



Soil nutrient availability as a mechanistic assessment of carbon addition and biological control of spotted knapweed (*Centaurea maculosa* Lam.)
by Michel Rene Brockington

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Land Resources and Environmental Sciences
Montana State University
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Abstract:

Invasive plant infestations alter indigenous plant communities, and have serious ecological and economic consequences. Management strategies aim to alter the processes driving plant communities to favor desired species. The goal of this research was to investigate a mechanistic approach to large-scale weed problems. Two studies examined: 1) the ability of carbon to reduce soil nutrients and invasive plant growth, and 2) insect biological control agent impacts on a spotted knapweed population and associated soil nutrient availability changes. The objective of the first study was to lower soil nutrients by adding a carbon source. We hypothesized that increasing the amount of sucrose would reduce soil nutrients and spotted knapweed growth. Sucrose addition reduced spotted knapweed biomass, yet led to soil nitrogen increases. This result may be attributed to a flush of nutrients after initial microbial immobilization. Our results suggest that if sucrose is used in research, high amounts (>70 gC/m²) must be coupled with frequencies of 30 days or less in order to sustain immobilization of limiting nutrients. The second study investigated the effect of an insect biological control agent, *Cyphocleonus achates* Fahr. (Coleoptera: Curculionidae), on soil nutrients and spotted knapweed (*Centaurea maculosa* Lam.). We hypothesized that spotted knapweed growth would increase with N-addition and decrease with sucrose addition, and that soil N would increase with the addition of *C. achates* where N is most limited. With one year of introduction, spotted knapweed aboveground biomass increased in response to *C. achates*, suggesting spotted knapweed may have compensated for insect infection. In a long-term experiment, soil ammonium decreased and biomass increased in response to *C. achates*. Uninfected individual plants may have responded to available resources prevented from acquisition by infected plants. This may suggest that sufficient insect density must be established to infect a majority of plants for an overall growth decline. Natural enemy impact on a target weed population may potentially be predicted from its influence on soil resources. Prediction accuracy will likely be improved where either weevil densities or infection intensities are considered.

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

Invasive plant infestations alter indigenous plant communities, and have serious ecological and economic consequences. Management strategies aim to alter the processes driving plant communities to favor desired species. The goal of this research was to investigate a mechanistic approach to large-scale weed problems. Two studies examined: 1) the ability of carbon to reduce soil nutrients and invasive plant growth, and 2) insect biological control agent impacts on a spotted knapweed population and associated soil nutrient availability changes. The objective of the first study was to lower soil nutrients by adding a carbon source. We hypothesized that increasing the amount of sucrose would reduce soil nutrients and spotted knapweed growth. Sucrose addition reduced spotted knapweed biomass, yet led to soil nitrogen increases. This result may be attributed to a flush of nutrients after initial microbial immobilization. Our results suggest that if sucrose is used in research, high amounts ($>70 \text{ gC/m}^2$) must be coupled with frequencies of 30 days or less in order to sustain immobilization of limiting nutrients. The second study investigated the effect of an insect biological control agent, *Cyphocleonus achates* Fahr. (Coleoptera: Curculionidae), on soil nutrients and spotted knapweed (*Centaurea maculosa* Lam.). We hypothesized that spotted knapweed growth would increase with N-addition and decrease with sucrose addition, and that soil N would increase with the addition of *C. achates* where N is most limited. With one year of introduction, spotted knapweed aboveground biomass increased in response to *C. achates*, suggesting spotted knapweed may have compensated for insect infection. In a long-term experiment, soil ammonium decreased and biomass increased in response to *C. achates*. Uninfected individual plants may have responded to available resources prevented from acquisition by infected plants. This may suggest that sufficient insect density must be established to infect a majority of plants for an overall growth decline. Natural enemy impact on a target weed population may potentially be predicted from its influence on soil resources. Prediction accuracy will likely be improved where either weevil densities or infection intensities are considered.

CHAPTER 1

INTRODUCTION

Invasion by nonindigenous, undesirable species is a serious ecological and economic threat to North American rangeland ecosystems. Millions of hectares of grassland throughout western North America are dominated by monotypic stands of invasive weeds (Sheley and Petroff 1999). Large-scale infestations are associated with reduced livestock and wildlife habitat (Olson 1999), increased surface runoff, erosion, and stream sedimentation (Lacey et al. 1989), lower species diversity (Kedzie-Webb et al. 2002) and greatly compromised land use. These effects can have devastating economic costs. In fact, weeds in rangeland cost nearly \$2 billion annually in the U. S. alone, which is more than all other pests combined (DiTomaso 2000). In addition to reducing species diversity at various trophic levels, nonindigenous species infestations are deleterious to the organization, structure and function of plant communities (Olson 1999), and may be responsible for disruption of nutrient cycles (Olson 1999, but see Svejcar and Sheley 2001).

The ultimate goal of large-scale weed management is to develop and maintain healthy, weed-resistant plant communities, while providing for other land use objectives such as forage, wildlife habitat, or recreation (Sheley et al. 1996). Functionally diverse communities with maximum niche occupation capture a greater proportion of the system's resources (Carpinelli 2000). This preempts their use by invasive species, and maintains ecosystem health and productivity (Sheley et al. 1996). To address this goal,

ecologically-based management strategies must focus on directing weed-infested grasslands toward a more desired, functioning plant community. Understanding the influence of land management practices on the organization, structure, and function of plant communities is central to ecologically-based management (Sheley and Rinella 2001). The aim of invasive plant management research must be to understand and modify the mechanisms directing the organization, structure, and function of plant communities (Olson 1999). Mechanistic knowledge of trophic-level interactions should provide an ecologically-based framework for greater management efficacy (Sheley et al. 1996, Davis and Pelsor 2001).

Though complex and dynamic, plant ecology requires an understanding of the mechanisms that drive plant community dynamics that may be manipulated and used to predict management outcomes (Luken 1990, Tilman 1990, Kedzie-Webb et al. 2002). Management and research practices that alter the most important mechanisms driving plant community dynamics will have a greater probability for success (Toner and Keddy 1997). The overall goal of this research is to investigate soil nutrient availability as a mechanism driving an invasive plant population.

In western semiarid grasslands of North America, competition for limited soil resources, especially nitrogen (N) and phosphorus (P), is an important mechanism among interspecific plant interactions (Chapin 1980, Redente et al. 1992, Vitousek and Farrington 1997, Kolb et al. 2002). Competition for nutrients in limited supply may be an important factor driving plant community dynamics (Wedin and Tilman 1990, Redente et al. 1992). Competition models offer phenomenological, rather than

mechanistic, models that are useful for predicting site- and species-specific competitive outcomes. Tilman (1981) provides a more mechanistic theory for predicting the outcome of competition based on the unique abilities of plant species to acquire resources. He proposed that as the limiting resource diminishes below a critical level (R^*), only species capable of continuing to acquire that resource persist. Tilman and Wedin (1991b) studied a range of species with different successional niches and found that late seral species were very competitive for N. Early seral species were poor competitors for N, yet persisted by maintaining rapid growth rates and high seed production. When grown in pairwise competition experiments, the late seral species displaced early to mid seral species (Tilman and Wedin 1991a). Species populations, therefore, have differing abilities to lower soil quantities of extractable ammonium and nitrate, and this accounted for the results of the competition trials.

Herron et al. (2000) found in a greenhouse study that two early seral species, bottlebrush squirreltail (*Elymus elimoides* (Nutt.) Smith) and annual rye (*Secale cereale* L.), sequestered soil N in their tissue driving the total plant available soil N to a level lower than the R^* of spotted knapweed (*Centaurea maculosa* Lam.). Bluebunch wheatgrass (*Pseudoroegneria spicata* (Pursh) Scribn. & Smith), a late seral species, may have had a lower R^* for N than spotted knapweed, and, therefore, competitively dominated in the limited-resource environment (Herron et al. 2000). Field studies are needed to elucidate this and other mechanisms associated with invasive plant ecology (Williamson 1999).

Resource availability associated with invasive plant populations may be an integrator of climatic, abiotic, and plant resource allocation factors contributing to plant community dynamics. Generally, dominance by invasive plants is correlated with excess plant-available soil nitrogen (N) (Alpert and Maron 2000, Lejeune and Seastedt 2001, Kolb et al. 2002), either from human application or small-scale disturbances creating small areas of high N available (Tyser and Key 1988). Traits common to many invasive species such as rapid growth, early emergence, and high seed production allow them to preempt N and other important nutrients from slower-growing, competitive species adapted to low nutrient conditions (Grime 1977, Redente et al. 1992, Lambers et al. 1998, Paschke et al. 2000, Blicker et al. 2002). In addition, invasive and/or early seral species are thought to be poor competitors for nutrients compared to later seral species (Grime 1977, Chapin 1980, Lowe et al. 2002). Spotted knapweed is one of the most aggressive nonindigenous forbs, dominating millions of hectares of western grassland ecosystems (Sheley et al. 1999). Spotted knapweed exhibits many early seral characteristics, yet can invade late seral communities when small disturbances induce patches of high N (Tyser and Key 1988).

Specifically, the overall objective of this research was to investigate the availability of soil N and P as a mechanistic factor affecting spotted knapweed populations, and to assess the potential of using nutrient availability for greater prediction accuracy of management outcomes. Toward this objective, I investigated: 1) the ability of carbon (C) to reduce soil nutrients and invasive plant growth, and 2) the effects of an insect biological control agent on a population of spotted knapweed and associated soil

nutrient availability. In the first experiment, carbon (sucrose) was added to soil in a gradient of sucrose amounts and three application frequencies to better understand the response of spotted knapweed growth to nutrient limitation resulting from immobilization of available nutrients. In the second experiment, manipulations of nutrient supply were combined with an insect biological control agent. Spotted knapweed population growth and soil nutrient responses to insect infection were measured in both the short- and long-term.

The overall objective of study 1 was to manipulate soil resources using carbon (as sucrose) additions to reduce the growth and productivity of spotted knapweed. Carbon addition was used to reduce nutrients low enough to shift the competitive balance in favor of desired species (Paschke et al. 2000). Addition of labile C was expected to limit plant-available soil nutrients and growth via microbial immobilization. The specific objective was to determine the relationship between spotted knapweed growth, and frequency and amount of sucrose addition. We hypothesized that increasing amount and frequency of sucrose application would reduce spotted knapweed aboveground biomass. Additionally, we expected negative effects of both amount and frequency of sucrose on soil nitrate, ammonium, and phosphorus. Currently, there is no available information on whether nutrient manipulation with C addition might be used to manage weed populations (Alpert and Maron 2000). Understanding the effects of nutrient limitation on spotted knapweed populations may allow for better predictive ability of the outcomes of this management tool.

The overall objective of study 2 was to determine the effect of *Cyphocleonus achates* Fahraeus (Coleoptera: Curculionidae), N-addition, and sucrose addition on plant-available soil nutrient content and spotted knapweed growth. Biological control assumes the competitive ability of the target plant species is compromised by a natural enemy introduction (Maron and Vila 2001). A greater understanding is needed concerning biological control effects on the mechanism of limited nutrient competition. We hypothesized that spotted knapweed biomass, cover, and density would increase with N-addition and decrease with sucrose addition. We also hypothesized that plant-available soil N would increase with the addition of *C. achates*, especially in low-N conditions. As the natural enemy stresses the plant and decreases the competitive ability, spotted knapweed's ability to sequester N would decrease, especially where competition for N is a key factor directing community dynamics. This information may provide an initial indication of the potential to use soil nutrient availability as a mechanistic method for predicting the outcome of plant community dynamics prior to management.

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CHAPTER 2

SPOTTED KNAPWEED (*CENTAUREA MACULOSA* LAM.) AND SOIL NUTRIENT RESPONSES TO SUCROSE ADDITIONIntroduction

In western semiarid grasslands of North America, competition for limited nutrients is an important factor driving plant community dynamics (Chapin 1980, Wedin and Tilman 1990, Redente et al. 1992, Vitousek and Farrington 1997). Dominance by invasive plants is often correlated with high soil nitrogen (N) availability (Alpert and Maron 2000, Lejeune and Seastedt 2001, Kolb et al. 2002). Traits common to many invasive species such as early emergence, rapid growth, and high seed production allow them to preempt N from slower-growing, competitive species adapted to low nutrient conditions (Grime 1977, Redente et al. 1992, Lambers et al. 1998, Paschke et al. 2000, Blicher et al. 2002). Spotted knapweed (*Centaurea maculosa* Lam.) exhibits many early seral characteristics and rapidly infests disturbed soils high in N (Sheley et al. 1999b). Within *Centaurea*, a nonindigenous genus that has infested western North America, phosphorus (P) can also be an important nutrient directing population dynamics (Lejeune and Seastedt 2001). Late-successional grasslands of the West are often limited by N and/or P (Lambers et al. 1998, Blicher et al. 2002). One potential strategy to reduce the dominance of spotted knapweed would be to reduce soil nutrient content to favor intense competition with desired species. For example, Herron et al. (2000) found available N was sufficiently reduced to shift the competitive balance from spotted knapweed to bluebunch wheatgrass

(*Pseudoroegneria spicata* (Pursh) Scrib. & Smith) in the presence of a cover crop that reduced plant available soil N.

Carbon (C) addition is also used to similarly reduce nutrients low enough to shift the competitive balance in favor of desired species (Paschke et al. 2000). In low nutrient soils, invasive and early seral species are poor competitors with late seral species that evolved with this soil condition (Grime 1977, Chapin 1980, Lowe et al. 2002). Even small changes in nutrient supply can affect species productivity (Wedin and Pastor 1993), so manipulation of soil microorganisms that control mineralization has been used to achieve nutrient reduction (Jonasson et al. 1996, Paschke et al. 2000, Schmidt et al. 2000, Ruess et al. 2001). Soil microorganisms are mainly C limited and its addition stimulates expanding microbe populations to immobilize N, P, and other mineralized nutrients in their tissues. With C addition, microbial population growth may also become limited by N and/or P. Microbes act as both a source and a sink of these mineralized nutrients (Jonasson et al. 1996, Kaye and Hart 1997, Ruess et al. 2001). Microbial populations have been found to preempt plant uptake for N and P, and may be more competitive than plants for these nutrients (Harte and Kinsig 1993, Jonasson et al. 1996, Kaye and Hart 1997, Jonasson et al. 1999). Therefore, addition of labile C is expected to limit plant available soil nutrients and growth of ruderal plant species and invasive plants with similar growth characteristics.

Various forms of C addition have been used to immobilize N to favor indigenous plant communities (Schmidt et al. 1997, Reeve-Morghen and Seastedt 1999, Alpert and Maron 2000, Paschke et al. 2000, Schmidt et al. 2000, Ruess et al. 2001). Simple sugars

have been used for more rapid immobilization relative to sawdust or other more complex C forms. Jonasson et al. (1996) found that sugar addition stimulated microbial biomass production, and usually found a decline in inorganic N. In Colorado, Paschke et al. (2000) used sucrose to reduce plant available soil N via immobilization, and in four years, increased the rate of succession from cheatgrass (*Bromus tectorum* L.) to late seral, perennial species. They applied 1600 kg C/ha/yr of sucrose every three weeks during the growing season causing a decrease in plant available soil N. While sucrose has yielded a decrease in plant available N, few studies have specifically investigated effects of frequency and rate of sucrose application on soil nutrient content and invasive weed growth. This information could provide better prediction accuracy of plant community structure as a result of soil nutrient supply, and provide valuable information about using sucrose as an experimental method.

Wedin and Tilman (1993) found that relatively small changes in nutrient supply produced strong feedbacks on plant productivity and net mineralization. These responses were species-dependant, thus have consequences on competition and plant community structure. Because spotted knapweed is primarily associated with disturbed, high N environments (Blicker et al. 2002), it may be feasible to lower N and/or P to a level which severely impacts plant growth. Conversely, this plant may exhibit both early seral characteristics coupled with the competitive ability to survive and reproduce in low N conditions. An improved understanding of spotted knapweed's ability to compete for limiting resources, and the effect of C addition on nutrient supply could be important

where management aims to shift the competitive balance in favor of desired, late-seral species.

The overall objective of this study was to manipulate soil resources using C (as sucrose) additions to reduce the growth of spotted knapweed. The specific objective was to determine the impact of application frequency and rate of sucrose on spotted knapweed growth. We chose to add sucrose on a monthly application schedule to enable quick microbial immobilization that would persist throughout the experiment (Schmidt et al. 2000). We hypothesized that increasing application frequency and amount of sucrose would reduce spotted knapweed aboveground biomass. Additionally, we expected a decrease in availability of both frequency and amount of sucrose application on soil nitrate, ammonium, and phosphorus.

Materials and Methods

Plant system

Spotted knapweed is an extremely aggressive, nonindigenous forb that dominates millions of hectares of western grassland ecosystems (Sheley et al. 1999a). This taprooted, rosette-forming perennial in the Asteraceae family currently invades every county in Washington, Idaho, Montana, and Wyoming. Originating from central to eastern Europe, it was introduced to North America in the late 1800s as an alfalfa contaminant and from discarded ship ballast (Sheley et al. 1999b). Large-scale, monotypic stands of spotted knapweed have detrimental ecological impacts, including reduction of trophic-level diversity, decreased livestock forage, increased soil erosion and

stream sedimentation (Lacey et al. 1989); all of which can have serious ecological and economic impacts (DiTomaso 2000). Large monocultures of spotted knapweed are difficult to control (Sheley and Jacobs 1997), and an integrated approach is necessary for sustainable ecological management (Sheley and Petroff 1999).

Soil and plant materials

The greenhouse study was conducted from February to July of 2002. Field soil from a spotted knapweed-dominated *Festuca idahoensis/Agropyron spicatum* (Mueggler and Stewart 1980) habitat type was collected from the Red Bluff Agricultural Research Station (45° 34'N, 111° 40'W). The area has an elevation of 4960 m, and receives 305 mm annual precipitation. The soil is a Varney clay loam, and is a fine-loamy, mixed frigid Aridic Argiustoll (Table 1). The A horizon, which is the primary biologically active zone, was collected and used for this study. Soil was sieved to remove >10mm rocks and placed in pots. Prior to initiating the study, aqueous solutions from a variety of the sucrose treatments were analyzed for N, and were all found to have similar, trace quantities of the nutrient (data not shown).

Spotted knapweed seeds were collected from Missoula County, MT, in 2000. Tests revealed 95% seed germination. Twenty seeds were sown in 110 (30 x 20 cm) pots in a growth chamber at 21° C day, 10° C night, with 16 hr full spectrum light bulbs (350 $\mu\text{mol}/\text{m}^2/\text{sec}$) placed 15 cm above all pots. To better approximate natural conditions, the pots were moved from the growth chamber to a greenhouse 49 days after plant emergence. Conditions in the greenhouse were 14 hr daylight (natural photoperiod), and temperatures were kept at 22° C day, 18° C night.

Table 1. Soil profile characteristics at Red Bluff Agricultural Experiment Station. Profile was excavated at the site of soil collection, and mostly A horizon soil was used.

Horizon	Depth (cm)	Texture*	Clay (%)	Rock Frag. Kind† (Est. % by volume)	pH	Lime‡
A ₁	0-10	Gr SL	16	HV 15%	7.2	NE
A ₂	10-17	Gr SCL	20	HV 15%	7.0	NE
Bt	17-30	Gr SL	10	HV + QC 25%	7.2	NE
2Bt ₁	30-56	Light CL	28	Trace	7.2	NE
2Bt ₂	56-66	CL	24	Trace	-	NE
2Bk ₁	66-91	Gr L	22	HV 25%	-	SE
2Bk ₂	91-150+	Gr SCL	20	HV 25%	-	VE

*Texture: Gr=gravelly, S=silt, C=clay, L=loam.

†Rock Fragment Kind: HV=hard volcanic, QC=quartz crystals.

‡Lime classes: NE=not effervescent, SE=slightly effervescent, VE=violently effervescent.

Experimental Design

Treatments consisted of two factors: amount of sucrose and frequency of sucrose application. A spectrum of seven low to high sucrose amounts based on literature was added to monocultures of spotted knapweed. Sucrose amounts consisted of 0, 10, 70, 130, 190, 250, 310 and 370 pure C/m² (determined from calculating the percentage of pure C in sucrose and corrected for the amount pure C in table sugar). Each of the seven sucrose amounts was added once (frequency 1), twice (frequency 2), or three times (frequency 3) at approximately 30 day intervals. Treatments were arranged factorially (7 amounts x 3 frequencies) in a randomized complete block design of 21 sucrose treatments plus a control. The experiment was replicated five times.

Procedures

Seedlings were allowed to grow for 30 days prior to treatment application. Sucrose frequency treatments were applied on April 11 (all pots but controls, day 31). Treatments were reapplied on May 20 (day 70) for pots receiving two and three applications, and again on June 19 (day 100) in pots receiving three applications. To ensure that soil water was not a limiting factor, pots were watered slightly below pot capacity every two weeks. Pot water holding capacity was determined by weighing each pot after a 24 hr saturation and re-weighing after about 30 days. The difference in these weights was calculated, and pots were watered with a slightly lower amount (900 mL/pot) to prevent leaching. Sucrose treatments were dissolved in and applied with the water application. To minimize leaching during watering and treatment application, the water and/or sucrose amount was slowly poured into pots, and buckets were placed under each pot to collect any solution. The solution was re-added to the pot until retention was complete. Pots were randomly rotated within each of the 5 replicates (blocks) during watering to minimize position effects. To mimic natural field density, approximately 7 plants/pot were maintained by hand-pulling extraneous plants prior to the first treatment (day 30). Plants were grown for 139 days after emergence.

Sampling

Plant growth and nutrient content. In the highest sucrose amount and frequency treatments, all seven plants in 12 pots died 27 days prior to the scheduled harvest date (day 139); so extant plant material was harvested, dried (60 °C, 48 hr) and weighed on

day 112. Soil nutrients were measured in all 110 pots at this time (Table 2). The remaining 98 pots were harvested for above-ground biomass on July 21 (day 139). Plants were clipped at the soil surface, dried (60 °C, 48 hr), and weighed to determine biomass. Spotted knapweed plant tissue was ground to pass a 1 mm sieve, and analyzed on a Leco® CN2000 analyzer (Leco Corporation, St. Joseph, MO) for percent N by combustion at 1150 °C.

Soil and microbial sampling. Soil was analyzed for extractable nitrate and ammonium at the time of field collection ($\text{mean}_{\text{nitrate}}=1.2$ mg/kg soil, $\text{SD}=0.04$; $\text{mean}_{\text{ammonium}}=1.52$ mg/kg soil, $\text{SD}=0.51$). Soil was sampled on 1 July 2002 (day 112) for extractable nitrate and ammonium. At the end of the study (21 July 2002, day 139), soil was re-sampled for extractable nitrate, ammonium, and P. If not indicated, other soil data were obtained on day 139 at final harvest. Soil was sampled using a 1-cm diameter auger bored approximately 10 cm deep. Cores were placed in soil bags and transferred to a drying oven, where samples were kept at 60 °C for 48 hr. The samples were then ground and passed through a 2 mm sieve. Nitrate was determined from a KCl extraction using cadmium reduction, and ammonium was analyzed using flow-injection analysis (Clesceri et al. 1998). Plant available P was analyzed using NaHCO_3 extraction (Olsen et al. 1954). To investigate trends in microbial population differences, soil was also analyzed by treatment for total microbe numbers. At pot harvest, a single soil sample was obtained for each treatment by combining approximately 0.3 L soil from each of the five replications. These 21 samples were analyzed for plate counts of total colony-forming units. Agar plate counts of colony-forming units are commonly used to measure

microbial growth after C additions (Alden et al. 2001). Total heterotrophic plate counts were performed by soil extraction with sterile 0.1% tetrasodium pyrophosphate dispersant. Ten-fold serial dilutions in P-buffered water were plated on standard plate-count agar and incubated for 7-10 d at 25 °C, and colony-forming units (CFU) were counted (Montana Microbiological Services, Inc., Bozeman, MT). Final colonies were expressed in colony-forming units per gram of soil.

Data Analysis

To identify significant relationships between predictor variables, linear regression was conducted. A combination of model simplicity, Mallows' Cp values, R^2 and sum of squares values were used to assess the appropriateness of the models. Dependent variables were spotted knapweed biomass, nitrate and ammonium (2 sampling times), P, plant aboveground tissue N, and microbial counts, and independent variables were sucrose amounts and application frequency. Poor-fitting models were obtained for tissue N, N uptake, and soil nutrient variables; thus, analysis of variance was used to test the effects of sucrose amount and application frequency on these variables. Least squares means were separated using Fisher's protected LSD ($\alpha=0.05$). Data were log-transformed for nitrate, ammonium, and P variables to meet assumptions of normality. For transformed data, untransformed means are presented, with log-transformed mean comparisons at a significance of $p<0.05$. The relationship between biomass and sucrose amount and frequency is presented as a response surface. Because treatments were pooled for microbe analysis, pooled treatment means of independent variables were regressed with the microbe count data ($n = 22$).

Results

Spotted knapweed biomass, uptake, and tissue analysis

Early plant death. Twelve pots in the highest sucrose amounts and application frequencies resulted in complete plant death 27 days prior to final harvest. Per-pot biomass (mean=1.3 g/pot, SD=0.9, Table 2) and per plant biomass (mean=0.23 g/plant, SD=0.13) were low in these pots. Nitrate and ammonium in the 12 pots were variable (mean_{nitrate}=3.5, SD=4.1, mean_{ammonium}=3.9 mg/kg, SD=2.5). Soil P was within the range of the data for the rest of the pots (mean_P=14.1, SD=7.5). These 12 pots all received >250 g C/m² and 2 or 3 applications of sucrose. Five of the pots received 310 g C/m² with four of these receiving 3 applications, and six received 370 g C/m² with four receiving 3 applications.

Table 2. Actual data for 12 pots that died prior to termination of the experiment.

Pot #	Pure C added (g/m ²)	Application frequency	Biomass per pot (g)	Biomass per plant (g)	Soil nitrate (mg/kg)	Soil ammonium (mg/kg)	Soil phosphorus (mg/kg)
78	250	3	3.4	0.5	5.8	0.9	23.6
86	310	2	1.6	0.2	4.8	8.3	8.5
92	310	3	2.7	0.4	2.5	2.2	19.8
93	310	3	1.1	0.2	1.0	4.9	6.7
94	310	3	2.3	0.3	0.2	2.8	10.3
95	310	3	1.6	0.2	1.1	1.8	8.9
102	370	2	0.6	0.1	15.6	3.7	23.3
105	370	2	1.9	0.3	1.7	9.7	10.8
106	370	3	2.0	0.3	6.0	2.2	29.7
107	370	3	0.5	0.1	0.6	3.9	10.5
109	370	3	0.2	0.03	0.6	3.9	6.7
110	370	3	0.5	0.1	2.1	3.3	10.4

Biomass. Sucrose amount and application frequency were negatively related to aboveground per plant and total pot spotted knapweed biomass (Figure 1 and 2). For every 70 g C/m² added, spotted knapweed per-plant biomass decreased by <0.01 g/plant, and with each application frequency, per-plant biomass decreased by 0.18 g/plant (Figure 1). For every 70 g C/m² added, spotted knapweed total pot biomass decreased by 0.01 g/pot, and decreased by 1.20 g/pot with every application frequency (Figure 2).

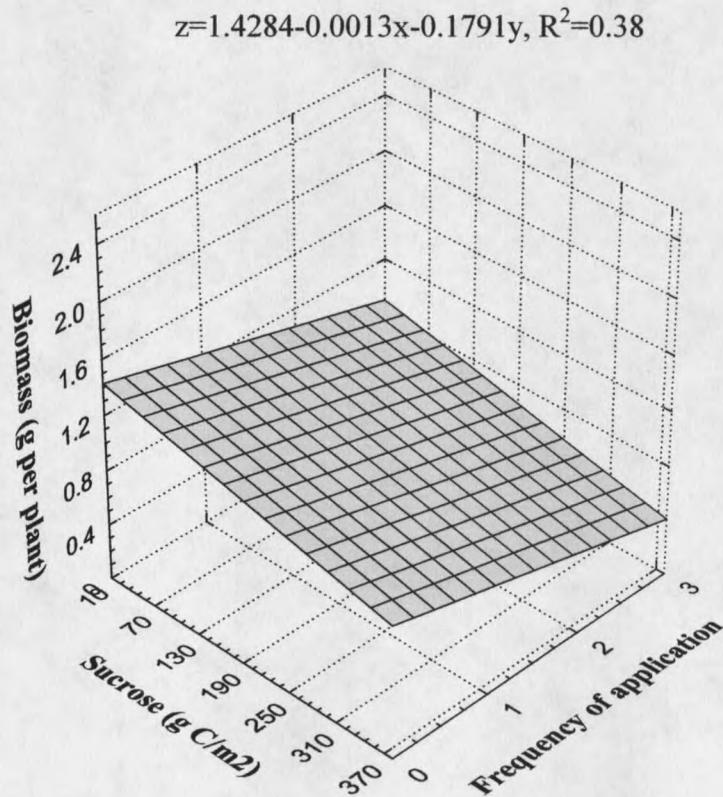


Figure 1. Relationship between spotted knapweed per plant biomass (g/plant, z-axis), frequency of application (0, 1, 2, or 3 applications, x-axis), and sucrose amount (g C/m², y-axis).

