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Native Plant Establishment Success Influenced by *Centaurea stoebe* Control Method

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Dedication

To all those who supported me during this endeavor, including: my parents, Brad and Jana, whose love and praise have been never-ending, who inspired my affection for nature, and who encouraged me to always follow my dreams; my mother-in-law, Betty, who has always had so much confidence in me, and who was always willing to critique yet another draft; and most of all to my wonderful husband, Chris, who has always been right by my side, and without whose love, support, and encouragement this would not have been possible.

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Abstract

Invasive species frequently hinder restoration efforts. While the effectiveness of differing control methods are often reported, the impacts these methods have on the establishment of a native plant community are often unknown. To determine methods that effectively reduce spotted knapweed (*Centaurea stoebe*) while enhancing native species establishment, we tested 12 treatment combinations consisting of an initial site preparation (mowing, mowing + clopyralid, or mowing + glyphosate), with combinations of annual adult knapweed hand pulling, and/or burning. We established 48 plots and applied site preparation treatments during summer 2008, seeded 23 native forbs and grasses during spring 2009, pulled adult knapweed annually from 2009-2012, and burned in the early spring 2012. During July of 2011 and 2012, percent cover of all species was visually estimated. By 2011, seeded species had established in all treatment plots, including plots that retained greater than 50% knapweed cover, indicating that native species successfully established despite knapweed dominance. Mowing alone had no long-term impacts on community development. Clopyralid favored non-native grass establishment, while glyphosate encouraged non-native forbs. Clopyralid had minimal impacts on native forb establishment, but did effectively control knapweed. Hand pulling reduced knapweed cover, increased non-native grass cover, and enhanced native species establishment. The established grasses helped prevent the resurgence of knapweed. Further, native graminoids were beginning to replace non-native grasses in the pulled plots. Burning had little impact, possibly due to low intensity and unseasonable weather. The application of knapweed control measures combined with seeding of native species resulted in successful native species establishment.

Table of Contents

List of Tables

List of Figures

Chapter I

Introduction

Ecological restoration is often hindered by the presence of invasive, non-native plant species, especially on degraded land. Following invasive plant establishment, restoration of a native plant community becomes very challenging (DiTomaso 2000). Invasive plants are capable of altering ecosystem function (Weidenhamer & Callaway 2010), nutrient cycles (Ehrenfeld et al. 2001; Allison & Vitousek 2004), and disturbance regimes (D'Antonio & Vitousek 1992; Brooks et al. 2004), resulting in a continuing need for long-term control efforts (D'Antonio & Meyerson 2002).

Control efforts targeting invasive species can also inhibit the development of a native community (Erskine Ogden & Rejmánek 2005; Rinella et al. 2009; Ortega & Pearson 2011). Successful control of one invasive plant may open the community to further invasion or surges in dominance of other non-natives already present (Zavaleta et al. 2001; Ortega & Pearson 2011). Unfortunately, it is infrequent for studies targeting invasive species control to report on the effects of these treatments on the restored plant community (Reid et al. 2009; Kettenring & Reinhardt Adams 2011). Thus, it is highly desirable to determine which methods can be used to control invasive plants while also allowing for either uninhibited or accelerated restoration of a native community.

Centaurea stoebe (spotted knapweed; hereafter knapweed) has invaded over 2.9 million hectares in North America (DiTomaso 2000), and can successfully invade plant communities on both remnant and disturbed sites. Following invasion, knapweed forms peripherally enlarging monocultures that seed profusely, have allelopathic effects on susceptible species, and negatively impact ecosystem richness and diversity (Watson & Renney 1974; Schirman 1981; Tyser 1992; Sheley et al. 1998; Kedzie-Webb et al. 2001; Thorpe et al. 2009). While it is known that knapweed can be controlled using a variety of methods including herbicide application (Rice et al. 1997), biological controls (Story et al. 2006), and controlled burning (MacDonald et al. 2007), resurgence of knapweed is very likely without the formation of a competitive, native community (Sheley et al. 1996), especially on sites in which seeds from previous infestations are present in the seed bank (Sheley et al. 1996; Carpinelli et al. 2004). As diverse native plant communities are more capable of resisting knapweed invasion (Kennedy et al. 2002; Bakker & Wilson 2004; Maron & Marler 2007), it is important during any restoration project to ensure that such diverse native systems are reestablished.

Targeting native plant establishment in combination with knapweed control requires knowledge on how community development will be impacted. For instance, the controlled burning of native, fire-adapted species stimulates flower stalk production, facilitates seedling establishment, enhances productivity and increases warm-season grasses (Old 1969; Abrams et al. 1986; Maret & Wilson 2005). In addition, carefully timed burns can reduce knapweed cover and seed viability (Emery & Gross 2005; MacDonald et al. 2007; Vermeire & Rinella 2009). Applying effective control methods, but relying on a remnant native seed bank or colonization from nearby sites for native species establishment may result in communities with lower species richness (Heslinga & Grese 2010), especially in isolated areas. Therefore, seeding with a diverse mix of native species immediately following control treatment will facilitate native species establishment and competition with knapweed (Tyser et al. 1998).

The objective of this study was to determine the most effective treatment for increasing native plant diversity while also reducing knapweed on an invaded site in western Michigan. To achieve this goal, twelve different treatment combinations were tested to determine which would result in reduced knapweed cover and increased native species diversity, cover, and floristic quality. These treatments included factorial combinations of mowing alone or mowing in combination with a single application of either a broadleaf-specific herbicide (clopyralid), or broad-spectrum herbicide (glyphosate), hand pulling of adult knapweed, and burning. We hypothesized that in treatment combinations where knapweed decreased, the presence of seeded native plants would increase. Also, we predicted that the development of the community would vary depending on the treatment combinations. Mowing may reduce knapweed seedfall prior to follow-up treatments, and will allow for ease of herbicide application (Packard & Mutel 1997). Use of clopyralid to control knapweed has been found to reduce knapweed cover and lead to increases in grass cover; however, knapweed can return to pre-treatment levels within six years (Rice et al. 1997), indicating a need for follow-up treatments. Additionally, the residual effects of clopyralid, which is particularly effective on the Asteraceae and Fabaceae families, may inhibit the establishment of native forbs (Enloe et al. 2005). Application of glyphosate may reduce competition during native species seedling establishment, but allows for rapid knapweed resurgence (MacDonald et al. 2003). While labor intensive, hand pulling of adult knapweed has been shown to reduce knapweed cover (Skurski et al. 2013; MacDonald et al. 2013), which should allow for increased native species establishment due to decreased competition. Controlled burns performed in mid spring can both reduce knapweed (MacDonald et al. 2007), and increase native warm-season grasses (Howe 1994; MacDonald et al. 2007). Based on these previous studies, we predicted that the broadleaf-specific herbicide treatment (clopyralid) would exhibit higher grass cover, while the broad-spectrum herbicide (glyphosate) + pulling + burning treatment and the mowed + pulling + burning treatment would exhibit the most native diversity. Finally, we hypothesized that burning would reduce knapweed cover, while encouraging the development of the native community.

Chapter II

Methods

Study Area

Our study site is within the Bass River Recreation Area, Ottawa County, Michigan (lat 43°00’49”N, long 86°01’47”W). Typical precipitation for the area, based on the 30-year average, is 369 mm (NCDC 2009). During the study, total precipitation from April through August was 510 mm during 2011 and 302 mm during 2012, as determined from the Muskegon, Michigan National Weather Service (NWS 2012). Average maximum temperatures for the area, based on the 30-year average, are 24.2 ˚C in June, 26.7 ˚C in July and 25.6 ˚C in August (NCDC 2009). Temperature averages during the study for June, July and August respectively were 25.3 ˚C, 29.2 ˚C, and 26.9 ˚C for 2011, and 26.8 ˚C, 31.1 ˚C and 26.6 ˚C for 2012 (NWS 2012). Thus, during 2011 there was above average precipitation, while low precipitation and high temperatures led to a severe drought lasting from mid- June through mid-October during 2012 (NDMC 2013).

Prior to establishment of the Bass River Recreation Area, the site was highly disturbed by extensive gravel mining in the mid 1900s (MacDonald et al. 2003), and subsequently colonized by several invasive species including knapweed. Knapweed was the dominant invasive plant at our study site prior to the initiation of our study with 60% to 70% cover based upon the total pre-treatment knapweed density (236 ± 16 m^{-2}, mean ± SE, MacDonald et al. 2013). Previous studies at this site demonstrated that the establishment of native grasses and fire reintroduction could successfully control knapweed (MacDonald et al. 2003; 2007), but these approaches resulted in a community with very low native diversity. The current study design expands on these works through the use of larger plots, incorporation of a more diverse seed mix, application of different combinations of knapweed control treatments, and an extensive evaluation of the developing community.

Experimental Design

This study evaluated 12 treatments consisting of three levels of initial site preparation, two levels of pulling, and two levels of burning (Table 1). The 5-m x 5-m treatment plots were arranged in a randomized complete block design with a factorial arrangement of treatments. Treatments were replicated four times for a total of 48 plots. Buffers were mowed yearly in late June, with plots separated by 2.5-m buffers, and blocks separated by 5-m buffers.

In the summer of 2008, the entire site was mowed to facilitate plot layout, reduce knapweed seedfall prior to other treatments and ease herbicide application (Packard & Mutel 1997). Randomly selected plots were subsequently treated with a single application of the broad-spectrum herbicide, glyphosate (Roundup Concentrate Plus®, Monsanto, Marysville, OH), at a rate of 9.9 kg ae ha^{-1} (n= 16), or the broadleaf-specific herbicide, clopyralid (Transline®, Dow AgroSciences, Indianapolis, IN), at a rate of 0.6 kg ae ha^{-1} (n= 16). The remaining 16 plots did not receive any additional site preparation. These mowing only plots allowed us to determine if native plants could be established simply by interseeding following minimal site preparation.

In the spring of 2009, we seeded all plots at a rate of 22 kg ha^{-1} with a mixture of native species representative of Michigan dry-mesic prairie, dry sand prairie and oak barrens (Kost et al. 2007). The seed mix included five native warm-season grasses, which comprised a total of 60% of the mix, and 18 native forbs, which made up the remaining 40% of the mix (Table 2). Pulling of adult, bolted knapweed on designated plots (*n*=24) began in July of 2009 and continued annually thereafter. Pulling entails removing adult knapweed, including the taproot, in advance of flowering to prevent seed production and dispersal within the plot with the aid of a handheld weed puller.

Controlled burning of the designated plots (*n*=24) took place on April 2, 2012 (MacDonald et al. 2013). Unseasonably warm weather during March of 2012 resulted in advanced plant phenology causing us to burn earlier than is optimal for knapweed control (Emery & Gross 2005; MacDonald et al. 2007). Fire temperature at ground level was measured with pyrometers constructed using Tempilaq G® indicator solutions (Tempil, South Plainfield, NJ). These solutions were painted on ceramic tiles and melt at specified temperatures. The 14 indicators used range from 79°C to 204°C, at 14°C intervals, and 232°C to 316°C, at 28°C intervals, as this has been shown to include the ranges of temperature in controlled burns (Kennard et al. 2005). Four pyrometers were installed per plot on the morning of the burns. Immediately following the burns, the pyrometers were collected and inspected to determine the highest temperature indicator that showed signs of changing during the fire.

Plant Surveys

To determine the impact that knapweed control methods were having on knapweed and the establishment of the native community, we visually estimated percent cover of all species in each plot in July of 2011 and 2012. This entailed dividing each 5-m by 5-m plot into quarters, with two researchers each estimating the cover of two quarters. During both years, each researcher consistently examined the same two quarters within each plot. In order to standardize visual estimates among researchers, we referred to published area charts (Anderson 1986), and used 0.1-m^2 PVC frames as a standard area reference. Following data collection, we calculated the relative percent cover (p_i) of each species on each plot by dividing the summed total cover of each species by the summed total cover of the plot.

Data Analysis

Using the relative percent cover calculated for each year, we determined plot diversity using the Shannon index of diversity:

$$1)\quad H' = -\sum p_i \log p_i$$

and Simpson's index of diversity:

$$2)\quad D = 1-\sum p_i^2$$

(McCune & Grace 2002). As recommended by Peet (1974), we used the exponential of H′ for analysis, as this indicates the functional number of species in the sample. Interpretations of the results remain the same, with higher values indicating higher diversity. Simpson's index has a range from zero, with a single species present, to one, maximum diversity (Peet 1974). Estimates of percent cover have been used successfully in previous studies to calculate these diversity indices (Potvin & Vasseur 1997, Tilman et al. 1997), and avoid errors resulting from miscounting clonal species if density had been used.

To evaluate community quality, we calculated the mean coefficient of conservatism ($\bar{C}$), and a floristic quality index (FQI) for each plot to distinguish among treatment combinations containing ubiquitous native plants and those containing more conservative species. These methods rely on coefficients of conservatism specific to Michigan (MDNR 2001), ranging from zero, representing ubiquitous native species, to ten, representing highly conserved native species (Taft et al. 1997). FQI was calculated for each plot by multiplying the $\bar{C}$ for the plot by the square root of the number of native species on the plot (Packard & Mutel 1997). Native tree and shrub species are not part of the target prairie community and were excluded from FQI and $\bar{C}$ analysis.

For analyses of community composition, we classified species into one of six groups: non-native forbs, knapweed, non-native grasses, native graminoids, native forbs and tree/shrub species. The non-native forbs group does not include knapweed; as the dominant invasive species and a focus of our research, knapweed was classified independently. Following this classification, we calculated the relative percent cover for each grouping by summing the relative percent cover of all species within that group. As the tree/shrub group accounted for less than 0.4% relative cover in both years, we did not include it in subsequent analyses. We performed simple linear (Pearson) correlations among the remaining five groups, in addition to total grass cover (sum of non-native grasses and native graminoids cover) using JMP 9.0.0 (n=48; JMP v. 9.0, SAS Institute, Cary, NC). In addition, the most prevalent native seeded species were analyzed to determine if the control treatments influenced their establishment.

We analyzed the 2011 data using three levels of initial site preparation, two levels of pulling, and eight replications, as pre-treatment burning effects were not significant in 2011 and therefore were pooled with error. The 2012 data were analyzed using the full factorial design, including the additional two levels of burning, with four replications. Data were analyzed using PERMANOVA, a nonparametric, permutational analysis of variance (Anderson 2001; McArdle & Anderson 2001; Anderson 2005). Post-hoc analyses were completed using nonparametric multiple comparison tests available in PERMANOVA (Anderson 2005). Significance was accepted at $p < 0.05$ for all tests.

In addition, based on soil testing performed at the onset of the study (MacDonald et al. 2013), we found that while soil properties did not differ significantly among the different treatment combinations, there was a trend toward lower gravel on plots assigned to the pulling treatments (p=0.09). Therefore, when a significant pulling effect was found, the data were re-analyzed using gravel as a covariate to ensure the effect was not related to soil properties.

Chapter III

Results

Seeding Effects

We observed native forbs and grasses on all plots three years after seeding, with 20 of the 23 native species established (Table 2). By 2011, *Andropogon gerardii, Schizachyrium scoparium* and *Sorghastrum nutans* had established in every plot (Table 2). Between 2011 and 2012, *Sorghastrum nutans* doubled in average relative cover from 1.8% to 3.6%. Coinciding with an increase in average native grass cover between 2011 and 2012 from 11.9% to 15.1%, there was a significant negative correlation between native and non-native grass cover in 2012 ($r = -0.47$, $p < 0.001$).

Several forbs also had high frequency of establishment, including *Asclepias tuberosa, Coreopsis lanceolata, Monarda fistulosa, Monarda punctata, Ratibida pinnata* and *Rudbeckia hirta* (Table 2). Between 2011 and 2012, *Coreopsis lanceolata* quadrupled in average relative cover from 0.2% to 0.8%, irrespective of treatment.

Both the FQI and $\bar{C}$ of the entire site were increased by seeding. Volunteer native species found on site had a $\bar{C}$ of 2.72 and an FQI of 13.6, while including seeded native species resulted in a $\bar{C}$ of 3.79 and an FQI of 26.0.

Initial Site Preparation Effects

Initial site preparation influenced both the diversity and quality of the plant community, with lower diversity, but a higher $\bar{C}$ in clopyralid treatments (Table 3, see also Table S1). Non-native species, including non-native grasses, non-native forbs, and knapweed exhibited the greatest response to site preparation (Fig. 1, Table S2). The clopyralid-only treatment maintained low knapweed cover through four years, while knapweed had resurged to near estimated pre-treatment levels on mowed-only and glyphosate-only treatments by 2011 (Fig. 1, Table S2). Clopyralid also consistently resulted in greater non-native grass cover (Fig. 1, Table S2). Glyphosate treatments contained the highest non-native forb cover in 2011 (29.4%), while non-native forb cover was lower and similar on the clopyralid and mowed treatments (7.7% and 7.4% respectively; Fig. 1, Table S2).

The initial site preparation also impacted the establishment of native species (Table 4). In 2012, *Andropogon gerardii* had the highest relative percent cover in the glyphosate and clopyralid treatments (Table 4, Table S3). While *Ratibida pinnata* had low establishment success, both this species and *Rudbeckia hirta* occurred most prevalently on glyphosate treatments (Tables 2 and 4, see also Table S3).

Pulling Effects

Pulling resulted in lower Simpson's diversity during 2012, but tended to increase FQI, however, this effect became non-significant when gravel was included as a covariate (Tables 3, Table S1). In 2011, plots showed higher native forb cover within the pulling treatment as compared to treatments that did not include pulling (6.3% compared to 2.3%; Fig.1, Table S2), but this effect was less pronounced in 2012 (6.9% compared to 3.9%). Knapweed cover was reduced to less than 0.6% after three years and 0.06% after four years in all pulling treatments (Fig. 1, Table S2). As adult knapweed was removed, the remaining cover represented the presence of knapweed juveniles or seedlings. Pulling also resulted in greater non-native grass cover (Fig. 1, Table S2). Increases in non-native grass cover corresponded to decreases in non-native forbs (2011: $r = -0.41$, $p = 0.004$;

2012: $r = -0.50$, $p < 0.001$), and knapweed (2011: $r = -0.67$, $p < 0.001$; 2012: $r = -0.58$, $p < 0.001$). Total grass cover had an increasing negative correlation with knapweed (2011: $r = -0.77$, $p < 0.001$; 2012: $r = -0.83$, $p < 0.001$).

Pulling favored the establishment of seeded native species, with *Asclepias tuberosa*, *Monarda punctata*, and *Schizachyrium scoparium* exhibiting greater cover on the pulling treatments during at least one year of the study (Table 4, Table S3). *Schizachyrium scoparium* had the greatest cover on the mowing-pulling treatment in 2012 (5.1%). While not significant with gravel included as a covariate, there was also a trend toward higher cover within pulling treatments for *Andropogon gerardii* during 2012, *Monarda fisulosa* during 2011, and *Rudbeckia hirta* during both years (Table 4, Table S3).

Burning Effects

Average plot temperatures during the 2012 burn ranged from <79°C to 159°C. Burning resulted in lower $\bar{C}$ compared to unburned treatments (3.57 to 3.94; Table 3, Table S1), however, $\bar{C}$ increased on both burned and unburned plots from 2011 to 2012 (3.49 and 3.59 respectively). Burning also resulted in reduced *Monarda punctata* cover, while *M. punctata* cover remained largely unchanged on unburned plots (Table 4, Table S3).

Chapter IV

Discussion

Seeding Effects

An unexpected finding in our study was the ease with which many seeded native species established on site. By seeding, we facilitated greater native forb diversity, which can assist in the prevention of knapweed reinvasion (Sheley & Half 2006), and avoided low recruitment that would have been expected if we had relied on the native seed bank or outside colonization (Heslinga & Grese 2010). While the FQI of the entire site remains lower than 35, which indicates floristic importance within Michigan (MDNR 2001), it is higher than would have been achieved by relying on volunteer species establishment alone. As with previous studies at this knapweed-infested site (MacDonald et al. 2003; 2007), *Andropogon gerardii*, *Schizachyrium scoparium* and *Sorghastrum nutans* established successfully (Table 2). Competition from these species should result in reduced knapweed cover over time. *S. nutans*, in particular, achieved uniform cover regardless of knapweed control treatments, demonstrating its establishment and persistence even within knapweed-dominated communities. The increasing negative correlation between total grass cover and knapweed is consistent with grass-induced suppression of knapweed (Lindquist et al. 1996; MacDonald et al. 2003). At the same time, a negative correlation between non-native grasses and native graminoids and increasing native graminoid cover (Fig. 1) illustrate competition that may lead towards a transition from non-native grass-dominated to native graminoid-dominated communities. Similar results have been found by Foster et al. (2007), in which sowing of native and naturalized prairie species decreased non-native grass, including the most common non-native grass species found during our study, *Poa pratensis*. Endress et al. (2012) found that while seeding native grasses into an invasive forb-dominated community lowered non-native grass cover within six years, cover of the dominant invasive forb was unaffected. In contrast, the native grasses seeded in our study have been shown to reduce knapweed dominance to 2.1% of total biomass or less through time (MacDonald et al. 2007).

While native grass dominance is likely to assist in knapweed suppression (MacDonald et al. 2003; 2007; Baer et al. 2004; McCain et al. 2010), this is not ideal given the goal of establishing a diverse community. Although there is no evidence of native forb suppression within the establishment stage of our study, it may be some time before we are able to determine whether competition with native grasses will have any undesirable effects on native forbs. Sluis (2002) found that increases in *Andropogon gerardii* dominance within prairie restorations paralleled reductions in species richness within 15 years, while Camill et al (2004) saw similar results three years into their restoration. After knapweed has been successful suppressed, however, utilization of methods such as burning during varying intervals and seasons can counteract decreases in native diversity due to competition from native grasses (Howe 1994).

Although species that are sensitive to the allelopathic effects of knapweed can persist in knapweed-infested areas (Perry et al. 2005), very little research has been published detailing which native forbs are capable of establishing in the presence of knapweed. We observed native forb establishment in all treatments, including those that retained high knapweed cover (Table 2). In addition, over 80% of the forbs studied had established within three years of seeding (Table 2). In particular, *Asclepias tuberosa*, *Coreopsis lanceolata, Monarda fistulosa, Monarda punctata*, and *Rudbeckia hirta* exhibited relatively high cover, with *C. lanceolata* having similar cover on all treatments indicating establishment success even in knapweed-dominated communities (Table 4). Several of these species share similar characteristics with knapweed, which may ensure greater competition with knapweed as they continue to increase in cover (Pokorny et al. 2005). Such was the case with *Achillea millefolium*, which shares similar phenology and rooting morphology with knapweed and is more able to resist knapweed invasion (Maron & Marler 2007). Within our study, *A. tuberosa, M. fistulosa, M. punctata,* and *R. hirta* share a similar flowering season with knapweed (Gleason & Cronquist 1991), and all have proven to be successful at establishing in the presence of knapweed. Seeded native forbs still have relatively low cover, however, and will require more time to demonstrate their competitive abilities. Once the native community becomes well-established, it is expected be more resistant to the resurgence of invasive plants, including knapweed.

Initial Site Preparation Effects

Of the three initial site preparation treatments tested, clopyralid had the most positive impacts on the development of the native plant community. Clopyralid increased *Andropogon gerardii* (Table 4) and non-native grass cover (Fig. 1), thereby helping to prevent knapweed resurgence. Use of broadleaf herbicide to control non-native forbs often results in increases in non-native grass cover (Sheley et al. 2004; Ortega & Pearson 2011; Endress et al. 2012; Skurski et al. 2013). While we were initially concerned that the persistence of clopyralid in the soils would reduce forb establishment (DiTomaso 2000; Enloe et al. 2005), we now believe the long-term trajectory of this community will trend towards a diverse blend of warm-season grasses and native forbs, as opposed to the grass-dominated community we had originally predicted. Clopyralid is particularly effective against the families Fabaceae and Asteraceae (Enloe et al. 2005), which includes knapweed, and 13 of the 18 forb species seeded (Table 2). Of the species we seeded, only *Rudbeckia hirta* exhibited reduced establishment on clopyralid treatments (Table 4), consistent with this known sensitivity. The minor impact of clopyralid on native forbs may have been modified by the sand to sandy-loam soil of our study site (MacDonald et al. 2013), as breakdown of clopyralid has been shown to be effected by soil type (Smith & Aubin 1989).

A single application of clopyralid provided knapweed control for at least four years post-treatment, even in the absence of pulling (Fig. 1). As knapweed cover has been found to be inversely related to diversity (Kedzie-Webb et al. 2001), we anticipated greater diversity in treatments that had reduced knapweed cover. Yet, we believe that the non-native species presence within the community remains too high for diversity indices to indicate long-term trajectory or the current condition of the restoration. Despite the effective knapweed control, clopyralid resulted in communities with lower diversity during 2012. The higher diversity within mowing and glyphosate treatments coincides with lower $\bar{C}$ and higher knapweed cover in the absence of pulling, and higher non-native forb cover (Fig. 1).

In contrast to the effectiveness of clopyralid, in absence of pulling the glyphosate-only treatment allowed for rapid knapweed resurgence from the seedbank (Fig. 1). Glyphosate in combination with seeding native grasses may provide limited short-term success in terms of grass establishment and knapweed control. Previous studies have shown that in as little as three years knapweed density may be greater than pre-herbicide treatment, ultimately resulting in low long-term grass establishment (Sheley et al. 2001). Glyphosate, in absence of pulling, also resulted in lower grassy fuel, leaving greater unburned areas within the plots (L. M. Martin, personal observation). Still, *Andropogon gerardii*, *Ratibida pinnata* and *Rudbeckia hirta* demonstrated higher cover within the glyphosate treatment (Table 4), indicating a positive change in the development of this community. Yet, it remains too early to know if the seeded native species will gain dominance within this developing community due to the persistence of non-native species, especially knapweed.

After a single mowing, we saw no major long-term impacts on the community trajectory, as was expected. Mowing allowed for comparable levels of seeded species cover as both herbicide treatments, with the exception of lower *Andropogon gerardii* cover (Table 4), which should be temporary due to the dominant nature of this species (McCain et al. 2010). Yet, the ease with which native grasses and forbs established following such minimal site preparation indicates that seed limitations may be preventing natural recovery of some degraded sites (Foster et al. 2007), and shows promise for the development of communities with this treatment.

Pulling Effects

Pulling provided both effective knapweed control (MacDonald et al. 2013) and favored native grass and forb establishment, in addition to encouraging the development of grassy fuels that will facilitate future burns. Greatly reduced knapweed cover was maintained throughout the study, resulting in non-native grass-dominated communities (Fig. 1). In turn, high non-native grass cover helps prevent knapweed resurgence

(Lindquist et al. 1996) until the native grasses become dominant. Additionally, the decreases in non-native forb cover associated with increases in non-native grasses, indicate that these grasses may be competitively excluding non-native forbs, similar to results found by Bosy and Reader (1995).

Pulling increased the total cover of native forbs and several individual species, including *Asclepias tuberosa*, *Monarda punctata*, and *Schizachyrium scoparium* (Table 4), consistent with the trend towards a higher FQI on the pulled treatment (Table 3). We attribute the higher establishment of native forbs on pulled plots to reduced competition with knapweed, since pulling has been shown to substantially reduce knapweed density and biomass (MacDonald et al. 2013). Knapweed, which can have detrimental impacts on native forb establishment and persistence (Lesica & Sheley 1996; Maron & Marler 2008), was highly dominant prior to the initiation of our study. Yet, while we saw increases in the establishment of native forbs and one native grass when knapweed was manually removed, we also saw establishment and persistence of native species even in treatments with high knapweed cover. This indicates that the allelopathic effects of knapweed (Thorpe et al. 2009) had little impact on native species establishment and persistence on our study site.

It is possible that rather than elimination of the non-native species, disturbance caused by pulling encouraged seedling establishment; however, Skurski et al. (2013) found that a single manual removal of knapweed and replicated soil disturbance exhibited no difference in variables affected, with the exception of knapweed cover. This provides evidence that it was the removal of knapweed, rather than the disturbance, that facilitated native forb establishment within our study.

Burning Effects

We believe that due to the extended drought period experienced during June and July, 2012, the effects of our burn on the native community may be delayed. Differences between burned and unburned sand prairies are less notable during drought years (Dhillion & Anderson 1994), and by reducing soil moisture (Anderson 1965), our burn may have exacerbated the effects of low soil moisture on productivity (Abrams et al. 1986; Briggs & Knapp 1995; Bowles & Jones 2013). In addition, burns conducted during the early spring result in lower soil moisture than mid- or late-spring burns (Anderson 1965). We believe that reduced soil moisture may have accentuated lower *Monarda punctata* cover on the burning treatments (Table 4), which contributed to the lower $\bar{C}$ (Table 3). There also was little change in *M. punctata* cover on unburned treatments (Table 4), indicating that unlike many other seeded native species which increased in cover, *M. punctata* may be more susceptible to drought stress. Other studies have found that burning encourages germination and flower stalk production in native species (Kucera & Ehrenreich 1962; Old 1969; Maret & Wilson 2005), increases warm-season grass dominance (MacDonald et al. 2007), and that, in combination with seeding of native species, fire can promote native species richness while decreasing non-native richness (Suding & Gross 2006).

While we had anticipated that burning would reduce knapweed cover, none of the plots reached an average fire temperature in excess of the 200˚C that has been shown to reduce knapweed germination rates in the laboratory (Abella & MacDonald 2000). Temperatures did exceed the 107 to 143 ˚C that has been shown to reduce knapweed germination under field conditions (Vermeire & Rinella 2009); however, weather conditions and timing were not optimal during our burn (MacDonald et al. 2013). Despite the low intensity of the initial burn, several of the treatment combinations should prove to be beneficial during future prescribed burns due to their high grass cover. The non-native grasses present within our study are mainly cool-season grasses that should be damaged by spring burns (Abrams et al. 1986), thereby reducing their cover. Additionally, spring burning has been shown to assist in the establishment of warm-season grasses in cool-season grass dominated communities (Doll et al. 2011), which would be beneficial in our pulling and clopyralid treatments that are non-native grass dominated (Fig. 1). We believe that future burns performed during this long-term study will result in greater knapweed control and native species establishment.

Chapter V

Conclusions

Our results show that the method used for knapweed control played a large part in determining the development of a restored plant community. Hand pulling of knapweed assisted in native plant community establishment. Both clopyralid application and pulling encouraged the accumulation of grassy fuel loads, which will facilitate future burns that may help to further control knapweed, suppress other non-native species, and encourage establishment and dominance by native species. Yet, the labor required for pulling may limit this treatment to small knapweed infestations, areas pre-treated with a broad-leaf specific herbicide such as clopyralid, or areas where herbicide use would damage a sensitive remnant native community. Our use of a more diverse seed mix allowed for a greater number of potentially competitive species to establish, which may further prevent knapweed from returning to the site. Seeding with a mix of selected native species takes little time to implement, and produces a more desirable native plant community over time as evidenced by the increased FQI and $\bar{C}$ achieved through seeding. Through time, the interactive effects of site preparation methods, pulling, and burning may become more pronounced as the native plant community continues to develop.

Implications for Practice

- Seeding of selected native grasses and forbs following minimal site preparation was worthwhile on the knapweed-infested site we studied as certain species established without intensive treatment, including *Andropogon gerardii, Asclepias tuberosa, Coreopsis lanceolata, Monarda fistulosa, Monarda punctata, Rudbeckia hirta,* *Schizachyrium scoparium*, and *Sorghastrum nutans*. While native species cover may be initially low, increased native dominance will facilitate future management to gradually suppress knapweed.

- Although labor intensive, pulling of knapweed, in concert with seeding native species, was an effective treatment for reducing knapweed cover and increasing native grass and forb establishment.
- A single application of clopyralid provided long-lasting control of knapweed and had little to no impact on seeded species establishment on this sandy site.
- A single treatment with glyphosate resulted in rapid resurgence of knapweed from the seedbank and increases in other non-native forbs. This approach would require intensive follow-up control measures to prevent continued knapweed dominance.
- Pulling of knapweed or a single application of clopyralid resulted in communities dominated by non-native grasses, which may prevent knapweed resurgence until seeded native grasses and forbs mature.
- To be effective, burning needs to be carefully timed to optimize knapweed control and encourage native species. In this study, a low-intensity early spring burn had minimal effects on either knapweed or native species cover.

Tables and Figures

Table 1. The twelve treatment combinations tested during study at Bass River Recreation Area, Ottawa County, Michigan.

Levels of Treatment			
Initial Site Preparation	Pulling Treatment	Burn Treatment	*n*
Mowed, 2008	None	None	4
Mowed, 2008	None	Burned, 2012	4
Mowed, 2008	Knapweed Pulling, Annually	None	4
Mowed, 2008	Knapweed Pulling, Annually	Burned, 2012	4
Mowed + Clopyralid, 2008	None	None	4
Mowed + Clopyralid, 2008	None	Burned, 2012	4
Mowed + Clopyralid, 2008	Knapweed Pulling, Annually	None	4
Mowed + Clopyralid, 2008	Knapweed Pulling, Annually	Burned, 2012	4
Mowed + Glyphosate, 2008	None	None	4
Mowed + Glyphosate, 2008	None	Burned, 2012	4
Mowed + Glyphosate, 2008	Knapweed Pulling, Annually	None	4
Mowed + Glyphosate, 2008	Knapweed Pulling, Annually	Burned, 2012	4

Table 2. Seeded species presence as a percent of the 48 total treatment plots at Bass River Recreation Area, Ottawa County, Michigan. Species are ordered in terms of presence during 2011. Column C corresponds to the coefficient of conservatism for each species specific to Michigan (MDNR 2001).

Family	Species	Common Name	C	2011	2012
Poaceae	*Andropogon gerardii*	Big Bluestem	5	100.0%	97.9%
Poaceae	*Schizachyrium scoparium*	Little Bluestem	5	100.0%	100.0%
Poaceae	*Sorghastrum nutans*	Indian Grass	6	100.0%	100.0%
Lamiaceae	*Monarda fistulosa*	Wild Bergamot	2	97.9%	95.8%
Asteraceae	*Coreopsis lancelota*	Sand Coreopsis	8	93.8%	93.8%
Lamiaceae	*Monarda punctata*	Horsemint	4	87.5%	66.7%
Asteraceae	*Rudbeckia hirta*	Black-eyed Susan	1	83.3%	85.4%
Apocynaceae	*Asclepias tuberosa*	Butterfly weed	5	77.1%	62.5%
Poaceae	*Elymus canadensis*	Canada Wild Rye	4	43.8%	16.7%
Asteraceae	*Ratibida pinnata*	Yellow Coneflower	4	39.6%	29.2%
Asteraceae	*Gnaphalium obtusifolium*	Old-Field Balsam	2	12.5%	18.8%
Fabaceae	*Lupinus perennis*	Wild Lupine	7	12.5%	0.0%
Poaceae	*Panicum virgatum*	Switch Grass	4	12.5%	8.3%
Asteraceae	*Solidago nemoralis*	Old-Field Goldenrod	2	10.4%	8.3%
Asteraceae	*Coreopsis tripteris*	Tall Coreopsis	7	8.3%	2.1%
Fabaceae	*Lespedeza capitata*	Round-headed Bush-Clover	5	6.3%	8.3%
Verbenaceae	*Verbena stricta*	Hoary Vervain	4	2.1%	4.2%
Asteraceae	*Coreopsis palmata*	Prairie Coreopsis	10	2.1%	2.1%
Asteraceae	*Helianthus occidentalis*	Western Sunflower	8	2.1%	2.1%
Asteraceae	*Solidago speciosa*	Showy Goldenrod	5	2.1%	0.0%
Asteraceae	*Solidago juncea*	Early Goldenrod	3	0.0%	0.0%
Fabaceae	*Tephrosia virginiana*	Goat’s Rue	10	0.0%	0.0%
Commelinaceae	*Tradescantia ohiensis*	Common Spiderwort	5	0.0%	0.0%

Table 3. Site preparation and pulling treatments effects on Simpson's diversity, Shannon diversity, mean coefficient of conservatism (C), and Floristic Quality Index (FQI), at Bass River Recreation Area, Ottawa County, Michigan. Means followed by different letters differ significantly.

	Initial Site Preparation Treatment			Pulling Treatment	
	Mow	Clopyralid	Glyphosate	None	Pulled
Simpson's Diversity					
2011	0.61 (0.11)	0.54 (0.21)	0.63 (0.13)	0.58 (0.17)	0.60 (0.15)
2012	**0.63 (0.12) a**[a]	**0.44 (0.15) b**	**0.66 (0.13) a**	**0.63 (0.15) x**	**0.53 (0.17) y**
Shannon Diversity					
2011	4.31 (1.03)	3.94 (1.56)	5.17 (1.75)	4.24 (1.42)	4.71 (1.64)
2012	**4.37 (1.46) a**	**3.13 (1.05) b**	**5.33 (1.90) a**	4.64 (1.81)	3.91 (1.61)
Mean C					
2011	**3.53 (0.29) ab**	**3.73 (0.51) a**	**3.34 (0.27) b**	3.52 (0.30)	3.56 (0.49)
2012	**3.61 (0.42) b**	**4.02 (0.47) a**	**3.64 (0.33) b**	3.66 (0.39)	3.85 (0.48)
FQI					
2011	13.51 (1.84)	13.31 (1.30)	13.88 (1.67)	13.05 (1.22)	14.09 (1.79)
2012	12.66 (2.39)	12.87 (1.53)	13.18 (1.41)	12.23 (1.55)	13.57 (1.82)

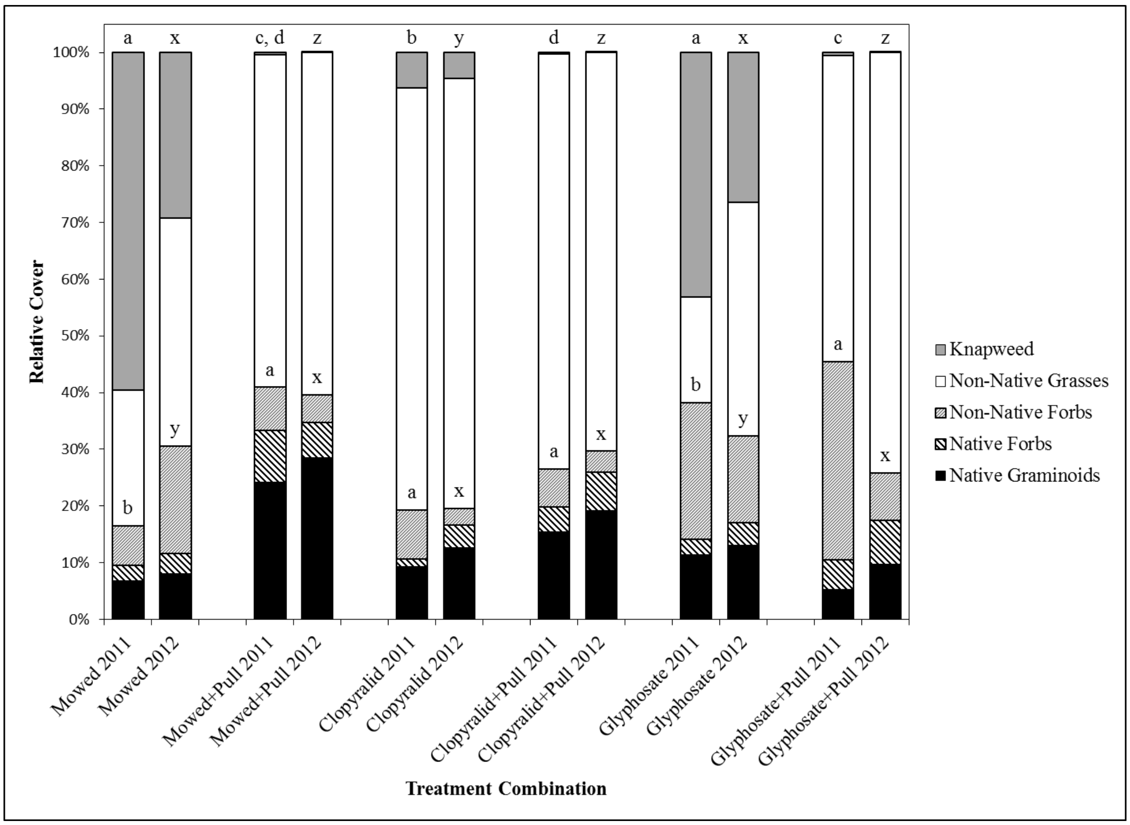
[a] Letters a, b compare initial site preparation means within a single year; x, y compare pulling means within a single year. See Table S1 for exact *p*-values. Standard deviation in parentheses.

Table 4. Site preparation, pulling and burning treatments effects on native seeded species at Bass River Recreation Area, Ottawa County, Michigan. All values given represent relative percent cover. Standard deviation in parentheses. Means followed by different letters differ significantly.

Variable	Initial Site Preparation Treatment			Pulling Treatment		Burning Treatment	
	Mow	Clopyralid	Glyphosate	None	Pulled	None	Burned
	%	%	%	%	%	%	%
Andropogon gerardii							
2011	0.77 (0.72)	1.06 (0.58)	1.05 (0.39)	0.81 (0.41)	1.11 (0.69)	0.99 (0.48)	0.93 (0.68)
2012	**1.17 b**[a] **(0.77)**	**1.83 a (0.96)**	**2.06 a (1.23)**	1.37 (1.03)	2.00 (1.00)	1.74 (1.07)	1.63 (1.06)
Asclepias tuberosa							
2011	0.06 (0.06)	0.05 (0.05)	0.07 (0.08)	**0.04 n (0.04)**	**0.08 m (0.08)**	0.05 (0.05)	0.07 (0.07)
2012	0.04 (0.06)	0.10 (0.11)	0.11 (0.17)	**0.04 n (0.07)**	**0.13 m (0.14)**	0.06 (0.07)	0.10 (0.16)
Monarda punctata							
2011	0.60 (0.63)	0.32 (0.37)	0.42 (0.41)	**0.24 n (0.31)**	**0.66 m (0.55)**	0.49 (0.53)	0.41 (0.46)
2012	0.27 (0.42)	0.16 (0.20)	0.47 (0.54)	0.20 (0.31)	0.40 (0.50)	**0.48 x (0.42)**	**0.12 y (0.34)**
Ratibida pinnata							
2011	**0.02 b (0.03)**	**0.01 b (0.04)**	**0.08 a (0.09)**	0.03 (0.06)	0.04 (0.07)	0.03 (0.06)	0.04 (0.07)
2012	**0.01 b (0.02)**	**0.01 b (0.03)**	**0.10 a (0.12)**	0.03 (0.07)	0.05 (0.10)	0.04 (0.07)	0.04 (0.09)
Rudbeckia hirta							
2011	**0.22 b (0.17)**	**0.11 b (0.16)**	**0.57 a (0.58)**	0.19 (0.30)	0.41 (0.47)	0.27 (0.25)	0.33 (0.52)
2012	**0.18 b (0.15)**	**0.07 c (0.08)**	**0.43 a (0.44)**	0.15 (0.21)	0.30 (0.37)	0.25 (0.34)	0.21 (0.27)
Schizachyrium scoparium							
2011	0.86 (0.64)	0.57 (0.26)	0.58 (0.45)	**0.50 n (0.35)**	**0.84 m (0.55)**	0.72 (0.50)	0.62 (0.48)
2012	**3.34 a (3.28)**	**1.37 b (0.80)**	**2.01 ab (1.58)**	**1.49 n (1.16)**	**2.99 m (2.82)**	2.55 (2.76)	1.93 (1.63)

[a] Letters a, b, c- compare initial site preparation means within a single year; m, n– compare pulling means within a single year; x, y- compare burn means within a single year. See Table S3 for exact *p*-values.

Figure 1. Plant community composition as affected by site preparation and pulling at Bass River Recreation Area. Treatments that differ significantly ($p < 0.05$) are indicated by different letters: a, b, c, and d compare means for 2011; x, y and z compare means for 2012. Letters within the non-native grasses bars indicate differences among these treatments, letters above the knapweed bars indicate differences among these treatments. See table S2 for exact *p*-values.



Supplementary Tables

Table S1**.** Significance probabilities from permutational analyses of variance for Simpson's diversity, Shannon diversity, mean coefficient of conservatism (C), and floristic quality index (FQI) data, at Bass River Recreation Area, Ottawa County, Michigan. Values in parentheses under pulling main effect are results with gravel included as a covariate if including the covariate removed the significance of the effect. Significant *p*-values (≤0.05) are given in bold face.

	Source of Variance[a]						
	Prep	Pull	Burn	Prep x Pull	Prep x Burn	Pull x Burn	Prep x Pull x Burn
Variable	*p*	*p*	*p*	*p*	*p*	*p*	*p*
Simpson's Diversity							
2011	0.23	0.76	-	0.20	-	-	-
2012	**<0.01**	**<0.01**	0.34	0.90	0.07	0.59	0.47
Shannon Diversity							
2011	0.06	0.29	-	0.25	-	-	-
2012	**<0.01**	0.11	0.73	0.91	0.37	0.67	0.31
Mean C							
2011	**0.02**	0.72	-	0.11	-	-	-
2012	**<0.01**	0.10	**<0.01**	0.45	0.79	0.93	0.70
Floristic Quality Index							
2011	0.58	**0.03** (0.08)	-	0.68	-	-	-
2012	0.72	**0.01** (0.06)	0.12	0.93	0.69	0.82	0.40

[a] Source of Variance includes: Prep = initial site preparation main effect; Pull = pulling main effect; Burn = burning main effect; Prep x Pull = initial site preparation and pulling interaction effect; Prep x Burn = initial site preparation and burning interaction effect; Pull x Burn = pulling and burning interaction effect; Prep x Pull x Burn = initial site preparation, pulling and burning interaction effect.

Table S2**.** Significance probabilities from permutational analyses of variance for percent cover data, at Bass River Recreation Area, Ottawa County, Michigan. Values in parentheses under pulling main effect are results with gravel included as a covariate if including the covariate removed the significance of the effect. Significant *p*-values (≤0.05) are given in bold face.

	Source of Variance[a]						
	Prep	Pull	Burn	Prep x Pull	Prep x Burn	Pull x Burn	Prep x Pull x Burn
Variable	*p*	*p*	*p*	*p*	*p*	*p*	*p*
Native Grass							
2011	0.42	0.20	-	0.10	-	-	-
2012	0.47	0.10	0.16	0.13	0.34	0.95	0.32
Native Forbs							
2011	0.15	**<0.01**	-	0.40	-	-	-
2012	0.58	**0.02** (0.06)	0.65	0.95	**<0.05**	0.39	0.25
Knapweed							
2011	**<0.01**	**<0.01**	-	**<0.01**	-	-	-
2012	**<0.01**	**<0.01**	0.97	**<0.01**	0.29	0.98	0.30
Non-native Grass							
2011	**<0.01**	**<0.01**	-	**0.02**	-	-	-
2012	**<0.01**	**<0.01**	0.34	**0.02**	0.16	0.58	0.36
Non-native Forbs							
2011	**<0.01**	0.49	-	0.47	-	-	-
2012	0.06	**<0.05** (0.11)	0.52	0.18	0.83	0.50	0.74

[a] Source of Variance includes: Prep = initial site preparation main effect; Pull = pulling main effect; Burn = burning main effect; Prep x Pull = initial site preparation and pulling interaction effect; Prep x Burn = initial site preparation and burning interaction effect; Pull x Burn = pulling and burning interaction effect; Prep x Pull x Burn = initial site preparation, pulling and burning interaction effect.

Table S3. Significance probabilities from permutational analyses of variance for most prevalent seeded native species relative percent cover, at Bass River Recreation Area, Ottawa County, Michigan. Values in parentheses under pulling main effect are results with gravel included as a covariate if including the covariate removed the significance of the effect. Significant *p*-values (≤0.05) are given in bold face.

	Source of Variance[a]						
	Prep	Pull	Burn	Prep x Pull	Prep x Burn	Pull x Burn	Prep x Pull x Burn
Variable	*p*	*p*	*p*	*p*	*p*	*p*	*p*
Andropogon gerardii							
2011	0.30	0.08	-	0.54	-	-	-
2012	**0.04**	**0.03** (0.10)	0.72	0.92	0.37	0.40	0.15
Asclepius tuberosa							
2011	0.55	**0.02**	-	0.49	-	-	-
2012	0.16	**<0.01**	0.16	0.29	0.16	0.65	0.18
Monarda fistulosa							
2011	0.24	**0.02** (0.06)	-	0.20	-	-	-
2012	0.05	0.26	0.36	0.40	0.75	0.60	0.91
Monarda punctata							
2011	0.20	**<0.01**	-	0.42	-	-	-
2012	0.08	0.08	**<0.01**	0.36	0.59	0.77	0.77
Ratibida pinnata							
2011	**<0.01**	0.35	-	0.65	-	-	-
2012	**<0.01**	0.52	0.82	0.86	0.88	0.49	0.94
Rudbeckia hirta							
2011	**<0.01**	**0.03** (0.09)	-	0.49	-	-	-
2012	**<0.01**	0.06	0.59	0.07	0.73	0.82	0.83
Schizachyrium scoparium							
2011	0.12	**<0.01**	-	0.09	-	-	-
2012	**0.01**	**<0.01**	0.24	**0.04**	0.18	0.16	0.07

[a] Source of Variance includes: Prep = initial site preparation main effect; Pull = pulling main effect; Burn = burning main effect; Prep x Pull = initial site preparation and pulling interaction effect; Prep x Burn = initial site preparation and burning interaction effect; Pull x Burn = pulling and burning interaction effect; Prep x Pull x Burn = initial site preparation, pulling and burning interaction effect.

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