THE EFFECTS OF SPOTTED KNAPWEED (*Centaurea stoebe* L.) ON GRASSLAND PLANTS AND SOILS IN BRITISH COLUMBIA

By

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ABSTRACT

In British Columbia, grasslands provide critical habitat for a wide variety of species and are a significant forage base for BC's ranching industry. Grasslands in BC are threatened from several human-caused stressors, including urban development, over-grazing, climate change and the introduction of non-native invasive species. Spotted knapweed (*Centaurea stoebe* L.), is an invasive plant introduced to North America from Europe. It can establish large monocultures in BC's grasslands which can cause a reduction in wildlife and livestock forage production, a lowering of native biodiversity, and an alteration in soil nutrient composition. I investigated the carry over effects immediately following removal of large and small spotted knapweed patches on soil mineral nutrients (nitrogen (N), phosphorus (P), carbon (C) and volumetric water content) and the growth of an important native grass, rough fescue (*Festuca campestris* Rydb.), in grasslands within Lac du Bois Provincial Park, located to the northwest of the city of Kamloops, British Columbia. The results of field experiments showed that soil total nitrogen, total carbon, volumetric water content and biomass of rough fescue were lower in former spotted knapweed patches, but there was no significant difference in soil total phosphorus. In addition, there was no measureable difference between large and small spotted knapweed patches in soil mineral nutrients and plant growth. In a greenhouse experiment I manipulated N:P ratios (1:1, 15:1 and 30:1) and biochar (10g/pot or none) to test their effect on competitive performance between spotted knapweed and rough fescue. The experimental design included five plant combinations: spotted knapweed alone, rough fescue alone, two spotted knapweed, two rough fescue, one spotted knapweed and one rough fescue. The results of the greenhouse experiment showed that total biomass of spotted knapweed was greater than rough fescue at each N:P ratio when they were grown alone or grown under intraspecific condition. Also, results showed that the competitive effect of spotted knapweed was -0.136 ± 0.052 SE; while rough fescue has a facilitation effect of 0.020 ± 0.007 SE. Biochar addition had no effect on plant growth of spotted knapweed or rough fescue, or competitive interactions within and between the two plant species.

Keywords: Soil properties, biomass, carryover effects, N:P ratio, biochar addition

TABLE OF CONTENTS

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LIST OF FIGURES

LIST OF TABLES

Chapter 1 – General Introduction

Non-native invasive species are a serious worldwide problem (Reid et al. 2009). Once established, these exotics can dominate an environment and are hard to control and eliminate. Invasive plant species can reduce biodiversity, change forage availability for domesticated and native herbivores and alter soil nutrient properties (Reid et al. 2009). Forests, rangelands, wetlands and valuable cropland in North America are threatened by non-native invasive plants, causing substantial economic lose (White & Schwarz 1998).

The success and dominance of invasive plants are suggested to be the result of two primary factors. First, exotic plant invasives may no longer be in the presence of their natural enemies from their native distribution; referred to as the enemy-free hypothesis (Andonian & Hierro 2011). Without their natural enemies, introduced species may quickly grow, reproduce and expand. The absence of herbivores, pathogens and competitive species in new habitats can provide an environment with less competition and disturbance for invaders to grow and multiply at a high rate. Second, the interaction between the soil biota and the invasive plant species may be mutualistic (Andonian & Hierro 2011). It is possible that invasive species encounter less inhibitory effects of soil biota where they are introduced than in their home range. Thus, soil microbes can promote invasion in recipient communities while inhibiting plants at home.

The management of invasive alien plants is challenging because it often requires multiple methods at a high cost. Even so, there is evidence of many successful eradication programs, or at least the maintenance of populations at low densities of exotics (Simberloff 2008). The different methods applied to control invasives include mechanical, physical, chemical and biological controls.

Grasslands

Grasslands are characterized by the dominance of grasses and forbs rather than large shrubs and trees, and cover about 3500 million ha of the Earth's surface (Carlier et al. 2009). Grasslands contribute to reducing soil erosion and improving soil and water quality (Carlier et al. 2009). Also, they provide food for livestock and serve as habitat to wildlife thus enhancing biodiversity (Miller 2013). From an economic perspective, grasslands provide livelihoods and a major source of income.

Despite the important role of grasslands, they are being threatened by several stressors, such as human activities, invasive species and climate change. Grasslands are converted for agricultural purposes and destroyed due to urban sprawl, thus reducing biodiversity through loss of habitat (Klaus 2013). Non-native invasive plants species are detrimental to grasslands through the alteration of disturbance processes, diminishment of the quality of feed for livestock and wildlife, and the displacement of native plants (Reid et al. 2009). Furthermore, the change of temperature, precipitation, and soil moisture caused by climate change can influence grassland function, including carbon storage, nitrate filtration, water quality, and forage for livestock and wildlife (Mannetje 2007).

Spotted knapweed (*Centaurea stoebe* L.)

Spotted knapweed (*Centaurea stoebe* L.) is an invasive plant introduced to North America from Europe. It is a short-lived perennial forb growing from a taproot to erect stems, ridged and laxly branched (Story et al. 1989). The height of spotted knapweed is 0.2-1.8 m, with solitary flowering heads at the ends of branches (Province of British Columbia 2002). The spotted appearance is given by stiff and tipped floral bracts with a dark, comb-like fringe (Province of British Columbia 2002). The flowers are pinkish purple or, rarely, cream colored. Its rosette leaves are up to 15 cm long and deeply lobed (Province of British Columbia 2002). The principal stem leaves are pinnately divided having smooth margins and getting smaller toward the top of the shoot (Province of British Columbia 2002).

Spotted knapweed is widespread at low- to mid-elevation grasslands and dry open forests in British Columbia, Canada (Province of British Columbia 2002). It also can be found on roadsides, fields, and disturbed areas. It is adapted to well-drained, light- to coarse-textured soils but is intolerant to dense shade. The plant is frequent in southern B.C. east of the Coast-Cascade mountains. It is present on the Mainland and Vancouver Island as well. Spotted knapweed has become a major concern in the Kootenay, Okanagan, Thompson, Cariboo, Omineca, and Peace River agricultural reporting regions (Province of British Columbia 2002).

Spotted knapweed reduces wildlife and livestock forage production, lowers native biodiversity, and increases loss of soil mineral nutrients (Sheley & Jacobs 1997; Fraser & Carlyle 2011). Through secreting allelochemicals, shifting microbial mechanism and affecting resource depletion mechanism, spotted knapweed influences soil properties and native grasses (Suding et al. 2004). Spotted knapweed contributes to reducing soil nitrogen availability (Hook et al. 2004; Story et al. 1989) and increasing soil phosphorus availability (Thorpe al. 2006). Hook et al. (2004) reports that spotted knapweed can increase or decrease, but mostly decrease, soil carbon pools in native grasslands. Spotted knapweed has been shown to have a greater ability to uptake soil water than native grass, such as *Pseudoregneria spicata and Pascopyron smithii* (Hill et al. 2006). In terms of influencing soil properties, spotted knapweed affects the growth of native plants since the essential nutrients for plant growth are changed. For example, the growth of rough fescue is restrained by the presence of spotted knapweed (Fraser and Carlyle 2011). It hinders rough fescue's growth through altering soil nutrients, reducing soil nitrogen, carbon and volumetric water content while increasing soil phosphorus and potassium.

Rough fescue (*Festuca campestris* Rydb.)

Rough fescue (*Festuca campestris* Rydb.) is a native, perennial, cool-season bunchgrass of northwestern North America (USDA-NRCS 2003). The large-diameter bunches of individual rough fescue plants usually average 30.5 – 35.5 cm in diameter, sometimes as large as 70.0 cm. The culms are erect, 30.5 to 137.2 cm. tall, glabrous, scabrous, naked below the panicle, and purplish at the base. Leaves are basal 30.5-76.2 cm long, 0.08-0.20 cm in diameter, folded, mostly erect, stiff, and pointed. The lower surface of the leaves are often scabrous (USDA-NRCS 2003).

Rough fescue occurs in grass-dominated and shrub-dominated plant communities. It also can occur in ponderosa pine woodlands, open ponderosa pine forests, subalpine forests, and in grassy areas within forests. For example, it occurs in southeastern British Columbia. Rough fescue grows slowly, which requires 3 to 5 years to become established and produces seed only every 2 to 10 years (Desserud & Naeth 2013). It prefers fertile mostly black soils (black chernozem) with ample moisture that is more than 33 cm annual precipitation, whereas it has been found growing in a 25 cm to 28 cm rainfall area in western Montana (Baldridge & Lohmiller 1990).

Rough fescue is an ecologically and economically important native plant species within grasslands of western Canada (Bogen et al. 2002). Rough fescue grassland is an important habitat for a variety of small mammals and provides forage for wildlife. From an agricultural and economic perspective, rough fescue grassland provides high quality forage for livestock (Desserud & Naeth 2013; Desserud et al. 2013). However, rough fescue grasslands are considered one of the most threatened grassland communities in western Canada due to residential development, livestock heavy grazing and non-native species invasion (Desserud et al. 2013; McInenly et al. 2010). Non-native plant species are particularly problematic when they are competitively superior to native plants.

Competition and soil nutrients

Competition occurs when resources are in short supply because they have been consumed by neighboring organisms (Keddy 2001). For plants, intra- or interspecific competition will arise when they are sharing resources with neighbors. Plants have the same basic requirements for light, water, and nutrients, and they inevitably shade their neighbors and/or have somewhat overlapping root systems (McGraw & Chapin 1989). Resource competition theory shows that a plant population in a monoculture with a single limiting resource would grow and reduce the concentration of that resource to an equilibria level where growth would be balanced by losses (Dybzinski & Tilman 2007). When species compete for a single resource, the species with the lower equilibria level is predicted to win, as demonstrated for rapidly growing organisms (Dybzinski & Tilman 2007). Rapid nutrient uptake and effective pre-emption of soil nutrients are critical for plant growth under competition. Competition among individuals and species of plants may be affected by nutrient availability and acquisition. The ratio of nitrogen to phosphorus (N:P) availabilities influence the productivity and species composition of plant communities (Ahmad-Ramli et al. 2013). Previous studies have shown that different outcomes of species competition under nitrogen- or phosphorus-limited conditions probably reflects the fact that some species compete most successfully for nitrogen, while others compete more successfully for phosphorus (Venterink and Güsewell 2010). A study from Leskovšek et al. (2012) demonstrates that higher nitrogen availability increases the competitive advantage of invasive grass (*Ambrosia artemisiifolia* L.) compared with native grass (*Lolium multiflorum* L.). Ahmad-Ramli et al. (2013) show that the availability of phosphorus is increasingly recognized as a key nutrient that limits productivity, either on its own or in combination with other mineral nutrients like nitrogen. These previous studies indicate that variations in nitrogen and phosphorus supply can influence the intensity of competitive interactions and could potentially affect the outcome of plant competition.

Biochar

Biochar is the use of charcoal as a soil amendment, which has been proposed to increase both soil organic carbon levels and soil fertility (Gathorne-Hardy et al. 2009). Biochar is able to improve soil productivity, carbon storage, or filtration of percolating soil water (Lehmann & Joseph 2009) because of its two key properties: a high affinity to nutrients and water, and a long residence time. The high affinity to nutrients and reduces onsite nutrient loss and offsite pollution from nutrient leaching. The long residence time leads to biochar's promotion for carbon sequestration (Gathorne-Hardy et al. 2009). Biochar's effects on soil properties may have direct impacts upon plant growth because of the penetration depth and availability of air and water within the root zone is determined largely by the physical components of soil (Lehmann & Joseph 2009).

Thesis Research Objectives

The overall objective of this thesis is to study methods to restore spotted knapweed infested grasslands in British Columbia. The first objective is to understand spotted knapweed invasion. My thesis examined the carry over effects of recently removed spotted knapweed on soil properties and vegetation at Lac du Bios Grasslands Provincial Park in chapter 2. The second objective is to test the ability of biochar to restore grasslands infested by spotted knapweed in British Columbia. As previous studies have shown positive results on plant growth with the use of biochar, I conducted a greenhouse experiment to examine competitive effects of spotted knapweed on rough fescue at different N:P ratios, and with a biochar addition treatment in chapter 3. The information gained through this study will help to move the spotted knapweed infested grassland restoration in British Columbia forward.

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Chapter 2 – Soil Residual Effects of Spotted Knapweed on Soil Properties and Vegetation at Lac Du Bios Grasslands Provincial Park

Introduction

Soil plays an important role by supporting the growth of plants, controlling the storage and flow of water in the hydrologic system, facilitating decomposition and nutrient cycling, providing habitat for living organisms, influencing the composition and physical condition of the atmosphere and being an engineering medium in human-built ecosystems (Brady &Weil 2008). Taking this significance into consideration, preserving soil properties is essential. Available soil nutrients are needed for plant growth; however, non-native invasive plants can alter soil nutrient availability (Reid et al. 2009).

Soil nitrogen is vital for plant growth and development. Nitrogen is an integral component of many essential plant compounds, such as amino acids and nucleic acids. Nitrogen is needed for protein development, photosynthesis and the processing of carbohydrates within plants (Bang 2007). Nitrogen stimulates root growth and development, as well as the uptake of other nutrients (Brady &Weil 2008). Soil phosphorus is also important for plant growth. It plays an important role in photosynthesis, nitrogen fixation, flowering, fruiting and maturation (Blackshaw & Brandt 2009). Phosphorus encourages root growth, especially development of lateral roots and fibrous rootlets (Brady &Weil 2008). Carbon is an essential element of soil organic matter; organic-matter-rich soils are generally better for plant growth. Organic matter improves soil physical and chemical properties, which provides suitable plant growing conditions (Smith & Stitt 2007). Volumetric water content is a key hydrological soil physical variable that influences the hydrological response of catchment (Dlamini & Chaplot 2012). It influences the partition between runoff and soil infiltration. It also controls energy and gas fluxes between the pedosphere, the biosphere and the atmosphere, which greatly influence land processes such as soil water denitrification (Grimaldi & Chaplot 2000), carbon sequestration, associated climate change and biodiversity (Asbjornsen et al. 2011). Thus, volumetric water content plays an important role in soil compaction and provides available water for plant growth.

Spotted knapweed has been shown to reduce soil nitrogen availability and increase soil phosphorus availability (Story et al. 1989; Hook et al. 2004; Thorpe et al. 2006; Fraser and Carlyle 2011). Hook et al. (2004) reported that spotted knapweed can increase or decrease, but mostly decrease, soil carbon pools in native grasslands. Fraser and Carlyle (2011) also reported a decrease in soil carbon in spotted knapweed patches compared to rough fescue grassland. Spotted knapweed has been shown to have a greater ability to uptake soil water than native grass (Hill et al. 2006). Spotted knapweed can influence soil properties but what is not known is whether there are residual effects of spotted knapweed on soil properties and plant growth after the spotted knapweed has been removed, and whether potential residual effects might affect the successful re-establishment of native species for restoration.

Spotted knapweed is a strong competitor with rough fescue and causes reduced biomass of native grasses (Press et al. 1998; Suding et al. 2004). Spotted knapweed has a larger leaf area and a higher rate of growth than rough fescue (Press et al. 1998). In addition, spotted knapweed secretes allelochemicals that negatively affect the growth of native plants (Suding et al. 2004). The question remains whether it is possible to restore rough fescue following invasion by spotted knapweed? Press et al. (1998) demonstrated that revegetation with fast-growing, tall-statured species can be a method of managing spotted knapweed invasion. Therefore, areas invaded by spotted knapweed may have the potential to be restored through the removal of spotted knapweed and transplanting rough fescue seedlings.

Fraser and Carlyle (2011) found that large patch size (>10 m^2) of spotted knapweed has been shown to have a greater negative effect on soil properties. Therefore, I hypothesize that if there are residual soil effects caused by spotted knapweed, these effects will likely be greater in sites where the knapweed patch size was larger. In my study, residual effects from spotted knapweed were compared between former large and small patches, and the biomass accumulation of planted rough fescue seedlings were compared between the different patch sizes.

The field site study was designed to test the following hypotheses:

(1) Soil nitrogen, carbon, volumetric water content and dry biomass accumulation of transplanted rough fescue seedlings will be lower in former spotted knapweed patches than in former native grass patches, but soil phosphorus will be higher.

(2) Soil nitrogen, phosphorus, carbon, volumetric water content and dry biomass accumulation of transplanted rough fescue seedlings will be lower in former large spotted knapweed than in former small spotted knapweed patches.

(3) Soil nitrogen, carbon and volumetric water content will have a negative linear relationship on biomass of transplanted rough fescue seedlings within former spotted knapweed patches, while soil phosphorus will be higher.

Materials and Methods

Site description

The research was done at Lac du Bios Grassland Provincial Park located to the northwest of the city of Kamloops, British Columbia, Canada (N50°47′, W120°26′). Soils of Lac Du Bois Park are classified as Chernozems (van Ryswyk et al. 1966) in accordance with the Canadian Soil Classification System (Soil Classification Working Group 1998). Within this park, the dominant grass species are *Festuca campestris* Rydb., *Achnatherum occidentale* (Thurb.) Barkworth ssp. *occidentale*, and S. *richardsonia* (Link) Barkworth described by van Ryswyk et al. (1996). Spotted knapweed first established in this grassland approximately 40 years ago (Fraser & Carlyle 2011).

The average annual precipitation is about 260 mm, but it increases up to 310 mm with greater precipitation partly from snow melting at higher elevation in the park (Carlyle et al. 2011). The highest rainfall is between June and August. The driest period occurs between March and April. Snow falls mainly in December and January (Ministry of Environment, Lands and Parks Report 2000). The average temperature in the valley bottom is 8.4℃, and decreases by approximately 0.5 degrees with an elevation increase every 500 m (Ministry of Environment, Lands and Parks Report 2000). The upper elevation grasslands in Lac Du Bois Park have higher annual precipitation and lower mean temperature compared to lower and middle grasslands.

Experimental design

The study was designed to test carry over effects from recently removed spotted knapweed. The experiment was established in May 2011 and ended in September 2011. Forty patches of spotted knapweed (twenty large and twenty small patches) were randomly selected within Lac Du Bois Provincial Park. A knapweed patch was identified as a group of at least 10 stems, with stems no further than 0.5 m from its neighbor. Patches were separated by at least 20 m. Patch size was classified according to a previous study, where a large patch had an area larger than 10 m^2 and a small patch had an area between 2 m^2 and 10 m^2 (Fraser

& Carlyle 2011). Paired patches of the neighboring native species were located 5 m due North from the edge of the knapweed patch. Within the knapweed and paired native patches, a 1 m^2 area of spotted knapweed and the paired 1 m^2 area of neighboring native species were respectively cleared by removing the above-ground vegetation in mid-May, 2011. Within each cleared area, nine seedlings of rough fescue were transplanted in a 3×3 array, spaced 30 cm apart (Figure 2.1). The seedlings were about 4 months old, between 5-10 cm tall, and had been propagated from seed in the Research Greenhouse at Thompson Rivers University, Kamloops, BC. Seedlings were randomly selected for transplant. Seedlings were watered to field capacity at the time of the transplant, but there was no further watering.

Figure 2.1 1 m^2 quadrat frame in place for rough fescue seedling transplants (left), and 9 rough fescue seedlings after transplanting (right).

Sampling, Measurements and Analysis

On the day of harvesting (mid-September, 2011) the transplanted rough fescue seedlings, the top 10 cm^3 of soil at each corner of the 1 m^2 plot was collected with the use of a soil corer and the four soil samples were combined, stored in zip-lock bags and transported to the laboratory where they were air dried. Dry soil samples were sieved with 2 mm mesh to separate coarse fragments, roots and small rocks.

All the transplanted rough fescue seedlings were clipped at the above-ground level in the last week of September. Biomass of each rough fescue seedling was stored in a paper bag and transported to the laboratory and oven dried at 75°C for at least 48h, and weighed.

Volumetric water content (%) was measured by using a TDR soil moisture meter fitted with a 20 cm soil rod (Spectrum Technologies, Inc.) at each corner of the 1 m^2 plot.

Dry and sieved soil samples were prepared for analysis for total nitrogen, total phosphorus and total carbon. Soil nitrogen (ppm) and carbon (ppm) were analyzed with a CE-440 Rapid Analysis Elemental Analyzer, Exeter Analytical, Inc. Total soil phosphorus ($mg \cdot l^{-1}$) was measured using Palintest© test kits. The mineral concentration of $mg \cdot l^{-1}$ is equivalent to mg kg^{-1} (i.e. ppm).

Statistical Analysis

First, paired *t*- tests were used to compare the biomass of rough fescue seedlings and soil properties within 40 former spotted knapweed patches and within 40 former native grass patches. Second, 2-sample *t*-tests were used to compare the biomass of rough fescue seedlings and soil properties in former large spotted knapweed patches and in former small spotted knapweed patches. Third, linear regressions were applied to predict the effect of soil properties on biomass accumulation of rough fescue seedlings. Statistical analysis of the data was performed using Minitab®16.2.4. All data followed a normal distribution under a Kolmogorov-Smirnov test or an Anderson-Darling test (Appendix A).

Results

Results from the paired *t*-test indicated that biomass of transplanted rough fescue seedlings was higher in the native grass patches compared to the spotted knapweed patches (Table 2.1). There was no difference in total phosphorus between spotted knapweed and native grass patches. Total nitrogen, total carbon and volumetric water content were found to have lower values in the spotted knapweed patches (Table 2.1).

Based on the 2 sample *t*-test biomass of rough fescue seedlings and all measured soil variables were found to be not significantly different between the large and small spotted knapweed patches (Table 2.2).

There were no significant linear regression results between rough fescue biomass and soil properties (total nitrogen, total phosphorus, total carbon and volumetric water content); or within former spotted knapweed patches that were large or small.

Table 2.1 Mean rough fescue biomass and soil parameters measured within the 40 spotted knapweed patches and 40 native grass patches with standard error in parentheses. Values in bold represent significant differences between the two patch size ($P<0.05$).

	Former spotted knapweed patches	Former native grass patches	P value
Rough fescue seedlings			
Biomass (g)	**0.336 (0.024)**	**0.384 (0.020)**	**0.052**
Soil variables			
Total nitrogen (ppm)	**4837 (239)**	**5838 (312)**	**0.011**
Total phosphorus (ppm)	1269.2 (28.3)	1263.6 (27.5)	0.858
Total carbon (ppm)	**5103 (279)**	**6287 (336)**	**0.008**
Volumetric water content (%)	**9.648 (0.349)**	**10.68 (0.370)**	**0.037**

Table 2.2 Mean rough fescue biomass and soil parameters measured within the 20 large spotted knapweed patches and 20 small spotted knapweed patches with standard error in parentheses. Values in bold represent significant differences between the two patch size ($P<0.05$).

	Former Spotted Knapweed Patches		P value
	(Large)	(Small)	
Rough fescue seedlings			
Biomass (g)	0.365 (0.031)	0.307 (0.035)	0.224
Soil variables			
Total nitrogen (ppm)	5026 (415)	4648 (242)	0.436
Total phosphorus (ppm)	1286 (32)	1252 (48)	0.554
Total carbon (ppm)	5295 (492)	4911 (272)	0.499
Volumetric water content (%)	9.90 (0.63)	9.39 (0.31)	0.473

Discussion

Soil nitrogen, carbon, volumetric water content and biomass of transplanted rough fescue seedlings were lower in former spotted knapweed patches compared to former native grass patches, which supports my first hypothesis. The result indicates that effects from spotted knapweed on these three measured soil properties and transplanted rough fescue seedlings' biomass accumulation still exist even though the above-ground parts of the spotted knapweed plants were cleared. In contrast, there was no difference in total soil phosphorus between former spotted knapweed patches and native grassland. However, Fraser and Carlyle (2011) showed that soils where spotted knapweed was present had higher concentrations of total phosphorus compared to grassland soils within the same Lac du Bois grasslands as studied here. My result suggests that the effects of soil phosphorus caused by spotted knapweed are relatively transient. Perhaps the planted rough fescue seedlings were able to uptake the extra available phosphorus in the soil within the former knapweed patches.

Soil nitrogen

Total soil nitrogen was higher in native grasslands compared to former knapweed patches. Harvey and Nowierski (1989) also found that nitrogen concentration was 62% lower on soils from a site infested with spotted knapweed than from a site dominated by grasses. The differentiation in total nitrogen between the former spotted knapweed patches and former native grass patches may be caused by the high mobility and multiple loss pathways of mineral soil nitrogen (Schulze 2000). Spotted knapweed is reported to reduce soil nitrogen availability (Hook et al. 2004; Story et al. 1989). Under this nutrient-deficient condition, bacteria, decomposer fungi and soil fauna will not mineralise organic nitrogen and not release ammonium (Schulze 2000). Organic nitrogen mineralization and ammonium release contribute to soil total nitrogen, so the decrease of them can cause a lower nitrogen level to knapweed-infested areas than non-infested areas. The lower nitrogen within former spotted knapweed patches was probably only temporary. Spotted knapweed decreases the rate of nitrification, which is driven by bacteria and the root exudate catechin (Thorpe and Callaway 2010). However, Thorpe and Callaway (2010) found that the inhibitory effects of catechin on nitrification decreased over time since some bacteria degrade catechin (Arunachalam et al. 2003) and catechin oxidizes rapidly in solution and in soils (Inderjit et al. 2008; Pollock et al. 2009).

Soil carbon

Soil carbon sequestration is limited by the availability of nitrogen and phosphorus (Goll et al. 2012). De Graaff et al. (2006) suggests that the main driver of soil carbon sequestration is soil carbon input through plant growth, which is strongly controlled by nutrient availability. Since spotted knapweed reduces soil nitrogen and increases phosphorus, the lower carbon within former spotted knapweed patches may be a decrease in sequestration of carbon. Soil carbon is a mixture of different organic molecules, ranging from very labile to very stable according to their residence time in the soil. Stable carbon is protected against decomposition by various mechanism including micro-aggregation, association with clay and silt particles and formation of recalcitrant compounds (Six et al. 2002). Labile carbon is by contrast affected by rates of decomposition (Epron et al. 2009). (±)Catechin, exudation secreted by spotted knapweed may accelerate the decomposition of soil organic matter and stimulate the dissolution of insoluble minerals by rhizosphere microorganisms (Haichar et al. 2014), which decreases carbon residence time. As a result, a lower carbon level reflected within infested areas may because of a decrease in carbon sequestration caused by spotted knapweed.

Soil phosphorus

There was no difference in total soil phosphorus between former spotted knapweed patches and native grassland. Stevenson and Cole (1999) found that spotted knapweed contains catechin which is important in the complexation of iron (Fe), aluminium (Al), and calcium (Ca), including the precipitation of Ca–P compounds. As a result, spotted knapweed can increase soil available phosphorus in the rhizosphere through chelation of metal elements. Furthermore, the amount of phosphorus taken up by plants is governed by the equilibria among the numerous phosphorus compounds in the soil and the differing abilities of plants to modify their rhizosphere environments. In this experiment, there may be three possibilities why phosphorus in former spotted knapweed infected area was the same as native grassland: the transplanted rough fescue seedlings may have taken up the soluble increased phosphorus; the soluble phosphorus was lost as surface runoff during rainfall events within the year the knapweed was removed and the soil was sampled; or there was no difference in phosphorus to begin with since the sites were not measured before the rough fescue was transplanted. The last point, though, is unlikely considering that Fraser and

Carlyle 2011) showed significantly higher soil phosphorus in knapweed patches compared to native grasslands within the same site two years earlier

Soil volumetric water content

Due to a greater access to soil water sources and a greater ability to extract water from deeper soils for invaders (Hill et al. 2006), spotted knapweed has a greater amount of water usage than native grasses. As a result, my experiment showed that soil volumetric water content was higher where the native grass formerly dominated. Gờrgens and Wilgen (2004) pointed out that it may take several years before streamflow recovery approaches pre-planting levels after clearing of dense and extensive stands of alien plants, which indicates that invasive plants deplete soil water resources and the water take time to be replenished. Similarly, loss of soil water within areas invaded by spotted knapweed may take time to recover. On the other hand, soil water is influenced by soil structure, the more well-aggregated soil the higher the water holding capacity (Liu et al. 2014). Spotted knapweed is able to change soil aggregation via changes in soil microbial properties or processes (Lutgen & Rillig 2004). While soil aggregation changed by spotted knapweed, soil structure in knapweed-infested areas has lower aggregate stability than that in non-infested area. This explains that the differences in soil volumetric water content may result from variance of soil aggregation between knapweed-infested and non-infested areas.

Biomass

Plant productivity is controlled by multiple nutrients and their interactions (Estate & Park 2013). Previous studies have shown that spotted knapweed reduces soil nitrogen and increases soil phosphorus (Fraser and Carlyle 2011; Thorpe et al. 2006; Olson & Blicker 2003); thus leading to nitrogen limitation for plant growth. In addition, decreased water availability within the former spotted knapweed patches likely caused a further limitation to the growth of rough fescue seedlings. Furthermore, spotted knapweed exudes large amounts of (±)-catechin from its roots, which is highly allelopathic to North American species (Newingham & Callaway 2006). Thus, it is possible that residual allelochemicals have effects on plants growth. As a result, the biomass of rough fescue was lower than in the former native grass dominated area.

Differences between large and small patches

There was no difference in soil nitrogen, phosphorus, carbon, volumetric water content and biomass of transplanted rough fescue seedlings between former large and small spotted knapweed patches, which does not support my second hypothesis. Fraser & Carlyle (2011) showed a trend that soil properties (nitrogen, carbon and volumetric water content) and plant biomass are negatively associated with patch size, while soil phosphorus is positively associated with patch size. That was a correlative study considering knapweed biomass as a covariate; it cannot say with certainty that differences in soil properties and plant biomass were caused by knapweed patch size. Taking this into consideration, the fact that carry over effects from patch size seemed to make no difference in soil properties and transplanted rough fescue seedling biomass may be because spotted knapweed was removed. Results from my experiment indicate that scale may not be an important consideration in the carry over effects of spotted knapweed on soil properties and rough fescue biomass following removal of spotted knapweed.

Linear regression

There was no relationship between soil properties (nitrogen, phosphorus, carbon and volumetric water content) and the biomass of transplanted rough fescue seedlings within former spotted knapweed patches, which does not support my third and last hypothesis. The observed differences in plant growth may be due to the complex interactions between soil properties caused by spotted knapweed, or allelochemicals (Newingham & Callaway 2006); or due to initial variances among rough fescue seedlings, small differences in initial size and growth rates between individuals could make differences in a long-term development (Mangla et al. 2011). Real effects on biomass of rough fescue seedlings can be hidden if variance in initial single soil nutrient availability (nitrogen, phosphorus, carbon and volumetric water content) is small compared to differences caused by spotted knapweed.

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Chapter 3 - Competitive Effects from Spotted Knapweed on Rough Fescue with or without Biochar Addition at Different N: P Ratios

Introduction

Competition

Competition is the negative effect from one organism on another by consuming a resource that is limited in availability or controlling access to that resource (Keddy 2001). Competition occurs when resources are in short supply because they have been consumed by neighboring organisms (Keddy 2001). For plants, intra- or interspecific competition will arise when they are sharing resources with neighbors. Plants have the same basic requirements for light, water, and nutrients, and they inevitably shade their neighbors and/or have somewhat overlapping root systems (McGraw & Chapin 1989).

Previous studies have shown that the non-native invasive spotted knapweed (*Centaurea stoebe* L. subsp. *micranthos* (Gugler) Hayek) can reduce biomass of native grasses, which indicates that competition occurs between spotted knapweed and native grasses, such as rough fescue (Press et al. 1998). The mechanism of spotted knapweed to successfully out-compete rough fescue is not clear. It could be that allelopathic root exudates restrain the growth of rough fescue (Suding et al. 2004); or the morphology of spotted knapweed (rosette leaves and stout taproot) is more competitive (Press et al. 1998); or spotted knapweed alters soil properties to the detriment of rough fescue growth rate: spotted knapweed reduces soil nitrogen, carbon, water and increases soil phosphorus, which influences growth of rough fescue (Story et al. 1989; Hook et al. 2004; Thorpe et al. 2006; Hill et al. 2006; Press et al. 1998; Fraser & Carlyle 2011). To understand how spotted knapweed competes with rough fescue I designed a pairwise interaction experiment in the greenhouse. In this study, N:P ratio was examined as one of factors influencing the growth of spotted knapweed and rough fescue. Blicker et al. (2002) reported that competition for nitrogen between spotted knapweed and native grasses is various, which means that spotted knapweed's growth response to nitrogen depends on which species it is growing with. Results from Suding et al. (2004) showed that reduction of soil phosphorus weakened the ability of *C. diffusa* to tolerate neighbor competition proportionately more than the other focal species. Similarly, under low phosphorus conditions, spotted knapweed may lose its competitive advantage.

N:P ratio

Soil nitrogen and phosphorus are vital for plant growth and development; however, they are often among the most limiting nutrients, especially in grassland soils (Olson & Blicker 2003; Goll et al. 2012). While the availability of nitrogen and phosphorus is often dynamic and changeable, the growth of spotted knapweed can influence soil nutrient availability. For example, Callaway et al. (2004) has shown that spotted knapweed increases phosphorus availability; but Hook et al. (2004) and Story et al. (1989) has shown that spotted knapweed reduces nitrogen availability. Fraser and Carlyle (2011) have also found that spotted knapweed patches have higher amounts of phosphorus and lower amounts of nitrogen in soils compared to areas where rough fescue grows.

Plants are usually exposed to multiple resource limitations, which is saying that they are limited by nitrogen, phosphorus, or other resources (e.g., light, CO_2, and water) at the same time. An increase in nitrogen limitation may create greater limitation in phosphorus (Baldwin 2013). In addition, individual plants within a diverse community may have different competitive abilities for various limiting resources. They can experience greater or lesser limitation to different resources depending on their morphology and physiology. For example, plants with larger roots may be competitively superior in soil mineral nutrient resource acquisition compared to plants with smaller roots (Baldwin 2013). On the other hand, the availability of nitrogen and phosphorus influences plant performance, plant species interactions and multi trophic interactions (Baeten et al. 2011). Consequently, altering the levels of nitrogen and phosphorus supply may have a significant impact on the growth of spotted knapweed and rough fescue and interactions between these two species. This study was conducted with three N:P ratios (1:1, 15:1 and 30:1) to determine how nutrient supply affects competition between spotted knapweed and rough fescue.

Biochar

Biochar is a soil amendment that is produced by thermal decomposition of organic material (forest residues such as bark, sawdust, and shavings; and agricultural wastes such as wheat straw and bagasse) under limited supply of oxygen and at relatively low temperatures (< 700 °C). Biochar is used to improve soil productivity, carbon storage, or filtration of percolating soil water (Lehmann & Joseph 2009). The study of biochar is growing due to its perceived ability to increase plant yields while at the same time reduce the need for fertilizer, to decrease soil nutrient runoff losses and reduce atmospheric carbon through the sequestration of carbon in the soil (America et al. 2007). Despite the positive effects attributed to biochar soil amendment, little research has been published elucidating the mechanisms and effects of biochar.

Biochar is considered to have positive effects on plants through influencing soil nutrient availability, such as potentially immobilizing plant-available nitrogen (Ventura et al. 2013). The added biochar is thought to stimulate nitrogen mineralization thereby improving nitrogen uptake by plants (Saarnio et al. 2013). Gathorne-Hardy et al. (2009) have shown that biochar appears to increase nitrogen use efficiency of crops when it is applied with N fertilizer. Similar to the impacts on soil nitrogen, biochar affects availability of phosphorus for plants (Biederman & Harpole 2013). Studies have shown that biochar can increase soil phosphorus availability through several mechanisms; for example, Parvage et al. (2012), Lehmann and Joseph (2009) demonstrate that biochar is a direct source of soluble phosphorus and can serve as a binder for positively charged metal complexes (Al^{3+}, Fe^{3+2+}, Ca^{2+}). These studies suggest that biochar has the potential to affect plant growth through increasing the availability of soil nitrogen and phosphorus for plant uptake. These changes of soil properties caused by biochar can influence plant growth (Devereux et al. 2013). However, the impact of biochar on plants is still not comprehensive since it is highly variable depending on the properties of the biochar and the soil, plant species and environmental conditions (Saarnio et al. 2013).

The availability of nitrogen and phosphorus influences plant performance, plant species interactions and multi trophic interactions (Baeten et al. 2011). The growth of spotted knapweed can influence soil nutrient availability. For example, Hook et al. (2004) and Story et al. (1989) have shown that spotted knapweed reduces nitrogen availability; but Callaway et al. (2004) have shown that spotted knapweed increases phosphorus availability. So, by manipulating nutrient supply through differing N:P ratios I can test whether spotted knapweed will have a greater growth response under a higher relative phosphorus concentration. Whereas, biochar is more likely to increase nitrogen availability and to bind phosphorus (Gathorne-Hardy et al. 2009; Parvage et al. 2012; Lehmann and Joseph 2009), which could reduce the growth potential of spotted knapweed.

The greenhouse study was designed to test three hypotheses:

(1) Biomass of spotted knapweed will be greater at 1:1 N:P ratio, and relatively less so at 15:1 and 30:1 N:P ratios; whereas rough fescue will show the opposite response.

(2) Biomass of spotted knapweed is less when grown in biochar, compared to grown in pure sand. In contrast, biomass of rough fescue is greater when grown in biochar, compared to grown in pure sand.

(3) Competitive importance decreases for spotted knapweed with increasing N:P ratios and biochar addition, whereas it increases for rough fescue with increasing N:P ratios and biochar addition.

Materials and Methods

Experimental design

The design was a 5 plant by 3 nutrient supply ratio by 2 biochar factorial combination replicated 8 times for a total of 240 treatment combinations. The five plant treatments included spotted knapweed alone, rough fescue alone, two spotted knapweed, two rough fescue and the two species in a pair wise interaction; the three nutrient supply ratios included N:P ratios of 1:1, 15:1 and 30:1; and the two biochar additions treatments consisted of a biochar added or not.

Species

Spotted knapweed is a short-lived perennial forb and an aggressive weed that reduces wildlife and livestock forage production, lowers native biodiversity, and increases loss of soil mineral nutrients (Sheley & Jacobs 1997; Fraser & Carlyle 2011) in British Columbia, Canada. Rough fescue (*Festuca campestris* Rydb.) is an ecologically and economically important native plant species within grasslands of western Canada (Bogen et al. 2002).

Greenhouse conditions

The climate, temperature and relative humidity, were electronically controlled during February and May in 2013. Daytime temperature was maintained at 25°C and night time temperature at 17°C (Hendry & Grime 1993). The relative humidity was held constant at 55% for both daytime and night time. Three 1000W halogen sulphide lamps supplied supplemental light for 14 hours in the day. Pots were divided into eight blocks and randomly arranged within each block.

Germination

Approximately 600 seeds of spotted knapweed and rough fescue were placed in separated plastic Petri dishes filled with a sand medium (fine-textured Home Depot© Play Sand) saturated with water. The Petri dishes were placed in the greenhouse receiving 16h of light. Rough fescue seeds were sown 4 days before spotted knapweed seeds to ensure germination of both species occurred within days of each other.

Treatments

Seedlings were transplanted from Petri dishes into 1.3 L round pots, (11.4 cm high, 15.2 cm diameter, with 8 drain holes). Each pot was filled with 1 L pure sand (Table 3.1& 3.2). Each pot received one or two transplants: spotted knapweed alone, rough fescue alone, two spotted knapweed together, two rough fescue together, spotted knapweed with rough fescue.

Each species combination that included a biochar addition were treated with 10 grams of biochar (Table 3.1 & 3.2). The application rate was biochar to soil ratio of 1:69 on a mass basis (Ameloot et al.2013). Biochar was mixed with sand medium one week before the sand was saturated with water. The sand medium was saturated with water immediately prior to transplantation. Three days after planting, each planting combination was watered with 100 mL modified Rorison’s nutrient solution (Hendry & Grime 1993) (Table 3.3) at three different ratios of nitrogen to phosphorus (N:P) every three days: 1:1, 15:1 and 30:1 N:P.

Table 3.1 Biochar and sand properties. Analysis done by Ministry of Environment, Environmental Sustainability and Strategic Policy Division Knowledge Management Branch – Laboratory using HNO_3/HCl Microwave Digest.

Element	Biochar	Sand	Element	Biochar	Sand
Ca (%)	2.129	1.042	Mo (ppm)	3.9	1.0
Mg (%)	0.243	0.718	Zn (ppm)	613.4	90.4
K (%)	0.532	0.261	Cu (ppm)	36.4	31.6
Fe (%)	1.641	2.022	Total P (ppm)	1004	446
Al (%)	0.323	1.489	Total N (%)	0.442	0.006
Na (%)	487	855	Total C (%)	79.35	0.13
Mn (ppm)	1106	368	pH	9.80	7.99
B (ppm)	23.1	5.0			

Table 3.2 Exchangeable Cations and Effective CEC (0.1 M Barium Chloride) of biochar and sand. Analysis done by Ministry of Environment, Environmental Sustainability and Strategic Policy Division Knowledge Management Branch – Laboratory.

Element(cmol+/Kg)	Biochar	Sand	Element(cmol+/Kg)	Biochar	Sand
Al	0.303	0.002	Mg	0.523	0.320
Ca	8.073	1.967	Mn	0.007	0.003
Fe	0.005	<0.001	Na	0.229	0.060
K	1.864	0.062	CEC	11.00	2.41

Table 3.3 Modified Rorison's nutrient solution as applied to the N: P ratio nutrient treatments (preparation of 100 mL; Hendry and Grime 1993). $^{\alpha}$ +, the solution was included; –, the solution was omitted from the treatment.

Element	mg. 100mL^{-1}	Stock solutions	N:P ratio$^{\alpha}$		
			1: 1	15: 1	30: 1
Ca/N	8.0/5.60	$Ca(NO_3)_2·4H_2O$	+	+	+
Mg	2.4	$MgSO_4·7H_2O$	+	+	+
K/P	14.2/5.60	$K_2HPO_4·3H_2O$	+	+	–
K/P	0.4/0.18	$K_2HPO_4·3H_2O$	–	–	+
Fe	0.3	Fe EDTA	+	+	+
Mn	0.06	$MnSO_4·4H_2O$	+	+	+
B	0.06	H_3BO_3	+	+	+
Mo	0.01	$(NH_4)_6Mo_7O_{24}·4H_2O$	+	+	+
Zn	0.01	$ZnSO_4·7H_2O$	+	+	+
Cu	0.01	$CuSO_4·5H_2O$	+	+	+
K	6.81	K_2SO_4 (0.5molL-1)	–	–	+

Harvesting

Harvesting occurred on Day 90. Individuals were separated by species and then by above-ground and below-ground biomass. The biomass samples were dried for 48 hours at 70°C and weighed.

Statistical analysis

To analyze mean potential biomass I used only the data for plants grown alone. A log transformation was done for these data; they follow a normal distribution under an Anderson-Darling test and have equal variances under a Levene's test (Appendix B). An ANOVA (Minitab®16.2.4) was conducted to test the effects of species, N:P ratios and biochar addition on the total plant biomass (α=0.05). A log transformation was done for the data when plants grown alone and two rough fescues grown together for using ANOVA (Appendix B). The data used for testing competitive importance with intraspecific competition had a normal distribution under an Anderson-Darling test and had equal variances under a Levene's test (Appendix B). A reciprocal transformation was done for the data when plants grown with interspecific competition. The data had a normal distribution under an Anderson-Darling test and had equal variances under a Levene's test (Appendix B). Competitive importance was calculated using the equation: $C_{imp} = \frac{P_{-N} - P_{+N}}{MP_{\pm N} - \min(P_{-N}, P_{+N})}$, where P_{-N} is plant grown without neighbors (alone) and P_{+N} is plant grown with neighbors, and $MP_{\pm N}$ is the maximum value of plant performance in the studied system, regardless of neighbors (Seifan et al. 2010— modified after Brooker et al. 2005). In this equation, the index has a limited range of -1 to1; where negative values represent competitive interaction and positive values represent facilitation. The importance of competition is a relative measure of the effect of competition at a point along the gradient relative to other processes, it can incorporate the role of other processes in describing the impact of competition (Gilbert & Fraser, 2013).

Results

Total biomass

Plants grown alone

The mean total dry biomass of plants when grown alone (without competition) was significantly affected by two of the three main effects (species and N: P ratio) but not by biochar addition (Table 3.4). Spotted knapweed had a greater mean biomass (4.83g ± 0.45 SE) than rough fescue (0.786 ± 0.094 SE). Biochar did not affect plant biomass.

An interaction between species and N:P ratios was shown to be not significant (Table 3.4). However, results showed that spotted knapweed had the greatest biomass at 1:1 N/P ratio, whereas rough fescue had the greatest biomass at 15:1 N/P ratio. Spotted knapweed's biomass was greater at 15:1 N/P ratio compared to 30:1 N/P ratio. Rough fescue's biomass was greater at 1:1 N/P ratio compared to 30:1 N/P ratio. Biomass of spotted knapweed was greater than that of rough fescue at each N/P ratio (Fig. 3.1).

The two-way interaction between species and biochar addition was not significant, neither the interaction between N: P ratio and biochar addition was significant (Table 3.4).

Table 3.4 Results of three-way ANOVA with blocking factor examining the effects of species, N: P ratio and biochar addition on the mean total biomass of spotted knapweed and rough fescue (species grown alone). Significant (α<0.05) values in bold.

Source	Mean squares	Degree of freedom	F ratio	P value
Block	0.115	7	1.71	0. 125
Species	10.823	1	160.97	**<0.001**
N:P ratio	1.625	2	24.17	**<0.001**
Biochar	0.067	1	0.99	0.323
Species × N:P ratio	0.131	2	1.94	0.153
Species × Biochar	0.037	1	0.56	0.459
N:P ratio × Biochar	0.071	2	1.05	0.356
Species × N:P ratio × Biochar	0.017	2	0.26	0.775
Error	0.067	56		

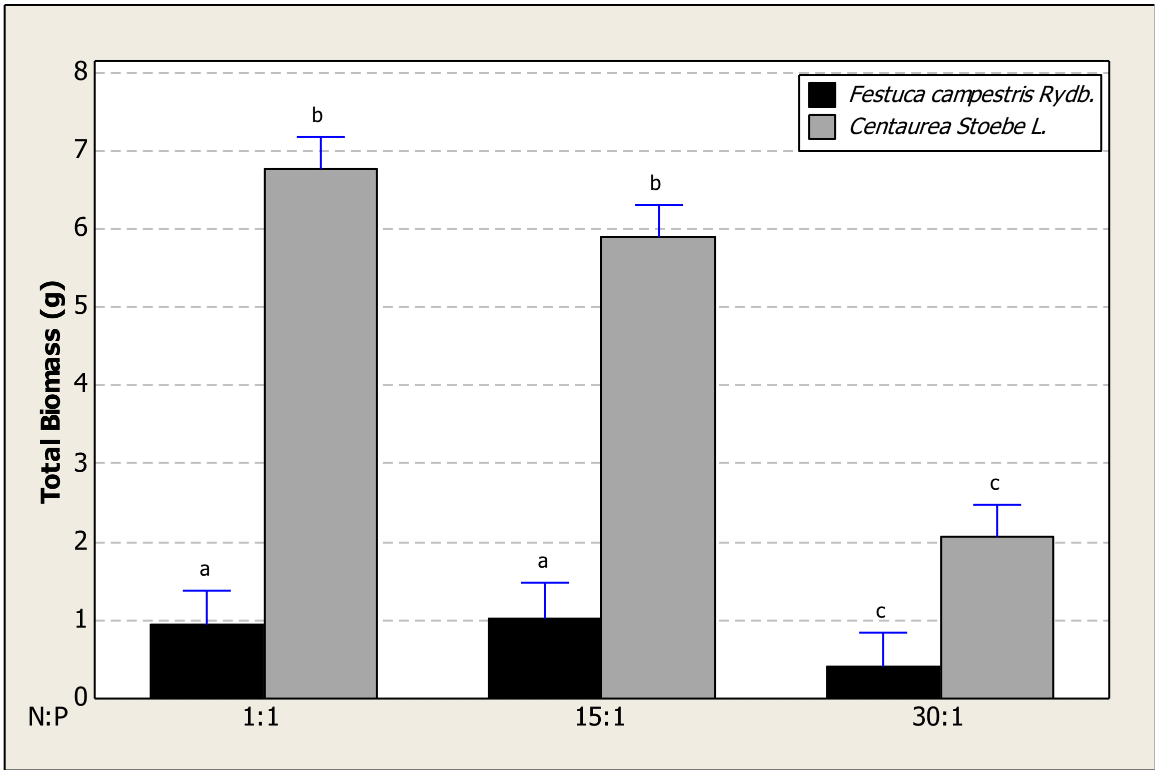

Figure 3.1 Mean total biomass (±1 SE) of *Festuca campestris* Rydb. and *Centaurea Stoebe* L. grown alone at three N/P ratio. Bars sharing the same letter are not significantly different using Bonferroni (P<0.05) between species.

Pairwise intraspecific interactions

The mean total dry biomass of plants when grown together (with intraspecific competition) was significantly affected by N:P ratio but not by biochar addition for both species (Table 3.5 & Table 3.6); the mean total dry biomass of rough fescue (with intraspecific competition) had significant block effect (Table 3.6). Spotted knapweed had a greater mean biomass (5.98g ± 0.54 SE) than rough fescue (1.104 ± 0.081 SE).

Results showed that spotted knapweed had the greatest biomass at 1:1 N/P ratio. Spotted knapweed's biomass was greater at 15:1 N/P ratio compared to 30:1 N/P ratio. On the other hand, N/P ratio did not result in any significant differences to rough fescue's biomass. The biomass of spotted knapweed was greater than that of rough fescue at each N/P ratio (Fig. 3.2).

The two-way interaction between N:P ratio and biochar addition was not significant for either species (Table 3.5 & Table 3.6).

Table 3.5 Results of two-way ANOVA with blocking factor examining the effects of N: P ratio and biochar addition on the mean total biomass of spotted knapweed (two same plants grown together). Significant (α<0.05) values in bold.

Source	Mean squares	Degree of freedom	F ratio	P value
Block	3.531	7	0.57	0.776
N:P ratio	145.331	2	23.36	**<0.001**
Biochar	2.582	1	0.41	0.524
N: P ratio × Biochar	4.557	2	0.73	0.489
Error	6.221	30		

Table 3.6 Results of two-way ANOVA with blocking factor examining the effects of N: P ratio and biochar addition on the mean total biomass of rough fescue (two same plants grown together). Significant (α<0.05) values in bold

Source	Mean squares	Degree of freedom	F ratio	P value
Block	0.055	7	3.03	**0. 013**
N:P ratio	0.103	2	5.65	**0.007**
Biochar	0.001	1	0.03	0.867
N: P ratio × Biochar	0.012	2	0.66	0.524
Error	0.018	35		

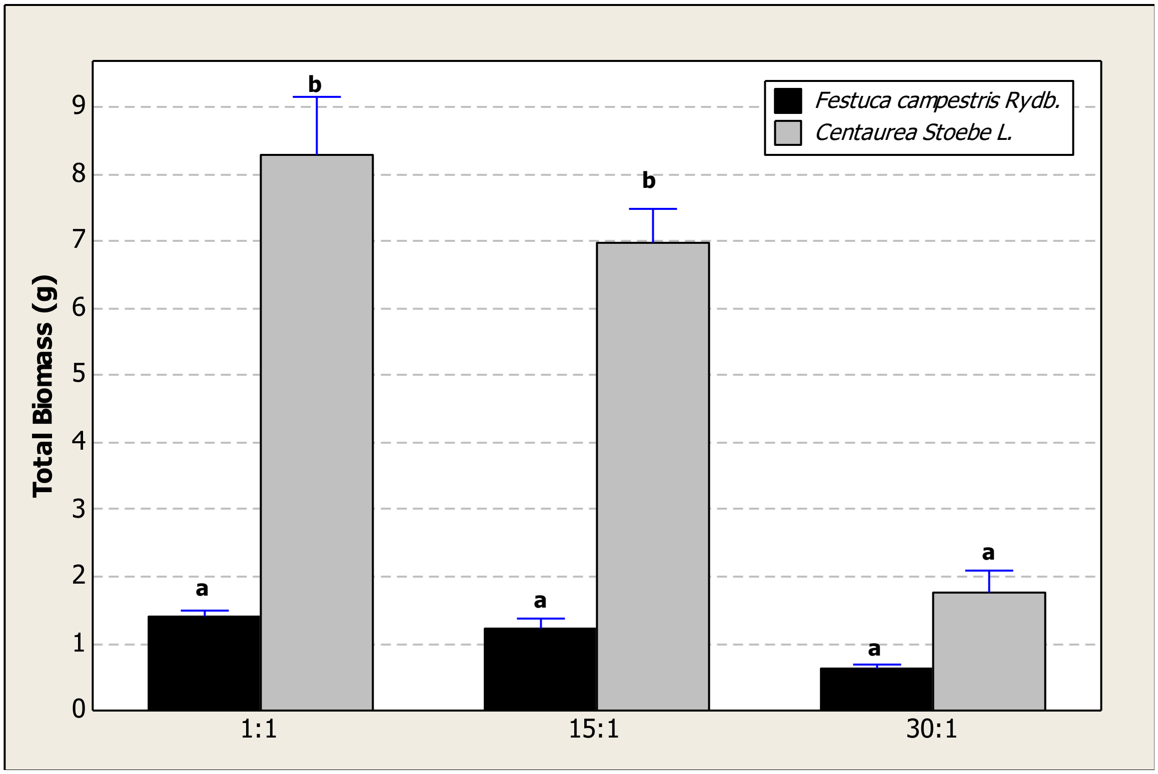

Figure 3.2 Mean total biomass (±1 SE) of *Festuca campestris* Rydb. and *Centaurea Stoebe* L. grown with conspecific plant at three N/P ratio. Bars sharing the same letter are not significantly different using Bonferroni (P<0.05) between species.

Competitive importance

For both species, competitive importance was not significant by any main effects (N:P ratio and biochar addition) when plants grown with intraspecific competition, neither was it significantly affected by a two-way interaction between N:P ratio and biochar addition (Table 3.7 & Table 3.8).

Table 3.7 Results of two-way ANOVA with blocking factor examining the effects of N: P ratio and biochar addition on competitive importance of spotted knapweed (intraspecific competition). Significant (α<0.05) values in bold.

Source	Mean squares	Degree of freedom	F ratio	P value
Block	0.059	7	0.60	0.751
N:P ratio	0.145	2	1.51	0.235
Biochar	0.179	1	1.86	0.182
N:P ratio × Biochar	0.264	2	2.75	0.078
Error	0.096	35		

Table 3.8 Results of two-way ANOVA with blocking factor examining the effects of N:P ratio and biochar addition on competitive importance of rough fescue (intraspecific competition). Significant (α<0.05) values in bold.

Source	Mean squares	Degree of freedom	F ratio	P value
Block	0.1946	7	1.32	0. 268
N:P ratio	0.0317	2	0.22	0.807
Biochar	0.0919	1	0.63	0.434
N: P ratio × Biochar	0.1735	2	1.18	0.319
Error	0.1469	35		

Competitive importance was significantly affected by one of the three main effects (species), but not by N: P ratio or biochar addition when plants grown with interspecific competition (Table 3.9). Results showed that there was a competitive interaction with spotted knapweed, the value of competitive importance was -0.136 ± 0.052 SE; whereas rough fescue demonstrated a facilitation response, the value of competitive importance was 0.0200 ± 0.0068 SE (Fig. 3.3). This shows that spotted knapweed had a stronger competitive ability than rough fescue when grown together.

The two-way interaction between species and N: P ratio was not significant, neither the interaction between species and biochar addition was significant, nor the interaction between N: P ratio and biochar addition was significant.

Table 3.9 Results of three-way ANOVA with blocking factor examining the effects of species, N: P ratio, and biochar addition on competitive importance (interspecific competition). Significant (α<0.05) values in bold.

Source	Mean squares	Degree of freedom	F ratio	P value
Block	0.07796	7	1.18	0. 323
Species	0.58144	1	8.82	**0.004**
N:P ratio	0.08055	2	1.22	0.300
Biochar	0.09861	1	1.50	0.225
Species × N: P ratio	0.12673	2	1.92	0.153
Species × Biochar	0.07089	1	1.08	0.303
N: P ratio × Biochar	0.02261	2	0.34	0.711
Species × N: P ratio × Biochar	0.00770	2	0.12	0.890
Error	0.06594	77		

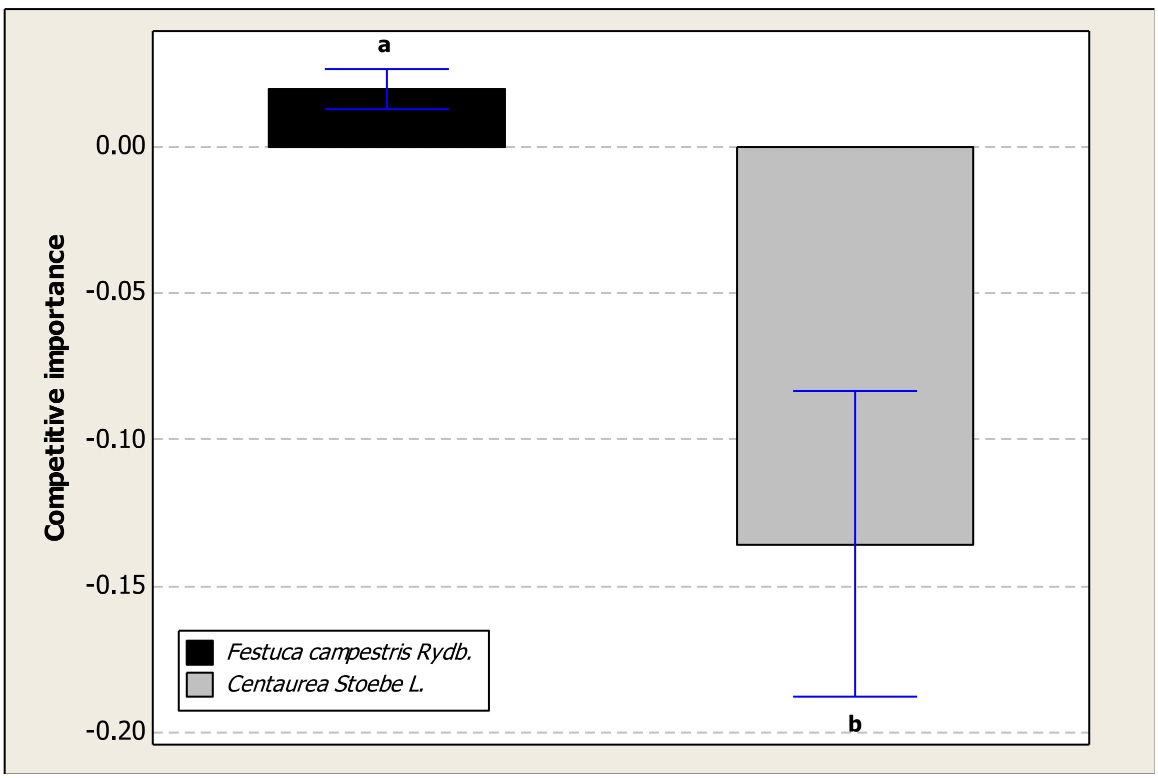

Figure 3.3 Competitive importance (±1 SE) of *Festuca campestris* Rydb. and *Centaurea Stoeb*e L. grown together. Bars sharing the same letter are not significantly different using Bonferroni (P<0.05).

Discussion

Total biomass

The results partially supported my first hypothesis because total biomass of spotted knapweed when grown alone was greatest at 1:1 N/P ratio and declined as N/P ratio increased. Total biomass for both species when two of the same plants grown together was greater at 1:1 N: P ratio, and relatively less so at 15:1 and 30:1 N: P ratios. Because a good supply of nitrogen stimulates root growth and development, and spotted knapweed prefers a high level of nitrogen (Blicker et al. 2002); spotted knapweed had its greatest biomass at 1:1 N/P ratio when grown alone and grown with its conspecific plant. Due to the polymorphism of spotted knapweed, intraspecific competition may not be strong enough to be shown under these N:P ratios. Güsewell (2005) suggests that all species seems to have maximum biomass at an N: P supply ratio of 15:1 within a level of light and nutrient supply. The result that rough fescue had greatest biomass at 15:1 N: P ratio when grown alone accords with Güsewell (2005). However, the results when two rough fescues grown together are different from when it is grown alone because the condition for growth has been changed. In the presence of a competitor, competition for nutrients generates a condition where plants must allocate more resources to acquisition of the limiting resource than is optimal for plants in the absence of competition (Craine 2006). Due to this condition, biomass of rough fescue may be influenced. As a consequence, the biomass of rough fescues grown together is different from when grown alone.

Differing from my prediction, biochar addition made no significant differences in total biomass of both species when plants grown alone or grown together. Desserud and Naeth (2013) suggest that smooth brome (*Bromus inermis*), another non-native introduced from Europe and Eurasia in the late 1880s, has a strong negative reaction to straw-amended soils. For native grasses, biochar addition increases biomass of plants regardless of variability introduced by soil and climate (Biederman & Harpole 2013). On the contrary, biomass of spotted knapweed and rough fescue were not significantly affected by biochar addition in my experiment. Nitrogen is volatilized in proportion to carbon and associated with the carbon in the retained fraction sharing its recalcitrance. Therefore, the fact that biochar addition did not work as expected might be that nitrogen is not always the limiting resource for plant growth.

Competitive importance

Neither N:P ratio, biochar addition nor the interaction between N:P ratio and biochar addition significantly influenced competitive importance for intraspecific interactions.. Previous research has indicated that increased nitrogen favors invasive and decreased nitrogen availability favors native species (Mangla et al. 2011). Considering this, spotted knapweed should have a stronger competition with increasing N:P ratio and rough fescue should have a stronger competition with decreasing N:P ratio. Intraspecific competition had no different response to biochar for spotted knapweed and rough fescue. Previous study indicated that spotted knapweed is able to avoid intraspecific competition since they display germination and emergence polymorphism (Interference et al. 2013). Intraspecific competition is strongly dependent upon the growth environment (Keddy 2001), for that reason, intraspecific competition of rough fescue might not show in this experiment. However, small differences in initial size and growth rates between individuals could potentially make differences in long-term development (Mangla et al. 2011). Since spotted knapweeds and rough fescues were transplanted into pots at early stage and they were affected by N: P ratio and biochar, there might be differences in competition between individuals for a longer period of experiment.

Competitive interactions drove spotted knapweed responses while facilitation drove the response of rough fescue when the two species were grown together regardless of N:P ratio and biochar addition, which means that spotted knapweed was a stronger competitor. Suding et al. (2004) reported that *Centaurea diffusa* remained the best competitor under low nitrogen conditions, while lost its competitive advantage under low phosphorus conditions. Therefore, *Centaurea stoebe L.* should have significant responses to various N:P ratios. Ridenour and Callaway (2001) showed that activated charcoal (which adsorbs organic compounds, similar to biochar) reduced the apparent allelopathic effect of *Centaurea stoebe L.* on *Festuca idahoensis* in sand culture. It suggests that the balance of competition in favor of *Centaurea* had been shifted by activated carbon. Biochar addition should have similar effects on rough fescue grown with spotted knapweed. However, the results from my study has shown that biochar addition had no effect, perhaps because the contact time between soil and biochar was not long enough or biochar was not activated before its application.

The availability of nitrogen and phosphorus influences plant performance, plant species interactions and multi trophic interactions (Baeten et al. 2011). In general, the growth of spotted knapweed and rough fescue had significant responses to N:P ratio but not to biochar addition. Various N:P ratios and biochar addition did not reduce competition of spotted knapweed on rough fescue.

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Chapter 4 – General conclusions, management implications and directions for future research

Conclusion

Lac du Bios grasslands are within the temperate grasslands of the southern interior British Columbia, which are a small but unique ecosystems. Unfortunately, they are being threatened by several factors, including the invasion of spotted knapweed. Invasive plants cause loss of biodiversity.

Carry over effects from spotted knapweed on soil properties and plant growth are important considerations when managing invasion. Previous studies have shown that spotted knapweed decreases some soil properties (nitrogen, carbon and water), but increases soil phosphorus (Hook et al. 2004; Story et al. 1989; Thorpe al. 2006; Hill et al. 2006). In addition, previous study has shown that the level of soil properties and biomass of plants are lower in large spotted knapweed patches than in small ones (Fraser and Carlyle 2011). While this evidence partially helps understand spotted knapweed invasion, my study on residual soil effects from spotted knapweed extends the understanding in spotted knapweed invasion.

This study has shown that soil properties (nitrogen, carbon and volumetric water content) and biomass of rough fescue are in a lower level because of spotted knapweed, even though spotted knapweed had been removed for three months. In contrast, soil phosphorus in former spotted knapweed patches was the same concentration as native grassland. Besides, there was no differences in soil properties (nitrogen, carbon, phosphorus and volumetric water content) and biomass of rough fescue between large spotted knapweed patches and small ones. This information on residual effects from spotted knapweed gives a general idea on how to restore areas infected by spotted knapweed.

Ridenour and Callaway (2001) showed that activated charcoal (which adsorbs organic compounds, similar to biochar) reduced the apparent allelopathic effect of *Centaurea stoebe L.* on *Festuca idahoensis* in sand culture. It suggests biochar can be considered as a potential material to restrain the ability of spotted knapweed to compete with native grasses. However, biochar used in my experiment seems to have no effect on competitive ability of spotted knapweed. N: P ratios were also manipulated to determine the effect on spotted knapweed. This study showed that biomass of plants is influenced by N: P ratio, but competitive importance is not. This information provides evidence that spotted knapweed growth may be restrained by increasing N: P ratio.

In conclusion, this short-term study identifies immediate impacts on specific processes that occur in soils and plants, but only long-term data provide confirmation that these effects are having a significant influence on soils and plants over time. Even so, this study extends the understanding of spotted knapweed invasion and offers a potential method to mitigate spotted knapweed invasion.

Management implications

Lac Du Bois grasslands are traditionally used for cattle grazing. To maintain biodiversity in this community, it is important understand the controlling factors. Invasion of spotted knapweed is one of those factors. This study has provided information on how to manage spotted knapweed.

Soil nitrogen, phosphorus, carbon and volumetric water content are essential elements of soil. The carry over effects from spotted knapweed on those soil properties were tested to provide directions for managing invasion. To restore infected areas, the solution can be improving soil properties which are altered by spotted knapweed. Biomass of plants grown in former infected areas are lower. Plant growth is poor in a soil environment with limited nutrients. Altering N: P ratios can be seen as a means of improving soil properties. Since N: P ratio plays a significant role in plant growth, manipulating N: P ratio in this study is a step to figure out the most unsuitable condition for spotted knapweed growth. To sum up, the key to recover spotted knapweed invasion is to improve soil properties.

Besides, there is no significant findings by comparing soil properties and biomass of plants between former large spotted knapweed patches and former small spotted knapweed patches. Even so, it is a worthy factor to be considered when managing spotted knapweed. It is common that a smaller problem is easier to solve.

Future research directions

In this study, residual soil effects from spotted knapweed were independently tested. Instead, current effects and carry over effect should be processed within the same study. To be specific, soil sampling should be done twice: once immediately after spotted knapweed is cleared; the second time after transplanted rough fescue seedlings are harvested. Through comparing these two data points, a more persuasive result could be obtained to better represent carry over effects from spotted knapweed. In addition, a long term study could provide evidence for residual effects from spotted knapweed.

This study has shown that N: P ratio affects spotted knapweed. It is possible to manage spotted knapweed invasion through improving infested soil properties. For example, a suitable N: P ratio for native grasses could enhance their competitive ability when they compete with spotted knapweed, helping to restrain spotted knapweed invasion.

Biochar is considered as a potential material to restore spotted knapweed. In this study, 10 g were used to each pot without making any difference in the results. In future study, different amounts should be tried to see if biochar really has no effects on spotted knapweed. Biochar made from different materials may have various influences on plant growth (Ameloot et al. 2013). If conditions allow, various biochars should tested. Last but not the least, biochar is more effective when mixed with soil for a long time. A biochar study needs to be longer to ensure the impact is properly tested.

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Appendix A

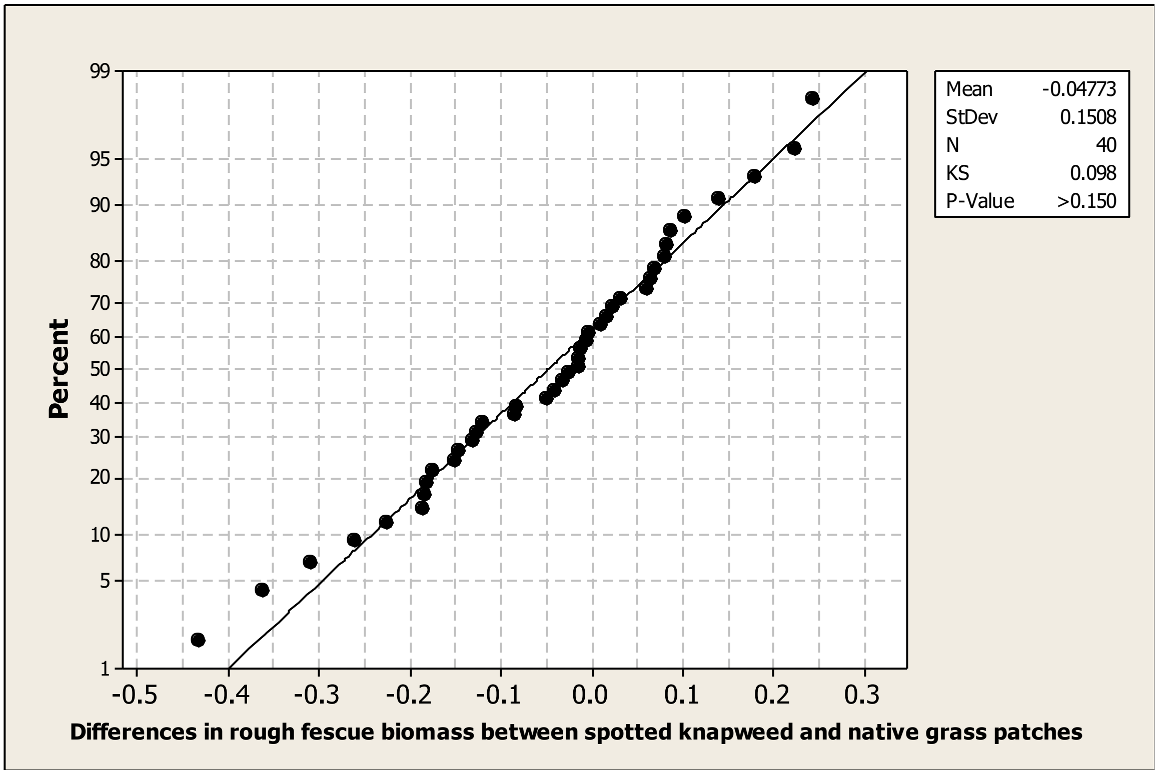

Figure A. 1 Normality of rough fescue seedlings' biomass in former spotted knapweed patches and native grass patches under a Kolmogorov-Smirnov test (P<0.05).

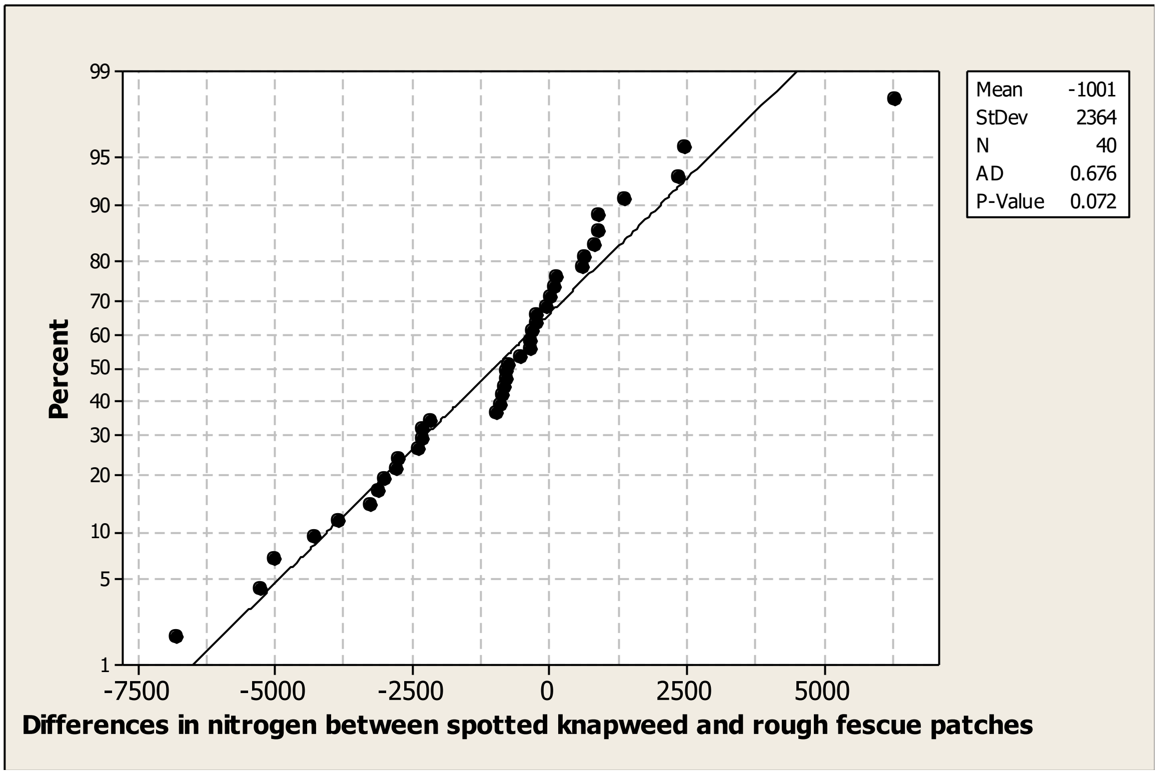

Figure A. 2 Normality of total nitrogen in former spotted knapweed patches and native grass patches under an Anderson-Darling test ($P<0.05$).

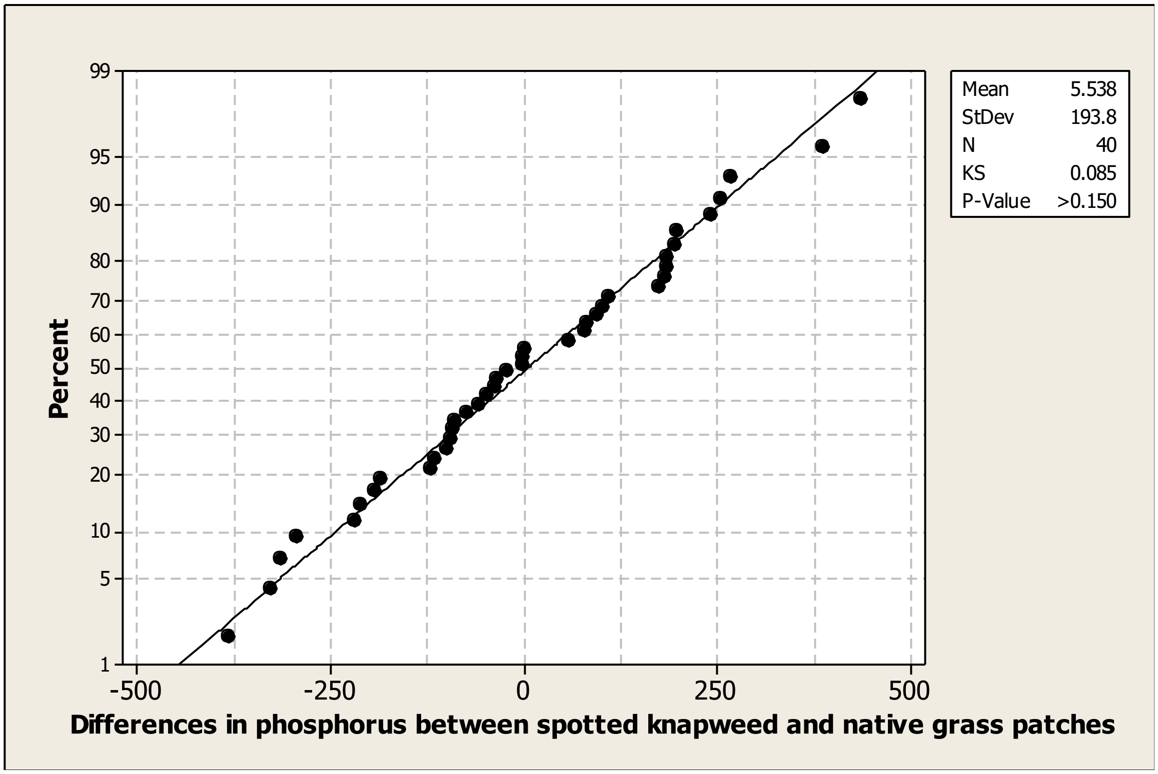

Figure A. 3 Normality of total phosphorus in former spotted knapweed patches and native grass patches under a Kolmogorov-Smirnov test ($P<0.05$).

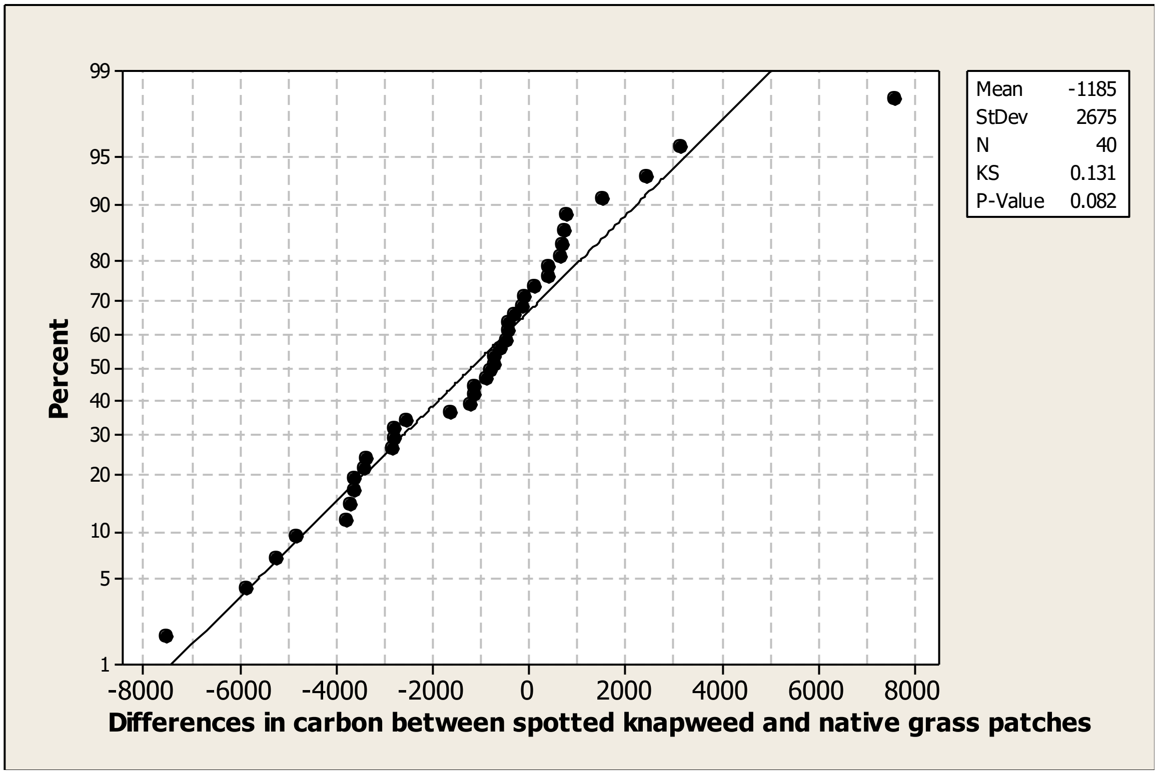

Figure A.4 Normality of total carbon in former spotted knapweed patches and native grass patches under a Kolmogorov-Smirnov test (P<0.05).

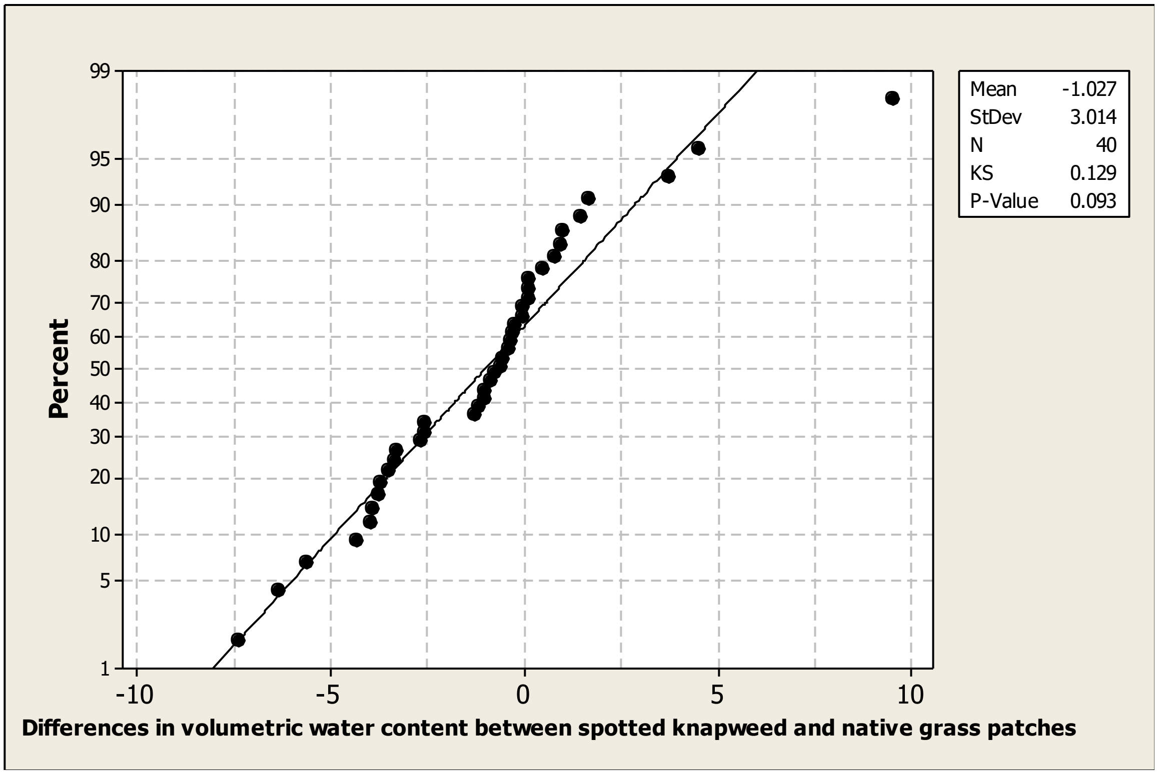

Figure A. 5 Normality of volumetric water content in former spotted knapweed patches and native grass patches under a Kolmogorov-Smirnov test (P<0.05).

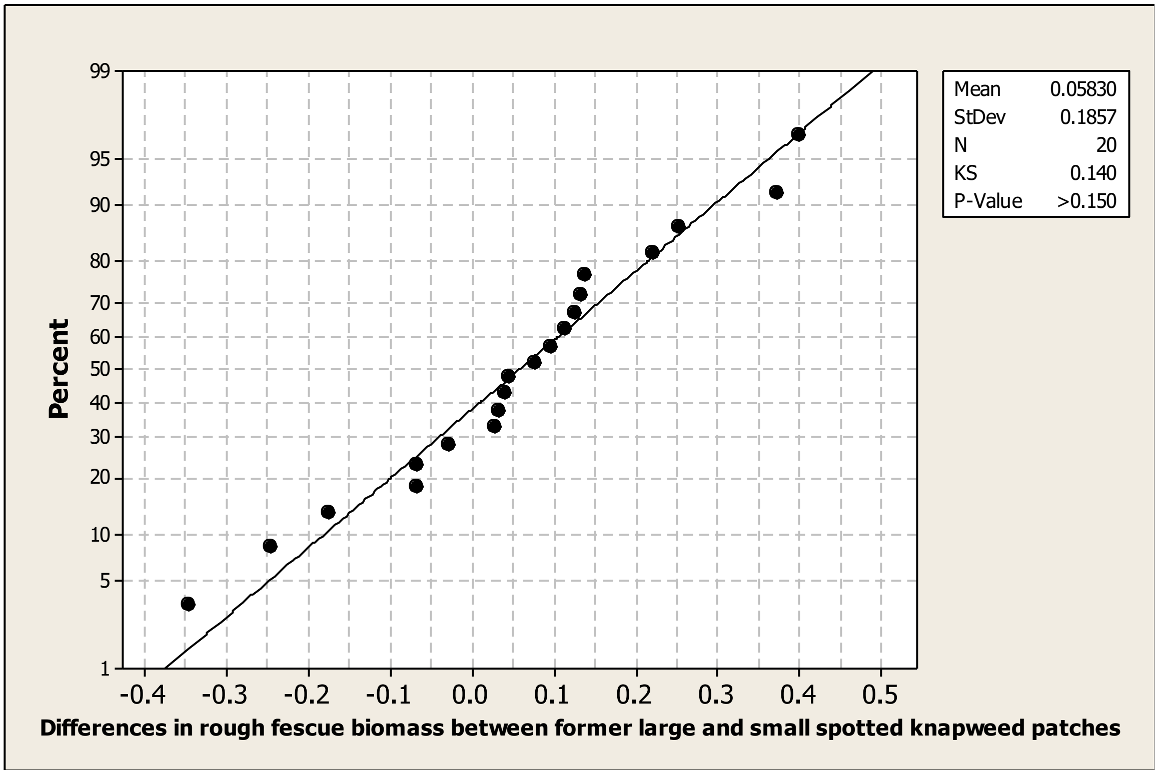

Figure A. 6 Normality of rough fescue's biomass in former large and small spotted knapweed patches under a Kolmogorov-Smirnov test ($P<0.05$).

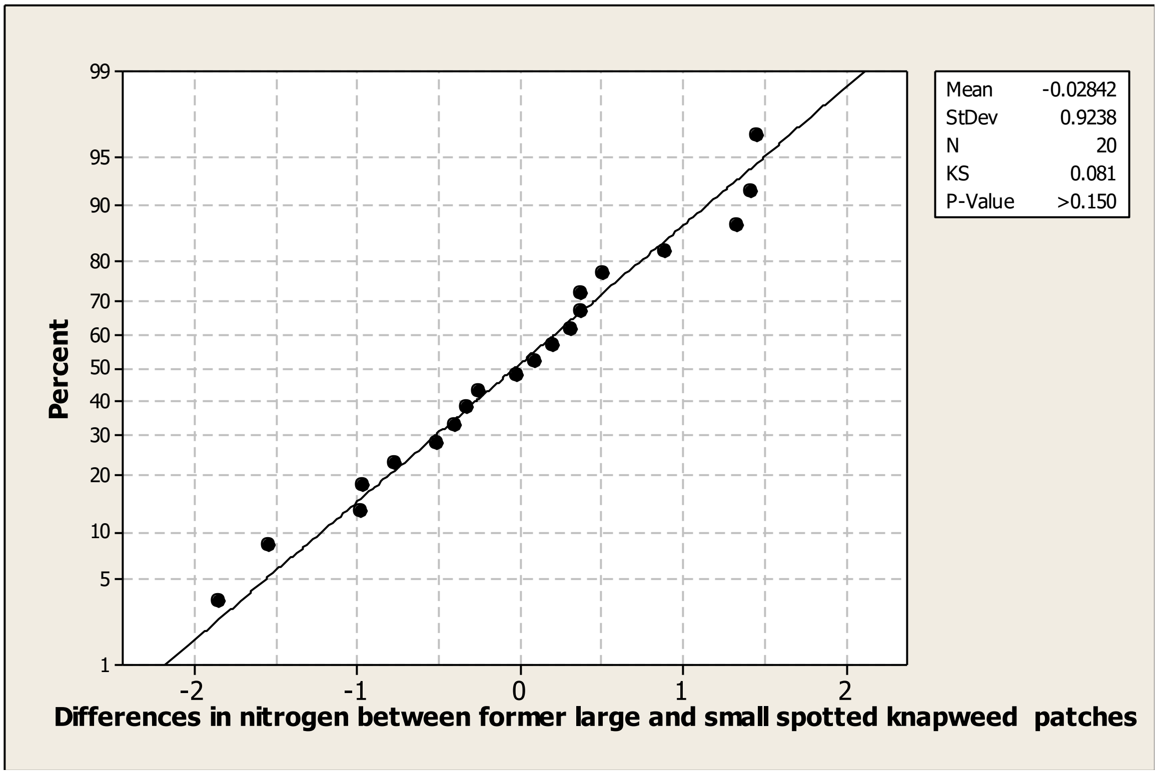

Figure A. 7 Normality of total nitrogen in former large and small spotted knapweed patches under a Kolmogorov-Smirnov test (P<0.05).

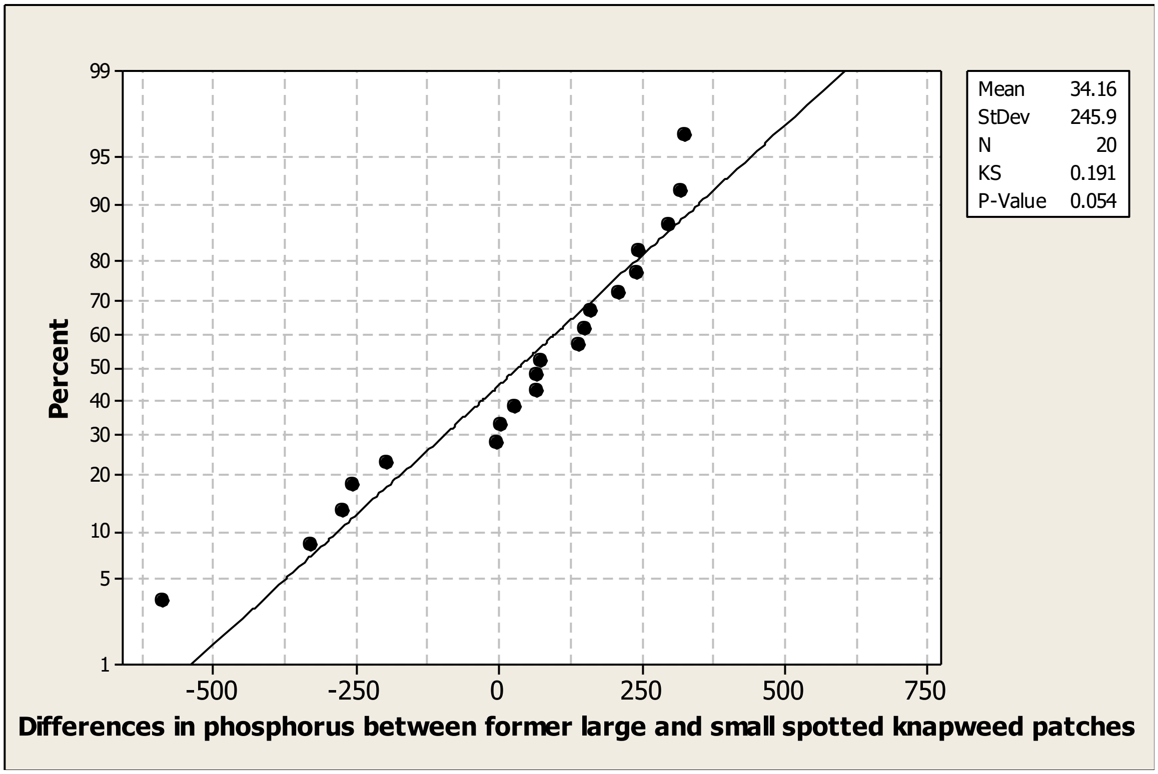

Figure A. 8 Normality of total phosphorus in former large and small spotted knapweed patches under a Kolmogorov-Smirnov test (P<0.05).

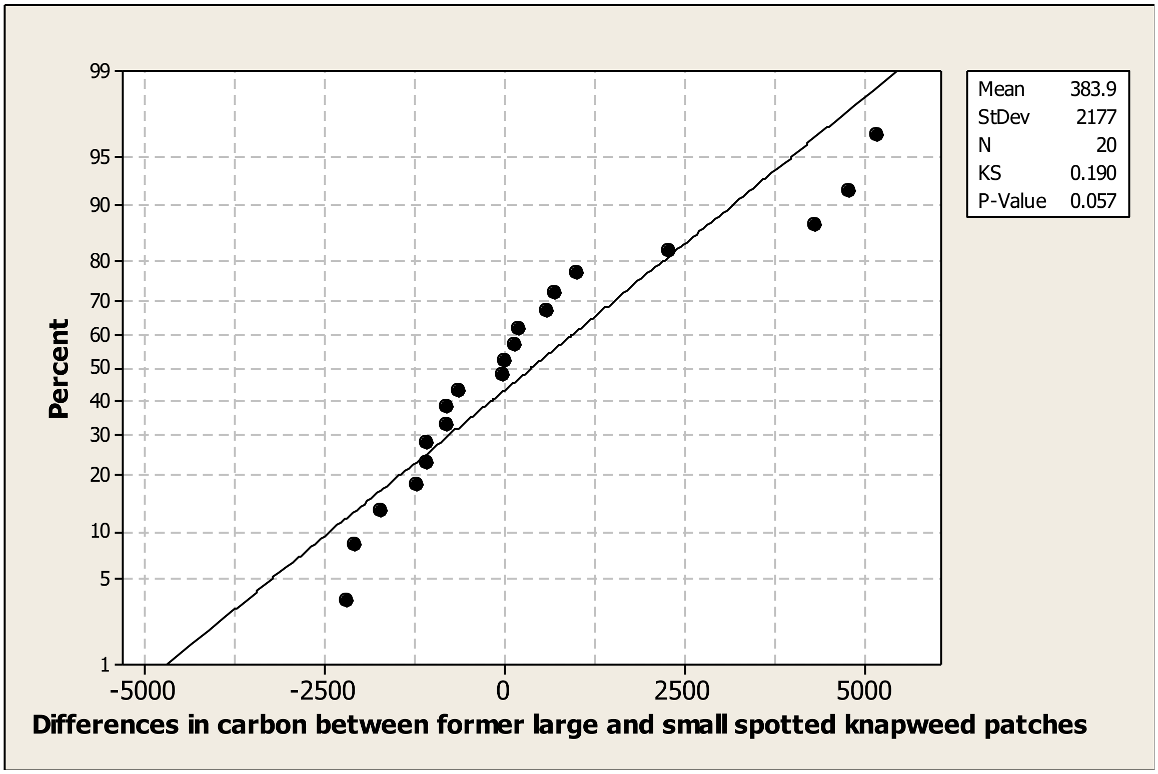

Figure A. 9 Normality of total carbon in former large and small spotted knapweed patches under a Kolmogorov-Smirnov test (P<0.05).

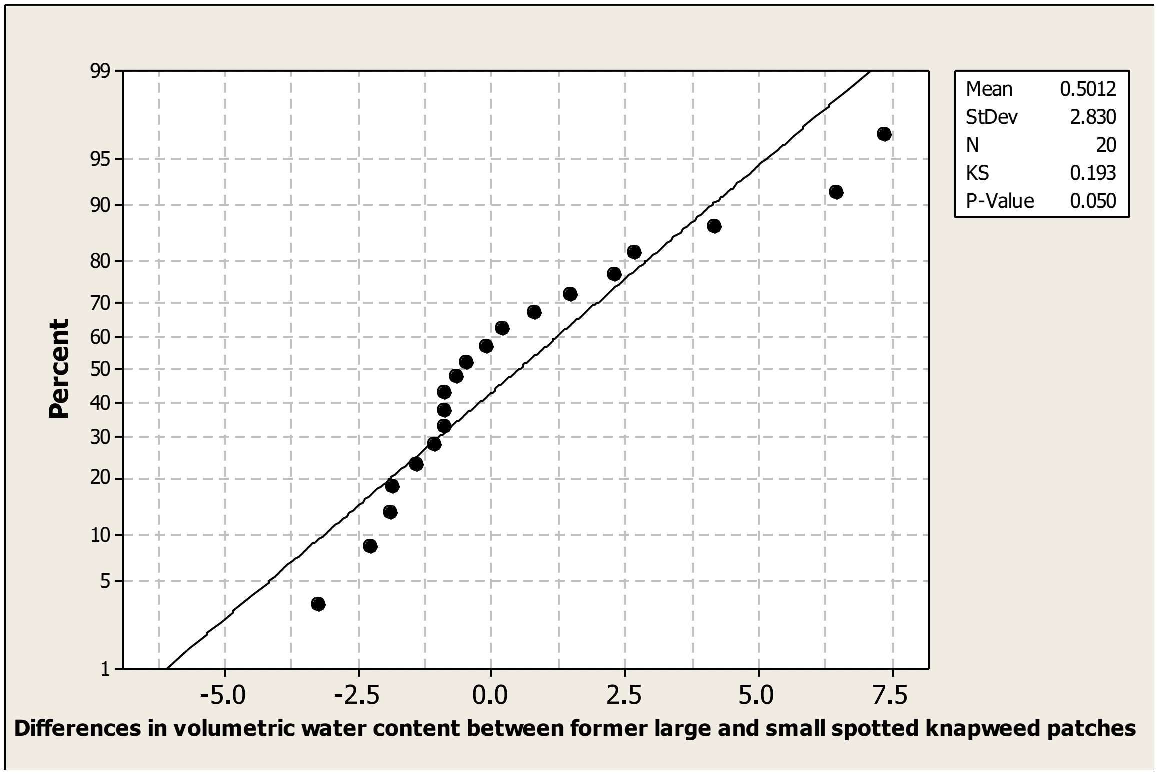

Figure A. 10 Normality of volumetric water content in former large and small spotted knapweed patches under a Kolmogorov-Smirnov test ($P<0.05$).

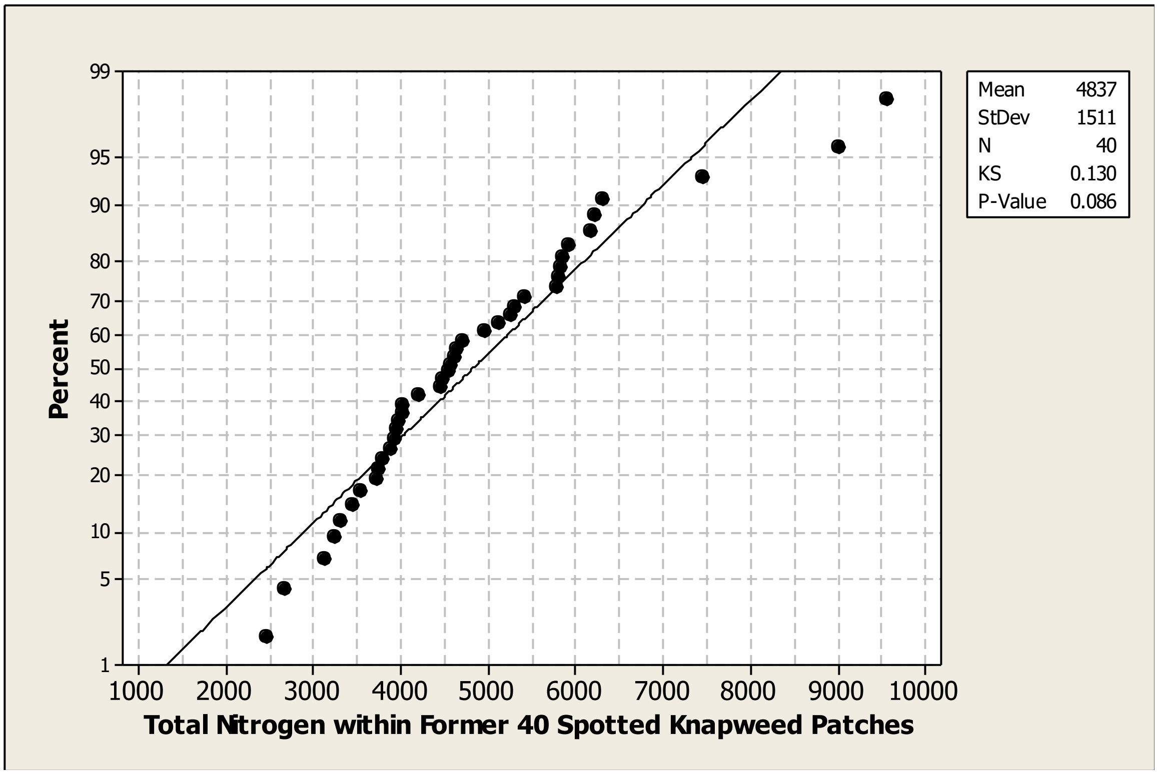

Figure A. 11 Normality of total nitrogen in former 40 spotted knapweed patches under a Kolmogorov-Smirnov test (P<0.05).

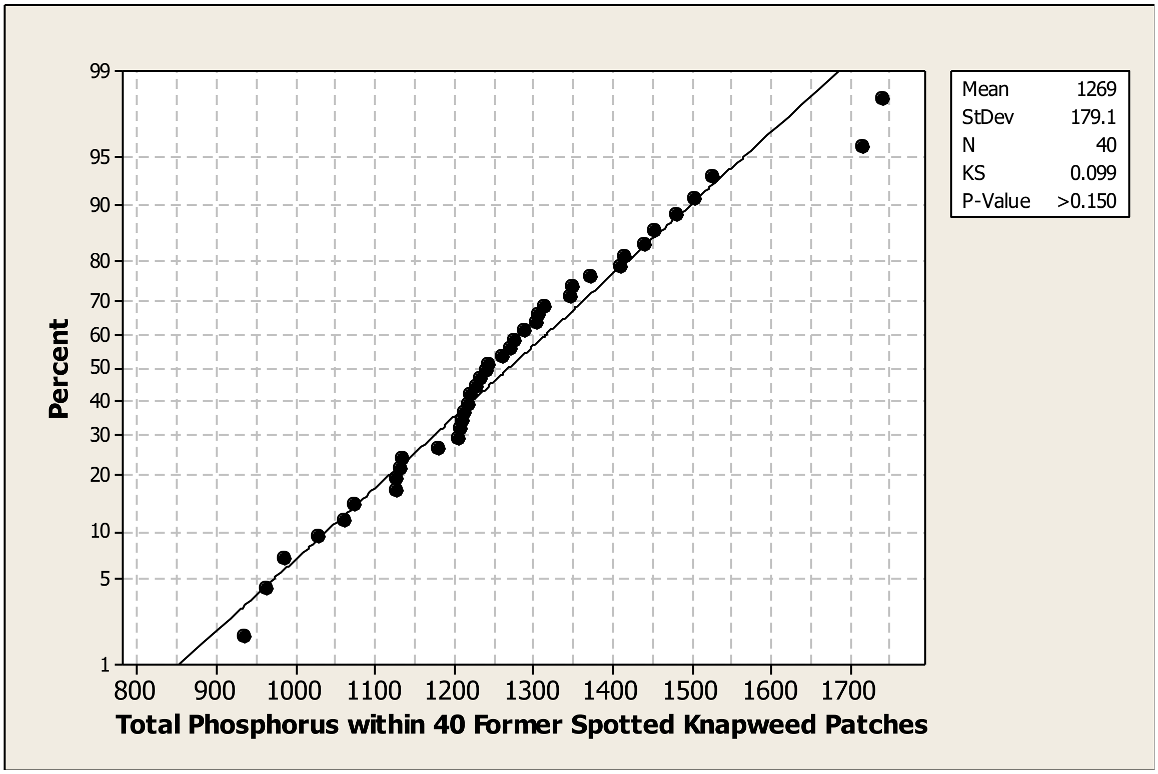

Figure A. 12 Normality of total phosphorus in former 40 spotted knapweed patches under a Kolmogorov-Smirnov test (P<0.05).

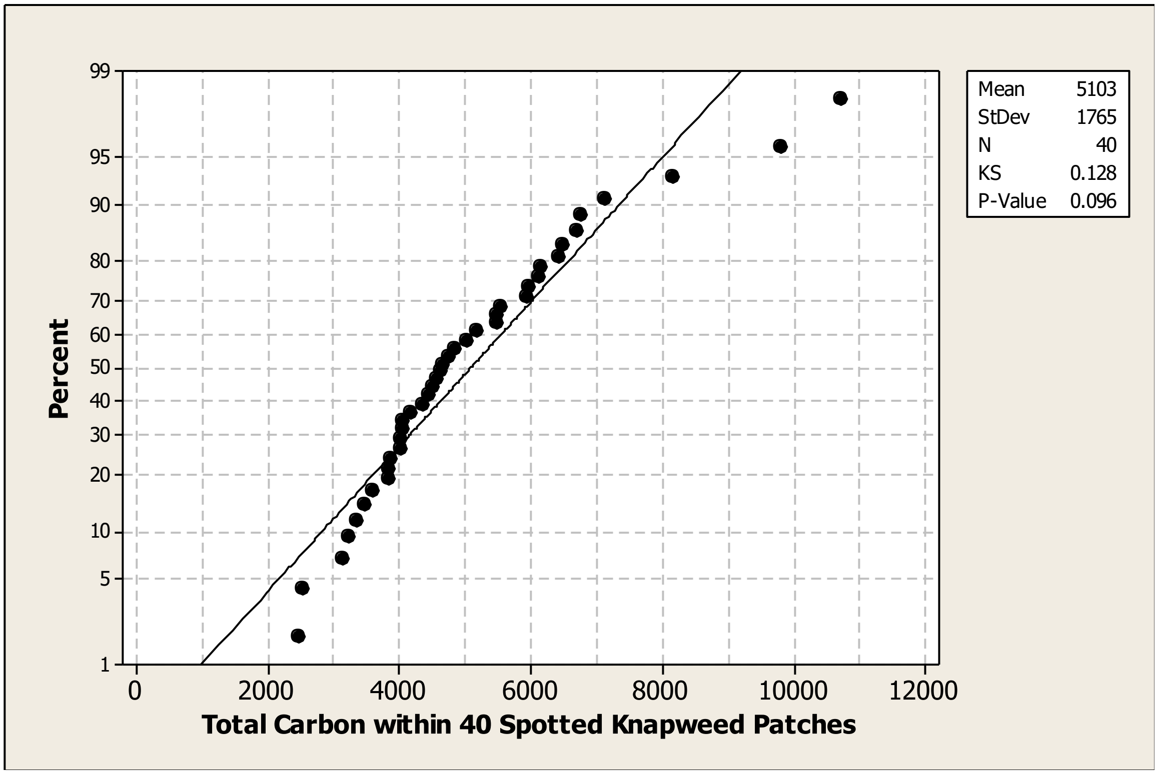

Figure A. 13 Normality of total carbon in former 40 spotted knapweed patches under a Kolmogorov-Smirnov test (P<0.05).

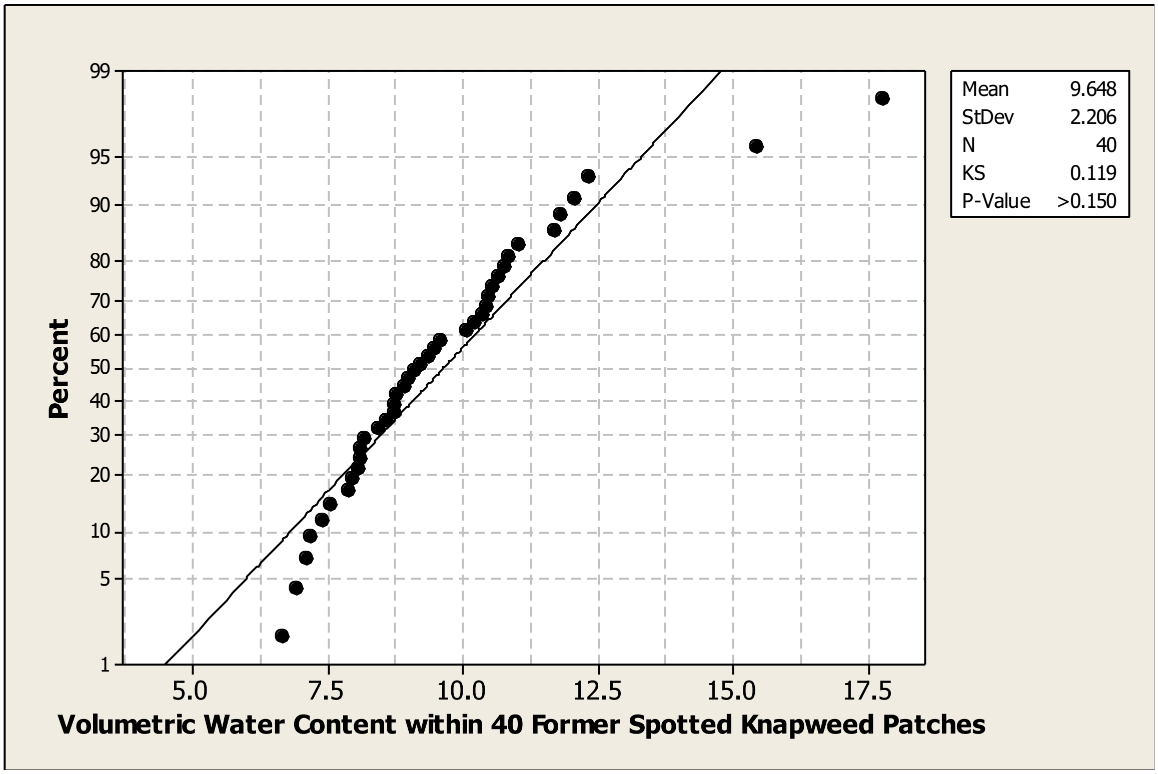

Figure A. 14 Normality of volumetric water content in former 40 spotted knapweed patches under a Kolmogorov-Smirnov test (P<0.05).

Appendix B

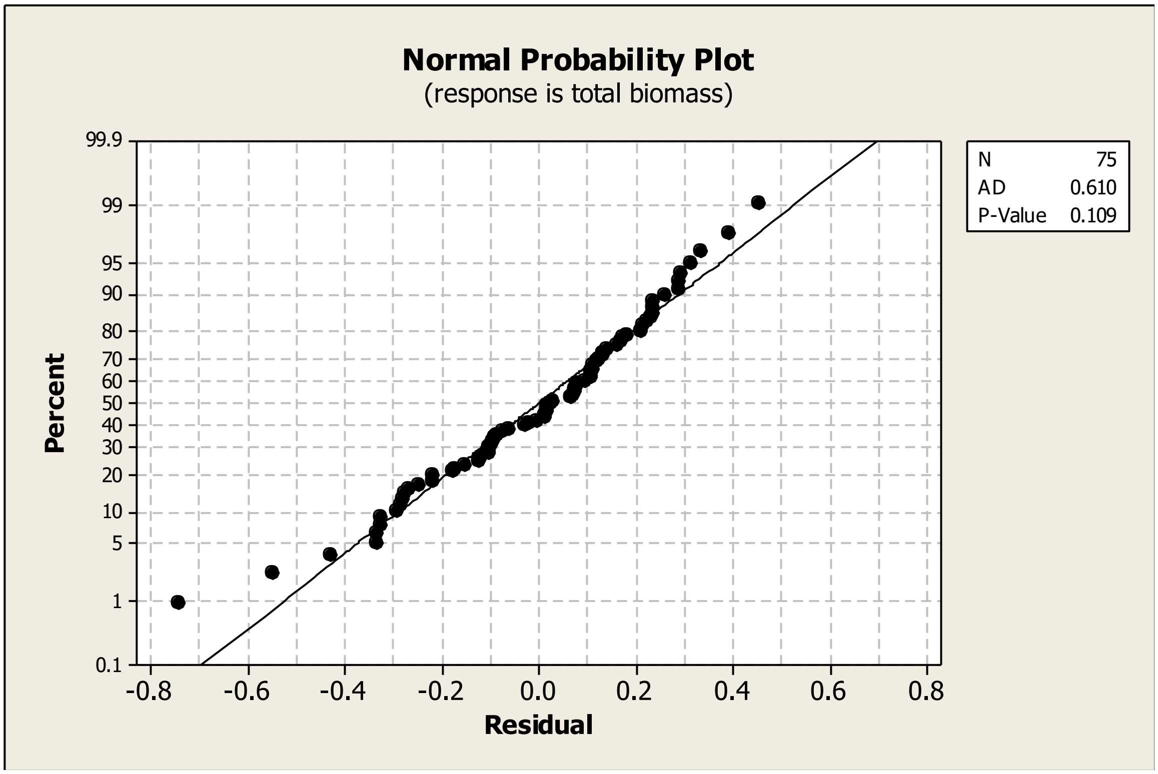

Figure B. 1 Normality of potential biomass using 3-way ANOVA when plants grown alone under an Anderson-Darling test (P<0.05).

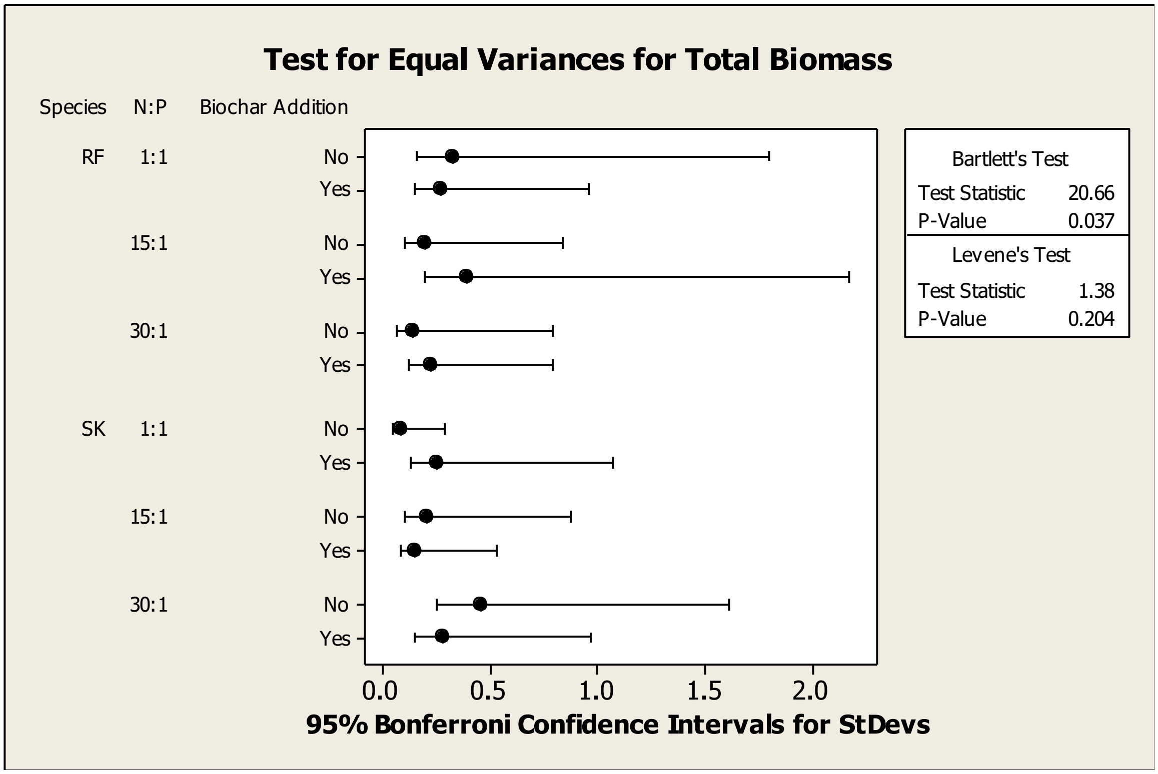

Figure B. 2 Equal Variances of potential biomass using 3-way ANOVA when plants grown alone under a Levene's test (P<0.05).

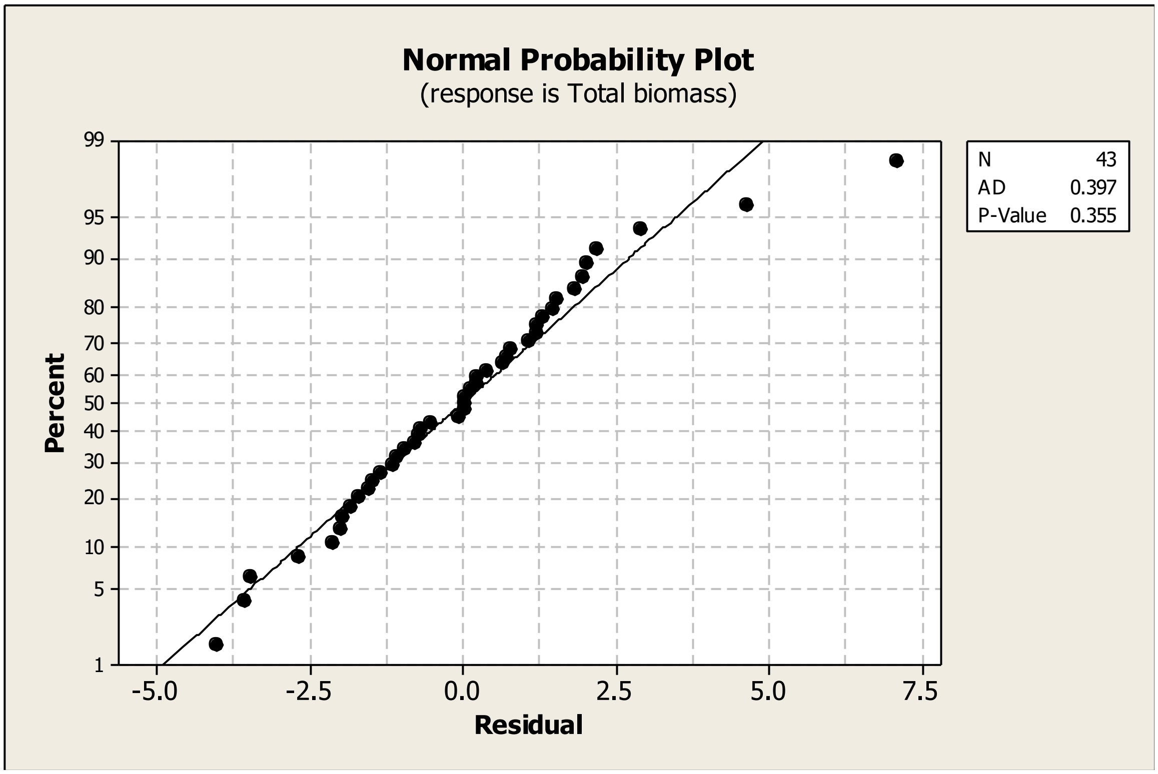

Figure B. 3 Normality of potential biomass using 3-way ANOVA when two spotted knapweeds grown together under an Anderson-Darling test (P<0.05).

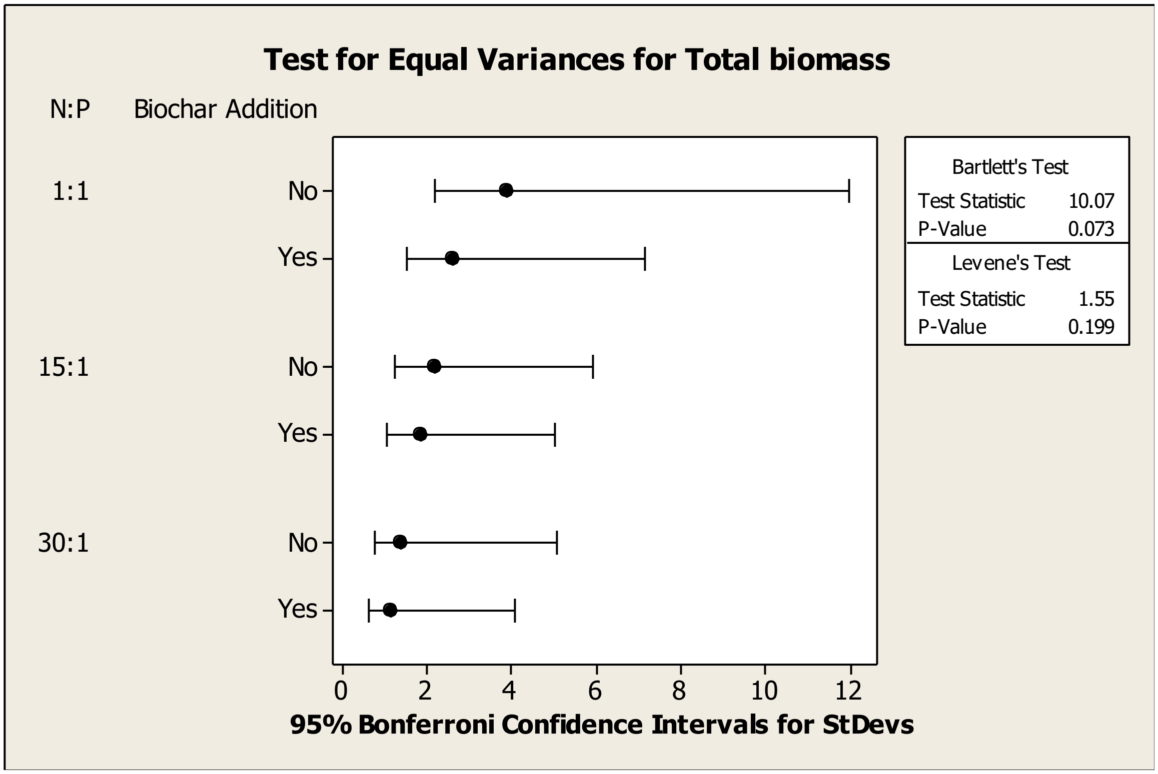

Figure B. 4 Equal Variances of potential biomass using 3-way ANOVA when two spotted knapweeds grown together under a Levene's test ($P<0.05$).

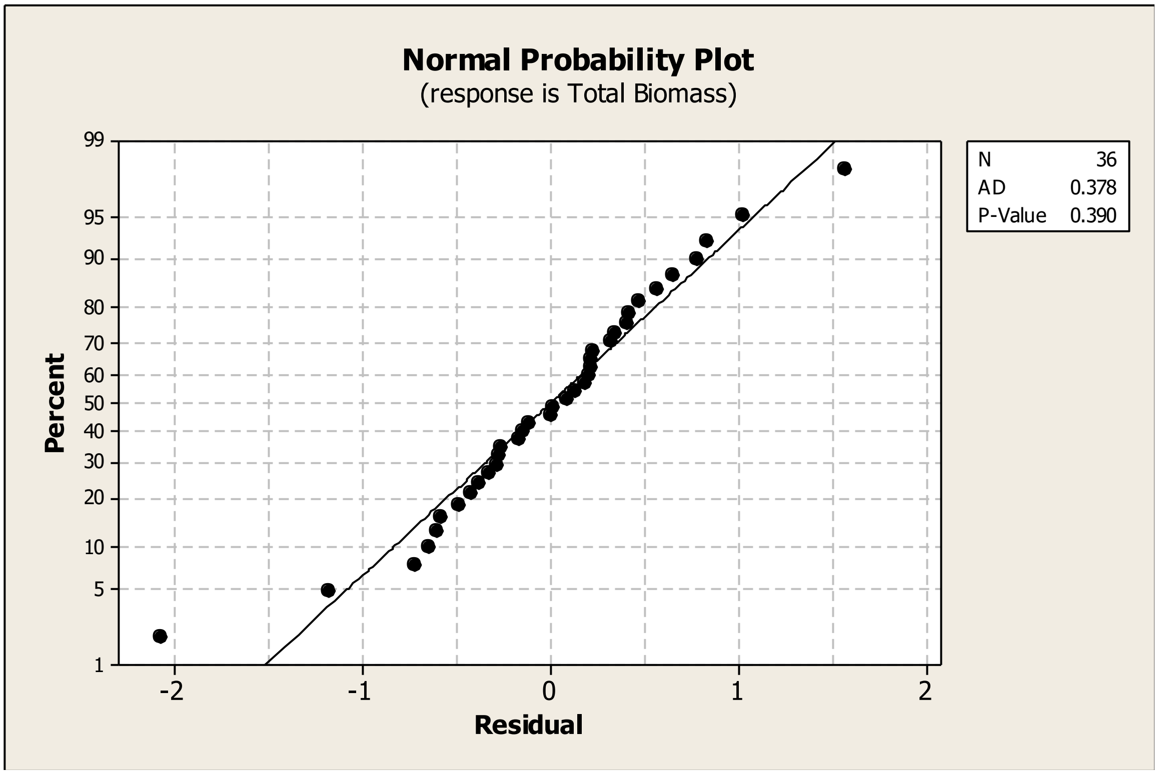

Figure B. 5 Normality of potential biomass using 3-way ANOVA when two rough fescues grown together under an Anderson-Darling test (P<0.05).

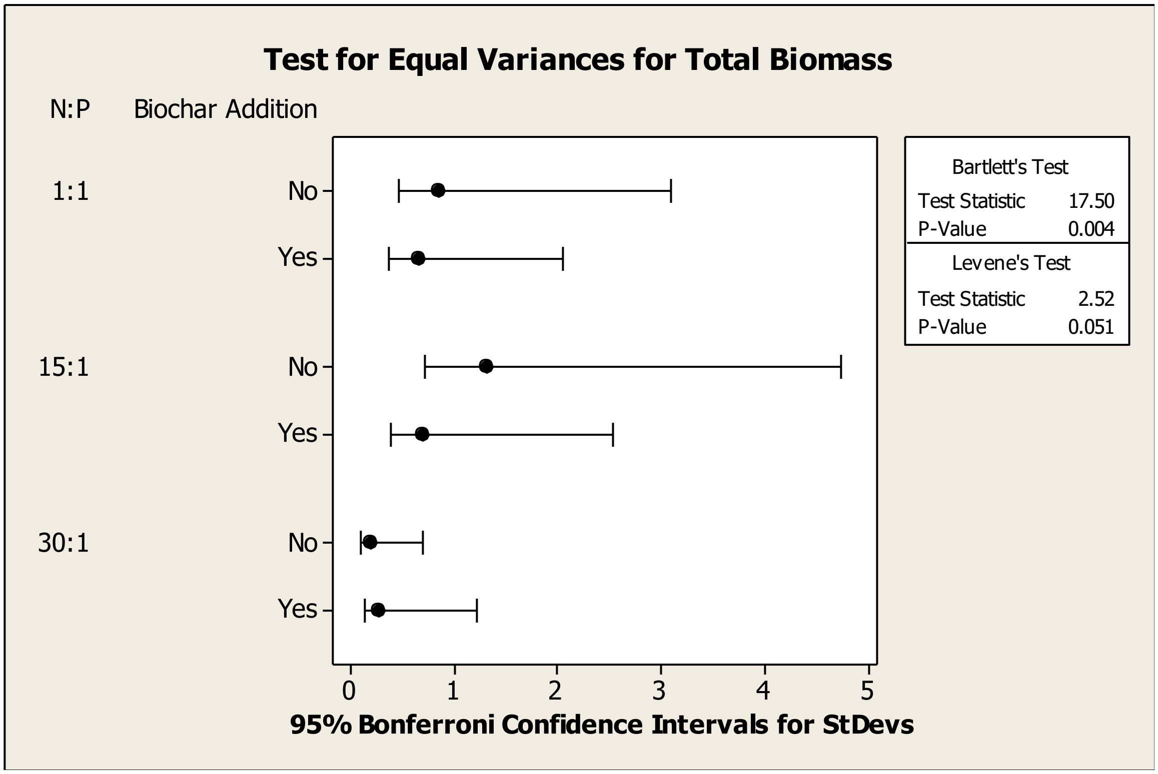

Figure B. 6 Equal Variances of potential biomass using 3-way ANOVA when two rough fescues grown together under a Levene's test (P<0.05).

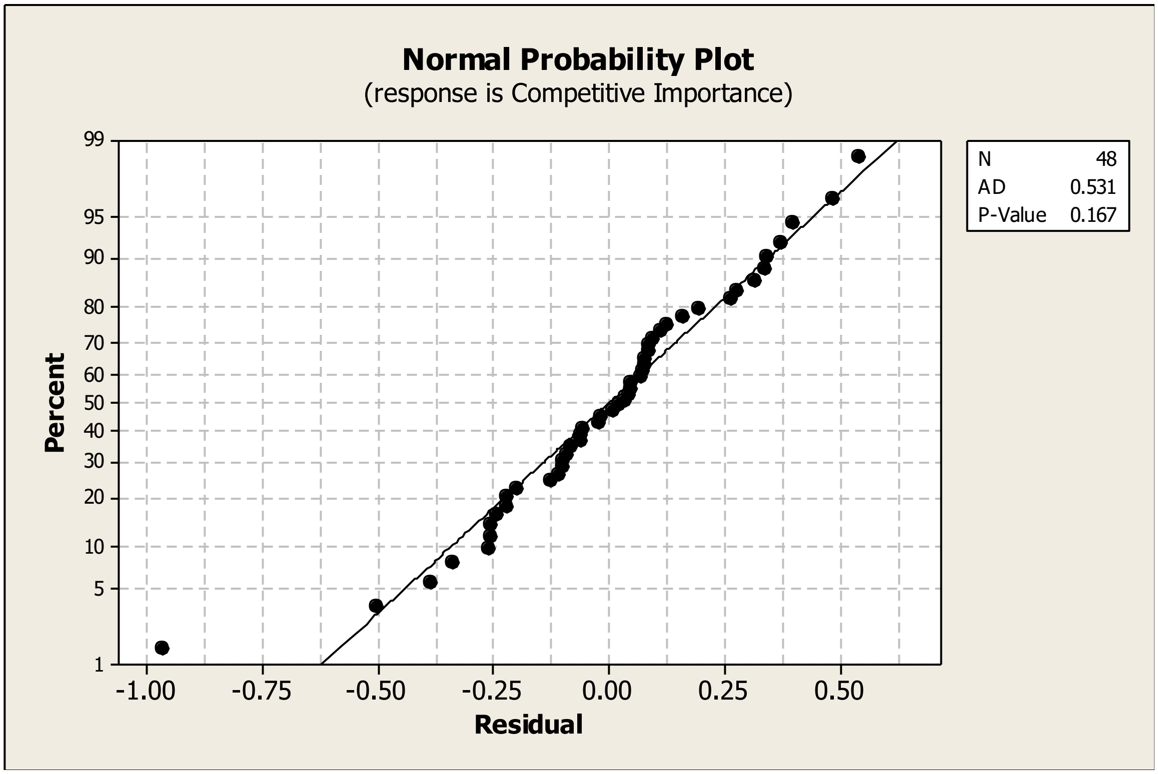

Figure B. 7 Normality of competitive importance using 3-way ANOVA when two spotted knapweeds grown together under an Anderson-Darling test (P<0.05).

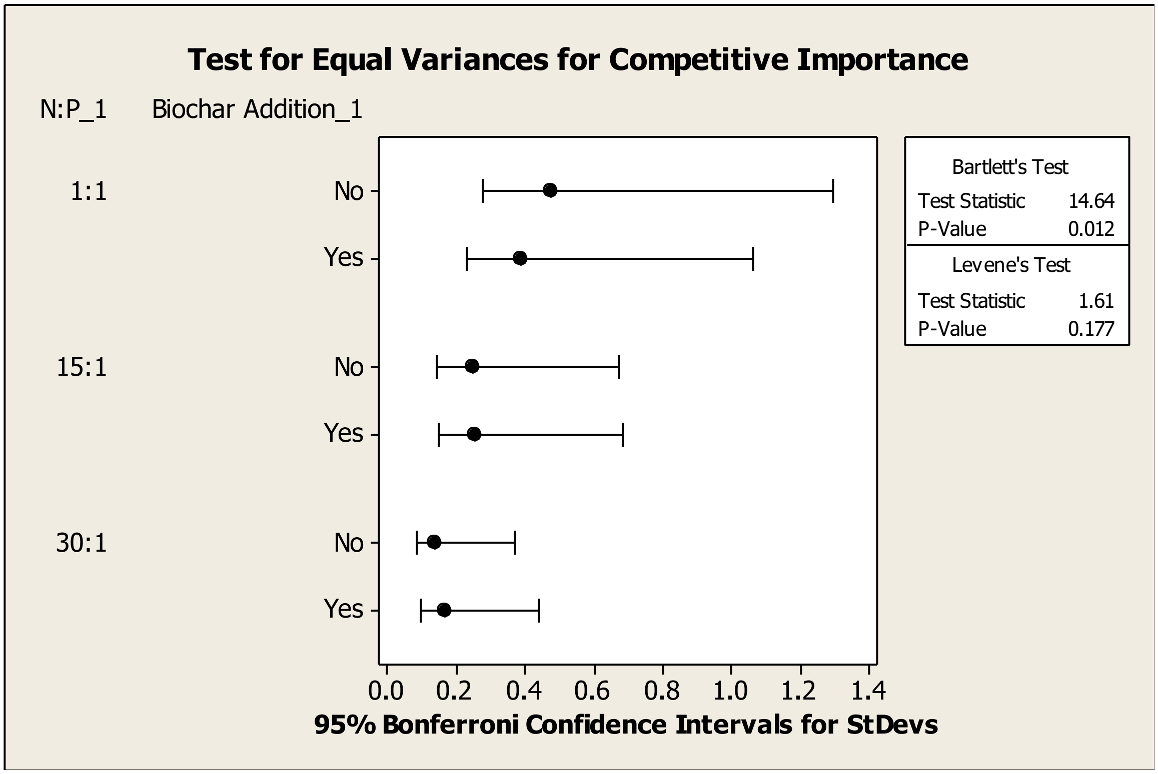

Figure B. 8. Equal Variances of competitive importance using 3-way ANOVA when two spotted knapweeds grown together under a Levene's test (P<0.05).

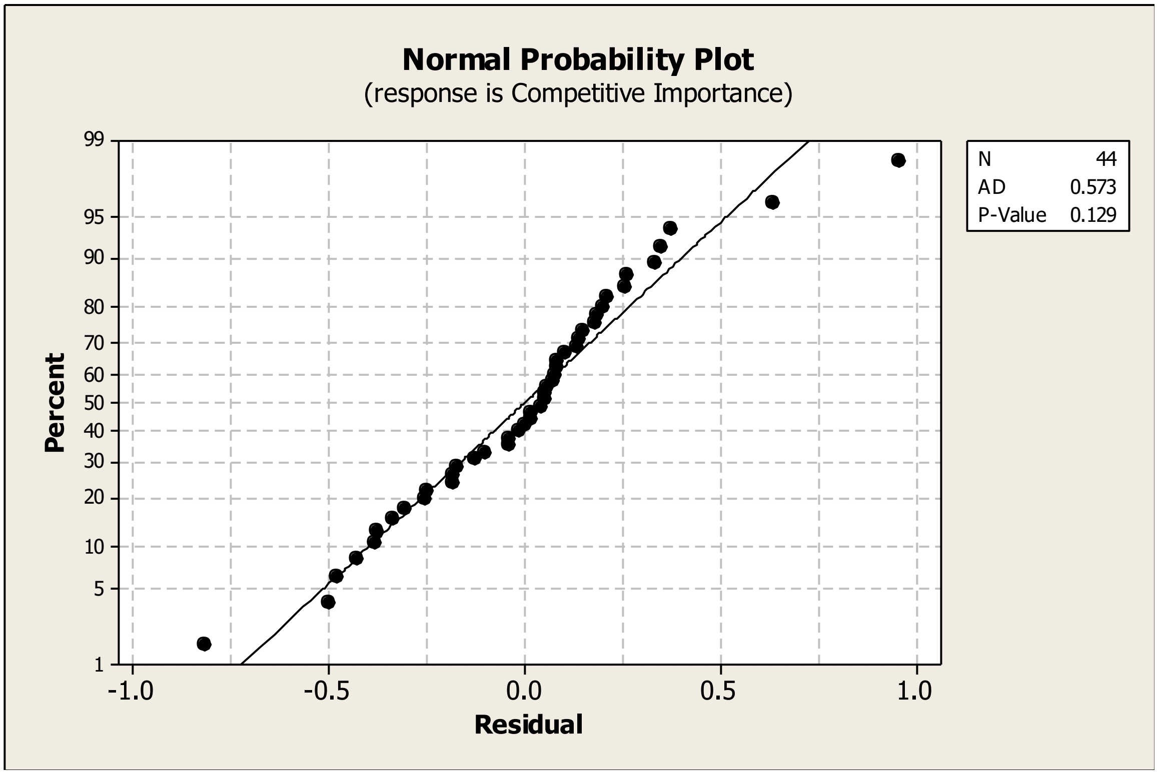

Figure B. 9 Normality of competitive importance using 3-way ANOVA when two rough fescues grown together under an Anderson-Darling test (P<0.05).

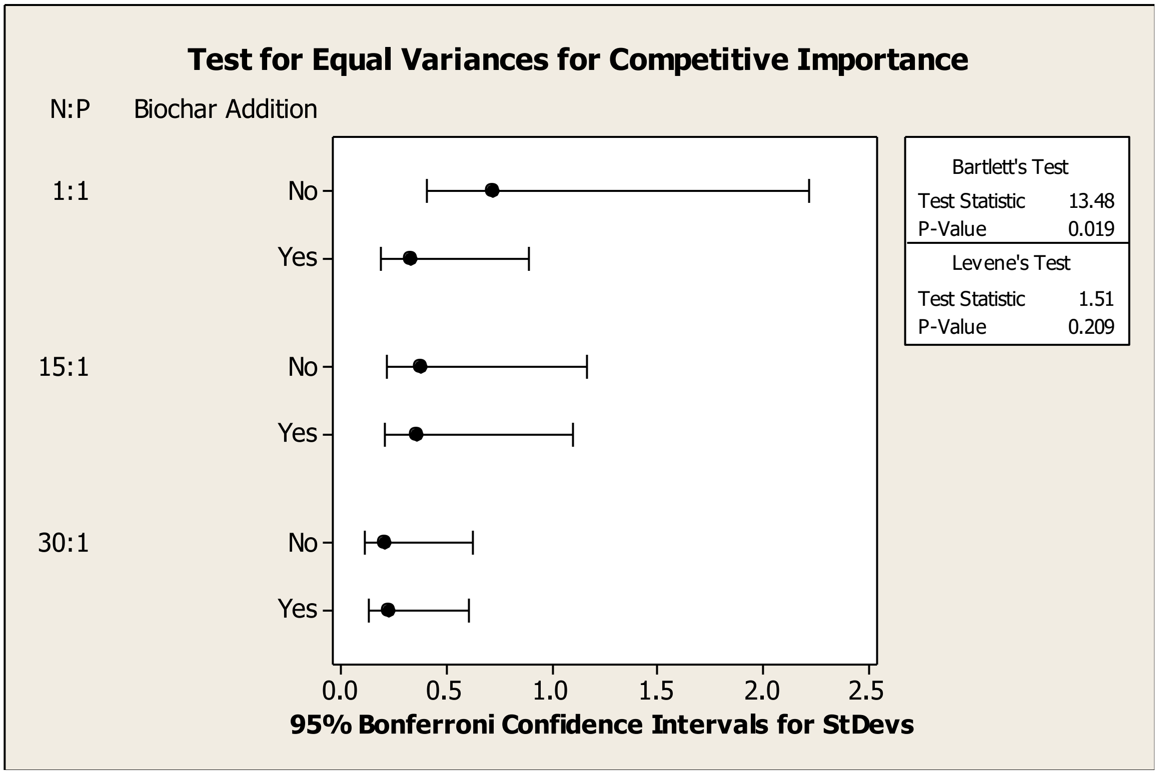

Figure B. 10 Equal Variances of competitive importance using 3-way ANOVA when two rough fescues grown together under a Levene's test (P<0.05).

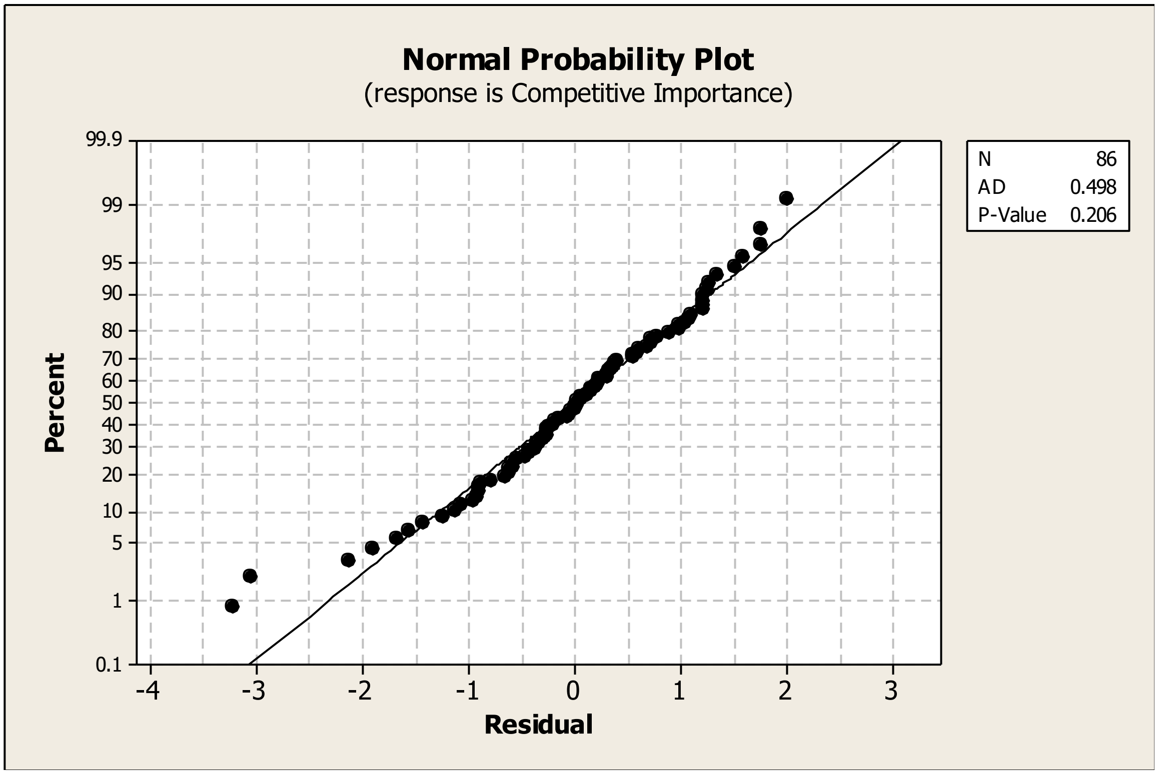

Figure B. 11 Normality of competitive importance using 3-way ANOVA when spotted knapweed and rough fescue grown together under an Anderson-Darling test (P<0.05).

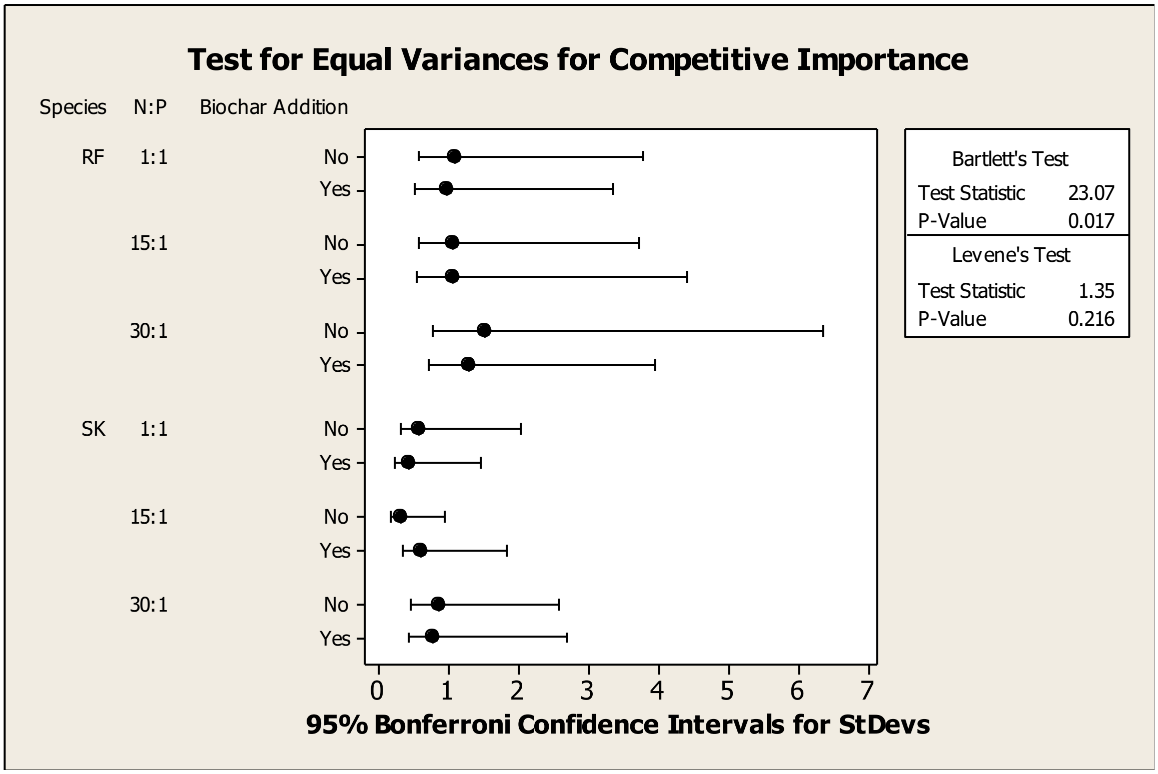

Figure B. 12 Equal Variances of competitive importance using 3-way ANOVA when spotted knapweed and rough fescue grown together under a Levene's test ($P<0.05$).