (Sakai et al. 2001; Dlugosch and Parker 2008; Yu et al. 2014). High genetic diversity is often thought to promote invasive ability by increasing plasticity and flexibility when colonizing new environments (Sakai et al. 2001) and has been supported by observational and experimental studies (Lavergne and Molofsky 2007; Kirk et al. 2011; Wang et al. 2012). Conversely, some research indicates that low genetic diversity indicates a single introduction and can increase invasiveness due to a single or few highly adapted and plastic genotypes (Le Roux et al. 2007; Dlugosch and Parker 2008; Yu et al. 2014). When comparing the genetic diversity of the invasive *Chromolaena odorata* between its native and invasive range in the Americas and Asia, Yu et al. (2014) found that all individuals sampled from 10 Asian countries existed as one haplotype with one central multilocus genotype. The authors' hypothesize that a single introduction occurred in Asia and the invader's success was attributed to the high competitive ability of this single genotype which allows for rapid invasion without a bottleneck period. Comparing genetic diversity across multiple populations may also explain an invasive plant's ability to disperse and establish new populations (Kirk et al. 2011; Wang et al. 2012; Yu et al. 2014). Additionally, these genetic analyses may describe effects of geographic barriers or pathways on dispersal ability and gene flow (Manel and Hoderegger 2013). Genetic diversity analyses might also indicate an invader's introduction rate and history (Dlugosch and Parker 2008; Kirk et al. 2011; Yu et al. 2014).

Understanding an invasive plant's functional connectivity and rate of introduction across the landscape could aid land management in prioritizing invasive plant control and understanding the role of new introductions in populations.

Little research has characterized community effects or genetic diversity of the invasive tree *Paulownia tomentosa* (princess tree). My study aims to increase understanding of *P. tomentosa* invasions with the following primary objectives: i) Characterize shift of native plant community composition and structure caused by new and older seed-producing *P. tomentosa* invasions across two fire regimes, ii) Compare community effects of *P. tomentosa* invasion between new and old invasions across two fire regimes, iii) Describe post-fire recruitment of *P. tomentosa* and co-occurring native plants across two fire regimes, and iv) Describe genetic diversity among and within closely located (~1.5 km apart) *P. tomentosa* populations located on opposite edges of a geographic barrier (large gorge).

## Materials and Methods

### *Study species*

*Paulownia tomentosa* is a widely planted ornamental and timber tree that was introduced to North America from Asia in the 1840s (Hu 1961; Tang et al. 1980). Like many other invasive plant species, *P. tomentosa* has many qualities of the classic ruderal species (Grime 1977) such as heavy production of seeds (as many as 20 million seeds per year from a single mature tree), fast growth rates, a strong re-sprouting response, and early maturation (Hu 1961; Carpenter et al. 1983; Longbrake 2001). Additionally, its small (2.25 mm diameter) seeds can disperse up to an estimated 10 km (Kuppinger 2008). *Paulownia tomentosa* often heavily invades areas following large-scale disturbances such as fire, surface mining, and timber harvest (Carpenter et al. 1983; Langdon and Johnson 1994; Longbrake 2001; Kuppinger 2008; Kuppinger et al. 2010).

Additionally, *P. tomentosa* is shade intolerant and benefits from competitor release following large scale disturbances (Kuppinger 2008; Kuppinger et al. 2010).

Post disturbance control efforts require significant time and expense (Jenkins and Johnson 2009), and eradication efforts are hampered by the propensity of individuals to inhabit difficult to access areas such as vertical cliffs (Lovenshimer, personal obs.). While *P. tomentosa* seedlings may initially invade disturbed systems, they are easily outcompeted by native canopy species and do not persist in shaded areas (Kuppinger et al. 2010). Thus, *P. tomentosa* persistence is observed as greatest on exposed areas such as rock outcrops and cliffs with high light and few competing tree species (Kuppinger 2008; Kuppinger et al. 2010).

### *Study Area*

The Linville Gorge Wilderness Area (LGWA; 4850 ha) is located in Burke County, NC within Pisgah National Forest. The LGWA lies on the eastern portion of the southern Appalachian Mountains and is defined by complex topography and extensive cliff systems with elevations ranging from 400-1250 m (Newell and Peet 1998). Approximately 5% of the LGWA experienced historic logging with the remainder of the LGWA containing original vegetation (Newell and Peet 1998).

Newell and Peet (1998) described 28 community types in the LGWA through extensive sampling across the gorge with the notable exception of inaccessible vertical cliffs. Prominent among these communities are thermic oak-pine forests which comprise 40% of total mapped vegetation. *Pinus pungens* (table mountain pine, Lamb.) is a dominant canopy species in these areas (Newell and Peet 1998) and is promoted by fire (Brose and Waldrop 2006). Also notable in frequency are rock outcrop communities which are limited in extent across the S. Appalachians but account for 8% of mapped vegetation in the LGWA (Newell and Peet 1998).

The LGWA experienced a period of fire suppression from the early 1950s-2000 (Newell and Peet 1998) ending with the Brushy Ridge Fire which burned approximately 4424 ha. Since the 2000 Brushy Ridge Fire, four additional fires have burned various areas of the LGWA. These include the Pinnacle (972 ha), Shortoff (1992 ha), and Table Rock fires (1044 ha) occurring in 2007, 2007, and 2013 respectively. These fires overlap large portions of the Brushy Ridge fire but do not share any burned areas (see Appendix 1).

### *Vegetation Sampling*

Vegetation sampling occurred in paired 4 $m^2$ plots occupying the eastern and western rims of the LGWA primarily within rock outcrops, thermic oak-pine communities, and cliff talus areas across two fire exposures (Appendix 1). Paired plots occupied nearby (< 45 m) invaded and non-invaded areas with similar abiotic factors (e.g., slope, aspect, percent soil cover, etc.; Hejda et al. 2009; Fried et al. 2014). In a few cases, a non-invaded plot contained low (< 5%) *P. tomentosa* cover (Hejda et al. 2009; Fried et al. 2014). Seven to eight plot pairs were located in three areas defined by fire regime and invader life stage (hereafter called burn/invasion treatments). Mature invasions were sampled in two areas burned twice (burned in 2000 and 2007, $n$ = 8; burned in 2000 and 2013, $n$ = 8) and immature invasions sampled in one area burned twice (burned in 2000 and 2013, $n$ = 8) for a total of 46 plots (See Appendix 1 for all plot locations). Mature invasions were classified by the presence of seed pods or flowers. In a few cases within the 2000/2013 burn area, mature individuals were identified by dead scorched stems with seed pods present. In these cases, a new re-sprouted stem without seed pods reached the approximate height of the scorched stem. All vegetation sampling occurred in June-July 2015.

All vascular plant and bryophyte species were documented in each plot. Species percent cover and soil cover were visually estimated in each plot. Woody stem height and woody stem

DBH for *P. tomentosa* were recorded in each plot. To compare post-fire recruitment and competitive ability of *P. tomentosa* with dominant native species, stem height and DBH were also recorded for *P. pungens*, and for the dominant native species (greatest cover) within each plot. Elevation, aspect, and slope (degrees) were recorded for each plot. Fire severity was visually estimated (1-5 scale) in each plot based on re-sprouting frequency, scorch height, and percent dieback of vegetation according to protocols used by Kuppinger et al. (2010).

As necessary, vascular plant samples were transferred to a plant press in the field and later identified to species. If species identification was not possible, vascular specimens were identified to genus, family, or morphotype. Sampled bryophytes were identified to morphotype. To determine age, tree cores or cross sections were collected 25 cm from the base of one representative *P. tomentosa* individual > 2 cm dbh for each size class present in each invaded plot. Because *P. tomentosa* has a high growth rate (approx. 1-2.5 m year$^{-1}$; Hu 1961), height classes occurred in 2 m increments.

### *Leaf Sample Collection*

I collected 19 leaf samples from different *P. tomentosa* individuals in September 2014 within two distinct patches in the LGWA for a total of 38 leaves (Appendix 1). These patches were located approximately 1.5 km apart on the eastern and western rims of the Linville Gorge. Leaf samples from the western rim were collected from an established patch approximately 20 m$^2$ large composed of mostly mature individuals on a rock outcrop. Leaf samples from the eastern rim were collected from an immature patch approximately 10 m$^2$ large on a rock outcrop. More than 90% of all present individuals in each patch were sampled. Collected leaves were placed in paper bags and refrigerated at approximately 2° C immediately after field work. All samples were freeze-dried within one day of collection. Following lyophilization, leaves were stored at -20$^{o}$C.

### *DNA extraction and Microsatellite analyses*

Genomic DNA was extracted from each leaf sample using the DNeasy Plant Mini kit reagents and protocol (Qiagen, Venlo, Netherlands). Five pairs of co-dominate, genus-specific microsatellite markers were used for genotyping analysis (Wang et al., 2013). PCR was completed with a final volume of 15 µL containing 7 µL Master Mix (Promega Corp, Madison, WI), 4 µL nuclease free water, 1 µL 100x BSA (New England BioLabs, Ipswich, MA), 1 µL DNA template diluted to 8 ng/µL, 1 µL 10 µM reverse primer, 0.5 µL 10 µM forward primer, and 0.5 µL 10 µM fluorescent dye (FAM, VIV, NED). PCR reactions were performed with a Mastercycler Nexus (Eppendorf, Hamburg, Germany) using protocol described by Wang et al. (2013). Reactions were multiplexed using the GeneScan Liz 600 size standard (Applied Biosystems, Foster City, CA). Genotyping was conducted at the Georgia Genomics Facility (Athens, GA) using an ABI 3730 sequencer (Applied Biosystems, Foster City, CA). The produced chromatograms were scored using Geneious 9.0.5 (Biomatters Limited, Auckland, NZ).

### *Plant Community Data Analysis*

Shannon diversity (H'), species richness (S), and species evenness (J) were calculated for each plot. These metrics were compared using paired *t*-tests of invaded and non-invaded plots in each burn/invasion treatment (Hejda et al. 2009). Differences in species composition between invaded and non-invaded plots were quantified with the Jaccard dissimilarity index ($D_J$; Fried et al. 2014). Analysis of similarities (ANOSIM) tests were used to test the significance of these community composition shifts within each burn/invasion treatment. *P*-values were calculated by permutation (999 permutations for $\alpha = 0.05$). Differences in H', S, and J of invaded and non-invaded plots were calculated as a Relative Impact (RI; Vilà et al. 2006) where $RI_V = (V_{NI} - V_I)/(V_{NI} + V_I)$, where V is the variable of interest, NI is the non-invaded plot, and I is the invaded plot. A positive RI represents a decrease in the variable of interest associated with *P. tomentosa* invasion and a negative RI represents an increase. Non-metric multidimensional scaling (NMDS) was fitted on $D_J$ values for invaded and non-invaded plots in each burn/invasion treatment. Indicator species analyses (using the multipatt function in the indicspecies R package; De Caeres and Legendre 2009) were used to highlight species associated with either invaded or non-invaded plots within each burn/invasion treatment

To assess the effect of *P. tomentosa* invasions on native community structure, species were grouped by growth form as trees, shrubs, herbs, graminoids, vines (Fridley et al. 2009), and bryophytes. Total percent cover within each growth form group was calculated for each plot. Using these groups, community structure was compared in invaded and non-invaded plots using ANOSIMs and NMDS ordinations using Jaccard dissimilarity values for each burn/invasion treatment. Paired *t*-tests compared growth form groups within invaded and non-invaded plots in all burn/invasion treatments.

One-way ANOVAs were used to assess differences of impacts to community composition by *P. tomentosa* invasion between fire regimes and *P. tomentosa* growth stages. Fire regime and *P. tomentosa* life stage were both used as factors. $D_J$ and differences in H', S, and J between each pairwise invaded and non-invaded plot were used as response variables. To assess differential impacts to community structure, each factor was tested with community structure data and $D_J$ as the response variable.

The effect of *P. tomentosa* invasion on post-fire recruitment and establishment of the *P. pungens* was evaluated through paired *t*-tests comparing *P. pungens* coverage and frequency in invaded and non-invaded plots. Paired *t*-tests were repeated in each burn/invasion treatments with seedling (< 1.5 m) data used for immature invasions in the 2000/2013 burn area. Frequency data for *P. tomentosa* age were compiled for each fire regime. All statistical analyses were performed using "R" version 3.1.2 (R Development Core Team 2014).

### *Comparison of genetic diversity and differentiation between two populations*

The 38 tree samples depicted 2 unique genotypes for further analysis. Individuals that did not amplify at a minimum of 4 out of 5 loci were excluded prior to analysis (~2.5%). Descriptive genetic statistics were calculated in Genalex 6.5 (Peakall and Smouse 2006, 2012). Within population statistics included allelic diversity (average number of alleles per locus), effective allelic diversity (average number of effective alleles per locus), average number of rare alleles per locus, expected heterozygosity, observed heterozygosity, and the fixation index.

## **Results**

### *Invasion impact on species composition*

Reductions in H' and S in invaded plots followed similar patterns in both the mature invasions in the 2000/2007 burn area and immature invasions (H': $p = 0.011$, S: $p = 0.007$, and H': $p = 0.050$, S: $p = 0.020$, respectively). Marginal reductions of J also occurred in invaded plots of mature invasions in the 2000/2007 burn area ($p = 0.089$). While diversity indices trended lower in invaded plots of mature invasions in the 2000/2013 burn area, impacts were not significant (H': $p = 0.205$, S: $p = 0.353$, J: $p = 0.279$) nor lower than those of the other sampling groups. Relative impacts of *P. tomentosa* invasion on each index indicate trends of progressively increasing impacts on J, H', and S respectively (Table 1).

ANOSIM results indicate community shifts in all burn/invasion treatments (2000/2007-mature $R = 0.436$, $p = 0.001$; 2000/2013-immature $R = 0.550$, $p = 0.001$; 2000/2013-mature $R = 0.414$, $p = 0.003$). NMDS analyses provide additional support of community differences in invaded and non-invaded plots (Fig. 1).

Indicator species analyses reveal the tree, *Nyssa sylvatica* (black gum; $p = 0.049$), and a woody ground cover species, *Gaultheria procumbens* (wintergreen; $p = 0.074$), to be associated with non-invaded plots in immature invasions in the 2000/2013 burn area. No other species were associated with invaded or non-invaded plots in any other burn/invasion treatment.

### *Invasion impact on community structure*

ANOSIM results indicate community structure is different between invaded and non-invaded plots in only immature invasions ($p = 0.002$). This observed difference is largely explained by the increase in tree coverage ($p = 0.040$) and marginal decreases in shrubs and vines in invaded plots ($p = 0.098$ and $p = 0.067$ respectively; Table 2). Mature invasions in both burn areas did not reveal overall shifts in community structure (2000/2007: $p = 0.585$; 2000/2013: $p = 0.132$). Each of these burn/invasion treatments did exhibit differences in individual life form groups with an increase in tree coverage in invaded plots of the 2000/2013 burn area ($p = 0.021$) and deceases in graminoid and bryophyte coverage in invaded plots of the 2000/2007 burn area ($p = 0.037$ and $p = 0.084$ respectively; Table 2). NMDS analyses mirror ANOSIM results for community structure shift in each burn/invasion treatment (Fig. 2).

### *Differences among burn/invasion treatments*

Results from one-way ANOVAs using $D_J$ as the response variable indicate that total differences in community structure shift in invaded plots were marginally different in mature and immature invasions within the 2000/2013 burn area ($F_{1,7} = 4.102$, $p = 0.064$). No other differential impacts were found between burn regimes or *P. tomentosa* life stages.

### *Post-fire recruitment of* P. pungens *and* P. tomentosa

The fire-promoted tree *P. pungens* was dominant in many plots within immature invasions in the 2000/2013 burn area (mean cover = 17.3%; mean seedling frequency = 36.3). *Pinus pungens* seedling frequency was not different between invaded and non-invaded plots in this group ($p = 0.348$). Age classes of *P. tomentosa* demonstrate that it is promoted by large-scale fire with the age of most collected specimens correlating with time since fire (Fig. 3). Some subsequent recruitment in mature populations also exists. Recruitment of *P. tomentosa* outside of a burn area is also supported by some ($n = 8$) individuals located in the 2000/2007 burn area with ages matching time since fire for the 2000/2013 burn regime (Fig. 3). Some individuals ($n = 4$) in the 2000/2013 burn area also have ages correlating to the 2007 fires.

### *Genetic Diversity*

From 38 samples, I scored 37 for each of the 5 microsatellite loci. Genetic diversity within and among the two sampled populations was very low with four of five loci characterized as monomorphic across populations. Only one locus (PT 187, Wang et al. 2013) contained one rare allele in only one sample; all other samples were identical.

## **Discussion**

Our results indicate that *P. tomentosa* may alter native plant community composition within mature and immature invasions and two distinct fire regimes. This shift varies in magnitude and may include decreases in diversity based on *P. tomentosa* life stage and burn history. Beyond community composition, *P. tomentosa* invasions also shifted community structure in different ways across each burn invasion treatment. These results indicate that *P. tomentosa* can be a significant plant invader capable of strongly impacting invaded communities.

### *Community Shift by P. tomentosa Invasions*

Invaded plots significantly differed from non-invaded plots in all three burn/invasion treatments based on $D_J$ values. While community shift was significant in each burn/invasion treatment, its scope and magnitude varied. Diversity indices show decreases in invaded plots within mature invasions in the 2000/2007 burn area and immature invasions in the 2000/2013 burn area. Negative effects on diversity were not detected in mature invasions within recently burned areas. These differences in observed diversity across burn/invasion treatments indicate recent fire that does not recruit *P. tomentosa* may diminish impacts of invasion (Table 1). Community structure was inconsistently affected by *P. tomentosa* invasion with only immature invasions exhibiting overall shifts. These overall shifts decreased in older invasions however, different life forms were variably impacted by invasion in each burn/invasion treatment (Table 2). Within the 2000/2013 burn area, tree coverage in mature and immature invasions increased in invaded plots likely due to the presence of *P. tomentosa* in these plots. Graminoids and bryophytes were negatively impacted in invaded plots within mature invasions of the 2000/2007 burn area. This decrease may be explained by reduced light levels for these ground cover species which, in the xeric and exposed areas they were largely found, are often light dependent (Newell and Peet 1998). This is supported by Gulezian and Nyberg (2011) who also found decreases in graminoid cover as stands of an invasive *Pinus sp*. became more established. The authors posited that reduced light levels are likely responsible for this progressive decrease in ground cover species.

Differences in community shift among burn/invasion treatments may be attributable to multiple factors including native species characteristics, *P. tomentosa* population characteristics, and time since fire (Keeley 2006; Hejda et al. 2009; Fried et al. 2014). The shifts in diversity, community composition, and community structure in invaded plots of recently burned immature invasions may be explained in these areas by the dense groundcover and large leaves of *P. tomentosa* that crowd out and shade competing native plants. These immature invasions also occur in fire-adapted communities and may decrease the post-fire recruitment of fire promoted native plants (Keeley 2006). Mature invasions in the 2000/2007 burn area also showed community composition shifts and diversity decreases with invasion. These impacts may stem from the more established nature of these invasions (Staska et al. 2014). While *P. tomentosa* cover did not change between mature burn treatments, invaded plots in the 2000/2007 burn area contained significantly more *P. tomentosa* individuals than did mature plots in the recent burn area. Additionally, multiple *P. tomentosa* age classes exist in this sampling area (Fig. 3), indicating that the invader occupies more community levels (e.g. overstory, midstory, understory) and may crowd out native species that would otherwise fill these niches. This explanation is supported by Gulezian and Nyberg's (2011) observation of increasing differences between invaded and non-invaded areas as age increased in invasive *Pinus nigra* stands likely due to decreased light levels.

Sampling invaded and uninvaded sites allows for large data sets that may cover a variety of environments and factors related to the invader (Hejda et al. 2009). However, this approach, coupled with the inherent constraints of observational studies, limits the confidence associated with accurately comparing pairwise plots since they may be different in factors beyond invasion status. Nonetheless, I argue that the community shifts shown here result primarily from *P.*

*tomentosa* invasion. This is evidenced by the high mean $D_J$ values (Table 1) and ANOSIM statistics (*R*) comparing invaded and non-invaded plots in each burn/invasion treatment. Also, each non-invaded plot in our study was closely located (< 45 m) to its corresponding invaded plots and contained matching habitat conditions. The geographic limitation of our study area was dictated by coinciding fire chronosequences and *P. tomentosa* invasions. While our results come from a limited geographic area, I again argue that because community shift associated with *P. tomentosa* invasion is consistent across varied study sites, our results provide a reasonable estimation of impact by an invasive tree for which none previously existed.

### *Post-fire recruitment of* P. tomentosa *and fire-promoted native species*

I found that fire promotes *P. tomentosa* recruitment, supporting earlier work by Kuppinger et al. (2010) and Dumas et al. (2007). In addition to high frequencies of *P. tomentosa* individuals correlating in age to the most recent fire, our data also indicate that recruitment is promoted beyond fire boundaries because the ages of some individuals (n = 4) in the 2000/2013 burn area correspond with the 2007 fires (Fig. 3). This observation is potentially explained by previous research supporting that fire related cues including light and liquid smoke promote *P. tomentosa* seed germination (Todorović et al. 2005, 2010). Working in the LGWA, Kuppinger et al. (2010) also found no subsequent *P. tomentosa* recruitment after the initial invasion following the 2000 fire. No mature individuals were present during their research. The multiple age classes I observed in the 2000/2007 burn area support that once a mature *P. tomentosa* population is established, it can successfully establish new individuals without a large-scale disturbance.

Recruitment of the dominant serotinous tree *P. pungens* was not affected by *P. tomentosa* invasion. While not significant, *P. pungens* seedling abundance in immature invasions was positively correlated with *P. tomentosa* presence, indicating that total post-fire recruitment may be higher in invaded plots. Indicator species analysis revealed that the *N. sylvatica* was associated with only non-invaded plots in immature invasions. Because *N. sylvatica* is long-lived and has fire-resistant properties (Abrams 2007; Silver et al. 2013), it is likely that many individuals survived the 2000 and 2013 fires in this area. Its strong association with non-invaded plots supports that its presence may decrease post-fire recruitment of *P. tomentosa*.

### *Genetic diversity*

Almost no genetic diversity existed in five microsatellite loci of two sampled *P. tomentosa* populations in the LGWA. Our results contrast sharply with previous research in the tree's native range which found between two and six alleles at each locus I studied (Wang et al. 2013). Our results, coupled with its high invasion success in the LGWA and other areas, supports the idea that *P. tomentosa* in the LGWA, like other plant invaders with low genetic diversity (Taylor and Hastings 2004; Poulin et al. 2005; Zhang et al. 2010; Yu et al. 2014), may be facilitated by a few highly competitive genotypes and likely resulted from a single introduction.

Colonizing novel environments presents genetic challenges to invading plants as they confront new selection pressures. These new pressures may actively select more adaptive and flexible genotypes (Davidson et al. 2011). Additionally, low genetic diversity is often thought to limit invasion success by hindering an invader's ability to evolve and match its new surroundings (Sakai et al. 2001; Allendorf and Lundquist 2003). Inbreeding may also limit the long term survival of genetically similar plant populations (Allendorf and Lundquist 2003). Our research, however, adds to the growing literature demonstrating that low genetic diversity may be associated with a successful plant invader (Taylor and Hastings 2004; Poulin et al. 2005; Zhang et al. 2010; Yu 2014). By possessing a highly competitive genotype with very little differentiation, rapid invasion may be facilitated by a non-native plant's increased competitive ability (Le Roux et al. 2007; Harrison and Mondor 2011; Yu et al. 2014). This highly competitive genotype may allow *P. tomentosa* and other invaders to avoid the genetic bottleneck commonly associated with lag times in many plant invasions (Dlugosch and Parker 2008). Additionally, selection for fast-growing and hardy *P. tomentosa* individuals in the timber and horticultural trades may have promoted more competitive genotypes in its exotic range.

Although additional sampled populations are necessary to better understand *P. tomentosa* gene flow, the low diversity in the LGWA suggests high functional connectivity between distinct populations. This finding supports research by Kuppinger (2008) suggesting that *P. tomentosa* seeds can disperse long distances (up to 10 km). The two almost genetically identical sampled populations also indicate that a large gorge does not serve as dispersal barrier. The apparent high dispersal ability of *P. tomentosa* demonstrates the importance of removal efforts prior to seed production. The low genetic diversity in the LGWA is also likely the result of a founder effect following a single introduction of *P. tomentosa* to the area (Dlugosch and Parker 2008; Yu et al. 2014). This possibility is not mutually exclusive with the competitive genotype hypothesis and indicates that one introduction of *P. tomentosa* may be sufficient to facilitate a large-scale invasion.

### *Management implications*

Our research provides a quantifiable understanding of the native plant community shift based on *P. tomentosa* invasion. Because *P. tomentosa* invasions significantly impact native plant communities, affected areas should be targeted for invasive plant control. Previous research recommended a post-establishment lag period for *P. tomentosa* control based on its poor competition with regenerating native vegetation and a prioritization towards exposed and xeric areas where its persistence is greatest (Kuppinger et al. 2010). Our results suggest that because immature post-fire invasions significantly impact community composition, structure, and diversity, immediate treatment to mitigate impacts may be warranted. It is possible that treating these immature populations will also encourage more effective overall treatment results and may reduce the total cost of control/eradication (Taylor and Hastings 2004; Miller et al. 2010). Treatment of immature invasions may also be more appropriate because hand pulling seedlings with their roots can effectively control invasions (Chapter 2). Treating longer lived populations limits the ability to hand pull individuals and the efficacy of cutting larger individuals may be limited by the vigorous re-sprouting ability of *P. tomentosa* (Longbrake 2001). However, it is unclear if this early treatment will reduce effects by *P. tomentosa* invasion, particularly because its impact may be tied to effects on post-fire recruitment. Regardless of treatment lag times, its high fecundity and dispersal coupled with an ability to effectively reproduce without a large-scale disturbance supports the notion that treatment before maturity should be emphasized.

The strong promotion of *P. tomentosa* invasion following fire disturbances may influence management decisions regarding fire. If fire is used as a management tool in or near invaded areas, pre- and post-fire treatment will be important to stem the abundance and effects of post-fire invasions. Treatment of *P. tomentosa* following wildfire should also be emphasized. Additionally, *P. tomentosa* can form a persistent seedbank and remain viable for an estimated 15 years (Longbrake 2001) indicating that invasions could return after a population is effectively controlled. Kuppinger (2008) however found that most *P. tomentosa* seeds germinate within the first year and that seeds have high mortality when exposed to fire. This indicates that fire may not promote seed bank germination of *P. tomentosa* seeds and that post-fire recruitment of *P.*

*tomentosa* stems from seeds dispersed following the fire. Thus, while fire promotes invasion and post-fire management should monitor and/or treat *P. tomentosa* invasions, its invasion success is likely not dependent on a persistent seed bank.

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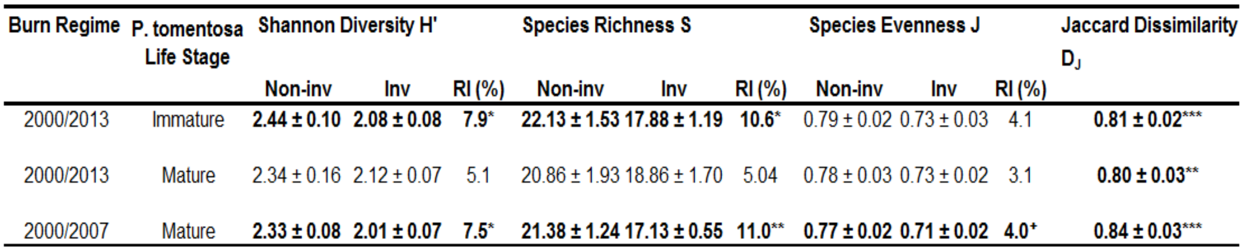
# Tables

**Table 1.** Results for Shannon diversity (H’), species richness (S), and species evenness (J) (mean ± se; n = 8, n = 7 for mature invasions in the 2000/2013 burn regimes, respectively). The relative impact (RI; $RI_V = (V_{NI} - V_I)/(V_{NI} + V_I)$, where V is the variable of interest, NI is the non-invaded plot, and I is the invaded plot) of these indices was calculated as the mean reduction in invaded plots compared to non-invaded plots. Jaccard dissimilarity ($D_J$) indicates differences in community structure between non-invaded and invaded plots (mean ± se). Significant differences are bold. $^{+}$ $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$



| Burn Regime | *P. tomentosa* Life Stage | Shannon Diversity H' | | | Species Richness S | | | Species Evenness J | | | Jaccard Dissimilarity $D_J$ |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Non-inv | Inv | RI (%) | Non-inv | Inv | RI (%) | Non-inv | Inv | RI (%) | |
| 2000/2013 | Immature | **2.44 ± 0.10** | **2.08 ± 0.08** | **7.9$^{*}$** | **22.13 ± 1.53** | **17.88 ± 1.19** | **10.6$^{*}$** | 0.79 ± 0.02 | 0.73 ± 0.03 | 4.1 | **0.81 ± 0.02$^{***}$** |
| 2000/2013 | Mature | 2.34 ± 0.16 | 2.12 ± 0.07 | 5.1 | 20.86 ± 1.93 | 18.86 ± 1.70 | 5.04 | 0.78 ± 0.03 | 0.73 ± 0.02 | 3.1 | **0.80 ± 0.03$^{**}$** |
| 2000/2007 | Mature | **2.33 ± 0.08** | **2.01 ± 0.07** | **7.5$^{*}$** | **21.38 ± 1.24** | **17.13 ± 0.55** | **11.0$^{**}$** | **0.77 ± 0.02** | **0.71 ± 0.02** | **4.0$^{+}$** | **0.84 ± 0.03$^{***}$** |

**Table 2.** Percent coverage of plant life forms in non-invaded and invaded plots. Values represent mean aggregates of total species percent covers within each life form. The relative impact (RI; $RI_V = (V_{NI} - V_I)/(V_{NI} + V_I)$, where V is the variable of interest, NI is the non-invaded plot, and I is the invaded plot ) of these indices was calculated as the mean reduction in invaded plots compared to non-invaded plots. Jaccard dissimilarity indicates differences in community structure between non-invaded and invaded plots (mean ± se; n = 8, n = 7 for mature invasions in the 2000/2013 burn regime). Significant differences are bold. $^{+}$ $p < 0.10$, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$

| Burn Regime | *P. tomentosa* Life Stage | Jaccard Dissimilarity DJ | Trees | | | Shrubs | | | Herbs | | | Graminoids | | | Vines | | | Bryophytes | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Non-inv | Inv | RI | Non-inv | Inv | RI | Non-inv | Inv | RI | Non-inv | Inv | RI | Non-inv | Inv | RI | Non-inv | Inv | RI |
| 2000/2013 | Immature | **0.58 ± 0.01****| **42.00** | **82.31** | **-32.43%** * | 16.07 | 7.13 | 38.57% | 35.19 | 27.94 | 11.49% | 10.50 | 12.25 | -7.69% | **6.38** | **3.50** | **29.11%** $^{+}$ | 7.81 | 11.75 | -20.13% |
| 2000/2013 | Mature | 0.43 ± 0.02 | **67.64** | **119.64** | **-27.77%** * | 21.50 | 25.21 | -7.95% | 34.93 | 32.50 | 3.60% | 17.71 | 11.43 | 21.57% | 6.43 | 17.57 | -46.43% | 4.21 | 4.57 | -4.07% |
| 2000/2007 | Mature | 0.48 ± 0.02 | 71.13 | 99.75 | -16.75% | 33.69 | 28.50 | 8.34% | 34.75 | 25.56 | 15.23% | **13.06** | **4.06** | **52.55%** * | 5.69 | 8.31 | -18.75% | **4.13** | **2.19** | **30.69%** $^{+}$ |

## Figure Legends

**Figure 1** NMDS ordinations of vegetation plots grouped as invaded (green) and non-invaded (red) areas with immature invasions burned in 2000/2013 (**a**), mature invasions burned in 2000/2013 (**b**), and mature invasions burned in 2000/2007 (**c**). Dispersion ellipses are based on the standard errors of the plots' weighted means at a 95% confidence limit.

**Figure 2.** NMDS ordinations of community structure for vegetation plots grouped as invaded (green) and non-invaded (red) areas with immature invasions burned in 2000/2013 (**a**), mature invasions burned in 2000/2013 (**b**), and mature invasions burned in 2000/2007 (**c**). Species within each plot were grouped as trees, shrubs, herbs, graminoids, vines, and bryophytes. Dispersion ellipses are based on the standard errors of the plots' weighted means at a 95% confidence limit.

**Figure 3.** Frequency data for *P. tomentosa* ages in two fire regimes in the LGWA. Ages were calculated from tree ring data collected from one representative *P. tomentosa* individual (> 2.0 cm dbh) in each 2 m height class present in invaded plots (n = 8 plots in 2000/2007 burn area; n = 15 plots in 2000/2013 burn area).

# Figures

## Figure 1

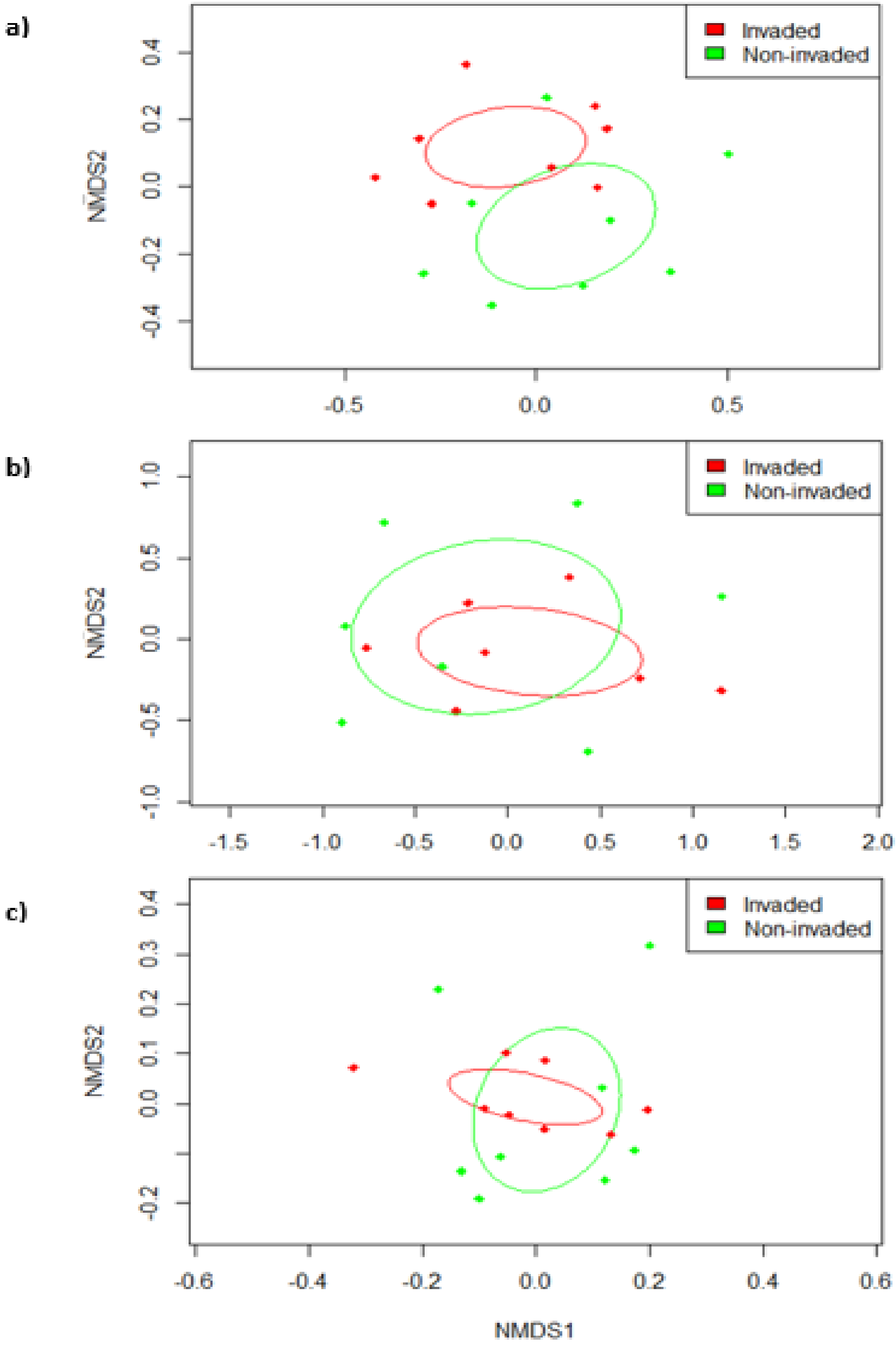


**Figure 2**

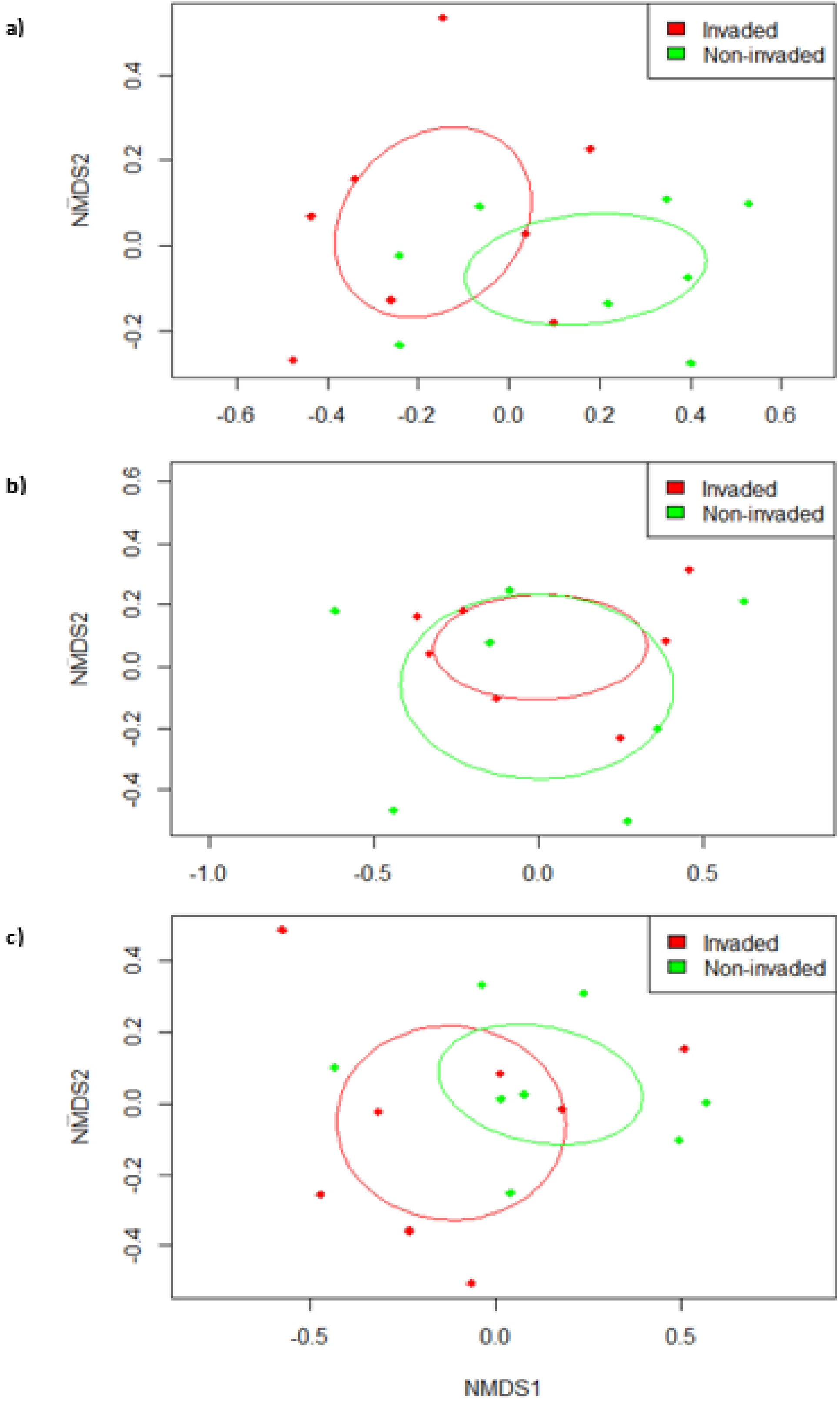
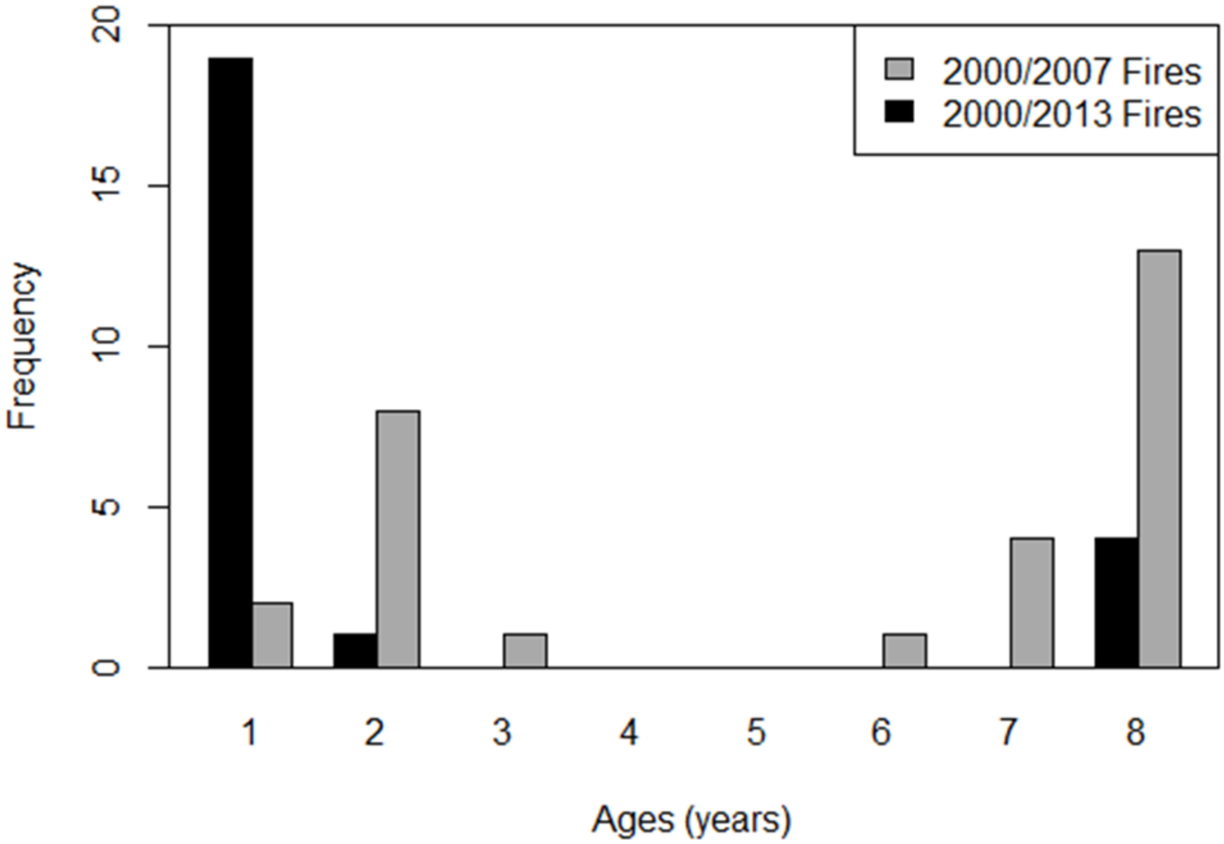
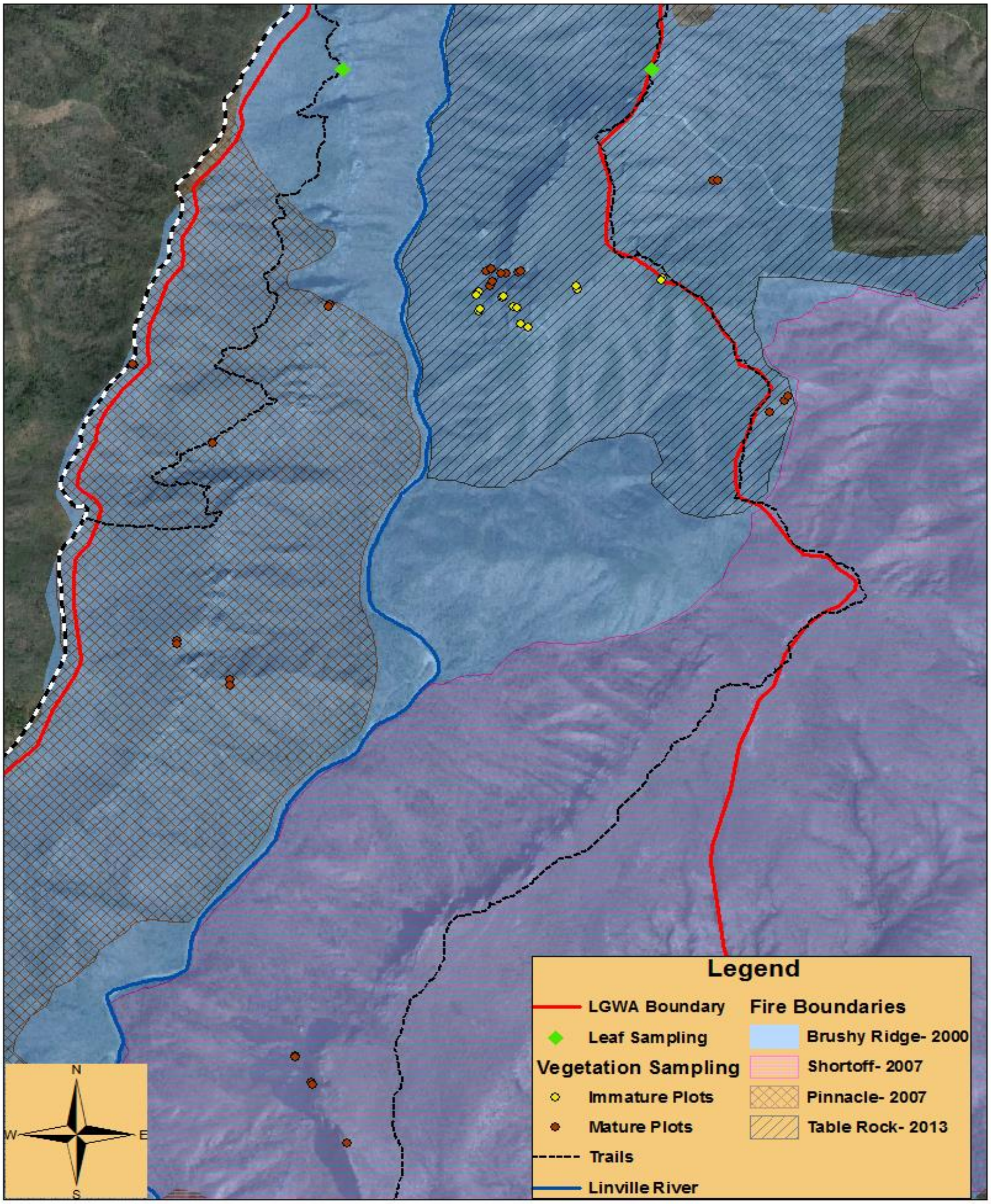

a)
Invaded
Non-invaded
NMDS2
0.4
0.2
0.0
-0.2
-0.6
-0.4
-0.2
0.0
0.2
0.4
0.6
b)
Invaded
Non-invaded
NMDS2
0.6
0.4
0.2
0.0
-0.2
-0.4
-1.0
-0.5
0.0
0.5
c)
Invaded
Non-invaded
NMDS2
0.4
0.2
0.0
-0.2
-0.4
-0.5
0.0
0.5
NMDS1

**Figure 3**



2000/2007 Fires
2000/2013 Fires
Frequency
0
5
10
15
20
1
2
3
4
5
6
7
8
Ages (years)

**Appendix 1.** Map of vegetation sampling and genetic material sampling sites.



**Appendix 2.** Plant species list

Plant samples were identified to species if possible. If species identification was not possible, samples were identified to genus, family, or morphotype. * indicates exotic-invasive species.

*Acer rubrum*
*Acer spicatum*
*Ageratina altissima*
*alternate spear leaf*
*Amelanchier arborea*
*Amphicarpaea bracteata*
*Andropogon virginicus*
*Antennaria plantaginifolia*
*Aralia spinosa*
*Asplenium montanum*
*Asplenium platyneuron*
*Asteraceae sp.*
*Asteraceae sp. 2*
*Athyrium aspleniodes*
*bamboo shrub*
*Betula lenta*
*blowing grass*
*blue tipped grass*
*Carya tomentosa*
*Cassia fasciculata*
*Celastrus orbiculatus**
*Circaea lutetiana ssp. Canadensis*
*Circea sp.*
*combination tree*
*Commelina diffusa*
*Companulla divaricata*
*Coreopsis verticillata*
*Corydalis sempervirens*
*Corylus cornuta*
*crusty ground moss*
*cucumber tree*
*dandy sedge*
*Danthonia compressa*
*Danthonia sericea*
*dark frilly moss*
*dark green rock moss*
*Dennstaedtia punctilobula*
*Dichanthelium commutatum*
*Dioscorea villosa*
*dry frilly moss*
*dry green moss*
*dry short green moss*
*Erigeron annuus*
*Erigeron canadensis*
*Eupatorium capillifolium*
*Eupatorium rotundifolium var. ovatum*
*Euribia divaricata*
*Eurybia spectabilis*
*flowering blue tipped grass*
*Fothergilla major*
*Fraxinus sp.*
*frilly green moss*
*frilly lespedeza*
*Galax urceolata*
*Gaultheria procumbens*
*Gaylussacia baccata*
*Geranium maculatum*
*Goodyera pubescens*
*green bean moss*
*green bundle grass*
*green flowering grass*
*green fruit grass*
*green fruiting moss*
*green ground feather*
*green normal grass*
*green spear grass*
*green stalk grass*
*hairy heath sp.*
*half-clasp aster*

*Hamamelis virginiana*
*Heuchera americana*
*Hieracium scabrum*
*Hydatica petiolaris*
*Hydrangea arborescens*
*Hypericum buckleyi*
*Ilex ambigua*
*Kalmia latifolia*
*kayah nut*
*Lactuca canadensis*
*Lamiaceae sp.*
*Laptortea canadensis*
*Lespedeza procumbens*
*Lillium michauxii*
*Liquidambar stryraciflua*
*Liriodendron tulipifera*
*Lobelia sp.*
*Lobilia inflata*
*long moss*
*Lycopodium digitatum*
*Lysimachia quadrifolia*
*Magnolia fraseri*
*Micranthes petiolaris*
*Microstegium vimineum**
*Mitchella repens*
*mixed leaf herb*
*mtn. blade grass*
*mtn. clump grass*
*mtn. grass 2*
*mystery grass*
*Nyssa sylvatica*
*onion grass*
*ovate goldenrod*
*Oxalis montana*
*Oxydendron arboreum*
*pale cliff moss*
*Parthenocissus quinquefolia*
*Paulownia tomentosa*
*Phytolacca americana*
*pine seedling*
*Pinus pungens*
*Pinus rigida*
*Pinus strobus*
*Poaceae sp. 1*
*Poaceae sp. 2*
*Poaceae sp. 3*
*Poaceae sp. 4*
*Polygala sanguinea*
*Polygala verticillata*
*Polygonatum biflorum*
*Polystichum acrostichoides*
*popcorn moss*
*Prunus serotina*
*Pteridium aquilinum*
*Pycnanthemum montanum*
*Pycnanthemum pycnanthemoides var. pycnanthemoides*
*Quercus coccinea*
*Quercus falcata*
*Quercus montana*
*Quercus rubra*
*Rhododendron catawbiense*
*Rhododendron maximum*
*Rhus copallina*
*Rhus glabra*
*Rhus typhina*
*Robinia hispida*
*Rubus allegheniensis*
*Solidago arguta var caroliniana*
*Solidago arguta var. arguta*
*Sanguinaria canadensis*
*Sassafras albidum*
*blue-vein grass*
*Selaginella tortipila*
*serrate ground violet*
*short blue grass*
*Smilacina racemosa*
*Smilax rotundifolia*
*smooth red opposite*
*Solidago altissima var. altissima*
*Solidago junceae*
*Solidago nemoralis*
*Solidago sp. 1 (squarrosa group)*
*Solidago sp. 3*

*Stellaria graminea*
*strange tooth shrub*
*Symphyotrichum pilosum*
*Symplocaceae tinctoria*
*Taraxacum officinale*
*Thalictrum coriaceum*
*Theypteris noveboracensis*
*tiny dry moss*
*toadstool sedge*
*Toxicodendron radicans*
*v leaf*
*Vaccinium pallidium*
*Vaccinium stamineum*
*velvet tree*
*Verbascum thapsus**
*Viola sp. 1*
*Viola sp. 2*
*Viola sp. 3*
*Viola sp. 4*
*Vitis labrusca*
*Waldsteinia fragaroides*
*yellowish flat grass*

# CHAPTER 2: POST-TREATMENT EFFECTS OF MANUAL AND CHEMICAL CONTROL METHODS FOR IMMATURE PAULOWNIA TOMENTOSA INVASIONS IN THERMIC OAK-PINE FORESTS

**Abstract.** *Paulownia tomentosa* invasions have a deleterious effect on native plant communities necessitating effective control measures. Here I compare the effectiveness and non-target effects of hand pulling and targeted foliar application of triclopyr for immature *P. tomentosa* invasions in thermic oak-pine forests of the Linville Gorge Wilderness Area in the Southern Appalachians. Both methods effectively removed *P. tomentosa* seedlings one year following treatment with ~ 1% cover in manually and chemically treated plots. Native plant community diversity, composition, and structure were not significantly different between treatment groups, indicating that either method is appropriate for immature *P. tomentosa* control. Based on these comparable post-treatment effects, land managers may factor treatment cost, proximity to sensitive/rare plant communities, and age of invasion into making management decisions for *P. tomentosa* control.

## Introduction

Plant invasions frequently drive environmental change with ecological and economic consequences (Pejchar and Mooney 2009; Vilá et al. 2011; Pyšek et al. 2012). Detrimental changes often lead land managers to pursue effective control of invasive plant populations; however, not all control options are equally effective. Herbicide application and manual removal are both common practices in invasive plant control (Miller et al. 2010; Kettenring and Adams 2011). The application methods, concentrations, and selectiveness of herbicides in invasive plant control are highly variable (Miller et al. 2010). Manual treatments also vary depending on the treatment site, target species, and available resources (Miller et al. 2010; Kettenring and Adams 2011). Each treatment option in its different forms has associated advantages and disadvantages including differing levels of negative effects on non-target species, effectiveness in controlling target invasives, and cost (Kettenring and Adams 2011; Lindenmayer et al. 2015). Thus, understanding the total effects of any treatment option is critical to inform management decisions and promote effective invasive plant management.

Herbicide application is a highly effective technique in invasive plant control (Kettenring and Adams 2011; Dohn et al. 2013; Souza-Alonso et al. 2013; Lindenmayer et al. 2015), yet is frequently accompanied by non-target effects. Consequently, numerous studies have demonstrated decreased native plant diversity following herbicide application (Newmaster et al. 1999; Power et al. 2013; Souza-Alonso et al. 2013; Aguilar-Dorantes et al. 2015; Lindenmayer et al. 2015; Kaiser-Bunberry et al. 2015). This deleterious effect on native plant communities may be explained by non-target herbicide application and/or the propensity of plants to take up residual herbicide (Cornish and Burgin 2005). Additionally, herbicide application may diminish subsequent seed germination and recruitment of natives, further complicating effective native plant community restoration (Wagner and Nelson 2014). However, the negative impacts of herbicide application on native plant communities may be short-lived as these effects have been documented to cease during long-term observation (Lindenmayer et al. 2015). Consequently, herbicide treatment of invasive plant populations may be appropriate depending on the short and long-term management goals.

Manual removal of invasive plants may provide an alternative to herbicide applications in invasive plant control efforts (Flory 2010; Oneto et al. 2010). This line of treatment may be particularly useful in areas where herbicide use is precluded by regulation (i.e., wilderness areas) or in sensitive plant communities where non-target effects would be detrimental (i.e., within rare plant communities). Manual treatments can be less effective in controlling invasive plant populations than herbicide application (Burch and Zedaker 2003; Kettenring and Adams 2011; Dohn et al., 2013; Souza-Alonso et al. 2013; Jones et al. 2015; Lindenmayer et al. 2015). This reduction in efficacy when compared to herbicide treatments has implications for the role of manual removal in invasive plant control and may be due to a variety of factors. Strong re-sprouting responses in many invasive plants diminishes post-treatment mortality particularly when the complete removal of belowground biomass is not feasible (Longbrake 2001; Burch and Zedaker 2003; Oneto et al. 2010). Additionally, soil disturbance and increased light levels that can accompany manual removal may create conditions that promote subsequent plant invasion, especially when treating invaders that produce persistent seed banks (Dohn et al. 2013). The lack of herbicide application may also fail to eliminate the invader's existing soil seed bank, allowing for subsequent invasion (Wagner and Nelson 2014). The variability of effects between chemical and manual treatments indicates that land managers should use available knowledge of invader and native plant community characteristics to inform invasive plant control decisions.

*Paulownia tomentosa* invasions cause significant native community shifts and are strongly promoted by disturbances such as fire and mining (Longbrake 2001; Kuppinger et al. 2010; Lemke et al. 2012; Chapter 1). Land managers often target *P. tomentosa* invasions for control efforts due to its impacts on native plant communities and large-scale invasions following disturbance (Gaydish, Pers Comm; Jenkins and Johnson 2009). However, no known research has compared treatment methods for this invader. My study compared the efficacy and non-target effects of manually hand pulling seedlings, and foliar application of triclopyr, a broadleaf specific herbicide, in immature post-fire invasions of *P. tomentosa* in thermic oak-pine forests.

## Methods

### *Study Area*

Vegetation sampling occurred along the eastern boundary of the Linville Gorge Wilderness Area (LGWA) within the LGWA and the adjacent Pisgah National Forest. This area burned during the Brushy Ridge (2000) and Table Rock (2013) fires, with the canopy experiencing dieback in some areas following the Table Rock fire. Forests within this area are characterized as thermic oak-pine. Dominant vegetation includes *Sassafras albidum*, *Pinus pungens*, *Kalmia latifolia*, and *Nyssa sylvatica*. This area was heavily invaded by *P. tomentosa* seedlings following the Table Rock fire with an average percent cover of 40-60% (Gaydish, pers comm).

This immature *P. tomentosa* invasion was treated using manual and chemical methods in adjacent, but separate and clearly defined areas from September to early November 2014. Manual treatment included hand pulling *P. tomentosa* seedlings with total root removal when possible. Chemical treatment employed targeted foliar application of 3% triclopyr 3A mix with

1% Agri-dex surfactant and 0.5% marking dye onto *P. tomentosa* individuals. All manual treatments occurred within the LGWA and chemical treatments took place in the adjacent national forest area.

### *Vegetation sampling*

Vegetation sampling occurred in eight pairs of 4 $m^2$ plots occupying thermic oak-pine forests (Appendix 1). Paired plots occupied nearby (< 15 m) manually and chemically treated areas with comparable abiotic factors (e.g., aspect, percent soil cover, slope, etc.). Each plot pair was separated by 50 m. Vegetation sampling occurred from August 28 to September 3, 2015.

I documented the estimated percent cover for soil and all vascular plant and bryophyte species in each plot. Woody stem height for *P. tomentosa* was recorded in each plot. I recorded the stem height and DBH for the dominant native species (greatest cover) and a dominant canopy species *Pinus pungens* (table mountain pine) within each plot to compare post-treatment competitive ability of *P. tomentosa* and a dominant native species. I recorded aspect, elevation, and slope (degrees) in each plot. I visually estimated fire severity on a 1-5 scale in each plot based on scorch height, re-sprouting frequency, and percent dieback of vegetation (Kuppinger et al. 2010).

Vascular plant samples were transferred as necessary to a plant press in the field and later identified to species. If species identification was not possible, I identified vascular specimens to genus, family, or morphotype. Sampled bryophytes were classified by morphotype.

### *Statistical Analysis*

Species richness (S), species evenness (J), and Shannon diversity (H’) were calculated for each plot. I compared these indices using paired *t*-tests of manually and chemically treated plots. Differences in species composition between manually and chemically treated plots were evaluated with the Jaccard dissimilarity index ($D_J$). I used an analysis of similarities (ANOSIM) test to evaluate the significance of these community composition shifts. The *p*-value was calculated by permutation (999 permutations for $\alpha = 0.05$). Differences in H', S, and J of manually and chemically treated plots were calculated as a Relative Impact (RI; Vilà et al., 2006) where $RI_V = (V_M - Vc)/(V_M + V_C)$, where V is the variable of interest, M is the manually treated plot, and C is the chemically treated plot. A positive RI represents a decrease in the variable of interest associated with chemical treatment and a negative RI represents an increase. Non-metric multi-dimensional scaling (NMDS) analysis was fitted on $D_J$ values using the two treatment methods as factors. Indicator species analysis (using the multipatt function in the indicspecies R package; De Caeres and Legendre 2009) highlighted species associated with either manually or chemically treated plots.

To assess differences in each treatment method's effect on native community structure, I aggregated species by growth form as trees, shrubs, herbs, graminoids, vines (Fridley et al. 2009), and bryophytes. The total percent cover within each life form group was calculated. Using these groups, I compared manually and chemically treated plots using ANOSIMs, and NMDS analyses. Paired *t*-tests also compared differences of growth form groups between corresponding manually and chemically treated plots.

Differences in effects of treatment methods on post-treatment recruitment of *P. pungens* seedlings (< 1.5 m) was evaluated using paired *t*-tests comparing *P. pungens* frequency in manually and chemically treated plots. Paired *t*-tests compared *P. tomentosa* percent cover in manually and chemically treated plots to evaluate the efficacy of each treatment method. All statistical analyses were performed using "R" version 3.1.2 (R Development Core Team 2014).

## Results

There were no differences in diversity indices between manually and chemically treated plots (S: $p = 0.446$, J: $p = 0.859$, H': $p = 0.661$). ANOSIM results did not indicate community compositional shifts between manually and chemically treated plots ($R = -0.030$, $p = 0.604$; Table 1). Consistent composition between plots in both treatment areas was also supported by NMDS analysis ($p = 0.835$; Fig. 1). Indicator species analysis revealed a tree species (*Quercus prinus*) was marginally associated with manually treated plots ($p = 0.053$).

ANOSIM testing indicated that community structure did not significantly change between manually and chemically treated plots ($p = 0.615$ respectively; Table 2). NMDS analysis supported the ANOSIM test results ($p = 0.487$; Fig. 2). While no overall structural differences were observed, percent cover of shrubs was lower in chemically treated plots ($p = 0.045$; Table 2).

Frequency of *P. pungens* seedlings did not differ between treatment groups ($p = 0.446$, RI = -12.3%). *Paulownia tomentosa* percent cover was low in both manually and chemically treated plots (mean = 1.3% and 0.6% respectively) with no difference between treatment groups ($p = 0.314$).

## Discussion

The goals of effective invasive plant control include more than simply the removal of the invader. Control efforts should also encourage restoration of the native plant community at the treated site (Sinclair et al. 1995; Holmes 2001). The low post-treatment *P. tomentosa* cover and comparable native plant communities in each treatment group indicate that hand pulling and targeted foliar application of triclopyr both deliver similarly effective results in controlling invasions of *P. tomentosa* seedlings in thermic oak-pine forests.

While no significant differences in post-treatment native plant community assemblages were observed, consistent trends indicate that chemically treated areas may be more negatively affected than manually treated areas (Tables 1 and 2). These negative trends associated with foliar application of triclopyr may be partially explained by residual effects of the herbicide persisting in the soil (Cornish and Burgin 2005; Kettenring and Adams 2011). This is especially possible because the amount of herbicide applied can vary up to tenfold based on the applicator (Cornish and Burgin 2005). Herbicide application may also sterilize native seeds existing in the seed bank preventing subsequent germination (Wagner and Nelson 2014). Additionally, non-target application of herbicide may be linked to a decreased native plant community. Although no significant decreases were detected in chemically treated plots, the observed negative trends may signal more research is necessary to elucidate differences in post-treatment plant communities.

Study sites with requisite conditions to evaluate our questions are rare and to our knowledge only existed within our study area. Based on study site limitations, only one transect of eight plot pairs allowed meaningful vegetation sampling. Sampling also only occurred within thermic oak-pine forests with recent *P. tomentosa* invasions. This narrow scope of our sampling efforts limits the ability to apply our results to treatment in other habitats or longer lived invasions (Kettenring and Adams 2011). Additionally, long-term post-treatment effects may vary more between manually and chemically treated areas than short-term effects (~ 1 year; Petrov and Marrs 2000; Price and Weltzin 2003; Cornish and Burgin 2005). Control plots were also not available in this area precluding comparison to plots without *P. tomentosa* control (but see chapter 1). However, our results are applicable for informing management of immature *P. tomentosa* invasions in thermic oak-pine forests which cover significant portions of the LGWA.

While expanded sampling efforts are likely necessary, the consistency of treatment efficacy and post-treatment native plant community composition and structure in both manually and chemically treated plots indicates that each method may be a viable option for controlling immature populations of P. *tomentosa* seedlings in thermic oak-pine forests.

Due to the similarity of effects in both treatment groups, selection of appropriate *P. tomentosa* control methods may be contingent on a variety of other factors. Available management resources play a large role in deciding management strategies (Miller et al. 2010) and favors herbicide application over manual removal (Kettenring and Adams 2011). For example, Dohn et al. (2013) found foliar herbicide application requires < 7% of the cost of manual removal efforts. While cost effectiveness frequently favors herbicide application, other factors may preclude its use in many areas. Management regulations in some areas, including the LGWA, prohibit herbicide use thus necessitating manual removal. *Paulownia tomentosa* invasions also persist well in some habitats occupied by rare plants (Kuppinger et al. 2010). The potential for non-target and residual effects of herbicide should discourage its use in these areas (Cornish and Burgin 2005; Miller et al. 2010; Kettenring and Adams 2011; Wagner and Nelson 2014). The age of *P. tomentosa* invasion is another important consideration for control efforts (Miller et al. 2010). When *P. tomentosa* individuals become too large to hand pull and remove below-ground biomass, manual removal utilizing cutting will be hampered by the invader's strong re-sprouting response (Longbrake 2001; Burch and Zedaker 2003). In these cases, herbicide application using the cut-treat or stem injection methods would be more appropriate (Miller et al. 2010). Follow-up restoration activities such as restoration plantings may also augment any negative post-treatment effects stemming from control efforts and should be considered regardless of which control method is utilized (Hartman and McCarthy 2004; Kettenring and Adams 2011; Lake et al. 2013).

*Paulownia tomentosa* invasions pose a threat to biodiversity in a variety of habitats (Chapter 1) and appropriate steps should act to diminish their impact. While I acknowledge that additional research would better evaluate post-treatment effects of *P. tomentosa* control methods, our results indicate that both hand pulling and targeted foliar application of herbicide may be appropriate for controlling immature invasions in thermic oak-pine forests. Following treatment, the regular monitoring of treated areas, quick response to newly invaded areas, and revegetation efforts will limit susceptibility to re-invasion (Miller et al. 2010; Kettenring and Adams 2011). Also because *P. tomentosa* can produce large-scale invasions following disturbances (Longbrake 2001; Kuppinger et al. 2010; Lemke et al. 2012; Chapter 1), control efforts should prioritize treatment of seed-producing individuals prior to any planned disturbance event such as a prescribed burn or timber harvest.

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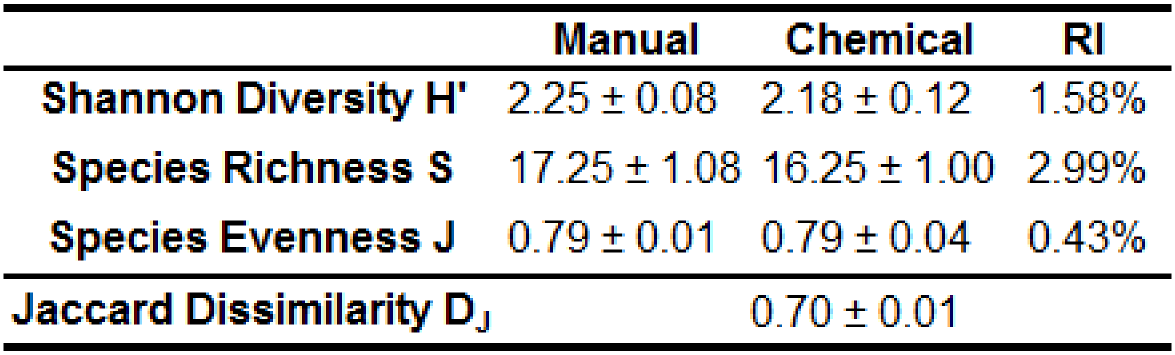
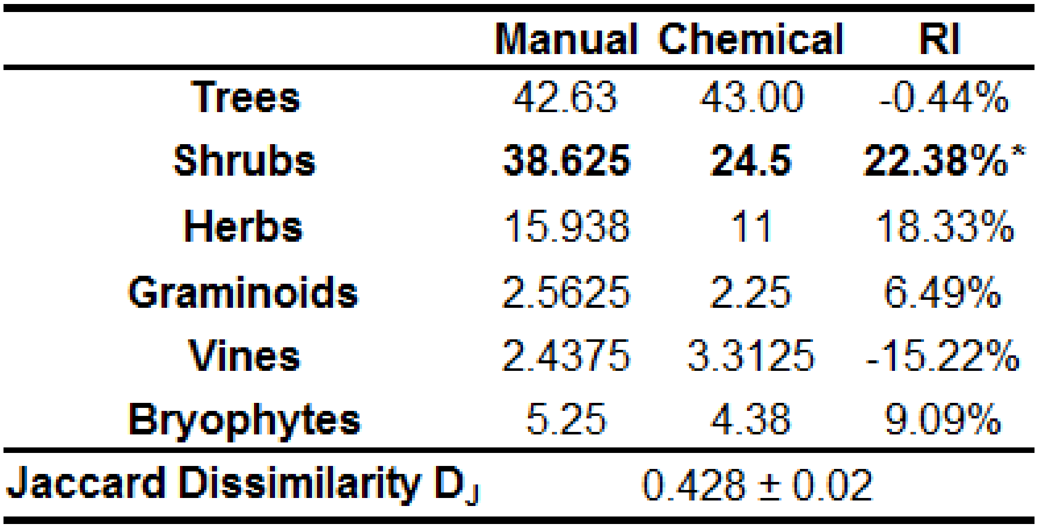
## Tables

**Table 1.** Shannon diversity (H'), species richness (S), and species evenness (J) values (mean ± se; n = 8). The relative impact (RI; $RI_V = (V_M - V_C)/(V_M + V_C)$, where V is the variable of interest, M is the manually treated plot, and C is the chemically treated plot) of differences in each index was calculated as the mean reduction in chemical plots compared to manual plots. A positive value represents a reduction. The Jaccard Dissimilarity Index ($D_J$) indicates overall differences in community composition (mean ± se).



| | Manual | Chemical | RI |
|---|---|---|---|
| **Shannon Diversity H'** | 2.25 ± 0.08 | 2.18 ± 0.12 | 1.58% |
| **Species Richness S** | 17.25 ± 1.08 | 16.25 ± 1.00 | 2.99% |
| **Species Evenness J** | 0.79 ± 0.01 | 0.79 ± 0.04 | 0.43% |
| **Jaccard Dissimilarity $D_J$** | | 0.70 ± 0.01 | |

**Table 2.** Percent coverage of plant life forms in manually and chemically treated plots. Values in each group represent the total percent coverage of all plants within each group. The relative impact (RI; $RI_V = (V_M - V_C)/(V_M + V_C)$, where V is the variable of interest, M is the manually treated plot, and C is the chemically treated plot) of differences in each index was calculated as the mean reduction in chemical plots compared to manual plots. A positive value represents a reduction. Each life form was compared between manually and chemically treated plots with paired t-test. The Jaccard Dissimilarity Index ($D_J$) indicates overall differences in community structure (mean ± sem). Bold font indicates statistical differences. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$



| | Manual | Chemical | RI |
|---|---|---|---|
| **Trees** | 42.63 | 43.00 | -0.44% |
| **Shrubs** | **38.625** | **24.5** | **22.38%*** |
| **Herbs** | 15.938 | 11 | 18.33% |
| **Graminoids** | 2.5625 | 2.25 | 6.49% |
| **Vines** | 2.4375 | 3.3125 | -15.22% |
| **Bryophytes** | 5.25 | 4.38 | 9.09% |
| **Jaccard Dissimilarity $D_J$** | 0.428 ± 0.02 | | |

# Figure Legends

**Figure 1.** NMDS ordination of vegetation plots grouped as manually treated (green) and chemically treated (red) areas. Dispersion ellipses are based on the standard errors of the plots' weighted averages at a 95% confidence limit.

**Figure 2.** NMDS ordination of community structure for vegetation plots grouped as manually treated (green) and chemically treated (red) areas. To account for community structure, species are grouped as trees, shrubs, vines, herbs, graminoids, and bryophytes. Dispersion ellipses are based on the standard errors of the plots' weighted averages at a 95% confidence limit.

# Figures

**Figure 1**

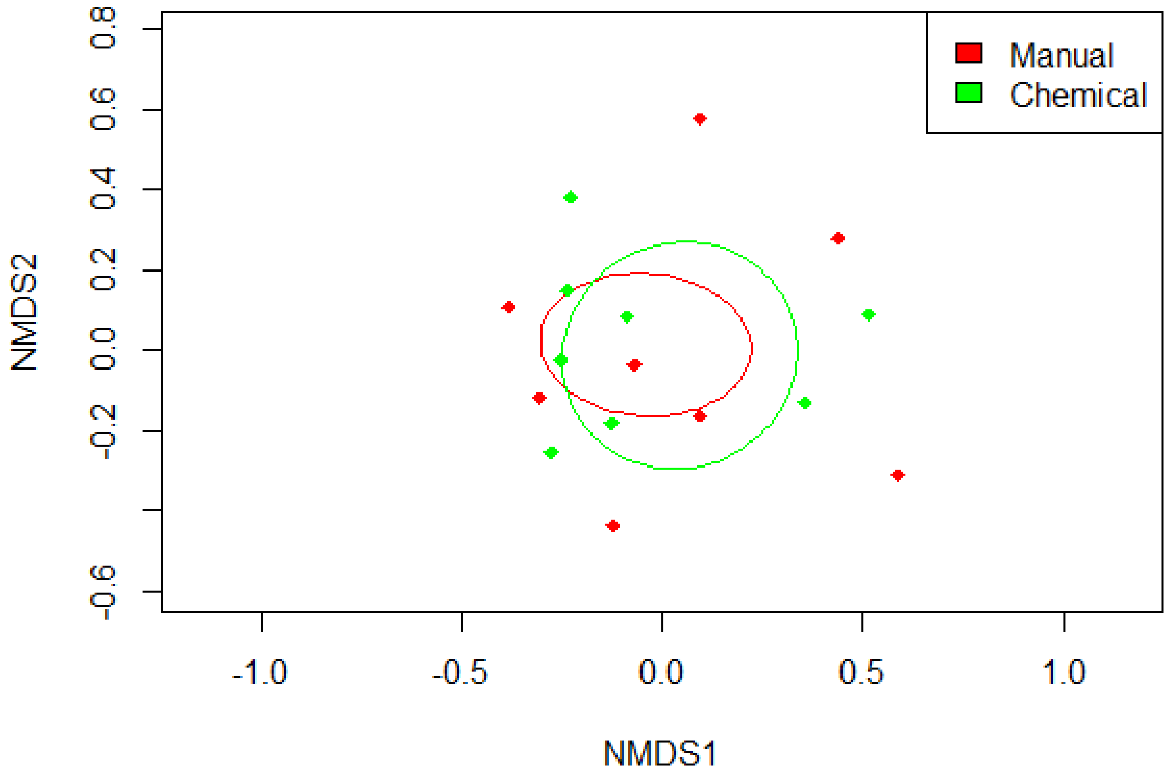
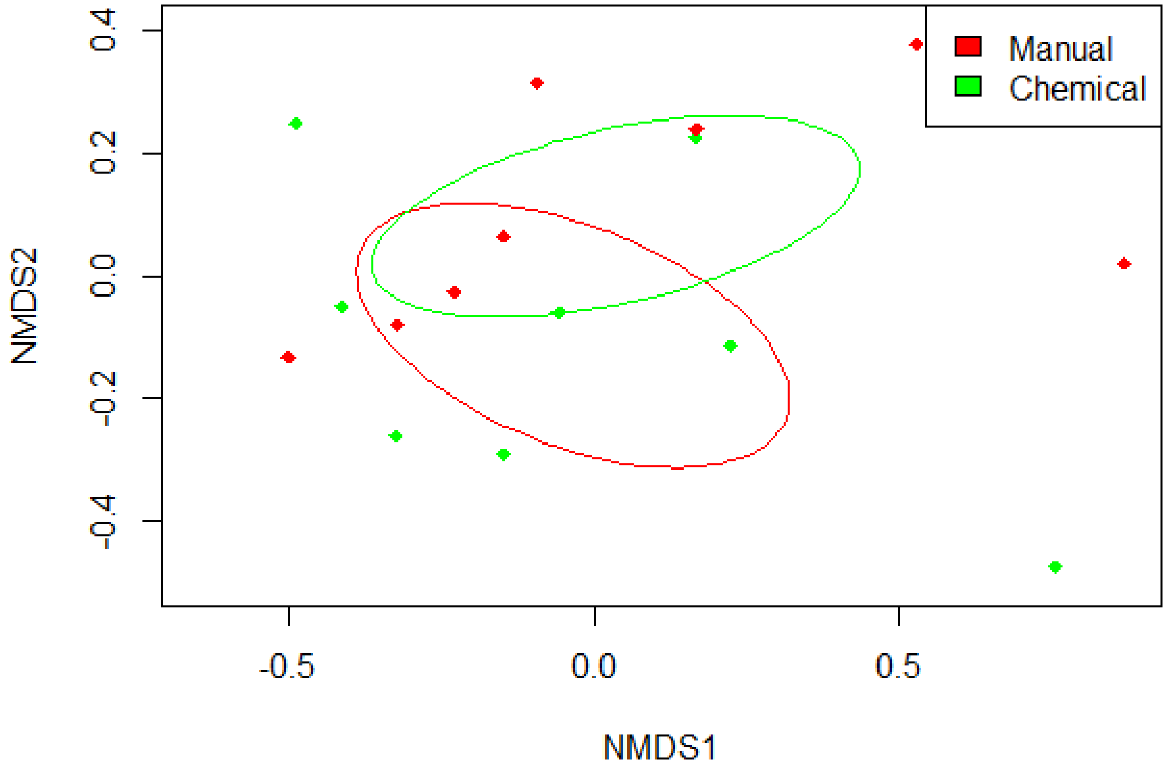
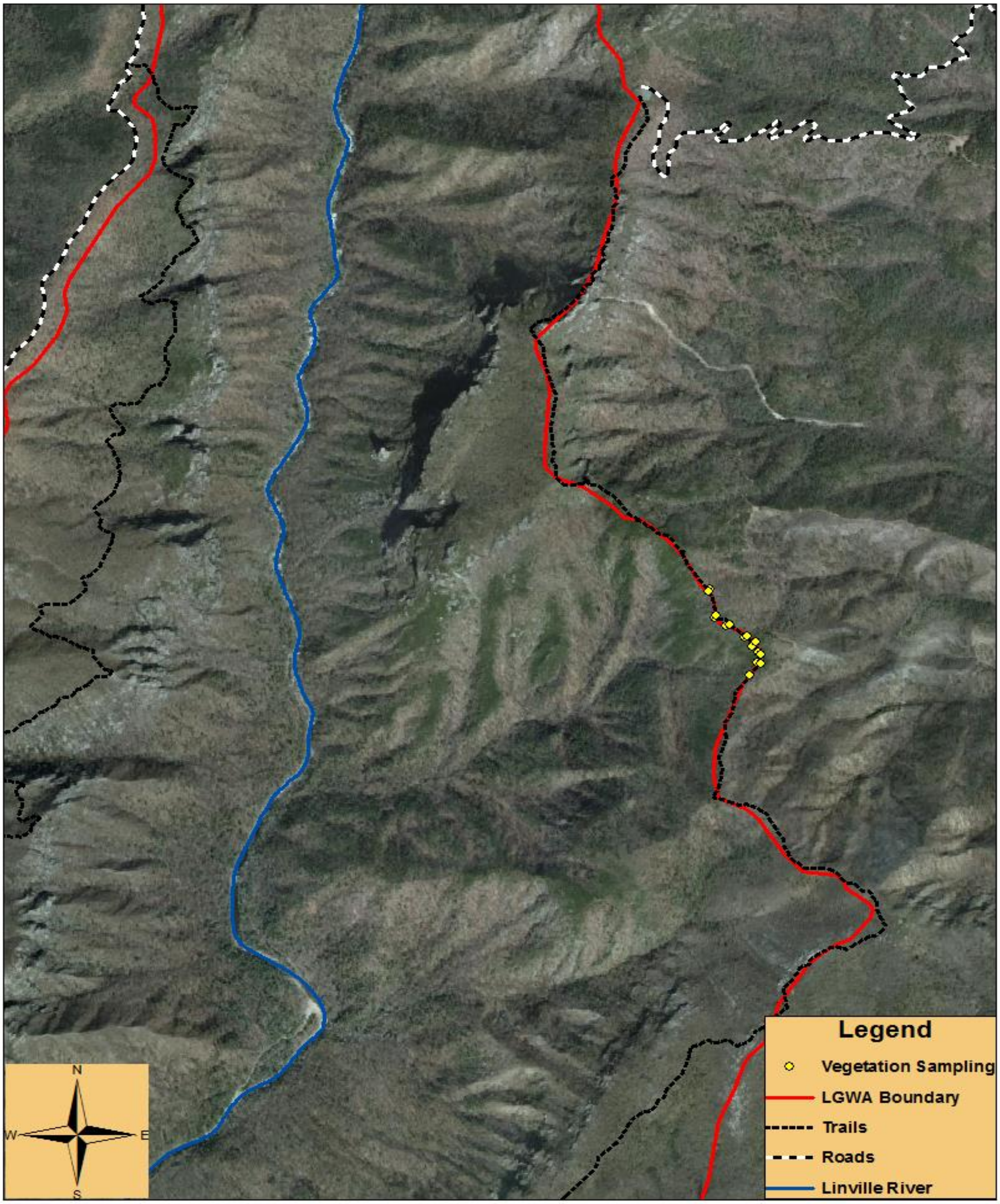


**Figure 2**



**Appendix 1.** Map of vegetation sampling sites



## **Appendix 2.** Plant Species List

Plant samples were identified to species if possible. If species identification was not possible, samples were classified by genus, family, or morphotype.

*Acer rubrum*
*Ageratina altissima*
*Antennaria plantaginifolia*
*Aralia spinosa*
*Aureolaria laevigata*
*Baptisia australis*
*Betula lenta*
*blowing grass*
*Carya tomentosa*
*Castanea dentatum*
*Chimaphila maculata*
*Companulla divaricata*
*Comptonia peregrina*
*Conium maculatum*
*Dennstaedtia punctilobula*
*Dichanthelium commutatum*
*Epigaea repens*
*Erigeron canadensis*
*Erigeron strigosus*
*Eubotrys recurva*
*Eubotrys sp.*
*Eupatorium capillifolium*
*flat blue grass*
*flat green grass*
*Fothergilla major*
*frilly green moss*
*Gaultheria procumbens*
*Hamamelis virginiana*
*Hexastylis sp.*
*Kalmia latifolia*
*Liriodendron tulipifera*
*Lobelia sp.*
*Lyonia ligustrina*
*Medeola virginiana*
*miscanthus mimic*
*Nyssa sylvatica*
*Oxydendron arboreum*
*Packera aurea*
*Paulownia tomentosa*
*Phytolacca americana*
*Pinus pungens*
*Poaceae sp. 3*
*Pteridium aquilinum*
*Quercus coccinea*
*Quercus falcata*
*Quercus montana*
*Quercus rubra*
*Rhododendron maximum*
*Rhus copallina*
*Rhus glabra*
*Robinia pseudoacacia*
*rounded plantain*
*Rubus allegheniensis*
*Sassafras albidum*
*Scleria triglomerata*
*silver leaf heath*
*Smilax rotundifolia*
*Solidago sp. 2*
*Symplocaceae tinctoria*
*Taraxacum officinale*
*Vaccinium pallidium*
*Vaccinium stamineum*
*Vitis labrusca*
*willow shrub*

# Vita

Joseph Lovenshimer was born in Morganton, North Carolina and developed a passion for the outdoors at an early age. Following this passion, he studied biology with a focus in ecology and natural history at Brevard College and was awarded a Bachelor of Science degree in 2011. While living in Brevard, he continued to explore the area's forests, rivers, and cliffs but also became deeply concerned by the threat posed by invasive species to these areas. This interest led him to Appalachian State University where he used his first wilderness playground, the Linville Gorge, as a study area while earning his Master of Science degree, which was awarded in May 2016.