

DISSERTATION

**INVASION ECOLOGY OF KNAPWEED (*Centaurea* spp. L.):
COMPETITION, ESTABLISHMENT AND INTERACTIONS WITH SOIL BIOTA**

Submitted by

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In partial fulfillment of the requirements

For the Degree of Doctor of Philosophy

Colorado State University

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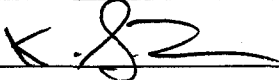
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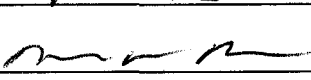
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ABSTRACT OF DISSERTATION

INVASION ECOLOGY OF KNAPWEED (*Centaurea* spp. L.): COMPETITION, ESTABLISHMENT AND INTERACTIONS WITH SOIL BIOTA

Knapweeds (*Centaurea* spp. L.) are damaging exotic plant invaders of North American rangelands. A two-part study was conducted to explore components of diffuse (*C. diffusa* Lam.) and spotted knapweed (*C. maculosa* auct. non Lam.) invasion ecology. A field experiment was conducted to study the resistance of a late-seral native foothills rangeland to invasion by diffuse knapweed and conditions that promote invasion (Part I). Greenhouse experiments were conducted to evaluate the relative benefit of native soil communities to two native plants and two knapweeds and to study plant growth in soil from knapweed infestations and adjacent native rangelands (Part II).

Diffuse knapweed was planted in a late-seral, native rangeland under treatment combinations that altered; 1) gap size, 2) competition, and 3) knapweed seed incorporation. Diffuse knapweed seeds were sown in fall 2001 and spring 2002. Greenhouse experiments were conducted with individual plants of two native species and two knapweed species grown in various soil treatments (core and perimeter of knapweed infestations, intact and autoclaved native soil).

In the field study (Part I), seed incorporation and adjacent live vegetation increased diffuse knapweed emergence, compared to unincorporated seed and dead adjacent vegetation. Emergence of incorporated seed increased with increasing gap size. Emergence approached 35%, but most knapweed plants died by late summer,

2002. In the greenhouse (Part II), the native soil community had negative effect on the growth of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), yarrow (*Achillea millefolium* L.) and diffuse knapweed, but a positive effect on spotted knapweed emergence. Plant growth was sometimes better in native soil than soil from knapweed infestations. However, growth of the native plants was not precluded, and knapweed growth was not promoted, in soils from knapweed infestations.

The native rangeland studied was not absolutely resistant to diffuse knapweed invasion. Disturbances that ensure diffuse knapweed seed incorporation and weaken native vegetation increase susceptibility to invasion. Greenhouse studies indicated that the native soil community is more beneficial to spotted knapweed than to the other plants studied, and that soil from diffuse and spotted knapweed infestations does not prevent growth of two native plants or promote knapweed growth.

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**PART I: DIFFUSE KNAPWEED (*Centaurea diffusa* Lam.) INVASION ECOLOGY:
COMPETITION AND ESTABLISHMENT IN A LATE-SERAL NATIVE RANGELAND
INTRODUCTION**

Invasive exotic plants currently occupy a large portion of the land area in North America (Sheley et al. 1998, DiTomaso 2000) and continue to spread rapidly. It has been estimated that the spread of invasive weeds in the United States causes \$34 billion in damage and control costs annually (Pimentel 2002). Pimentel et al. (2002) estimate the total annual costs associated with invasive, exotic weeds in pastures of the United States to be \$6 billion. Rangelands are defined as a type of land rather than a particular land use (Heady and Child 1994) while the term pasture implies livestock grazing as the specified use. Therefore, estimates of economic damages associated with invasive weeds in pastures likely underestimate the magnitude of the problem for the broader category of rangelands. The economic damage associated with invasive, exotic weeds on rangelands is expected to fall between \$6 billion and \$34 billion annually.

Invasions also result in damage that is difficult to express in economic terms. Common consequences of exotic plant invasions include increased runoff and erosion (Lacey et al. 1989), reduced forage and habitat values for wild and domestic animals, and reduced native biodiversity, aesthetic value and recreational value (DiTomaso 2000). Collectively, the negative impacts of noxious weed invasions have resulted in documented land value reductions of 60-80% in Montana, North Dakota and Oregon (Westbrooks 1998). Similar un-documented reductions in land value have likely occurred throughout the west.

Two of the most economically and ecologically damaging invasive plants on North American rangelands are diffuse knapweed (*Centaurea diffusa* Lam.) and spotted knapweed (*C. maculosa* auct. non Lam.) (Lacey et al. 1989, Roche 1994, Sheley et al. 1998). According to the National PLANTS Database (USDA, NRCS 2002), the present distribution of spotted knapweed in the United States includes all states except Texas, Oklahoma, Mississippi and Georgia. Diffuse knapweed is present in the 12 westernmost states as well as Nebraska, Iowa, Missouri, Michigan, Wisconsin, Illinois, Tennessee, Kentucky, Indiana, Massachusetts, Connecticut and, New Jersey (USDA, NRCS 2002). As of 1998, spotted knapweed had been reported in 326 counties of the western United States, including all counties in Washington, Idaho, Montana and Wyoming and diffuse knapweed occupied 204 counties in the western U.S. (Sheley et al. 1998). Diffuse knapweed may be more prevalent in relatively xeric regions, and spotted knapweed in relatively mesic regions (Sheley et al. 1998), but the present distribution of these two knapweeds in the western United States may be more representative of the patterns of introduction and spread and less indicative of suitable habitats for these plants (K. George Beck, personal communication).

A variety of control strategies exist that may be used to successfully manage noxious weeds in specific areas, but throughout the west, invasive exotics continue to spread at an unprecedented rate (Westbrooks 1998). Therefore, it is crucial to continue the search for economically and ecologically effective control methods. Identification of improved control methods for invasive plants relies on the integration of ecological knowledge of the invader and the site being invaded. Often, this information and an understanding of the processes leading to invasion are lacking (Mullin et al. 2000). While much is known regarding the biology and life history of knapweeds (Watson and Renney 1974, Schirman 1981, Roze et al 1984, Lacey et al. 1989, Davis et al. 1993, Jacobs et al. 1996, Jacobs and Sheley 1998, Sheley et al. 1998, Jacobs and Sheley

1999, Marler et al. 1999a, Marler et al. 1999b), it remains unknown whether these plants have the ability to establish and subsequently invade otherwise undisturbed late-seral native rangeland or whether invasion relies on some level of physical soil disturbance, removal of competition from native vegetation, or both. Manipulative field experiments that identify whether or not late-seral native rangelands resist invasion, and the conditions under which they are susceptible to invasion would greatly improve the present understanding of knapweed ecology and be valuable for prioritization of weed control efforts. Several authors have identified this as a critical precursor to improved management and prevention of noxious weed invasions (Bergelson et al. 1993, Levine and D'Antonio 1999, Davis et al. 2000, Jesson et al. 2000, Prieur-Richard and Lavorel 2000).

The role of disturbance has been discussed extensively in the invasion ecology literature, but is one of the least studied components of knapweed ecology. Based on an observational study, Lacey et al. (1990) suggested that, in the absence of disturbance, a climax rough fescue community in Montana appeared to be fairly resistant to knapweed invasion. After this rather tentative conclusion, the authors expressed a need for manipulative experiments to clarify the relationships among ecological classes, disturbance, and noxious weed invasions. The literature on spotted knapweed (closely related to diffuse knapweed) also includes accounts of this plant's ability to invade relatively undisturbed rangelands in good condition (Mauer et al. 1987, Tyser and Key 1988).

The lack of experimental tests to clarify the role of disturbance in plant invasions is a problem that is not unique to the knapweeds. Rather, it is a problem common to the invasion ecology literature with only a few exceptions (see Bergelson et al. 1993, Burke and Grime 1996 and Larson et al. 2000) and has been repeatedly identified as a hurdle to improved prevention and management of invasion (Bergelson et al. 1993, Jesson et

al. 2000, Mullin et al. 2000, Prieur-Richard and Lavorel 2000). Although it is reasonable to accept the generalization that disturbance favors invasion, it is important to consider that the wide range of possible disturbances result in a variety of different impacts on systems (Prieur-Richard and Lavorel 2000). Therefore, the role of disturbance in invasion can be expected to vary depending on the system being invaded, disturbance regimes involved and the species of invader. The two most commonly discussed mechanisms by which disturbance is believed to facilitate invasion include the creation of germination and establishment sites through physical impact to the soil surface (Hobbs and Huenneke 1992, Bergelson et al. 1993, Prieur-Richard and Lavorel 2000) and the reduction of competition from established vegetation (Burke and Grime 1996, Lonsdale 1999, Jesson et al. 2000). The few studies that have experimentally manipulated disturbance to determine its effects on invasion have not attempted to partition between these two mechanisms.

This research was conducted to evaluate conditions under which a late-seral, native foothills rangeland in northern Colorado either resists, or is susceptible to invasion by diffuse knapweed. More specifically, this study provides information related to safe sites for diffuse knapweed emergence and establishment and the role of disturbance in diffuse knapweed invasion. Treatments were designed to elucidate whether or not disturbances that incorporate knapweed seed into the soil and/or reduce competition from established native vegetation are required for diffuse knapweed invasion. The objectives of this study and the associated hypotheses were as follows:

1. Determine the ability of diffuse knapweed seedlings to emerge and establish in a late-seral, undisturbed, semi-arid, native foothills rangeland.

Hypothesis 1.1: *A Late-seral, undisturbed, native foothills rangeland will prevent diffuse knapweed seedling emergence and establishment.*

Hypothesis 1.2: *A Late-seral, undisturbed, native foothills rangeland will not prevent emergence, but will prevent establishment of diffuse knapweed.*

Hypothesis 1.3: *A Late-seral, undisturbed, native foothills rangeland will not prevent emergence or establishment of diffuse knapweed.*

2. Determine how the removal of competition from established, native vegetation, method of seeding and gap size affect seedling emergence and establishment of diffuse knapweed in a late-seral, semi-arid, native foothills rangeland.

Hypothesis 2.1: *Removal of competition from established vegetation will increase diffuse knapweed seedling emergence in a late-seral, native foothills rangeland regardless of seed incorporation or gap size.*

Hypothesis 2.2: *Removal of competition from established vegetation will increase diffuse knapweed establishment in a late-seral, native foothills rangeland regardless of seed incorporation or gap size.*

Hypothesis 2.3: *Diffuse knapweed seedling emergence in a late-seral native foothills rangeland will be higher from incorporated seed than from un-incorporated seed regardless of gap size or competition from native vegetation.*

Hypothesis 2.4: *Diffuse knapweed establishment in a late-seral native foothills rangeland will be higher from incorporated seed than from un-incorporated seed regardless of gap size or competition from native vegetation.*

Hypothesis 2.5: *Diffuse knapweed emergence in a late-seral native foothills rangeland requires both the removal of competition from native vegetation and seed incorporation regardless of gap size.*

Hypothesis 2.6: *Diffuse knapweed establishment in a late-seral native foothills rangeland requires both the removal of competition from native vegetation and seed incorporation regardless of gap size.*

Hypothesis 2.7: *The combination of seed incorporation and removal of competition from native vegetation will result in greater diffuse knapweed emergence and establishment than either treatment alone.*

Hypothesis 2.8: *Diffuse knapweed emergence in a late-seral native foothills rangeland will increase with increasing gap size regardless of seed incorporation or competition from established vegetation.*

Hypothesis 2.9: *Diffuse knapweed establishment in a late-seral native foothills rangeland will increase with increasing gap size regardless of seed incorporation or competition from established vegetation.*

METHODS

Study Area: The objectives of this study were addressed by planting seeds of diffuse knapweed in a late-seral, native foothills rangeland under combinations of treatment factors that altered; 1) the location of the seed relative to established native plants (gap size), 2) competition from established native vegetation, and 3) knapweed seed incorporation. This field experiment was conducted in a native foothills rangeland at the Colorado State University Foothills Research Campus at an elevation of 1,540-m (Fort Collins, Colorado, USA, UTM 13 487150E 4492800N). Annual precipitation averages 365 mm, more than half of which typically falls between April and August. The site is dominated by native perennial plants including needle and thread (*Hesperostipa comata* (Trin. & Rupr.) Barkworth), New Mexico feathergrass (*Hesperostipa neomexicana* (Thurb. ex Coult.) Barkworth), green needlegrass (*Nasella viridula* (Trin.) Barkworth), little bluestem (*Schizachrium scoparium* (Michx.) Nash), big bluestem (*Andropogon gerardii* Vitman), prairie flax (*Linum lewisii* Pursh), white sagebrush (*Artemisia ludoviciana* Nutt.) and yucca (*Yucca glauca* Nutt.). Exotic species are a very

minor component of the vegetation on the study site, and no diffuse knapweed was found anywhere on the site.

Field plots were established in the summer of 2001. Three blocks measuring approximately 30-m x 60-m were randomly located at the study site leaving 10- to 30-m buffers between blocks. Two hundred and forty diffuse knapweed introduction plots were systematically located at 2-m intervals along 20 transects spaced 2 m apart in each block (720 introduction plots total). Ten-centimeter diameter by 5-mm thick PVC rings centered at each introduction plot and secured with small aluminum stakes (fence ties) were used as markers. Each introduction plot was randomly assigned to one of twelve treatment groups and one of two runs of the experiment (fall 2001 or spring 2002).

A perimeter fence was constructed around the study site containing the three blocks of introduction plots to minimize disturbance. Two rain gauges were installed at the study site and monitored from 17 September through 19 November 2001 and 2 April through 2 October 2002. Rain gauges were not monitored over winter. One rain gauge was placed at ground level and the other was mounted approximately 1.5-m above the vegetation. A composite soil sample (approximately 20 subsamples from a 0-20-cm depth) was collected in April, 2002 for particle size analysis by the hydrometer method (modified from Day 1965). Plant species composition by weight was determined by clipping 10, 0.25-m² quadrats in each of the 3 blocks in early July, 2002. Clipped plant material was oven dried to a constant weight at 55-60° C and weighed. *Yucca* (*Yucca glauca* Nutt.) abundance was determined in November 2002 using the line intercept method to measure canopy cover along ten transects in each of the three blocks.

Treatments: Treatments consisted of a full factorial arrangement of three gap sizes (0-cm, 5-cm, and 15-cm), two levels of competition from existing native vegetation (live and killed) and two methods of knapweed seeding (seed incorporated and seed not incorporated) yielding 12 treatment groups (3x2x2) (Table 1.1). The entire experiment

Table 1.1. Treatment combinations including three gap sizes, two seed incorporation methods and whether native vegetation surrounding introduction plots was killed or left alive.

Gap Size	Knapweed Seed Incorporation	Native Vegetation
0-cm (bunchgrass)	Incorporated	Live
0-cm (bunchgrass)	Incorporated	Killed
0-cm (bunchgrass)	Not-Incorporated	Live
0-cm (bunchgrass)	Not-Incorporated	Killed
5-cm	Incorporated	Live
5-cm	Incorporated	Killed
5-cm	Not-Incorporated	Live
5-cm	Not-Incorporated	Killed
15-cm	Incorporated	Live
15-cm	Incorporated	Killed
15-cm	Not-Incorporated	Live
15-cm	Not-Incorporated	Killed

was conducted twice (fall 2001 and spring 2002) using each introduction plot only once. Gaps were defined as existing interspaces among the bases of established native plants. In other words, the gaps used in this study were a product of the plant spacing in the community where the study was conducted and were not created by disturbance. Gap treatments were achieved by moving the introduction plot from each 2 meter mark to the nearest existing gap of the appropriate size. A 0-cm gap was defined as the center of the base of an existing needle and thread plant. Five- and 15-cm gaps were located where plastic discs of either 5- or 15-cm diameter fit among the bases of existing, established plants. Knapweed seed was sown using two different methods. One method involved dropping the seed from the air immediately over the introduction plot, and leaving it where it fell without ensuring seed-to-soil contact (seed not incorporated). The other method involved careful removal of litter or any other objects that would prevent seed contact with the soil, pressing the seed firmly into the soil and covering with approximately 5 mm of mineral soil from within the introduction plot (seed incorporated). Any litter moved prior to placement of the seed was carefully returned to its original location. Removal of competition from established native vegetation was achieved through application of a 3% solution of glyphosate sprayed onto the foliage of

plants within a 50-cm diameter circle centered at the introduction plot ('killed' native vegetation treatment). This treatment served to eliminate competition for resources by the native vegetation in and around the selected introduction plots. All plots receiving this treatment for both runs (fall and spring) were sprayed on 10 September 2001. A small hole was cut in the bottom of a plastic garbage can with a 50-cm diameter opening at the top. The garbage can was then inverted, and centered over the introduction plot prior to spraying. The wand of a hand sprayer was inserted through the small hole cut in the garbage can to spray the plot. This resulted in a consistent 50-cm diameter sprayed area and also eliminated herbicide drift. Nothing was done to the vegetation in the 'live' native vegetation treatments.

Seed Collection, Preparation and Planting: Diffuse knapweed seed was collected on 31 August 2001 from plants growing at the Foothills Campus of Colorado State University approximately 2-km from the study site. Plants were mature and seeds were hard at the time of collection. Seed was kept dry and stored at room temperature for 30 days before being cleaned. A South Dakota Seed Blower was used to separate chaff and light seed from heavy seed. Seed cleaning consisted of three phases. In the first phase (1 minute) airflow was adjusted to separate chaff from seeds. For the second phase (3 minutes) airflow was manipulated to sort off approximately the lightest 10% of the seeds. The heavy portion of seed from the second phase was subjected to a third phase (2 minutes) to remove another 10% of the lightest seeds. The heavy seed remaining after 3 phases of cleaning was used for this study.

Two hundred seeds were randomly selected for germination trials prior to seeding each run of the experiment. Germinators were constructed by placing 4 pieces of filter paper (Whatman[®] #2) in each of 16 Petri dishes. Fifty seeds were placed in each

germinator and kept covered. Germinators were placed in the greenhouse, kept moist and monitored for 3 weeks.

The cleaned diffuse knapweed seed used for this study was fairly large (750 seeds g^{-1}) and had high germination values. Germination was $93\% \pm 2\%$ and $93\% \pm 3\%$ at the time of the fall seeding and spring seeding, respectively (mean \pm SE, $n=4$). Between the fall and spring seeding, seed was stored in a cool, dark location with low humidity. Diffuse knapweed seed was planted in the field by sowing five seeds in the plastic ring centered at each introduction plot. Seeding took place on 17 September 2001 for the fall run and on 2 April 2002 for the spring run. Although knapweed seeds can germinate over a wide range of environmental conditions, each introduction plot was watered lightly every day for the first 30 days after seeding if the soil in introduction plots was dry and less than 2-mm of natural precipitation was received. Each watering consisted of adding 60-ml of water to each 10-cm diameter introduction plot. This amount of water was adequate to keep soil in the introduction plots moist between watering events.

Following the emergence determinations (3 June and 28 June), thinning or transplanting was used to ensure the presence of exactly one live diffuse knapweed plant per introduction plot. Fall seeded plots were thinned / transplanted on 6-8 June 2002. Spring seeded plots were thinned / transplanted on 1 July 2002. Introduction plots supporting more than one diffuse knapweed plant were thinned to one plant per introduction plot by randomly selecting plants to pull until only one live plant remained. Container-grown seedlings were transplanted into introduction plots where no live diffuse knapweed plants were present on 3 June and 28 June 2002 for the fall and spring runs, respectively. Seedlings used for transplants were grown in the greenhouse for 30 days in Hiko™ plug trays (Stuewe and Sons, Corvallis, OR, USA) filled with soil from the field site that had been passed through a 5.6-mm sieve. Each cavity of the plug trays had a

depth of 4.9-cm and a top diameter of 2.1-cm. One week prior to transplanting, plug trays containing seedlings were moved out of the greenhouse to the field site for an acclimation period. Seedlings had 2 – 5 leaves at the time of transplanting. Transplants were watered immediately after being planted in introduction plots, and every day thereafter for 2 weeks (60-ml water per transplant per day).

Data Collection: The response variables measured in this study included diffuse knapweed seedling emergence and establishment. Seedling emergence was quantified in two ways. One expression of emergence was the percentage of successful introduction plots, and was defined as the percent of introduction plots in each treatment that supported one or more diffuse knapweed seedlings in the 30 days after seeding. The second expression of emergence was the percentage of diffuse knapweed seeds that developed into seedlings in each treatment combination within 30 days of seeding. For the fall-seeded run, emergence was monitored between 17 September and 15 October 2001 and checked again on 3 June 2002. For the spring-seeded run, emergence was monitored between 2 April and 28 June 2002.

Diffuse knapweed establishment was determined in September, 2002 by recording whether or not each introduction plot supported a live diffuse knapweed plant with 13 or more total leaves (live and dead). Thompson and Stout (1991) determined that greater than 50% of diffuse knapweed plants with 13 or more leaves can be expected to bolt after cold vernalization indicating that the juvenile stage ends at about the 13- leaf stage.

Experimental Design and Statistical Analyses: Treatments represent a factorial arrangement of three gap sizes (0-cm, 5-cm and 15-cm), two levels of competition from surrounding native vegetation (live and killed) and two levels of knapweed seeding method (seed incorporated, seed not incorporated). The experiment was replicated 3 times (blocks) using a randomized complete block design, and

repeated twice (fall 2001 and spring 2002). Blocks consisted of ten sub-samples of each treatment combination for each of the two runs (fall 2001 seeding and spring 2002 seeding). Analysis of Variance was used to test for effects of gap size, method of seeding, presence or absence of competition from established native vegetation, and season of seeding on diffuse knapweed seedling emergence and establishment. Where appropriate, establishment data was analyzed, both including and excluding, the introduction plots that received a transplanted diffuse knapweed seedling. Pairwise comparisons of treatment means were conducted using Tukey's Method ($\alpha=0.05$).

RESULTS

Tables of basic data and outputs from the analyses conducted for Part I of this dissertation are presented in Appendix I.

Characterization of Study Area: The moisture record for the study site including natural precipitation and supplemental watering for the duration of the study is presented in Figure 1.1. Notice that consistent periods of available moisture were provided following seeding and transplanting dates (indicated on graph). Soils at the study site were classified as sandy clay loam (54% sand, 16% silt and 30% clay). Total above ground current years plant production (oven dry basis) in early July, 2002 was $97 \pm 5\text{-gm}^{-2}$ (mean \pm SE, $n=30$). Plant species composition by weight is presented in Table 1.2. Twenty native species were encountered and accounted for approximately 97% of the above ground plant biomass on the site. Two exotic species were encountered, but only in trace amounts. Absolute canopy cover of yucca was $7\% + 1\%$ (mean \pm SE, $n=30$).

Application of Treatments: Appropriately sized gaps were easily located among established plants at the study site. Less than 10 out of 720 introduction plots had to be re-assigned to treatment combinations due to the inability to find the

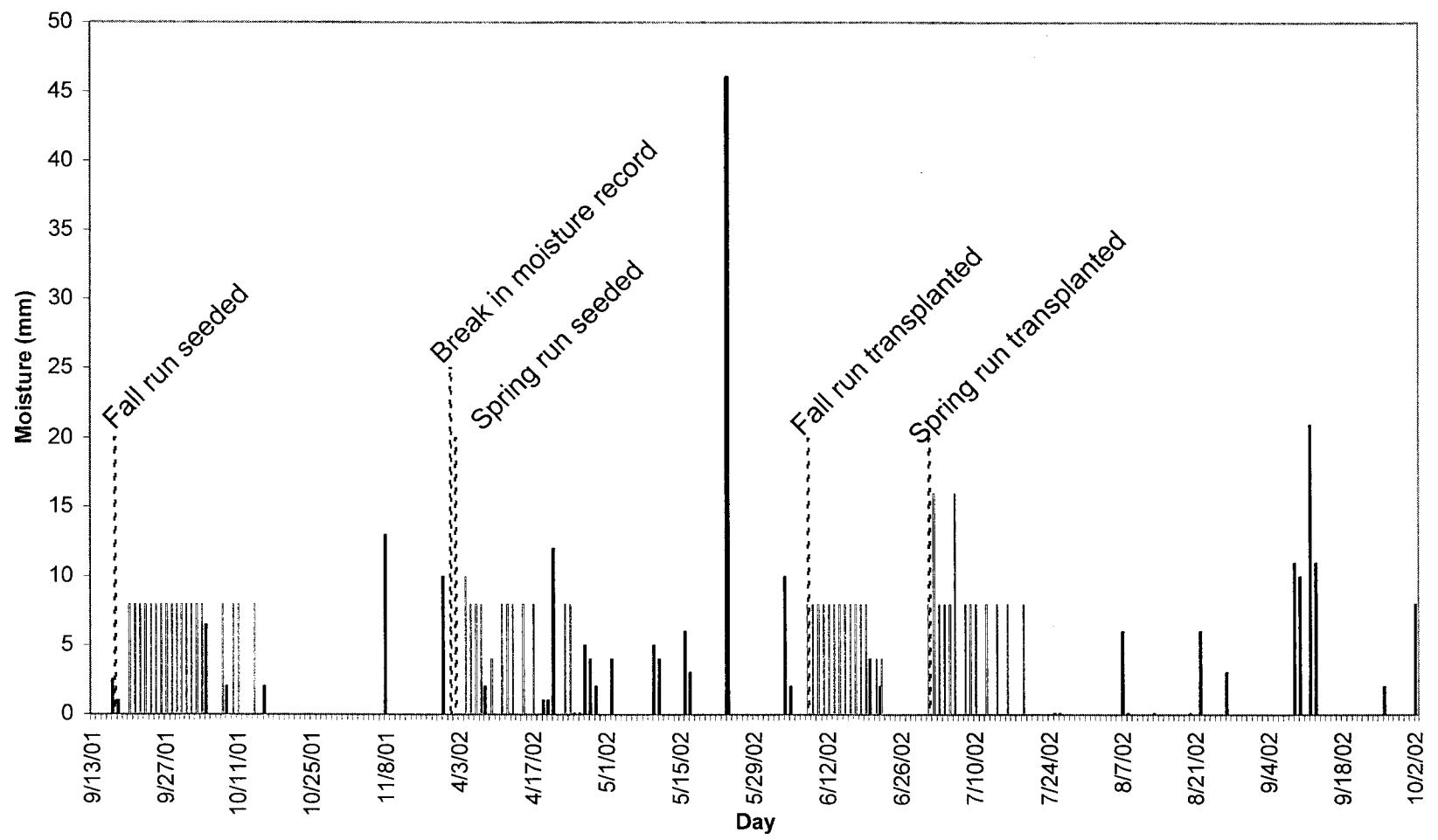


Figure 1.1. Moisture record for diffuse knapweed introduction plots located in a native foothills rangeland west of Fort Collins, Colorado, U.S.A. Period of record is from 17 September 2001 through 2 October 2002 (excluding the break in record during winter of 2001-2002). Natural precipitation (heavy lines) and supplemental water (fine lines) are represented. Broken lines indicate important events during the study.

Table 1.2. Plant species composition by weight (mean \pm SE, n=30) of the foothills rangeland study site located on the western edge of Fort Collins, Colorado, U.S.A. Species composition values are based on oven dried, above ground, current year plant biomass obtained from clipping 0.25-m² circular plots.

Plant Name	%Comp. by Wt.
Grasses and Grasslike Plants	
Needle and thread (<i>Hesperostipa comata</i> (Trin. & Rupr.) Barkworth)	46 \pm 4
Little bluestem (<i>Schizachyrium scoparium</i> (Michx.) Nash)	9 \pm 3
Big bluestem (<i>Andropogon gerardii</i> Vitman)	7 \pm 4
Green needlegrass (<i>Nassella viridula</i> (Trin.) Barkworth)	4 \pm 2
Fendler threeawn (<i>Aristida purpurea</i> Nutt. var. <i>longiseta</i> (Steud.))	4 \pm 1
New Mexico feathergrass (<i>Hesperostipa neomexicana</i> (Thurb. ex Coult.) Barkworth)	2 \pm 2
Western wheatgrass (<i>Pascopyrum smithii</i> (Rydb.) A.Löve)	1 \pm 1
Threadleaf sedge (<i>Carex filifolia</i> Nutt.)	1 \pm 1
Blue grama (<i>Bouteloua gracilis</i> (Wild. ex Kunth) Lag. ex Griffiths)	1 \pm 1
Japanese brome (<i>Bromus japonicus</i> Thunb. ex Murr.) EXOTIC	trace ¹
Forbs and Shrubs	
Prairie flax (<i>Linum lewisii</i> Pursh)	8 \pm 2
White sagebrush (<i>Artemisia ludoviciana</i> Nutt.)	6 \pm 3
Nuttall's sunflower (<i>Helianthus nuttallii</i> Torr. & Gray)	5 \pm 3
Threadleaf ragwort (<i>Senecio flaccidus</i> Less. var. <i>flaccidus</i>)	2 \pm 1
Rabbitbrush (<i>Chrysothamnus</i> Nutt. spp.)	1 \pm 1
Fleabane (<i>Erigeron</i> L. spp.)	1 \pm 1
Slimflower scurfpea (<i>Psoralidium tenuiflorum</i> (Pursh) Rydb.)	1 \pm 1
Hairy false goldenaster (<i>Heterotheca villosa</i> (Pursh) Shinnery)	trace
Winged buckwheat (<i>Eriogonum alatum</i> Torr.)	trace
Dotted blazing star (<i>Liatrix punctata</i> Hook.)	trace
Common pepperweed (<i>Lepidium densiflorum</i> Schrad.) EXOTIC	trace
Scarlet globemallow (<i>Sphaeralcea coccinea</i> (Nutt.) Rydb.)	trace

¹trace = < 1%

appropriate gap size based on the initial randomization. The glyphosate treatment was very effective at killing the existing established vegetation in the treated plots.

The 50-cm diameter round treated areas were visible throughout the summer of 2002 indicating effective control of existing vegetation and no off-site effects.

Experimental diffuse knapweed seedlings and seedlings of non-experimental plants were observed in treated as well as untreated plots, but sprayed plots remained essentially void of live vegetation for the duration of the study.

Diffuse Knapweed Emergence: The two methods for quantifying emergence (percent of successful introduction plots and percent of seeds emerged) resulted in the same conclusions. Therefore, only the results of the analyses on data for the percent of

seeds emerged is presented here. Information on the percent of successful introduction plots is included in Appendix I. Residual plots confirmed that the assumptions of analysis of variance were met. There was a slight tendency toward increasing variance with increasing predicted values, but because it was merely a slight deviation, transformation of the data was deemed unnecessary. Further, F-tests are robust with respect to departures from this assumption (Dowdy and Wearden 1991).

Emergence of diffuse knapweed in the field plots ranged from 10% to 35% of seeds sown, and patterns were explained by four, two-way interactions of treatment factors. The effects of seed incorporation varied by season ($P < 0.0001$) (Figure 1.2). Incorporation of diffuse knapweed seeds into the soil increased emergence in both runs of the experiment, but the effect was much greater in the fall-seeded run. The effect of removing competition from established native plants also varied by season ($P = 0.013$) (Figure 1.3). Killing the plants in and around introduction plots reduced emergence of diffuse knapweed in the fall-seeded run, but had no effect on emergence in the spring-seeded run of the experiment. Gap size interacted with competition from native vegetation ($P = 0.0355$) (Figure 1.4) and relatively weakly with seed incorporation ($P = 0.0808$) (Figure 1.5). There was no effect of gap size where natives were left alive, but emergence in the 5- and 15-cm gaps was slightly higher than in the 0-cm gap where established native vegetation had been killed (Figure 1.4). Emergence of incorporated seed was higher than un-incorporated seed in all gap sizes (Figure 1.5). Further, emergence of incorporated seed tended to increase with increasing gap size while gap size had no effect on emergence of un-incorporated seed.

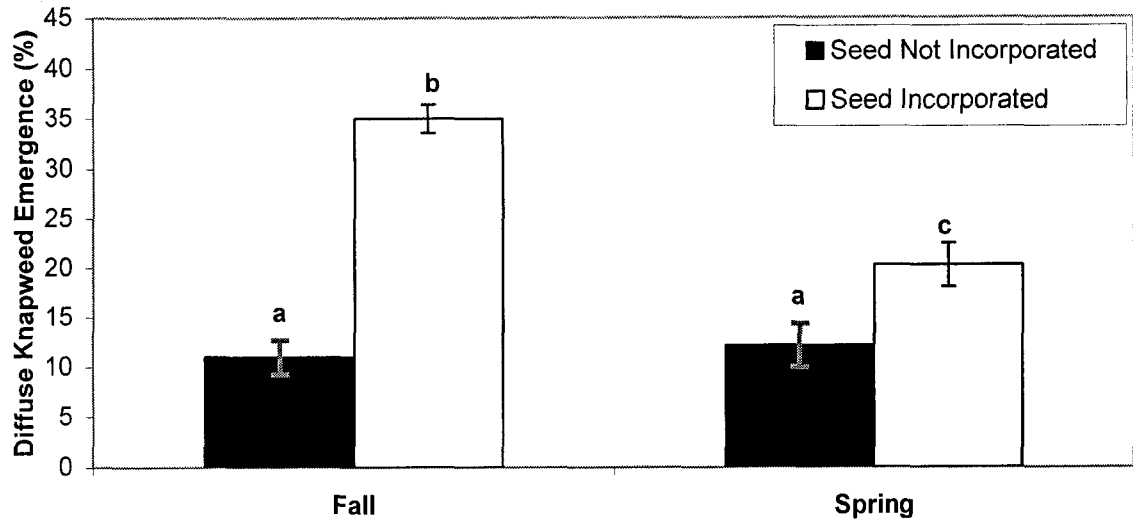


Figure 1.2. Two-way interaction plot showing the effects of seed incorporation and season of seeding on the percent of diffuse knapweed (*Centaurea diffusa* Lam.) seeds that emerged (LS mean \pm SE, n=18) in a native foothills rangeland west of Fort Collins, Colorado, U.S.A.

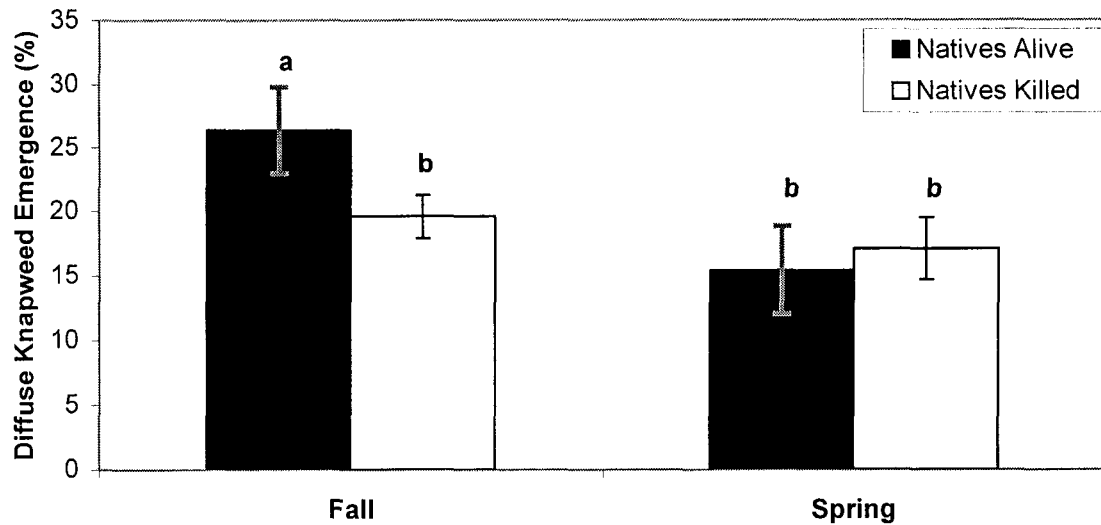


Figure 1.3. Two-way interaction showing the effects of killing native vegetation and season of seeding on the percent of diffuse knapweed (*Centaurea diffusa* Lam.) seeds that emerged (LS mean \pm SE, n=18) in a native foothills rangeland west of Fort Collins, Colorado, U.S.A.

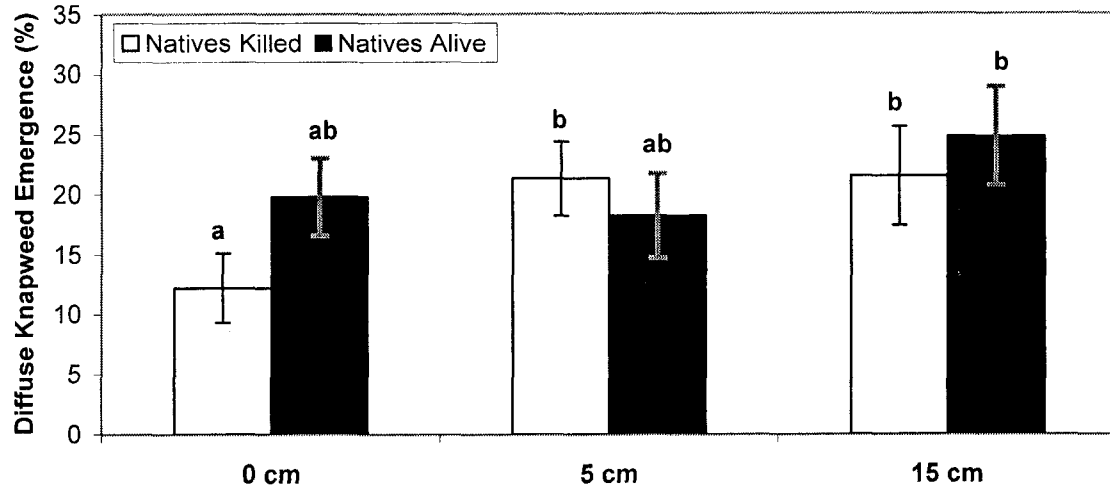


Figure 1.4. Two-way interaction plot showing the effects of killing native vegetation and gap size on the percent of diffuse knapweed (*Centaurea diffusa* Lam.) seeds that emerged (LS mean \pm SE, n=12) in a native foothills rangeland west of Fort Collins, Colorado, U.S.A.

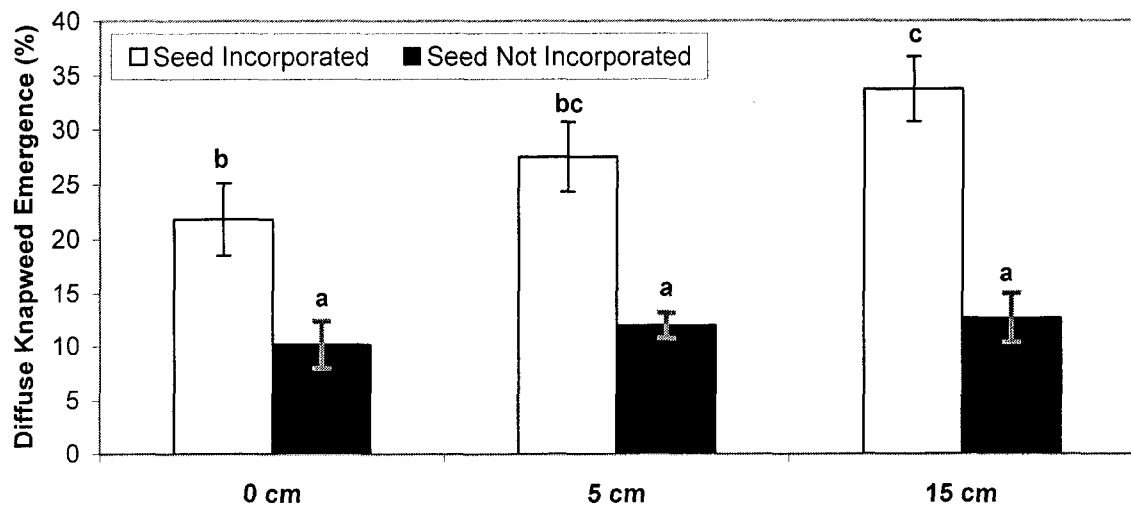


Figure 1.5. Two-way interaction plot showing the effects of gap size and seed incorporation on the percent of diffuse knapweed (*Centaurea diffusa* Lam.) seeds that emerged (LS mean \pm SE, n=12) in a native foothills rangeland west of Fort Collins, Colorado, U.S.A.

Diffuse Knapweed Establishment: Despite emergence values of up to 35%, very few diffuse knapweed seedlings survived. Out of 360 introduction plots in the fall-seeded run, 339 received transplants in early June of 2002 because they contained no live diffuse knapweed seedlings. Similarly, 327 of 360 introduction plots in the spring-seeded run received transplants on 1 July 2002. Survival of transplanted seedlings was also very low. By the fall of 2002 only 7 plants remained alive from seed and transplants combined (Table 1.3). All plants that survived from seed were seeded in the spring run. All plants that survived from transplants were in introduction plots included in the fall run. Out of all plants alive on 11 October 2002, only 2 met the establishment criteria adopted (at least 13 leaves). Due to the very low survival of diffuse knapweed plants from seed and transplants, no analyses were run on establishment data.

Table 1.3. Live diffuse knapweed plants and their size (number of live and dead leaves) as of 15 September 2002 and 11 October 2002. No seed incorporation designation is provided for transplants because it is irrelevant.

Gap (cm)	Natives	Seed Incorporation	Run	Survived from	Plant Size 9/15/02	Plant Size 10/11/02
15	killed	n/a	fall	transplant	3 leaf	3 leaf
5	killed	n/a	fall	transplant	14 leaf	17 leaf
0	killed	n/a	fall	transplant	7 leaf	9 leaf
5	killed	incorporated	spring	seed	11 leaf	11 leaf
15	killed	incorporated	spring	seed	8 leaf	10 leaf
15	killed	incorporated	spring	seed	14 leaf	19 leaf
0	alive	not incorporated	spring	seed	5 leaf	6 leaf

DISCUSSION

Characterization of Study Area: Due to the absence of natural precipitation events immediately following the fall seeding (Figure 1.1), introduction plots were watered the same amount every day for the majority of the month following the fall seeding. Much of this period was characterized by sunny, hot days. Figure 1.1 suggests that moisture inputs for the month following the spring seeding were less

consistent and included several relatively low-magnitude moisture inputs. However, natural precipitation events were more common in this period resulting in cooler and moister conditions than occurred in the 30 days following seeding of the fall run of the experiment. Despite these differences, moisture conditions of the introduction plots were fairly similar between the two runs because watering decisions were based on daily qualitative assessments of soil moisture in introduction plots.

The study area was clearly dominated by native vegetation. Essentially all of the above ground plant biomass that grew on the study area in 2002 was contributed by native plants (Table 1.2). Two species of exotic plants were encountered but only in trace amounts. In terms of composition by weight, the dominant species included needle and thread, little bluestem, big bluestem, green needlegrass and Fendler threeawn. All of these grasses with the exception of Fendler threeawn are typical late-seral dominants. Fendler threeawn is considered a mid-seral species (USDA 1988), but was not overly abundant on the study site. Given the abundance of late-seral native perennial grasses, the diversity of native forbs, and the small amount of Fendler threeawn encountered, the site does appear to be in a late-seral stage. The variety of native forbs encountered suggests that the area has not been treated with broadleaf selective herbicides in the recent past.

Application of Treatments: The ease of locating appropriate gap sizes for location of introduction plots suggests that 5- and 15-cm gaps are common in the foothills system where this study was conducted. The 5- and 15-cm gap sizes fall within the range of naturally occurring gaps in grasslands and are similar to gap sizes used in other studies of disturbance and plant invasions (Bergelson et al. 1993, Burke and Grime 1996, Larson et al. 2000).

Seedlings of native plants and experimentally planted diffuse knapweed were commonly observed in plots that had been sprayed with glyphosate at the beginning of

the study. This suggests that the spraying treatment only had negative effects on plants that were alive at the time of spraying and did not prevent subsequent seedling growth. This is not surprising given that glyphosate is tightly bound by soil particles (Ahrens 1994) and is readily degraded by soil microbes (Ahrens 1994, Haney et al. 2000) resulting in very little to no soil activity on plants.

Diffuse Knapweed Seed Characteristics: Seed lots used in this study were composed of fairly large seed. One gram of cleaned seed used for this study contained 750 individual seeds. Watson and Renney (1974) reported that the average weight ($n=500$) of diffuse knapweed seed that had not been cleaned was $1.099\text{-mg seed}^{-1}$. This translates to a value of 910-seeds g^{-1} . All seed lots used in this study had germination values of 93%; therefore seed viability was not a limitation to emergence or establishment of diffuse knapweed.

Diffuse Knapweed Emergence: All emergence values observed in this study (10%-35%) were much lower than the results of the greenhouse germination trials (93%), indicating that conditions in the field plots limited diffuse knapweed emergence. Seed incorporation increased diffuse knapweed emergence in both the fall and spring runs of the experiment, but the effect was more dramatic for the fall run (Figure 1.2). Watson and Renney (1974) found that diffuse knapweed emergence was highest from seeds at the soil surface that had good contact with mineral soil. Berube and Myers (1982) observed two to four times more diffuse knapweed seedlings and rosettes in plots that had been physically disturbed compared to undisturbed plots. Similarly, Larson et al. (2000) observed emergence of hoary cress (*Cardaria draba* (L.) Desv.) in plots where the soil had been physically disturbed but no emergence in undisturbed plots. The results of this study are consistent with the findings of others in that diffuse knapweed emergence is highest from seed that has good contact with mineral soil and is near the soil surface.

Killing the native plants in, and around the introduction plots reduced diffuse knapweed emergence in the fall seeded run but had no effect on emergence in the spring seeded run (Figure 1.3). At first, reduced diffuse knapweed emergence in killed plots of the fall run may seem contrary to expectations. However, it is important to remember that the response being discussed is that of seedling emergence in areas surrounded by live versus dead vegetation. The effects of this treatment on microclimate conditions for emergence are probably much more important than its effects on competition between the diffuse knapweed seedling and surrounding vegetation. Close proximity to live, transpiring plant material may provide a cooler and moister environment than would be expected adjacent to dead, dry plant material. Further, newly emerged seedlings would not be expected to compete with established vegetation for water and nutrients, especially in the present study where supplemental water was added. In the fall seeded run, killed plots were dry and yellow from being sprayed, while the plots in the live native vegetation treatments supported live, green vegetation. Other researchers have observed similar responses. Larson and McInnis (1989) found that growing conditions for early seedling growth of diffuse knapweed were more favorable in close proximity to crowns of established grasses than in the interspaces between established grass plants.

Killing native vegetation surrounding the introduction plots had no effect on diffuse knapweed emergence in the spring-seeded run and this may have been driven by the drought of 2002. Precipitation for the 6-month period immediately prior to seeding of the spring run was 57% of average. Precipitation for the months of April through September of 2002 was only 48% of average. Although there were periods of cool, cloudy weather in the spring, precipitation was not sufficient to promote normal spring green-up. Precipitation for the 30-day period corresponding to the emergence monitoring for the spring-seeded run was approximately 15% of average. The

vegetation surrounding all introduction plots in the spring seeded run was dry and yellow regardless of whether the surrounding vegetation had been sprayed or not. Therefore, the micro climatic conditions in plots that were dry and yellow because of drought were likely similar to plots that were dry and yellow because they had been sprayed the previous fall. In other words, it is believed that the drought conditions of 2002 masked the effects of spraying in the spring-seeded run. Supplemental water was added to all introduction plots in both the fall- and spring-seeded runs of the experiment. However, water was added only to the area encompassed by the 10-cm diameter rings used to mark the locations where diffuse knapweed seed was sown. The amount of water added (60-ml at each watering) was not sufficient to allow surrounding plants to overcome the effects of the drought year. In contrast, for the fall-seeded run, unsprayed plots supported live, green vegetation throughout the 30-day period following the fall seeding and only the sprayed plots were yellow and dry. Precipitation for the six month period immediately prior to the fall seeding was 87% of average. During the 30-day period when emergence was monitored for the fall seeded run, precipitation was 68% of average.

The effect of killing the native vegetation in and around introduction plots also varied by gap size (Figure 1.4). Where surrounding vegetation was left alive, diffuse knapweed emergence was around 20% regardless of gap size. However, in plots where surrounding vegetation had been killed, diffuse knapweed emergence was reduced to 12% in the 0-cm gap compared to 20% in the 5- and 15-cm gaps. Therefore, the interaction between gap size and the status of surrounding native vegetation was driven completely by the low emergence in the 0-cm gap where surrounding native vegetation had been killed. These emergence patterns seem to be consistent with the discussion offered above regarding the importance of microclimate effects. It makes sense that the locations where diffuse knapweed emergence is most affected by killing the surrounding

vegetation are those in closest proximity to surrounding native vegetation (0-cm gap). Diffuse knapweed seedlings in the 5- and 15-cm gaps appear to have been far enough away from surrounding vegetation that killing it did not influence emergence.

The interaction between gap size and seed incorporation (Figure 1.5) was marginally significant statistically ($P=0.0808$) but is ecologically interesting. Gap size had no effect on the emergence of diffuse knapweed seed that was not incorporated into the soil. On the other hand, emergence of incorporated diffuse knapweed seed tended to increase slightly with increasing gap size. This suggests that seed contact with the soil is more limiting to diffuse knapweed emergence than gap size, because the benefits of larger gap sizes were only observed in treatment groups where diffuse knapweed seed was incorporated. Low emergence of non-incorporated seed may have been driven by unfavorable conditions for emergence or by seed predation. Rodent utilization of knapweed seed has been observed (Watson and Renney 1974), and other granivores likely use knapweed seed as well. It is reasonable to assume that seed loss to predation would be higher for seed lying on the soil surface as compared to seed located just below the soil surface.

Diffuse Knapweed Establishment: Only 0.1% of diffuse knapweed seeds planted in this experiment (4 out of 3,600) survived to the rosette stage (Table 1.3). Even fewer (1 out of 3,600 seeds) reached the 13-leaf stage, which was the size criteria selected for establishment. Survival of the transplanted seedlings (0.45%) was higher than survival from seed, but was still very low and only one out of more than 650 transplanted seedlings reached the 13-leaf stage. Based on these results, it appears that the late-seral native foothills rangeland studied is not absolutely resistant to diffuse knapweed invasion. However, there are four important points to consider with respect to the resistance of the study area to diffuse knapweed invasion.

First, six of the seven total diffuse knapweed plants alive at the end of the study grew in plots that had been sprayed to eliminate competition from established native vegetation. This suggests that some level of disturbance to weaken established native plants promotes diffuse knapweed invasion of the late-seral foothills rangeland studied. Only one of the seven diffuse knapweed plants alive at the end of the study survived from seed that was dropped (not incorporated) in the center of a live needle and thread grass plant (0-cm gap) in a plot that had not been sprayed. The fact that only one diffuse knapweed plant survived in a plot without experimental disturbances suggests that in the absence of disturbances that weaken native vegetation and ensure seed-to-soil contact, diffuse knapweed invasion may occur, but would likely proceed much slower than in disturbed areas.

A second consideration that influences the interpretation of how resistant the study area is to invasion by diffuse knapweed relates to the drought of 2002. The drought of 2002 seems to have had an effect on diffuse knapweed emergence in the spring 2002 run and on diffuse knapweed establishment in both the fall 2001 and spring 2002 runs. Speculation offered above suggested that most of the differences in emergence observed between the fall and spring runs of this study were driven by the drought conditions during the spring emergence determination. Although drought is the most likely explanation for these differences, nothing in this study allows separation of drought conditions from other differences between fall and spring diffuse knapweed seedling emergence. Similarly, drought is believed to be a large part of the reason diffuse knapweed establishment was low, but this study did not monitor diffuse knapweed establishment in a favorable year to confirm the effects of drought. However, in years with more precipitation, one might speculate that diffuse knapweed establishment would be higher. In this case, the native rangeland studied would appear

to be even less resistant to invasion by diffuse knapweed than was observed in this study.

Third, any conclusion regarding the resistance of the studied rangeland to invasion by diffuse knapweed should be viewed with some level of caution and one must establish a definition of invasion. A total of seven diffuse knapweed plants survived from seed or transplants until the end of this study. If these plants had been left alive, all would have likely survived to produce seed. Annual seed production in diffuse knapweed infestations has been estimated to be 11,000- to 48,000-seeds m^{-2} (Schirman 1981). An assumption that each flowering plant occupies approximately $0.25\text{-}m^2$, suggests that each plant is capable of producing 2,800 to 12,000 seeds per year, a very high percentage of which can be expected to be viable (Watson and Renney 1974). Based on seed production studies, Schirman (1981) estimated that only about 0.1% survival from seed was necessary to maintain the diffuse knapweed stands he studied. It appears that 0.1% survival from seed, as observed in the present study, may also be sufficient to start an infestation of diffuse knapweed. Considering the seed production estimates offered above and a survival rate from seed of 0.1% per year, the 7 plants that survived until the end of the study could multiply into between 100 and 1,200 diffuse knapweed plants on the site in less than 5 years. Therefore, an area with only 0.1% survival of diffuse knapweed seedlings that may appear *relatively* resistant to invasion may in fact be invasible. This calculated rate of spread would easily exceed the criteria offered by Richardson et al. (2000) for defining invasive plants that reproduce by seed (spread $>100\text{-}m$ in less than 50 years).

As mentioned earlier, one of the seven plants alive at the end of the study grew in a plot that had not been sprayed, from un-incorporated seed in a 0 cm gap. This might suggest that even in the absence of the disturbance factors used in this study, the potential for invasion by diffuse knapweed still exists. Calculations similar to those

outlined above but using a survival rate of 0.028% (1 divided by 3,600) suggest that one live diffuse knapweed plant has the potential to multiply into somewhere between 14 and 170 plants within 5 years in the native rangeland studied.

Survival of diffuse knapweed observed in the present study was much lower than suggested by Watson and Renney (1974), and toward the low end of what has been reported by others. Watson and Renney (1974) suggested that field seedling mortality could be as high as 55%, but is more commonly around 12%. Application of these seedling mortality estimates to the present study would suggest that 5 to 31% of seeds sown should have developed into rosettes, yet only 0.1% survival from seed to rosette was observed in the present study. Although no description of the field site used by Watson and Renney (1974) was offered, it is assumed that their seedling mortality estimates apply to existing knapweed infestations in disturbed areas rather than to a native, late-seral rangeland like the one used in the present study. Myers and Berube (1983) observed 73% survival from seedling to rosette and 74% survival from rosette to flowering along a road in a lightly grazed rangeland in the dry interior of British Columbia. Survival figures offered by Myers and Berube (1983) correspond to about 54% survival from seedling to flowering and would represent 46% mortality. These same authors observed that seed numbers were 100 to 1,000 times higher than the density of flowering plants suggesting that survival from seed to flowering was between 0.1 and 1.0%. Roze et al. (1984) also worked in disturbed plots located in the dry interior of British Columbia and reported that 0.6 to 0.9% of seeds developed and survived to produce rosettes.

The low survival of diffuse knapweed observed in the late-seral native foothills rangeland where the present study was conducted may be linked to interactions between diffuse knapweed and soil biota. Klironomos (2002) recently demonstrated a relationship between plant abundance and feedback with soil biota for 61 species. That

study suggested that plants with positive feedback interactions with soil biota tended to be the most abundant while those with negative feedback values tended to be least abundant (Klironomos 2002). It has recently been reported that diffuse knapweed emergence and production were negatively influenced by the soil biota associated with a native sagebrush steppe rangeland in central Washington (Part II of this dissertation). If a similar relationship exists between diffuse knapweed and the soil biota associated with the native foothills rangeland where the present study was conducted, it could help explain the low survival of diffuse knapweed observed in this study relative to studies conducted in more intensively disturbed habitats (Watson and Renney 1974, Myers and Berube 1983, Roze et al. 1984).

A fourth point that warrants consideration is propagule pressure. The total number of seeds sown in this experiment was 3,600 and a solitary diffuse knapweed plant is capable of producing between 2,800- and 12,000-seeds $m^{-2}yr^{-1}$. Therefore, the propagule supply used in this experiment amounts to 30-128% of the annual seed production of one individual diffuse knapweed plant. This level of propagule pressure is low and represents what might be expected in the initial stages of invasion. As more plants become established, or for areas close to large infestations, propagule pressure would be much higher and the rate of invasion would likely be much faster than observed in the present study. This study was not designed to determine the importance of propagule pressure for diffuse knapweed invasion, but the results observed suggest that high propagule pressure is important for successful diffuse knapweed invasion of late-seral rangelands. Although prolific seed production is often highlighted as a major factor contributing to the aggressiveness of knapweeds (Watson and Renney 1974), manipulative experiments designed to determine the relationships between propagule pressure, disturbance, seral stage and diffuse knapweed invasion of native rangeland systems are lacking from the invasion ecology literature.

CONCLUSIONS

The native foothills rangeland where the present study was conducted is not absolutely resistant to invasion by diffuse knapweed. In the absence of disturbance, this native foothills rangeland did not prevent the emergence or the establishment of diffuse knapweed. However, only 0.028% of seeds survived to the rosette stage in plots with no experimental disturbance suggesting that although invasion is possible under these conditions, it would be expected to proceed relatively slowly.

Removal of competition from established vegetation did not increase diffuse knapweed emergence in this study. Rather, this treatment reduced diffuse knapweed emergence in the fall-seeded run, and had no effect on emergence in the spring-seeded run. Removal of competition from established native vegetation appears to have increased establishment based on the observation that six of the seven diffuse knapweed plants alive at the end of the study occurred in plots that had been sprayed with glyphosate prior to planting. Diffuse knapweed emergence was higher from seed that was incorporated into the soil than from unincorporated seed. Seed incorporation may also have increased diffuse knapweed establishment because three of the four plants that survived from seed until the end of the study were from incorporated seed. Despite the observed effects of seed incorporation and removal of competition, neither of these disturbance treatments was required for diffuse knapweed seedlings to emerge and establish in this study. The combination of seed incorporation and removal of competition from native vegetation did not result in higher emergence than either treatment alone. Rather, the effects of seed incorporation were the same regardless of how the surrounding vegetation was treated. It does appear that the combination of seed incorporation and removal of competition from native vegetation results in higher establishment than either treatment alone because three of the four plants that survived

until the end of the study from seed occurred in plots that had been sprayed and grew from incorporated seed.

Diffuse knapweed emergence from incorporated seed increased slightly with increasing gap size, but there was no effect of gap size on emergence of non-incorporated seed. Finally, there was no apparent effect of gap size on diffuse knapweed establishment.

Overall, diffuse knapweed establishment in this study was only 0.1% even though the diffuse knapweed seed used in this study had been cleaned to select for filled, viable seeds with over 90% germination in greenhouse trials. Although 0.1% survival seems low, it appears to be sufficient to initiate diffuse knapweed invasion of the study area.

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PART II: THE ROLE OF THE NATIVE SOIL COMMUNITY IN THE INVASION

ECOLOGY OF SPOTTED (*Centaurea maculosa* auct. non Lam.)

AND DIFFUSE (*C. diffusa* Lam.) KNAPWEED

INTRODUCTION

Two of the most economically and ecologically damaging invasive plants on North American rangelands are diffuse knapweed (*Centaurea diffusa* Lam.) and spotted knapweed (*C. maculosa* auct. non Lam.) (Lacey et al. 1989, Roche 1994, Sheley et al. 1998). According to the National PLANTS Database (USDA, NRCS 2002), the present distribution of spotted knapweed in the United States includes all states except Texas, Oklahoma, Mississippi and Georgia. Diffuse knapweed is present in the 12 westernmost states as well as Nebraska, Iowa, Missouri, Michigan, Wisconsin, Illinois, Tennessee, Kentucky, Indiana, Massachusetts, Connecticut and, New Jersey (USDA, NRCS 2002). As of 1998, spotted knapweed had been reported in 326 counties of the western United States, including all counties in Washington, Idaho, Montana and Wyoming and diffuse knapweed occupied 204 counties in the western U.S. (Sheley et al. 1998). Diffuse knapweed may be more prevalent in relatively xeric regions, and spotted knapweed in relatively mesic regions (Sheley et al. 1998), but the present distribution of these two knapweeds in the western United States may be more representative of the patterns of introduction and spread and less indicative of suitable habitats for these plants (K. George Beck, personal communication).

Efforts to control and manage invasive species are often designed to kill or damage target plants using biological, physical or chemical means (Sheley et al. 1998, DiTomaso 2000). Although existing weed management techniques can successfully

reduce negative impacts of knapweeds, these plants continue to spread in a largely unpredictable manner. Therefore, it is crucial to continue the search for economically and ecologically effective control methods. Identification of improved methods for the control and management of invasive plants and the ability to predict invasions relies on the integration of knowledge regarding the ecology of the invader and of sites being invaded. Often, this information and an understanding of mechanisms leading to invasion are lacking (Mullin et al. 2000). Much is known of the biology and life history of spotted and diffuse knapweed (Schirman 1981, Lacey et al. 1990, Davis et al. 1993, Jacobs et al. 1996, Jacobs and Sheley 1998, Sheley et al. 1998, Jacobs and Sheley 1999, Marler et al. 1999a, Marler et al. 1999b), but a comprehensive understanding of the conditions and interactions facilitating knapweed invasions in North America is still lacking. This may be due to the fact that much of the information we currently have on knapweeds focuses on plant-centered mechanisms such as seed production and growth characteristics, while the invasive ability of knapweeds may actually be driven by its interactions with organisms other than the plants it out competes.

Recently, the role of the soil community in the invasion process has received increased attention from ecologists. Klironomos (2002) studied feedback relationships with soil biota for rare native plants and exotic invasive plants. In that greenhouse study, five rare native species displayed negative feedbacks driven by pathogens while positive feedbacks were common for five invasive (exotic) species, and appeared to have been driven by mycorrhizal fungi. Slower accumulation of pathogens was suggested as a mechanism that might afford invasive species an advantage over rare, native plants (Klironomos 2002). Brown knapweed (*C. jacea* L.) was included in that study, and displayed a weak negative feedback response with the soil biota (Klironomos 2002), but spotted and diffuse knapweeds have not been studied in this manner. A few studies have looked at the role of specific soil organisms, namely mycorrhizal fungi, in

knapweed invasions (Marler et al. 1999a, Marler et al. 1999b) while others have speculated that knapweeds chemically alter their rooting zone in a manner that affects their interactions with soil microbes (Callaway et al. 1999). Still, the relative benefit of the entire soil community to native plants and exotic knapweeds remains largely unknown. One possibility is that successful exotic invaders, such as knapweed, alter the native soil community and disrupt mutualisms that are important to native plant persistence. Alternatively, native soil communities may be more beneficial to exotic, invasive plants than to native plants. Although these are related scenarios that would result in similar outcomes, they constitute very different mechanisms of invasion. An improved understanding of knapweed invasion relies on clarification of the role of native soil communities in the invasion process.

For a long time, scientists have suspected that spotted and diffuse knapweeds are allelopathic (Fletcher and Renney 1963), but early efforts were not able to identify the growth inhibitor. Later, cnicin was identified as a compound produced by glandular trichomes on the surface of the aboveground portions of spotted knapweed (Locken and Kelsey 1987). Pure cnicin has been shown to significantly inhibit seedling root growth of a variety of test plants including spotted knapweed (Kelsey and Locken 1987). Olson and Kelsey (1997) found that cnicin in the above ground portions of spotted knapweed fed to sheep resulted in strong depressant effects on rumen microbial activity. A review by the same authors suggests that cnicin's antimicrobial activity has been confirmed elsewhere. However, the importance of phytotoxic effects from this compound does not appear to be great (Locken and Kelsey 1987).

Many still suspect that chemicals produced by spotted and diffuse knapweed play an important role in their ability to invade, either through direct effects on surrounding plants (Ridenour and Callaway 2001, Callaway and Aschehoug 2000) or through chemical interactions with soil microbes (Callaway et al. 1999). These authors,

however, have not attempted to identify compounds believed to be important in the invasion of spotted and diffuse knapweed. Recently, Bais et al. (2001) reported allelopathic effects of root exudates from spotted knapweed on Dalmatian toadflax (*Linaria dalmatica* L.) and common mullein (*Verbascum thapsus* L.). Further work by this group of researchers indicates that two isomers of catechin isolated from root exudates of spotted knapweed may be important to this plant's ability to invade (Bais et al. 2002, Bais et al. 2003). (+) Catechin was found to have antibacterial properties and was active against root infesting pathogens. (-) Catechin was shown to be a soil active herbicide that resulted in death of eight plant species tested in the lab, including diffuse knapweed. Spotted knapweed, however, was resistant to its own root exudates and to purified (-) catechin. If the responses discussed above hold true in field conditions, one might expect the growth of native plants to be inhibited or prevented in soil from knapweed infestations, but the growth of knapweed to be unaffected.

The greenhouse experiments conducted in this study used 100% field collected soil to provide information on two components of spotted and diffuse knapweed invasion ecology. The first component focused on the relative benefit of the native soil community to native plants versus exotic knapweeds. The second component addressed the ability of native plants and exotic knapweeds to grow in soil from knapweed infestations compared to soil from native rangelands. The objectives of this study were to:

1. Compare the effects of the native soil community on seedling emergence and production of two native plant species versus two exotic knapweed species.

Hypothesis 1.1: *Emergence and production of native plants and exotic knapweeds will be higher when grown in soil with the native soil community intact, than when grown in autoclaved native soil.*

Hypothesis 1.2: Autoclaving native soil to suppress the soil community will result in greater reductions in emergence and production of exotic knapweeds compared to two native plant species.

2. Compare seedling emergence and production of two native plant species and two exotic knapweed species in soil from knapweed infestations versus soil from the adjacent native rangeland.

Hypothesis 2.1: Knapweed emergence and production will be higher when grown in soil from the uninvaded native rangeland than in soil from knapweed infestations.

Hypothesis 2.2: Knapweed emergence and production will be lower when grown in soil from the core versus the perimeter of a knapweed infestation.

Hypothesis 2.3: Emergence and production of native plants will be higher when grown in soil from the uninvaded native rangeland than in soil from a knapweed infestation.

Hypothesis 2.4: Emergence and production of native plants will be higher when grown in soil from the core versus the perimeter of a knapweed infestation.

Hypothesis 2.5: Emergence and production of exotic knapweeds in soil from uninvaded native rangeland will be higher than the emergence and production of native plants in soil from uninvaded native rangeland.

Hypothesis 2.6: Emergence and production of exotic knapweeds in soil from the core of knapweed infestations will be greater than the emergence and production of native plants in soil from the core of knapweed infestations.

MATERIALS AND METHODS

Four greenhouse experiments were conducted using 100% field collected soil to address the two objectives of this study. The first objective dealt with the benefit of the soil community associated with uninvaded native rangeland to native plants and exotic knapweeds. This objective was addressed using soil from uninvaded rangeland adjacent to knapweed infestations. Treatments for the first objective included soil community (intact or autoclaved) and fertilizer (fertilized or not fertilized). The purpose of the fertilizer treatment was to distinguish between biological and chemical effects of autoclaving. The second objective was addressed using soil from the core and perimeter of knapweed infestations and from the uninvaded rangeland adjacent to knapweed infestations. Treatments for the second objective consisted only of soil source (core, perimeter or adjacent native). Two experiments were conducted for each objective: one with soil from the diffuse knapweed site and the other with soil from the spotted knapweed site. Bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve) and yarrow (*Achillea millefolium* L.) were grown in all experiments. Diffuse knapweed was grown in experiments using soil from the diffuse knapweed site and spotted knapweed was grown in experiments using soil from the spotted knapweed site.

Soil Collection Sites: The soil used in this study was collected from areas in, and adjacent to knapweed infestations in semi-arid sagebrush steppe rangelands of Kittitas County, in central Washington, U.S.A. Two soil collections sites were identified; a diffuse knapweed site (UTM 10 711506E 5198830N) and a spotted knapweed site (UTM 10 710492E 5199345N). The two sites are less than 1-km apart and occur at an elevation of 760-m on loam-textured soils within the United States Army, Yakima Training Center. Average annual precipitation is 230-mm. Hot, dry summers and cool moist winters characterize the area. The uninvaded native rangeland at the spotted knapweed site (adjacent to the spotted knapweed infestation) is dominated by basin

wildrye (*Leymus cinereus* (Scribn. & Merr.) A. Löve) and basin big sagebrush (*Artemisia tridentata* Nutt. ssp. *tridentata*). The uninvaded native rangeland at the diffuse knapweed site (adjacent to the diffuse knapweed infestation) is dominated by Wyoming big sagebrush (*Artemisia tridentata* Nutt. ssp. *Wyomingensis* Beetle & Young), bluebunch wheatgrass and native perennial bluegrasses (*Poa* spp. L.).

Plant species composition was determined in May of 2002 for all soil collection areas (core and perimeter of the knapweed infestation, and the adjacent native rangeland) at both sites. In each area, four, 25-m transects were randomly located. The line intercept method (Bonham 1989) was used to determine canopy cover by species.

Soil Collection and Preparation: Soil was collected on 30 November 2001 from three source areas (core, perimeter and adjacent uninvaded native rangeland) at each of the two sites (spotted knapweed site and diffuse knapweed site). In each source area, a spade was used to collect soil and associated roots from thirty locations to a depth of 15-cm. Prior to collection in each source area, spades were sterilized with denatured alcohol. Soil was collected only from areas under canopies of the dominant plant species. The 30 subsamples from each source area were mixed in sterilized buckets and then transferred into sterilized coolers. A single subsample from each of the source area composite samples was sent to a commercial lab for soil community assays. Root samples were collected from three randomly selected knapweed plants at each site and sent to a commercial lab for determination of mycorrhizal colonization. Coolers full of soil were loaded into a pickup insulated above and below with 1.2- x 2.4-m sheets of 4-cm thick, white styrofoam, and transported to Fort Collins, Colorado on 1 December 2001. Fall collected soil was then placed in a cool (5-10° C), moist and dark location for storage until use. Coolers with lids propped open were covered with cloth sheets to minimize contamination. Additional soil was collected 23 May 2002 from the same source areas at the same sites using the techniques described above.

Prior to planting experimental plants on 24 June 2002, all soil was moved to the greenhouse at Colorado State University, where it was sieved (5.6-mm) to remove large roots, rocks and debris. Spring and fall collected soils from each source area at each site were thoroughly mixed. Soil texture was later determined by particle size analysis using the hydrometer method (modified from Day 1965). Sieved soil was placed in aluminum pans and loosely covered with aluminum foil. Covered pans of soil were placed in the greenhouse and kept moist. Half of the pans containing soil from the native rangeland adjacent to each of the knapweed infestations were randomly selected for autoclaving (objective 1). None of the core or perimeter soil was autoclaved (objective 2). Soil in the autoclaved treatments was subjected to three, 60-minute autoclaving periods (pressurized steam at 121° C at 0.10-MPa) (Wolf and Skipper 1994), and incubated for four to eight days between autoclavings. Autoclaving half of the native rangeland soil from each site resulted in a total of eight soil types (core, perimeter, intact native and autoclaved native soil from each site). A single subsample was collected from each of the eight soil types on 24 June 2002 and sent to a commercial lab for analysis of chemical and physical properties. In addition, a single sample was collected from each of the autoclaved and intact native rangeland soils and sent to a commercial lab for soil community assays to provide supporting information on the effects of autoclaving.

Planting and Growing Conditions: Glass wool was used to plug the bottom of 3.8- x 21-cm plastic Cone-tainer™ pots (Stuewe & Sons, Corvallis, OR, USA) to prevent soil from leaking out of the holes at the bases of the pots, while still allowing them to drain. Pots were filled to the top with field soil and then agitated to cause settling. After settling, the soil surface was approximately 2-cm below the top rim of the pot.

Spotted knapweed seeds that had been collected in 1998 were obtained from J.M. Story at Montana State University. Diffuse knapweed seeds were collected on 31

August 2001 from plants growing at the Foothills Campus of Colorado State University (Fort Collins, CO, USA). Plants were mature and seeds were hard at the time of collection. Knapweed seed (spotted and diffuse) was cleaned using a South Dakota Seed Blower to separate chaff and light seed from heavy seed. Seed cleaning consisted of three phases. In the first phase (1 minute) airflow was adjusted to separate chaff from seeds. For the second phase (3 minutes) airflow was manipulated to sort off approximately the lightest 10% of the seeds. The heavy portion of seed from the second phase was subjected to a third phase (2 minutes) to remove another 10% of the lightest seeds. The heavy seed remaining after 3 phases of cleaning was used for this greenhouse study. Seeds of bluebunch wheatgrass and yarrow were obtained from Pawnee Buttes Seed Company (Greeley, Colorado, U.S.A.) and hand sorted to obtain a consistent lot of filled and unbroken seeds for use in this study. Two hundred seeds of each species were randomly selected for germination trials. Germinators were constructed by placing four pieces of filter paper (Whatman® #2) in each of 16 petri dishes. Fifty seeds were placed in each germinator (4 germinators per species, 50 seeds per germinator) and kept covered. Germinators were placed in the greenhouse, kept moist and monitored for three weeks.

Cone-tainer™ pots were randomly assigned to combinations of soil treatment and plant species. Two days prior to seeding, all pots (containing soil) were watered to the point of runoff so that soil would be wet at the time of planting. All pots were planted on 24 June 2002 by sowing one seed per pot. Bluebunch wheatgrass seed was planted to a depth of 0.5-cm. Seeds of yarrow and the two knapweeds were firmly pressed into the surface of the soil. A thin layer (5-mm) of vermiculite was placed on the soil surface in each pot to help retain moisture in the pots. Racks of pots were randomly placed on a greenhouse bench and watered daily with a misting nozzle for 24 days. From that point on, plants were checked daily and watered as needed with a gentle sprinkling nozzle.

Each rack of 98 pots contained only one type of soil but all plant species. Racks were randomly arranged on the greenhouse bench and randomly rearranged every two to three weeks throughout the experiment. Fertilizer was applied only to the fertilized treatment groups in the experiments associated with objective 1. Plants in fertilized treatments were irrigated with a dilute, liquid complete fertilizer (Table 2.1) two times per week from 8 July through 30 August 2002.

Plants were grown in a greenhouse for 16 weeks. Throughout this period, greenhouse temperatures were maintained at $18 \pm 7^\circ \text{C}$. Supplemental light was supplied by 430-W high-pressure sodium bulbs to provide a 14-hour photoperiod.

Table 2.1. Composition of the complete fertilizer solution (Miracle-Gro™ Nutriblend 21-18-18, 1:100 dilution of stock solution) added to fertilized treatment groups in soil community benefit experiments (objective 1).

Nitrogen	50.7-mg L ⁻¹
Phosphorus	43.5-mg L ⁻¹
Potassium	43.5-mg L ⁻¹
Magnesium	121.0- μg L ⁻¹
Boron	16.4- μg L ⁻¹
Copper	10.1- μg L ⁻¹
Iron	130.0- μg L ⁻¹
Manganese	67.6- μg L ⁻¹
Molybdenum	4.8- μg L ⁻¹
Zinc	5.8- μg L ⁻¹

Data Collection: Throughout the 16-week growing period, plants were monitored for emergence. Emergence was determined simply by determining whether or not the one seed in each pot developed into a live plant. At the end of the 16-week growing period, above ground material was clipped, oven dried at 55°C to a constant weight and then weighed. Roots were separated from soil using a hydropneumatic elutriator with the air pressure set at 0.28-MPa and water pressure of 0.28 - 0.34-MPa. Root elutriation consisted of three, 5-minute cycles (agitation only, agitation and washing, washing only).

Elutriated roots were weighed after being oven dried at 55° C to a constant weight. Ash content of root material was determined for 10% of samples by mass loss on ignition at 550° C for four hours.

Experimental Design and Statistical Analyses: All experiments were conducted using a split-plot design with sub sampling. Racks of pots were treated as whole plots and whole plot treatments were soil community and fertilizer for objective 1, and soil community for objective 2. Sub plots were portions of racks and the sub plot treatment was plant species. Duplicate individuals of a given species of plant in a rack were sub samples. The soil community benefit experiment with soil from the diffuse knapweed site and both core-perimeter-native experiments used 49 replicate plants for each treatment combination. A shortage of soil resulted in 23 replicate plants for each treatment combination in the soil community benefit experiment conducted with soil from the spotted knapweed site.

Root, shoot and total plant biomass data were analyzed using analysis of variance. Pairwise comparisons of treatment means were conducted using Tukey's Method ($\alpha=0.05$). These data were analyzed in terms of biomass per pot and biomass per plant. Because only one seed was planted in each pot, biomass per pot is affected by emergence and plant size. Analyses of biomass per pot included zeros for all pots that did not support a live plant and were therefore balanced analyses. Biomass per plant was determined using only non-zero data and resulted in unbalanced analyses because of exclusion of the 0's from the dataset. Biomass per plant is not affected by emergence, but rather indicates how large emerged plants grew. Emergence data were analyzed using logistic regression. This expresses emergence as odds of emergence, which is defined as the ratio of the probability of emergence to the probability of no

emergence. Pairwise comparisons of treatment means were conducted using Wald chi-square tests ($\alpha=0.05$).

RESULTS

Tables of basic data and outputs from the analyses conducted for Part II of this dissertation are presented in Appendix II.

Soil and Soil Collection Site Characteristics: Plant species composition data for the three soil collection areas at each site are presented in Table 2.2 (diffuse knapweed site) and Table 2.3 (spotted knapweed site). At both sites, knapweed was present only in the core and perimeter areas and was more abundant in the core than the perimeter of the infestation. No knapweed was encountered in the native rangeland adjacent to either knapweed infestation. Rather these plant communities were dominated by native, perennial late-seral vegetation.

Soil texture in all areas from both sites was classified as loam, and most chemical and physical properties were similar (Table 2.4). Nitrate nitrogen was slightly higher in the native and perimeter soils than the core soil from the diffuse knapweed site. Similarly, nitrate nitrogen was higher in the perimeter and native soils than in the core soil from the spotted knapweed site. Autoclaved native soil from both sites was much higher in manganese than non-autoclaved native soil. Autoclaving also appears to have increased sulfur availability.

The results of the soil community assays based on the single subsample collected from each soil collection area and analyzed at the time of the fall soil collection are presented in Appendix II, Table A2.23. At the time of the fall soil collection, active bacterial and fungal biomass appeared to be similar across all soil collection areas at both sites. Flagellate protozoa were least abundant in the core soil, intermediate in the perimeter and most abundant in the native rangeland soils at both sites. Amoebae protozoa trends were the reverse of flagellates. Ciliate protozoa were only detected in

Table 2.2. Relative canopy cover (mean \pm SE, n=4) of major species at the diffuse knapweed soil collection site, central Washington, U.S.A.

	-----Relative Canopy Cover (%)-----		
	Core of Infestation	Perimeter of Infestation	Adjacent Native Rangeland
Introduced species			
diffuse knapweed (<i>C. diffusa</i> Lam.)	16 \pm 2	3 \pm 1	0
bur buttercup (<i>Ceratocephala testiculata</i> (Crantz) Bess.)	4 \pm 1	1 \pm 1	0
bulbous bluegrass (<i>Poa bulbosa</i> L.)	66 \pm 3	64 \pm 3	t ¹
cheatgrass (<i>Bromus tectorum</i> L.)	0	5 \pm 1	1 \pm 1
blue mustard (<i>Chorispora tenella</i> (Pallas) DC.)	t ¹	2 \pm 1	0
Native Species			
Wyoming big sagebrush (<i>Artemisia tridentata</i> Nutt. ssp. <i>Wyomingensis</i> Beetle & Young)	0	7 \pm 2	38 \pm 1
bluebunch wheatgrass (<i>Pseudoroegneria spicata</i> (Pursh) A. Löve)	0	0	24 \pm 4
Sandberg bluegrass (<i>Poa secunda</i> J. Presl.)	6 \pm 4	3 \pm 1	9 \pm 2
Leiberg's bluegrass (<i>Poa leibergii</i> Scribn.)	0	t ¹	13 \pm 1
bottlebrush squirreltail (<i>Elymus elymoides</i> (Raf.) Swezey)	4 \pm 2	5 \pm 2	3 \pm 1
rabbitbrush (<i>Chrysothamnus</i> Nutt. spp.)	2 \pm 2	1 \pm 1	0
longleaf phlox (<i>Phlox logifolia</i> Nutt.)	0	2 \pm 2	0
yarrow (<i>Achillea millefolium</i> L.)	0	0	t ¹

¹t=trace

Table 2.3. Relative canopy cover (mean \pm SE, n=4) of major species at the spotted knapweed soil collection site, central Washington, U.S.A.

	-----Relative Canopy Cover (%)-----		
	Core of Infestation	Perimeter of Infestation	Adjacent Native Rangeland
Introduced species			
spotted knapweed (<i>C. maculosa</i> auct. non Lam.)	67 \pm 5	18 \pm 5	0
cheatgrass (<i>Bromus tectorum</i> L.)	10 \pm 2	10 \pm 1	5 \pm 1
Native species			
basin big sagebrush (<i>Artemisia tridentata</i> Nutt. ssp. <i>tridentata</i>)	13 \pm 3	9 \pm 1	30 \pm 5
basin wildrye (<i>Leymus cinereus</i> (Scribn. & Merr.) A. Löve)	1 \pm 1	55 \pm 6	56 \pm 6
rabbitbrush (<i>Chrysothamnus</i> Nutt. spp.)	2 \pm 2	0	4 \pm 1
yarrow (<i>Achillea millefolium</i> L.)	t ¹	t ¹	t ¹
silky lupine (<i>Lupinus serecioides</i> Pursh.)	1 \pm 1	1 \pm 1	0

¹t=trace

Table 2.4. Chemical and physical properties of the soil collected 30 November 2001 from areas in and adjacent to knapweed infestations in central Washington, U.S.A. for use in the greenhouse experiments. Timing of soil analysis corresponded to the time of planting for the greenhouse studies, 24 June 2002.

	-----DIFFUSE KNAPWEED SITE-----				-----SPOTTED KNAPWEED SITE-----			
	Core	Perimeter	Native	Autoclaved	Core	Perimeter	Native	Autoclaved
pH	6.9	7.0	7.2	6.9	7.2	7.1	6.5	6.7
sol. salts (mmho·cm ⁻¹)	0.23	0.20	0.28	0.32	0.28	0.52	0.39	0.42
Organic Matter (%)	2	2	2	2	3	5	5	5
NO ₃ -Nitrogen (mg·kg ⁻¹)	26	27	56	45	38	87	64	46
Phosphorus (mg·kg ⁻¹)	39	39	36	46	95	102	109	95
Potassium (mg·kg ⁻¹)	565	633	414	445	981	1,130	1,070	915
Sulfur (mg·kg ⁻¹)	7	11	9	20	8	16	13	32
Calcium (mg·kg ⁻¹)	1,672	1,436	1,383	1,416	2,532	3,048	1,981	1,997
Magnesium (mg·kg ⁻¹)	429	384	341	350	383	305	321	366
Sodium (mg·kg ⁻¹)	17	15	16	29	17	13	9	26
Zinc (mg·kg ⁻¹)	0.8	0.6	1.0	1.5	1.8	4.1	5.2	7.2
Iron (mg·kg ⁻¹)	20	17	29	21	20	27	53	35
Manganese (mg·kg ⁻¹)	8	7	8	131	6	6	10	145
Copper (mg·kg ⁻¹)	1.7	1.6	1.6	1.6	0.8	0.9	1.4	1.5
Cation Exchg. Cap.	13	12	11	11	18	21	15	15

the soil from the core of the spotted knapweed site. Total nematode numbers were lowest in the core soil, intermediate in the perimeter soil and highest in the soil from the native rangeland at the spotted knapweed site. There was no apparent difference in total nematode numbers in the core, perimeter and native soils collected from the diffuse knapweed site. Mycorrhizal colonization of diffuse and spotted knapweed roots was $11\% \pm 5\%$ and $28\% \pm 13\%$, respectively (mean \pm SE, n=3).

Biomass and Emergence: Analyses of above ground, below ground and total biomass resulted in essentially the same patterns of response; therefore only total plant biomass is presented in Figures 2.1 through 2.6 and in Tables 2.5 and 2.6. Similarly, analyses of biomass per plant only provided additional meaningful insight in the soil community benefit experiment with soil from the spotted knapweed site and will only be reported there. All other biomass values reported are in terms of total plant biomass per pot. Information on analyses not presented in the text can be found in Appendix II.

Greenhouse germination trials indicated that consistent seed lots were selected for use in this experiment. Germination of bluebunch wheatgrass, yarrow, diffuse and spotted knapweed seed was $92\% \pm 3\%$, $94\% \pm 1\%$, $93\% \pm 3\%$ and $97\% \pm 2\%$, respectively (mean \pm SE, n=4). Ash contents of roots were as follows (mean \pm SE); bluebunch wheatgrass, $30\% \pm 2\%$, n=26; knapweeds, $23\% \pm 2\%$, n=30; yarrow, $23\% \pm 2\%$, n=34; and all plants combined, $25\% \pm 1\%$, n=90.

Plots of residuals versus predicted values from the biomass analyses suggested a slight tendency toward increasing variance with increasing predicted values for some analyses, but transformations were not deemed necessary. All emergence analyses resulted in converging algorithms. Deviance and Pearson goodness of fit statistics for all analyses ranged from 1.16 to 1.19, and 1.02 to 1.03, respectively, indicating that the assumption of binomial variability was valid. One degree of freedom X^2 tests on these

statistics resulted in $0.2022 < P < 0.2370$, indicating that all models fit the data sets reasonably well.

Soil Community Benefit – Diffuse Knapweed Site: Total plant biomass per pot was explained by two, two-way interactions. The effect of fertilizer on total plant biomass per pot varied by species ($P=0.0067$). Biomass of both yarrow and diffuse knapweed increased in response to fertilizer but fertilizer did not affect bluebunch wheatgrass (Figure 2.1). The interaction between fertilizer and autoclaving was marginally significant ($P=0.0777$) but worth exploring (Figure 2.2). Total plant biomass per pot was smallest for all three plants in the intact native soil without fertilizer and increased in response to fertilizer. Autoclaving resulted in a greater increase in biomass than fertilizer, and the largest biomass values occurred in the treatments that were both autoclaved and fertilized.

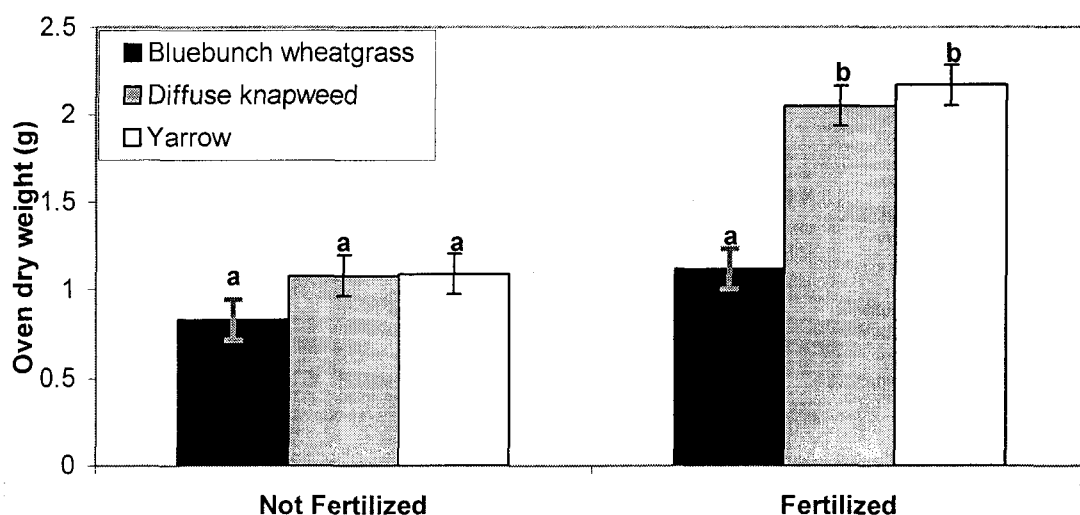


Figure 2.1. Two-way interaction plot showing the effects of a complete fertilizer on total plant biomass per pot (LS mean \pm SE, 16 d.f.) of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), diffuse knapweed (*Centaurea diffusa* Lam.) and yarrow (*Achillea millefolium* L.) grown in soil from native rangeland adjacent to a diffuse knapweed infestation in central Washington, U.S.A. Means with a different letter are different, Tukey's HSD, $\alpha=0.05$.

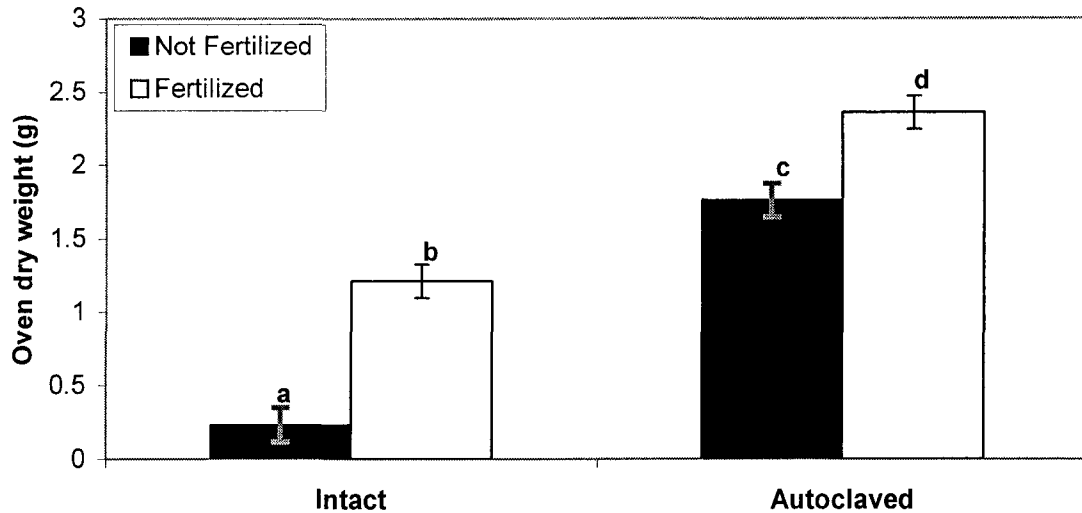


Figure 2.2. Two-way interaction plot showing the effects of complete fertilizer and autoclaving on total plant biomass per pot (LS mean \pm SE, 8 d.f.) of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), diffuse knapweed (*Centaurea diffusa* Lam.) and yarrow (*Achillea millefolium* L.) grown in soil from native rangeland adjacent to a diffuse knapweed infestation in central Washington, U.S.A. Means with a different letter are different, Tukey's HSD, $\alpha=0.05$.

Results of the logistic regression for this experiment suggest that emergence varied by species ($P<0.0001$) and the effects of autoclaving differed between fertilized and unfertilized treatments ($P=0.0475$). Averaged across all treatments, the odds of emergence for diffuse knapweed and yarrow were 3.0 and 3.2, respectively. Odds of emergence for bluebunch wheatgrass were 1.0, which was significantly lower than the odds of emergence for the other two species ($P<0.001$). Odds of emergence averaged across all three plant species were higher in autoclaved native soil than intact native soil, but the effect of autoclaving was more pronounced in the unfertilized treatments compared to the fertilized treatments (Table 2.5).

Soil Community Benefit – Spotted Knapweed Site: Total plant biomass per pot was again explained by two, two-way interactions. In this case, the effects of autoclaving varied by fertilizer treatment ($P=0.0229$) (Figure 2.3) and by species ($P=0.0023$) (Figure 2.4). As before, the smallest biomass per pot was observed in the

Table 2.5. Odds of emergence (least squares means from two-way interactions) for the soil community benefit experiments conducted using soil from native rangeland adjacent to a diffuse knapweed infestation and a spotted knapweed infestation in central Washington, U.S.A. A separate analysis was conducted for each of the two soil collection sites.

	Intact Native Soil	Autoclaved Native Soil
a) Soil from the diffuse knapweed site		
Not Fertilized	1.0 ^{**1,a}	5.2 ^{1,b}
Fertilized	1.0 ^{1,a}	2.5 ^{2,b}
b) Soil from the spotted knapweed site		
Bluebunch wheatgrass	1.0 ^{1,a}	1.0 ^{1,a}
Spotted knapweed	1.0 ^{1,a}	0.2 ^{2,b}
Yarrow	14.5 ^{2,a}	1.9 ^{1,b}

*Odds of emergence = probability of emergence ÷ probability of no emergence

**Means in a column with a different number are significantly different, and means in a row with a different letter are significantly different using Tukey's HSD, $\alpha=0.05$.

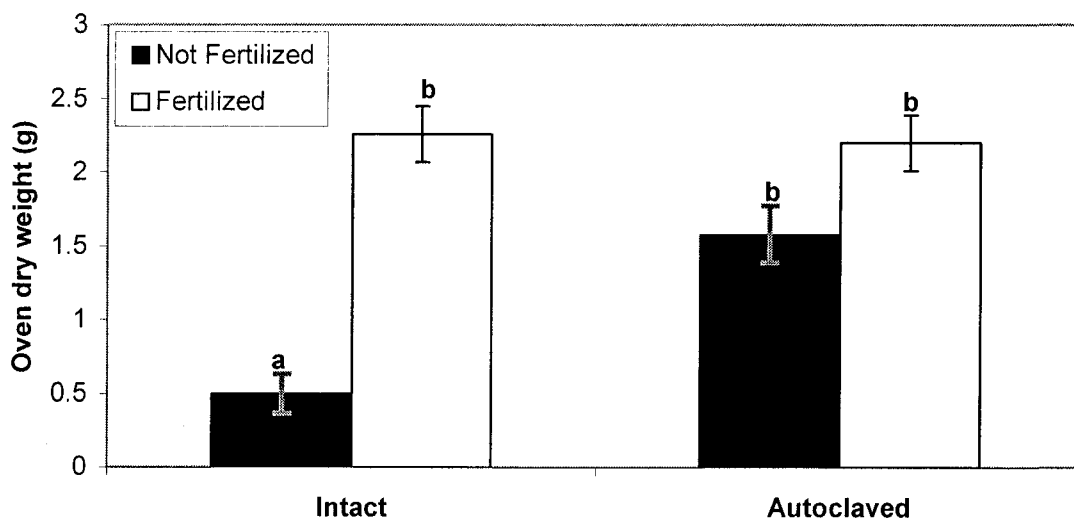


Figure 2.3. Two-way interaction plot showing the effects of complete fertilizer and autoclaving on total plant biomass per pot (LS mean \pm SE, 5 d.f.) of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), spotted knapweed (*Centaurea maculosa* auct. non Lam.) and yarrow (*Achillea millefolium* L.) grown in soil from native rangeland adjacent to a spotted knapweed infestation in central Washington, U.S.A. Means with a different letter are different, Tukey's HSD, $\alpha=0.05$.

intact native soil without fertilizer, but in this experiment there were no differences

among the fertilized, autoclaved, and autoclaved plus fertilizer treatments (Figure 2.3).

Bluebunch wheatgrass biomass increased in response to autoclaving and, while not

statistically significant, there appeared to be a slight upward trend in yarrow biomass and

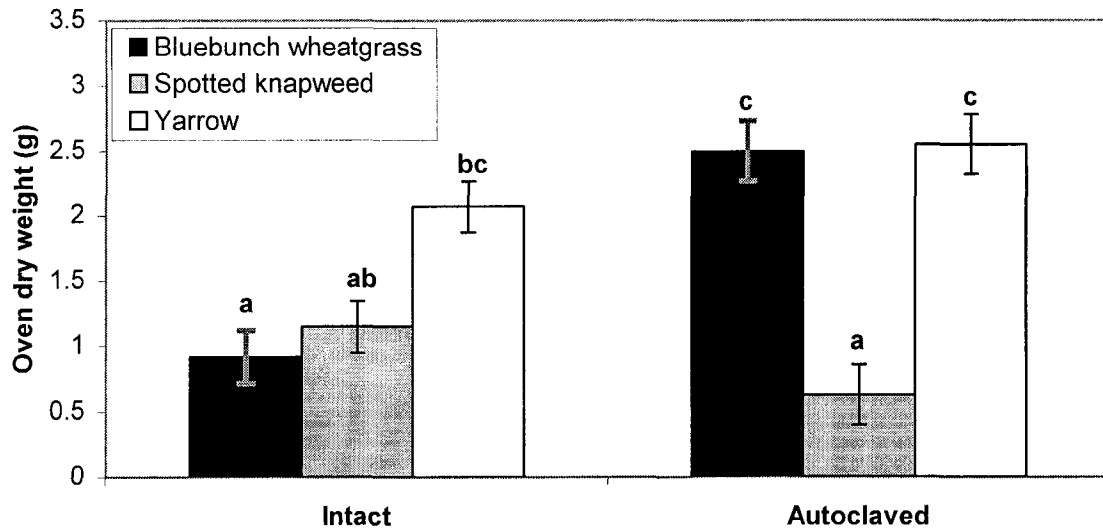


Figure 2.4. Two-way interaction plot showing the effects of autoclaving on total plant biomass per pot (LS mean \pm SE, 10 d.f.) of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), spotted knapweed (*Centaurea maculosa* auct. non Lam.) and yarrow (*Achillea millefolium* L.) grown in soil from native rangeland adjacent to a spotted knapweed infestation in central Washington, U.S.A. Means with a different letter are different, Tukey's HSD, $\alpha=0.05$.

a slight downward trend in spotted knapweed biomass in response to autoclaving (Figure 2.4). Interestingly, total biomass per plant for spotted knapweed increased in response to autoclaving (Appendix II, Table A2.8) and this increase was marginally significant ($P=0.0780$). Finally, the effect of fertilizer on total biomass per pot did not vary by species ($P=0.1789$), and all three species appeared to grow larger when fertilized (Figure 2.5).

Results of the emergence analysis for this experiment are presented in Table 2.5. Emergence was explained by the interaction of autoclaving and plant species ($P=0.0023$). Odds of emergence for bluebunch wheatgrass were 1.0 in both the intact native soil and the autoclaved native soil. Yarrow and spotted knapweed emergence both decreased significantly in response to autoclaving but the probability of yarrow emergence in the autoclaved soil was still two times greater than its probability of no

emergence. Odds of emergence for spotted knapweed in the autoclaved native soil were 0.2 which is very low and indicates that the probability of spotted knapweed emergence in the autoclaved native soil was 5 times lower than its probability of no emergence.

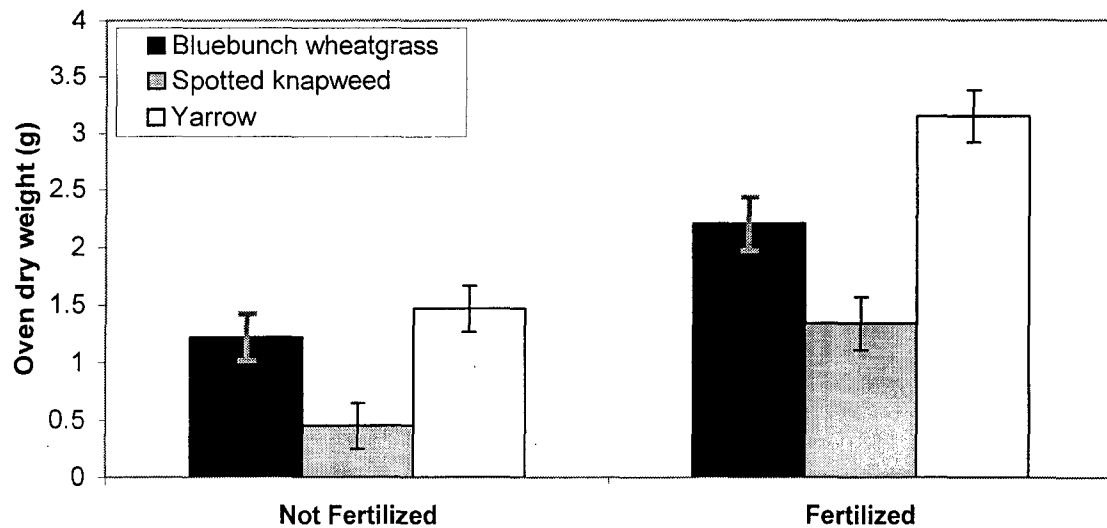


Figure 2.5. Two-way interaction plot showing the effect of complete fertilizer on total plant biomass per pot (LS mean \pm SE, 10 d.f.) of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), spotted knapweed (*Centaurea maculosa* auct. non Lam.) and yarrow (*Achillea millefolium* L.) grown in soil from native rangeland adjacent to a diffuse knapweed infestation in central Washington, U.S.A.

Core, Perimeter, Native Soil – Diffuse Knapweed Site: Total plant biomass per pot varied by soil ($P=0.0132$) and by plant species ($P<0.0001$), but the interaction of these factors was not significant. Averaged across all species, total plant biomass per pot was slightly higher when grown in the native soil (0.23 ± 0.02 g, $n=6$) than when grown in soil from the core (0.16 ± 0.02 g, $n=6$) or perimeter (0.15 ± 0.02 g, $n=6$) of the diffuse knapweed infestation. Across all soil types, total plant biomass per pot for diffuse knapweed (0.22 ± 0.02 g, $n=12$) and yarrow (0.23 ± 0.02 g, $n=12$) was higher than that of bluebunch wheatgrass (0.10 ± 0.02 g, $n=12$).

Emergence was explained by the interaction of soil and plant species ($P=0.0033$). The odds of emergence for bluebunch wheatgrass and diffuse knapweed were not affected by soil source, but yarrow emergence was much higher in soil from the core of the diffuse knapweed infestation than in perimeter or native soil (Table 2.6). Further, the plant with the highest odds of emergence in soil from the core of the diffuse knapweed infestation was yarrow, which was 11 times more likely to emerge than not to emerge.

Core, Perimeter, Native Soil – Spotted Knapweed Site: The effects of core, perimeter and native soil on total plant biomass per pot again varied by species ($P=0.0072$). Biomass per pot of bluebunch wheatgrass and spotted knapweed was unaffected by soil source, but yarrow biomass was slightly higher when grown in the perimeter and native soil than when grown in soil from the core of the spotted knapweed infestation (Figure 2.6).

Emergence also varied by soil and species, simultaneously ($P=0.0003$) (Table 2.6). Odds of emergence for bluebunch wheatgrass were higher in the core and native soil than in the soil from the perimeter of the spotted knapweed infestation. Spotted knapweed seed was five times more likely to emerge than not emerge in the soil from the core of the spotted knapweed infestation, while in the perimeter and native soil the probability of emergence was the same as the probability of no emergence. Odds of emergence for yarrow were 3 in the core and perimeter soils and 9 in the native soil.

Table 2.6. Odds of emergence (least squares means from two-way interactions) for the core, perimeter, native soil experiments conducted using soil collected in and adjacent to spotted and diffuse knapweed infestations in central Washington, U.S.A. A separate analysis was conducted for each of the two soil collection sites.

	Core Soil	Perimeter Soil	Native Soil
a) Soil from the diffuse knapweed site			
Bluebunch wheatgrass	0.2 ^{**1,a}	0.4 ^{1,a}	0.5 ^{1,a}
Diffuse knapweed	2.1 ^{2,a}	1.3 ^{2,a}	1.5 ^{2,a}
Yarrow	11.3 ^{3,a}	1.9 ^{2,b}	2.2 ^{2,b}
b) Soil from the spotted knapweed site			
Bluebunch wheatgrass	1.0 ^{1,a}	0.4 ^{1,b}	1.0 ^{1,a}
Spotted knapweed	5.1 ^{2,a}	1.0 ^{2,b}	1.0 ^{1,b}
Yarrow	3.1 ^{1,2,a,b}	2.8 ^{3,a}	8.8 ^{2,b}

^{*}Odds of emergence = probability of emergence ÷ probability of no emergence

^{**}Means in a column with a different number are significantly different, and means in a row with a different letter are significantly different using Tukey's HSD, $\alpha=0.05$.

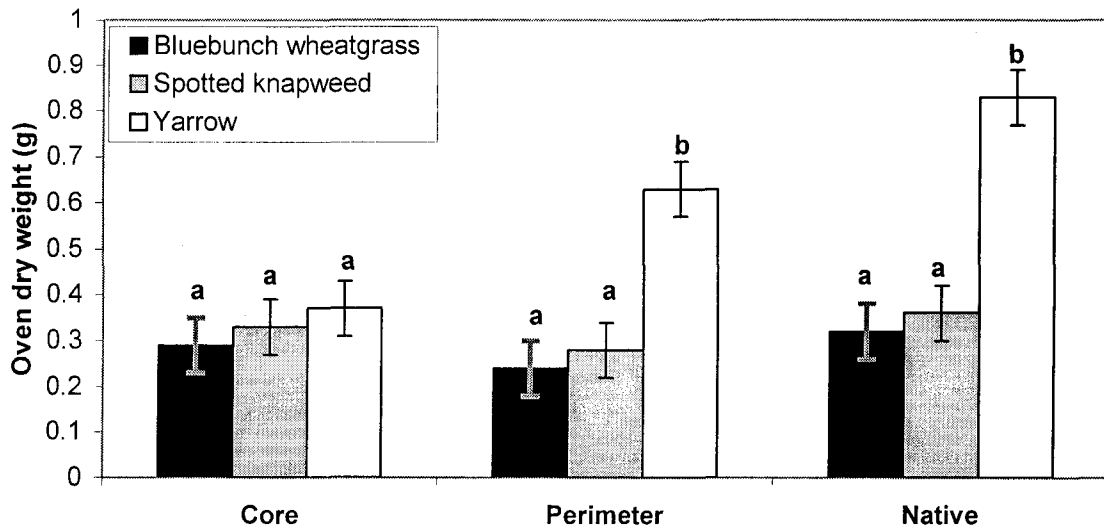


Figure 2.6. Two-way interaction plot of total plant biomass per pot (LS mean \pm SE, 12 d.f.) of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), spotted knapweed (*Centaurea maculosa* auct. non Lam.) and yarrow (*Achillea millefolium* L.) in soil from a spotted knapweed infestation and adjacent native rangeland in central Washington, U.S.A. Means with a different letter are different, Tukey's HSD, $\alpha=0.05$.

DISCUSSION

Biomass and Emergence: The fact that above ground, below ground and total biomass all responded similarly to treatment combinations was most likely a result of growing experimental plants for only 16 weeks from seed. In older, established plants, differences between root growth and shoot growth may have been observed.

Ash content of roots for all species combined was estimated to be $25\% \pm 1\%$ (mean + SE, n=90). This value is low and consistent relative to estimates discussed in the literature (Hunt et al. 1999) and suggests that correction of root biomass for ash content was not necessary.

Emergence responses of the experimental plants were driven by soil treatments, and not differences in seed viability because germination values for all plant species used were consistent and above 90%. Ninety percent emergence would correspond to an odds ratio of 9, in other words a probability of emergence nine times greater than the probability of no emergence. Odds of emergence observed in this study were as low as 0.2 and as high as 14.5 suggesting substantial departures from a value of 9, which would be expected had there been no effect of the treatments on emergence.

Soil Community Benefit – Both Sites: The lack of a response of bluebunch wheatgrass to fertilizer when grown in native rangeland soil from the diffuse knapweed site (Figure 2.1) suggests that nutrients were not the most limiting factor for bluebunch wheatgrass in that soil. Soil from the diffuse knapweed site may have contained one or more factors that were more limiting to the growth of bluebunch wheatgrass than available nutrients. Interestingly, when grown in native rangeland soil from the spotted knapweed site, biomass per pot of bluebunch wheatgrass, yarrow and spotted knapweed all increased in response to fertilizer (no species by fertilizer interaction) (Figure 2.3). This suggests that in soil from the spotted knapweed site, nutrients were a limiting factor for growth of bluebunch wheatgrass and that no other factor precluded the

ability of bluebunch wheatgrass to benefit from the applied nutrients. Given that bluebunch wheatgrass biomass increased in response to fertilizer when grown in soil from the spotted knapweed site but not when grown in soil from the diffuse knapweed site, one might assume that available nutrients were lower in the soil from the spotted knapweed site when compared to soil from the diffuse knapweed site, but this was not the case. Nutrient content of soil from the spotted knapweed site was the same or slightly higher than that of the diffuse knapweed site (Table 2.4). Therefore, it appears that nutrients were not the most limiting factor for bluebunch wheatgrass growth in soil from the diffuse knapweed site and that one or more factors existed that were more limiting and precluded the ability of this grass to benefit from applied nutrients. Further, the factors that precluded the ability of bluebunch wheatgrass to benefit from applied fertilizer did not appear to be present in soil from the spotted knapweed site. Bluebunch wheatgrass was abundant in the native rangeland at the diffuse knapweed site, and while present, was not encountered in the canopy cover sampling of the native rangeland at the spotted knapweed site (Tables 2 and 3). Recently, Klironomos (2002) linked feedbacks with soil biota to plant abundance and suggested that accumulation of pathogens in the soil increases with the time of occupation for a given native plant. If this type of relationship holds true for the sites used in the present study, it may help explain additional limiting factors for bluebunch wheatgrass in the soil from the diffuse knapweed site where bluebunch wheatgrass was abundant.

In both soil community benefit experiments, yarrow and the knapweeds increased in response to fertilizer. Although yarrow is a perennial plant that can be considered native to North America, it exhibits some behavior of an early-seral plant including high seedling vigor, moderately abundant seed, fairly rapid rate of spread by seed and a rapid rate of spread through rhizomes. Therefore it is not surprising that yarrow is often described as a plant that increases in abundance on cultivated or

otherwise disturbed sites (Sampson 1919, Harrington 1954, USDA 1988, Stubbendieck et al. 1989). In fact, yarrow is included on the invasive plant list of the National PLANTS Database (USDA, NRCS 2002).

Diffuse and spotted knapweeds have many characteristics of early-seral plants including very high seed production, high seed viability, high seedling vigor, rapid growth rate and relatively short life spans (Watson and Renney 1974, Schirman 1981). Early-seral habitats are generally characterized by high nutrient availability in the soil (Barbour et al. 1987). McLendon and Redente (1991) identified ample nitrogen supply as the key factor driving the ability of annual plants to achieve and maintain dominance in disturbed sites. Similarly, Paschke et al. (2000) found that reducing soil nitrogen availability resulted in plant and soil communities with late-seral characteristics and increasing soil nitrogen availability resulted in plant and soil communities with early-seral characteristics. Even though yarrow and the two species of knapweed are not annual plants, they do have characteristics of early-seral plants and similar responses to nutrients might be expected.

Autoclaving and fertilizer did not have the same effect on total biomass per pot in the experiment using soil from the diffuse knapweed site (Figure 2.2). Rather, autoclaving resulted in a greater increase in biomass than fertilizing. Statistically, in the experiment using soil from the spotted knapweed site, there was no difference between fertilized treatments, autoclaved treatments and those that were both fertilized and autoclaved (Figure 2.3). However, had the biomass of the autoclaved treatments been higher, Figure 2.3 would be very similar to Figure 2.2. The lack of an increase in biomass per pot of the autoclaved treatments over the intact treatments in the experiment using soil from the spotted knapweed site was driven by the response of spotted knapweed to autoclaving, which was different from the responses of the other two plants. Total plant biomass per pot of bluebunch wheatgrass was low in the intact

native soil and significantly higher in the autoclaved native soil (Figure 2.4). Biomass per pot of yarrow was relatively high in both intact native soil and autoclaved native soil. Spotted knapweed biomass was low and similar to that of bluebunch wheatgrass in the intact native soil but did not increase in response to autoclaving. Rather, spotted knapweed accumulated only about half as much biomass in the autoclaved native soil as compared to the intact native soil. This apparent reduction in biomass was not statistically significant, presumably because of the large variances associated with means caused by the inclusion of 0's for biomass where no live plant developed from the one seed in each pot. Because only one seed was planted in each pot, low values of biomass per pot can be a result of low emergence or small plants. Bluebunch wheatgrass had low biomass per pot in the intact native soil relative to the autoclaved native soil (Figure 2.4), however its odds of emergence in both treatments were 1.0 (Table 2.5). Therefore, the response of bluebunch wheatgrass to autoclaving was driven by differences in plant size and not differences in emergence. Spotted knapweed biomass per pot was low in the intact native soil and appeared to decrease in response to autoclaving. Unlike bluebunch wheatgrass, this response was driven by reduced emergence and not reduced plant size. The odds of emergence for spotted knapweed were 1.0 in the intact native soil and 0.2 in the autoclaved native soil. Odds of emergence of 0.2 suggest that the probability of emergence was five times lower than the probability of no emergence. Further, spotted knapweed biomass per plant increased in response to autoclaving (no 0-biomass values included) (Appendix II, Table A2.8). Soil tests suggest that autoclaving only resulted in minor changes in nutrient availability. Manganese was the only nutrient that was substantially increased by autoclaving (Table 2.4), but all levels observed were above those believed to be deficient and below those thought to be toxic (Aubert and Pinta 1977). Further, if nutrient dynamics were responsible for the response of spotted knapweed to

autoclaving, one might expect effects of fertilizer and autoclaving to be similar and they were not (Figure 2.5). Spotted knapweed biomass per pot increased in response to fertilizer but appeared to decrease in response to autoclaving. Autoclaving has been reported to result in drastic increases in water-soluble organic carbon (Shaw et al. 1999). Although it is possible that organic compounds may have affected plant growth in the present study, it seems unlikely. The results of Shaw et al. (1999) and the studies they reviewed suggested that when increases in water-soluble organic carbon occurred, a decrease in pH was also observed and attributed to the formation of organic acids. The observations of the present study were more consistent with Wolf and Skipper (1994) in that no change in pH was observed in response to autoclaving. Rather, it is believed that the effects of autoclaving in these experiments were driven by suppression of soil organisms.

Patterns of emergence by species in the experiment using soil from the diffuse knapweed site are not surprising. Averaged across all treatments, emergence of diffuse knapweed and yarrow was higher than that of bluebunch wheatgrass. Again, both of these forbs are early-seral plants while bluebunch wheatgrass is a late-seral species. Early-seral plants typically have shorter life spans and allocate relatively large amounts of energy to sexual reproduction (Barbour et al. 1987). Reproduction from seed is more important for shorter lived, early-seral plants so it is not surprising that emergence is higher. Late-seral plants, on the other hand, are typically longer lived and allocate relatively small amounts of energy to reproduction in any given year (Barbour et al. 1987). Reproduction from seed is probably less important for most late-seral plants compared to early-seral plants. In fact, for most herbaceous late-seral perennial plants, the importance of regeneration from seed is largely unknown, but it is widely accepted that reproduction from seed is less critical for these plants than for short lived, early-seral plants. The increase in emergence in response to autoclaving native soil from the

diffuse knapweed site may be a result of reductions in plant pathogens that affect newly germinated plant tissue. Autoclaving has been reported to increase emergence of flax (*Linum usitatissimum* L.) by reducing pathogenic soil microorganisms (Saeidi and Rowland 2000). The different effects of autoclaving in fertilized versus unfertilized treatments are surprising and the factors driving this response are unknown. Al-Mudaris and Jutzi (1999) reported that priming seeds of sorghum and millet with ammonium or micronutrient solutions increased germination but in the present study, nearly all germination took place before fertilizer application began. Further, the small amount of germination that occurred after the fertilizer application began was common to fertilized and unfertilized treatment groups. A review by Wolf and Skipper (1994) indicates that both ammonium- and nitrate-nitrogen can be expected to increase in response to autoclaving soil. Our soil test indicated no increase in nitrate nitrogen, but ammonium nitrogen was not quantified. It is assumed that since no increase in nitrate was observed, ammonium also did not increase in response to autoclaving. However, if this assumption is not valid, it is possible that increased ammonium caused by autoclaving contributed to higher emergence of all plants in the autoclaved versus intact native soil from the diffuse knapweed site.

In the experiment using soil from the spotted knapweed site, emergence varied by species and soil treatment, simultaneously (Table 2.5). Patterns of emergence for spotted knapweed and bluebunch wheatgrass were discussed above. The highest odds of emergence observed in all experiments were those of yarrow in the intact native soil from the spotted knapweed site. Here, odds of emergence were over 14, suggesting that the probability of no emergence was very small. Autoclaving reduced the odds of emergence for yarrow significantly to around 2.0. Although it is a significant reduction from 14.5, a value of 2.0 still suggests that the probability of emergence was two times greater than the probability of no emergence. Interestingly, both yarrow and spotted

knapweed emergence was reduced significantly by autoclaving the native soil from the spotted knapweed site. This suggests that the net effect of the native soil community from the spotted knapweed site on spotted knapweed and yarrow is positive. The lowest odds of emergence observed in these experiments (0.2, Table 2.5) were associated with spotted knapweed in the autoclaved native soil. This extremely low value suggests that the native soil community may be more important to the success of spotted knapweed than any other plant tested.

One obvious example of how the soil community represents a net positive effect on plant growth is when the effects of beneficial organisms such as mycorrhizal fungi overshadow the effects of pathogenic or otherwise damaging soil organisms. Another possibility would occur where one or more components of the soil community interfere with plant pathogens. Ishibashi and Choi (1991) found that pre-emergence damping-off disease of cucumber was significantly reduced by the presence of fungal-feeding nematodes. In the present study, autoclaving was very effective at eliminating nematodes (Appendix II, Tables A2.23-A2.25). Fungal-feeding nematodes were detected in the intact native soil from both sites, but no nematodes were detected in the autoclaved native soil from either site at the time of planting or when plants were harvested. Similar dynamics have been described among other components of the soil community. Patrick (1954) concluded that the micro flora of nine "virgin" soils contained an abundance of microorganisms capable of antagonizing most of the 28 bacterial pathogens tested. More recently, rhizosphere bacteria have been shown to reduce damping-off disease in safflower (Liang et al. 1996) and corn (Hebbar et al. 1998), fungal tan spot disease in wheat (da Luz et al. 1998), and to increase emergence of field pea (Xi et al. 1996) and two species of pine (Enebak et al. 1998).

In a study using five invasive exotic plants and five rare native plants, Klironomos (2002) found that the invasive exotic plants typically displayed positive feedbacks with

soil biota and the rare, native plants displayed negative feedbacks. Using the same ten plants, that author was able to link positive feedbacks to arbuscular mycorrhizal fungi and negative feedbacks to pathogens and saprobes. In the present study, the observed responses of bluebunch wheatgrass and spotted knapweed were consistent with the findings of Klironomos (2002). The native soil community had a net negative effect on the native bluebunch wheatgrass, and a net positive effect on the exotic, invasive spotted knapweed.

The relationship of yarrow with the native soil community was less obvious. Both biomass and emergence of yarrow were negatively affected by the native soil community in the experiment using soil from the diffuse knapweed site. However, in the experiment using soil from the spotted knapweed site, the native soil community had a fairly strong positive effect on emergence and little to no effect on biomass. This observation seems to be consistent with another experiment reported by Klironomos (2002) that determined feedback with soil biota and abundance in a field for 61 plant species. Yarrow displayed a very slight positive feedback in that experiment (Klironomos 2002). Interestingly, seven of the 11 plant species that displayed positive feedbacks with soil biota in that experiment were natives, including the five with the highest positive feedback scores. Seven of the 11 positive feedback scores reported by Klironomos (2002) were also associated with plants belonging to the composite family (Asteraceae), and five were both native to North America and members of the composite family.

Yarrow was selected as one of two North American native plant species for the greenhouse experiments, but there is no way to confirm the origin of the seeds planted in the greenhouse. The seed was identified as *A. millefolium*, but this name has been used to refer to both native and introduced yarrow. There is no doubt that some varieties of yarrow are native (Pollard 1899) or at least indigenous (Tyr1 1975, Gervais 1977) to North America. In fact, according to Pollard (1899), the type specimens for two

early ancestors of yarrow were both found in North America. It has also been suggested that the Pacific Northwest region in North America is a center of evolutionary activity for the genus *Achillea* (Pireh and Tyrl 1980). In addition to the native yarrows, there are also Eurasian varieties, some of which have been introduced to North America (Clausen et al. 1940, Warwick and Black 1982). However, it is interesting to note that according to Ehrendorfer (1973) most of the yarrow that dominates eastern North American habitats is clearly native, but has usually been mistaken for introduced yarrow, which is quite rare. Given its wide distribution, it may be more appropriate to consider yarrow as circumpolar (Pollard 1899) or at least a circumboreal (Clausen et al. 1940, Pireh and Tyrl 1980).

Over a century ago, Pollard (1899) proposed an appended key to the species of *Achillea* that included both North American native plants and introduced ballast weeds from Eurasia as *A. lanulosa* Nutt. In later years, native yarrow found in rangeland systems of western North America was referred to as *A. lanulosa* Nutt., while the introduced, Eurasian yarrow was referred to as *A. millefolium* L. (Clausen et al. 1940). More recently, several attempts have been made to separate yarrow into species that are native versus those that are introduced to North America based on morphological (Pollard 1899, Clausen et al. 1940), cytological (Ehrendorfer 1973, Tyrl 1975, Gervais 1977, Pireh and Tyrl 1980) or growth (Clausen et al. 1940) characteristics. However, the most common recommendation of these efforts is that such separation is not possible (Pollard 1899, Clausen et al. 1940, Ehrendorfer 1973, Tyrl 1975). For example, Clausen et al. (1940) conducted transplant studies using *A. millefolium* L. (Eurasian), *A. borealis* Bong. (native), and *A. lanulosa* Nutt. (native) and concluded that all three of these taxa showed different reactions at transplant stations, but noted that variation among clones within each taxon was appreciable. Actually, variation of growth characteristics among clones of a given taxon often appeared greater than differences between taxa. In

addition, there was consistently one clone of what Clausen et al. (1940) referred to as *A. millefolium* L. (Eurasian) that responded very similar to one or more clones of the ecotype they referred to as *A. lanulosa* Nutt. (native). Ehrendorfer (1973) suggested that differentiation among the yarrows found in North America should be in the form of varietal names subordinated to *A. millefolium*. Similarly, Tyrl (1975) stated that ecotypic and morphologic variation in the *Achillea* complex in North America is clinal and subject to constant change making taxonomic classification problematic. The present convention appears to follow the recommendation of Ehrendorfer (1973), where varietal names subordinated to *A. millefolium* L. are used to differentiate among yarrows found in North America (USDA NRCS 2002).

The inability to distinguish between native and introduced members of the *A. millefolium* L. complex as discussed above makes less important the ability to determine the origin of the seeds used in the greenhouse experiments. The fact that yarrow has circumpolar or circumboreal distribution also offers some explanation for the observation that yarrow and spotted knapweed were both positively influenced by the soil community associated with native rangeland soil from the spotted knapweed site.

Finally, our findings suggest that the native soil community had a net negative effect on diffuse knapweed which is both exotic and invasive. Based on information found in the National PLANTS Database (USDA NRCS 2002), 26 of the 61 plant species considered in the experiment conducted by Klironomos (2002) were classified as both introduced to, and invasive in, North America. Of these, only three displayed positive feedbacks while 23 displayed negative feedbacks. Some of the introduced invasive plants that displayed negative feedbacks in that experiment were brown knapweed, Canada thistle (*Cirsium arvense* (L.) Scop.), bull thistle (*C. vulgare* (Savi) Ten.), bindweed (*Convolvulus arvensis* L.), St. Johnswort (*Hypericum perforatum* L.) and yellow toadflax (*L. vulgaris* P.Mill.). So, while positive feedbacks may contribute to

invasiveness for some species (Klironomos 2002), it does not appear that this is an overwhelmingly common mechanism for invasiveness. Further, it is not uncommon for native, noninvasive plants to display positive feedbacks with soil communities.

The observation of a negative influence of the native soil community on diffuse knapweed emergence and biomass may help explain the low emergence and establishment of that invasive plant in a native foothills rangeland located in northern Colorado (Part I of this dissertation). Emergence and establishment of diffuse knapweed in a late-seral, native foothills rangeland did occur in the absence of experimental disturbance, but survival was very low. Disturbances that weakened established native vegetation and incorporated diffuse knapweed seed appear to have increased the chance of diffuse knapweed establishment in that native rangeland system (Part I of this dissertation). Therefore, diffuse knapweed invasion appears to be facilitated more by disturbance than by beneficial interactions with native soil communities. In fact, the native soil community may help protect late-seral, native rangelands from diffuse knapweed invasion.

Following is a summary of the soil community experiments relative to the hypotheses presented under the first objective. Emergence and production of diffuse knapweed, bluebunch wheatgrass and yarrow were higher in autoclaved versus intact native rangeland soil collected from the diffuse knapweed site. When grown in native rangeland soil from the spotted knapweed site, production of spotted knapweed appeared to decrease in response to autoclaving, and emergence clearly decreased in response to autoclaving. Yarrow production was unaffected by the autoclaving treatment, but like spotted knapweed, yarrow emergence decreased in response to autoclaving. Neither emergence nor production of bluebunch wheatgrass was higher when grown in intact versus autoclaved native soil. Autoclaving native soil from the spotted knapweed site to suppress the soil community resulted in greater reductions in

emergence and production of spotted knapweed than of yarrow and bluebunch wheatgrass. However, this was not the case in the experiment using native rangeland soil from the diffuse knapweed site. In that experiment, emergence and production of all three species increased in response to autoclaving.

Core, Perimeter, Native Soil – Both Sites: In soil from the diffuse knapweed site, averaged across all three soil treatments, biomass per pot of yarrow and diffuse knapweed was slightly greater than that of bluebunch wheatgrass. This is not surprising considering the discussion above that yarrow and diffuse knapweed have many characteristics of early-seral plants and bluebunch wheatgrass is typically considered a late-seral plant. Biomass per pot averaged across all three species was slightly greater in the native soil than soil from the core or perimeter of the diffuse knapweed infestation. Emergence of bluebunch wheatgrass and diffuse knapweed were relatively insensitive to soil treatment, while that of yarrow was much higher in the core soil compared to perimeter or native soil. Considering only soil from the core of the diffuse knapweed infestation, odds of emergence for yarrow were five times higher than diffuse knapweed. When biomass and emergence are considered together, it becomes apparent that for bluebunch wheatgrass and diffuse knapweed, roughly the same number of plants emerged in all three soil treatments, but that plants grew larger in the native soil than in soil from the core or perimeter of the knapweed infestation. More yarrow plants emerged in the core soil than the perimeter or native soil but plants in the core and perimeter soil were smaller than those in the native soil. These patterns may be a result of nitrate-nitrogen availability, which was higher in the native soil compared to the core and perimeter of the diffuse knapweed infestation (Table 2.4). The very high emergence of yarrow in the core soil and the fact that it is much higher than that of diffuse knapweed is important. As discussed above, yarrow is circumboreal and is often described as an invader of cultivated or otherwise disturbed sites (Sampson 1919, Harrington 1954,

USDA 1988, Stubbendieck et al. 1989). The core of the diffuse knapweed infestation has an obvious history of past disturbance. It is located in a pasture corner not far from a shipping corral. There has been no livestock grazing in the area for nearly ten years, but other forms of disturbance continue to affect the site. The area is not far from the John Wayne Trail, which is a popular recreational attraction. Also, this area provides a convenient place for vehicles to pull off the road and park or turn around. Finally, this area also appears to be a location where troops congregate from time to time when carrying out training exercises in this portion of the Yakima Training Center. Disturbance history may help explain the high emergence of yarrow in the soil from the core of the diffuse knapweed infestation, but one must also consider that soil collection and preparation for these experiments resulted in the same degree of physical disturbance for all soil.

Disturbance does not explain the fact that yarrow emergence was higher than diffuse knapweed emergence in the core soil. An allelochemical has recently been identified in root exudates of diffuse knapweed that is a different compound than \pm catechin, the allelochemical found in root exudates of spotted knapweed (J.M. Vivanco, personal communication). Based on the work done with \pm catechin (Bais et al. 2001, Bais et al. 2002), one might expect that chemicals in the root exudates of diffuse knapweed also affect plants and soil organisms. Chemicals in root exudates may be the primary mode of protection from soil-borne pathogens for diffuse knapweed. If these chemicals also reduce non-pathogenic and beneficial soil organisms, their presence may also represent a cost from the plants perspective. Some substances used to suppress fungal soil pathogens (Abu-Qamar and Al-Raddad 2001) and bacterial pathogens have been shown to negatively affect non-pathogenic soil bacteria (Siemann et al. 1999, Lan et al. 2002, Mladenova et al. 2002) as well as other bacteria and fungi (Kenawy 2001). Many non-pathogenic soil organisms are believed to be antagonistic to soil borne

pathogens (Patrick 1954, Liang et al. 1996, Xi et al. 1996, da Luz et al. 1998, Enebak et al. 1998, Hebbar et al. 1998) and this may be the main mode of pathogen protection for yarrow in the soil used in these experiments. Such a strategic difference for protection from pathogens, if it exists, would help explain the observed difference in emergence patterns of yarrow and diffuse knapweed in soil from the core of the diffuse knapweed infestation.

In the experiment using soil from the spotted knapweed site, yarrow was the only plant for which biomass per pot varied by soil treatment (Figure 2.6). Biomass per pot for yarrow growing in the core soil was significantly lower than that of yarrow growing in perimeter or native soil. Bluebunch wheatgrass and spotted knapweed biomass per pot was relatively low and consistent across soil treatments. Nitrate-nitrogen, zinc, iron and total nematodes all appeared to be slightly higher in the perimeter and native soil than the core soil (Table 2.4) and may be partly responsible for the observed patterns of yarrow biomass.

Emergence of bluebunch wheatgrass was slightly higher in core and native soils than the perimeter soil, while that of spotted knapweed was higher in the core soil than the perimeter and native soil (Table 2.6). As with biomass, emergence of yarrow was higher when grown in the native soil than in core or perimeter soil from this site. Yarrow emergence patterns in soil from the spotted knapweed site are the reverse of those observed with soil from the diffuse knapweed site. Following the discussion offered above, it may be that chemicals in root exudates of spotted knapweed have different effects on soil biota than chemicals in the exudates of diffuse knapweed. +Catechin, found in root exudates of spotted knapweed as well as in the soil from a spotted knapweed infestation, had antimicrobial properties and was active against soil borne root infesting bacterial pathogens (Bais et al. 2002). The effects of +catechin on non-pathogenic soil bacteria were not evaluated but it is likely that this compound affects

non-pathogenic as well as pathogenic soil bacteria. Because \pm catechin has been found in soil from spotted knapweed infestations (Bais et al. 2002) it has the potential to affect both pathogenic and non-pathogenic soil bacteria in the soil that all species were grown in, including yarrow. If yarrow does in fact rely on antagonistic relationships among non-pathogenic and pathogenic soil organisms for protection from disease, +catechin in soil from the core of the spotted knapweed infestation may help explain the difference between the emergence of yarrow in the core soils from the two different knapweed sites.

The following paragraphs summarize the core, perimeter native soil experiments relative to the hypotheses presented under the second objective. Diffuse knapweed emergence was not higher when grown in soil from uninvaded native rangeland compared to soil from a diffuse knapweed infestation. However, production of diffuse knapweed was slightly higher when grown in native soil versus soil from the core or perimeter of a diffuse knapweed infestation. Neither emergence nor production of spotted knapweed was higher when grown in the native rangeland soil than when grown in soil from a spotted knapweed infestation. Neither emergence nor production of diffuse and spotted knapweed was lower when grown in the core versus the perimeter soil from knapweed infestations.

Production of the native plants was higher when grown in native rangeland soil than soil from a diffuse knapweed infestation. However, emergence of bluebunch wheatgrass was the same for native rangeland soil and soil from the diffuse knapweed infestation. Yarrow emergence was actually higher when grown in soil from the core of the diffuse knapweed infestation than when grown in perimeter or native soil. In the experiment using soil from the spotted knapweed site, production of yarrow was slightly higher when grown in the native and perimeter soils than in soil from the core of the spotted knapweed infestation. Yarrow emergence was higher when grown in the native

rangeland soil than in soil from either the core or perimeter of the spotted knapweed infestation. Bluebunch wheatgrass production and emergence were consistent across core, perimeter and native soils.

Yarrow emergence was higher in soil from the core versus the perimeter of the diffuse knapweed infestation. However, yarrow production as well as emergence and production of bluebunch wheatgrass were the same in core and perimeter soils. Yarrow production and bluebunch wheatgrass emergence were higher when grown in soil from the core than in soil from the perimeter of the spotted knapweed infestation, but bluebunch wheatgrass production and yarrow emergence were the same in the two soils.

Considering only the native rangeland soil collected adjacent to the diffuse knapweed infestation, diffuse knapweed production was not greater than production of the native plants. Rather, production of all three species was the same. Diffuse knapweed emergence was higher than bluebunch wheatgrass emergence, but about the same as yarrow emergence in soil collected from the native rangeland adjacent to the diffuse knapweed infestation. Neither production nor emergence of spotted knapweed was greater than that of the native plants in soil from the native rangeland adjacent to the spotted knapweed infestation.

Emergence of both knapweed species was greater than bluebunch wheatgrass, but not yarrow, in soil from the core of their respective knapweed infestations. However, production of each knapweed was about the same as the native species in soil from the core of their respective knapweed infestations.

CONCLUSIONS

The observed responses of plants to the autoclaving treatments appear to have been driven by suppression of soil organisms rather than by changes to chemical and physical properties of the soil. Based on these experiments, it appears that the native soil community is more beneficial to spotted knapweed than to diffuse knapweed, bluebunch wheatgrass or yarrow. In fact, the native soil community consistently represented a negative influence on bluebunch wheatgrass and diffuse knapweed, but had a positive influence on spotted knapweed emergence. The interactions between yarrow and the native soil community were variable, and intermediate between the responses of spotted knapweed and bluebunch wheatgrass, possibly because yarrow is a circumboreal plant that has some characteristics of invasive species.

It appears that two, closely related knapweeds have very different interactions with soil biota. The native soil community appears to benefit spotted knapweed, and this plant seems to rely more heavily on soil biota for emergence in native rangeland soil than diffuse knapweed.

Despite the fact that biomass or emergence or both were sometimes higher in native soil than soil from knapweed infestations, it appears that bluebunch wheatgrass and yarrow are capable of growth in soil from the core and perimeter of knapweed infestations. Therefore, it does not appear that soils from the core or perimeter of the spotted and diffuse knapweed infestations studied have any properties that preclude the emergence and growth of bluebunch wheatgrass or yarrow. There was also no indication that soil from the knapweed infestations promoted the growth of either spotted or diffuse knapweed.

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APPENDIX I

Basic data and analyses for Field Study (Part I)

Table A1.1. Analysis of diffuse knapweed (*Centaurea diffusa* Lam.) emergence in terms of the percent of seed sown in introduction plots under various treatment combinations in a native foothills rangeland west of Fort Collins, Colorado, U.S.A.

Source	DF	Sum of Squares	Mean Square	F Value	P
Model	25	8678.1	347.1	6.99	<0.0001
Error	46	2284.6	49.7		
Total	71	10962.6			
Block	2	131.4	65.7	1.32	0.2762
Gap ¹	2	616.8	308.4	6.21	0.0041
Natives ²	1	122.7	122.7	2.47	0.1228
Season ³	1	813.4	813.4	16.40	0.0002
Seed ⁴	1	4640.1	4640.1	93.43	<0.0001
Gap*Natives	2	356.8	178.4	3.59	0.0355
Season*Gap	2	105.4	52.7	1.06	0.3542
Gap*Seed	2	264.1	132.1	2.66	0.0808
Season*Natives	1	329.4	329.4	6.63	0.0133
Natives*Seed	1	53.4	53.4	1.07	0.3052
Season*Seed	1	1136.1	1136.1	22.87	<0.0001
Season*Gap*Natives	2	48.1	24.1	0.48	0.6192
Gap*Natives*Seed	2	8.1	4.1	0.08	0.9217
Season*Gap*Seed	2	8.8	4.4	0.09	0.9156
Season*Natives*Seed	1	12.5	12.5	0.25	0.6183
Season*Gap*Natives*Seed	2	31	15.5	0.31	0.7334

¹Gap = gap size (0-, 5- and 15-cm)

²Natives = treatment of native vegetation surrounding introduction plot (killed, alive)

³Season = season of seeding (fall 2001 run or spring 2002 run)

⁴Seed = seed incorporation (seed incorporated, seed not incorporated)

Table A1.2. Diffuse knapweed (*Centaurea diffusa* Lam.) emergence (mean \pm SE, 2 d.f.) in terms of the percent of seed sown in introduction plots under various treatment combinations in a native foothills rangeland west of Fort Collins, Colorado, U.S.A.

Season ¹	Gap ²	Natives ³	Seed ⁴	Mean Emergence (%)	SE
Fall	0	Killed	Incorporated	24.7	6.4
Fall	0	Killed	Not Incorporated	5.3	2.9
Fall	0	Alive	Incorporated	34.0	6.9
Fall	0	Alive	Not Incorporated	16.0	6.0
Fall	5	Killed	Incorporated	35.3	2.4
Fall	5	Killed	Not Incorporated	12.0	3.1
Fall	5	Alive	Incorporated	36.7	3.5
Fall	5	Alive	Not Incorporated	12.7	1.8
Fall	15	Killed	Incorporated	36.0	1.2
Fall	15	Killed	Not Incorporated	4.0	2.0
Fall	15	Alive	Incorporated	43.3	6.7
Fall	15	Alive	Not Incorporated	16.0	5.0
Spring	0	Killed	Incorporated	14.0	3.1
Spring	0	Killed	Not Incorporated	4.7	0.7
Spring	0	Alive	Incorporated	14.7	2.7
Spring	0	Alive	Not Incorporated	14.7	1.8
Spring	5	Killed	Incorporated	24.0	5.0
Spring	5	Killed	Not Incorporated	14.0	2.0
Spring	5	Alive	Incorporated	14.0	4.2
Spring	5	Alive	Not Incorporated	9.3	2.7
Spring	15	Killed	Incorporated	29.3	6.6
Spring	15	Killed	Not Incorporated	16.7	4.4
Spring	15	Alive	Incorporated	26.0	4.0
Spring	15	Alive	Not Incorporated	14.0	3.5

¹Season = season of seeding (fall 2001 run or spring 2002 run)

²Gap = gap size (0-, 5- and 15-cm)

³Natives = treatment of native vegetation surrounding introduction plot (killed, alive)

⁴Seed = seed incorporation (seed incorporated, seed not incorporated)

Table A1.3. Analysis of diffuse knapweed (*Centaurea diffusa* Lam.) emergence in terms of the percent of introduction plots in which at least one diffuse knapweed seedling emerged from seed when sown under various treatment combinations in a native foothills rangeland west of Fort Collins, Colorado, U.S.A.

Source	DF	Sum of Squares	Mean Square	F Value	P
Model	25	27218.1	1088.7	5.42	<0.0001
Error	46	9247.2	201.0		
Total	71	36465.3			
Block	2	352.8	176.4	0.88	0.4227
Gap ¹	2	2852.8	1426.4	7.10	0.0021
Natives ²	1	401.4	401.4	2.00	0.1644
Season ³	1	501.4	501.4	2.49	0.1211
Seed ⁴	1	14734.7	14734.7	73.30	<0.0001
Gap*Natives	2	1102.8	551.4	2.74	0.0749
Season*Gap	2	1002.8	501.4	2.49	0.0937
Gap*Seed	2	586.1	293.1	1.46	0.2433
Season*Natives	1	2334.7	2334.7	11.61	0.0014
Natives*Seed	1	168.1	168.1	0.84	0.3653
Season*Seed	1	2568.1	2568.1	12.77	0.0008
Season*Gap*Natives	2	286.1	143.1	0.71	0.4962
Gap*Natives*Seed	2	2.8	1.4	<0.01	0.9931
Season*Gap*Seed	2	36.1	18.1	0.09	0.9143
Season*Natives*Seed	1	112.5	112.5	0.56	0.4582
Season*Gap*Natives*Seed	2	175.0	87.5	0.44	0.6497

¹Gap = gap size (0-, 5- and 15-cm)

²Natives = treatment of native vegetation surrounding introduction plot (killed, alive)

³Season = season of seeding (fall 2001 run or spring 2002 run)

⁴Seed = seed incorporation (seed incorporated, seed not incorporated)

Table A1.4. Diffuse knapweed (*Centaurea diffusa* Lam.) emergence (mean \pm SE, 2 d.f.) in terms of the percent of introduction plots in which at least one diffuse knapweed seedling emerged from seed when sown under various treatment combinations in a native foothills rangeland west of Fort Collins, Colorado, U.S.A.

Season ¹	Gap ²	Natives ³	Seed ⁴	Mean Emergence (%)	SE
Fall	0	Killed	Incorporated	53.3	12.0
Fall	0	Killed	Not Incorporated	20.0	10.0
Fall	0	Alive	Incorporated	76.7	6.7
Fall	0	Alive	Not Incorporated	36.7	16.7
Fall	5	Killed	Incorporated	73.3	6.7
Fall	5	Killed	Not Incorporated	33.3	6.7
Fall	5	Alive	Incorporated	76.7	3.3
Fall	5	Alive	Not Incorporated	46.7	3.3
Fall	15	Killed	Incorporated	66.7	3.3
Fall	15	Killed	Not Incorporated	16.7	8.8
Fall	15	Alive	Incorporated	86.7	3.3
Fall	15	Alive	Not Incorporated	36.7	6.7
Spring	0	Killed	Incorporated	40.0	5.8
Spring	0	Killed	Not Incorporated	20.0	0.0
Spring	0	Alive	Incorporated	40.0	5.8
Spring	0	Alive	Not Incorporated	36.7	3.3
Spring	5	Killed	Incorporated	66.7	6.7
Spring	5	Killed	Not Incorporated	50.0	5.8
Spring	5	Alive	Incorporated	46.7	12.0
Spring	5	Alive	Not Incorporated	33.3	12.0
Spring	15	Killed	Incorporated	76.7	6.7
Spring	15	Killed	Not Incorporated	46.7	8.8
Spring	15	Alive	Incorporated	60.0	5.8
Spring	15	Alive	Not Incorporated	43.3	13.3

¹Season = season of seeding (fall 2001 run or spring 2002 run)

²Gap = gap size (0-, 5- and 15-cm)

³Natives = treatment of native vegetation surrounding introduction plot (killed, alive)

⁴Seed = seed incorporation (seed incorporated, seed not incorporated)

APPENDIX II

Basic data and analyses for greenhouse experiments (Part II)

Table A2.1. Analysis of total plant biomass per pot of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), diffuse knapweed (*Centaurea diffusa* Lam.) and yarrow (*Achillea millefolium* L.) grown in soil collected from native rangeland adjacent to a diffuse knapweed infestation in central Washington, U.S.A. Analyses of fixed effects are presented for root, shoot and total biomass.

Source	Numerator DF	Denominator DF	F Value	P
Root Biomass				
Soil ¹	1	8	193.2	<0.0001
Fertilizer ²	1	8	51.3	<0.0001
Soil*fertilizer	1	8	7.8	0.0233
Species ³	2	16	18.1	<0.0001
Soil*species	2	16	3.4	0.0573
Fertilizer*species	2	16	6.2	0.0103
Soil*fertilizer*species	2	16	2.1	0.1611
Shoot Biomass				
Soil	1	8	189.5	<0.0001
Fertilizer	1	8	100.0	<0.0001
Soil*fertilizer	1	8	<0.1	0.8385
Species	2	16	20.5	<0.0001
Soil*species	2	16	0.4	0.6539
Fertilizer*species	2	16	7.5	0.0050
Soil*fertilizer*species	2	16	0.1	0.9494
Total Biomass				
Soil	1	8	204.8	<0.0001
Fertilizer	1	8	69.7	<0.0001
Soil*fertilizer	1	8	4.1	0.0777
Species	2	16	19.7	<0.0001
Soil*species	2	16	1.4	0.2684
Fertilizer*species	2	16	7.0	0.0067
Soil*fertilizer*species	2	16	1.1	0.3545

¹Soil = Native soil community treatment (autoclaved native soil or intact native soil)

²Fertilizer = Complete fertilizer treatment (fertilized or not fertilized)

³Species = Species of plant grown (bluebunch wheatgrass, diffuse knapweed or western yarrow)

Table A2.2. Analysis of total plant biomass per plant of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), diffuse knapweed (*Centaurea diffusa* Lam.) and yarrow (*Achillea millefolium* L.) grown in soil collected from native rangeland adjacent to a diffuse knapweed infestation in central Washington, U.S.A. Analyses of fixed effects are presented for root, shoot and total biomass.

Source	Numerator DF	Denominator DF	F Value	P
Root Biomass				
Soil ¹	1	8	212.7	<0.0001
Fertilizer ²	1	8	217.2	<0.0001
Soil*fertilizer	1	8	20.0	0.0021
Species ³	2	16	0.2	0.7861
Soil*species	2	16	21.6	<0.0001
Fertilizer*species	2	16	1.0	0.3839
Soil*fertilizer*species	2	16	9.47	0.0019
Shoot Biomass				
Soil	1	8	310.4	<0.0001
Fertilizer	1	8	460.6	<0.0001
Soil*fertilizer	1	8	1.4	0.2749
Species	2	16	3.1	0.0733
Soil*species	2	16	1.0	0.4015
Fertilizer*species	2	16	2.4	0.1196
Soil*fertilizer*species	2	16	0.5	0.6018
Total Biomass				
Soil	1	8	396.2	<0.0001
Fertilizer	1	8	461.5	<0.0001
Soil*fertilizer	1	8	20.4	0.0020
Species	2	16	0.2	0.7959
Soil*species	2	16	14.5	0.0003
Fertilizer*species	2	16	1.7	0.2107
Soil*fertilizer*species	2	16	6.8	0.0074

¹Soil = Native soil community treatment (autoclaved native soil or intact native soil)

²Fertilizer = Complete fertilizer treatment (fertilized or not fertilized)

³Species = Species of plant grown (bluebunch wheatgrass, diffuse knapweed or western yarrow)

Table A2.3. Oven dry weight (g) of root, shoot and total plant biomass per pot (LS mean \pm SE, 16 d.f.) of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), diffuse knapweed (*Centaurea diffusa* Lam.) and yarrow (*Achillea millefolium* L.) as affected by soil community treatment (autoclaved or intact) and fertilizer (fertilized or not fertilized) when grown in soil collected from native rangeland adjacent to a diffuse knapweed infestation in central Washington, U.S.A.

Soil	Fertilizer	Species	Roots (g)	Shoots (g)	Total (g)
Intact native	Fertilized	Bluebunch wheatgrass	0.38 \pm 0.11	0.17 \pm 0.05	0.55 \pm 0.16
Intact native	Fertilized	Diffuse knapweed	1.14 \pm 0.11	0.50 \pm 0.05	1.64 \pm 0.16
Intact native	Fertilized	Yarrow	0.94 \pm 0.11	0.49 \pm 0.05	1.43 \pm 0.16
Intact native	Not fertilized	Bluebunch wheatgrass	0.12 \pm 0.11	0.03 \pm 0.05	0.15 \pm 0.16
Intact native	Not fertilized	Diffuse knapweed	0.21 \pm 0.11	0.08 \pm 0.05	0.28 \pm 0.16
Intact native	Not fertilized	Yarrow	0.19 \pm 0.11	0.09 \pm 0.05	0.27 \pm 0.16
Autoclaved native	Fertilized	Bluebunch wheatgrass	1.12 \pm 0.11	0.57 \pm 0.05	1.70 \pm 0.16
Autoclaved native	Fertilized	Diffuse knapweed	1.50 \pm 0.11	0.95 \pm 0.05	2.45 \pm 0.16
Autoclaved native	Fertilized	Yarrow	2.01 \pm 0.11	0.90 \pm 0.05	2.92 \pm 0.16
Autoclaved native	Not fertilized	Bluebunch wheatgrass	1.09 \pm 0.11	0.43 \pm 0.05	1.51 \pm 0.16
Autoclaved native	Not fertilized	Diffuse knapweed	1.30 \pm 0.11	0.57 \pm 0.05	1.87 \pm 0.16
Autoclaved native	Not fertilized	Yarrow	1.39 \pm 0.11	0.52 \pm 0.05	1.91 \pm 0.16

86 **Table A2.4.** Oven dry weight (g) of root, shoot and total plant biomass per plant (LS mean \pm SE, 16 d.f.) of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), diffuse knapweed (*Centaurea diffusa* Lam.) and yarrow (*Achillea millefolium* L.) as affected by soil community treatment (autoclaved or intact) and fertilizer (fertilized or not fertilized) when grown in soil collected from native rangeland adjacent to a diffuse knapweed infestation in central Washington, U.S.A.

Soil	Fertilizer	Species	Roots (g)	Shoots (g)	Total (g)
Intact native	Fertilized	Bluebunch wheatgrass	1.45 \pm 0.14	0.63 \pm 0.07	2.07 \pm 0.18
Intact native	Fertilized	Diffuse knapweed	1.93 \pm 0.10	0.84 \pm 0.04	2.77 \pm 0.12
Intact native	Fertilized	Yarrow	1.31 \pm 0.09	0.69 \pm 0.04	2.00 \pm 0.11
Intact native	Not fertilized	Bluebunch wheatgrass	0.38 \pm 0.13	0.10 \pm 0.06	0.48 \pm 0.16
Intact native	Not fertilized	Diffuse knapweed	0.35 \pm 0.10	0.13 \pm 0.04	0.48 \pm 0.12
Intact native	Not fertilized	Yarrow	0.29 \pm 0.09	0.13 \pm 0.04	0.42 \pm 0.11
Autoclaved native	Fertilized	Bluebunch wheatgrass	2.20 \pm 0.10	1.12 \pm 0.05	3.32 \pm 0.13
Autoclaved native	Fertilized	Diffuse knapweed	1.89 \pm 0.09	1.20 \pm 0.04	3.08 \pm 0.10
Autoclaved native	Fertilized	Yarrow	2.53 \pm 0.09	1.14 \pm 0.04	3.67 \pm 0.10
Autoclaved native	Not fertilized	Bluebunch wheatgrass	1.61 \pm 0.09	0.63 \pm 0.04	2.25 \pm 0.11
Autoclaved native	Not fertilized	Diffuse knapweed	1.42 \pm 0.08	0.62 \pm 0.04	2.04 \pm 0.09
Autoclaved native	Not fertilized	Yarrow	1.62 \pm 0.08	0.60 \pm 0.04	2.23 \pm 0.10

Table A2.5. Analysis of total plant biomass per pot of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), spotted knapweed (*Centaurea maculosa* auct. non Lam.) and yarrow (*Achillea millefolium* L.) grown in soil collected from native rangeland adjacent to a spotted knapweed infestation in central Washington, U.S.A. Analyses of fixed effects are presented for root, shoot and total biomass.

Source	Numerator DF	Denominator DF	F Value	P
Root Biomass				
Soil ¹	1	5	6.6	0.0503
Fertilizer ²	1	5	35.9	0.0019
Soil*fertilizer	1	5	14.7	0.0122
Species ³	2	10	22.1	0.0002
Soil*species	2	10	12.9	0.0017
Fertilizer*species	2	10	2.6	0.1249
Soil*fertilizer*species	2	10	0.6	0.5576
Shoot Biomass				
Soil	1	5	12.3	0.0172
Fertilizer	1	5	65.6	0.0005
Soil*fertilizer	1	5	1.5	0.2714
Species	2	10	16.7	0.0006
Soil*species	2	10	6.9	0.0131
Fertilizer*species	2	10	1.2	0.3532
Soil*fertilizer*species	2	10	2.2	0.1613
Total Biomass				
Soil	1	5	8.5	0.0333
Fertilizer	1	5	46.0	0.0011
Soil*fertilizer	1	5	10.5	0.0229
Species	2	10	21.9	0.0002
Soil*species	2	10	11.8	0.0023
Fertilizer*species	2	10	2.1	0.1789
Soil*fertilizer*species	2	10	1.0	0.4146

¹Soil = Native soil community treatment (autoclaved native soil or intact native soil)

²Fertilizer = Complete fertilizer treatment (fertilized or not fertilized)

³Species = Species of plant grown (bluebunch wheatgrass, spotted knapweed or western yarrow)

Table A2.6. Analysis of total plant biomass per plant of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), spotted knapweed (*Centaurea maculosa* auct. non Lam.) and yarrow (*Achillea millefolium* L.) grown in soil collected from native rangeland adjacent to a spotted knapweed infestation in central Washington, U.S.A. Analyses of fixed effects are presented for root, shoot and total biomass.

Source	Numerator DF	Denominator DF	F Value	P
Root Biomass				
Soil ¹	1	5	54.5	0.0007
Fertilizer ²	1	5	39.0	0.0015
Soil*fertilizer	1	5	16.2	0.0101
Species ³	2	10	1.2	0.3388
Soil*species	2	10	5.8	0.0209
Fertilizer*species	2	10	2.7	0.1156
Soil*fertilizer*species	2	10	1.3	0.3173
Shoot Biomass				
Soil	1	5	159.0	<0.0001
Fertilizer	1	5	184.5	<0.0001
Soil*fertilizer	1	5	0.2	0.7184
Species	2	10	3.0	0.0970
Soil*species	2	10	1.6	0.2557
Fertilizer*species	2	10	1.1	0.3657
Soil*fertilizer*species	2	10	<0.1	0.9666
Total Biomass				
Soil	1	5	93.1	0.0002
Fertilizer	1	5	79.3	0.0003
Soil*fertilizer	1	5	12.0	0.0181
Species	2	10	0.3	0.7477
Soil*species	2	10	4.5	0.0416
Fertilizer*species	2	10	2.2	0.1630
Soil*fertilizer*species	2	10	1.1	0.3601

¹Soil = Native soil community treatment (autoclaved native soil or intact native soil)

²Fertilizer = Complete fertilizer treatment (fertilized or not fertilized)

³Species = Species of plant grown (bluebunch wheatgrass, spotted knapweed or western yarrow)

Table A2.7. Oven dry weight (g) of root, shoot and total plant biomass per pot (LS mean \pm SE, 10 d.f.) of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), spotted knapweed (*Centaurea maculosa* auct. non Lam.) and yarrow (*Achillea millefolium* L.) as affected by soil community treatment (autoclaved or intact) and fertilizer (fertilized or not fertilized) when grown in soil collected from native rangeland adjacent to a spotted knapweed infestation in central Washington, U.S.A.

Soil	Fertilizer	Species	Roots (g)	Shoots (g)	Total (g)
Intact native	Fertilized	Bluebunch wheatgrass	1.12 \pm 0.25	0.41 \pm 0.09	1.53 \pm 0.33
Intact native	Fertilized	Spotted knapweed	1.43 \pm 0.25	0.51 \pm 0.09	1.94 \pm 0.33
Intact native	Fertilized	Yarrow	2.50 \pm 0.25	0.81 \pm 0.09	3.31 \pm 0.33
Intact native	Not fertilized	Bluebunch wheatgrass	0.24 \pm 0.17	0.07 \pm 0.06	0.32 \pm 0.22
Intact native	Not fertilized	Spotted knapweed	0.29 \pm 0.17	0.07 \pm 0.06	0.36 \pm 0.22
Intact native	Not fertilized	Yarrow	0.61 \pm 0.17	0.22 \pm 0.06	0.83 \pm 0.22
Autoclaved native	Fertilized	Bluebunch wheatgrass	1.99 \pm 0.25	0.88 \pm 0.09	2.87 \pm 0.33
Autoclaved native	Fertilized	Spotted knapweed	0.43 \pm 0.25	0.31 \pm 0.09	0.74 \pm 0.33
Autoclaved native	Fertilized	Yarrow	2.13 \pm 0.25	0.87 \pm 0.09	3.00 \pm 0.33
Autoclaved native	Not fertilized	Bluebunch wheatgrass	1.74 \pm 0.25	0.38 \pm 0.09	2.12 \pm 0.33
Autoclaved native	Not fertilized	Spotted knapweed	0.37 \pm 0.25	0.16 \pm 0.09	0.53 \pm 0.33
Autoclaved native	Not fertilized	Yarrow	1.58 \pm 0.25	0.52 \pm 0.09	2.10 \pm 0.33

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Table A2.8. Oven dry weight (g) of root, shoot and total plant biomass per plant (LS mean \pm SE, 10 d.f.) of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), spotted knapweed (*Centaurea maculosa* auct. non Lam.) and yarrow (*Achillea millefolium* L.) as affected by soil community treatment (autoclaved or intact) and fertilizer (fertilized or not fertilized) when grown in soil collected from native rangeland adjacent to a spotted knapweed infestation in central Washington, U.S.A.

Soil	Fertilizer	Species	Roots (g)	Shoots (g)	Total (g)
Intact native	Fertilized	Bluebunch wheatgrass	2.11 \pm 0.31	0.78 \pm 0.09	2.90 \pm 0.35
Intact native	Fertilized	Spotted knapweed	2.68 \pm 0.31	0.97 \pm 0.09	3.66 \pm 0.35
Intact native	Fertilized	Yarrow	2.61 \pm 0.26	0.85 \pm 0.07	3.46 \pm 0.29
Intact native	Not fertilized	Bluebunch wheatgrass	0.48 \pm 0.23	0.15 \pm 0.06	0.62 \pm 0.26
Intact native	Not fertilized	Spotted knapweed	0.71 \pm 0.24	0.17 \pm 0.07	0.88 \pm 0.28
Intact native	Not fertilized	Yarrow	0.68 \pm 0.20	0.25 \pm 0.05	0.93 \pm 0.22
Autoclaved native	Fertilized	Bluebunch wheatgrass	3.28 \pm 0.29	1.44 \pm 0.08	4.72 \pm 0.33
Autoclaved native	Fertilized	Spotted knapweed	2.49 \pm 0.46	1.75 \pm 0.13	4.23 \pm 0.53
Autoclaved native	Fertilized	Yarrow	3.45 \pm 0.29	1.42 \pm 0.08	4.87 \pm 0.33
Autoclaved native	Not fertilized	Bluebunch wheatgrass	3.61 \pm 0.31	0.79 \pm 0.09	4.41 \pm 0.35
Autoclaved native	Not fertilized	Spotted knapweed	2.14 \pm 0.45	0.91 \pm 0.13	3.05 \pm 0.52
Autoclaved native	Not fertilized	Yarrow	2.27 \pm 0.28	0.75 \pm 0.08	3.02 \pm 0.32

Table A2.9. Analysis of total plant biomass per pot of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), diffuse knapweed (*Centaurea diffusa* Lam.) and yarrow (*Achillea millefolium* L.) as affected by soil from different locations in and adjacent to a diffuse knapweed infestation in central Washington, U.S.A. Analyses of fixed effects are presented for root, shoot and total biomass.

Source	Numerator DF	Denominator DF	F Value	P
Root Biomass				
Soil ¹	2	6	11.0	0.0100
Species ²	2	12	14.3	0.0007
Soil*species	4	12	1.7	0.2074
Shoot Biomass				
Soil	2	6	5.2	0.0484
Species	2	12	52.4	<0.0001
Soil*species	4	12	2.6	0.0862
Total Biomass				
Soil	2	6	9.7	0.0132
Species	2	12	23.4	<0.0001
Soil*species	4	12	2.1	0.1424

¹Soil = Soil treatments correspond to area from which soil was collected (core of knapweed infestation, perimeter of knapweed infestation or adjacent uninvaded native rangeland)

²Species = Species of plant grown (bluebunch wheatgrass, diffuse knapweed or western yarrow)

Table A2.10. Analysis of total plant biomass per plant of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), diffuse knapweed (*Centaurea diffusa* Lam.) and yarrow (*Achillea millefolium* L.) as affected by soil from different locations in and adjacent to a diffuse knapweed infestation in central Washington, U.S.A. Analyses of fixed effects are presented for root, shoot and total biomass.

Source	Numerator DF	Denominator DF	F Value	P
Root Biomass				
Soil ¹	2	6	40.1	0.0003
Species ²	2	12	4.1	0.0451
Soil*species	4	12	1.2	0.3725
Shoot Biomass				
Soil	2	6	43.7	0.0003
Species	2	12	7.8	0.0068
Soil*species	4	12	1.0	0.4607
Total Biomass				
Soil	2	6	49.7	0.0002
Species	2	12	1.9	0.1861
Soil*species	4	12	1.1	0.4110

¹Soil = Soil treatments correspond to area from which soil was collected (core of knapweed infestation, perimeter of knapweed infestation or adjacent uninvaded native rangeland)

²Species = Species of plant grown (bluebunch wheatgrass, diffuse knapweed or western yarrow)

Table A2.11. Oven dry weight (g) of root, shoot and total plant biomass per pot (LS mean \pm SE, 12 d.f.) of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), diffuse knapweed (*Centaurea diffusa* Lam.) and yarrow (*Achillea millefolium* L.) as affected by soil community treatment (autoclaved or intact) and fertilizer (fertilized or not fertilized) when grown in soil collected from native rangeland adjacent to a diffuse knapweed infestation in central Washington, U.S.A.

Soil	Species	Roots (g)	Shoots (g)	Total (g)
Core of diffuse knapweed infestation	Bluebunch wheatgrass	0.03 \pm 0.02	0.01 \pm 0.01	0.04 \pm 0.03
Core of diffuse knapweed infestation	Diffuse knapweed	0.11 \pm 0.02	0.06 \pm 0.01	0.18 \pm 0.03
Core of diffuse knapweed infestation	Yarrow	0.16 \pm 0.02	0.09 \pm 0.01	0.25 \pm 0.03
Perimeter of diffuse knapweed infestation	Bluebunch wheatgrass	0.07 \pm 0.02	0.02 \pm 0.01	0.10 \pm 0.03
Perimeter of diffuse knapweed infestation	Diffuse knapweed	0.13 \pm 0.02	0.06 \pm 0.01	0.19 \pm 0.03
Perimeter of diffuse knapweed infestation	Yarrow	0.10 \pm 0.02	0.06 \pm 0.01	0.16 \pm 0.03
Adjacent native rangeland	Bluebunch wheatgrass	0.12 \pm 0.02	0.03 \pm 0.01	0.15 \pm 0.03
Adjacent native rangeland	Diffuse knapweed	0.21 \pm 0.02	0.08 \pm 0.01	0.28 \pm 0.03
Adjacent native rangeland	Yarrow	0.19 \pm 0.02	0.09 \pm 0.01	0.27 \pm 0.03

Table A2.12. Oven dry weight (g) of root, shoot and total plant biomass per plant (LS mean \pm SE, 12 d.f.) of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), diffuse knapweed (*Centaurea diffusa* Lam.) and yarrow (*Achillea millefolium* L.) as affected by soil community treatment (autoclaved or intact) and fertilizer (fertilized or not fertilized) when grown in soil collected from native rangeland adjacent to a diffuse knapweed infestation in central Washington, U.S.A.

Soil	Species	Roots (g)	Shoots (g)	Total (g)
Core of diffuse knapweed infestation	Bluebunch wheatgrass	0.17 \pm 0.04	0.08 \pm 0.01	0.25 \pm 0.05
Core of diffuse knapweed infestation	Diffuse knapweed	0.17 \pm 0.02	0.09 \pm 0.01	0.26 \pm 0.02
Core of diffuse knapweed infestation	Yarrow	0.17 \pm 0.02	0.10 \pm 0.01	0.27 \pm 0.02
Perimeter of diffuse knapweed infestation	Bluebunch wheatgrass	0.23 \pm 0.03	0.07 \pm 0.01	0.30 \pm 0.03
Perimeter of diffuse knapweed infestation	Diffuse knapweed	0.20 \pm 0.02	0.08 \pm 0.01	0.28 \pm 0.02
Perimeter of diffuse knapweed infestation	Yarrow	0.15 \pm 0.02	0.09 \pm 0.01	0.23 \pm 0.02
Adjacent native rangeland	Bluebunch wheatgrass	0.38 \pm 0.03	0.10 \pm 0.01	0.48 \pm 0.03
Adjacent native rangeland	Diffuse knapweed	0.35 \pm 0.02	0.13 \pm 0.01	0.48 \pm 0.02
Adjacent native rangeland	Yarrow	0.29 \pm 0.02	0.13 \pm 0.01	0.42 \pm 0.02

Table A2.13. Analysis of total plant biomass per pot of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), spotted knapweed (*Centaurea maculosa* auct. non Lam.) and yarrow (*Achillea millefolium* L.) as affected by soil from different locations in and adjacent to a spotted knapweed infestation in central Washington, U.S.A. Analyses of fixed effects are presented for root, shoot and total biomass.

Source	Numerator DF	Denominator DF	F Value	P
Root Biomass				
Soil ¹	2	6	5.9	0.0385
Species ²	2	12	21.4	0.0001
Soil*species	4	12	5.8	0.0079
Shoot Biomass				
Soil	2	6	3.3	0.1104
Species	2	12	79.2	<0.0001
Soil*species	4	12	6.3	0.0059
Total Biomass				
Soil	2	6	4.9	0.0549
Species	2	12	34.5	<0.0001
Soil*species	4	12	5.9	0.0072

¹Soil = Soil treatments correspond to area from which soil was collected (core of knapweed infestation, perimeter of knapweed infestation or adjacent uninvaded native rangeland)

²Species = Species of plant grown (bluebunch wheatgrass, spotted knapweed or western yarrow)

Table A2.14. Analysis of total plant biomass per plant of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), spotted knapweed (*Centaurea maculosa* auct. non Lam.) and yarrow (*Achillea millefolium* L.) as affected by soil from different locations in and adjacent to a spotted knapweed infestation in central Washington, U.S.A. Analyses of fixed effects are presented for root, shoot and total biomass.

Source	Numerator DF	Denominator DF	F Value	P
Root Biomass				
Soil ¹	2	6	39.2	0.0004
Species ²	2	12	2.3	0.1392
Soil*species	4	12	9.2	0.0012
Shoot Biomass				
Soil	2	6	126.4	<0.0001
Species	2	12	112.2	<0.0001
Soil*species	4	12	1.6	0.2270
Total Biomass				
Soil	2	6	56.1	0.0001
Species	2	12	7.1	0.0093
Soil*species	4	12	8.6	0.0016

¹Soil = Soil treatments correspond to area from which soil was collected (core of knapweed infestation, perimeter of knapweed infestation or adjacent uninvaded native rangeland)

²Species = Species of plant grown (bluebunch wheatgrass, spotted knapweed or western yarrow)

Table A2.15. Oven dry weight (g) of root, shoot and total plant biomass per pot (LS mean \pm SE, 12 d.f.) of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), spotted knapweed (*Centaurea maculosa* auct. non Lam.) and yarrow (*Achillea millefolium* L.) as affected by soil community treatment (autoclaved or intact) and fertilizer (fertilized or not fertilized) when grown in soil collected from native rangeland adjacent to a spotted knapweed infestation in central Washington, U.S.A.

Soil	Species	Roots (g)	Shoots (g)	Total (g)
Core of diffuse knapweed infestation	Bluebunch wheatgrass	0.23 \pm 0.05	0.06 \pm 0.01	0.29 \pm 0.06
Core of diffuse knapweed infestation	Spotted knapweed	0.24 \pm 0.05	0.09 \pm 0.01	0.33 \pm 0.06
Core of diffuse knapweed infestation	Yarrow	0.24 \pm 0.05	0.13 \pm 0.01	0.37 \pm 0.06
Perimeter of diffuse knapweed infestation	Bluebunch wheatgrass	0.19 \pm 0.05	0.05 \pm 0.01	0.24 \pm 0.06
Perimeter of diffuse knapweed infestation	Spotted knapweed	0.17 \pm 0.05	0.10 \pm 0.01	0.28 \pm 0.06
Perimeter of diffuse knapweed infestation	Yarrow	0.42 \pm 0.05	0.21 \pm 0.01	0.63 \pm 0.06
Adjacent native rangeland	Bluebunch wheatgrass	0.24 \pm 0.05	0.07 \pm 0.01	0.32 \pm 0.06
Adjacent native rangeland	Spotted knapweed	0.29 \pm 0.05	0.07 \pm 0.01	0.36 \pm 0.06
Adjacent native rangeland	Yarrow	0.61 \pm 0.05	0.22 \pm 0.01	0.83 \pm 0.06

Table A2.16. Oven dry weight (g) of root, shoot and total plant biomass per plant (LS mean \pm SE, 12 d.f.) of bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), spotted knapweed (*Centaurea maculosa* auct. non Lam.) and yarrow (*Achillea millefolium* L.) as affected by soil community treatment (autoclaved or intact) and fertilizer (fertilized or not fertilized) when grown in soil collected from native rangeland adjacent to a spotted knapweed infestation in central Washington, U.S.A.

Soil	Species	Roots (g)	Shoots (g)	Total (g)
Core of diffuse knapweed infestation	Bluebunch wheatgrass	0.41 \pm 0.04	0.11 \pm 0.01	0.52 \pm 0.05
Core of diffuse knapweed infestation	Spotted knapweed	0.28 \pm 0.04	0.11 \pm 0.01	0.40 \pm 0.04
Core of diffuse knapweed infestation	Yarrow	0.31 \pm 0.04	0.17 \pm 0.01	0.48 \pm 0.04
Perimeter of diffuse knapweed infestation	Bluebunch wheatgrass	0.70 \pm 0.06	0.19 \pm 0.01	0.89 \pm 0.07
Perimeter of diffuse knapweed infestation	Spotted knapweed	0.37 \pm 0.05	0.22 \pm 0.01	0.59 \pm 0.05
Perimeter of diffuse knapweed infestation	Yarrow	0.57 \pm 0.04	0.29 \pm 0.01	0.86 \pm 0.04
Adjacent native rangeland	Bluebunch wheatgrass	0.48 \pm 0.05	0.14 \pm 0.01	0.62 \pm 0.05
Adjacent native rangeland	Spotted knapweed	0.72 \pm 0.05	0.17 \pm 0.01	0.89 \pm 0.05
Adjacent native rangeland	Yarrow	0.68 \pm 0.03	0.25 \pm 0.01	0.93 \pm 0.04

Table A2.17. Number of pots in which the one seed either emerged or did not emerge in the greenhouse experiments conducted with soil collected in, and adjacent to a diffuse knapweed infestation in central Washington, U.S.A.

Soil	Species ¹	Fertilizer ²	Number Emerged	Number Not Emerged
Soil Community Benefit Experiment				
Native ³ , Autoclaved	Diffuse knapweed	Not fertilized	45	4
Native, Autoclaved	Yarrow	Not fertilized	42	7
Native, Autoclaved	Bluebunch wheatgrass	Not fertilized	33	16
Native, Autoclaved	Diffuse knapweed	Fertilized	39	10
Native, Autoclaved	Yarrow	Fertilized	39	10
Native, Autoclaved	Bluebunch wheatgrass	Fertilized	25	24
Native, Intact	Diffuse knapweed	Not fertilized	28	21
Native, Intact	Yarrow	Not fertilized	32	17
Native, Intact	Bluebunch wheatgrass	Not fertilized	15	34
Native, Intact	Diffuse knapweed	Fertilized	29	20
Native, Intact	Yarrow	Fertilized	35	14
Native, Intact	Bluebunch wheatgrass	Fertilized	13	36
Core, Perimeter, Native Soil Experiment				
Core ⁴	Diffuse knapweed	N/A	33	16
Core	Yarrow	N/A	45	4
Core	Bluebunch wheatgrass	N/A	8	41
Perimeter ⁵	Diffuse knapweed	N/A	33	16
Perimeter	Yarrow	N/A	34	15
Perimeter	Bluebunch wheatgrass	N/A	16	33
Native	Diffuse knapweed	N/A	28	21
Native	Yarrow	N/A	32	17
Native	Bluebunch wheatgrass	N/A	15	34

¹Plant Species included diffuse knapweed (*Centaurea diffusa* Lam.), yarrow (*Achillea millefolium* L.) and bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve).

²Fertilizer treatments consisted of either fertilized with a complete nutrient solution or not fertilized

³Native = soil from native rangeland adjacent to knapweed infestation. For the soil community benefit experiment, soil was either autoclaved to suppress soil organisms or left intact. For the core, perimeter, native soil experiment, all soil was left intact

⁴Core = oldest part of knapweed infestation

⁵Perimeter = outside edge of knapweed infestation where it interfaces with adjacent native rangeland

Table A2.18. Number of pots in which the one seed either emerged or did not emerge in the greenhouse experiments conducted with soil collected in, and adjacent to a spotted knapweed infestation in central Washington, U.S.A.

Soil	Species ¹	Fertilizer ²	Number Emerged	Number Not Emerged
Soil Community Benefit Experiment				
Native ³ , Autoclaved	Spotted knapweed	Not fertilized	4	19
Native, Autoclaved	Yarrow	Not fertilized	16	7
Native, Autoclaved	Bluebunch wheatgrass	Not fertilized	11	12
Native, Autoclaved	Spotted knapweed	Fertilized	4	19
Native, Autoclaved	Yarrow	Fertilized	14	9
Native, Autoclaved	Bluebunch wheatgrass	Fertilized	14	9
Native, Intact	Spotted knapweed	Not fertilized	20	29
Native, Intact	Yarrow	Not fertilized	44	5
Native, Intact	Bluebunch wheatgrass	Not fertilized	25	24
Native, Intact	Spotted knapweed	Fertilized	13	12
Native, Intact	Yarrow	Fertilized	24	1
Native, Intact	Bluebunch wheatgrass	Fertilized	13	12
Core, Perimeter, Native Soil Experiment				
Core ⁴	Spotted knapweed	N/A	41	8
Core	Yarrow	N/A	37	12
Core	Bluebunch wheatgrass	N/A	28	21
Perimeter ⁵	Spotted knapweed	N/A	24	25
Perimeter	Yarrow	N/A	36	13
Perimeter	Bluebunch wheatgrass	N/A	13	36
Native	Spotted knapweed	N/A	20	29
Native	Yarrow	N/A	44	5
Native	Bluebunch wheatgrass	N/A	25	24

¹Plant Species included spotted knapweed (*Centaurea maculosa* auct. non Lam.), yarrow (*Achillea millefolium* L.) and bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve).

²Fertilizer treatments consisted of either fertilized with a complete nutrient solution or not fertilized

³Native = soil from native rangeland adjacent to knapweed infestation. For the soil community benefit experiment, soil was either autoclaved to suppress soil organisms or left intact. For the core, perimeter, native soil experiment, all soil was left intact

⁴Core = oldest part of knapweed infestation

⁵Perimeter = outside edge of knapweed infestation where it interfaces with adjacent native rangeland

Table A2.19. Logistic regression analysis of emergence for bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), diffuse knapweed (*Centaurea diffusa* Lam.) and yarrow (*Achillea millefolium* L.) as affected by soil community treatment and fertilizer when grown in soil collected from native rangeland adjacent to a diffuse knapweed infestation in central Washington, U.S.A.

Source	DF	Chi-Square	P
Soil ¹	1	41.2	<0.0001
Species ²	2	52.8	<0.0001
Soil*species	2	2.3	0.3109
Fertilizer ³	1	2.9	0.0899
Soil*fertilizer	1	3.9	0.0475
Species*fertilizer	2	0.9	0.6534
Soil*species*fertilizer	2	0.5	0.7902

¹Soil = Native soil community treatment (autoclaved native soil or intact native soil)

²Species = Species of plant grown (bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), diffuse knapweed (*Centaurea diffusa* Lam.) and yarrow (*Achillea millefolium* L.))

³Fertilizer treatments consisted of either fertilized with a complete nutrient solution or not fertilized

Table A2.20. Logistic regression analysis of emergence for bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), spotted knapweed (*Centaurea maculosa* auct. non Lam.) and yarrow (*Achillea millefolium* L.) as affected by soil community treatment and fertilizer when grown in soil collected from native rangeland adjacent to a spotted knapweed infestation in central Washington, U.S.A.

Source	DF	Chi-Square	P
Soil ¹	1	16.9	<0.0001
Species ²	2	58.7	<0.0001
Soil*species	2	12.1	0.0023
Fertilizer ³	1	0.9	0.3414
Soil*fertilizer	1	0.6	0.4344
Species*fertilizer	2	<0.1	0.9929
Soil*species*fertilizer	2	1.8	0.3993

¹Soil = Native soil community treatment (autoclaved native soil or intact native soil)

²Species = Species of plant grown (bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), spotted knapweed (*Centaurea maculosa* auct. non Lam.) and yarrow (*Achillea millefolium* L.))

³Fertilizer treatments consisted of either fertilized with a complete nutrient solution or not fertilized

Table A2.21. Logistic regression analysis of emergence for bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), diffuse knapweed (*Centaurea diffusa* Lam.) and yarrow (*Achillea millefolium* L.) as affected by soil collected from different locations in, and adjacent to a diffuse knapweed infestation in central Washington, U.S.A.

Source	DF	Chi-Square	P
Soil ¹	2	2.7	0.2589
Species ²	2	85.6	<0.0001
Soil*species	4	15.8	0.0033

¹Soil = Soil treatments correspond to area from which soil was collected (core of knapweed infestation, perimeter of knapweed infestation or adjacent uninvaded native rangeland)

²Species = Species of plant grown (bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), diffuse knapweed (*Centaurea diffusa* Lam.) and yarrow (*Achillea millefolium* L.))

Table A2.22. Logistic regression analysis of emergence for bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), spotted knapweed (*Centaurea maculosa* auct. non Lam.) and yarrow (*Achillea millefolium* L.) as affected by soil collected from different locations in, and adjacent to a spotted knapweed infestation in central Washington, U.S.A.

Source	DF	Chi-Square	P
Soil ¹	2	15.9	0.0004
Species ²	2	40.7	<0.0001
Soil*species	4	21.1	0.0003

¹Soil = Soil treatments correspond to area from which soil was collected (core of knapweed infestation, perimeter of knapweed infestation or adjacent uninvaded native rangeland)

²Species = Species of plant grown (bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) A. Löve), spotted knapweed (*Centaurea maculosa* Lam.) and yarrow (*Achillea millefolium* L.))

Table A2.23. Results of soil community assay on soil collected for the greenhouse experiments from the spotted knapweed (*Centaurea maculosa* auct. non Lam.) site and the diffuse knapweed (*C. diffusa* Lam.) site in central Washington, U.S.A. This table includes results of the assay performed 4 December 2001 on soil collected from the field sites on 30 November 2001.

	-----Spotted Knapweed Site-----				-----Diffuse Knapweed Site-----			
	Core	Perimeter	Intact Native	Autoclaved Native	Core	Perimeter	Intact Native	Autoclaved Native
Active Bacterial Biomass ($\mu\text{g}\cdot\text{g}^{-1}$)	48.2	34.5	43.1	N/A	39.0	30.0	63.3	N/A
Total Bacterial Biomass ($\mu\text{g}\cdot\text{g}^{-1}$)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Active Fungal Biomass ($\mu\text{g}\cdot\text{g}^{-1}$)	7.7	8.1	11.3	N/A	9.8	5.0	13.4	N/A
Total Fungal Biomass ($\mu\text{g}\cdot\text{g}^{-1}$)	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Flagellate Protozoa ($\#\cdot\text{g}^{-1}$)	1,592	1,669	5,598	N/A	548	541	5,104	N/A
Amoebae Protozoa ($\#\cdot\text{g}^{-1}$)	1,752	555	185	N/A	684	89	259	N/A
Ciliate Protozoa ($\#\cdot\text{g}^{-1}$)	37	0	0	N/A	0	0	0	N/A
Total Nematodes ($\#\cdot\text{g}^{-1}$)	2.3	4.3	5	N/A	1.4	1.3	1.4	N/A
Bact. Feeding Nem. ($\#\cdot\text{g}^{-1}$)	0.45	1.30	1.91	N/A	0.22	0.17	0.46	N/A
Fungal Feeding Nem. ($\#\cdot\text{g}^{-1}$)	0.88	0.47	1.00	N/A	0.68	0.59	0.55	N/A
Fung./Root Feeding Nem. ($\#\cdot\text{g}^{-1}$)	0.49	0.55	0.73	N/A	0.32	0.34	0.12	N/A
Root Feeding Nem. ($\#\cdot\text{g}^{-1}$)	0.17	1.24	0.45	N/A	0	0	0.08	N/A

Table A2.24. Results of soil community assay on soil collected for the greenhouse experiments from the spotted knapweed (*Centaurea maculosa* auct. non Lam.) site and the diffuse knapweed (*C. diffusa* Lam.) site in central Washington, U.S.A. This table includes results of the assay performed 3 July 2002 (at the time of planting, and after autoclaving treatments) on soil collected from the field sites on 30 November 2001.

	-----Spotted Knapweed Site-----				-----Diffuse Knapweed Site-----			
	Core	Perimeter	Intact Native	Autoclaved Native	Core	Perimeter	Intact Native	Autoclaved Native
Active Bacterial Biomass ($\mu\text{g}\cdot\text{g}^{-1}$)	N/A	N/A	15.6	13.2	N/A	N/A	30.8	27.9
Total Bacterial Biomass ($\mu\text{g}\cdot\text{g}^{-1}$)	N/A	N/A	55.6	49.4	N/A	N/A	62.2	57.7
Active Fungal Biomass ($\mu\text{g}\cdot\text{g}^{-1}$)	N/A	N/A	3.9	32.1	N/A	N/A	5.3	25
Total Fungal Biomass ($\mu\text{g}\cdot\text{g}^{-1}$)	N/A	N/A	66.9	94.2	N/A	N/A	153.0	71.3
Flagellate Protozoa ($\#\cdot\text{g}^{-1}$)	N/A	N/A	1,609	0	N/A	N/A	514	6
Amoebae Protozoa ($\#\cdot\text{g}^{-1}$)	N/A	N/A	3,219	3,872	N/A	N/A	475	612
Ciliate Protozoa ($\#\cdot\text{g}^{-1}$)	N/A	N/A	0	0	N/A	N/A	7	162
Total Nematodes ($\#\cdot\text{g}^{-1}$)	N/A	N/A	2.81	0	N/A	N/A	3.73	0.01
Bact. Feeding Nem. ($\#\cdot\text{g}^{-1}$)	N/A	N/A	0.99	0	N/A	N/A	1.99	0
Fungal Feeding Nem. ($\#\cdot\text{g}^{-1}$)	N/A	N/A	1.42	0	N/A	N/A	0.46	0.01
Fung./Root Feeding Nem. ($\#\cdot\text{g}^{-1}$)	N/A	N/A	0.06	0	N/A	N/A	0.61	0
Root Feeding Nem. ($\#\cdot\text{g}^{-1}$)	N/A	N/A	0	0	N/A	N/A	0.38	0

Table A2.25. Results of soil community assay on soil collected for the greenhouse experiments from the spotted knapweed (*Centaurea maculosa* auct. non Lam.) site and the diffuse knapweed (*C. diffusa* Lam.) site in central Washington, U.S.A. This table includes results of the assay performed 15 October 2002 (at the end of the experiment when greenhouse plants were harvested) on soil collected from the field sites on 30 November 2001.

	-----Spotted Knapweed Site-----				-----Diffuse Knapweed Site-----			
	Core	Perimeter	Intact Native	Autoclaved Native	Core	Perimeter	Intact Native	Autoclaved Native
Active Bacterial Biomass ($\mu\text{g}\cdot\text{g}^{-1}$)	16.4	20.6	28.2	48.3	21.2	35.6	35.2	38
Total Bacterial Biomass ($\mu\text{g}\cdot\text{g}^{-1}$)	112	138	183	153	143	141	127	104
Active Fungal Biomass ($\mu\text{g}\cdot\text{g}^{-1}$)	4.7	6.7	16.1	4.3	10.7	11.6	11.5	9.8
Total Fungal Biomass ($\mu\text{g}\cdot\text{g}^{-1}$)	125	173	147	31	70	61	149	90
Flagellate Protozoa ($\#\cdot\text{g}^{-1}$)	684	3,329	7,545	30,412	553	1,592	5,560	302
Amoebae Protozoa ($\#\cdot\text{g}^{-1}$)	684	1,664	3,636	50,523	52	41,019	5,146	46,482
Ciliate Protozoa ($\#\cdot\text{g}^{-1}$)	38	38	8	392	0	32	56	464
Total Nematodes ($\#\cdot\text{g}^{-1}$)	0.98	1.80	1.25	0.04	0.23	0.68	0.64	0
Bact. Feeding Nem. ($\#\cdot\text{g}^{-1}$)	0	0.04	0.09	0.02	0.03	0.01	0.10	0
Fungal Feeding Nem. ($\#\cdot\text{g}^{-1}$)	0.98	1.72	0.34	0.01	0.15	0.64	0.39	0
Fung./Root Feeding Nem. ($\#\cdot\text{g}^{-1}$)	0	0.04	0	0	0.04	0.03	0.02	0
Root Feeding Nem. ($\#\cdot\text{g}^{-1}$)	0	0	0	0	0.02	0	0.15	0